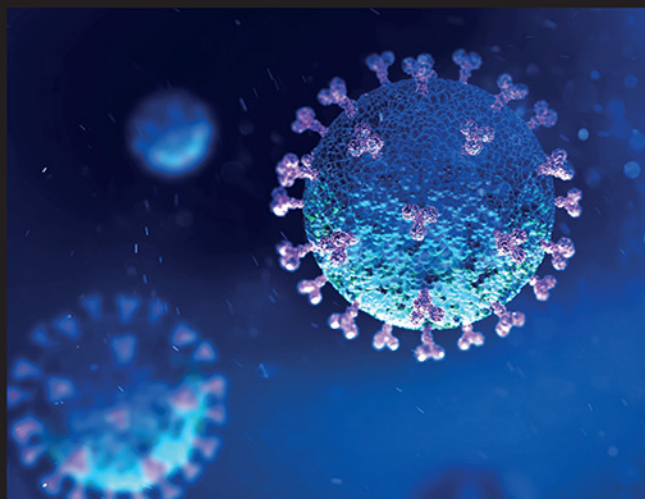




PATHOGENIC CORONAVIRUSES OF HUMANS AND ANIMALS

SARS, MERS, COVID-19, and Animal
Coronaviruses with Zoonotic Potential

Lisa A. Beltz



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Coronaviruses with Zoonotic Potential

LISA A. BELTZ, Ph.D.
Microbiology and Public Health



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Dedication

*This book is dedicated to all of those who lost their lives to SARS, MERS, or
COVID-19.*

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Introduction

1.1 Of viruses and men

The best-laid schemes of mice and men oft to go askew —*Robert Burns (1785)*

Many species of coronaviruses infect humans and animals, including livestock (pigs, cattle, dromedary camels, alpacas, llamas, horses, sheep, goats), wild and semi-domesticated animals (bats, rodents, ferrets, minks), and companion animals (cats and dogs). Some of these coronaviruses, including feline enteric coronavirus, ferret enteric coronavirus, canine enteric coronavirus, and alpaca enteric coronavirus, primarily cause disease in the digestive system. Other coronaviruses, such as severe acute respiratory syndrome virus (SARS-CoV), canine respiratory coronavirus, and porcine respiratory coronavirus, primarily cause respiratory disease. Additionally, several coronaviruses, including ferret systemic coronavirus and feline infectious peritonitis virus (FIPV), cause lethal, immune-mediated, inflammatory systemic disease. Furthermore, many coronaviruses can cause severe central nervous system (CNS) diseases. Since humans have closer contact with companion and agricultural animals than with bats or rodents, it would be wise to invest greater resources into investigating the potential of microbes infecting these animals to undergo zoonotic transmission that results in pathology in humans.

The ancestral coronavirus(es) is believed to have arisen and mutated primarily in bats. Bats host a greater and more diverse range of coronaviruses than other animal species.¹ Such natural reservoir hosts of RNA viruses usually have the greatest viral genetic diversity among the possible host species and infection of the reservoir host is asymptomatic in most circumstances.² Dating of various coronavirus lineages indicates that bat coronaviruses are also older than those of other animals.¹ Additionally, the population size of bat coronaviruses is stable but is undergoing exponential growth in other animal groups.¹ Changing virus population size from a stable to an exponential growth state is indicative of interspecies transmission of viruses from their natural reservoir host to alternate hosts.³ It has been suggested by Woo et al.⁴ that bat coronaviruses are the ancestors of most alpha- and beta-coronaviruses, including the human coronaviruses HCoV-NL63, HCoV-229E, MERS-CoV, and SARS-CoV and SARS-CoV-2. The ancestors of HCoV-OC43 and HCoV-KHU1, however, may have originally been present in rodents.^{4,5} Bird coronaviruses are the ancestors of gamma- and delta-coronaviruses.⁴

1.1.1 Coronaviruses of humans

Seven coronaviruses are known to infect humans (Table 1.1). These coronaviruses, including SARS-CoV-2, may cause mild to severe, life-threatening respiratory illness. While some of them may also cause liver, intestinal, cardiovascular, and neurological diseases, they often produce no detectable illness (**asymptomatic**). HCoV-229E, HCoV-OC43, HCoV-HKU1, and HCoV-NL63 typically produce mild respiratory disease, however, they are responsible for about 10% of all hospitalizations of children with respiratory tract infections.^{6,7} HCoV-NL63 infections are generally more severe than those caused by HCoV-OC43 and HCoV-229E.⁸

The first two known human coronaviruses are HCoV-229E, discovered in 1966, and HCoV-OC43, discovered in 1967. They typically cause mild upper respiratory tract illnesses and cause 10%–30% of the cases of the common cold.⁹ Co-infection occurs between HCoV-229E and other respiratory disease viruses, such as human metapneumovirus or respiratory syncytial virus, and the combination of viruses may increase the extent of lung damage.¹⁰ It should be noted that these “respiratory system disease” viruses infect and damage the human CNS to a greater or lesser extent. For example, HCoV-229E may act as an autoimmune trigger for CNS diseases, including multiple sclerosis (MS).^{11,12} Aminopeptidase N (APN), the host cell receptor used by HCoV-229E, is expressed on nerve synapse membranes and may provide a means for this virus to enter the CNS.

Two other human coronaviruses, HCoV-NL63 and HCoV-HKU1, discovered in 2004 and 2005, respectively, generally are also responsible for mild respiratory illness. Occasionally, however, these human coronaviruses may cause serious diseases, especially in immunosuppressed patients and infants. Additionally, HCoV-NL63 is a major cause of **croup** in children and HCoV-OC43 may contribute to the production of pneumonia¹³ and

TABLE 1.1 Coronaviruses of humans.

Coronavirus	Type of coronavirus	Host cell receptor	Disease
HCoV-229E	Alphacoronavirus	APN ^a	Common cold
HCoV-OC43	Betacoronavirus Lineage A	N-acetyl-9-O-acetylneuraminic acid receptor	Common cold Rarely neurological disease
HCoV-HKU1	Betacoronavirus Lineage A	N-acetyl-9-O-acetylneuraminic acid receptor	Common cold Rarely pneumonia
HCoV-NL63	Alphacoronavirus	ACE2 ^b	Croup
SARS-CoV	Betacoronavirus Lineage B	ACE2	Severe acute respiratory syndrome (SARS)
MERS-CoV	Betacoronavirus Lineage C	DPP4 ^c	Middle East respiratory syndrome (MERS)
SARS-CoV-2	Betacoronavirus Lineage B	ACE2	COVID

^aAminopeptidase N.

^bAngiotensin-converting enzyme 2.

^cDipeptidyl peptidase 4.

severe neurological diseases, such as **chronic demyelinating disease** and **acute encephalomyelitis**.¹⁴ HCoV-HKU1 is also able to cause inflammation of the bronchial tubes of the lungs and pneumonia.¹⁵ These coronaviruses appear to be spread by the human-to-human respiratory route.

The other three known human coronaviruses are more likely to cause severe to fatal respiratory system disease as well as attack other organ systems. SARS-CoV caused a major outbreak of severe acute respiratory syndrome (SARS) in 2002–2003.¹⁶ The outbreak began in China and then spread worldwide before disappearing entirely in 2004. Some of the final cases were laboratory-associated. Middle East respiratory system coronavirus (MERS-CoV) was first reported in Saudi Arabia in 2012.¹⁷ It is currently endemic in the Middle East and parts of Africa and Europe. SARS-CoV-2 is the causative agent of COVID-19. It appears to have first emerged in China in late 2019 and remains, at the time of this writing in September 2021, a pandemic that has killed millions of people throughout the world. COVID-19 was designated by the World Health Organization to be a public health emergency of international concern on January 30, 2020, and as a **pandemic** on March 11, 2020.¹⁸

While human coronaviruses are transmitted primarily via respiratory secretions, some of these viruses appear to be currently transmitted solely between humans. Coronaviruses entered human populations via zoonotic transmission from animal intermediate host species, including several species of wild cats, wild dogs, dromedary camels in the Arabian Peninsula, and perhaps pangolins in China. A more detailed, comparative description of human and animal coronaviruses is found in [Appendix I](#).

1.1.2 Factors affecting zoonotic transmission of coronaviruses

The numbers of known human and animal coronavirus have been increasing recently, such as SARS-CoV-2, with rapid increases in the numbers of those testing positive for viral antibodies and deaths. This increase is partially due to increased concern, testing, and detection of zoonotic transfer of animal viruses into people, and partially due to increased human-to-animal contact in many regions of the world that have been transformed from forest to crop production. While the risk of zoonotic transmission affects countries throughout the world, it is a particularly great concern for people living in crowded conditions in low-income regions that lack proper sanitation and clean water supplies.¹⁹ Some coronaviruses are shed in the feces or urine of infected animals that then enter the food and drinking water of people who may already be ill or malnourished. Many of those living in low-income areas do not have the fuel to cook their food or treat or pasteurize their water or milk. These populations are also at much greater risk of exposure to rodents and the viruses that they may carry.

Infections are also transmitted by consumption of food and liquids as nutrient sources or for medicinal purposes. In Asia, live animal markets (“**wet markets**”) sell many different species of animals in small and often nonhygienic places that may allow exposure of people and many species of animals to infected animals by the respiratory route via droplets and aerosols as well as by inhalation of dust containing dried fecal, salivary, or urinary material. The latter route of infection is also found in the transmission of pulmonary hantaviruses, another type of respiratory virus found in the Americas.¹⁹

Many people live in low-income or remote areas which are not readily accessible to outside help, especially during the wet season, and may lack access to vaccines, medications, healthcare providers, and medical equipment, including ventilators. Masks and gloves are also in short supply. This is not only a problem in **developing countries** but is also a threat to those who live in these unsanitary conditions in the inner-cities and tent-cities of developed countries, including those in North America and Europe.¹⁹ Some populations worldwide are subjected to discrimination and inadequate healthcare, including people living in parts of Appalachia in the United States and the Roma (“gypsies”) in Europe. Those living in refugee camps are also at risk, not only due to the relative lack of doctors and medicines but also from the influx of infected people displaced by war or civil unrest.¹⁹

Some cultural practices also may increase the risk of zoonotic transmission. These practices include shopping for food in live animal markets, drinking raw camel milk, or using camel urine to wash the hair and skin.²⁰ Many people are drawn to cultural traditional medicines or healers that often are ineffective. Many people in the **developed world** also use supplemental vitamins or alternative medicinal compounds of unknown efficacy and whose contents vary among batches rather than medications that have been tested for safety and efficacy and whose components are consistent between batches. Large gatherings of people at weddings and funerals as well as traditional means of preparing the dead for burial also increase the risk of person-to-person contact and the spread of infection as the participants travel back to their homes.²¹ Some increasingly popular practices in the developed world, including drinking unpasteurized milk and juices, also place people at risk of acquiring infectious disease agents.

In the developed world, people living in crowded metropolitan areas, such as Hong Kong and New York City, are more likely to be exposed to infected people. This was seen in Hong Kong during the SARS epidemic and presently in New York City, with its high numbers of COVID-19 cases and deaths. Public transportation, such as buses, airplanes, and subways, may also increase the risk of transmission due to prolonged contact with respiratory emissions of their fellow passengers and indirect contact, as exemplified by people holding onto the poles in buses and subways whose seats are full. It is impractical to sterilize these points of potential infection at each stop. Cruise ships are potential incubators for infectious agents, including SARS-CoV-2 and other groups of viruses such as the “cruise ship” norovirus. Many poorer areas of the world are experiencing increases in workers who are employed in urban centers, followed by regular weekend returns to their villages, bringing novel microbes along with them. This was a major factor in the transmission of human immunodeficiency virus-1 (HIV-1) in parts of sub-Saharan Africa.²²

People living in agricultural regions are also at risk of infection due to their proximity to pest animals, such as rodents and monkeys in the fields that eat the crops, as well as rodents and insects inhabiting human dwellings and releasing their excreta (feces, urine, and saliva) or biting the inhabitants, respectively. Dried saliva may become aerosolized by sweeping the floors and then being inhaled by the human residents. This is not only a concern in rural areas where the dwellings have dirt floors but also in vacation homes in wealthier regions and people camping in trail shelters.²³ Dwellings in low-income areas of the **developing world** may also have thatch roofs that are inhabited by rodents or insects and may lack doors or intact screened windows. Even people in developed regions may

also choose to leave their doors or windows open, as is seen in restaurants in parts of the southern and coastal United States. Rodents may still be found in kitchens of restaurants or homes that do close intact windows and doors. (Install a cheap and effective rodent control system—buy a cat, they'll enjoy the hunt!)

It has been postulated that SARS-CoV-2 may display seasonality, since many respiratory infections occur during the winter, decline during warmer seasons, and then repeatedly reappear during the following winter(s) for multiple years. This was not the case for the other two highly pathogenic human coronaviruses. During the 2002–2003 SARS epidemic, many disease cases with a high fatality rate were seen that winter, but only four mild cases of SARS were reported in 2004 and no cases have been reported since that time.²¹ The seasonal epidemic pattern is also not applicable to the ongoing MERS epidemic which continues throughout the year and is normally found in the arid, hot region of the Arabian Peninsula. Other notable exceptions among noncoronavirus epidemics include the 2012 Zika outbreak in Brazil, which infected large numbers of people, causing tragic neurological diseases in both fetuses during pregnancy and **Guillain-Barré syndrome** in some adults. Zika virus-associated illness did not return in large numbers the following year or afterward.²⁴ Even the 1918 Spanish influenza, which killed tens of millions of people worldwide, did not return in 1919.

1.2 A brief introduction to viruses

1.2.1 Characteristics of viruses

Almost all viruses are extremely small—small enough to pass through 0.2 μm pore-size filters that remove bacteria and other microbes. Unlike bacteria and all other forms of life, they are not composed of cells and lack **plasma membranes**. Some viruses, including coronaviruses, are surrounded by an **envelope**. Drugs that kill bacteria, the antibiotics, do not kill or inactivate viruses. A small number of antiviral drugs have been produced, but they are often toxic to human cells. Viruses that mutate quickly are also able to change their properties enough so that a given antiviral drug or vaccine is no longer effective, as discussed below.

The genetic information of **prokaryotes** (such as bacteria) and the **eukaryotes** is **deoxyribonucleic acid (DNA)**. Viruses belong to neither of the above groups and some categories of viruses, including coronaviruses, HIV, and hepatitis C virus, use **ribonucleic acid (RNA)** as their genetic material. Another difference between prokaryotes and eukaryotes is in the location of DNA, which is found in the nucleus of eukaryotes and the nucleoid region of cytoplasm in bacteria. DNA in eukaryotes and some viruses take the form of a “double-helix” whose two strands resemble a spiral staircase. The nucleus of eukaryotes contains the vast majority of cellular genes. The genes are composed of the **nucleotides** adenine, thymine, guanine, and cytosine. During the process of **transcription**, eukaryotic and prokaryotic genes serve as blueprints for the production of several types of RNA and occur within the nucleus or the nucleoid region, respectively. The various types of RNA form single strands that fold into highly complex structures. RNA is composed of the **nucleosides** adenosine, uracil, guanosine, and cytosine, which are very similar to the

nucleotides of DNA. After their production, some types of RNA travel into the **cytoplasm** of the cell where, in association with **ribosomes**, they produce proteins by the process of **translation**. Proteins provide much of the structure of cells and, via **enzymes** and **hormones**, direct most of the cells' functions. By contrast, the genetic information of viruses may be single- or double-stranded DNA or single- or double-stranded RNA and exist within a viral nucleocapsid. Viral DNA or RNA contain only a small number of genes necessary for them to infect cells and **replicate** (reproduce).

The coronaviruses RNA genome encodes a variable number of structural and nonstructural proteins (Nsp's). Most of the 5' terminal of their genomic RNA encodes the RNA polymerase protein and the remainder of the genomic RNA encodes four to five structural proteins, the S (spike), E (envelope), M (membrane), and N (nucleocapsid) proteins, together with several nonstructural and accessory proteins which vary in number among various viral species and even within the lineages of individual virus species.²⁵ Some beta-coronaviruses have the fifth structural protein, haemagglutinin esterase (HE).²⁶

1.2.2 Overview of mutations and recombination in viruses

To be able to successfully function and replicate, viruses must find ways to evade the immune cells, antibodies, drugs, and vaccines. Often mutations of the viral DNA or RNA allow this evasion to occur. Mutations, in general, are detrimental to eukaryotic cells, either resulting in their death or altering their functions. However, in the case of RNA viruses, mutations may be beneficial since they decrease the ability of the host's adaptive immune response to recognize them. Additionally, mutations may reduce the effectiveness of drug therapy. This high mutation rate is possible since viruses multiply very rapidly. Due to the large degree of diversity in the genome of some RNA viruses, individual people may not be infected by only one variant of a viral species but by a "swarm" of closely related variants, many of which are nonfunctional or are rapidly killed by the host immune response. A small minority of these mutated viruses, however, are better suited for survival and can be more pathogenic to their hosts. This appears to be the case for HIV and may be true for other rapidly mutating RNA viruses as well, including coronaviruses.

RNA viruses, in general, mutate at a greater rate than DNA viruses, partially due to the high error rate of the **RNA-dependent RNA-polymerase (RdRp)** enzyme used during replication. Examples of RNA viruses that mutate rapidly include HIV, influenza viruses, and, to a somewhat lesser extent, coronaviruses. Nucleoside substitutions are typically four times more common than insertions or deletions.²⁸

Relative to other single-stranded RNA viruses, coronavirus mutation rates are moderate to high (1 error per 1000–10,000 nucleosides replicated), even though coronaviruses are the only known category of RNA viruses that have a mechanism for proofreading their genomes and correcting mistakes made during replication. This permits coronaviruses to escape producing excessive numbers of genetic errors that would otherwise render their progeny nonviable.^{29,30}

Part of the reason that coronaviruses have relatively high mutation rates is that they contain unusually large RNA strands whose length increases the chance that mutations may occur. Coronaviruses also undergo a high degree of genetic recombination events in

which they swap pieces of their genomic RNA with that of other viruses of the same or different species.^{4,31,32} The high degree of genetic recombination³³ may result from a unique random **template switching** process during genomic replication. Alteration in their genetic material by coronaviruses is described in greater detail later in this chapter.

1.2.3 Viruses and their host receptors

Viruses are **obligate intracellular parasites** that must infect cells to function. Viruses have proteins on their surfaces that bind to **receptors**, specific molecules on the surface of the virus' target cells. Different species of viruses use different molecules that bind to different receptors present only on specific cell types. SARS-CoV uses its S protein to bind to its cellular receptor, **angiotensin-converting enzyme 2 (ACE2)**, found on a subset of human cells, especially several cell types found in the terminal air sacs of the lungs (**alveoli**), cells lining the airways, and cells lining the blood vessels (**endothelial cells**). MERS-CoV uses its S protein to bind to its receptor, **dipeptidyl peptidase IV (DPP4)**, on a different subset of human or animal cells.

Viral binding to their target cell receptors is vital for the virus to enter the cell and direct it to produce more viruses that are then released to infect another cell in a process that is repeated multiple times. Mutations of the genes that code for the viruses' binding molecules or the target cells' receptors affect the ability of the virus to enter the cell. If viruses cannot enter cells, they are rendered **inactive** and cannot function or replicate. Some drugs and **neutralizing antibodies** reduce the binding of viruses to their target cells and thus inactivate the viruses, reducing the severity of the disease and may cure an infected person. Other drugs inactivate viruses by attacking other vital viral functions or by killing infected cells before the viruses contained within them can reproduce.

1.2.4 Baltimore class IV viruses

The **Baltimore Classification System** divides viruses into seven classes based on their type of DNA or RNA genetic information (Table 1.2). Coronaviruses are **Class IV** viruses.³⁴ Class IV viruses use positive single-stranded RNA as their genetic material. To reproduce, Class IV viruses use the virus-encoded enzyme RdRp to produce negative single-stranded RNA and then more positive single-stranded RNA to serve either as the genetic information for progeny viruses or in the production of proteins. Other related viruses in Class IV include: (1) German measles virus, which often kills fetuses if the mother becomes infected while pregnant, (2) polioviruses, which affect muscular activity, crippling people or making them unable to breathe on their own, (3) dengue virus, which causes excruciating bone pain or deadly **hemorrhagic fever**, (4) West Nile virus, which may cause **encephalitis** or **meningitis**, (5) Zika virus, which causes deformities in fetuses if the mother is infected while pregnant, and (6) hepatitis C virus, which may cause chronic liver disease or liver cancer.

1.2.5 Viruses, diseases, and pandemics—victories and failures

Throughout most of human history, smallpox was among the leading causes of death and disfigurement. The causative virus, **variola**, caused a multitude of deep skin lesions

TABLE 1.2 Baltimore classification system.

Baltimore class	Genome	Example(s)
Class 1	dsDNA ^a	Herpesviruses, Smallpox virus
Class 2	ssDNA ^b	Parvoviruses
Class 3	dsRNA ^c	Rotavirus
Class 4	Positive ssRNA ^d	Coronaviruses, Dengue viruses, Zika virus
Class 5	Negative ssRNA	Influenza virus, Rabies virus, Ebola virus
Class 6	ssRNA retroviruses with DNA intermediate	HIV ^e HTLV ^f I and II
Class 7	dsDNA retroviruses with RNA intermediate	Hepatitis virus B

^aDouble-stranded DNA.^bSingle-stranded DNA.^cDouble-stranded RNA.^dSingle-stranded RNA.^eHuman immunodeficiency Virus.^fHuman T Lymphotropic Virus I.

on an infected person. Variola major is the more common and deadly virus, killing approximately 30% of those infected.³⁵ Variola minor causes less severe disease with a fatality rate of about 1%. Unlike **varicella**, the chickenpox virus, smallpox lesions are more numerous on the head and limbs.¹⁹ These lesions scarred people's faces, often permanently. The lesions were the obvious signs of infection and initially are found in the infected person's mouth and throat. Under the surface, however, the virus was attacking the internal organs, often resulting in death. The virus spread rapidly through inhalation when the infected person coughed or sneezed. Variola is also transmitted by contact with scabs or dried scab material on clothing, bedding material, coats, and other objects that contact uninfected people.³⁵

Smallpox altered the course of history in the Americas, killing vast numbers of Native Americans and aiding in the European conquest of the Western hemisphere. The smallpox vaccine, developed at the end of the 18th century, was the first successful vaccine.³⁶ While smallpox vaccines were available for hundreds of years, they were not commonly used. George Washington almost succumbed to smallpox. The Continental Army was hard-hit as well.

Skip ahead to the 1900s, when the vaccine began to be widely used and smallpox was vanishing or eliminated in most parts of the world.¹⁹ Some religious beliefs forbade the use of the vaccine and large pockets of smallpox remained in those areas, acting like smoldering embers that occasionally reignited in areas that had previously eliminated the disease. In some regions of the world, governments forced their populations to take the vaccine. This draconian strategy was difficult to enforce in democracies or republics, including the United States, but enough people were forcibly vaccinated in viral hot spots that **herd immunity** reduced the number of new cases to the point at which even the unvaccinated people remained uninfected.¹⁹ The number of smallpox cases dropped

dramatically and eventually become very rare. When a person did become infected, **ring vaccination** was used, vaccinating people living in the area surrounding the patient and vaccinating the person's contacts as well.

Between the use of mass vaccinations and ring vaccination, the smallpox virus was eliminated from natural transmission. The last natural smallpox outbreak in the United States occurred in 1949. In late 1975, Rahima Banu from Bangladesh was the last person in the world to be naturally infected by variola major and the last person naturally infected by variola minor was Ali Maalinin in Somalia in 1977.^{35,36} In 1980, the World Health Organization declared that smallpox was eradicated from natural transmission.³⁶ This was the first time that any microbe was eradicated from nature worldwide and the virus only remains in very few laboratories that maintain stocks of the virus.³⁵ The eradication of this scourge ranks among the greatest accomplishments of humans. It was possible to eradicate smallpox since the virus did not infect animals and was only transmitted between people.

Poliovirus infection may result in paralysis or death, crippling many survivors throughout the world, some of whom remained wheelchair-bound permanently, including President Franklin Roosevelt of the United States. Other infected people were even more unfortunate and needed to be placed in "**iron lungs**" since they could not breathe on their own.¹⁹ In the mid-1900s, two antipolio vaccines were produced: first, the Salk vaccine that used killed viruses, and later, the Sabin vaccine that used a live, **attenuated virus** (a harmless form of the virus). Poliovirus type 2 was eradicated in 1999, and wild poliovirus type 3 has not been reported since November 2012.³⁷ The United States has not reported wild poliovirus type 1 cases since 1979.³⁸ These two vaccines eliminated poliovirus type 1 from most of the world, with the exception of some "hot zones" in Pakistan and Afghanistan as of 2020.³⁷ Unfortunately, due to unvaccinated people, the virus occasionally spread from its remaining strong-holds back into regions that had been disease-free. While poliovirus Type I has been almost eradicated, as long as one person is infected, the possibility exists that the disease will return with a vengeance to new areas in addition to those that it had previously occupied.

Several influenza pandemics have been formally declared since the beginning of the 20th century, in 1918, 1957, 1968, and 2009–2010. Before 2009, a disease outbreak was declared to be a pandemic only if it was found in most areas of the world AND led to either great **morbidity** or great **mortality** (suffering or death). The first of these influenza pandemics was the "Spanish Flu" of 1918. It was the single most destructive infectious disease event in history, as about 500 million people became infected, resulting in at least 50 million deaths worldwide and 675,000 in the United States within 2 years.¹⁹ While the death toll of plague, "The Black Death" in the Middle Ages may have killed greater numbers of people, it did so over a much longer period of time. Part of the reason for the rapid spread of 1918 influenza was that the world was in the midst of the First World War. From the virus's origin in a military base in Kansas, United States, infected military personnel were taken to New York before being transported to fight in Europe. This highly contagious virus did not discriminate between the combatants, killing as many soldiers as died during combat.³⁹ The virus spread throughout the world, aided by travel on ships and railroads. It was formed by **interspecies genetic recombination**, in which genes from a bird influenza virus swapped genes with a pig influenza virus and a human influenza virus, forming a **hybrid virus** that was so different from previous influenza viruses that

the immune system did not even partially recognize it.³⁹ Once a person was infected, the virus met no resistance as it quickly traveled to and attacked the lungs.

Influenza viruses bear on their surfaces two proteins, the H (hemagglutinin) and N (neuraminidase) proteins. There are several major types of both the H and N proteins and the name of the influenza strain includes the kind of H and N that it uses.³⁹ The 1918 influenza virus was an H1N1 strain. While this H1N1 variant killed tens of millions of people and caused much suffering to many more, it did not return during the next influenza season.

The year 1957 saw the next influenza pandemic—the “Asian Flu,” an H2N2 influenza strain. Approximately 1.1 million deaths occurred worldwide and 116,000 in the United States.⁴⁰ The degree of suffering was very high as people throughout the world become extremely ill. In 1968, the “Hong Kong Flu” hit and resulted in approximately 1 million cases worldwide and 100,000 in the United States, typically in people over the age of 65 years.⁴⁰ Again, the suffering was very great although the number of deaths was not extremely high. This strain of the virus was of the H3N2 group. While both these influenza strains caused a great degree of suffering, they also did not return to do so the following flu season.

In 1976, another H1N1 flu variant appeared, known as the “Swine Flu.” While the epidemic was not declared to be a pandemic, because the virus was an H1N1 variant, infectious disease experts and healthcare providers assumed the worst. Throughout the world, leaders planned to battle a virus that was predicted to kill tens of millions of people.³⁹ Fortunately, the predictions were wrong. Unfortunately, the experts, working together with governmental agencies, rushed to prepare a vaccine that was specific for this influenza variant. In the United States, huge quantities of the vaccine were produced with a plan to vaccinate every person in the country. They promptly began this vaccination program, immunizing large numbers of people in a very short time period before abruptly halting the program, partially because this H1N1 flu variant was not unusually severe and partially because the hastily produced vaccine was not safe. Over 200 people developed a serious neuromuscular disease, **Guillain-Barré syndrome**, which left many people wheelchair-bound. Because of the panic and in the rush to produce the vaccine, there was no time to adequately test whether it was safe for human use or if it was even effective.³⁹ The dire predictions of massive influenza-related deaths were greatly overestimated, resulting in people being crippled as a result of panic that demanded a vaccination program be implemented quickly by “cutting the red tape” and by-passing the normal safety studies. This important lesson was forgotten just as most people soon forgot that the whole incident had occurred.

In 1961, a new variant of **avian** (bird) influenza began to circulate in terms in South Africa. Much later, in 1997, the first human cases were reported in Asia and, later, several cases in eastern Africa and the Middle East.⁴¹ This H5N1 influenza strain infected and killed millions of Old World birds, including wild birds, chickens, ducks, and geese. Very rarely, humans in close contact with sick or dead birds were infected. About a third of the infected people died.³⁹ Fortunately, this “H5N1 bird flu” was not transmitted from one person to another and the only route of transmitting the virus to people was by contact with sick or dead birds. Due to the huge numbers of dead birds and the high mortality rate in the few humans who did become infected, disease models and infectious disease

experts predicted that this bird flu would spread throughout the world, resulting in 100 million human deaths. Even though the bird flu never infected humans in Europe, the Americas, Australia, and almost all of Africa, people panicked. To lessen the predicated human death toll, millions of uninfected chickens, ducks, and geese were killed in Hong Kong, China, and some other areas of Asia.³⁹ Fortunately, the models again overestimated the number of deaths and instead of 100 million human deaths, less than 700 people, primarily in Asia, have been infected in the ensuing years.⁴¹ Unfortunately, the domestic birds that had provided eggs and meat, as well as a source of income for many impoverished people in Asia, were destroyed.⁴¹ The fear and panic soon subsided and, for most people, the incident was again forgotten.

In 2009–2010, another H1N1 influenza strain rapidly spread throughout the world. Since the 2009 influenza virus was of the same general type as the 1918 influenza, a pandemic was declared. Panic ensued as healthcare providers, infectious disease experts, and governmental institutions assumed the worst, vastly overpredicting the amount of suffering and number of deaths. The models used to predict the impact of this H1N1 variant again proved to be very erroneous since this influenza variant itself did not typically cause excessive suffering and the number of deaths was close to that caused by a typical influenza strain.³⁹ During the first 12 months of the 2009 influenza pandemic, it is estimated that less than 0.007% of the world's population died of H1N1-associated respiratory complications.⁴⁰ The predicted 100 million deaths worldwide did not occur, however, but the ensuing panic threatened to close down public schools and universities if a single person was found to be infected. In the case of this pandemic, while the virus was present throughout the world, it never approached the degree of either morbidity or mortality that was predicted in 2009–2010 or since then. The people soon forgot their fear again and life continued.

Acquired immunodeficiency syndrome (AIDS) is an ongoing pandemic caused by HIV-1, another single-stranded RNA virus.⁴² Since the early 1980s, 79.3 million people have been infected and 36.3 million have died of AIDS.⁴³ HIV has the highest mutation rate of any known microbe, allowing it to circumvent the immune system and defeat researchers who struggle to produce an effective and safe vaccine or drugs. When no single drug is effective against the virus for very long, combination antiviral drug treatment has greatly increased the life span of those infected.⁴⁴

COVID-19 results from infection by a newly emerged coronavirus which has infected over 225 million people and led to the death of over 4.6 million people worldwide as of mid-September 2021.⁴⁵ It is the subject of one of the chapters in this book and its story continues to rapidly change. [Table 1.3](#) compares the modern pandemics.

1.2.6 Vaccination—then and now

The roots of **vaccination** began as long ago as 1000 BC in the battle against smallpox. For centuries, certain parts of Asia practiced **variolation**, a process in which a person is deliberately infected with a mild form of smallpox virus to induce immunity to and decrease the risk of being infected by the more dangerous form of the virus.⁴⁶ This process occasionally killed the recipient.

TABLE 1.3 Human pandemics in modern times.

Organism	Virus class	Virus family	Diseases	Year(s)
H1N1 (Spanish) Influenza	5	Orthomyxoviridae	Severe respiratory disease	1918
H2N2 (Asian) Influenza	5	Orthomyxoviridae	Severe respiratory disease	1957
H3N2 (Hong Kong) Influenza	5	Orthomyxoviridae	Severe respiratory disease	1968
H1N1 2009 Influenza	5	Orthomyxoviridae	Respiratory disease	2009–2010
HIV	6	Retroviridae	Immunosuppression Increased susceptibility to microbes Cancer Dementia	1981-present
COVID-19	5	Coronaviridae	Primarily respiratory	2019-present

In 1796, Edward Jenner noticed that milkmaids rarely developed smallpox lesions or facial disfigurement, but were frequently infected with **vaccinia**, the cowpox virus, a close relative of variola. He began to infect people with cowpox virus, followed several weeks later by infecting the inoculated people with live variola major. His procedure was proven to be effective in preventing smallpox.⁴⁶ He called the process “vaccination” after the Latin word for cow, “vacca.” Vaccination changed the course of infectious disease history forever, leading the way to decreasing infection rates for many microbes and saving countless lives. As described earlier in this chapter, the last naturally occurring case of smallpox was reported in 1977 and, after several years went by with no smallpox cases, vaccinia was declared to be eradicated in 1980, although vials of the virus remain in several select Biosafety Level IV laboratories.

Vaccination has played a leading role in the eradication of smallpox and types 2 and 3 poliomyelitis viruses as well as dramatically decreasing the numbers of deaths from other severe infectious diseases. Nevertheless, many people hesitate or refuse to be vaccinated due to a myriad of reasons. One of the most prominent examples of vaccine-hesitancy is playing out currently with the COVID-19 vaccine. This subject is described in [Chapter 7](#).

1.2.7 Comparison of viruses, bacteria, and eukaryotic cells

There are several general types of living organisms, eukaryotes, bacteria, archaea, and, perhaps, viruses. All living organisms, with the possible exception of viruses, are composed of cells that are surrounded by a plasma membrane; perform complex chemical reactions, such as reproduction and other metabolic functions; and use double-stranded DNA as their genetic information.

Eukaryotes contain membrane-bound organelles, including a nucleus. Members of this group include single-celled protists and multicellular fungi, plants, and animals. Eukaryotes are very diverse, differing greatly in many respects, yet similar in others. Eukaryotic cells are

almost always larger than bacteria and viruses. Most eukaryotes are free-living and can replicate independently, although some are parasitic and require some sort of aid from the host in order to survive. Eukaryotes are usually not killed by antibiotics but are often sickened by antiviral compounds.

Bacteria and **archaea** are single-celled organisms that congregate their double-stranded DNA genome in a nucleoid area. They are much smaller than cells and yet much larger than almost all viruses. Although most bacteria are capable of independent reproduction, a small number of them are parasitic and infect and live within host cells. Many bacteria are killed by at least one type of antibiotic, however, antibiotic resistance is becoming ever more problematic.

Viruses may or may not be considered living organisms. They are not composed of cells and lack a plasma membrane. They use either double- or single-stranded DNA or RNA as their genetic material, but lack a nucleus or nucleoid area, organelles, and cytoplasm. Since they are incapable of replication or performing almost any metabolic function independently, they must infect cells and rely on them to replicate and perform chemical reactions or almost any other activity. Viruses are extremely small and most of them can pass through pores in membranes (“**filterable agents**”). A rare number of viruses are large enough to be seen by a conventional light microscope. Viruses infect the plant and animal cells, bacteria, and even other viruses. They are not inactivated (“killed”) by antibiotics, but most viruses are susceptible to at least some antiviral compounds, in addition to other “**repurposed drugs**” that were produced and approved for activity against some form of life, for example, chloroquine which is approved to treat malaria but also is active against SARS, MERS, and COVID-19. Some microbiologists classify viruses as a form of life, while other microbiologists do not. Whether or not they are classified as alive, viruses infect living organisms, and some cause life-threatening diseases. For a comparison between eukaryotes, bacteria, and viruses see [Table 1.4](#).

1.3 A brief introduction to the immune system

The immune system is divided into several major categories: the **innate** and the **adaptive immune systems**. Both categories are composed of several types of **leukocytes** (white

TABLE 1.4 Eukaryotes, bacteria, and viruses.

Characteristic	Eukaryotes	Bacteria	Virus
Cellular?	Single- or multicellular	Single-celled	Not composed of cells
Has nucleus?	Yes	No	No
Genetic material	DNA	DNA	DNA or RNA
Relative size	Large	Small	Extremely small
Independent replication?	Usually	Yes	No
Killed by antibiotics?	No	Yes	No
Lifestyle	Free-living or parasitic	Free-living or parasitic	Parasitic
Living organism	Yes	Yes	?

blood cells), their secretions, and immune organs (bone marrow, spleen, thymus, tonsils, appendix, and lymph nodes) as well as small groupings of immune cells in the intestine and respiratory tract.

1.3.1 Introduction to the innate immune system

People are born with a functional innate immune system. While its responses are weaker than those of the adaptive immune system, innate immunity rapidly responds to microbial threats. Innate immunity is nonspecific—the same leukocyte may respond to many types of microbial threats as well as cancerous growths. Since innate immunity does not produce **immunological memory**, it does not improve the next time(s) that it encounters the same microbe. Some of the cell types that are part of the innate immune system are **neutrophils**, **monocytes** and **macrophages**, **natural killer (NK) cells**, **basophils** and **mast cells**, and **dendritic cells**, as discussed later.

The complement cascade of the innate immune system is important in killing coronavirus-infected cells. It produces large holes in cells and releases **chemotactic** molecules that draw other leukocytes into the area. Several molecules in the complement cascade also, however, cause a strong proinflammatory, antiviral response that may be pathogenic if excessive or chronic. SARS-CoV infection activates complement and contributes to disease.⁴⁷

1.3.2 The cells of the innate immune system

Neutrophils are the most common type of leukocyte and are our first line of defense against bacterial infections. They are **phagocytes** and ingest and digest unwanted materials, including both microbes and cell debris. They also release powerful digestive enzymes and toxic **reactive oxygen species (ROS)** into the surrounding area to kill material that they do not ingest. ROS are extremely chemically-active derivatives of oxygen, including hydrogen peroxide and the active component of bleach. While these toxic materials kill many extracellular bacteria, they also damage healthy neighboring cells. Neutrophils accumulate in infected areas and contribute to inflammation.

Similar to neutrophils, monocytes are phagocytes that are present in the blood and release toxic materials into infected areas, contributing to inflammation. Monocytes migrate out of the blood and into a wide variety of tissues after about 8 hours. While in the tissues, they mature into macrophages (“large eaters”) and differentiate in a manner that best serves their tissue location (**microglia** are brain macrophages, **osteoclasts** are bone macrophages). Macrophages are larger than monocytes and produce a much more powerful response. These cells activate **CD4⁺ T helper cells** and produce **cytokines** and **chemokines**, which increase or decrease the activity of other immune system components or draw other leukocytes into infected areas, respectively (discussed below). There are two forms of macrophages, M1, and M2, with opposing purposes. **M1 macrophages** are proinflammatory, while **M2 macrophages** are antiinflammatory. M1 macrophages function in the defense against microbial invasion, but may also damage the human cells and tissues surrounding the area, especially if they are chronically active. M2 macrophages help to

prevent excessive inflammatory responses and cell damage, but downregulate antiviral activity as well.

NK cells eliminate microbes indirectly by killing infected cells rather than by killing the microbes directly. By eliminating infected cells, microbes, especially viruses, are killed before they can replicate and infect new cells. NK cells do so by releasing **perforins**, molecules that form pores in the infected cells, and **granzymes**, molecules that induce the infected cells to undergo apoptosis, an orderly self-destructive process. NK cells are among our best cellular defenses against viral infections and their activity is superior to that of most antibodies as well as longer-lasting.

Basophils and mast cells are similar types of cells in the blood and tissues, respectively. They are responsible for inducing some forms of allergies by releasing molecules, such as **histamines** and **leukotrienes**, which produce a variety of reactions, including runny noses, coughing, sneezing, vomiting, asthma, itching, and hives. In large amounts, they may induce fatal **anaphylactic shock**. In response to microbial or other threats and when the level of response is not excessive, however, these reactions are important in the removal of microbes and other inappropriate material from the body, flushing out the sinuses, throat, lungs, and digestive system. Their activity aids in the removal of viruses that cause upper and lower respiratory tract infections, including coronaviruses. While allergic responses range from irritating to deadly, mice lacking functional mast cells are more susceptible to infections since the microbes are not flushed out of their body as efficiently as normal mice.

The primary function of dendritic cells is to activate immature CD4⁺ T helper cells. Without proper dendritic cell functioning, the T helper cell response is low. Since this type of T cell directs most of the antimicrobial activity, much of the rest of the immune response also operates much less effectively, as described later. Unfortunately, some viruses can bind onto dendritic cells and use them to travel to other parts of the body via the circulatory or **lymphatic systems**.

1.3.3 Introduction to the adaptive immune system

The adaptive immune response is not present immediately after birth and only becomes functional in humans at about 6 months of age. While it is much more powerful than the innate immune response, it requires 7–14 days to respond to a threat. Adaptive immunity is extremely specific—a single leukocyte responds to only one small part of a single microbe or cancer cell. This means that a single cell responds to one component of one species of coronavirus and not to other coronavirus species, except in cases of **cross-reactivity**. In these situations, a single immune cell that is specific to one microbe is also partially active against a similar structure on another microbe. An example of this phenomenon was seen in the case of the immune response to the vaccinia virus, which cross-reacted with and provided protection against infection with the virulent variola virus, as discussed earlier. In most cases, however, if the targeted region of the microbe is altered by mutations, as in the case of the influenza virus, the previously effective leukocyte is no longer able to recognize the altered microbe. This is the reason why people may be repeatedly infected by influenza virus variants and need to be vaccinated yearly due to minor or

major shifts in the predominant virus variants. This is also true for the various species and variants of coronaviruses since people who are immune to SARS are not well-protected against other, similar coronavirus diseases, such as MERS or COVID-19.

Since the adaptive immune response does produce immunological memory, its response is much more powerful and longer-lasting the next time(s) that it encounters the same microbe. **T and B lymphocytes** (T and B cells) are the cell types that comprise the adaptive immune system. Vaccines are administered to stimulate the adaptive immune response to produce memory cells and, in so doing, produce a much stronger, more rapid, and longer-lasting response upon exposure to that microbe, typically preventing a person from infection or, in the case of previously infected people, from reinfection. Vaccines inducing virus-specific memory T killer cells show the greatest promise against viral infections.⁴⁸

1.3.4 The cells of the adaptive immune system

The adaptive immune system is comprised of B and T lymphocytes and their products. B cells produce various types of **antibodies** with specialized functions. B cells also help to stimulate T cells to become active. The antibody tests that are used to screen people for current or past COVID-19 infection detect the **IgM** and **IgG** classes of antibodies, as described later.

There are several major types of T cells, including **CD4⁺ T helper cells** and **CD8⁺ T killer cells**. There are many types of T helper cells, some of which conflict with the activity of other T helper cell types. **Th1 cells** are generally proinflammatory and produce inflammatory cytokines that, in excessive amounts, may induce a deadly “**cytokine storm**,” such as that seen during severe COVID-19 infection.⁴⁹ Th1 cells are most active against viruses and cancer cells. **Th2 cells** and their cytokines often work in opposition to Th1 cell activity. Th2 cells are more active against bacterial, rather than viral, infections and trigger the production of certain antibacterial classes of antibodies. **T regulatory cells (Treg)** are a type of T helper cell that downregulates the immune response, preventing excessive, potentially life-threatening allergic or autoimmune reactions.

All types of CD4⁺ T helper cells release cytokines, immune messenger molecules that direct the other parts of the immune system, increasing or decreasing their activity. Some cytokines, the **interferons (IFNs)**, also have direct antiviral activity, while other cytokines induce the production of additional leukocyte types in the bone marrow. CD4⁺ T helper cells also produce chemokines, inflammatory molecules that attract other leukocytes into the infected area. CD4⁺ T helper cells are the keystones of the immune response, directing the activity of all other leukocytes. When they are killed, as occurs during HIV infection, the whole immune response is weakened.

CD8⁺ T killer cell activity in many ways resembles that of NK cells of the innate immune system. They also eliminate viruses indirectly, by killing virus-infected cells. They also release perforins and granzymes to produce pores in infected cells and induce apoptosis. CD8⁺ T killer cells differ from NK cells in that they are very specific, with each T killer cell only active against one small part of one species of virus. Unlike NK cells, T killer cells produce memory cells, so their activity becomes stronger each time that they contact the same virus. They are also one of our best defenders against viral diseases,

including those caused by coronaviruses, and comprise about 80% of inflammatory cells in coronavirus-infected livers.⁵⁰ As an example of the importance of CD8⁺ T killer cells, depletion of these cells, but not CD4⁺ T helper cells, in mouse hepatitis virus (MHV)-infected mice significantly increases viral numbers in the liver early after infection.⁴⁸ Additionally, adoptive transfer of specific, anti-MHV antibodies protects mice from developing encephalitis.⁵¹ The importance of CD8⁺ T killer cell-mediated immunity has also been demonstrated in other CoV infections.⁵² IFN- γ , working in concert with T killer cells, is also important in viral elimination since mice lacking this cytokine do not eliminate the virus from the infected host, but instead develop a chronic, subacute infection.⁴⁸ Unfortunately, CD8⁺ T killer cells also play an important role in lung damage in coronavirus-infected people.⁵⁰ The functions of innate and adaptive immune cells are presented in [Table 1.5](#).

1.3.5 Cytokines and chemokines

Cytokines and chemokines are important immune messenger molecules. Cytokines direct many processes that activate and regulate both innate and adaptive immune system processes. Chemokines are chemotactic and recruit immune system and nonimmune system cells to the areas of infection. [Tables 1.6 and 1.7](#) list the functions of the cytokines and

TABLE 1.5 Types of immune cells and their functions.

Cell type	Type	Form memory cells	Function
Neutrophil	Innate	No	Phagocytic Release toxins outside of cell
Monocyte/ Macrophage	Innate	No	Phagocytic Release toxins outside of cell Activate T helper cells
Natural killer cell	Innate	No	Release molecules that create large pores in cells Release molecules that cause cell to self-destruct Kill virally-infected cells
Basophil/ Mast cell	Innate	No	Release material to flush out nasal and digestive tracts Release material that narrows diameters of airways
Dendritic cell	Innate	No	Prime activator of T helper cells
Plasmacytoid dendritic cell	Innate	No	Secrete large amounts of type I IFN in response to viral infections
B cell	Adaptive	Yes	Produce antibodies
T helper cell	Adaptive	Yes	Release many kinds of cytokines
T regulatory cell	Adaptive	Yes	Downregulate many immune system functions Protects against autoimmune diseases Promotes homeostasis
T killer cell	Adaptive	Yes	Release molecules that produce large pores in cells Release molecules that cause cell to self-destruct Kill virally-infected cells

TABLE 1.6 Cytokine Functions.

Cytokine	Function
G-CSF ^a	↑ production of neutrophils, eosinophils, basophils, mast cells
GM-CSF ^b	↑ production of monocytes, neutrophils, eosinophils, basophils, mast cells
IL-1 ^c	Proinflammatory Induces fever and malaise
IL-4	↑ differentiation to Th2 cells Activation B cell and T cell proliferation ↑ differentiation of B cells into antibody secreting cells ↑ IgE production ↑ MHC class II production
IL-5	↑ B cell growth and secretion of antibodies, especially IgA Activates eosinophils
IL-6	Proinflammatory ↑ production of acute phase proteins such as C-reactive protein and fibrinogen
IL-8	↑ chemotaxis of neutrophils and phagocytosis ↑ endothelial cell survival and proliferation ↑ matrix metalloproteinases production
IL-10	Antiinflammatory Protect against allergy and autoimmune responses Regulate growth and differentiation of lymphocytes, NK cells, mast cells, dendritic cells, keratinocytes, and endothelial cells ↑ differentiation and regulates function of Treg cells
IL-11	Growth factor for megakaryocytes
IL-12	Primes T helper cells and NK cells for high IFN- γ production ↑ Th1 cell differentiation ↑ production of IgG2a and IgG2b antibodies
IL-13	↑ Th2 cell responses Regulates airway hyper-reactivity ↑ tissue remodeling and fibrosis
IL-15	↑ proliferation of NK cells ↑ neutrophil phagocytosis and chemokine production
IL-17	↑ chronic inflammatory responses during autoimmune diseases and allergies ↑ production of chemokines such as CXCL1 and CXCL2
IL-18	Proinflammatory ↑ IFN- γ production
IFN ^d - α	Strong antiviral activity ↓ production of viral proteins
IFN- β	Strong antiviral activity ↓ production of viral proteins
IFN- γ	Strong antiviral activity by inhibiting viral replication Activates macrophages ↑ Class II major histocompatibility complex molecules
IFN- λ	↓ growth of intestinal viruses
TNF ^e - α	Proinflammatory molecule ↑ body temperature Induces excessive weight loss Shock

(Continued)

TABLE 1.6 (Continued)

Cytokine	Function
TGF ^f -β	Antiinflammatory ↑ Treg differentiation ↓ B cell activation ↑ IgA production

^aGranulocyte-colony stimulating factor.^bGranulocyte monocyte-colony-stimulating factor.^cInterleukin.^dInterferon.^eTumor necrosis factor.^fTransforming growth factor.

TABLE 1.7 Chemokine Functions.

Chemokine	Cell type for which chemotaxis occurs	Receptor
IL-8 ^a	Neutrophils	IL-8RA, IL-8RB
CCL ^b 1	Monocytes, NK cells, immature B cells, DC	CCR8
CCL2 (MCP-1) ^c	Monocytes, memory T cells, DC	CCR3, CCR4
CCL3 (MIP-1α) ^d	Neutrophils	CCR1, CCR4 and CCR5
CCL4 (MIP-1β) ^e	Monocytes	CCR5
CCL5 (RANTES) ^f	T cells, inflammatory eosinophils, and basophils	CCR5
CCL6	Resting T cells and monocytes	CCR1
CCL11 (Eotaxin-1)	Eosinophils	CCR3 (CCR2, CCR5)
CCL20	DC, T cells, and B cells	CCR6
CCL22	Monocytes, DC, and natural killer cells	CCR4
CXCL ^g 8	Neutrophils and basophils	CXCR1, CXCR2
CXCL2	Neutrophils	CXCR2
CXCL9 (MIG) ^h	T killer cells, natural killer cells, NKT cells, macrophages.	CXCL9/CXCR3
CXCL10 (IP-10) ⁱ	Monocytes/macrophages, T helper and T killer cells, NK cells, and DC	CXCR3

^aInterleukin-8.^bChemokine (C-C motif) ligand^cMonocyte chemoattractant protein 1.^dMacrophage inflammatory protein-1α.^eMacrophage inflammatory protein-1β.^fRegulated upon Activation, Normal T Cell Expressed and Presumably Secreted.^gChemokine (C-X-C motif) ligand.^hMonokine induced by gamma interferon.ⁱInterferon-inducible protein 10.

chemokines, respectively, that are most relevant in defense against coronavirus infection and diseases.

T helper cells are our primary source of cytokines, although other leukocytes and some other types of cells also produce them. Some of the cytokines cause inflammation, while others decrease it. Some cytokines direct the killing of bacteria. Other cytokines direct the killing of viruses or kill viruses themselves. Tumor necrosis factor- α (TNF- α), interleukin (IL)-6, and IL-1 are among the major proinflammatory cytokines. TNF- α and IL-1 also stimulate the production of fever. TNF- α additionally can cause **wasting** (extreme weight loss) as well as a life-threatening drop in blood pressure that may result in fatal shock. When SARS-CoV-19 binds to microbe-sensing molecules on the surface of immune cells, it stimulates the secretion of IL-1, which indirectly causes the secretion of IFN- β outside of the cells.⁵⁰ IL-1 is a major cause of immune-induced diseases, including damage to the lungs.

IFNs are a group of cytokines so named because they interfere with viral replication. They are very active in the defense against viral diseases, including those caused by coronaviruses. IFN- α and IFN- β are the **type I IFNs** and are produced by both immune and nonimmune cells. High doses of type I and **type III IFNs** are effective against SARS-CoV, MERS-CoV, and perhaps other coronaviruses in cultured cells in vitro and animals in vivo.⁵³ MERS-CoV is much more IFN-sensitive than SARS-CoV in vitro.⁵⁴

IFNs have been used to treat a variety of viral infections, including infection by coronaviruses, either alone or in combination with other compounds, such as chloroquine and hydroxychloroquine. The coronaviruses' N protein indirectly inhibits the production of IFN- α and IFN- β .^{55,56} Several coronavirus Nsp's also interfere with the innate immune response. These include viral proteins nsp3b, 6, and 9b of SARS-CoV; nsp4a and 4b of MERS-CoV; and nsp2 of MHV.⁵⁷ Nsp2 of some coronaviruses encodes the enzyme **phosphodiesterase** which indirectly blocks activation of the host cell's antiviral **2'-5' oligoadenylate-RNase L RNA pathway**. RNase L is an **endoribonuclease** that cleaves viral and cellular single-stranded RNA and is important in the activation of antiviral IFN responses. MHV-nsp2 plays a major role in the liver damage produced by this coronavirus.⁵⁸ HCoV-OC43 encodes a nsp2a that has a high degree of similarity to the corresponding MHV enzyme. MERS-CoV Nsp4b also encodes a phosphodiesterase.²⁵

SARS-CoV and MERS-CoV induce production of only small amounts of IFN in most cell types in vitro, except for plasmacytoid DCs which produce large amounts of type I IFNs.^{59,60} Delayed production of IFNs during SARS and MERS leads to activation of proinflammatory monocyte-macrophages and cytokines in the lungs, resulting in vascular leakage and impaired adaptive immune responses in the lungs of infected people.⁵³

Type II IFN contains only one member, IFN- γ , which is produced by several leukocytes. A growing number of type III IFNs are also found in humans. High doses of type I and type III IFNs decrease levels of SARS-CoV and MERS-CoV in vitro in selected cell lines and also in vivo in animal models of infection.

1.3.6 Antibodies

Five major classes of antibodies are produced by stimulated B cells about 10–14 days after microbial infection. Two of the antibody classes contain multiple subclasses. The antibody classes and subclasses have specialized functions ([Table 1.8](#)).

TABLE 1.8 Antibody classes.

Class	Location	Number of antibody units	Characteristics
Ig ^a G	Blood	1	Most abundant blood Ab in blood Attack blood-borne pathogens Trigger complement cascade Protect fetuses by crossing placenta
IgM	Blood Attached to B cells	5 in blood 1 on B cells	1st Ab produced in primary immune response 1st Ab produced by infants Triggers complement cascade Acts as microbial receptor on cells
IgA	Secretions from mucus membranes	2	Most abundant Ab in secretions Protects against microbes in mucus membranes Present in milk to protect newborns
IgD	Attached to B cells	1	Acts as microbial receptor on cells
IgE	Bound to surface of mast cells and basophils Blood	1	Triggers allergies Stimulates removal of pathogens in mucus membranes

^aImmunoglobulins (antibodies).

IgM is the first type of antibody produced after infection. Later, **IgG** antibodies are made. Testing for the class(es) of antibody that is present in a person helps a health-care professional to determine whether a person is newly infected (IgM) or is late in the course of infection or recovery (IgG). This testing is a major factor in determining infection status for COVID-19 and other coronaviruses. IgM and IgG are present primarily in blood **plasma** and **serum**. **IgA** antibodies, by contrast, are found in areas with **mucus membranes**, including those of the respiratory, digestive, and urogenital systems. IgA is also present in mucus, milk, saliva, and tears. It helps to prohibit microbes from entering through various body openings, including blocking coronaviruses from entry through the nose, mouth, eyes, urinary bladder, urethra, and vagina.

In the body, antibodies have several functions, some are more important during bacterial infections and others, during viral infections. The most important function of an antibody during a viral infection is to prevent the virus from binding to and entering its host's target cells. Neutralizing antibodies perform this function. During coronavirus infection, these antibodies block the viral S protein from binding to its receptor on the host cell before entering the cell.⁶¹ Genetic variation in the part of the S protein that binds to its receptor may help different viral strains to evade the hosts' adaptive immune responses. Neutralizing antibodies are only present for a short time in the blood after the initial infection or vaccination, but B memory cells rapidly produce and return antibodies to high levels after exposure to that specific virus during the second and subsequent exposures to that virus.

High levels of IgG antibodies remain in the blood for months after the person has recovered from a viral infection. Because of this, one treatment option is to administer either plasma or serum from a person who has recently recovered from an infection (**convalescent plasma/serum**)

to an ill person. The antibodies in these fluids may help to decrease the severity of the recipient's disease or reduce the length of infection. Administration of convalescent serum has been used to treat several viral diseases, including those caused by coronaviruses.

1.4 Introduction to coronaviruses

Coronaviruses are Baltimore Class IV viruses of the order Nidovirales, suborder Cornidovirineae, family Coronaviridae, and subfamily Orthocoronavirinae. These viruses are members of four virus genera: *Alpha-*, *Beta-*, *Gamma-* and *Deltacoronavirus*.³⁴ SARS-CoV and SARS-CoV-2 belong to the subgenus *Sarbecovirus* and MERS belongs to the subgenus *Merbecovirus*.⁶² Almost all mammalian coronaviruses are either alphacoronaviruses or betacoronaviruses. Porcine deltacoronavirus is an exception to this rule. Betacoronaviruses have been divided into several lineages: A, B, and C.

Coronaviruses are spherical and have a diameter of 70–80 nm. They utilize several different host cell receptors, but all bind to their target cells via the viral S protein, a transmembrane protein that spans the viral envelope. Three S proteins interact to form the characteristic corona (“crown”) structure for which these viruses are named (Figs. 1.1 and 1.2).⁶³

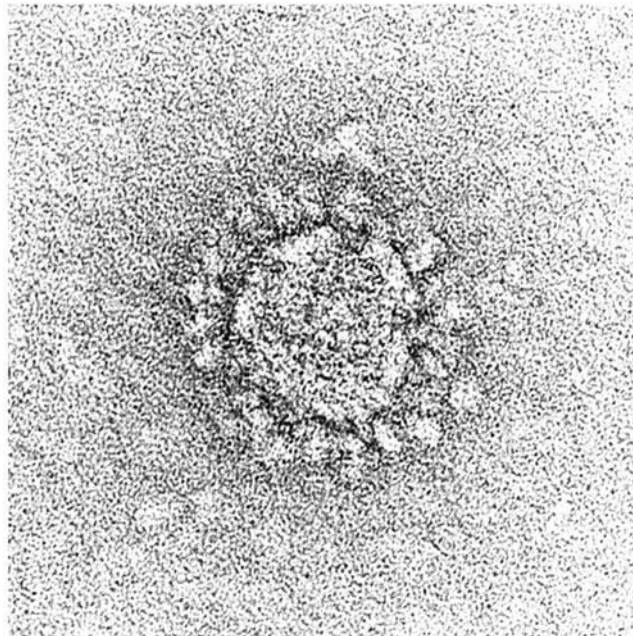


FIGURE 1.1 Photomicrograph of a coronavirus. This figure depicts a particle of a coronavirus. The prominent spikes give the virus a crown-like image. *Photo credit: Cynthia S. Goldsmith and A. Tamin.*

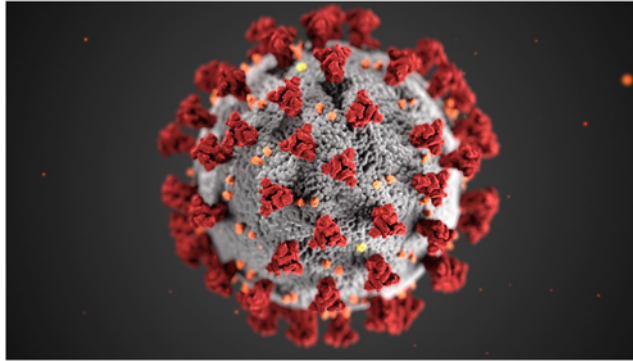


FIGURE 1.2 Illustration of a coronavirus. This image of a coronavirus shows several structural proteins studing the cell. The largest and most abundant projections are trimers of the spike protein. The smaller projections are the envelope and membrane proteins. *Photo credit: Alissa Eckert, MSML; Dan Higgins, MAMS.*

1.4.1 Coronavirus genomic and subgenomic RNA

The single-stranded coronavirus **genomic RNA** is the largest among RNA viruses (27.6 to 31 kilobases) and includes a **5'-cap** and a **3'-poly-A tail**.²⁹ The genome encodes 4–5 structural and a variable number of Nsp's, some of which serve partially redundant functions to counteract the host's innate immune response. For example, SARS-CoV has at least 11 viral proteins that antagonize type I IFN.⁶⁴

In addition to genomic RNA, coronaviruses produce **subgenomic mRNAs (sgmRNAs)**. The sgmRNA8 present early during the 2002–2003 SARS-CoV outbreak produced a single 8ab protein, while sgmRNA8 found late during the outbreak instead contained a deletion of 29 nucleotides that lead to the production of two smaller proteins, 8a and 8b.⁶⁵ In the absence of the 8a protein, the 8b protein is quickly degraded by **proteasomes**. When glycosylated, protein 8ab is stabilized.⁶⁵ The importance of proteins 8a, 8b, and 8ab protein progeny are described in greater detail in [Chapter 2](#).

1.4.2 Increasing genetic diversity by mutation and recombination in coronaviruses

Increasing genetic diversity is a major factor in allowing all forms of life to adapt to changing conditions as well as to expand their niche, permitting them to survive and thrive in new environments. It also permits at least some members of a species to avoid the ever-changing host immune response and disease treatment and prevention measures. There are multiple means of increasing diversity which vary among groups of organisms. One of the most familiar processes that are used by many forms of life is sexual reproduction in which progeny receives genetic information from both the male and female parents. Some of the asexual means of increasing diversity in bacteria involve acquiring exogenous DNA from the external environment (**transformation**) or **plasmids**. Bacteria also use **transduction**, a process in which they receive new DNA during infection by **bacteriophages** (viruses that infect bacteria). Additionally, bacteria exchange DNA with other bacteria of the same species via specialized

pili during **conjugation**. Viruses increase their genomic diversity by having a very high mutation rate and by genetic recombination.

As stated earlier in this chapter, coronaviruses have both a very large genome and a relatively high mutation rate due, to a large degree, to the large number of errors made by RdRp during viral replication.⁴ It should be noted that the mutation rate slowed throughout the 2002–2003 SARS-CoV epidemic and the new progeny viruses became less pathogenic.⁶⁶ Perhaps at some point in time, this decrease in pathogenicity will also occur during the ongoing COVID-19 pandemic. Members of the *Nidovirales* order of viruses, to which coronaviruses belong, exclusively possess ExoN, a 3′ to 5′ **exoribonuclease** that acts as a proofreader to boost replication accuracy and prevent an excessive and detrimental mutation rate. This proofreading enzyme is only present in nidoviruses with genomes larger than 20 kilobases.³⁰ Coronaviruses have genomes of 26.3 to 31.7 kilobases.⁶⁷ Variants of MHV and SARS-CoV that lack a functional ExoN have a greater number of mutations.³⁰

The “**Eigen paradox**” suggests that replication faithfulness, genome size, and genetic complexity trap each other in a low state of primitive self-replicating molecules.⁶⁸ With the acquisition of ExoN, nidoviruses may have solved the Eigen paradox, so that in members of this order, controlling genome size may be more complex than was previously realized.³⁰ This complexity appears to be related to viral architecture, including an exceptionally large size range of the 3′ open reading frame (ORF) in large nidoviruses.³⁰ The acquisition of ExoN by coronaviruses tightly correlates with the acquisition of two adjacent replicative **methyltransferases**, nsp14 and nsp16. The size of genomic RNA viruses is generally associated with a corresponding increase in the average size of proteins involved in the replication process,⁶⁹ a decrease in numbers of overlapping genes, and a strong correlation between the presence of **helicase** and ExoN domains and genome size.³⁰

Since more than one coronavirus can simultaneously infect a single host, the viruses may increase their genomic diversity using genetic recombination.^{1,4} During this process, virions exchange sections of their genomic RNA with other coronaviruses. This genetic exchange may occur between viruses of different variants of the same virus species (**homologous recombination**). During **heterologous recombination**, coronaviruses switch portions of their genome with that of other viral groups, resulting in large genetic changes⁷⁰ that may be able to evade the existing host adaptive immune response; adapt to a new organ, tissue, or host species, or undergo a change in virulence. For example, at least one of the strains of SARS-CoV that infect **palm civets** appears to be the product of recombination of genomic RNA from two horseshoe bat coronaviruses, WIV16 and Rf4092.⁷¹

Recombination occurs between different HCoV-OC43 isolates. This human coronavirus species is composed of at least five genotypes, A–E, whose emergence may have been driven by recombination.²⁵ In addition, recombination between different strains of HCoV-OC43 appears to have produced a more pathogenic form of human coronavirus as recently as 2004.¹³ Interestingly, of 18 HCoV-OC43 strains isolated from 2004, only 1 belonged to the recombinant genotype D that arose from recombination between genotypes B and C. All 8 strains of HCoV-OC43 isolated from 2008 to 2011, however, belonged to this recombinant genotype, which is now the dominant genotype in East Asia. Genotype D also appears to be

associated with increased disease severity, including causing pneumonia in the elderly. This recombination-associated increase in pathogenicity echoes that found in pandemic influenza strains. Evidence of recombination has also been found in other coronaviruses of humans (HCoV-HKU1) and animals, including various coronaviruses of bats, MHV of mice, porcine transmissible gastroenteritis virus of pigs, and feline coronavirus of cats.¹³ The rapid and continuing emergence of SARS-CoV-2 variants may also involve recombination events.

Furthermore, while the genomes of coronaviruses possess a fixed cohort of structural and nonstructural genes, they also contain differing numbers of genes for accessory proteins. Coronaviruses thus utilize a gene gains and losses mechanism for altering their genetic composition.^{25,72,73} Coronaviruses may also use template switching to increase their genetic diversity. Template switching leads to a high rate of homologous RNA recombination among the genomes of different coronaviruses.⁷⁴

1.4.3 Production of recombinant, chimeric coronaviruses

In order to better understand the potential of recombinant coronaviruses to infect and cause pathology in other animal species or humans, a multinational group performed “**gain-of-function**” research and constructed a chimeric virus that contained the S protein of the Chinese horseshoe bat coronavirus SHC014 in a mouse-adapted SARS-CoV.⁷⁵ This chimeric coronavirus was named SHC014-MA15 and can bind to and use the human ACE2 **ortholog** to replicate in primary human airway cells in vitro. In in vivo studies, this virus causes disease in the lungs of mice that were not protected by available antibody therapy or vaccines.⁷⁵ In another study, Bat-SCoV RNA was able to replicate in African green monkey kidney cells in vitro but was not infectious. By contrast, replacing the bat **receptor-binding domain (RBD)** of the S protein with the human RBD enabled these viruses to replicate in the cultured monkey cells.⁷⁶ Additionally, changing a single amino acid significantly increased replication and pathogenicity in mice.⁷⁷

1.4.4 Coronaviruses’ structural proteins

Structural proteins encoded by coronaviruses’ genomic RNA are found in the following 5′-to-3′ order in all coronaviruses: the S, E, M, N proteins, and, in some beta-coronaviruses, the HE protein as depicted in Table 1.9.⁷⁸ The HE protein is not present in alpha-coronaviruses. Of the structural proteins, the N protein and genomic RNA compose the nucleocapsid, the M and E proteins span the envelope, and the S and HE proteins project outward from the envelope.⁷⁹ The catalytic sites in coronavirus enzymes are highly conserved among the three highly pathologic human coronaviruses. The main drug-binding pockets in these viruses are probably conserved as well, allowing the most promising of the therapeutic drug candidates for SARS-CoV and MERS-CoV to be screened for activity against SARS-CoV-2.⁸⁰ In some MHV-infected mice, the HE protein increases neurovirulence and the extent of HE expression is linked with the severity of CNS pathology.³³ Interestingly, the HEs in HCoV-OC43 and HCoV-HKU1 lost their sugar-binding functions.⁸¹

The S protein binds to its receptor on the target cell and is involved in fusion prior to entering the cell’s cytoplasm. It also determines what cell types may serve as viral targets

TABLE 1.9 Coronavirus Structural Proteins.

Structure protein	Location	Function
Spike	Surface	Bind to cellular receptor Fuse to endosomal membrane Entry into cytoplasm Determines host species and cells
Envelope	Surface	Viral assembly Produce envelope Bud viral progeny from cell membrane
Membrane	Surface	Integration
Nucleocapsid	Around genomic RNA	Regulate transcription Package RNA into virions
Hemagglutinin esterase	Surface	Attach to sialic acid receptor during entry Cleave sialic acid receptor during viral release

and the species that different coronaviruses may use as hosts.⁸² ACE2 serves as the receptor for several pathogenic coronaviruses, including SARS-CoV and SARS-CoV-2. Since ACE2 orthologs from multiple animal species can mediate SARS-CoV-2 entry in cells, it appears that this virus may have a broad host range. Interestingly, ACE2 orthologs from several New World nonhuman primates (marmosets and tufted capuchin and squirrel monkeys) do not permit infection with this virus, even though the human ACE2 differs from those in the other primates by only two amino acids. Changing these two primate ACE2 amino acids to the human form of ACE2 allows SARS-CoV-2 to infect the primate cells. This emphasizes the ability of the S protein to discriminate between ACE2 proteins from even similar host species if certain key amino acids differ.⁸³

The importance of the S protein in expanding coronaviruses' host range is seen in an MHV variant isolated from mouse brain tumor cells. The S protein of MHV uses **carcinoembryonic antigen-related cellular adhesion molecule (CEACAM)** as its host cell receptor. This MHV variant binds better to human CEACAM than to murine CEACAM and may acquire mutations that allow zoonotic transmission in the future.⁶⁶

The M protein is the most abundant coronavirus protein in the viral membrane. It is involved in the assembly and budding of virus particles before their exit from the target cell (Fig. 1.3). The external portion of the M and S proteins contains regions that stimulate the production of neutralizing antibodies as well as inducing an IFN response.⁸⁴

The N protein is composed of two functional domains, each of which binds the virus' genomic RNA. It regulates transcription and packages the encapsulated RNA genomes into the virions. Unlike the other structural proteins, the N protein is not glycosylated. Multiple copies of the N protein associate closely with the genomic RNA to form a long, flexible, helical nucleocapsid.⁷⁹

The E protein is responsible for coronavirus assembly, forming the viral envelope, and budding and release of the progeny viruses from the cell. It also helps to determine viral pathogenesis.⁸² The E protein is an integral membrane protein with a transmembrane domain that functions as an ion channel and is necessary for coronavirus replication.⁸⁵ The E protein has a large α -helix in its N-terminal region. This helix appears to be

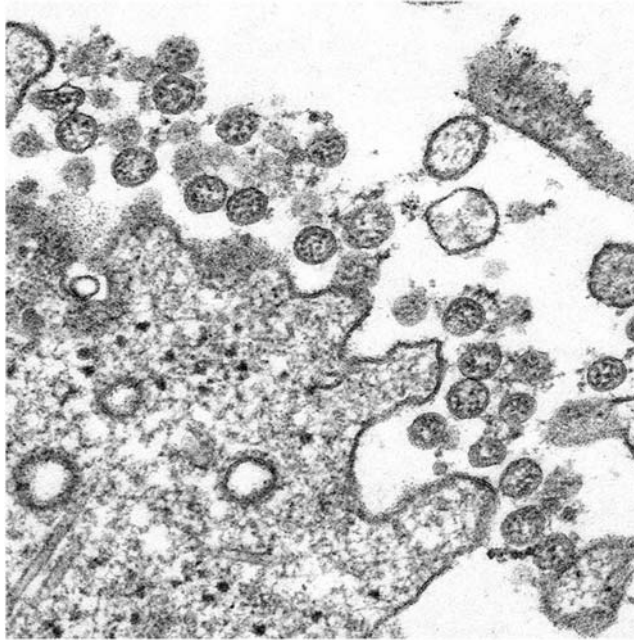


FIGURE 1.3 Coronavirus exiting infected cell. This is a photomicrograph of coronaviruses budding off of an infected cell's membrane as it exists the cell. During this process, the virus removes part of the membrane, which subsequently is used in the viral envelope. Photo credit: Cynthia S. Goldsmith and A. Tamin.

involved in the protein's ion channel activity, especially since the N-terminal of the E protein alone is an active ion channel.⁸⁶ Ion channel blockers inhibit coronaviruses' E protein activity in several human and animal viruses, including HCoV-229E, MHV, and FIPV from cats, interfering with their replication.⁸⁷

Coronavirus E protein forms a pentamer containing a central pore that is characteristic of **viroporins**. Viroporins are small hydrophobic viral proteins that assemble into **oligomeric** ion channels that form pores in the host cell's membrane that facilitate the release of viral progeny from infected cells. Viroporins also affect the host cell's vesicles, glycoprotein trafficking, and membrane permeability. Viroporins are also present in HCoV-OC43, HCoV-NL63, and HCoV-NL63.^{88,89} While not required for viral replication, viroporins increase viral growth.⁹⁰ It should be noted that it is controversial whether the E protein is a viroporin.

HE exists as a viral membrane, disulfide-linked dimer found in betacoronaviruses of lineage A, including HCoV-HKU1 and HCoV-OC43.⁷⁹ Interestingly, HE may have originally arisen through heterologous recombination with the similar HE gene of influenza C virus.^{71,91} Both the S and HE proteins bind to cell surface receptors containing the sialic acid Neu 5, 9A_{C2}. HE also acts as a receptor destroying enzyme that cleaves this sialic acid and releases 9-O-acetyl residues.⁷⁹ The destruction of sialic acid is needed for the detachment of the progeny viruses from carbohydrates present on infected cells since, without it, the newly produced viruses will bind back to their former host cell.⁹²

1.4.5 Coronaviruses' nonstructural proteins

While coronavirus nsp's have important roles in the viral life cycle and avoidance of the host immune response, their number and functions differ among coronavirus species. Nsp's are produced by proteolysis of two primary **polyproteins**, pp1a and pp1ab, which together encode up to 16 nsp's.⁸² Polyproteins, produced during translation of viral sgRNA, are composed of multiple joined proteins and must be processed to allow the release of functional individual proteins. Polyproteins are unique to viruses. During processing, polyproteins are cleaved by several cellular and viral **proteases**, including the virus-encoded **papain-like proteinase (PL^{Pro})** and **3C-like protease (3CL^{Pro})** which cleave the central and C-terminal regions of pp1a and pp1ab at 11 conserved sites. Dimerized 3CL^{Pro} is the active form of this protease.^{93,94}

Nsp1 suppresses signaling via the IFN- α and IFN- β pathways by degrading the IFN mRNAs and decreasing the translation of cellular proteins by binding to and inactivating the 40S ribosomal subunit.⁹⁵ Nsp1 is only present in alphacoronaviruses and betacoronaviruses.⁹⁶ The structure of **replication-transcription complexes** relies upon nsp2, which also alters cellular differentiation and the cell cycle and death pathways.⁹⁷ Nsp3 is responsible for the breakdown of pp1a and pp1ab,⁹⁸ alters the host cell cycle, and blocks the early IFN and proinflammatory response, thus inhibiting the innate immune activity.^{82,99} The macrodomain of nsp3 is an ADP-ribose 1'-**phosphatase**⁹⁶ that removes the covalently attached ADP-ribose tail from proteins. Nsp4 is involved in the development, arrangement, and function of replication-transcription complexes.¹⁰⁰

Nsp5 (3CL^{Pro} or main protease) is an enzyme involved in generating some of the other nsp's by cleaving viral polyproteins. It also disrupts IFN signaling pathways.⁸² Nsp6 triggers cell **autophagocytosis**, in which cells undergo self-digestion by the cell's enzymes.^{101,102} Nsp7 and nsp8 together produce a **heterodimer** that stabilizes the RNA binding site on nsp12. Excessive levels of 7a and 3b may result in the host cell undergoing apoptosis and cell cycle arrest.^{103–105} Nsp8 also has RNA polymerase replicase subunits that are unique to coronaviruses.^{82,106} Nsp9 is also unique to coronavirus and protects the viral genome from degradation during replication.¹⁰⁷ Nsp10 is a 2' **O-methyltransferase**¹⁰⁸ that works together with nsp14 and nsp16 to regulate viral replication.^{82,109} In the presence of nsp10, the N-terminal of nsp14 acts as a proofreading exoribonuclease of genomic RNA in a 3' to 5' direction.^{29,109} As discussed earlier, proofreading is vital to prohibit the excessive production of mutations. It also decreases the activity of nucleoside analogs used to treat coronavirus-associated disease. The C-terminal is a guanine-N7 methyltransferase used for mRNA capping.¹⁰⁹ Nsp11 is an **endonuclease**, an enzyme that cuts the interior regions of double- and single-stranded RNA.¹¹⁰ Nsp11 and nsp1b decrease signaling by the proinflammatory cytokines TNF- α and IL-1.¹¹¹

ORF1 encodes the nsp12 polymerase and the nsp13 helicase. Nsp12 and nsp13 are the two most conserved enzymes of coronaviruses. Nsp12 is the error-prone RdRp and is located in autophagosomal membranes.¹¹² It works together with nsp7 and nsp8 as an RNA polymerase that replicates the coronavirus' genome. During the process of viral replication, the nsp13 helicase unwinds double-stranded RNA and DNA in a 5'-to-3' direction, an unusual direction for RNA viruses. It obtains its energy by the hydrolysis of any of the common naturally occurring deoxyribonucleotide and ribonucleotide triphosphates.^{94,113,114} The presence of nsp12 increases nsp13's efficiency twofold.⁹⁴

Other protein products of ORF1 include the RNA-processing enzymes nsp10 and nsp14.⁵³ Some of the accessory proteins span the membrane multiple times and form a molecular scaffold that is used during the assembly of the **replicase-transcriptase complex**.¹¹⁵ Nsp14 and nsp15 are endonucleases.⁸² The activity of nsp15 (NendoU) is similar to nsp14, but nsp15 removes sections of RNA at **uridine** sites in a manganese ion-dependent manner.¹¹⁶ Nsp16 is an S-adenosyl methionine-dependent ribose 2'-O-**methyltransferase**.⁹³ It works together with nsp10 to alter the IFN- β signaling pathway.^{29,82}

1.4.6 A brief summary of the coronavirus life cycle

While a few bacteria abide within cells, it is critical for viruses to infect their proper host cells since viruses have relatively simple structures that lack the enzymes that they need to replicate their DNA or RNA genomes, produce and modify viral proteins, and assemble these viral components into a form that can exit the current host cell and enter the next target cell. The newly formed viruses often rupture and destroy the original cell during their exit.

The viral S protein is composed of S1 and S2 domains. The S1 domain is located on the distal part of the spikes that bind specifically to receptor proteins on the host cell via its RBD. The S2 domain anchors the spikes in the virus envelope and fuses with the target cell's membrane. The S protein is the most divergent part of all known coronavirus proteins, particularly the S1 portion, and has low sequence similarities across different coronavirus genera.^{117,118}

The two domains of the coronaviruses' S protein allow them to bind to, fuse with and enter host cells through one of two routes: the "early pathway" via the plasma membrane or the "late pathway" by **clathrin-dependent endocytosis**. In the latter process, viruses are transported into the cell in endosomal vesicles that are formed by invagination of **clathrin-coated pits** into vesicles that later bind to acidic lysosomes with digestive enzymes and other toxic compounds, including ROS. Coronaviruses generally use early cell surface pathways rather than the late endosomal pathways during cell entry.^{78,119}

In addition to binding to their receptor, the entry of most coronaviruses into the cytoplasm of the cell requires two sequential cleavages of the S protein, one in the S1/S2 cleavage site that separates it into the S1 and S2 domains and another at the S2 cleavage site that is exposed following the S1/S2 cleavage and that primes the RBD.¹²⁰ These two cleavages require both viral and host **proteases**. Different coronaviruses use different enzymes to accomplish these two cuts in the S protein and they determine whether the virus can enter via the safer "early" pathway or the more dangerous "late" pathway.

When coronaviruses enter the cell via the early, plasma membrane route, they use host cell surface serine proteases, such as **transmembrane protease**, and **serine 2 (TMPRSS2)**. SARS-CoV-2 has a four amino acid insertion at the S1/S2 site that can be cleaved by the host cell protease **furin**. The furin cleavage site is also present in MERS-CoV and HCoV-HKU1, however, SARS-CoV does not have this site.^{120,121} Coronaviruses with the furin cleavage site can use the early pathway, giving them a selective advantage in lung and primary human airway epithelial cells since using this pathway avoids exposure to the anti-viral **interferon-inducible transmembrane proteins** (IFITMs) that are present in

endosomes/endolysosomes that are used when viral entry is via the late pathway.¹²⁰ IFITM2 blocks entry of SARS-CoV-2 variants which lack the insertion in the S protein and, therefore, are not cleaved by furin. Furin-mediated SARS-CoV-2 cleavage may be at least partially responsible for its increased transmissibility in comparison to SARS-CoV.¹²² The selective advantage of viruses with the S protein insertion is dependent not only on furin cleavage but also upon the expression and activity of TMPRSS2, making this enzyme a potential drug target. Drugs targeting TMPRSS2 would not prevent infection via the late, endosomal route, however.¹²⁰

When viruses enter the cytoplasm via clathrin-dependent endocytosis, lysosomal cysteine proteases, such as the **cathepsin** enzymes, are utilized for viral cleavage at sites that differ from those used by TMPRSS2.¹²³ SARS-CoV, porcine epidemic diarrhea virus, and several other coronaviruses use the host cell's cathepsin L and B enzymes in the lysosomes to perform this cleavage. The host serine protease **trypsin** is also needed for S protein cleavage and cell entry via the late pathway.¹²⁴

Differences among the coronaviruses' S proteins are responsible for determining which cells or organs the coronaviruses can infect as well as which animal species can host a given coronavirus. Both the S1 and S2 subunits are interchangeable among various coronaviruses and replacing an S protein subunit from one coronavirus with that of another coronavirus could alter the host range. An example of this is the replacement of the S protein of a bat coronavirus with that of a mouse coronavirus resulting in a gain-of-function hybrid coronavirus that infects mice and has much greater pathogenicity in these animals as well as increased virulence.⁷⁵

A similar situation could occur if a human S protein were to be inserted into a highly pathogenic bat coronavirus. If the change of host species and increase in virulence were similar to the above work in mice, it could result in a novel, highly pathogenic virus in humans. Since such human-generated chimeric viruses gain in pathogenesis, such studies need to carefully consider whether they are too risky to pursue, especially those based on strains of coronaviruses that are currently circulating in nature, particularly viruses from horseshoe bats. Due to these concerns, the Obama administration banned such research.

During the late entry pathway, after S protein-mediated fusion to its cellular receptor has taken place, the target cell brings coronaviruses into its cytoplasm in the membrane-surrounded endosomes. SARS-CoV, MHV, FIPV, and potentially other coronaviruses enter target cells by such clathrin-mediated endocytosis.¹²⁵ The endosomes then become acidic and, in the case of many viral infections, fuse with **lysosomes**, other cellular vesicles that contain powerful digestive enzymes as well as some toxic ROSs. Together, the fused endosomes and lysosomes usually digest the microbes contained within them. Coronaviruses, however, are not killed within these vesicles, but instead, require the acidic conditions to enter the cell's cytoplasm. Drugs, such as chloroquine, that prevent this endosomal acidification thus block coronavirus entry into the cytoplasm of the cell.

The **ubiquitin-proteasome system** is critical for cellular endocytosis and maturation of at least some coronaviruses, such as MHV. Ubiquitin typically tags proteins for degradation in the proteasome. In the case of MHV, proteasome inhibitors block replication at an early point in the virus' life cycle, forcing them to accumulate in both endosomes and lysosomes.¹²⁶ Cellular protein synthesis is not affected by the proteasome inhibitors nor is cell

surface expression of the MHV receptor. The neurotropic MHV strain JHM enters cells by both the late endosomal and early nonendosomal pathways, dependent on the target cell type and viral strain. There was no difference in infectious MHV-JHM internalization into endosomes in the presence or absence of the proteasome inhibitors.¹²⁶ Unexpectedly, none of the viral structural proteins, virus receptors, or cellular proteins associated with the MHV virion in the endosome appear to be ubiquitinated.¹²⁶ Since ubiquitination is believed to be necessary for the proteasomal degradation pathway to operate, the precise role of the ubiquitin-proteasomal pathway in coronavirus entry into the cell's cytoplasm is currently unknown.

Upon reaching the cell's cytoplasm and the release of the viral nucleocapsid, the coronavirus' positive-sense strand genomic RNA acts as a messenger RNA and translates the viral **replicase gene complex** which is composed of two large ORFs, ORF1a and ORF1b.¹²⁷ These encode polypeptides 1a and 1ab, which, following cleavage by viral proteases form the nsp's, including those viral proteins involved in replication, as described earlier.

RdRp works in concert with some other nsp's to produce complementary full-length, negative-sense RNAs which serve as templates that are used in the production of full-length, positive-sense genomic RNA for progeny viruses as well as the smaller, sgRNA molecules. In coronaviruses, the first ORF encodes a large pp1 that, upon processing, yields a series of nsp's that are directly or indirectly involved in viral replication. These proteins include the helicase, several proteases, and metal-binding proteins.¹²⁸ In addition to viral enzymes, several cellular proteins are used during coronavirus replication. These proteins include heterogeneous nuclear ribonucleoprotein A1, polypyrimidine-tract-binding protein, poly(A)-binding protein, and mitochondrial aconitase.¹²⁸ While the overall scheme of replication is similar in all mammalian coronaviruses, differences exist among the individual viruses. A detailed description of replication as performed by different coronaviruses is beyond the scope of this book, however, Shi's excellent review describes the process and the role of both viral and cellular proteins.¹²⁸

The small, negative-sense, single-stranded RNAs produced by RdRp are copied into the positive-sense mRNAs used in the translation of coronavirus proteins.²⁹ These newly formed proteins are inserted into the **rough endoplasmic reticulum** (RER) and taken to a viral assembly site in the cell's endoplasmic reticulum-Golgi intermediate compartment. Virus assembly occurs during budding in the membranes of this compartment. The N protein interacts with newly synthesized, full-length, viral genomic RNA to form fragile nucleocapsids that align on the cytoplasmic surface of the membranes of the RER and **Golgi apparatus** by interacting with the viral M protein. In these membranes, the host cell proteins in the RER are replaced by the viral surface glycoproteins. The immature viral progeny appropriate some of the host cell's lipid bilayer. This bilayer comprises most of the viral envelope. Afterward, the intact virions are pinched off of the RER membrane and released into the lumen of the Golgi apparatus. The virions then enter **secretory vesicles** that are taken to the plasma membrane and released from the infected cell via **exocytosis**.^{29,99}

1.4.7 Viral transmission

The primary route of infection with SARS-CoV, SARS-CoV-2, and MERS-CoV is by inhalation of the virus found in aerosols, such as respiratory secretions from infected

people, camels, palm civets, or raccoon dogs, that are inhaled by an uninfected person. The droplets expelled by coughs or sneezes may be able to contaminate indoor air for as many as 2 or 6 m away, respectively, at least for some microbes.¹²⁹ Coronaviruses travel over 1 m from the infected person and survive for extended amounts of time in aerosols with droplet nuclei less than 5 mm in diameter.¹³⁰ For example, CoV-229E present in aerosols are infectious for up to 6 days at 20°C and relative humidity of 50%.¹³¹ Larger droplets travel less than a meter.¹³⁰ This should call attention to the risks associated with transmission of coronaviruses by bioaerosols generated by coughing or sneezing, but does not address the possibility of infection posed by normal breathing or talking. Transmission by the inhalation route is partially dependent on the density of people in an area. The quality of air exchange, circulation, and filtration affect the risk of infection in unvaccinated people.

The use of proper infection control procedures and **personal protective equipment (PPE)** are important factors in reducing viral transmission. This is particularly important in the light of the growing number of human-to-human hospital-based transmissions of MERS-CoV in the Middle East, as well as the healthcare-associated transmission that occurred during the MERS outbreak in South Korea. Transmission between people is rare and involves close contact, such as unprotected healthcare workers giving care to a MERS patient or the patient's family members.¹⁷

Pathogenic coronaviruses may persist on the surface of materials found in healthcare facilities, such as waiting, treatment, and patient rooms; emergency departments; intensive care facilities; and on medical instruments. A study of locations used to care for SARS patients in Bangkok and Taipei discovered that 38.1% of these sites were contaminated with SARS-CoV RNA.¹³² By contrast, in a Canadian hospital, 3.5% of tested surfaces in SARS units contained SARS-CoV RNA.¹³³ It should be noted that no viable viruses were found on any surfaces in either study.

Although many enveloped viruses remain infectious for only a short time in the external environment, this may not be the case for some coronaviruses. SARS-CoV and MERS-CoV may survive on dry surfaces better than other human coronaviruses, including HCoV-229E, HCoV-OC43, and HCoV-NL63.¹³⁰ SARS-CoV and MERS-CoV survive on a wide range of porous surfaces, such as metals, woods, glass, tiles, and Formica as well as on plastic (including light switches, telephones, electronic devices), and fabrics (including cotton, polyester, and handkerchiefs); paper (including magazines and paper money) and soft toys. They also persist on medical devices, such as stethoscopes, and PPE, including gowns, gloves, and masks.¹³⁰ SARS-CoV and MERS-CoV can survive on at least some of the above surfaces for weeks to months, depending upon environmental conditions. They remain viable on dry surfaces much longer than several other human coronaviruses (HCoV-229E, HCoV-OC43, and HCoV-NL63).¹³⁰ Dried MHV and droplets containing MERS-CoV are stable on glass coverslips for at least 60 and 30 minutes in the laboratory, respectively.¹³⁴

Strain variation, initial levels and concentration of the virus, surface type, material in which the virus is suspended, how the virus is deposited on the surface, temperature, and relative humidity all affect how long the virus remains infectious.^{130,135} The importance of transmission of coronaviruses from dry **fomites** is unknown, but may result in viral transfer onto people's hands and, from there, to the mucous membranes of the facial

region.¹³⁰ People in office environments were observed to touch their faces an average of 15 times per hour.¹³⁶ To transmit coronaviruses by this route, the number of viruses must at least equal a concentration above the infectious dose and must be able to be infectious after contacting the eyes, nose, or mouth.¹³⁷

Placement of viruses from clinical specimens on surfaces generally has shorter survival times than viruses suspended in a cell culture medium. The method used to determine whether the virus remains infectious is also important. For example, tests which detect the presence of viral RNA may not be indicative of infectiousness. Studies have found that while SARS-CoV RNA is present on surfaces, it is not infectious to cultured cells *in vitro*.¹³² Furthermore, the coronavirus that is infectious to such cultured cells may not be infectious to animals or humans *in vivo*.

On plastic or steel surfaces, MERS-CoV and SARS-CoV remain viable for relatively long periods, especially when the relative humidity and temperature are low. The ability to survive better in relatively dry and hot conditions is especially important for MERS-CoV since it primarily infects people in the Arabian Peninsula. Perhaps the higher levels of humidity inside hospitals are harmful to this coronavirus, raising the possibility that increasing humidity in the vicinity of infected people may decrease the severity of the disease and reduce the risk of transmission to other people.

A study on the susceptibility of coronaviruses to various types of surfaces was performed using another less pathogenic, human coronavirus, HCoV-229E. This virus can infect cultured human lung cells *in vitro* after as long as 5 days on the surface of many materials. These surfaces include Teflon, polyvinyl chloride, ceramic tiles, glass, silicone, rubber, and stainless steel.¹³⁸ It is important to note that the virus preparations used in this study contained some lung cell debris, to protect the virus from **desiccation** (drying out). Desiccation typically rapidly inactivates viruses.

Using copper alloys on the surface has been found to decrease the number of infectious organisms as well as reduce **nosocomial** (healthcare-associated) infections in medical facilities.¹³⁹ HCoV-229E is inactivated within several minutes when placed on copper alloy surfaces and copper/zinc surfaces were effective at lower copper concentrations. Copper destroys the viral envelope as well as HCoV-229's RNA. The antiviral activity of copper is more effective in the presence of ROS, such as hydrogen peroxide and the **hydroxyl radical**, which are formed by the interaction of water in the respiratory droplet with oxygen in the air. Ultraviolet light speeds up this process as well, even at levels found in normal sunlight. Since HCoV-229E is susceptible to copper, either copper or copper/zinc alloys could be used on surfaces in places where other, more pathogenic, coronaviruses may be present, such as doctor's offices, and clinics, hospitals, and nursing homes.¹³⁸ It can be incorporated into commonly touched surfaces, including doorknobs and handrails. Exposure of HCoV-229E to copper alloy surfaces for several minutes destroys its RNA and causes massive structural damage to the virus.¹³⁸ It should be mentioned, however, that HCoV-229E is an alphacoronavirus, while SARS-CoV, SARS-CoV-2, and MERS are betacoronaviruses. It is therefore important to repeat this study using these more pathogenic betacoronaviruses and to test whether the viruses are still able to infect mice.

1.5 Coronaviruses and disease

While infection with some of the more pathogenic human coronaviruses is generally considered by the public to result in respiratory illness, these and other human coronaviruses may also attack the nervous system. Since many animal coronaviruses primarily affect the brain, this chapter focuses on coronavirus-induced diseases in the CNS, especially those human coronaviruses that are generally known for causing mild, cold-like respiratory illness, since they are not the focus of any of the other chapters of this book. Some of these coronaviruses may directly or indirectly lead to nervous system diseases of unknown origin, including MS and Guillain–Barré syndrome.^{12,140,141}

1.5.1 Coronaviruses and respiratory disease

Many respiratory viruses, including human coronaviruses, infect the human upper respiratory tract, typically resulting in mild diseases. However, in high-risk populations, the viruses may affect the lower respiratory tract as well and cause severe diseases, including pneumonia.¹⁴² The effects of SARS-CoV, SARS-CoV-2, MERS-CoV, and animal coronaviruses are discussed in detail in [Chapters 2–6](#).

1.5.2 Coronaviruses and central nervous system disease

Some animal coronaviruses are neuroinvasive, including porcine hemagglutinating encephalitis virus, feline coronavirus, and MHV. These viruses enter the CNS and induce different types of neuronal disease.¹⁴² While the olfactory bulb is usually highly efficient at controlling neuroinvasion by coronaviruses, nevertheless, several viruses enter CNS via the olfactory route.^{143,144} In mice, HCoV-OC43 might use **anterograde axonal transport** to convey the viruses to the cell body, between neurons, or from neurons to glial cells in the CNS and then on to the spinal cord.¹⁴²

All seven human coronaviruses have been implicated in some type of CNS dysfunction.⁷⁸ In infants and young children, the most common CNS symptom is **febrile seizures**. A wide range of mild to severe diseases is found in adults infected with SARS-CoV, SARS-CoV-2, or MERS-CoV, including **acute flaccid paralysis**,¹⁴⁵ Guillain–Barré syndrome,¹⁴⁶ and perhaps MS as well.¹⁴⁰ One study found that as many as 84% of hospitalized COVID-19 patients displayed neurologic manifestations in China and France.⁷⁸ In mice infected with some neuropathogenic strains of MHV, the HE protein increases neurovirulence.⁷⁸ Of note: the HEs in the relatively low pathogenicity HCoV-OC43 and HCoV-HKU1 viruses appear to lack receptor sugar-binding activity.⁸¹

In transgenic mice expressing human ACE2 and infected with SARS-CoV, a large fraction of cells, predominantly neurons, contain viral antigen by 4 days postinfection.¹⁴⁷ Neuronal death in this model system occurs in the absence of inflammation, apoptosis, or necrosis. It should be noted that not all areas of the brain are infected to the same extent. While the cerebellum has only a small amount of viral antigen, the thalamus and cerebrum (including the cortex, hippocampus, basal nuclei, and amygdala) are heavily infected. The three regions of the brainstem, hypothalamus, and olfactory bulb have an intermediate

level of viral antigen.¹⁴⁷ It will be interesting to learn whether regional differences of infection are also produced by other coronaviruses and in other host species, including humans.

Chronic infection of the brain by human coronaviruses may cause long-term sequelae.¹⁴² In a BALB/c mouse model of HCoV-OC43 infection, the virus persists in the CNS of animals that survive acute encephalitis. These mice have smaller hippocampi with a loss of neurons.¹⁴⁸ This decrease in hippocampus neurons may affect learning and memory. Other surviving mice develop an abnormal four limb clasp reflex and decrease in motor activity, beginning several months postinfection. These results suggest that similar chronic CNS diseases may occur in humans infected by this relatively mild human coronavirus due to viral persistence in susceptible people.¹⁴⁸ It should be noted, however, that an animal model may be more or less susceptible to coronavirus infection and disease in the CNS than humans. Nevertheless, HCoV-OC43 has been shown to persist in the human CNS and may, over time, develop specific molecular adaptation to the CNS environment.¹⁴⁹ Anti-human coronavirus antibody synthesis also occurs in the intrathecal region (within the spinal canal).¹⁵⁰ The severity of CNS pathology is related to the genetics of both host and virus, dose and route of inoculation, and the host's age and immunological status.¹⁵¹ It should be kept in mind that the mere presence of coronaviruses in the CNS does not necessarily correlate with disease.¹⁴⁹

Under normal conditions, coronaviruses invade peripheral nerve terminals, then travel to the brain across the synapsis to the CNS, where viral antigens are detectable in brain-stem nuclei.¹⁵² Once in the CNS of experimentally infected rodents, coronaviruses stimulate the production of the proinflammatory cytokines IFN- β and IL-6, the chemokines IL-8 and CCL2, and **granulocyte-colony stimulating factor** and **granulocyte-monocyte-colony-stimulating factor**.¹¹⁸ The latter two cytokines direct the bone marrow to produce more neutrophils and monocytes/macrophages. When mice lacking functional IFN receptors are infected with a normally nonlethal coronavirus, they die in less than two weeks, demonstrating the importance of these antiviral, proinflammatory cytokines to survival.¹⁵³

Various coronaviruses, including SARS-CoV-2, are associated with one or more of the following CNS diseases: **meningoencephalitis**, **acute necrotizing encephalopathy**, **acute ischemic stroke**, **acute disseminated encephalomyelitis**, acute flaccid paralysis, **anorexia**, **anosmia** (loss of the sense of smell), and **hypogeusia** (decreased sense of taste).¹⁵² The latter two manifestations are associated with SARS-CoV-2 as well as HCoV-229E infection.¹⁵⁴

Coronavirus infection rarely triggers encephalitis in humans but does so frequently in animals.⁷⁸ Meningoencephalitis and febrile seizures occur in children, while general seizures occur in both children and adults. In children hospitalized for coronavirus respiratory illness caused by HCoV-NL63 or HCoV-OC43, approximately one-fourth had febrile seizures.¹⁴ Febrile seizures were also found in about half of hospitalized children infected with HCoV-HKU1.^{78,155,156} Acute meningoencephalitis or meningitis is also rarely present in infants and children.⁷⁸

Several of these conditions are linked to **demyelination** associated with coronavirus infection. Upon autopsy, the brains of patients with either MS or normal control people found HCoV-OC43 RNA in 35.9% of MS patients and 13.7% in controls.¹⁴⁹ The virus was present in normal **white and gray matter** as well as in the plaques of the MS patients' brains.¹⁴⁹ In general, T cells entering the CNS play a major role in demyelination, as is the

case in MS. Additionally, cross-reacting T cells that recognize both coronaviruses and myelin are found in the brains of people infected by HCoV-OC43 or HCoV-229E.^{12,157} Autoreactive T cells that recognize myelin are also present in the brains of uninfected people, but their activity is kept in check by Tregs.¹²

The association of coronaviruses with MS, however, is controversial, since some studies have found HCoV-229E in the CNS of MS patients, including coronavirus RNA in demyelinating plaques, while other studies have not detected differences in coronavirus RNA in MS patients compared to controls.¹⁵² Nevertheless, experimental infection of mice with two strains of MHV results in fatal encephalitis or paralysis and severe demyelination.¹⁵⁸ The S protein of HCoV-OC43 plays a role in disease manifestation since a mutation in this protein alters CNS pathology from chronic encephalitis to flaccid paralysis. CD8⁺ T killer cells, IFN- γ , anticoronavirus antibodies, chemokines, and the complement cascade have been linked to demyelination in some mouse models.¹⁵² HCoV-OC43 RNA was detected in the cerebrospinal fluid of a child with the rare demyelinating disease, **acute disseminated encephalomyelitis**.¹⁵⁹ Other studies indicate a possible link between MERS-CoV and SARS-CoV-2 and this CNS disease manifestation.^{152,160,161} Guillain–Barré syndrome has also been reported to be related to MERS-CoV¹⁶² and SARS-CoV-2 infection.¹⁴¹

In the CNS, coronaviruses infect all neuroglial cell types. When primary cell cultures of microglia derived from the brains of newborn rats are infected with HCoV-OC43, microglia are productively infected but only have a very low viral titer, as is the case with infected primary human adult microglia.¹⁶³ Primary cell cultures of **astrocytes** and **oligodendrocytes** only develop an abortive HCoV-OC43 infection.¹⁶³ Microglia and astrocytes might, however, secrete cytokines or chemokines that affect viral entry and productive replication.¹⁴⁸ Uninfected neurons and glial cells that are in close proximity to infected cells undergo apoptosis, perhaps caused by secretions from infected cells. For example, activated microglia are the major source of TNF- α in the CNS and this cytokine can induce neuronal apoptosis. HCoV-OC43-infected microglia cultures secrete high levels of TNF- α that may cause apoptosis in neighboring cells.¹⁴⁸

Demyelination correlates with viral-associated cytopathogenic effect in oligodendrocytes, the neuroglia cell that produces myelin sheets around some types of **axons** in the CNS.¹⁶⁴ Additionally, cross-reactive T cells that recognize coronavirus proteins, including those of HCoV-229E, and myelin, contribute to the host assault on myelin.^{12,152,157} From the neuroglia, neurotropic coronaviruses spread to the neurons. Under normal conditions, most immune cells, except microglia, are not permitted to enter the CNS due to the presence of the **blood:brain barrier**. During infection with a neurotropic coronavirus, however, several types of immune cells pass through this barrier.

In MHV-infected mice lacking functional IFN signaling, neutrophils are the primary immune cell type that enters the brain, while macrophages are the primary immune cell type in the brains of normal, infected mice. In addition, these mice have greater levels of TNF- α , IL-6, and IFN- γ .¹⁵³ Coronavirus-specific CD8⁺ T killer cells typically provide some of the most powerful protection against viral infection. Their activity requires the presence of major histocompatibility complex (MHC) class I molecules on infected cells. While T killer cells are still present in the CNS of infected mice, they are not able to prevent disease or death in the absence of type I IFN,¹⁵³ perhaps because these mice have reduced levels of MHC class I molecules. Of note: mice without a functional type I IFN system also die upon infection with a normally nonpathogenic neurotropic coronavirus.¹⁵³

1.5.3 Other coronavirus disease manifestations

Under some circumstances, coronaviruses of humans and animals infect multiple organs and tissues. In some patients, MERS-CoV not only causes severe lower respiratory tract disease, but also causes gastrointestinal pathology and renal failure.¹⁶⁵ SARS-CoV is also found in the gastrointestinal tract, where it may cause diarrhea and lead to environmental spread. SARS-CoV levels in the nose and throat and those in fecal material may be high ($\sim 10^6$ per mL). MERS-CoV is also present in feces at about the same levels.¹³⁰ SARS-CoV-2 may also cause diarrhea and the patient's feces contain small amounts of the virus. Some coronaviruses also cause myocarditis, meningitis, severe diarrhea, and multi-organ failure, especially in children.¹⁴² Details about infection and pathology of different coronaviruses in various organ systems will be described in [Chapters 2–6](#).

1.6 Categories of coronaviruses

Four genera of coronaviruses belong to the family Coronaviridae: *Alpha-*, *Beta-*, *Gamma-*, and *Deltacoronavirus*. Each genus contains a variety of species, most of which do not infect people ([Table 1.10](#)). Alphacoronaviruses and betacoronaviruses lineages A, B, and C have only been reported in mammals, except for one known bat coronavirus. At least one viral species from both *Alpha-* and *Betacoronavirus* sickens humans to some extent.⁷⁹ The highly pathogenic coronaviruses of humans are members of betacoronavirus lineages B and C.

1.6.1 Coronavirus genera

HCoV-229E and HCoV-NL63 are alphacoronaviruses, as are several pig, canine, and feline coronavirus. In general, alphacoronaviruses are five times more likely to switch host species than betacoronaviruses.²⁶ The remaining human coronaviruses are betacoronaviruses. Betacoronaviruses are further divided into four lineages (A–D). HCoV-OC43 and HCoV-HKU1

TABLE 1.10 Genera and lineages of human coronaviruses.

Alphacoronavirus	Betacoronavirus			
	Lineage A	Lineage B	Lineage C	Lineage D
		SARS-CoV		
			MERS-CoV	
		SARS-CoV-2		
HCoV-229E				
	HCoV-OC43			
HCoV-NL63				
	HCoV-HKU1			

belong to lineage A; SARS-CoV, SARS-CoV-2, civet SARS-related CoVs, and the SARS-related horseshoe bat coronaviruses belong to lineage B; and MERS-CoV belongs to lineage C. Betacoronavirus lineages C and D include bat viruses which are very similar to human coronaviruses, such as the lineage C viruses HKU4 bat coronavirus from the lesser bamboo bat (*Tylonycteris pachypus*) and HKU5 bat CoV from the Japanese pipistrelle (*Pipistrellus abramus*), and the lineage D horseshoe bat CoV HKU9 from Leschenault's rousette bat (*Rousettus leschenaultia*). Other members of *Betacoronavirus* include MHV, a cattle coronavirus, and coronaviruses of rats, pigs, horses, and dogs. A factor opposing intraspecies virus transmission is the location of the RBD within the S protein. MHV's RBD is found at the tip of the N terminus of the S1 domain, while that of HCoV-229E is found at the C terminus.⁶⁶

Within the *Betacoronavirus* group, HCoV-OC43 is most closely related to bovine coronavirus (BCoV), except in the S protein.⁸ The alphacoronavirus HCoV-229E is more closely related to camel coronaviruses than to bat coronaviruses.¹⁶⁶ HCoV-229E uses the human enzyme APN as its host cell receptor, however, its ability to serve as the receptor varies depending on the APN protein's **glycosylation** status.¹⁶⁷

Gammacoronaviruses primarily infect birds, while deltacoronaviruses infect birds and some mammals. The diversity of bird species is similar to that of bats. Although birds and bats belong to separate classes of vertebrates, they have some similarities that make them excellent agents of virus diversity and geographical spread. Both groups are composed of large numbers of diverse species, providing many different "host ecosystems." Additionally, both birds and bats fly and many species from both groups migrate and congregate in large numbers, enabling them to disperse their microbes over very large territories and readily pass microbes throughout large colonies or flocks.¹⁶⁸ Furthermore, a single bat colony may house multiple bat species and families. They also have a long life span that allows coronaviruses to infect several generations of bats.¹⁶⁹ Differences also exist in the coronaviruses of bats and birds, so while *Alphacoronavirus* and *Betacoronavirus* contain a great diversity of bat coronaviruses, they do not contain coronaviruses of birds. The situation is reversed in *Gammacoronavirus* and *Deltacoronavirus* in which bird, but not bat, coronaviruses are very diverse.

1.6.2 Coronaviruses of animals and zoonotic disease potential

A large survey of journal articles found that RNA from coronaviruses in some species of Chinese bats is similar to those of SARS-CoV and SARS-CoV-2. MERS-CoV also has a high degree of genetic homology with bat coronavirus species worldwide, including bats from Egypt, Africa, Italy, and China.¹⁶⁹ Interestingly, travel-related MERS is not reported in humans in some of these locations, perhaps because few of the proper camelid vectors (dromedary camels, alpacas, and llamas) are found in these regions. If MERS-CoV were to adapt and use other animals as intermediate hosts, such as Bactrian camels or Guanacos, the virus could, perhaps, further adapt and undergo zoonotic transmission to humans as well as increase the virus' geographical range. Most Bactrian camels have been domesticated and are present in the steppes of Central Asia. Guanacos are wild ungulates found throughout many regions of South America.

The two bat viruses which appear to be the closest relatives to human SARS-CoV have important differences in their RBD that do not allow their S protein to bind to human

ACE2. Therefore, these bat coronaviruses may be unable to directly cause disease in people.¹⁶⁸ A recent study, however, found two SARS-like bat coronaviruses that enter human airway cells in vitro and kill chimeric mice engineered to express human ACE2 in vivo.⁷⁵ It is thus possible that one of these bat coronaviruses may mutate enough to infect and cause serious disease in humans. If such a mutation were ever to occur, other differences between bat and human coronaviruses would probably decrease the ability of the highly specific adaptive immune system's memory response from recognizing the newly formed bat coronavirus.⁷⁵ This could leave the new human hosts vulnerable to infection that might result in mild to severe disease or merely asymptomatic infection. This potential vulnerability speaks of the need for broad-spectrum anticoronavirus drugs that target **conserved regions** of both human and SARS-like bat viruses. Drugs against conserved proteins might recognize many species of coronaviruses, even those originating in other animals, and decrease the risk of zoonotic transmission.¹⁷⁰

The inability of most bat coronaviruses' S protein to bind to human ACE2 is unlike the situation occurring in SARS-CoVs derived from humans and civets, since civet coronaviruses do bind to human ACE2, allowing them to infect human cells and continue their life cycle. It should be noted that coronaviruses are found in many other animal species in addition to bats. The "human" HCoV-OC43 virus, for example, appears to have originated from a cattle coronavirus.²⁶ It may well be that a large number of known bat coronaviruses, including SARS- and MERS-like coronaviruses, is due to the unusually strong emphasis that has been placed on the study of bat viruses and the relative lack of similar attention to coronaviruses present in other animal groups. For example, humans are believed to have been directly infected by both SARS-CoV and SARS-CoV-2 through contact with animals in wet markets, in the case of SARS-CoV, by contact with **palm civets** and **raccoon dogs**. Human infection with MERS-CoV occurs by direct contact with **dromedary camels** or by consumption of their unpasteurized milk or urine.

Evidence suggests that bats, especially horseshoe bats (*Rhinolophus* species) may have served as reservoirs for the ancestors of pathogenic human coronaviruses after these bat viruses mutated into a form that was able to infect intermediate hosts, including civets, raccoon dogs, and dromedaries. Analysis of complete genomic sequences of newly identified bat SARS-related coronaviruses suggests that the direct ancestor of SARS-CoV arose from multiple genomic recombination events between different bat coronaviruses before they entered into an intermediate host.¹⁷¹ Multiple species of horseshoe bats coinhabit caves in China and may be breeding sites for such recombinant viruses, especially bat species from the Guangdong and Yunnan provinces. Special attention should be placed upon the bat coronaviruses WIV1 and WIV16 which have 90% to 97% amino acid sequence identities in their S proteins to those of civet and human SARS-CoVs.¹⁷¹

Mutated bat coronaviruses may have infected humans via contact with intermediate hosts. Nevertheless, the role of bat coronaviruses in causing severe human diseases is indirect. It might be wise for the agencies funding large amounts of research on coronaviruses of bats to increase their attention to the study of coronaviruses of other mammalian species as well, especially the coronaviruses that infect other animals that are sold in wet markets and may play a much more direct role in infecting humans. Additionally, while studies of coronaviruses of shrews and hedgehogs are very limited, these small mammals harbor a diverse group of alpha- and beta-coronaviruses, including viruses that are closely

related to MERS-CoV. Perhaps further studies on hedgehog and shrew coronaviruses will help to fill in the many gaps in the coronaviruses' family tree.

Individual coronavirus species often infect more than one animal species. In addition to cattle, BCoV and bovine-like coronaviruses are present in domestic and wild ruminant species, including water buffalos, sheep, goats, dromedary camels, llamas, alpacas, deer, wild cattle, antelopes, and giraffes, as well as dogs and humans.³²

1.7 Treatment of coronavirus diseases

1.7.1 Chloroquine

Chloroquine is one of the most effective and widely used antimalarial drugs. It is also very effective against many RNA viruses, including rabies virus, poliovirus, HIV, hepatitis A and C viruses, influenza A and B viruses, dengue virus, Zika virus, and Ebola virus.¹⁷² As described below, it is also active against at least some coronaviruses, including SARS-CoV and MERS-CoV. Several studies and case reports indicate that chloroquine may also be beneficial in treating SARS-CoV-2 infection, while other studies indicate that it does not do so. The use of chloroquine to treat COVID-19 is described in greater detail in [Chapter 4](#).

Chloroquine inhibits coronavirus replication in HCoV-OC43-infected cell lines in vitro.⁸ While usually causing mild, cold-like disease in humans, HCoV-OC43 typically kills very young infected mice. Chloroquine protects these newborn mice from a lethal dose of this virus in vivo. HCoV-OC43 may be acquired transplacentally or via infected mother's milk. When infected, pregnant mice were given a high dose of chloroquine (15 mg/kg), 98.6% of their pups survived.⁸ This drug appears to only decrease the entry of HCoV-OC43 into the target cell's cytoplasm and not later stages of infection, so this drug must be administered to the dam before birth. In untreated mothers, all the pups die within 6 days after infection with HCoV-OC43. Additionally, all newborn mice that received chloroquine exclusively by the transplacental route died, perhaps due to low drug levels reaching the fetuses by this route.⁸ Chloroquine has several mechanisms of action against coronavirus infection or disease. It inhibits the maturation of ACE2. Chloroquine is also a weak base and concentrates within endosomes, increasing endosomal pH and decreasing the ability of SARS-CoV to enter the cytoplasm. Additionally, chloroquine suppresses the production and release of the proinflammatory cytokines TNF- α and IL-6, thus reducing immunopathology.⁸ A relative of chloroquine, hydroxychloroquine, is used for the long-term treatment of several autoimmune diseases, such as lupus and rheumatoid arthritis, in addition to malaria. It is also effective in decreasing the development of potentially fatal respiratory disease as well as the number of deaths in people infected with severe, highly pathogenic human coronaviruses, especially when used in combination with an antibiotic. The antibiotics decrease the risk of developing a secondary bacterial infection that may progress to pneumonia.

Chloroquine has been administered to millions of people for decades and has been proven to generally have a good safety record with only mild side effects in most people. Hydroxychloroquine has an even better safety record, even though with prolonged use, it

can cause mild gastrointestinal, dermatological, neurological, and retinal side effects. Nevertheless, hydroxychloroquine may QT prolongation, a serious cardiac condition.¹⁷³ It should be noted, however, that most drugs, including penicillin and aspirin, may lead to disease in at least some people. The relative risk of administering any drug should take into consideration the severity of the condition being treated as well as the proportion of people who may develop serious adverse drug effects. Steps may then be taken to mitigate potential threats by identifying individuals at risk and avoiding giving them the drug in question.

1.7.2 Nucleic acid analogs

The proofreading capacity of coronaviruses confers resistance to some nucleoside analogs. Several analogs, such as β -D-N4-hydroxycytidine (NHC) and remdesivir (GS-5734), still inhibit the replication of multiple coronaviruses of humans, including MERS-CoV, HCoV-NL63, SARS-CoV, as well as MHV and several bat coronaviruses with zoonotic potential, including WIV1.¹⁷⁴ NHC is mutagenic in at least some of these coronaviruses. Remdesivir also has prophylactic and therapeutic efficacy in SARS-CoV-infected mice in addition to its in vitro activity against other human and animal coronaviruses, including those with functional proofreading activity.¹⁷⁵ Two mutations in coronaviruses' RdRp, however, confer sixfold greater resistance to remdesivir in comparison to wild-type coronaviruses. However, resistance to remdesivir emerges slowly, is only partial, and leads to a lack of fitness that attenuates resistant coronaviruses.¹⁷⁶

1.7.3 Traditional medicinal compounds

Cultural groups throughout the world use traditional medicinal products to combat coronaviruses and other respiratory viruses either exclusively or in combination with Western medicine. The efficacy of these compounds is often dependent upon the coronavirus strain and the host species or cell lines in which they are tested. An example of this is a compound isolated from the paper mulberry (*Broussonetia papyrifera*) which inhibits the PL^{pro} from SARS-CoV, but not against the PL^{pro} from MERS-CoV. Griffithsin, an algae-derived sugar-binding molecule, has different degrees of efficacy against SARS-CoV strains, being higher for the Urbani and Tor-II strains and low for the Frank strain.¹⁷⁷

Some well-known notable plants used in the treatment of respiratory problems include the common mugwort from the daisy family (*Artemisia vulgaris*), red spiderling of the 4 o'clock flower family (*Boerhavia procumbens*), the caper bush (*Capparis spinosa*), ajwain (*Carum copticum*), the desert hyacinth (*Cistanche tubulosa*), dove milk (*Euphorbia hirta*), and black henbane (*Hyoscyamus niger*).¹⁷⁸ Some of the active compounds of traditional medicinal plants that have produced promising results in the treatment of coronaviruses include emodin, reserpine, aescin, myricetin, scutellarin, apigenin, luteolin, and betulonic acid.¹⁷⁸ For an excellent review of traditional medicinal plants, their active compounds, their antiviral activities, and mechanisms of action, see Khan et al.¹⁷⁸ The mechanisms of action include blocking the activity of 3CL^{pro} and RdRp, decreasing nsp13 ATPase activity, blocking the expression of the S and N proteins, and reducing viral attachment and penetration

during entry into the target cells.¹⁷⁸ Plant **lectins**, **flavonoids**, and **terpenoids** often have antiviral activity as well.¹⁷⁸

Some of the most promising anticoronavirus candidates, primarily polyphenols, contain a conjugated fused ring structure.¹⁷⁷ The bioactive polyphenols include quercetin, myricetin, scutellarein, isobavachalcone, psoralidin, and are discussed below.¹⁷⁷ Polar compounds extracted using ethanol or an ethanol/water combination are the most commonly tested drug candidates.¹⁷⁷ Polar extracts, especially the highly polar glycosylated compounds, typically contain higher levels of bioactive and antimicrobial compounds than nonpolar compounds.¹⁷⁹ Additionally, when polar compounds are administered orally, they are more likely to be compartmentalized within the body, which decreases their rate of elimination.¹⁷⁷

The cardiotonic steroids ouabain and bufalin inhibit infection by several murine and feline coronaviruses as well as MERS-CoV. They block the activity of the ATP1A1 subunit of $\text{Na}^+ \text{K}^+$ -ATPase, a major ion transporter in cells that also acts as a signaling transducer. This ion transporter plays a role early in coronavirus infection that involves clathrin-mediated endocytosis and intracellular signaling via the **Src intracellular signaling pathway**.¹²⁵ Ouabain is a plant-derived toxin used in poison arrows in eastern Africa. In lower doses, it is used to treat hypotension and heart arrhythmias. Bufalin is derived from Chinese toad venom and is used in many Chinese traditional medications.

Some other traditional medicinal compounds have anticoronavirus activity. Reserpine from the root of Indian snakewood (*Rauvolfia serpentina*) is typically used to treat high blood pressure and schizophrenia. Escin from horse chestnuts (*Aesculus hippocastanum*) has antiinflammatory activity.¹⁸⁰ Care must be used when taking these drugs since reserpine is toxic at high doses. The bark of the tropical tree *Aglaia foveolate* is used to produce silvestrol, which is active against SARS-CoV and MERS-CoV, as well as Ebola and Zika viruses.¹⁸¹ It blocks the production of coronavirus replication and transcription complexes by inhibiting the formation of coronaviruses' structural and Nsp's, including nsp8.

Two flavonoids, scutellarein from the bald and blue skullcaps, *Cutellaria barbata* and *Scutellaria lateriflora*, respectively, and myricetin from grapes, onions, walnuts, herbs, berries, wine, and tea, are reported to inhibit the viral helicase enzyme. Nobiletin, derived from citrus peels, inhibits the binding of the S protein to ACE2. The flavonoids hesperetin from citrus fruits, herbacetin from flaxseed; quercetin from red onions and kale; rhoifolin from China grass, Canton lemon, bitter orange, grapefruit, onions, and sabal fruit; pectolinarin from nakai and some citrus fruits; apigenin from celery, parsley, grapes, and cherries, wine, and chamomile tea; and aloe emodin from aloes and rhubarb, inhibit the viral 3CL^{pro} enzyme. The triterpenes iguesterin from the woody climbing plant *Salacia madagascariensis*; pristimerin and celastrol from the thunder duke vine; the polyphenol curcumin found in turmeric; the phlorotannin dieckol from the brown algae *Ecklonia cava*; the glucosinolate sinigrin from Brussels sprouts and broccoli also inhibit this viral enzyme.¹⁸⁰

The following compounds also may be active against coronavirus infection: lycorine, monensin sodium, mycophenolate mofetil, mycophenolic acid, and baicalin.¹⁸⁰ Lycorine is derived from lilies and daffodils of the Amaryllidaceae family and is used as an antimalarial compound. Monensin sodium is an antibiotic from the bacterium *Streptomyces cinnamomensis* that is often present in ruminant animal feeds. Mycophenolate mofetil and mycophenolic acid, derived from several *Penicillium* species of fungi, are immunosuppressants that decrease

virus-induced inflammation but may cause miscarriage and birth defects if used by pregnant women. Baicalin is an antiinflammatory molecule derived from the Indian trumpet flower (*Oroxylum indicum*) and thyme.

Some medicinal compounds are also active against other infectious diseases^{177,180} Emetine from the ipecac root (*Psychotria ipecacuanha*) is used to treat ameba infections and induce vomiting. Pyrvinium pamoate is a quinoline-derived cyanine dye used to treat parasitic worm infections and cancer. Saikosaponin b2 is a terpenoid from the roots of the sickle-leaved hare's-ear (*Bupleurum falcatum*) that efficiently inhibits hepatitis C virus entry into cells.¹⁸⁰

Several natural components inhibit the activity of the viral chymotrypsin-like protease.¹⁸² These include flavonoids from citrus fruits; **polyphenols** from artichoke heads, chicory, spinach, onions, broccoli, asparagus, and green tea; glucosinolates from cabbage, broccoli, Brussels sprouts, watercress, horseradish, capers, mustard, and radishes; and some steroid hormones. The activity of the viral nsp13 helicase enzyme is blocked by flavonoids, including myricetin, that is found in tomatoes, oranges, nuts, berries, tea, and red wine.¹⁸³ Other several natural components, such as nicotianamine from soybeans that are used to decrease blood pressure and glycyrrhizine from the black licorice root (*Glycyrrhiza glabra*), block the viral S protein from binding to ACE2.¹⁸⁰ The proteases 3CL^{pro} and PL^{pro} are targeted by the pentacyclic triterpenoid iguesterin from the woody climbing plant (*Salacia madagascariensis*) and the red sage (*Salvia miltiorrhiza*) compound cryptotanshinone, respectively. The flavonoid sotetsuflavone from Asian sago palms (*Cycas revoluta*) targets the viral RNA polymerase.¹⁸¹ Care must be taken when using these plant-based medicinal compounds to avoid dose-related toxicity or adverse effects of interaction between these compounds. For example, consumption of excessive amounts of black licorice decreases blood potassium levels, increases blood pressure, and may lead to irregular heartbeat.

Some natural compounds that have activity against the mildly pathogenic human coronaviruses are listed below. Compounds that inhibit HCoV-229E include blancoxanthone and pyranojacareubin from the roots of the evergreen *Calophyllum blancoi* and silvestrol from the bark of mahogany (*Khaya* species), which targets helicase. The following have activity against HCoV-N63: tryptanthrin and indigodole B from the leaf of the herbaceous tropical plant *Strobilanthes cusia* which block viral genomic RNA synthesis and caffeic acid, chlorogenic acid, and gallic acid from the stem of the elderberry (*Sambucus formosana*), and griffithsin from *Griffithsia* species of red algae which affects binding of the S protein. The latter also is active against HCoV-229E and HCoV-OC43. The following compounds are active against HCoV-OC43: tetrandrine, fangchinoline, and cepharanthine from *Stephania tetrandra*, a herbaceous vine found in China and Taiwan, which inhibit viral replication and the expression of the viral S and N proteins.¹⁷⁷ A more comprehensive review of other natural medicinal proteins with activity against SARS-CoV, SARS-CoV-2, and MERS-CoV may be found in the excellent review by Zhou.¹⁸⁰

Psoralidin, a phenolic compound from the seeds of *Psoralea corylifolia*, a Chinese medicinal compound, may be active against several types of cancer as well as coronaviruses. Isobavachalcone, derived from *Psoralea corylifolia* Linn, is an anticancer agent that has antibacterial activity as well as acting against coronaviruses.¹⁷⁷ It is also a major component of Chinese medicinal treatments.

1.8 Prevention of coronavirus infection

For respiratory infections, wearing surgical or N95 masks helps to protect from infection by the respiratory route from droplets, while gloves, gowns, and eye protection help to prevent droplet contact of the facial mucous membranes as well as decrease contamination of clothing and hands from touching the nose or eyes (Fig. 1.4).¹³⁰ Correct removal of the protective equipment is important to prevent viruses from being transferred to the hands.¹⁸⁴ Safe practices for removing PPE may be exacting and not always practiced¹⁸⁵ as was evidenced by several nurses becoming infected with Ebola virus after treating a travel-associated case in the United States. While Ebola is transmitted primarily by contact with contaminated feces and blood and human coronaviruses are usually acquired by the respiratory route, all hospital personnel, including the custodial staff and volunteers, need to know and practice proper infection control techniques, including consistently using and properly removing gloves and gowns. Proper mask usage should also be stressed, including constant covering of the nose and not touching face masks when in use since that would contaminate the person's fingers and increase, rather than decrease, the risk of transmission.

Studies about SARS-CoV and other coronaviruses suggest that they may be able to survive on external environmental surfaces long enough to permit transmission via fomites.^{186,187} Coronaviruses, like other enveloped viruses, are quite sensitive to detergents and lipid solvents, such as ether and chloroform, and heat.⁷⁹ Contaminated surfaces may be disinfected with 70% alcohol, diluted bleach, quaternary ammonium compounds, and 3% hydrogen peroxide. These decontaminants must be in contact with the contaminated materials for a sufficient time period to inactivate the amount of virus on the surfaces, taking into account the type of surface being decontaminated and the amount of fluid and organic material present.¹³⁰



FIGURE 1.4 Personal protective equipment (PPE). Coronavirus researcher wearing PPE consisting of a disposable full-body garment, a facemask, and latex gloves. During the SARS-CoV-2 pandemic, the general public was often required to wear one to two masks while indoors. Some people wore face shields.

Incidence of the coronavirus-mediated disease is often highest during winter since these viruses survive best at low temperatures with low levels of ultraviolet light.⁷⁹ Direct use of ultraviolet light (254 nm), while having effective anticoronaviral activity is hazardous to skin and eyes. Far-UVC light (207–222 nm), however, kills microbes without harming exposed humans since it only penetrates a few micrometers into biological materials. UV light inactivates 99.9% of HCoV-229E and HCoV-OC43 when aerosolized in droplets similar to those produced by sneezing or coughing and may have protective activity against other coronaviruses as well. Based on the results with HCoV-OC43, continuous far-UVC exposure from inexpensive excimer lamps could inactivate approximately 90% of coronaviruses within 8 minutes, 95% in 11 minutes, and 99% in 16 minutes, and 99.9% in 25 minutes in occupied public spaces.¹⁸⁸

Whole room UVC disinfection systems greatly reduce numbers of at least some species of infectious coronaviruses, including SARS-CoV, on surfaces after 5–10 minutes.¹³⁴ UVC disinfection works best when combined with a standard cleaning of hard surfaces. It should be noted that UV light damages plastics, so care must be taken not to damage plastic-containing materials in these treated rooms.

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Severe acute respiratory syndrome (SARS)

2.1 Introduction

2.1.1 A brief overview of the 2002–2003 severe acute respiratory syndrome-coronavirus outbreak

Beginning in late 2002, an outbreak of a new, deadly respiratory illness, later named severe acute respiratory syndrome (SARS), struck first Asia, and later, countries throughout the world until 2003, when the epidemic essentially ended, except for a few cases in 2004. The 2002–2003 outbreak led to large numbers of cases with a high mortality rate.¹ SARS results from infection by SARS-CoV (SARS coronavirus), a lineage B betacoronavirus, subgenus *Sarbecovirus*. This virus was present in the lungs of all tested patients with the fatal disease. A traveler from Asia brought SARS-CoV into Toronto, Canada, which also experienced a high rate of infection and death. Travelers from these areas then spread the infection into many other countries throughout the world, leading to scattered, smaller outbreaks.² Unlike the far smaller outbreak of H5N1 avian influenza in humans which is acquired by contact with live or dead domestic birds, SARS-CoV was easily transmitted between humans by causal contact, similar to the spread of the common cold viruses, to which it is related, but with much more serious consequences.¹ While raising public and professional fears of a possible pandemic with a highly pathogenic, readily transmissible virus, SARS was never classified as such

2.1.2 Phases of the 2002–2003 outbreak

The 2002–2003 SARS epidemic may be divided into early, middle, and late phases. During the early phase of the epidemic, limited numbers of cases occurred in localized areas in China and Hong Kong and were believed to have been acquired from live wild animals sold in “wet markets.” During the middle phase of the epidemic, person-to-person transmission increasingly became the primary means of SARS-CoV transmission. This change in the route of transmission was accompanied by increases in the viruses’ geographical range.

“Superspreaders,” people who transmitted SARS-CoV to an unusually high number of other people, played a large role in increasing the number of people infected.³ One such superspreader was infected in China and hospitalized in a general ward in Hong Kong. Within 2 weeks, 138 people were infected following exposure to this patient. During the late phase of the epidemic, the spread of SARS-CoV was international.

Genetic analysis revealed that SARS-CoV isolated from patients infected early during the epidemic developed a much more severe form of the disease than SARS-CoV isolates from patients who were infected later. The SARS-CoV Urbani strain is a less virulent variant isolated in the later stages of the 2002–2003 epidemic. Viable viruses of the Urbani strain alter their host cells’ immune responses in vitro to a greater extent than the more pathogenic SARS-CoV strains from the initial stages of the epidemic.⁴ These immune system alterations, however, are not caused by inactivated SARS-CoV.

Part of the change in pathogenicity over time appears to be due to alterations in viral protein 8, encoded by two overlapping **open reading frames (ORFs)**, ORF8a/8b. SARS-CoV circulating during the later phases of the epidemic typically contained a large 29-nt deletion in ORF8 that led to the production of two smaller proteins, 8a, and 8b, or an 8ab fusion molecule.^{5,6} This deletion decreases viral replication by 23-fold and is dependent on **type I interferons (IFNs)**, some of the most powerful antiviral immune molecules.⁷ The generation of two overlapping ORFs may have played a major role in the adaptation of SARS-CoV from animals to humans.⁶

2.1.3 “Wet Markets” and wild cats and dogs

The SARS outbreak triggered an intensive research effort that rapidly traced the disease to infection with a previously unknown coronavirus that was found in several animals sold in live animal (“wet”) markets and was responsible for initiating the human epidemic. **The zoonotic transmission** was linked to contact with live Himalayan **palm civet** cats (*Paguma larvata*), **raccoon dogs** (*Nyctereutes procyonoides*), or Chinese ferret badgers (*Melogale moschata*) either in wet markets or in restaurants.^{8,9} See [Fig. 2.1](#) to view palm civets in a live animal market.

Civets experimentally infected with SARS-CoV have the virus in their lungs, liver, kidneys, heart, lymph nodes, and spleen in addition to their respiratory tracts. Civets infected with SARS-CoV isolates that contain the 29-nucleoside deletion had a higher body temperature than those infected with the full-length genomic RNA.¹⁰

2.1.4 The severe acute respiratory syndrome-coronavirus spike protein and its angiotensin-converting enzyme 2 receptor

Angiotensin-converting enzyme 2 (ACE2) was identified as the host cell receptor for SARS-CoV.¹¹ Differences in key viral genes and how well the spike (S) protein of different viral variants bind to the human form of ACE2 affect the ability of SARS-CoV to infect various types of cells as well as disease severity. Other human coronaviruses, such as the generally far less pathogenic HCoVNL63 and the current pandemic human coronavirus, SARS-CoV-2, also use ACE2 as their receptor. SARS-CoV-2 also binds to ACE2 of civet



FIGURE 2.1 Civet cats in animal meat market. Civet cats, raccoon dogs, and Chinese ferret badgers from live animal meat markets are believed to serve as intermediate hosts that transmitted SARS-CoV to humans. This market is located in Guangzhou, China. Bats are also sold in these markets. Paul Hilton. University of East Anglia. Free under Creative Commons.

cats, raccoon dogs, rhesus monkeys, ferrets, minks, and cats, but not the ACE2 of the horseshoe bat (*Rhinolophus pearsonii*) or rats (*Rattus* species).¹² Of these, SARS-CoV-2 possesses the greatest binding affinity for human ACE2.¹³ This may at least partially explain why SARS-CoV-2 is transmitted so readily among humans and may cause severe disease in susceptible human populations.¹⁴ Viral variants that bind well to the ACE2 receptor typically caused more severe disease than those viruses that do not bind well to it.

ACE2 RNA is found in at least some cells in almost all organs of the human body, but production of the ACE2 protein is restricted. Cell surface ACE2 is found on the **endothelial cells** of the **capillaries** around the **alveoli** (the small, terminal sacs of the airways in which gas exchange between the blood and lungs occurs), on the **enterocytes** of the digestive system which absorb material passing through the small intestine, and in the **vasculature** of the brain, but not in brain tissue proper. SARS-CoV infects enterocytes from all regions of the small intestine, but not the stomach or colon. ACE2 is also found on the surface of cells lining the **lumen** of the arteries and veins and the smooth muscle cells of the arteries. Interestingly, even though SARS-CoV causes primarily respiratory disease, the highest levels of ACE2 expression are present in the small intestine, gall bladder, kidneys, testes, and heart.¹⁵

With several exceptions, ACE2 is not detectable on the surface of **leukocytes**.^{16,17} Nevertheless, SARS may directly infect leukocytes, including **macrophages**, **dendritic cells** (DCs), **T lymphocytes** (T cells), and **neutrophils**. **B lymphocytes** (B cells) and **natural killer cells** (NK cells) are infected to a lesser extent.^{4,13,18} While DCs and NK cells can be infected by SARS-CoV and the virus remains viable within them, they do not support SARS-CoV replication. Interestingly, some ACE2-expressing endothelial cells and intestinal cell lines are not infected by SARS-CoV, while other cells lacking ACE2, such as liver cells, are infected as shown in [Table 2.1](#).¹⁹ Taken together, these findings suggest that ACE2 alone is not sufficient to allow SARS-CoV entry into host cells.

TABLE 2.1 Cells infected by SARS-CoV and the presence or absence of ACE-2.

Cell type	Body region	SARS-CoV replication	ACE2 presence
Endothelial cells	Lungs Brain Vascular cells	Positive	Positive
Enterocytes	Small intestine	Positive	Positive
Enterocytes	Stomach	Negative	Positive
Epithelial cells	Gall bladder Kidneys Heart Pituitary gland Thyroid gland	Positive	Positive
Smooth muscle cells	Arteries	Negative	Positive
Type I and II pneumocytes	Lungs	Positive	Positive
Leukocytes	T cells Macrophages Neutrophils	Positive	Negative
Leukocytes	B cells ^a Dendritic cells ^a	Negative	Negative
Hepatocytes	Liver	Positive	Negative

^aInfected by SARS-CoV without viral replication.

Other compounds have been found to bind to the S protein and may help to facilitate viral entry. **Vimentin**, an intracellular filamentous **cytoskeletal protein**, directly interacts with the SARS-CoV S protein and is necessary for binding to and entering host cells.¹⁹ Vimentin is part of the SARS-CoV S protein-ACE2 complex and is similar to complexes necessary for the replication of other viruses as well.¹⁹ Vimentin typically serves as a receptor involved in triggering the host cell's **extracellular-signal-regulated kinase (ERK)** signaling pathway. This pathway carries messages from the outside of the cell to the nucleus and, in this case, ultimately results in increasing the numbers of **monocytes** and neutrophils to fight microbial infections.²⁰ Several other molecules also bind the S protein of SARS-C-V, including **dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN)**, a molecule expressed on the surface of DC and macrophages, and a related molecule, **liver/lymph node-specific intercellular adhesion molecule-3-grabbing integrin (L-SIGN)**, that is expressed on endothelial cells of liver and lymph nodes.^{19,21}

2.2 The history of severe acute respiratory syndrome

The first reported cases of severe, atypical pneumonia, later identified as SARS, occurred in mid-November 2002, in the Guangdong Province in southern, coastal China. The illness is known to have led to 305 cases with five deaths. Almost a third of the cases

occurred in healthcare workers.^{22,23} Evidence from stored blood later indicated that 1.8% of healthy members of the Hong Kong population had developed antibodies to an animal form of the virus by 2001.

In December 2002, a second outbreak began with the infection of a Chinese chef. Subsequent outbreaks early during the disease's history were often traced to contact with live exotic or game animals in restaurants or wet markets. By mid-March 2003, SARS was found in healthcare workers and household members of SARS patients in several Asian countries. Contract tracing linked the outbreak to a doctor from Guangdong Province of China who had traveled to a conference in Hong Kong, where he entertained guests in his hotel room. Sixteen people on the same floor of his hotel were later found to be infected.²² Days later, the guests left and sowed the seeds of outbreaks of cases in the hospital systems of Hong Kong, Viet Nam, and Singapore. SARS began to spread throughout the world along international air travel routes.²¹

By the end of April 2003, over 4300 SARS cases and 250 SARS-related deaths were reported from more than 25 countries.²³ When the epidemic ended later in 2003, over 8000 people had been infected. In the United States, eight people had laboratory-confirmed SARS-CoV infection and all of these were travel-related.¹ A rapid and concerted effort led to the identification of a novel coronavirus as the causative agent of this disease by March 2003. The decoding of the complete content of the SARS-CoV RNA genome was finished on April 12th.

A SARS-like-CoV was isolated from nasal or fecal swabs of six palm civet cats and a **raccoon dog** from a wet market in Shenzhen, China, following the removal of a ban on serving palm civets in these venues. In these markets, many different species of animals from different geographical locations are brought into proximity to each other and humans. Only civets from wet markets, and not those from farms or wild-caught, had antibodies against SARS-CoV in their blood. Some ferret badgers from Chinese markets also had evidence of infection with a SARS-like-CoV. Given the unusually high interest in bats as the source of a SARS-like-CoV, it should be mentioned that bats are often sold and served in wet markets and restaurants in China as well. Genetic analyses have shown that some of the coronaviruses from bats have a high degree of similarity to two other severely pathogenic human coronaviruses, Middle East respiratory syndrome coronavirus (MERS-CoV) and SARS-CoV-2, which are subjects of other chapters. While isolation of SARS-like-CoV strains from bats has often failed,²⁴ some studies have found bats in which the S protein has a remarkably high level of amino acid identity with the 2002–2003 epidemic of SARS-CoV strain from humans.^{7,25–27}

Several other early clusters of human cases in Guangdong and the Guangxi Province, located immediately to its west, were traced to human-to-human spread from infected individuals to uninfected family members and healthcare workers. Fortunately, SARS-CoV appears to only have been able to undergo several rounds of transmission among people. Nevertheless, the virus spread rapidly to seven other Chinese provinces. The virus and disease spread from there to several other parts of Asia and Toronto, Canada, eventually involving a total of 29 countries from five continents and was responsible for a cumulative number of 8,096 cases and 774 deaths, a number that was later reported as 8437 cases with 812 deaths.^{22,23}

A particularly high mortality rate (21%) was seen in hospital personnel. Moreover, a study of SARS patients during this epidemic revealed that 50% of patients required

supplemental oxygen, 20%–36% were admitted to an intensive care unit, and 13%–26% developed **acute respiratory distress syndrome (ARDS)**, which requires invasive ventilatory support.²⁸ Public health measures, such as issuing travel advisories, screening international travelers for fever, and quarantining contacts of infected people, appear to have played important roles in halting the spread of SARS-CoV throughout the world by July 2003, except for several laboratory-related cases later in 2003 and four mild, naturally occurring cases in China in January 2004. It should be noted, however, that the extent to which these preventative measures affected the course of the disease is not known, especially since the SARS-CoV strain that was predominant in the later part of the pandemic was less pathogenic than that prevalent during the initial stage.

This epidemic, like other severe disease epidemics throughout history, had an associated societal and financial impact.²⁹ Due to its geographical distribution, the SARS epidemic led to fear of contact with Chinese and people from Toronto, leading to discrimination against Asians and their communities. Decreased tourism and travel during the SARS epidemic resulted in economic losses of tens of billions of dollars in the affected areas of the world. This served as a precursor to the much more severe global economic consequences, political upheaval within and between nations, and shredding of traditional cultural practices and human-to-human contact that have and are continuing to be seen during the far larger and longer COVID-19 pandemic of 2019–the present.

2.3 Severe acute respiratory syndrome—the disease

2.3.1 An overview of severe acute respiratory syndrome

SARS results from infection with a highly contagious virus that causes a severe to life-threatening respiratory disease. The incubation period is 2–7 days. Initial symptoms include fever, headache, and malaise, followed by a dry, nonproductive cough and shortness of breath. Diarrhea develops in 10%–20% of patients.²³ SARS predominantly affects the lower respiratory tract, with the lungs being its main target. Progressive respiratory failure leads to death in 3%–10% of the cases.²³ Lung tissues from deceased SARS patients have diffuse alveolar damage (**DAD**) with pneumocytic **hyperplasia** (excessive cell growth) and accumulation of alveolar and interstitial macrophages as well as other symptoms of ARDS. Interestingly, much of the lung damage occurred after the clearance of the virus from the blood.⁴ Small blood vessels and immune organs are other sites often attacked by SARS-CoV. The infection leads to widespread inflammation of the lungs' vasculature, decreased immunity, and severe respiratory distress.¹⁶ SARS-CoV infection in some cases led to massive **necrosis** of cells in the immune system organs, including the spleen and lymph nodes.¹⁶

SARS-COV also infects other organs and tissues as well, leading to mild to severe disease, especially in the **central nervous system (CNS)**.^{30–32} Infection may also result in **anemia** (low numbers of red blood cells), **myocarditis** (inflammation of the heart muscles), and secondary infections. Gastrointestinal disorders, including nausea, vomiting, and watery diarrhea, are present in about 25% of infected people.³³ Cells of the liver, heart and

the endocrine and urinary systems may also be infected, followed by inflammation. The inflammation is believed to be due to a dysfunctional and excessive immune response.¹⁶ In fatal cases of SARS, the events proceeding death include severe respiratory or multiorgan failure, **viremia** (viral infection of the blood), and acute **myocardial infarction** (death of heart cells). **Hemophagocytosis** may also occur in which red and white blood, **platelets**, and their precursors in the bone marrow are ingested and destroyed by other immune cells, such as macrophages.³⁴

The amount of virus present in an individual affects the course of the disease. People with virus levels of less than one million viruses/gram of lung tissue were more likely to have a short illness that lasted less than three weeks. Transmission to other people usually occurred after the fifth day of infection as viral numbers in the **upper respiratory tract** and feces gradually increased. In people who succumbed to the disease, cytokine levels increased at the onset of ARDS. The persistence of high levels of inflammatory cytokines indicated a high risk for poor outcome, implicating proinflammatory cytokines as having played a major role in the extent of pathology.^{35,36} Additionally, up to 64% of patients with ARDS subsequently developed **pulmonary fibrosis** (scarring of the lungs) during the recovery period.³⁷

Unlike the situation with many respiratory diseases, including the common cold and influenza, relatively few children became infected with SARS-CoV, and those who were infected developed less severe disease manifestations than adults, similar to that later seen in SARS-CoV-2 infection. While disease incidence was highest in people between the ages of 20–39 years, only 1% of those infected were under the age of 10 years. The older population was at higher risk of developing serious, primarily early- and late-stage ARDS and pulmonary fibrosis. Survivors had reduced lung elasticity which decreased the lungs' ability to expand and contract as one inhales and exhales.²⁸ One year after infection, evidence of pulmonary fibrosis was still detectable in 27.5% of SARS survivors and many patients also had poor general health in comparison with the general population.³⁸

Mortality rates were age-related. All SARS patients under the age of 24 years survived, compared to a fatality rate of 6%, 15%, and >50% in patients aged 25–44, 45–64, and greater than 65 years, respectively.³⁹ SARS-CoV infected children or adolescents only had mild decreases in lung functioning 6 months postinfection.⁴⁰ This could be due in part to the relative absence of other risk factors in most children under the age of 12 years, who generally have a lower rate of **comorbidities** (the presence of other disease states). Fewer children also receive hospital care, use supplemental oxygen, or are treated with methylprednisolone, an immunosuppressant and antiinflammatory drug, all factors which are associated with more severe disease in adults. Another possible factor in the increased survival of younger people with SARS is an age-dependent increase in the antiinflammatory enzyme phospholipase A2. This enzyme lowers both inflammatory and antiviral immune responses. This latter activity is linked to worse disease outcomes in mice and in cultured human macrophages in vitro.⁴¹

Other conditions that increased morbidity and mortality rates include extensive lung infection, increased levels of certain liver enzymes in the blood, acute kidney failure, and pregnancy. These factors were associated with more severe forms of SARS-CoV infection and poor prognosis, even in younger people. See [Table 2.2](#) for a comparison of SARS-CoV-mediated pathology in various organ systems.

TABLE 2.2 SARS effects on the circulatory, skeletal, digestive, urinary, nervous, endocrine, and reproductive systems.

Organ system	Pathology
Respiratory	Cough Difficulty breathing Rapid breathing rate Low pulse oxygenation Atypical pneumonia Pulmonary fibrosis
Cardiovascular	Thrombocytopenia followed by thrombocytosis Disseminated intravascular coagulation Decreased hemoglobin levels Coagulation and formation of fibrin thrombi Rapid heart rate Decreased isovolumic relaxation time in heart Lower cardiac output Cardiac arrhythmia Cardiac failure Systolic and diastolic blood pressure abnormalities Decreased hematopoiesis
Skeletal	Large joint pain Avascular necrosis and osteonecrotic death (femoral head)
Digestive	Diarrhea Balloon degeneration of hepatocytes Lymphocyte infiltration of the liver Necrotic and apoptotic cell death of hepatocytes Fatty degeneration of the liver
Urinary	Benign hypertensive nephrosclerosis Acute renal failure
Nervous	Seizures Severe meningitis Meningoencephalitis Acute flaccid paralysis Guillain–Barré syndrome
Endocrine	Lethargy, malaise, fatigue, general weakness, anxiety Dizziness Anorexia Apathy and depression
Reproductive	Infertility and destruction of sperm Low levels of immature sperm in the seminiferous tubules Damage to eggs
Fetal Pathology	Thrombi in placenta Intrauterine growth delay Small fetal size Miscarriage and preterm delivery

2.3.2 Severe acute respiratory syndrome and the respiratory system

2.3.2.1 Severe acute respiratory syndrome-induced damage to the respiratory system

While the SARS incubation period in humans is less than a week, up to 2 weeks may pass before a person begins to develop readily observable symptoms. Asymptomatic infections are uncommon in SARS-CoV infected individuals, unlike infection with SARS-CoV-2 in which asymptomatic or relatively mild disease cases are common in some populations. After entering the **pharynx** (throat) of the upper respiratory tract, SARS-CoV infects the **epithelial cells** lining this cavity, including the cells lining the salivary glands and the tonsils. In Chinese macaques, within 2 days after infection, the virus spreads into the lower respiratory system via the **trachea** and the **bronchial tubes** of the lungs.⁴² Early respiratory symptoms include cough, sore throat, and difficulty breathing. During the final stage of SARS in the lungs, 6–8 weeks postinfection, pulmonary fibrosis develops as **collagen** is deposited in the damaged area and cells begin to reproduce in both the alveoli and the **interstitial spaces**. Interestingly, severe lung pathology usually occurs after the clearance of detectable levels of SARS-CoV from the blood and in the absence of the virus.⁴

In severe cases, SARS-CoV-induced lower respiratory tract infection progresses to atypical pneumonia. Rapid breathing and heart rate and low levels of blood oxygen were present in 40%–75% of patients admitted to hospitals. About 20%–30% of all patients required intensive care and many needed **mechanical ventilation** to help them breathe.^{43,44} The severity of the disease may be increased by SARS-CoV-associated shedding of ciliated cells lining the bronchi. A thin layer of mucus lining much of the respiratory tract traps and prevents unwanted material from passing into the lower passages and alveoli. The beating of the **cilia** (small hairlike projections present on the surface of many epithelial cells in the lungs) sweeps the mucus up the respiratory system and away from the lungs. The mucus traps many types of microbes as well as other material passing down the respiratory system via the trachea. Loss or damage to the cilia allows microbes to pass down to the lower regions of the respiratory tract. Loss of the cilia allows the establishment of secondary bacterial diseases, including pneumonia. X-ray evidence of pneumonia involving one or both lungs was found in all tested individuals.

Alveolar pneumocytes line the alveoli. During SARS, some of these cells develop **cytomegaly** (abnormal enlargement of cells) with granules in their **cytoplasm**.³¹ There are two types of alveolar pneumocytes, with the vast majority being type 1. **Type 1 pneumocytes** are flattened cells that exist as a single flat layer (**simple squamous epithelium**). Their thin structure allows rapid exchange of oxygen and carbon dioxide between the alveoli and blood within the lung capillaries. The much less common **type 2 pneumocytes** are larger and cube-shaped (**simple cuboidal epithelium**) that secrete **surfactant**, a slippery fluid that decreases surface tension within the alveoli so that these tiny air sacs do not close when one exhales. Type 1 pneumocytes are infected by SARS-CoV early during the disease (day 4 postinfection). Studies performed in rhesus monkeys suggest that type 1 pneumocytes may be the primary target of the virus during early infection.⁴⁵ Infection of type 2 pneumocytes results in inflammatory damage. Since ACE2 is highly expressed in these cells, they are good targets for SARS-CoV infection.¹⁶ Extensive hyperplasia (expansion of a region due to cellular proliferation) is present by day 6 of infection.^{45,46}

A study of autopsy tissue from the lungs of 32 people from Hong Kong and Toronto who died during the 2002–2003 epidemic found that SARS-CoV N protein or RNA was present in type 1 alveolar pneumocytes in slightly over half of those who died during the first two weeks of illness, but were not found in any patients who died later. Macrophages, but not lymphocytes, were also infected, but to a lesser extent and without spread to regional lymph nodes. Bronchiolar pneumocytes were rarely infected in the study population. Viral replication appears to occur early after detectable illness, but widespread replication halts after two weeks. This makes treatment early after symptom onset a matter of great importance since many treatment options act by stopping or slowing viral replication.⁴⁷ Anti-SARS-CoV antibodies appear at approximately day 10 postinfection, followed by a decline in viral levels in the nasopharyngeal aspirates, urine, and stool samples 10–15 days postinfection.⁴⁸ In disease survivors, 23.7% still had decreased lung functions (low levels of **total lung capacity**, **vital capacity**, and **residual volume**) after 1 year.²⁸

While most patients eliminate the virus after 1–2 weeks, about a third of those infected develop ARDS or other serious lung injuries that require respiratory support and hospitalization. This may be followed by the development of acute **DAD**, an important contributor to lung damage during SARS. By the fourth day of infection, there are extensive changes in the epithelial cells lining the **bronchioles** and alveoli as the cells begin to proliferate. **Squamous metaplasia** develops during which the elongated, rectangular epithelium lining the bronchioles is replaced by multiple layers of flattened cells (**stratified squamous epithelium**).³¹ Early during DAD, protein-rich **edema** is present in the alveoli. During this phase of DAD, **exudation** (fluid slowly oozing from a wound or pores) and inflammation occur. By day 10, dysfunctional surfactant and severe **hypoxia** (decreased oxygen levels) are found.⁴⁹ Following its exudative phase, DAD is accompanied by increased macrophage numbers as these cells proliferate in the alveoli and interstitial regions of the lungs.⁵⁰ Later pathological manifestations include lung hemorrhaging and the formation of **hyaline membranes** in the alveoli which impair gas exchange.⁵¹ After 2–5 weeks, DAD expands to involve 25%–100% of the lungs and the interstitial areas between them. DAD pathology is shown in Fig. 2.2.

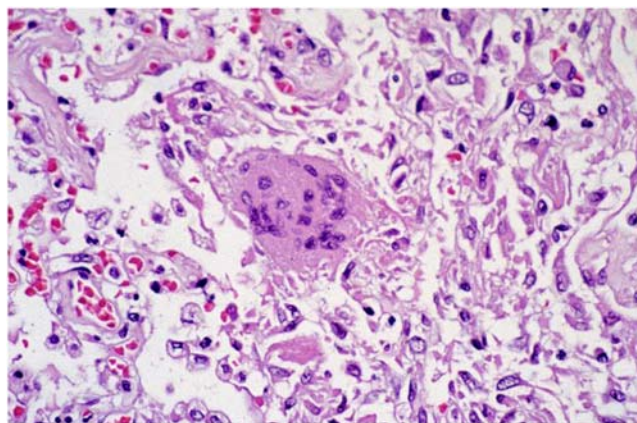


FIGURE 2.2 SARS-CoV-induced diffuse alveolar damage DAD in the lungs. This photomicrograph shows DAD in the lungs of a SARS-CoV infected person. The center of the image contains a multinucleated giant cell.

2.3.2.2 *Severe acute respiratory syndrome and co-infection with other respiratory microbes*

As mentioned previously, some SARS patients are coinfecting with human metapneumovirus, a respiratory virus that is also emerging since its first report in 2001. Coinfection with human metapneumovirus may hasten SARS disease progression or increase its severity.⁵² Coinfections with SARS-CoV and cytomegaloviruses, and reoviruses, were also common. People infected with HCoV-229E also may be coinfecting with human metapneumovirus or respiratory syncytial viruses.⁵³

2.3.2.3 *Pulmonary fibrosis and wound healing*

Alveoli may develop pulmonary fibrosis, which is especially present in the lungs of older patients. This condition typically begins 2–8 weeks after infection. This condition is typified by large-scale scarring of lung tissue which impairs their proper functioning and may result in potentially fatal difficulty in breathing.⁵¹ Radiographic analysis of the lungs revealed multiple areas with a “ground glass” appearance in 38% of the patients.²⁸ One of the key characteristics of fibrosis is the presence of excessive numbers of cells in the affected area, due either to enhanced cellular proliferation or decreased **apoptosis** (programmed cell death). During SARS, both processes occur in the lungs of infected people.

Proper wound healing involves a complex series of events that are necessary for the lungs to regain their normal functioning after SARS-CoV-mediated acute lung injury. Healing begins with infected cells sending out damage signals which recruit inflammatory cells and **keratinocytes** into the damaged region. These cells, in turn, induce the secretion of growth factors, such as **epidermal growth factor (EGF)**, and the chemokines **CXCL10** and **CCL2** that attract monocytes, NK cells, T cells, and DC into the infected region. The binding of growth factors to their receptors stimulates the proliferation of cells that repair and replace injured tissue and its underlying **basement membrane**.⁵⁴ After the repair is completed, the wound healing response is normally terminated and the levels of the proteins involved in this process return to baseline by approximately day 9 postinfection.⁵⁵ When the wound healing response is not properly regulated, pulmonary fibrosis may occur, leading to decreased lung functioning.⁵⁶

The process of wound healing is required for the organized replacement of dead or injured cells and, in the lungs, is generally necessary for survival. Tissue injuries that trigger this process may be acute or chronic and include infections and mechanical or chemical traumatic events. However, this crucial repair process may also be pathogenic if it continues unchecked and may result in the formation of permanent scar tissue that may impede vital bodily functioning and lead to organ failure.⁵⁴ Interestingly, the extent of fibrosis is not always linked to the extent of inflammation, suggesting that different regulatory systems may control these conditions. The outcome may depend upon which of the several types of T helper cell response are active during the disease.⁵⁴

The development of fibrosis appears to be strongly linked to profibrotic **T helper 2 (Th2)** activity that involves secretion of the cytokines **IL-4**, **IL-5**, and **IL-13**.⁵⁴ Th2 responses are double-edged swords in that they are important to both wound healing during an acute disease but also to the development of fibrosis during chronic infections. IL-4 is almost twice as active in inducing fibrosis as the powerful profibrotic cytokine

transforming growth factor- β (TGF- β) that is discussed later⁵⁷. Part of IL-4's wound healing/fibrotic activity lies in its ability to upregulate the production of the **extracellular matrix (ECM)** protein types I and III collagen and **fibronectin**. The ECM proteins form networks of large extracellular proteins that are often composed of collagen, fibronectin, enzymes, and glycoproteins. They are present between cells and normally aid in cell adhesion and intracellular communication. Genes upregulated during a Th2 response also include precursors to collagen (procollagen-I and procollagen-III), **matrix metalloproteinases 2 and 9**, and **tissue inhibitor of matrix metalloproteinase 1**.⁵⁴ While IL-4 and IL-13 share many functions and bind to the same cellular receptor on **fibroblasts**, IL-13 appears to be the more powerful mediator of pulmonary fibrosis, perhaps because its levels are 10–100 times higher than the levels of IL-4 in the lungs. Additionally, IL-13 induces **CCL6**, a chemokine that recruits resting T cells and monocytes into the infected area; **CCL11** which recruits eosinophils; **CCL20** which recruits DCs, T cells, and B-cells; and **CCL22** which recruits monocytes, DCs, and NK cells.⁵⁴ IL-5 also aids in the activation and recruitment of eosinophils, which may serve as an important source of other pro-fibrotic cytokines, including TGF- β and IL-13. IL-5 acts together with eosinophils during tissue repair in pulmonary fibrosis.⁵⁴

The production of the potent antifibrotic cytokine **IFN- γ** by the **T helper 1 (Th1)** cells leads to inflammatory responses and reduction of tissue fibrosis. Chronic inflammation thus does not necessarily lead to the deposition of fibrosis-related tissue elements in the damaged area. During chronic inflammation, a predominately Th1 response occurs in the tissues of mice. This then leads to the transcription of the many genes associated with IFN- γ activity, but without significantly triggering a fibrotic response. Instead, genes involved in the **acute-phase reactions** and those genes involved in apoptosis are upregulated which may help to induce the large amounts of cell death and tissue damage that are found when Th1 cell responses are unrestrained.⁵⁴

Normal wound healing following infection occurs in several steps. During the first step, SARS-CoV damages epithelial and endothelial cells, triggering an antifibrinolytic cascade that forms a temporary patch on the wounded area. Next, neutrophils, macrophages, eosinophils, and **fibrocytes** are drawn to the sites where they release the proinflammatory, profibrotic cytokines IL-1, **tumor necrosis factor- α (TNF- α)**, IL-13, and TGF- β . Phagocytic macrophages and neutrophils remove debris from the injured cells and eliminate the infecting microbes. In elderly people with SARS, the cellular infiltration into the lungs is also accompanied by large levels of fibrous protein **fibrin**.⁵⁸ The presence of greater levels of neutrophils in the lungs of infected elderly people is also found in aged BALB/c mice and suggests that infiltrating neutrophils may make a major contribution to the increased severity of pathology in the aged humans and mice.⁵⁹

Fibroblasts then proliferate and differentiate into **myofibroblasts**. Myofibroblasts are a critical cell type that coordinates wound repair and secretes new ECM components upon which the replacement tissue is rebuilt.⁶⁰ This occurs under normal wound healing conditions. Afterward, the wound healing process is terminated and the myofibroblasts contract and decrease the size of the wound. The levels of many of the now unneeded repair cell types are normally reduced by apoptosis.³⁷

Dysfunctional wound repair, however, often leads to more SARS-related severe disease, including the development of pulmonary fibrosis, especially in the elderly, primarily due

to a hyperactive response to virus-induced lung injury. It is mediated by **epidermal growth factor receptor (EGFR)** signaling through a complex process that involves binding to at least one of its seven known **ligands** (compounds that bind to a specific receptor).³⁷ Depending upon local circumstances, EGFR signals may increase or decrease wound healing activity, thus increasing or decreasing the risk of developing fibrosis. Factors that may affect the outcome of EGFR signaling include which of its seven ligands it binds and the timing of this signal (during the initial stage of wound healing vs later times in the wound repair process). Levels of two of the EGFR ligands, **amphiregulin**, and **heparin-binding EGF-like growth factor**, are increased during SARS. Inoculation of SARS-CoV-infected aged mice with these ligands increases the wound healing process in a manner that enhances lung damage.³⁷ Activation of the intracellular signaling molecule **STAT1 (signal transducer and transcription activator)** decreases the excessive cell growth triggered by EGFR signaling. Mice lacking STAT1 have dysregulated wound healing and increased cell growth which results in fibrosis as discussed later.³⁷ Treatment strategies that block EGFR signals, therefore, might be beneficial or harmful to the patients, depending upon only partially understood conditions that are further complicated by the SARS-CoV strain involved.³⁷

One of the consequences of dysregulated wound healing is the infiltration of inflammatory cells into the region, followed by the deposition of excessive amounts of EM.⁶¹ Fibrin is activated to form a fibrous network that traps blood cells, producing a clot. When fibrin is deposited at the correct time and levels, it is an important part of wound healing. After the wound is healed, however, the clot normally is removed by another protein, **plasmin**, that helps to degrade fibrin when it is no longer needed, thus preventing fibrosis.⁶¹ Plasmin is produced by the cleavage of **plasminogen** by the **urokinase** and **tissue plasminogen activator** enzymes and takes part in the removal of clots once the wound healing process is complete. The actions of plasmin are blocked by **plasminogen activator inhibitor-1 (PAI-1)**, which promotes fibrosis, including pulmonary fibrosis as discussed in greater detail later.

2.3.2.4 SARS, coagulation, and extracellular matrix proteins

SARS causes excessive **coagulation** (formation of blood clots) and hematologic dysfunction, including the production of edema, **fibrin thrombi** (fibrous blood clots) in the vasculature of the lungs, **pulmonary embolisms**, **deep vein thrombosis**, multiorgan **infarctions**, and **ischemic strokes**.⁶² These are described in greater detail in Chapter 1 where coagulation-related issues among various human coronaviruses will be compared and contrasted with one another. See Giannis⁶² for an excellent review of coagulation pathology during SARS.

The coagulation process may either aid in acute and beneficial wound repair or may instead contribute to chronic and excessive formation of clots and fibrosis. Coagulation involves complex interactions between multiple clotting factors, proteases, and their precursors, as well as fibrous proteins that act together to form fibrin thrombi. One of the “clotting factors” is factor Va, a cofactor for the enzyme factor Xa, which together form the prothrombinase complex. This complex rapidly converts inactive **prothrombin** to the active form of the **thrombin** enzyme.⁶³ In its active form, thrombin converts soluble **fibrinogen** into insoluble strands of fibrin. Tissue plasminogen activator is an anticlotting agent

which aids in the removal of clots after the completion of wound repair, as stated previously. Blood levels of urokinase and PAI-1 are increased in SARS-CoV-infected patients as well as in the lungs of SARS-CoV-infected macaques.⁵¹

A study of genes that are differentially expressed before and after SARS-CoV infection found that coagulation-related pathways, such as the antifibrotic pathway, strongly influence whether the infection will be lethal or result in recovery.⁵¹ Activation of the urokinase pathway leads to the degradation of proteins present in the blood clots that are lodged in the lungs. In the presence of fibrin, urokinase increases plasmin levels by initiating the fibrinolytic pathway. Plasmin degrades fibrin and thus aids in the destruction of fibrin thrombi. Plasmin also inactivates the blood clotting factor Va, as discussed above, and VIIIa, a nonenzymatic part of the coagulation pathway.⁶⁴ Malfunction of the urokinase pathway may result in fibrotic lung disease or hemorrhage.⁵¹ Alterations in the urokinase, coagulation and fibrinolytic pathways occur during SARS and result in thrombi that clog small airways in the lungs, thus preventing carbon dioxide and oxygen gas exchange. SARS-mediated alteration of these pathways disturbs the hemostatic balance produced by coagulative and antifibrinolytic factors. SARS induces a high-fibrin state within the alveoli, leading to fibroblast growth and adherence to the clot, followed by the deposition of collagen. The importance of the urokinase pathway in regulating SARS severity was confirmed by comparing the decreased pathology in normal mice versus mice in which the urokinase pathway is inactive. In the latter mice, the fibrotic clot remains, resulting in chronic lung pathology.⁵¹

2.3.2.5 The severe acute respiratory syndrome-coronavirus N protein, transforming growth factor- β , and lung damage

The viral nucleocapsid (N) protein increases TGF- β levels. TGF- β induces the production of PAI-1, thus increasing clot formation.⁶⁵ TGF- β induces the production of fibrin and collagen that compose part of the fibrotic scar, but can instead trigger the secretion of matrix metalloproteinase enzymes which break down damaged proteins before their removal by phagocytic cells and thus decrease fibrosis. The source of TGF- β may help to determine whether this cytokine induces or suppresses fibrosis since macrophage-derived TGF- β 1 is often pro-fibrotic and T-cell-derived TGF- β 1 is antifibrotic.⁵⁴ The release of TGF- β by injured cells during the normal lung repair process helps to clear the lung of infectious microbes. During SARS, however, the fibrin-forming macrophage-derived TGF- β 1 pathway is often hyperactivated, in part due to the actions of the viral N protein, thus promoting pulmonary fibrosis. In animal models of TGF- β , rodents that overexpress TGF- β also develop pulmonary fibrosis. The presence of TGF- β in the lungs stimulates the maturation of lung fibroblasts into wound repair myofibroblasts as well.³⁷ By thus increasing the amount of TGF- β to abnormally high levels, the viral N protein dysregulates the wound repair process and contributes to the production of pulmonary fibrosis. Several **chemokines**, CCL2 and CCL3, play an important role in wound repair/fibrosis since they recruit mononuclear phagocytes into the affected area.⁵⁴ The levels of these two chemokines are regulated by IL-13.

In vitro, the binding of activated **Smad3** to **Smad4** causes apoptosis of lung cells that are no longer needed, decreasing fibrosis.⁶⁵ The viral N protein binds to Smad3 and competitively inhibits Smad3-Smad4 binding. This reduces the formation of apoptotic Smad3/Smad4

complexes while increasing the formation of TGF- β /Smad3 complexes. The latter complexes lead to higher levels of PAI-1, thereby indirectly increasing fibrosis by blocking plasmin formation.⁶⁵ The N protein also inhibits the expression of several other proapoptotic genes (*Bax* and *Bim*). Taken together, the N protein decreases host cell apoptosis while contributing to pulmonary fibrosis via the fibrinogenic actions of TGF- β .⁶⁵

2.3.3 Severe acute respiratory syndrome and the cardiovascular system

Some SARS patients develop circulatory disease manifestations due to **platelet** activation, malfunction of blood vessels, and excessive inflammation. As platelet activation occurs, these blood elements are depleted. Platelet numbers at first decrease (**thrombocytopenia**) in 55% of the patients, followed by **thrombocytosis** (elevated platelet numbers) in 49% of the patients.⁶⁶ Potentially fatal **disseminated intravascular coagulation** (DIC) was present in a 2.5% of patients during the SARS epidemic. The early stage of DIC is characterized by excessive production of blood clots that reduce blood flow. This leads to the depletion of platelets and clotting factors which, in the later stages of SARS, results in excessive bleeding. Hemoglobin levels are also reduced in many patients.⁶⁶ Differences between patients who died and those who survived include DIC in 71% of patients who died and 0.6% among the survivors.

Circulatory system defects present in the respiratory tract include the generation of fibrin thrombi, the ultimate products of blood coagulation, as described previously. The thrombi consist of aggregated platelets and red blood cells that form a plug together with a mesh of cross-linked fibrin proteins. They are produced in the vascular system of the **bronchial tubes**, lung tissues, and small lung veins. One study found thrombocytopenia in about half of the SARS cases, with the lowest platelet count occurring one week after symptom onset and **reactive thrombocytosis** (elevation of platelet counts in response to low platelet levels) peaking at week three.⁶⁶ Convalescing patients have higher than normal platelet counts. **Cerebral infarction** (lack of adequate oxygen supply to the brain that results in cell death) found during SARS is present in severe cases of COVID-19 as well and may also cause ischemic strokes.⁶⁷

Injury to fetuses of infected mothers is linked to clotting abnormalities. These injuries include intrauterine growth delay and small fetal size due to abnormal blood circulation in the placenta, resulting from fibrin deposition and the formation of small clots in the placenta. High incidences of miscarriage and preterm delivery are also reported.⁶⁸

In addition to the above effects of SARS-CoV on the vascular system, the heart of infected people is also affected. In a small study, subclinical cardiovascular manifestations of SARS were detected that include a longer **isovolumic relaxation time** (the time of the relaxation phase of the heartbeat) and lower **cardiac output** (the amount of blood ejected from the left ventricle in a single heartbeat) during the acute phase of infection in comparison with those found 30 days after recovery. Heart valves were not damaged and the blood pressure in the lungs remained normal.¹¹ Those patients who required mechanical respiration had a more vigorous immune response and worse **diastolic** dysfunction than the patients with the less overall disease. Nevertheless, following the resolution of the initial acute inflammatory response, people who had experienced more severe disease

entered the **convalescent** phase in which cardiac abnormalities were reversed. These findings indicate that during the acute phase of SARS, the left ventricle of the heart may experience a low level of abnormal functioning during the heart's resting phase and that this condition is reversible.⁶⁹

Pulmonary damage may be associated with fatal SARS-CoV infection of the heart tissue as well. This leads to age-dependent cardiac dysfunction, including **arrhythmia**, sudden cardiac failure, and **systolic** and **diastolic** blood pressure abnormalities. Upon autopsy, viral RNA was found in 35% of the heart samples from these patients and they died earlier than patients whose heart was not infected. Infected hearts also had large levels of macrophages, but not lymphocytes, infiltrating the region and causing myocardial damage. Expression of ACE2 RNA and levels of ACE2 protein is also decreased in the heart during SARS. Loss of ACE2 from the heart cells may contribute to **cardiomyopathy** as well as increasing pulmonary and kidney disorders.⁷⁰

2.3.4 Severe acute respiratory syndrome and the skeletal system

Approximately 53% of SARS survivors reported pain in their large joints for up to six months after recovery.³⁸ This may be related to SARS-induced bone cell death as well as the death of **hematopoietic** cells in the red bone marrow that produce all types of blood cells and platelets, some bone cells (**osteoclasts**), and marrow fat cells.⁷¹ Joint damage may cause **avascular necrosis of the femoral head** and **osteonecrotic death** of the bone cells in the head of the **femur** (the "ball" of the ball and socket joint of the hip) caused by the lack of an adequate blood supply to the region. The hip region is of great importance not only for its role in weight-bearing but also for being one of the major sites of hematopoiesis. In a study of 29 SARS patients, the damage was also found in the following regions: the hips and knees, knee(s), the proximal fibula of the lower leg, and the talus (an ankle bone).⁷¹

Corticosteroids are antiinflammatory immunosuppressants given to some SARS patients to alleviate virus-induced inflammation. Avascular necrosis in the hip and lower extremities is primarily found during the first year after high doses of short-term intravenous **corticosteroid** therapy.⁷² In a study of male patients, 39.5% were diagnosed as developing avascular necrosis, as opposed to 19.3% of the female patients, however, more male than female patients had this treatment regimen which may contribute to the differences between the sexes.⁷³ The total and maximum doses of steroids and the length of treatment were higher in patients with avascular necrosis in comparison to those with normal hip structure. Interestingly, the necrotic disorder is primarily found in young adults aged 20–49 years (25.9%) than in older adults aged 50–59 years (6.3%).⁷³ A small number (5%) of the patients received surgical treatment. In addition to the use of the corticosteroids, extensive alcohol use, smoking, diabetes, and autoimmune diseases are risk factors for developing skeletal disease manifestations.^{74,75}

2.3.5 Severe acute respiratory syndrome and the digestive system

In addition to respiratory system disease, some SARS patients develop concurrent gastrointestinal disorders in the lower digestive tract, but not the esophagus or the stomach.

Near the beginning of the SARS outbreak, aerosolized fecal material from a faulty sewage system in the Amoy Gardens housing estate in Hong Kong appears to have played a role in the infection of greater than 300 residents, at least in part due to contact with fecal contamination on elevator buttons and door handles.⁷⁶ Infection in these cases appears to have been by the fecal-oral route and may have involved rats. In addition, some pet cats from that estate were found to be infected with SARS-CoV.⁷⁷

Biopsies of both small and large intestines detected viral replication. Additionally, SARS-CoV has been isolated from the intestines and its RNA is shed in the stool of patients for over 10 weeks.³³ The viral load in the small intestine may be even higher than that seen in the lungs. Interestingly, other than minimal destruction of some intestinal cells, the intestines have a normal appearance under endoscopic and microscopic examination.³³ In one study of 138 patients, 38% had watery diarrhea at some time throughout the illness, particularly during the first week. Diarrhea generally lasted for an average of 3.7 days and, in most patients, the diarrhea was self-limiting.³³

During SARS, the liver tissues also contain many dividing **hepatocytes** (liver cells) along with balloon degeneration of these cells. Small to moderate amounts of lymphocytes infiltrated the liver.⁷⁸ Both necrotic and apoptotic cell death were present in this organ as well. **Fatty degeneration** of the liver (the accumulation of small fat droplets in the cytoplasm of hepatocytes) also occurs in some patients.⁷⁹

2.3.6 Severe acute respiratory syndrome and the urinary system

SARS-CoV is found in the cells lining the kidney's **distal renal tubules**.⁸⁰ A considerable number of the cells in this region of the kidney underwent necrotic death. Some areas of the kidneys also hemorrhaged. Benign **hypertensive nephrosclerosis** may also be present in which chronic high blood pressure leads to hardening and thickening of the kidney tissues.^{78,81} SARS-CoV has also been isolated in urine, as discussed previously.

Patients with SARS-related **acute renal failure (ARF)** had a mortality rate of 77%. This condition was more common among older patients, males, and diabetics.⁸² Patients with ARF usually had multiple organ system failures as well. ARF was also linked to gastrointestinal bleeding and **rhabdomyolysis** (destruction of striated muscle cells) elsewhere in the body. **Myoglobin**, a breakdown product of these muscles, can damage the kidneys and lead to ARF.

2.3.7 Severe acute respiratory syndrome and nervous system

Many of the human “respiratory” coronaviruses, including the human coronaviruses HoCV-229E, HCoV-OC43, SARS-CoV, SARS-CoV-2, and MERS-CoV, have been detected in the CNS. These coronaviruses and SARS-CoV cause mild to severe neurological illnesses in humans.^{83–85} SARS-CoV-2 and MERS-CoV will be further discussed in separate chapters. Of the SARS patients from Hong Kong in 2002–2003, 56% reported headache and 43% reported dizziness, while in Toronto, 35% reported headache and 4% dizziness.^{86,87} Seizures and **meningoencephalitis** were occasionally reported in SARS patients, although SARS-CoV-related **encephalitis** was extremely rare in people of all ages.⁸⁸

In very young children, no SARS-CoV may be present in cerebrospinal fluid and all children receiving acetaminophen therapy recover.¹³ Neuronal infection with SARS-CoV occurred without inflammatory infiltrates. In adults, CNS receptors for SARS-CoV, SARS-CoV-2, and MERS-CoV are primarily located in the brain vasculature. This includes ACE2, the receptor for SARS-CoV and SARS-CoV-2. L-SIGN may also serve as a SARS-CoV receptor.¹³ It is found in the human brain microvascular endothelial cells as well as endothelial cells in the liver and lymph.²¹ The relevance of L-SIGN in SARS-CoV-mediated CNS pathology is currently unknown.

SARS-CoV can cross the **blood-brain barrier**, a structure formed by **astrocytes**, a type of **glial cell** which normally prohibits microbes, immune cells, toxins, and many other substances from entering the brain. During SARS, T cells can leave the brain vasculature and infiltrate the brain tissue itself.⁷⁹ The entry of viruses and lymphocytes into the brain tissue may result from the interaction of SARS-CoV with the endothelial cells that line the brain capillaries,⁷⁸ making them more permeable and subject to **edema**.

The human coronavirus HCoV-OC43 may enter the mouse brain using **axonal transport** to travel back along the axon to the cell body in **olfactory nerves**. These nerves are in the nasal area which is close to the brain.⁸⁹ SARS-CoV also enters the brain via the olfactory nerves and, from there, spreads throughout the brain and spinal cord during the next 6–12 hours.^{90,91} SARS-CoV protein and nucleic acids have been detected in the cytoplasm of brain neurons, particularly in the gray matter of the **cerebral cortex** and in the **hypothalamus**, but not in the **cerebellum**.^{78,92,93} In addition to edema, the brains of SARS patients had degenerative changes. The neurons underwent necrosis, while the glia, the neurons' support cells, divided, leading to glial hyperplasia.^{79,93}

In animal models, both the animal species' coronavirus and several human coronaviruses cause **demyelination** (loss of the fatty covering surrounding some of the nerves), necrosis of the neurons, and meningoencephalitis when these viruses are inoculated into nasal cavities or directly into the brain.¹³ The animal coronaviruses that infect the CNS include porcine hemagglutinating encephalitis virus, feline coronavirus, and mouse hepatitis virus which will be discussed in a separate chapter.⁹⁴

In **transgenic** mice which express the human form of ACE2, the brain is a major site of SARS-CoV in the CNS.⁹⁰ In these mice, as in humans, the virus enters the brain primarily via the olfactory nerve and olfactory bulb and rapidly spreads between connected neurons. Even low doses of the "respiratory" SARS-CoV kills all infected mice, even though infection of the lungs is minimal. Death appears to be due to the lack of living and functional neurons, especially those found in the cardiorespiratory centers of the **medulla oblongata**, part of the brainstem that controls vital functions. Severe brain pathology can occur with only minimal viral infection or cellular infiltration.⁹⁰ Excessive levels of IL-1, TNF- α , and IL-6 are also found in the brains of these infected transgenic mice. IL-6 is primarily generated by infected neurons even though astrocytes normally produce this proinflammatory cytokine.⁹⁰ Co-infection with SARS and noncoronavirus respiratory viruses, such as respiratory syncytial virus and human metapneumovirus, may be responsible for the enhancement of neurological diseases, including severe meningitis, encephalitis, **acute flaccid paralysis**, and possibly **Guillain-Barré syndrome**, and may lead to long-term sequelae.^{94,95}

It should be noted that severe pathology occurs in both the lungs and the brain in all transgenic mice expressing human ACE2 following infection by the intranasal route.⁹⁶ Mice with the highest level of human ACE2 expression lost 20% of their body weight before death between 3–5 days postinfection. Extensive virus replication was observed in the lungs of these mice along with inflammatory cell infiltration, hemorrhaging, and the sloughing of the lung epithelial cells.⁹⁰

Neuropathology is not only present in SARS patients, but people infected with other human coronaviruses as well. SARS-CoV-2 in the cerebrospinal fluid and the brain produces several neurological disorders in about one-third of the patients and 88% of those with severe COVID.⁹⁷ The nsp2 and nsp5 from HCoV-OC43 increase virulence and pathogenesis in the CNS of experimentally infected mice.⁹⁸ Acute disseminated encephalomyelitis was reported in a teenager infected with HCoV-OC43, but the disease was self-resolved.⁸³ T cell responses to infection may also be partially responsible for causing virus-induced encephalitis and demyelinating **multiple sclerosis**-like lesions, as is the case during mouse hepatitis virus infection.^{99,100} In fact, a study of coronaviruses in patients with multiple sclerosis detected RNA of HCoV-229E in 36% of the patients' CNS tissue but was not detectable in patients with other neurological disorders or normal control subjects.¹⁰⁰ HCoV-229E RNA was detected not only in the white matter of the brain and spinal cords but was also found in gray matter. Human aminopeptidase N and N-acetyl-9-O-acetylneuraminic acid, which serve as the cellular receptors for HCoV-229 and HCoV-OC43, respectively, are present in the brain's synaptic membranes.

2.3.8 Severe acute respiratory syndrome and the endocrine system

The **endocrine system** is composed of organs and glands that produce hormones. Several key endocrine organs and hormones are affected during SARS and, of these, the virus has been shown to infect at least the **pituitary** and **adrenal glands**.¹⁰¹ The effects may play a major role in the lethargy, malaise, fatigue, general weakness, dizziness, anorexia, apathy, anxiety, and depression that may be seen for months or up to a year after recovery from SARS.¹⁰² The pituitary gland is of great importance since it controls the release of many of the hormones from other endocrine organs. Among other hormones, the adrenal gland produces epinephrine and norepinephrine (adrenalin and noradrenalin) which stimulate the “fight-or-flight” response. Decreased levels of these hormones may be responsible for many of the above-listed symptoms.

The **hypothalamic–pituitary–thyroid (HPT) axis** is dysfunctional following SARS.¹⁰³ The pituitary gland may be inflamed during and after SARS. **Thyroid glands** from SARS patients have extensive damage to the **follicular epithelium** whose hormones regulate metabolism. The **parafollicular cells** whose hormones help to regulate blood calcium levels may be lost during infection. Many cells in the thyroid glands undergo apoptotic death.⁷⁸ Among survivors of SARS-CoV infection, 3.3% of those studied had transient mild **hyperthyroidism**, which leads to increased cellular metabolism, while 6.7% had **hypothyroidism**.¹⁰³ SARS reduces levels of **thyroid-stimulating hormone**, a hormone produced by the pituitary gland which activates the thyroid gland to release its hormones.

During SARS, a reduction is observed in the serum levels of two thyroid hormones that regulate metabolism, **triiodothyronine (T3)** and **thyroxine (T4)**, as well as **calcitonin**, a thyroid hormone that decreases calcium levels in the blood.¹⁰² Altered blood calcium levels affect not only bone density, but the activity of the nervous and muscular systems.

Impairment of the **hypothalamic–pituitary–adrenal (HPA) axis** occurs late postinfection.¹⁰³ Other endocrine organs and hormones are also impacted by SARS-CoV infection. During SARS, the **adrenal glands** produce low levels of the adrenal hormone **dehydroepiandrosterone** in 24% of the survivors and 39.3% had low levels of **cortisol**.¹⁰³ The former hormone affects the production and release of the sex hormones, testosterone and estrogen, while cortisol is a stress hormone that acts in an antiinflammatory and immunosuppressive manner.

The pituitary gland may be inflamed during and after SARS as well.¹⁰³ SARS affects the correct functioning of the **pituitary** endocrine cells. Both the number of cells producing **human growth hormone** (somatotropin), thyroid-stimulating hormone and **adrenocorticotrophic hormone** and the amount of these hormones produced or released per cell are decreased during SARS.¹⁰² Among its many functions, human growth hormone regulates metabolism, production of red blood cells, bone growth, and muscle mass. The **adrenocorticotrophic hormone** triggers the release of cortisol from the adrenal glands in response to stress.

2.3.9 Severe acute respiratory syndrome, the reproductive system, and sex-related disease severity

Despite the lack of SARS-CoV and its RNA in the testes, male SARS patients experience **orchitis** (inflammation of the testicles and groin pain) that may lead to infertility, destruction of sperm, very small amounts of immature sperm in the **seminiferous tubules** (the part of the testes which produces sperm), and an influx of lymphocytes and macrophages into the testes.^{78,80,92,104} Large amounts of IgG antibodies are present in the lining of the seminiferous tubules, suggesting that immunopathology may contribute to the reproductive abnormalities.¹⁰⁴ In women, the eggs may be damaged.

As is the case in humans, male mice are more susceptible to SARS than females of the same age. The difference between the sexes becomes greater with age in studies of mice and men. Removal of the ovaries from infected females or blocking the female hormone **estrogen** from binding to its receptor increases the numbers of inflammatory monocytes/macrophages in the lungs, accompanied by an increased mortality rate. Estrogen, therefore, appears to play an important role in protecting females from SARS-related diseases.¹⁰⁵ Since normal males tend to have slightly weaker innate and adaptive immune responses than females, they are more susceptible to infections.¹⁰⁶ Moreover, while mRNA levels of IFN- β are similar in SARS-CoV-infected male and female mice, the levels of the pro-inflammatory cytokine IL-6 and the chemokines CCL-2 and CXCL-1 remain elevated longer in the lungs of male mice.¹⁰⁵

Reproductive hormone levels are altered by SARS infection. Serum levels of the predominately female hormones **prolactin**, **follicle-stimulating hormone**, **luteinizing hormone**, and **gonadotropic hormone** are higher than normal, while estradiol levels are lower.¹⁰² Prolactin

stimulates the development of mammary glands and secretion of milk in women. Follicle-stimulating hormone activates the maturation of ovarian follicles which produce estrogen and progesterone in females and stimulates the development of the gonads and sperm production in men. **Luteinizing hormone** triggers ovulation and the development of the **corpus luteum** (an endocrine organ produced by follicles following ovulation) in women. Luteinizing hormone acts together with follicle-stimulating hormone to produce testosterone in men. The gonadotropic hormone triggers the secretion of follicle-stimulating hormone and luteinizing hormone in women and the production of testosterone in men.¹⁰² Similar to the case in the testes, no evidence of SARS-CoV has been found in the ovaries.¹⁰¹

2.4 The causative virus

2.4.1 An overview of severe acute respiratory syndrome-coronavirus

SARS-CoV is a betacoronavirus lineage B that causes SARS, an acute and often fatal respiratory system pathology that inflicts damage on several other organ systems as well. It is closely related to SARS-CoV-2, the causative agent of COVID-19.¹⁰⁷ The SARS epidemic lasted for only two years after which only several cases were reported in the last part of 2003 and early 2004. Some of the cases in late 2003 were laboratory-associated. The reasons for the disappearance of this disease are unknown since no effective vaccines or cures were available. COVID-19, by contrast, continues to persist, despite the development of several very effective vaccines and treatments. This persistence may be due to the ability of SARS-CoV-2 to rapidly produce highly contagious variants.

SARS-CoV and SARS-CoV-2 have many similarities and differences. Both coronaviruses use ACE2 as their primary cellular receptor. SARS-CoV may also use DC-SIGN, L-SIGN, and vimentin, an intermediate filament protein that is a component of cells' cytoplasm.^{19,21,108} Both SARS-CoV and SARS-CoV-2 are believed to have originated in bats and may use reservoir hosts present in wet markets in China. SARS-CoV is associated with palm civets and raccoon dogs, while SARS-CoV-2 might have entered the human population via pangolins,^{8,77} although the initial cases may have been associated with a laboratory accident. Transmission of both viruses is primarily via inhalation of large droplets of infected respiratory secretions of the above wild animals initially and humans soon after. Infection with both viruses may also occur to a much lesser extent by contact with contaminated surfaces.¹ SARS-CoV-2 can undergo many more generations of human-to-human transmission than SARS-CoV and has caused a long-lasting pandemic that, as of June 2022, will continue for an unknown period of time. It also has produced more variants and has infected and killed many more people than SARS-CoV.

Overall, the SARS-CoV genomic sequence conservation with other coronaviruses is low, except for SARS-CoV-2, and is thus considered to be a distinct species that differs in many aspects from other known coronaviruses.²³ Another large difference between known betacoronaviruses and SARS is in the gene encoding nsp3, the largest replicase subunit. SARS-CoV does not have an ortholog of papain-like proteinase 1 (PL1^{Pro}) that is found in almost all other coronaviruses. The activities of this enzyme are performed by PL2^{Pro}, a paralog

of PL1^{Pro}.¹⁰⁹ The 3' region of the SARS-CoV genome also has five ORFs (6, 7a, 7b, 8a, and 8b) that are lacking in most betacoronaviruses.¹⁰⁹ Such differences between coronaviruses suggest that caution should be used when attempting to predict characteristics of SARS-CoV with other coronaviruses. Nevertheless, due to the greater degree of similarity, a comparison of SARS-CoV and SARS-CoV-2 may prove to be useful during the current COVID-19 pandemic. A recent study divided betacoronavirus lineage B into SARS1 and SARS2 classes based on six key insertions and deletions. Interestingly, the ORF8 from the bat betacoronavirus RmYN02 appears to have arisen by recombination in a bat that was coinfecting with coronaviruses of the SARS1 and SARS2 classes.¹¹⁰ ORF8 is only found in betacoronavirus lineage B and may have a major role in the adaptation of exogenous coronaviruses to their new human hosts due to alterations in viral replication.^{7,111}

SARS-CoV genomic RNA has a 5' cap and 3' polyadenylation tract. Its 5' terminal ORF is translated into a large polypeptide that consists of several nonstructural proteins (nsp's) which are separated and released upon enzymatic cleavage. These nsp's include an **RNA-dependent RNA polymerase (RdRp)** and an adenosine triphosphatase **helicase**, both essential for replication.⁵ The helicase (nsp13) unwinds double-stranded RNA and DNA in a 5' to 3' direction. It also hydrolyzes any naturally occurring nucleotides or deoxynucleotides, especially ATP, dATP, and GTP, and may aid in the formation of the RNA 5' cap as well.¹¹² SARS-CoV genomic RNA also encodes the S, envelope (E), membrane (M), and N structural proteins that are typical for coronaviruses.¹¹³ The S and M proteins associate during the formation of the viral envelope.

Nsp protein 3a (also known as U274) is a minor transmembrane structural protein localized in the plasma membrane and perinuclear region of infected cells as well as in intracellular viral particles in vitro.¹¹³ The 3a protein interacts with the SARS-CoV M, E, and S membrane proteins. In addition to the full-length protein, two other processed forms are also found in these locations.¹¹⁴ Some nsp's are usually not required for viral replication in cell cultures but may be involved in virus-host interactions in vivo, so care must be taken when interposing the results of in vitro studies with in vivo conditions.

Some of the unusual features of the SARS-CoV genome include a 12 amino acid overlap between ORFs 10 and 11, while other ORFs are located entirely within another ORF, such as ORF 4 and ORFs 3 and the E protein gene.⁵ ORFs that produce predicted proteins of greater than 50 amino acids are present within some of the structural genes of SARS-CoV, including the N and S proteins, especially in the latter.²³

During virion assembly, the N protein binds to a specific packaging signal on SARS-CoV during the formation of the nucleocapsid. The M protein is found in specialized intracellular membrane structures. After the addition of the M and E proteins, the nucleocapsids bud through the membrane. Next, the S protein is packaged into the viral envelope by interacting with M proteins. The mature virions are then released from smooth vesicles.²³

SARS-CoV also contains subgenomic mRNA3, mRNA6, mRNA7, mRNA8, and mRNA9 which encode 5–8 accessory proteins, of which at least proteins 3a, 7a, and 9b are expressed. Interestingly, these subgenomic mRNAs have little sequence similarity with proteins present in other known coronaviruses, with the possible exception of SARS-CoV-2.^{5,6}

2.4.2 Entry of severe acute respiratory syndrome-coronavirus into cells

The S protein that studded the outer part of the human SARS-CoV envelope during the 2002–2003 outbreak binds to the ACE2 receptor on the surface of several types of human and civet cells. The binding of the SARS-CoV (and SARS-CoV-2) S protein to the host cell's ACE2 enzyme is the first step in viral entry into its various target cell types. The normal S protein of the civet form of SARS-CoV binds to human ACE2 poorly, but over time, specific mutations in two of the S protein's amino acids increased viral binding to human ACE2 by 1000-fold, resulting in an epidemic strain that passed very readily between people without an animal intermediate host.^{14,115} The increased ability of this more humanized SARS-CoV to bind more strongly or rapidly to cells may be a very important **virulence factor**, increasing disease spread in humans. The S protein present in viral isolates from people with mild disease in late 2003 is similar to that of the civet virus but differs from the more virulent form of SARS-CoV that was present in humans between 2002 and early 2003. The later, less pathogenic form of the virus only binds to human ACE2 weakly when compared to the binding affinity of earlier SARS-CoV strains.

ACE2 normally plays a vital role in human health. It is part of the hormonal **renin-angiotensin-aldosterone response**, which will be described later. This response reduces the diameter of blood vessels and normally raises blood pressure levels during periods of **hypotension**. This activity helps to return low levels of blood pressure up to their optimal functioning range. The binding of the viral S protein to ACE2 on human cells can be blocked by **neutralizing antibodies** or several medicinal compounds already used to treat high blood pressure, including ACE2 inhibitors. ACE2 also helps to defend against acute lung injury, so the binding of this enzyme to SARS-CoV may additionally aggravate lung damage. Several other molecules on the host cell surface also bind to the SARS-CoV S protein, including the immune system proteins DC-SIGN and L-SIGN, as discussed earlier.

The binding of the SARS-CoV S protein to ACE2 leads to the **phosphorylation** of the latter.¹⁹ Phosphorylated ACE2 plays a role in triggering the production of the fibrosis-associated chemokine CCL2 as well as in **intracellular signaling** (signaling pathways in the cell's interior). Phosphorylated ACE2 helps to activate the intracellular **Ras-ERK-AP-1 signaling** pathways,¹¹⁶ which aids in cellular division, differentiation into more specialized cells, and survival. Stimulation of these pathways is advantageous to the virus, since it increases the number of potential host cells, allowing the virus greater opportunity to replicate.

2.4.3 Viral polyproteins and proteases

Translation of the RNA of many viruses, including coronaviruses, produces polyproteins which must be enzymatically cleaved into their protein components before they can function. One-third of the SARS-CoV's genome encodes mRNAs for viral structural proteins and nsp. The remainder of the genome contains the overlapping genes ORF 1a and ORF 1ab. ORF 1a is directly translated into the polyprotein pp1a that encodes nsp1-nsp11, while ORF 1ab is translated into the polyprotein pp1ab which encodes nsp1-nsp16. Which

polyprotein is produced depends on a ribosomal (-1)-frameshift, so 11 or 16 individual nsp's are produced.¹¹⁷ These polyproteins are subsequently cleaved and processed into either 11 or 16 individual proteins by two viral proteases: the papain-like protease (PL^{pro}; nsp3) and 3CL^{pro}, the main chymotrypsin-like protease (also known as M^{pro} and nsp5).¹¹⁷ Following cleavage of the two polyproteins, a series of further protein cleavages and processing occur. Some of the nsp's form a replication/transcription complex, a dynamic protein-RNA complex that aids in replication.¹¹⁷ After processing, nsp12 acts as an RdRp which replicates the SARS-CoV RNA genome. Drugs that inhibit the action of cysteine proteases, including 3CL^{pro}, strongly suppress the early stages of SARS-CoV and SARS-CoV-2 replication.¹¹⁸

2.4.4 Severe acute respiratory syndrome-coronavirus and the ubiquitin-pathway

Ubiquitination is the addition of **ubiquitin**, one of whose functions is to mark proteins for degradation in **proteosomes**.⁶ To be degraded by proteosomes, molecules need to be attached to multiple molecules of ubiquitin. Proteosome activity is necessary for the replication of several coronaviruses, such as murine hepatitis virus and feline infectious peritonitis virus.^{119,120} Unlike other coronaviruses, the ubiquitin-proteosome pathway is not required for the replication of SARS-CoV and perhaps SARS-CoV-2 as well.¹¹⁷ The **interferon-stimulated gene 15 (ISG15)** is a ubiquitin homolog that regulates intracellular signal transduction and immunity to viruses.

Both **deubiquitination** and **deISGylation** (removal of ubiquitin and ISG15, respectively) help coronaviruses to evade host immune responses and proteasomal degradation. SARS-CoV produces several deubiquitination enzymes that decrease the levels of ubiquitin on viral proteins. These enzymes include PL^{pro}, the N and M proteins, and products of ORF6 and ORF3.^{121,122} PL^{pro} also removes ubiquitin from the cellular proteins **STING**, **RIG-I**, **TBK1**, **IRF3**, and the ISG15.¹²² PL^{pro} also removes ISG15 from host proteins.¹²³

Some thiopurine analogs block ubiquitin and ISG15 removal by PL^{pro} and inhibit viral degradation.¹²¹ Most proteasomal inhibitors have little effect on viral replication. MG132, however, is a drug that inhibits proteasomal activity and decreases SARS-CoV survival. This compound appears to act in a manner by which ER stress utilizes lysosomes to degrade proteins, **autophagy** (the natural, orderly degradation of the cell that removes unnecessary or dysfunctional components), or the **Unfolded Protein Response (UPR)**, as discussed later.^{120,124} MG132 inhibits SARS-CoV degradation via reducing the activity of the cellular cysteine protease **m-calpain** which plays a role in apoptosis and in inhibiting SARS-CoV replication.¹²⁰

Another m-calpain inhibitor, MDL28170, blocks SARS-CoV replication by greater than 7 orders of magnitude in comparison with MG132. M-calpain is active early during SARS-CoV's life cycle.¹²⁰ In addition to their actions of m-calpain, MG132 and MDL28170 inhibit **cathepsin L**-mediated proteolytic processing of the S1 subunit of SARS-CoV and SARS-CoV-2, which is needed for viral entry into target cells.^{125,126} Cathepsin L is a protease found in endosomes and has a wide variety of cellular functions, including decreasing B-cell production.

2.4.5 Severe acute respiratory syndrome-coronavirus and the unfolded protein response

The endoplasmic reticulum (ER) is not merely a membranous network upon which ribosomes transcribe secreted or membrane-bound proteins, it is also essential for post-translational modifications of the nascent proteins, particularly their correct folding, without which the proteins are unable to correctly function. Unfolded or incorrectly folded proteins are typically degraded by **lysosomes**, but excessive amounts of these proteins trigger the UPRs to alleviate ER stress due to the presence of large numbers of proteins to be processed. The UPR is activated by the excessive production of coronavirus proteins, particularly the SARS-CoV S1 protein, which requires large amounts of modification before it leaves the ER.¹²⁷ The accumulation of these proteins overwhelms the ER's ability to correctly fold newly produced proteins. If the levels of misfolded proteins continue to increase to a critical level, ER stress induces the cell to undergo apoptosis.¹²⁸ SARS-CoV normally derives some of its envelope components from the membranes of the host cell's ER and, in so doing, the viruses take some of the ER membranes with them as they pass into the cytoplasm, leading to ER depletion. The virus also modifies the membranes of the other cellular organelles. These extensive changes in the cell's membranes may also lead to ER stress and activate the UPR.

The UPR triggers mechanisms that temporarily halt the production of new proteins, destroy misfolded proteins, or set in motion the means of increasing the production of correctly folded proteins. There are three branches of the UPR: the PKR-like ER kinase (PERK), the **inositol-requiring enzyme 1 (IRE-1)**, and the **activating transcription factor 6 (ATF6)** branches.¹²⁷ **ER-associated degradation (ERAD)** is the process by which unfolded or misfolded proteins are escorted by various **ER chaperones** to the **proteosomes**, the site of their ultimate degradation. This degradative process is activated by the IRE1 and ATF6 branches of the UPR.¹²⁹ The PERK branch, by contrast, decreases the transcription of some cellular proteins while increasing the production of ER chaperones that promote correct protein folding.¹²⁹ The bat coronaviruses Rf4092 and WIV1 contain different genotypes of ORF8 (described later), yet both stimulate ATF6-mediated activity. WIV1 contains the complete, intact form of ORF8 and stimulates the strongest activation of ATF6. By contrast, the ORF8a protein derived from SARS-CoV strains isolated during the later phase of the epidemic induces apoptosis in infected cells¹³⁰ as does the ORF8a from the bat SARS-like-CoV Rs4084, despite the latter having an eight amino acid insertion.²⁷

To avoid chronic stress in the ER and the premature death of their host cells, viruses have devised a variety of mechanisms to regulate the UPR. The S protein of the virulent GZ50 SARS-CoV strain stimulates transcription of several of the UPR proteins in vitro in monkey kidney cell lines. The form of SARS-CoV present during the early phase of the 2003–2004 epidemic modulates the UPR in an unusual manner that facilitates viral replication by stimulating only the PERK branch of the UPR.¹²⁷ The S protein of the less pathogenic coronavirus HCoV-HKU1 similarly stimulates the UPR via the PERK pathway.¹³¹ However, while the S1 subunit of SARS-CoV's S protein alone can activate the UPR, the S1 subunit of HCoV-HKU1 cannot. This may be due to the actions of the **TMPRSS2 protease** which cleaves the SARS-CoV's S protein into S1 and S2 subunits. TMPRSS2 does not

cleave the S protein of HCoV-HKU1. The failure to cleave the S protein may contribute to the lower pathology associated with HCoV-HKU1 infection.¹³¹ Host proteases, including trypsin, thermolysin, elastase, and factor Xa cleave SARS S protein as well, which increases viral infectivity.¹²

In addition to the S protein, the protein products of SARS-CoV ORF3a, ORF6, ORF7a, ORF8ab, and ORF8b activate the UPR.¹³² The S protein and ORF3a activate the UPR solely via PERK,¹²⁹ while ORF8ab activates ATF6.¹³³ By only triggering the PERK branch of the UPR, protein 3a potentially protects itself and other viral proteins from ERAD, while promoting the correct folding of the accumulated proteins, including viral proteins, within the ER.¹²⁹ By contrast, the SARS-CoV E protein alters the type of UPR that is active by decreasing the IRE-1, but not the PERK or ATF6, branch of the UPR. This limits the stress response and decreases apoptosis in infected cells, which may allow for increased virus production and dissemination.¹³⁴

SARS-CoV-induced activation of the UPR by the PERK branch alone not only decreases ER stress and apoptosis but also suppresses host type 1 IFN signaling. The viral protein 3a modifies one of the IFN- α receptor subunits, which increases the receptor's degradation in lysosomes. The loss of type I IFN receptors eliminates one of the host's most effective mechanisms for eliminating viruses.¹²⁹ Hepatitis C virus and cytomegalovirus also modulate ER stress and the UPR using PERK, however, both viruses also trigger one of the other UPR branches. Since this research was performed in vitro, it would be interesting to determine whether SARS-CoV also utilizes this approach to ER stress management in vivo, particularly in lung and intestinal cells, and whether this method is used to the same extent by both SARS-CoV-infected young and aged mice.

2.4.6 Severe acute respiratory syndrome-coronavirus open reading frame 8

The products of ORF8 are important in pathology and the type of UPR used. Mutation or deletion of ORF8 from SARS-CoV and SARS-CoV-2 correlates with mild disease.¹³² ORF8 and the associated protein 8 differ in SARS-CoV isolated from the early and late stages of the 2003–2004 human epidemic. These differences appear to be very important for zoonotic transmission.¹¹¹ Protein 8 of two SARS related-CoV (SARSr-CoV) from *Rhinolophus ferrumequinum* horseshoe bats, Rf-BatCoV YNLF_31C and YNLF_34C, have amino acid identities of 80.4% and 81.3%, respectively, to the human and civet SARSr-CoV protein 8, respectively. This is much higher than the degree of identity found in SARSr-BatCoVs from other horseshoe bats, such as RsSHC014 and Rs3367. These two bat coronaviruses have 95% genomic identity to human and civet SARSr-CoVs, but their ORF8 proteins have only 32.2%–33% amino acid identities.¹¹¹ Additionally, potential recombination events in the area around ORF8 in coronaviruses derived from *R. ferrumequinum* and *Rhinolophus sinicus* may have led to the production of civet SARSr-CoVs, such as the SZ3 strain, which appears to have acquired its ORF8 from SARSr-Rf-BatCoVs.¹¹¹

ORF8a and the overlapping ORF8b encode two small proteins, 8a (39 amino acids) and 8b (84 amino acids), and a fusion protein, 8ab (122 amino acids).⁶ The ORF8ab and ORF8 differ greatly, sharing only 26% nucleoside and 20% amino acid identities. Both ORF8 and SARS-CoV ORF8ab appear to be of bat origin.⁶⁷ ORF8a alone generates protein 8a, while

ORF8b alone does not generate either the 8a or 8b proteins since, in the absence of protein 8a, protein 8b undergoes rapid degradation by proteasomes because the latter protein is believed to contain a ubiquitin-binding domain.⁶ ORF8 may also generate 8ab. The form of 8ab found early during the 2003 epidemic is modified by N-linked **glycosylation** (the addition of sugar that stimulates protein activity) and by ubiquitination.⁶

The different forms of proteins produced by ORF8 have different functions.¹¹¹ Protein 8ab is found on the luminal membrane of the ER from where it increases the production of chaperon molecules that are used during protein folding via stimulation of ATF6.¹³³ The 8a protein increases SARS-CoV replication. It is localized in mitochondria where excessive levels lead to increased production of **reactive oxygen species** (ROS) and **cas-pase 3** activity which is used during apoptosis.¹³⁰ The viral 8b protein decreases the production of the viral E protein.¹³⁵ The 8b and 8ab proteins also affect the host ubiquitin-proteasome pathway.⁶ Interestingly, proteins 8b and 8ab bind covalently and noncovalently to monoubiquitin (a single ubiquitin molecule) and polyubiquitin. The region of 8a and 8b that binds to ubiquitin differs from all other known ubiquitin-binding domains, suggesting that they contain a novel type of ubiquitin-binding protein that appears to be found in the 8b region.⁶

As mentioned early, ORF8s derived from during the later stage of the epidemic in humans have a 29-nt deletion that is lacking from that found in civets and some human SARS-CoV present early during the 2003 epidemic. The 29-nt deletion interferes with the expression of functional expression of ORF8 in humans but not in animals.¹³⁶ Some of the early human SARS-CoV strains, as well as a strain from a farmed civet, contain an 82-nt deletion in ORF8. Other deletions which arose in the area around the ORF8 region were present in less pathogenic viral strains found very late during the 2003–2003 pandemic. Some human isolates from the late stage of the epidemic contain a 415-nt deletion which removes ORF8 entirely.¹¹⁵ Additionally, the less virulent European SARS-like coronaviruses from horseshoe bats from Spain, Bulgaria, Italy, and Slovenia also lack protein 8, while almost all SARS-related coronaviruses from Asia produce one continuous protein 8.⁷ Furthermore, restoration of the full ORF8 to the genome increases viral replication in SARS-CoV strains isolated late during the epidemic.⁷

2.4.7 Severe acute respiratory syndrome and small non-coding RNAs

The genome of coronaviruses, including SARS-CoV, contains regions of **small noncoding RNAs** (areas of the genomic RNA that does not produce proteins). Three **small viral RNAs (svRNAs)** composed of 18–22 nucleosides are derived from the regions of the SARS-CoV genome that encode nsp3 and the N protein. Production of these svRNAs is unusual since it appears to depend on the activity of the human RNA processing enzyme **Argonaute 2**, but not **Dicer** and **Drosha**.¹³⁷ The three svRNAs are found in the cytoplasm of lung cells and are cell-type specific. One of these svRNAs is also present in the blood. The three svRNAs contribute to lung pathology, including edema and cellular infiltrates, yet do not decrease viral load.¹³⁷ They also enhance the production of pro-inflammatory cytokines and chemokines, including IL-6, CCL2, and CXCL10, in the lungs of mice. Inhibition of these svRNAs decreases lung damage and levels of pro-inflammatory

cytokines.¹³⁷ The svRNAs from SARS-CoV are highly conserved in the bat SARS-like-CoVs LYRa11, RF1, Rm1, Rs3367, and Cp/Yunnan as well, but not in the human MERS-CoV. In addition to regulating RNA activity and the production of viral proteins, they also regulate RNA of infected humans and in animal models.¹³⁷

2.4.8 Severe acute respiratory syndrome-coronavirus and bats

In addition to bats, palm civets, and raccoon dogs, other animals can also be infected with SARS-CoV, including macaques, ferrets, exotic and domestic cats, exotic dog species, bats, pigs, and chickens. Both infected ferrets and domestic cats can transmit SARS-CoV to other ferrets and cats which come into close contact with them.¹³⁸ Some of these animals may act as intermediate hosts that acquire the virus from infected bats and then pass it on to humans. Key mutations in the viral S protein are believed to enable the virus to enter and adapt to new host species.

Bats are believed to be the most likely reservoir species for SARS-CoV. Mutant forms of bat coronaviruses may have infected civets and raccoon dogs, followed by additional mutations that then rendered SARS-CoV infectious to humans, as described later. This chapter includes a short section that highlights findings that are believed to be most relevant to human infection. Coronaviruses of bats and other animals that have the potential for zoonotic transmission to humans are the subjects of another chapter.

SARSr-CoVs have been detected in more than eleven species of horseshoe bats (*Rhinolophus* species) from Asia, Africa, and Europe as well as free-tailed (*Chaerephon*) and round leaf (*Hipposideros*) bats from Africa and China.¹¹¹ Bats host a large range of coronavirus species, which are listed in tabular form by bat and coronavirus species by Beltz.¹³⁹ A five-year study of multiple species of *Rhinolophus* bats residing in a cave in Yunnan, China found a high prevalence of SARS-like-CoVs.²⁷ This province is immediately west of the Guangxi Province which had an outbreak of SARS in humans. While the majority of new bat coronaviruses were derived from anal swabs or fecal material from *Rhinolophus sinicus* (Chinese rufous horseshoe bat), several of the isolates were from *R. ferrumequinum* (greater horseshoe bat) or *Aselliscus stoliczkae* (Stoliczka's trident bat). Comparison of the Yunnan viruses' full-length genomes revealed the presence of eleven new SARS-like-CoV strains in the bats in this cave that have a high degree of genetic diversity, primarily in the S1 portion of the S protein gene, ORF3, and ORF8.¹³⁰ Additionally, this cave contained bats infected with coronaviruses having receptor binding regions that are closely related to Rs672, HKU3, and Rf1 bat viruses from other regions of China.²⁷

The ORF3b proteins of bat SARS-like-CoVs are almost always smaller than those of SARS-CoV due to the early termination of translation of the 3b protein. Bats from the Yunnan cave were the first known to possess an ORF3b that did not produce a truncated 3b protein.²⁷ The product of ORF3b of the novel bat Rs7327 strain differs from that found in the human SARS-CoV GZ02 strain at only one amino acid.²⁷

In a separate study, Yang et al. found that the greatest genetic differences between bat SARS-like-CoVs and human SARS-CoV strains were present in the S protein gene and ORF8. The ability of the receptor-binding domain (RBD) of the S protein to bind to ACE2 has a strong effect upon which animals and cell types different coronavirus species may

use as hosts.¹⁴⁰ Small differences in the RBD may dramatically alter potential host species. Mutations in ORF8 alter SARS-CoV's virulence.

In the late phase of the 2002–2003 epidemic, a mutation in the human SARS-CoV ORF 8 produced ORF8a and 8b. A complete ORF8 that is similar to that of early civet/human SARS-CoV (> 97% nucleotide identity) was present in some of the bat viruses from the aforementioned cave, such as strain Rf4092. Another bat SARS-like-CoV from this cave, Rs4084, contains both ORF8a and ORF8b that are highly similar to those found in human SARS-CoVs. This suggests that Rs4084 might have acquired its ORF8 from strain Rf4092 or a closely related virus strain through genetic recombination and later underwent a five-nucleoside deletion which led to the splitting of ORF8 into ORF8a and ORF8b.²⁷

Additionally, some of the bat coronaviruses' ORF1a and 1b are closely related to SARS-CoV.²⁷ A separate study of the ORF1a/1b from SARS-like-CoVs conducted elsewhere in Yunnan found that viruses isolated from *R. ferrumequinum*, rather than *R. sinicus*, are more closely related to SARS-CoV than other known bat virus strains known at that time.¹¹¹ The E, M, and N protein genes had greater than 98% amino acid identities with human/civet SARS-CoVs. It appears that frequent recombination events had occurred within the S protein gene and around ORF8 in the bat coronaviruses from this cave. Three of these new bat SARS-like-CoVs, having different S protein sequences, can use human ACE2 to enter cells in vitro, similar to the S proteins from human SARS-CoV.²⁷ Taken together, these studies suggest that several sequential genetic recombination events may have produced the direct progenitor of either the civet SARS-CoV or the human SARS-CoV strains circulating early during the 2002–2003 epidemic in China. This progenitor virus may have then undergone interspecies or zoonotic transmission. It should be kept in mind that many SARS-like-CoVs were found in a single cave in China, suggesting that many more SARS-like-CoV strains are present in bat colonies in caves or other structures throughout China. Some of the caves are potentially home to numerous SARS-like-CoVs that are constantly recombining their genetic material and altering their host species range. After entering human populations, similar recombination events may have continued over time, producing the less pathogenic SARS-CoV strains that predominated later during the epidemic as the virus strains adapted themselves to their human hosts.

Bats are infected by and serve as reservoir hosts for diverse alphacoronaviruses in addition to lineage B, C, and D betacoronaviruses.¹⁴¹ Regardless of the reservoir species, SARS-CoV is believed to have entered the human population by contact with a form of animal coronavirus that has even greater similarity to the human strains of the virus than do the bat coronaviruses. The SARS-like-CoV virus is present in Himalayan palm civets and raccoon dogs. Interestingly, the severe respiratory disease does not occur in either of those animals. The strain of SARS-CoV which infected people during the late phases of the 2003 outbreak had several large, specific deletions in their RNA that were not present in the strains isolated from humans during the early phase of the epidemic. These deletions are not found in most of the viral relatives of SARS-CoV that are present in bats, palm civets, or raccoon dogs. The deletions in "humanized" forms of SARS-CoV appear to make the virus better adapted to living in humans rather than animals as well as decreasing their virulence.

The bat coronavirus SHC014 is similar to SARS-CoV, but differs from the human coronaviruses in critical regions of their S proteins. In Kenya, viruses similar to human HCoVNL63 are found in *Triaenops* African trident bats and viruses similar to human HCoV229E are

found in *Hipposideros* roundleaf bats. Furthermore, the S protein gene in HCoVNL63 appears to have arisen through recombination of the two bat virus S protein genes.¹⁴² The bat SARS-like-CoVs Rs672 and HKU3 from *R. sinicus* and Rf1 from *R. ferrumequinum* are also similar to SARS-CoV.²⁵ Another SARS-like-CoV strain, LYRa11, has been isolated from *Rhinolophus affinis* (intermediate horseshoe bats) in Yunnan. This strain has 90% amino acid identity to SARS-CoV in the S protein gene.¹⁴³ Two other studies reported additional SARS-like-CoVs (YNLF 31C/34C and GX2013/YN2013) whose ORF8 bears high similarity to that present in SARS-CoV (Wu 2016).¹¹¹ SARS-like-CoV evolution appears to be more strongly correlated with their geographical origin than with their host species.¹⁴⁴

Whole-genome sequences from two Chinese bat coronaviruses, RsSHC014 and Rs3367 from *R. sinicus*, reveal that their RNA is much more closely related to SARS-CoV than any previously known bat coronaviruses, especially in the RBD of the S protein.²⁵ These new bat viruses have 95% overall nucleotide sequence identity to civet coronaviruses and the Tor2 strain of human SARS-CoV, isolated from a person with a fatal case of SARS. By contrast, other bat SARS-like-CoVs have overall sequence identities with SARS-CoV of 88–92% and 76% for Chinese and European bats, respectively. Higher amino acid identities are present between bat RsSHC014 and WIV1-CoV with SARS-CoV (85% and 96%, respectively).²⁵ Additionally, bat WIV1 can grow in human alveolar basal epithelial cells and in pig and *R. sinicus* kidney cell lines in vitro.²⁵ Nevertheless, of the five key SARS-CoV S protein amino acids that have pivotal roles in receptor binding, all five differ between bat RsSHC014 and human SARS-CoV and only two of these five amino acids are shared by this region between WIV1 and SARS-CoV.²⁵

In 2013, a new SARS-like-CoV strain, bat SL-CoV WIV16, was isolated from feces of *R. sinicus* in Yunnan Province of China.²⁶ It is nearly genetically identical to WIV1 and differs slightly from civet SARS-CoV and other bats SARS-like-CoVs. WIV16 has the same host cell range as WIV1 and these two viruses replicate at approximately the same rate.²⁶ Notably, WIV16 contains an additional ORF between its ORF6 and ORF7. Overall, WIV16 has 96% identity to civet and human SARS-CoVs.²⁶ The WIV16 S protein gene also has a 95% nucleoside and 97% amino acid sequence identity to that found in SARS-CoV. Additionally, one of the SARS-like-CoVs, Rs4874, from the Yunnan bat cave has a S protein gene that is almost identical to that of WIV16 and can also bind to human ACE2.²⁷ Looking more closely, the **N-terminal binding domain (NBD)** of the S1 portion of WIV16's S protein has 94% amino acid sequence identity to that of SARS-CoV, while only 50%–75% to another bat SARS-like-CoV.²⁶ Given the high degree of identity of the RBD of WIV16 to that of SARS-CoV and to that of WIV1, it is possible that the WIV16 S protein gene arose from a recombination event between WIV1's S protein gene and that of a recent ancestor of SARS-CoV.²⁶ The Rs4231 strain from the Yunnan bat cave could be this ancestor. Recombination might have occurred in the RNA at the junction between the S protein's NBD and RBD in Rs4231 and WIV1 gave rise to WIV16.²⁷

2.4.9 Transmission of severe acute respiratory syndrome between humans

After its initial entry into humans in Asia, SARS-CoV spread rapidly among people by inhalation of large droplets of infectious respiratory secretions, by rubbing their eyes, or

by touching their faces. SARS-CoV was also present in bodily secretions such as feces, urine, sweat, and tears. Additionally, SARS-CoV was detected in the following organs: the lungs, trachea, and bronchus of the respiratory system; the small intestine and liver of the digestive system; the distal convoluted renal tubules of the kidneys; sweat glands; skeletal muscles; the **parathyroid gland**, pituitary gland, **pancreas**, and adrenal gland of the endocrine system; the cerebrum of the brain; the spleen; and some leukocytes.⁸⁰ This study also found SARS-CoV in the stomach. It was not found in the esophagus (digestive tract); lymph nodes and bone marrow (immune system); cerebellum (CNS); or the testes, ovary, and uterus (male and female reproductive systems) or muscle.⁸⁰ ACE2 is expressed on at least some cell types in most of these body areas as well⁷⁰.

Transmission occurred in airplanes in the 2002–2003 epidemic, perhaps due to inhalation of aerosolized infectious particles or contact with contaminated surfaces in areas such as the lavatory. A population of superspreaders appeared to have played a major role in human-to-human transmission during the SARS epidemic. Superspreaders generally had more severe disease symptoms, a higher fatality rate were older, and contacted a greater number of people than most infected people.

Most common human coronaviruses typically cause a mild form of the common cold. They grow best at temperatures found in the nasal cavity and throat of the upper respiratory tract, which is slightly less than 37°C (98.6°F), the average human internal body temperature. By contrast, SARS-CoV withstands and grows at the slightly higher temperatures found deeper inside the human body, particularly the temperature present in the lungs. This ability to survive at normal internal body temperatures allows the virus to invade the **lower respiratory tract**, leading to more serious symptoms, including potentially fatal pneumonia. This relative loss of temperature sensitivity also permits the virus to infect other internal body organ systems.

2.4.10 Severe acute respiratory syndrome-coronavirus in the external environment

SARS-CoV may also be found in the environment, where it remains in an inert state until entering a host cell. SARS-CoV survives in an infectious form in the external environment longer than MERS-CoV does, however, the average survival rate outside of the body is 4 hours.²³ The inert viruses that are present on surfaces may be factors of unknown importance in transmitting both these pathogenic human viruses, as well as SARS-CoV-2, between people. SARS-CoV remains infectious in water and on foods for extended periods of time that are determined by a large variety of factors including temperature, relative humidity, the nature of the surface, and the material which surrounds the virus.^{145–147}

When in suspension in a fluid, SARS-CoV remains infectious for up to 9 days, far longer than its less pathogenic relative, HCoV-229E. In serum, **sputum**, and feces, the virus remains active for at least 96 hours and even longer in the less acidic diarrheic feces of patients. It should be noted that drying occurs slowly in these materials and that the organic material that these materials contain also protects the virus from dehydration and chemical or physical decontamination. In a dehydrated state, SARS-CoV survives for 6 days. The virus is also stable at 4°C (39°F), 20°C (68°F), and 37°C (98.6°F) for over 2 hours. UV light eliminates all infectious viruses within one hour and is also active

against SARS-CoV-2. SARS-CoV is also stable for 30 minutes at 75°C (167°F), for 60 minutes at 67°C (153°F), and 90 minutes at 56°C (133°F) in the absence of protective protein and much longer in the presence of material containing at least 20% protein. During the 2002–2003 epidemic, it was recommended that contaminated areas be heated for at least 30 minutes at 60°C (140°F) to eliminate infectious SARS-CoV on surfaces.^{145–147}

2.5 The immune response

2.5.1 Introduction to severe acute respiratory syndrome-coronavirus and the immune system

2.5.1.1 *Severe acute respiratory syndrome-coronavirus and leukocyte numbers*

SARS is responsible for several changes in leukocytes and their secreted immune effector molecules that are responsible for most of our innate and adaptive immune responses to viruses. During the first five weeks of illness, the total numbers of leukocytes in the blood remain normal, however, 70%–98% of people who are infected had **lymphopenia** (low blood B cell and T cell counts), including CD19⁺ B cells, CD4⁺ T helper cells, and CD8⁺ T killer cells as well as CD16⁺/CD56⁺ NK cells. The T cell loss is greater in those patients with more severe illness or in those who later died than it is in survivors.^{66,143}

2.5.1.2 *Severe acute respiratory syndrome-coronavirus, leukocytes, and lymphoid organs*

Some lymphoid organs are infected and damaged by SARS-CoV. These organs include the lymph nodes and spleen which contain areas of hemorrhagic necrosis.⁷⁹ In the **secondary follicles** of the lymph nodes, the **germinal centers** which contain activated B cells are destroyed. Additionally, in the spleen, the **white pulp** which contains leukocytes shrinks and the numbers of T and B cells decrease, while the **red pulp** which contains red blood cells undergoes massive hemorrhaging and necrotic cell death.⁷⁸ T and B cell numbers also decline in the **Peyer's patches**, clusters of lymphoid cells in the walls of the small intestine.

Many blood leukocytes are infected in SARS patients, as many as 30% of the monocytes and 50% of the lymphocytic cells. These are primarily T cells, but B cells and NK cells may also be infected.⁷⁸ Interestingly, T cells, B cells, and some types of macrophages are infected by SARS-CoV despite their lack of the ACE2 receptor. Lowered numbers of lymphocytes are also present in the lymph nodes and spleen, even though both organs are enlarged.³⁴ Infected B and T cells in the tracheobronchial lymph nodes and spleen undergo apoptosis, which is one of the major causes of the decrease in lymphocyte numbers in these organs. Other potential causes for SARS-CoV-associated lymphopenia maybe that infected lymphocytes may be destroyed by the virus, sequestered in the lungs, or be subjected to altered chemokine-mediated lymphocyte trafficking. Suppression of lymphocyte maturation in the **red bone marrow** or **thymus** may also play a role.¹⁴³ Red bone marrow is the primary site of **hematopoiesis** and B cell maturation, while the thymus is the site of T cell maturation.

Myeloid DCs, while not themselves infected by SARS-CoV, play a role in transmitting the virus to host target cells using a synapse-like structure.¹⁴⁴ In this manner, myeloid DCs thus may act as SARS-CoV reservoirs and aid in persistent, chronic infection. Membrane engagement by the S protein is followed by S protein proteolysis in the acidic environment of the endosomes and is needed for membrane fusion to occur.^{125,144}

2.5.2 Severe acute respiratory syndrome-coronavirus and the adaptive immune response

2.5.2.1 Severe acute respiratory syndrome-coronavirus and lymphocytes

The adaptive immune response plays a critical role in protection against SARS-CoV. CD4⁺ T helper and CD8⁺ T killer cells' responses play important roles in defense against SARS-CoV by producing cytokines, especially IFN- γ produced by T helper cells, or by T killer cells directly killing infected cells¹⁴⁸. Levels of anti-SARS-CoV CD4⁺ T helper and CD8⁺ T killer cells in the lungs correlate with better protection against SARS. However, numbers of CD4⁺ T helper and CD8⁺ T killer cells are reduced in SARS-CoV-infected patients.¹⁴⁶

SARS-CoV-specific neutralizing antibodies produced by B cells are also important to block viral binding to and entering target cells and in viral clearance.¹⁴⁴ High levels of anti-SARS-CoV neutralizing antibodies and CD8⁺ T killer cell activity are found in people who recover from SARS. Additionally, the levels of these responses inversely correlate with disease severity. Although not part of the adaptive immune system, NK cells are also among our best lines of defense against viral diseases.

The most immunogenic SARS-CoV protein (most likely to generate an immune response) is the M protein, the most abundant protein on the surface of SARS-CoV virion. It activates both B cell and CD8⁺ T killer cell activity. The S protein is also a major stimulus for both B and T cell responses.¹⁴⁹

2.5.2.2 Severe acute respiratory syndrome-coronavirus and memory cells

During recovery from SARS, memory B and T cells are produced. The memory B cells can rapidly produce large amounts of neutralizing antibodies upon subsequent exposures to SARS-CoV.¹⁵⁰ The antibody levels are much higher than those produced during the first encounter with SARS-CoV and they last for much longer periods of time, preventing reinfection by the same or similar SARS-CoV strains. Neutralizing antibodies interfere with the virus's ability to bind to its ACE2 receptor, thus blocking the virus from entering its target cells. Antibody production by memory B cells, including anti-SARS-CoV antibodies, has been reported in some studies to be short-lived.¹⁵⁰ Other studies, however, report the production of antibodies against the N and S proteins that began about 2 weeks after infection were still detectable for at least 210 days after symptom onset.¹⁵¹ These antibodies were of the IgM, IgG, and IgA classes.

Memory T cell responses are generally present for much longer periods of time than are the B cell responses. Memory T cells produced during the first exposure to SARS-CoV may remain active and protect against SARS-CoV reinfection for a decade or more.¹⁵² While B cell responses tend to target variable regions of viruses found on the target cell

surface, T cells are more likely to target highly conserved internal proteins that are common to multiple species of coronaviruses.¹⁵³

Memory CD4⁺ T helper cells are more numerous at the site of infection than are memory CD8⁺ T killer cells.¹⁵⁴ T cell activity is especially important for protection against SARS-related damage to the lungs. CD8⁺ T killer cells, in particular, play a very important role in protection against infection by closely related betacoronaviruses, including SARS-CoV-2. Memory T killer cells primarily target the SARS-CoV M and N proteins.¹⁵² Many vaccines target regions of the S protein, but this protein differs among the various human coronavirus species and therefore is active against a smaller number of coronavirus species. The N protein, however, is conserved among human coronaviruses. Vaccines that target this viral protein induce cross-reacting antibodies that are active against at least some other human coronaviruses, but these vaccines do not induce the production of neutralizing antibodies. Anti-N protein antibodies and CD8⁺ T killer cells against SAR-CoV do not cross-react with or protect mice against challenges with MERS-CoV.^{152,153,155} Nevertheless, those blood-borne memory T helper cells that are active against one or more portions of the coronavirus N protein do induce protective responses against SARS-CoV and MERS-CoV and perhaps SARS-CoV-2 as well.¹⁵³ IFN- γ is produced by these long-lived memory T helper cells.¹⁵² Airway memory CD4⁺ T helper cells that are specific for the coronavirus N protein also induce protective responses during infection with SARS-CoV or MERS-CoV.¹⁵³ Other small discrete portions of viral proteins (**epitopes**) which are recognized by CD4⁺ T helper and CD8⁺ T killer cells lie within the SARS-CoV M protein and thus may be targeted by T cells.^{26,156} SARS-CoV-specific memory T cell responses in SARS survivors are present at least for four years after recovery from SARS. Approximately 29% of these responses target portions of the viral M protein.¹⁵⁷

In the respiratory tract, memory T helper cells are present among cells of the airway and lung **parenchyma** and are also found adhering to the vasculature of the lungs. The memory CD4⁺ T helper cells present in these three lung locations differ in several ways. Some memory T cell markers and molecules used to retain cells in a location are present in the lower levels of the airway. The expression of several cell surface markers on memory T helper cells in the parenchyma has a greater similarity to those found on T resident memory cells than to the airway T memory cells. More importantly, while other memory T helper cells in the region are multifunctional, the airway T helper cells are even more so. These T cells contain a higher proportion of cells that produce multiple cytokines as well as producing higher levels of cytokines per cell.¹⁵³ During infection with respiratory system viruses, the memory T helper cells in the airways are the first to contact the viruses.

Vaccines that activate the airway memory CD4⁺ T helper cells may help to swiftly eliminate the virus before it can damage lung tissues. A vaccine that contains a region of SARS-CoV that is recognized by T helper cells protects mice against subsequent exposure to a lethal strain of SARS-CoV. This region of the virus is conserved in both SARS-CoV and MERS-CoV and, perhaps SARS-CoV-2, as well as in various bat coronaviruses and may thus provide some degree of protection against other coronaviruses as well. Protection relies upon the rapid production and release of IFN- γ by T helper cells, together with vigorous nonspecific innate immunity and SARS-CoV-specific CD8⁺ T killer cell responses, along with the migration of respiratory DCs to the region.¹⁵³ This vaccine is effective when administered intranasally via mucosal tissue but not subcutaneously.

Vaccines of this type and route of inoculation have the potential of acting as pancoronavirus vaccines that cross-react with MERS-CoV, SARS-CoV-2, and perhaps other respiratory coronaviruses that may emerge in the future. These cross-reacting cells may provide some partial protection against other potentially pathogenic zoonotic coronaviruses as well.¹⁵³ Other potential vaccines are described later.

2.5.2.3 Severe acute respiratory syndrome-coronavirus and antibodies to the viral N and spike proteins

Almost 90% of SARS patients produced anti-N protein IgG. Even though these antibodies were not neutralizing, they may have been protective via another, yet unknown means, as is the case for mouse hepatitis virus. Interestingly, levels of IgG specific to the N protein are higher than IgM levels early after infection, even though IgM antibodies are generally the first to be produced. Early production of IgG may result from a strong CD4⁺ T helper cell response to the virus.¹⁵⁸ An excessive T cell response, however, may be pathogenic and contribute to the SARS-CoV-induced pneumonia.⁴⁸

Antibody responses to the SARS-CoV N protein play an important role in regulating disease severity. Those people who survive SARS have antibodies with higher **binding affinity** against the viral N protein and more sustainable levels of neutralizing antibodies to the S protein than patients with fatal disease outcome.¹⁵¹ In survivors, anti-N protein antibodies may be present by 10–15 days after the onset of symptoms. These antibodies reach peak levels during the next week and high levels are still present on day 87 postinfection. In patients with a fatal disease, the anti-N protein antibodies are either absent or first appear by 14–21 days after infection and soon undergo a large decrease in titer. This emphasizes the importance of producing anti-N protein antibodies soon after infection. In all tested SARS patients, the anti-S protein antibody was also detectable at 10–15 days postinfection. Interestingly, when compared to survivors, SARS patients with fatal outcomes produced larger levels of neutralizing antibodies against the S protein, but these high levels were transient and dropped soon thereafter.¹⁴⁷

2.5.3 Severe acute respiratory syndrome-coronavirus, cytokines, and chemokines

During infection with SARS-CoV, the immune system may be protective or pathogenic, depending on immune activation level, location in the body, and duration of the response. For a favorable outcome, coronavirus infection of the lungs in humans needs the immune system to eliminate the virus rapidly while preventing the development of an inflammatory condition that may lead to serious or lethal respiratory system damage, such as ARDS.¹⁵⁹ SARS patients have high levels of IFN- α and IFN- β . These IFNs trigger the release of ISGs, including the chemokine IL-8, which attracts neutrophils into an area. The proinflammatory cytokines IL-6, TNF- α , IL-1, IL-12, and IL-18 are also present.¹⁰⁷

IL-6 levels correlate with increased disease severity. This cytokine is important to the development of some aspects of adaptive immunity against pathogens, such as stimulation of antibody production by B cells and the activity of regulatory T cells (Tregs). IL-6's effects on the innate immune response include the regulation of macrophage and DC

differentiation and increased **Toll-like receptor** (TLR) 6 expression.¹⁷ TLRs are a class of several different host proteins that detect specific features of different types of microbes and initiate an immune response that is effective against that particular kind of microbe. Sera from deceased patients in the early stage of infection had greater amounts of IL-6, IL-8, and CCL2 than that present in the sera from survivors.¹⁵⁹ CCL2 is a chemokine that attracts monocytes, memory T cells, and DCs into infected areas. Immune mediator molecules act together with TNF- α and IL-1 β and are associated with ARDS.

IL-6 is also a primary inducer of the **acute-phase response** which enhances the release of stress compounds, especially **C-reactive protein** that is normally present in small amounts in the blood serum but increases during acute inflammation.³¹ The acute-phase response occurs soon after infection and is accompanied by fever and increased numbers of circulating neutrophils. In addition to the increase in C-reactive protein levels, increased levels of creatine phosphokinase and lactate dehydrogenase are also present.¹⁰⁷

TNF- α has multiple biologic effects, including triggering the release of other proinflammatory cytokines and chemokines, as well as enhancing the migration of eosinophils and neutrophils to the sites of infection. Higher levels of TNF- α are detected in the sera of SARS patients than in the sera of normal, healthy people. The numbers of inflammatory eosinophils and neutrophils in the lungs of the patients subsequently also increase.^{17,160} Both TNF- α and IL-1 protect the body from infection by causing fever that kills heat-sensitive microbes. If the body temperature becomes too high due to excessive levels of these cytokines, however, the body begins to damage its cells, potentially causing death. IL-1 also gives people a feeling of **malaise** (a general feeling of unwellness). In excessive amounts, TNF- α may lead to **wasting** (dangerously large weight loss), shock resulting from an extreme and often lethal drop in blood pressure, and inappropriately large levels of acute or chronic inflammation.

Inflammation plays an important role in halting microbial growth and killing invading organisms. Acute inflammation is a generalized defense mechanism that at proper levels is protective, but in excess or if present chronically, may cause great pain and damage to the affected organs or tissues. In the presence of appropriate levels of IL-1 β and TLR4, SARS-CoV viral load and tissue damage decrease.¹⁶¹ By contrast, compounds that reduce inflammation during infection with a mouse-adapted SARS strain can reduce viral pathogenicity in mice.¹⁶² Given the beneficial or detrimental effects of TNF- α and other proinflammatory cytokines, it is necessary to have an accurate regulatory system that restricts inflammation, keeping it at appropriate levels and ending it at the appropriate time. **Protein phosphatase 1 (PP1)** inhibits TNF- α signaling, decreasing tissue damage but may lead to increased SARS-CoV replication and, ultimately, pathology. PP1 is inhibited by **Kepi**, one of its subunits. Kepi serves to increase the inflammatory response to levels that decrease viral load and the associated tissue damage. A complex regulatory system with interacting components relies upon optimal levels of TNF- α , PPI, and Kepi activity to kill SARS-CoV while minimizing SARS-CoV-mediated inflammatory pathology.¹⁶¹

The blood levels of the chemokines **monokine induced by IFN- γ (Mig)** and the IFN- γ -**inducible protein 10 (IP-10)** were also highly elevated. These chemokines attract T and NK cells into the region. The levels of most other cytokines and chemokines are nearly normal.⁷⁸

2.5.4 Severe acute respiratory syndrome and interferons

In SARS-CoV-infected normal BALB/c mice, the levels of T cell apoptosis are higher than those seen in mice that lack type I IFN responses. Increased apoptotic T cell death is likely to play a role in the suboptimal T cell responses in the infected mice. Since some types of T cells reduce cytokine storms by suppressing the innate immune response, the lower levels of T cell responses may be at least partially responsible for unchecked innate immune responses that are not tempered by a switch to an adaptive response later in infection.¹⁶³

In this context, it is interesting that corticosteroids have been considered for the treatment of SARS-CoV since they decrease inflammation in several diseases, including the autoimmune diseases lupus and rheumatoid arthritis. These antiinflammatory hormones were the mainstay of SARS treatment in Hong Kong, but not elsewhere.¹⁶⁴ They slightly decreased the levels of proinflammatory mediators during acute-phase SARS, however, they also decreased levels of protective antiviral ISGs and IFN- α .¹⁶⁵ Corticosteroids may thus delay the onset of protective adaptive immunity if administered during acute-phase SARS.¹⁶⁶ Another study found that SARS patients who received corticosteroids had worse outcomes than those who did not (37.9% and 16.7%, respectively) despite their younger age and fewer comorbidities. Patients receiving corticosteroids also had a 20.7-fold greater risk of admission to intensive care units or death than patients not receiving this treatment.¹⁶⁴ The association of corticosteroids with skeletal system disease during SARS was discussed previously in this chapter. Taken together, these findings suggest that the treatment with corticosteroids during SARS needs to be considered carefully before being implemented, especially during the early stages of infection.

Somewhat different responses are seen in some mouse models of infection. As mentioned previously, a delayed-type I IFN response is associated with strong and rapid virus replication. In these animals, SARS-CoV concentrations in the lungs reach their peak during the first day of infection, when the IFN response is just beginning. **Plasmacytoid dendritic cells (pDC)**, however, induce rapid, but delayed, production of IFN after these cells are infected by coronaviruses.¹⁶⁷ These cells are rare but are the major source of IFN- α during viral infections. By stimulating the production of IFN- γ by Th1 cells, pDCs link the innate and adaptive immune systems. TLR7, a pathogen receptor that recognizes the presence of single-stranded RNA viruses, recognizes intracellular coronaviruses, including both SARS-CoV and mouse hepatitis virus, and alerts the immune system to the presence of these viruses. While high levels of inflammatory cytokines and chemokines are produced by macrophages or monocyte-derived DCs during SARS-CoV infection of humans, these cells do not produce type I IFN, nor do fibroblasts or lung epithelial cells.^{167,168} IFN- α and IFN- β are very important in controlling SARS-CoV infection. They reduce SARS-CoV replication in cell cultures in vitro as well as the severity of SARS in mice in vivo.^{45,167} In response, some variants of human SARS-CoV produce as many as five proteins that block either IFN production or interfere with their signaling pathways. MERS-CoV, like SARS-CoV, blocks the activation of type I IFN by inhibiting the activity of **interferon regulatory factor 3 (IRF-3)** in cultured primary human airway epithelial cells. When IFN is administered to cells in vitro, MERS-CoV is much more sensitive than SARS-CoV.¹⁶⁹

2.5.5 The severe acute respiratory syndrome-coronavirus E protein and the immune response

In a mouse-adapted strain of SARS-CoV, viral mutants that lack the E protein have reduced levels of proinflammatory cytokines and have lower amounts of neutrophils in lung infiltrates of infected mice. These mutated viruses also stimulate a higher stress response and apoptosis, activating a self-destruct mechanism in infected cells.¹³⁴ Additionally, the viral E protein is a **viroporin** that forms ion channels using pores in the host cell's surface that allow viruses to exit the cell and subsequently infect new ones.¹⁷⁰ SARS-CoV without viroporin activity is not virulent but maintains the same levels of viruses as **wild-type** (nonmutated) SARS-CoV. Other respiratory viruses, including influenza viruses, also have viroporin activity.¹⁶² Taken together, SARS-CoV that lack the E protein have decreased lung injury and increased survival in infected mice.

Mutant SARS-CoV lacking the E protein have decreased activity of the immune system transcription factor **NF- κ B** than that seen in wild-type viruses.¹⁶² SARS-CoV nsp1, nsp3a, nsp7a, and S and N proteins increase NF- κ B activation. Activation of this transcription factor appears to be involved in SARS-CoV pathogenesis.^{17,171–173} Addition of a **truncated** SARS-CoV S protein that lacks the S1 RBD to a mouse macrophage cell line in vitro still activates NF- κ B. NF- κ B, in turn, induces the release of the proinflammatory cytokines IL-6 and TNF- α , but not the chemokine IL-8.¹⁷ Drugs that inhibit NF- κ B activation decrease the levels of proinflammatory cytokines and lung injury in vitro and in vivo in mice, increasing their survival.¹⁶²

2.5.6 Severe acute respiratory syndrome-coronavirus and the innate immune response

2.5.6.1 Severe acute respiratory syndrome-coronavirus, macrophages, and dendritic cells

Macrophages and DCs express ACE2 and are infected by SARS-CoV, however viral replication occurs in neither. Monocytes freshly isolated from human blood, however, do allow self-limited SARS-CoV replication.¹⁷⁴ The maturation state of monocytes and macrophages may play a role in these differing results. Nevertheless, these cells may play an important role in SARS-CoV-related disease by serving as reservoir host cells that disseminate the virus throughout the body via the circulatory system. SARS-CoV also increases the viability of infected macrophages and DC, increasing the amount of time in which the cells can spread the infection.

When DCs are incubated with live viruses, but not with a radiation-inactivated virus, they become more phenotypically and functionally mature and displayed increased expression of the cell surface **major histocompatibility (MHC)** class II molecules and the CD4⁺ T helper cell **costimulatory molecules** CD40, CD83, and CD86. Together, these molecules stimulate CD4⁺ T helper cell activity and cytokine production. Live viruses also prime Th1 responses to low doses of the powerful immunostimulant **lipopolysaccharide** found on the surface of Gram-negative bacteria. This results in a dangerously excessive and potentially fatal release of **interleukin (IL)-6** and IL-12. Cytokines are also produced by SARS-CoV-infected macrophages and low-to-moderate production by DCs.⁴

The lungs of patients with severe disease who died soon after infection contained large numbers of alveolar and **interstitial macrophages**. **Alveolar macrophages** normally act as

TABLE 2.3 SARS and the innate immune system.

Cell type	Pro-or antiinflammatory	Effect
Dendritic cells	Proinflammatory	Increased lifespan and maturation Act as reservoirs that disseminate virus throughout body Increased expression of T helper cell costimulatory molecules Stimulate Th1 cytokine production (IL-6 and IL-12)
Mature macrophages in general	Proinflammatory	Increased lifespan Act as reservoirs that disseminate virus throughout body Numbers in lungs decrease Decreased phagocytosis
M1 macrophages	Proinflammatory	Vascular leakage and edema Produce toxic nitric oxide and reactive oxygen species Produce IL-1, IL-6, and TNF- α
M2 macrophages	Antiinflammatory	Homeostatic activities Promote recovery from acute respiratory distress syndrome Wound healing Produce growth factors Produce IL-10 and TGF- β
Natural killer cells	Neither	Numbers in lungs decrease Strong antiviral activity

part of the nonspecific first line of defense against microbes entering via the respiratory route, while interstitial macrophages normally cooperate with interstitial lymphocytes to produce a stronger and more specific immune response.³⁴ Two types of interstitial macrophage populations exist and loss of either of these populations worsens experimental lung fibrosis.¹⁷⁵ Numbers of lung DCs and NK cells are also decreased. The reduction in DC numbers impacts their ability to activate the remaining CD4⁺ T helper cells. The decrease in levels of CD8⁺ T killer cells and NK cells is also significant since these two cell types provide some of the greatest levels of protection against viral diseases in general.¹⁵⁰

SARS-CoV decreases macrophage **phagocytic** activity in vitro.⁴ Porcine reproductive and respiratory syndrome virus, a virus of pigs, also decreases macrophage phagocytosis.¹⁷⁶ Reduced phagocytosis may open the door to secondary infection by bacteria and fungi that cause respiratory system diseases. It will be important to determine whether these results are also found in blood and lung macrophages and DCs isolated from infected patients in vivo. See Table 2.3 for an overview of SARS and the innate immune system.

2.5.6.2 Severe acute respiratory syndrome-coronavirus and M1 and M2 macrophages

As stated previously, sustained high levels of some chemokines, including CXCL10, are associated with disease severity. CXCL10 is produced by several types of cells that participate

in wound healing, including monocytes, endothelial cells, and fibroblasts. The excessive or chronic presence of active fibroblasts, however, causes pathogenic fibrosis, in this case leading to the scarring of the lungs. CXCL10 recruits monocytes/macrophages, T cells, NK cells, and DCs into the affected area. It also stimulates monocyte production of proinflammatory cytokines, turning these cells into **M1 macrophages**. M1 macrophages are pathogenic in some mouse strains, even though this type of macrophage is protective in other mouse models of SARS. Similarly, differences in the roles of M1 macrophages in increasing or decreasing disease severity vary among people. M1 macrophages may accumulate in the lungs of SARS patients, promoting **vascular leakage**, in which fluid leaves the circulatory system and enters the tissues. T cell responses are inadequate to prevent tissue damage.¹⁶³ In patients with severe disease, high levels of CXCL10 persist in the lungs until death.¹⁶⁵

M2 macrophages are important in dampening inflammation during SARS and assisting in recovery from ARDS.¹⁵⁹ Interestingly, when activated, alveolar macrophages have both proinflammatory and wound-healing properties simultaneously, characteristics of M1 and M2 macrophages, respectively. The balance between these two macrophage subsets is important to disease outcome. Soon after infection, M1 cells produce toxic **nitric oxide (NO)**, ROS, IL-1, IL-6, and TNF- α . While these molecules are important in eliminating SARS-CoV, they may also cause lung injury when present in excess or for prolonged periods of time. The excessive release of inflammatory mediators during the SARS-CoV-induced cytokine storm may trigger ARDS and systemic inflammatory response.¹⁵⁹ When SARS-CoV is eliminated, M1 cell activities diminish. Activated M2 cell activity then begins to produce growth factors, the antiinflammatory regulatory cytokine TGF- β , and matrix metalloproteins that help to remodel (degrade and repair) the wounded area. Still, later, macrophages secrete another regulatory cytokine, IL-10, to restore **homeostasis**.¹⁵⁹ See [Table 2.3](#) for an overview of SARS and M1 and M2 macrophages.

The presence of anti-S protein IgG antibodies in the lungs indirectly leads to severe to fatal pulmonary disease.¹⁵⁹ The production of anti-S protein neutralizing antibodies is more rapid and of a higher titer (larger amount) in patients with a fatal illness in comparison to survivors.¹⁵¹ These antibodies decrease the wound-healing M2 cell activity while supporting M1 macrophage production of the proinflammatory chemokines CCL2 and IL-8. The former chemokine attracts monocytes, memory T cells, and DCs, while the latter attracts neutrophils to the sites of inflammation.¹⁵⁹ The lungs of patients with fatal cases of SARS contain M1 macrophages in the absence of M2 macrophages. The above-listed results appear to differ from other studies that report that neutralizing antibodies to the S protein prevents SARS-CoV replication in macaques, mice, ferrets, and hamsters.¹⁷⁷ These antibodies also prevent death in mice and ferrets during what would otherwise be a lethal SARS-CoV challenge.^{178,179} Nevertheless, SARS-CoV-neutralizing antibodies induced by different types of vaccines have been reported to enhance proinflammatory responses following viral challenges.^{59,159} This is also the case in **seropositive** cats upon re-exposure to the cat coronavirus feline infectious peritonitis virus following prior infection and either passive or active immunization.¹⁷⁷ Since healthy children under the age of 12 years have much less risk of symptomatic infection following SARS-CoV infection than adolescents and adults,⁴⁰ prior coronavirus infections among the latter two groups may increase pulmonary inflammation.¹⁵⁹ This exposure could take the form of infection with one of the less pathogenic human coronaviruses or could result from prior vaccination.

2.5.6.3 Severe acute respiratory syndrome-coronavirus, angiotensin converting enzyme, and angiotensin converting enzyme2

The relative levels of ACE and ACE2 affect blood pressure and vascular leakage. ACE is an enzyme that normally regulates the renin-angiotensin-aldosterone system in a manner that raises blood pressure systemically. In excess, however, this system may promote vascular leakage into the lungs.⁴⁹ The normal function of ACE2 (the receptor of SARS-CoV and SARS-CoV-2) is to counterbalance the effects of ACE, thus lowering blood pressure while protecting against acute lung failure.¹⁸⁰ During experimental SARS-CoV infection, ACE2 levels decrease in mice. This decrease correlates with fatal disease outcomes since ACE's activity is less impeded by ACE2, resulting in increased blood pressure and vascular leakage. Administration of the viral S protein by itself also worsens the disease course and may cause acute lung failure in mice. The extent of lung pathology, however, is lessened by compounds that block the renin-angiotensin-aldosterone pathway, thus returning blood pressure in the lungs to a more optimal operating range.¹⁸¹ ACE inhibitors that are currently available for the treatment of high blood pressure may decrease SARS-CoV-induced lung pathology by decreasing vascular leakage. There is a chance, however, that these ACE inhibitors might increase the expression of ACE2, thereby facilitating the entry of SARS-CoV (and perhaps SARS-CoV-2 as well) into the lung cells, increasing lung damage. The potential beneficial and pathogenic effects of ACE inhibitors on lung pathology during coronavirus infection will be discussed in the COVID chapter.

2.5.7 Animal models and the immune response to severe acute respiratory syndrome

2.5.7.1 Severe acute respiratory syndrome-coronavirus and the immune response of nonhuman primates

Having a proper animal model(s) of SARS is important in the search for an effective treatment as well as for evaluating the safety and efficacy of vaccine candidates. Nonhuman primates are sometimes used to model SARS. There are negative and positive aspects to the use of these animals. They reproduce more slowly and are more expensive to raise than rodents. Additionally, bonobos and chimpanzees, our closest animal relatives, are endangered. However, when compared to rodents, nonhuman primates are much more closely related to humans genetically and physiologically, may or not be **inbred**, live longer, and have lungs whose structure has greater similarity to those of humans. Oftentimes, macaques and other monkeys are used instead of great apes. Since some of these monkey species have been used extensively, their genomes, tissues and organs, immune systems, and responses to many varied types of microbes are well-defined. Both **inbred** strains and **outbred** monkeys are available for research. In addition, many monkey cell lines are available for in vitro testing, including Vero cells which are cloned kidney cells from African green monkeys (*Chlorocebus sabaeus*). Vero cells are used extensively in microbial research, including many of the in vitro studies of the effects of SARS-CoV on cells.

Rhesus macaques (*Macaca mulatta*) and African green monkeys are some of the more well-studied primates. African green monkeys experimentally infected with SARS-CoV display age-dependent differences in pathology as is the case in humans. Several parameters of immune functioning have been tested in the blood/serum and **mucosal regions**,

such as the mucus linings of the nasal cavity and lungs, and immune organs, such as the spleen and lymph nodes, of these SARS-CoV-infected animals. Testing from a variety of sites in the body is important since testing blood levels of viruses, immune cells, and immune-modulatory molecules alone may not be indicative of the immune response in other regions of the host, including their levels and activities in the infected organs or tissues themselves. Nevertheless, many studies continue to rely solely on evidence gathered from the serum of infected animals since the collection of blood is noninvasive and can be performed repeatedly and relatively easily. Information based only on blood work may also provide the foundation for further studies of tissue or fluid from other parts of the host's body.

Blood levels of CD8⁺ T killer cells and B cells of the adaptive immune system are lower in aged monkeys in comparison with younger animals regardless of whether the monkey is infected with SARS-CoV. However, innate immune responses, such as blood neutrophil and monocyte numbers, generally do not change with age or whether the host is infected or not.¹⁸² While serum levels of proinflammatory cytokines are similar in young and old monkeys, lower levels of proinflammatory IL-1 β , IL-18, IL-6, IL-12, and IL-15 are found in the lungs of SARS-CoV-infected aged monkeys at days 5 and 10 postinfection.¹⁸² These findings differ from those reported in aged SARS-CoV-infected mice in which proinflammatory responses increase respiratory system pathogenesis when compared to that occurring in younger animals.⁴⁹ Part of the difference in these findings may be due to the use of different animal species or could be the result of sampling on different days postinfection.

In uninfected monkeys, the total numbers of lung leukocytes are lower in aged animals as are the relative frequencies of CD8⁺ T killer cells, B cells, macrophages, and DCs despite the presence of chemokines in the lungs. Interestingly, no differences are seen in the numbers of these cells in the lymph nodes of juvenile and aged primates. DCs and monocytes/macrophages from older monkeys have an altered state of activation and numbers of cell surface chemokine receptors. Following experimental infection with SARS-CoV, higher levels of virus are present in nasal swabs of aged as compared to juvenile monkeys 1 day after infection, but a much smaller difference is seen by day 3.¹⁸² In uninfected monkeys, **immunosenescence**, the gradual deterioration of the immune system resulting from the normal aging process, appears to occur earlier in mucosal tissue than it does in the blood. The proportion of specific T cell subsets decreases as well as the number of B cells in the lungs. In infected monkeys, levels of SARS-CoV-specific IgA, the major antibody found in mucosal tissue, is lower in the lungs of aged than younger animals. The reduction in the amount of IgA in mucosal tissues may increase the risk of infection by inhalation, eating, and drinking as well as microbial entry via the urinary and reproductive systems in older monkeys. The level of antiviral neutralizing antibodies is also lower in the blood of older animals.^{182,183}

Anti-SARS-CoV neutralizing antibodies provide complete immunity in several animal models of infection and appear to reduce disease severity during human infections as well. While neutralizing anti-S protein antibodies may be detectable for up to a year following vaccination in mice, anti-N protein antibodies are not neutralizing in nonhuman primates and instead appear to decrease the longevity of other, more protective antibodies.⁵⁹ Unfortunately, the viral N protein is both the most abundant SARS-CoV protein during infection and is the most strongly **immunogenic**. Immunization of the animals

with the N protein appears to skew the T helper cell response in an antiinflammatory Th2 direction instead of towards the antiviral Th1 response. This skewing of the type of T helper cell response may be a major factor in coronavirus vaccine-stimulated disease.¹⁸⁰ Vaccination using inactivated (“killed”) viruses, especially those produced by formalin-inactivation, also tend to encourage Th2 responses and may, in some of those vaccinated, interfere with SARS-CoV.¹⁸⁴

2.5.7.2 Severe acute respiratory syndrome-coronavirus and the immune response of mice

Using rodents as animal models of human coronavirus disease has several advantages compared to the use of nonhuman primates. SARS-CoV infection of some strains of aged mice mimics the symptoms of SARS in humans. Given the small size of the animals and their fast reproduction rate, rodents are easy to care for and inexpensive enough to permit studies that use large numbers of animals. With their short lifespans, it is easy to test both young, adult, and aged animals. As stated above, a large amount of information is known about the genomes, tissues and organs, immune systems, and antimicrobial responses of nonhuman primates. In general, however, much greater amounts of such information are available for mice and rats. Many more inbred strains of mice and rats are also available that have well-defined genetic and phenotypic traits. Outbred rodents are also available but are used far less often than the specialized inbred mice and rat strains. Importantly, a very large range of antibodies has been produced against various mouse and rat proteins. These antibodies may be used to study the relative levels of different rodent cells or rodent molecules as well as their functioning. Such antibodies may also be used in the diagnosis of microbial diseases and to track pathogenesis or healing over time. The availability of such antibodies against nonhuman primates is much more limited by comparison.

While SARS-CoV replicates in both the upper and lower respiratory tracts of young mice, the virus is rapidly eliminated and the mice develop only short-term, mild **pneumonitis** (inflammation of the lungs) with little to no evidence of proinflammatory cytokines in their lungs.¹⁷³ No clinical disease is evident in C57BL/6 strain mice when they are infected with the human SARS-CoV Urbani strain isolated during the later stages of the 2002–2003 epidemic, a time period in which the disease generally was less severe than that present earlier in the epidemic.¹⁸⁵ Accordingly, the Urbani SARS-CoV strain appears to be less virulent than the initial SARS-CoV strains. The Urbani strain still, however, replicates in the lungs of mice, where it induces the production of proinflammatory chemokines that eliminate the virus even in the absence of T and B cells and NK cells. In this mouse model, it appears that innate immunity is sufficient to prevent disease in adult mice.¹⁸⁶

A mouse-adapted strain of SARS-CoV, MA15, was developed that differs from the human Urbani strain by only six amino acids. Both viruses are found in the spleen and liver of STAT1-deficient BALB/c mice experimentally infected with SARS-CoV MA15. Both young and old mice lacking this key intracellular signaling molecule produce high virus levels in the lungs, leading to severe disease and a 100% mortality rate. This model system reproduces lung disease that is similar to that seen in the most severe human cases of SARS-CoV and may be used to study the underlying mechanisms of SARS-CoV-induced pathology at cellular and molecular levels.^{47,187}

Differences in SARS severity among members of the same animal species may be due in part to differences in the timing of the onset of virus production as well as the length of time that various components of the host's immune responses are functional, especially if the animals differ in age.⁴⁹ Unlike surviving mice, animals with the fatal disease have increased levels of IL-1 α , IL-6, and TNF- α at the beginning of ARDS and these levels remain high until death.⁴⁹ Mice with defective IL-1 receptors usually survive infection with a normally lethal mouse SARS-like-CoV strain.¹⁸⁵ These findings suggest that the proinflammatory cytokine IL-1 plays a major role in pathogenesis, especially when present early during the course of infection.

Aged mice have a significantly higher risk of SARS-related death than younger animals. This risk is due at least partially to the development of early- and late-stage ARDS and pulmonary fibrosis.⁴⁹ Aged mice tend to mount later innate immune responses than do younger mice and have greater levels of proinflammatory cytokines in their blood. Peak viral replication in older mice also occurs very early after infection. SARS-CoV targets different host cells in older than younger animals. Older BALB/c mice that are experimentally infected with human coronaviruses, including SARS-CoV, develop a rapidly progressive disease that is in many ways similar to the disease manifestations displayed by elderly humans.

In a study by Rockx et al.,⁴⁹ young or aged mice were infected with one of two strains of human SARS-CoV that had been isolated either late or early during the 2003 SARS outbreak, the aforementioned Urbani or the more virulent GZ02 strain, respectively. Some animals were also infected with viruses isolated directly from palm civets. These viral isolates differ in the composition of their S proteins. While both human strains of SARS-CoV and the civet viral isolate were replicated in young mice, the animals did not develop the clinical symptoms of SARS.^{49,188} Infection by either the GZ02 or the civet-derived virus strain in these young mice is linked to strong control over the ER's UPR signaling pathway. Infection of older mice with these three SARS-CoV strains led to many different results.⁴⁹ While only mild disease and transient weight loss were seen in aged Urbani-infected animals, aged mice infected with GZ02 or the civet-derived virus strain developed severe lung disease and acute-onset DAD, ARDS, and death. Infection of the older animals increased the expression of several apoptotic genes within the first 24 hours of infection. By day 4 postinfection, 75% of the mice infected by the virulent human GZ02 strain died, while all mice infected with the palm civet-derived SARS-CoV strain died on or before day 3 of infection.⁴⁹

Infection of mice also increased the expression of the Kruppel-like factor 6 (Klf6) transcription factor. Among its many functions, KLF6 decreases cell division and cooperates with the transcription factor NF- κ B to regulate macrophage differentiation towards the proinflammatory M1 type and away from the M2 type.¹⁸⁹ KLF6 also regulates the transcription of genes involved in the wound repair process via activation of the TGF- β pathway of tissue repair. KLF6, together with STAT3, additionally coregulate the activity of genes involved in the regeneration of axons and thus might have a role in mitigating some SARS-associated damage to the nervous system.¹⁸⁹ Another important function of KLF6 is to increase the production of the toxic molecule nitric oxide (NO).

2.5.7.3 Severe acute respiratory syndrome-coronavirus and nitric oxide in mice

NO, a reactive nitrogen species, is known to stimulate apoptosis of cells infected by influenza and respiratory syncytia viruses, thereby helping to eliminate lung infection by these viruses. Exposure to NO inhibits replication of SARS-CoV as well as viral protein and RNA synthesis in vitro.¹⁹⁰ This inhibitory activity is not due to the formation of **peroxynitrite**, a product of the interaction of NO with the ROS **superoxide**.¹⁹¹ NO or one of its derivatives reduces chemical modification (**palmitoylation**) of the S protein, which appears to interfere with a cell-to-cell fusion of infected cells in vitro. In addition to its effects on SARS, proper levels of NO appear to also inhibit COVID-19-induced pathology. Due to its antiviral effects in SARS-CoV-2 infected patients, inhaled NO has been proposed as a treatment option for patients with COVID-19. Interestingly, few asthma patients have been found to develop severe COVID-19-related diseases in China. NO production is abnormally high in asthmatic patients and so may contribute to this unexpected finding.¹⁹²

Besides its antimicrobial activity, NO decreases blood pressure and increases blood flow to the lungs, heart, and CNS as well as other organs by dilating blood vessels in those regions, contributing to unregulated proinflammatory responses, and altering neurological activity. The levels of NO must therefore be carefully regulated since excessive levels result in hyperinflammation and substantial death of lung tissue.

2.5.7.4 Severe acute respiratory syndrome-coronavirus and chemokines in mice

Another mouse model system uses aged BALB/c mice that are infected through the nasal route. SARS-CoV rapidly replicates to high levels in the rodents' lungs, inducing proinflammatory cytokines by 24 hours of infection, followed by a peak in viral numbers 1–2 days postinfection.¹¹⁶ In animals that survive, the IFN responses increase early during infection, while animals that undergo a fatal outcome have a delayed response.⁴⁹ NK cells, macrophages, and DCs migrate into the affected site. This leads to increased production of the proinflammatory cytokine IL-6 and chemokines, including CXCL10, CCL2, CCL3, and CCL5. CXCL10 and CCL2 are **chemotactic** for monocytes/macrophages, CD4⁺ T helper and CD8⁺ T killer cells, NK cells, and DCs. CXCL10 binds to the cell surface chemokine receptor CXCR3, while CCL3 and CCL4 serve as the receptor for CCL2. The cell surface expression of these chemokine receptors also increases during SARS-CoV infection.¹¹⁶ CCL5 recruits T cells, inflammatory eosinophils, and basophils. The expression of one of its receptors, CCR5, is also increased during SARS-CoV infection. The innate immune system also plays an important role in decreasing viral load in the lungs early in the course of infection in these aged mice.¹¹⁶ The absence of normal innate immune signaling makes mice highly susceptible to SARS-CoV infection.

Pneumonitis is present by day 7 postinfection in mice and is accompanied by a second wave of proinflammatory cytokines. These include TNF- α , IL-6, IFN- γ , IL-2, and IL-5. Additional chemokines are also produced, such as CCL2, CCL3, CCL5, CXCL9, and CXCL10.¹¹⁶ The lungs are infiltrated by T cells and neutrophils and a strong T helper cell response is elicited. The virus is cleared from the lungs by day 9 postinfection.¹¹⁶ Removal of Th1, but not CD8⁺ T killer cells, increases the interstitial pneumonitis and delays clearance of the virus from the lungs. This suggests that Th1 cells may be more important than

CD8⁺ T killer cells in controlling SARS-CoV-associated respiratory disease, at least in the lungs in this mouse model. This is unusual for respiratory diseases, including influenza, in which CD8⁺ T killer cells play a major role in controlling the disease.¹¹⁶

2.5.7.5 Severe acute respiratory syndrome-coronavirus and Toll-like receptors in mice

Genetic differences between inbred mice populations may lead to differing results that are dependent on the strain of mouse used. **Collaborative Cross** is a large panel of new inbred mouse strains produced by crossing several different established mouse strains. Crossing several of these mouse strains produces offspring with a wide range of susceptibility to SARS. By examining the differences in the proteins between two of these strains of mice, five regions of the genome were identified that may contribute to SARS-CoV pathology, including weight loss, virus load, and pulmonary hemorrhage. One region of chromosome 18 controls multiple SARS-CoV responses. The gene for **Ticam2** is located within this region and alterations in this gene affect the severity of SARS in mice.¹⁹³ Ticam2 assists the host cell to sense the presence of SARS-CoV by facilitating the binding of the TLR4 to TRIF (TIR-domain-containing adapter-inducing interferon- β) typically after recognition of lipopolysaccharide on Gram-negative bacteria. TRIF is an adapter protein for TLR4 and is required for TLR4-mediated production of type 1 IFN. TRIF activity may act in a protective or pathologic manner in inflammatory diseases and is vital to wound-repair processes.¹⁹⁴ TLR4 recognizes some viruses as well, including SARS-CoV and respiratory syncytial virus, probably due to TLR4's ability to recognize alterations in the ECM. Extensive changes in the ECM occur during SARS-CoV infection and may result in TLR4 activation.^{51,193} TLR4-deficient mice are at a much higher risk of developing severe SARS than are wild-type mice.¹⁹⁵ Variation in the gene for TLR4 is also associated with increased disease severity in humans.

In addition to its recognition by TLR4, SARS-CoV is also detected by **TLR3** and the **cell's melanoma differentiation-associated protein 5 (MDA5)** in mice. TLR3 and MDA5 sense the presence of intracellular double-stranded RNA, which is produced during the replication of SARS-CoV and other coronaviruses. SARS-CoV nsp6 allows the virus to evade detection by MDA5.¹⁹³

2.5.7.6 Severe acute respiratory syndrome-coronavirus and interferon in mice

The genes encoding the innate immune system molecules **MyD88** (myeloid differentiation primary response 88) and **STAT1** are linked to the severity of SARS.⁵¹ Both are also associated with IFN activity. Type I IFNs induce the phosphorylation of STAT1. Afterward, STAT1 joins a transcription complex that enters the nucleus and induces the expression of ISGs that produce an antiviral state.¹⁹⁶ MyD88 is an adapter protein that aids in the communication of TLRs and IL-1 pathways from the outside of the cell to protein signaling entities inside the cell, eventually activating NF- κ B.¹⁹⁷

The mouse-adapted SARS-CoV MA15 strain is lethal in the BALB/c mice. Intranasal infection of young C57BL/6 mice, however, does not result in lethal infection, however, they do have high levels of viral replication in the lungs which is accompanied by inflammation and destruction of lung tissue and a 12%–15% loss of body weight by day 3

postinfection. Early after infection of C57BL/6 mice, MyD88 induces production of the antiviral, but proinflammatory cytokines IL1- β , TNF- α , and IL-6 as well as the chemokines CCL2, CCL3, and CCL5. CCL2 draws inflammatory M1 macrophages into the lungs early after infection.¹⁸⁵ The above cytokines and chemokines may act in either a protective or destructive manner, depending on when they are produced following infection. The chemokine receptors CCR1, CCR2, and CCR5 also may stimulate either protection or progression of disease caused by the neurovirulent mouse hepatitis virus.^{198,199} MA15-infected C57BL/6 mice recover by day 6 in a manner that does not appear to depend on T or B cell activity or induction of type I IFNs.¹⁸⁵

By contrast, when young C57BL/6 mice lacking functional MyD88 are infected by the SARS-CoV MA15 virus, they often develop severe lung damage. The mortality rate in these mice exceeds 90% by day 6 postinfection. Infected MyD88-negative mice have higher viral loads in the lungs in addition to multiple proinflammatory cytokines and chemokines, including those that recruit M1 monocytes/macrophages to the lungs. M1 macrophage number is greatly reduced in the MyD88-deficient mice when compared to SARS-CoV-infected normal C57BL/6 mice.¹⁸⁵ In addition to mice lacking MyD88, mice lacking the receptors for monocyte-recruiting chemokines, including CCL2, also have severe lung damage, underscoring the importance of both MyD88 and monocytes/macrophages in the recovery of mice after infection with SARS-CoV MA15.¹⁸⁵

Type I, II, and III IFNs often are critical to survival in mice infected with respiratory viruses. These types of IFN differ in several ways, including utilizing different cell surface receptors. Type I IFN uses IFNAR1, type II uses IFNGR, and type III uses IL28Ra/IL10Rb. IFN- λ , the type III IFN, plays a major role in defense against SARS-CoV by inhibiting viral replication in both lungs and gastrointestinal tracts in normal mice.²⁰⁰ Accordingly, expression of functional IFN- γ complexes is restricted almost exclusively to the lungs and the intestinal tract epithelial cells. Since type I IFN and type III IFN stimulates the expression of similar genes, these IFN types might have overlapping functions.²⁰⁰

Infection of mice lacking type I, type II, or type III IFN with either Urbani or MA15 viruses resulted in clinical disease outcomes and recovery that is almost identical to that seen in infection of wild-type mice. This suggests that type I, II, and III interferon signaling do not play a major role in regulating SARS pathogenesis in at least these mouse strains. However, infection of STAT1-negative mice leads to severe disease, with high numbers of viruses and extensive lesions in the lungs that are similar to that seen in late-stage human disease. All of these mice infected with MA15 or Urbani SARS-CoV variants died.⁵⁶ Taken together, these findings suggest that SARS-CoV pathogenesis is regulated by a STAT1-dependent, but type I, II, and III interferon-independent, manner. This is unusual since IFNs are typically one of the primary mediators of protection during many respiratory system viral infections, including SARS-CoV, influenza virus, respiratory syncytial virus, and human metapneumovirus.⁵⁸

Separate from its roles in IFN signaling, STAT1 also is involved in regulating cell cycle arrest and cell division, including decreasing tumor formation in several types of cancers, including lung cancer. SARS-CoV infected mice lacking STAT1 are at higher risk of developing pulmonary fibrosis due to STAT1's role in restricting cell proliferation and wound healing responses.^{58,201} The low degree of macrophage responses to the IFN- γ in the lungs of aged mice has also been linked to impaired signaling through STAT1.

2.5.8 Severe acute respiratory syndrome-coronavirus and escape from the immune response

The N protein is the most abundant viral protein in some coronavirus-infected cells. It functions as an innate immunity antagonist in several coronaviruses, including SARS-CoV and mouse hepatitis virus. The N proteins in these coronaviruses directly interact with the **protein activator of protein kinase R (PACT)**, a cellular dsRNA-binding protein that stimulates type I IFN via competitive binding to RIG-I/MDA, and thus inhibits IFN- β production. ORF4a of MERS-CoV and the N protein of mouse hepatitis virus also act in this function. However, the N proteins from the porcine epidemic diarrhea virus and porcine reproductive and respiratory syndrome viruses do not bind or interact with PACT in this manner.²⁰²

Other SARS-CoV molecules, including ORF6, inhibit the translocation of STAT1 into the nucleus, thus blocking the subsequent activation of IRF-3, nsp7, and nsp 15. Nsp7 increases the activity of the RdRp during replication and nsp15 blocks the production of type I IFN and its signaling in vivo.²⁰³ In addition to the N protein, viral ORF3b is also a type I IFN antagonist.²⁰⁴ The actions of the N protein are confined to SARS-CoV-infected, specific, permissive cells in the lungs.^{58,205}

2.5.9 Severe acute respiratory syndrome immunopathology

SARS-CoV and MERS-CoV may induce fatal acute lung injury in mice via **hypercytokinemia** (large release of proinflammatory cytokines) and intensive inflammation, leading to DAD, as well as **epithelial necrosis** of the lungs (death of cells lining the airways), and deposition of fibrous material, including fibrin, in the lungs.³¹ Proper functioning of the innate immune response in the epithelium of the airways is vital to keep respiratory viruses, including SARS-CoV, from entering the lungs until the production and arrival of elements of the adaptive immune response into the lower respiratory tract.²⁰⁶

IgM and IgG antibodies initiate one of the three pathways of the multifunctional **complement cascade**, which produces pores in cells that harbor SARS-CoV, allowing fluid to enter and lyse the infected cells before they release more viruses. Some of the activated components of the complement cascade enhance the phagocytic activity of monocytes and macrophages, while other components are chemotactic and draw other leukocytes into the site of infection. This results in acute inflammation that kills viruses but may play a pathogenic role by inducing the production of ARDS during SARS. Complement is thus typically an important part of host defense against viral, bacterial, and fungal infections, but may also contribute to pathology in the lungs or other organs. Following intranasal infection of mice with SARS-CoV, the complement cascade is activated in the lungs and the blood during day 1 postinfection.¹⁹³ Some of the products of an activated complement cascade are the **anaphylatoxins** C4a, C3a, and C5a, which recruit proinflammatory cells into the area. C3a and C5a also lead to the degranulation of mast cells of the innate immune system, initiating a “cytokine storm.”¹⁹⁶

In mice lacking C3, a central component of all three complement activation pathways, SARS-CoV replication in the lungs is similar to that occurring in normal mice.

Nevertheless, C3-deficient infected mice lose less weight and have less severe respiratory symptoms when compared to infected mice with a fully functional complement system. The numbers of neutrophils and inflammatory monocytes in the lungs of the C3-deficient mice are also decreased, leading to reduced lung pathology and lower levels of the cytokines and chemokines IL-5, IL-6, CXCL1, and the cytokine and growth factor **granulocyte colony-stimulating factor** in the lungs and blood sera. This growth factor stimulates the red bone marrow to increase the production of selected leukocytes that contribute to inflammation, including neutrophils and mast cells. Activation of the complement cascade may, therefore, play an important role in enhancing SARS-related lung disease.¹⁹³

2.6 Treatment options

Treatment for SARS may require intensive care and respiratory support using ventilators. Care is needed to prevent the spread in healthcare facilities, especially nursing homes. Antibiotics are not generally useful against viruses, including SARS-CoV, however, several antiviral compounds are available that are effective against some viral diseases. It should be noted that many antiviral compounds have serious side effects, which include pancreatitis, abdominal pain, diarrhea and nausea, liver dysfunction, and anemia.⁷² Remdesivir is one such antiviral compound. In experimentally infected mice, remdesivir inhibits the replication of human coronaviruses, including SARS-CoV and MERS-CoV.²⁰⁷ It improves lung function and decreases SARS-CoV levels and severe pathology in the lungs. Additionally, remdesivir appears to decrease the length of COVID-19 by four days. Early during the COVID pandemic, remdesivir was given an emergency use authorization by the United States Food and Drug Administration for treatment of COVID-19 patients with severe disease. It was later approved for use in all hospitalized COVID-19 patients.²⁰⁸

Remdesivir is a nucleotide analog that inhibits viral replication by blocking the activity of the viral RdRp. In general, drugs that cause premature termination of transcription are less effective in coronaviruses than in many other single-stranded RNA viruses since the coronaviruses' nsp12 encodes a proofreading enzyme that corrects mistakes in the viral RNA that are made during transcription. Remdesivir, however, decreases coronavirus-associated pathology without affecting nsp12's proofreading activity, implying that it targets a different viral function.²⁰⁷

Ribavirin is another widely used antiviral agent, but it does not prevent SARS-CoV infection *in vitro*. Nevertheless, during the SARS outbreak, high doses of ribavirin were administered to infected people in the hope of decreasing disease severity. While SARS patients had normal levels of IFN- γ -producing cells, levels of IL-2 and IL-4 were decreased by approximately 50%, however, T cells stimulated *in vitro* had higher levels of IL-6 and TNF- α .²⁰⁹ Ribavirin also produced many adverse side effects, even at relatively low drug doses.

Other general antiviral treatments were more promising when tested *in vitro*, including a form of type I IFN.⁴⁵ This IFN eliminates 99.5% of SARS-CoV from cultured cells *in vitro* within 24 hours of administration. Moreover, *in vivo* studies indicate that SARS-CoV

infects type 1 pneumocytes in the airways of cynomolgus macaques at an early stage of the disease. A chemically altered form of IFN (pegylated-IFN) administered to these animals before infection reduces infection of type 1 pneumocytes as well as decreases the extent of viral replication and lung damage.⁴⁵ Administration of this drug **prophylactically** (used to prevent infection) or early after exposure to a SARS-CoV infected person may help to lessen the extent of viral disease in healthcare workers, family members, friends, or other people spending time with at-risk populations. When administered at later times postinfection, however, IFN is less effective in preventing severe disease.⁴⁵

Other compounds found to protect against SARS include chloroquine, glycyrrhizin, reserpine, niclosamide, and several anti-HIV drugs. Many of these drugs are repurposed (developed for the treatment of other illnesses) but have activity against SARS-CoV as well.

As introduced in Chapter 1, chloroquine is an antimalarial drug that was discovered in 1934. It has been repurposed to prevent or treat various viral infections in cultured cells in vitro or in mice in vivo.²¹⁰ This drug blocks viral entry into uninfected cells in vitro and decreases disease in previously infected cells. Chloroquine alters the structure of ACE2 by interfering with this receptor's **terminal glycosylation** (a process that attaches sugars to the end of proteins), thus interfering with SARS-CoV's ability to bind to and enter cells. Chloroquine also decreases the release of proinflammatory cytokines. Additionally, chloroquine raises the pH of the endosomal vesicles, lysosomes, and the Golgi apparatus. During the process of infecting the target cell, SARS-CoV is internalized into endosomes. These endosomes must be acidic for the viruses to establish successful escape into the cells' cytoplasm and replicate. The viral progeny are later transported to the Golgi apparatus, from which they bud as they exit the cell.²¹⁰

Chloroquine is also active against the human CoV-229E coronavirus. Since this drug has been used for decades to treat malaria, it is known to be safe for use in most people at the appropriate dose, is relatively inexpensive, and is readily available. Care must be used when administering this drug to people with heart conditions since they are at risk for potentially severe side effects. Nevertheless, chloroquine or its derivatives are widely used to treat human diseases other than malaria, such as infection with amebas, HIV, and autoimmune diseases, without significant side effects.²¹¹ Importantly, chloroquine is effective against SARS-CoV at plasma concentrations present when administered to malaria patients.^{210,212}

Chloroquine appears to be active against MERS and COVID-19, as described in later chapters. It should be noted that despite its positive results when tested in vitro, when used alone, chloroquine was not able to prevent SARS-CoV replication in infected mice. Nevertheless, chloroquine may reduce lung inflammation in animals and appears to be more effective when used in combination with drugs that decrease virus replication, such as IFN derivatives. These derivatives can greatly reduce the amount of SARS-CoV present in the lungs of infected mice. It also should be noted that laboratory rodents, including mice, are more sensitive to disease than humans. Drug trials that use mice, therefore, may produce results that do not reflect the situation in primates, including humans, great apes, and monkeys. The fact that chloroquine alone does not decrease SARS-CoV replication in mice, therefore, does not necessarily mean that it is not effective in humans. This drug is very effective in primate cells in vitro and is active when administered before or after exposure to SARS-CoV, suggesting both preventative and therapeutic activity.²¹⁰ Followup

studies *in vivo* using monkeys or smaller primates, such as marmosets, while expensive, perhaps are needed to clarify drug and vaccine effectiveness, especially when they are used in combination with other drugs.

Other medications are being repurposed to treat SARS-CoV and other human coronavirus infections. Glycyrrhizin, derived from licorice root, has been used to decrease the risk of liver cancer in people infected with hepatitis B and C. When repurposed, it also decreases replication of several SARS-CoV isolates from infected patients.²¹³ Reserpine, derived from the dried root of Indian snakeroot, is typically used to treat high blood pressure, while niclosamide is used to treat tapeworm infections. Other promising drug candidates include amiodarone, typically used to treat irregular heartbeats, and luteolin, an extract from Chinese herbs.^{214–217} All these drugs have a high degree of activity against SARS-CoV. Niclosamide has also been shown to be active against MERS.

Several anticancer agents have anticoronavirus activity and may be used to treat SARS-CoV and MERS-CoV. One of these drugs, thalidomide, is notorious for causing severe birth defects in fetuses and so should not be used by pregnant women. Another anticancer drug, imatinib, is an **Abelson kinase (Abl)** inhibitor. Abl2 kinase regulates several cellular pathways, including pathways that control adhesion. During SARS and MERS, imatinib blocks the viruses from adhering to and fusing with the endosomal membrane, thus inhibiting viral entry into the cell.²¹⁸

Since lung damage during SARS may at least partially be due to an inappropriate immune response, the **corticosteroid** prednisone has also been tested for anti-SARS activity. Corticosteroids reduce the production and release of proinflammatory cytokines by Th1 cells, so these compounds decrease inflammatory reactions in the lungs of those with SARS. However, the administration of corticosteroids has also been associated with psychosis, diabetes, and avascular necrosis of the femoral head. Corticosteroids should perhaps be used very carefully and in combination with antiviral drugs, such as ritonavir and lopinavir, since when used alone, corticosteroids may increase viral numbers.⁷² See Table 2.4 for an overview of treatment options.

Since the duration of the SARS epidemic was short, there was little time for the drugs to be tested for safety or effectiveness in controlled, randomized human clinical trials, but were instead judged for their safety and efficacy in SARS-CoV-infected animals. This proved to be difficult since most species of animals experimentally infected with SARS-CoV do not develop lesions and did not mimic SARS in humans. Animals that were tested include monkeys, hamsters, marmosets, and ferrets. Obvious disease was only seen in ferrets.

2.7 Diagnosis

Researchers from 17 countries discovered SARS-CoV as the cause of SARS and sequenced its complete genome within six months of the first reported human case. Diagnostic tests were also rapidly developed.²¹⁹ SARS-CoV may be cultured from droplets contained in respiratory secretions (aerosols from coughs and fluids in the mouth), feces, and urine. While viral SARS-CoV RNA was detected in the respiratory secretions and feces by 11–12 days postinfection, it may be detected more readily in samples from an infected person's respiratory tract, which contain higher levels of virus. Immune-based screening techniques became the gold standard for confirmation of SARS-CoV infection.²²⁰ However, since this

TABLE 2.4 Comparison of several SARS-CoV treatment options.

Intervention category	Several treatment options	Mode of action
Supportive care	Supplemental oxygen Respirators	Increase oxygen levels
General antiviral medications	Ribavirin	Broad-spectrum antiviral guanosine analog Decreases activity of viral RdRp ^a Decreases guanosine triphosphate levels Inhibit capping of viral mRNA Ineffective against SARS-CoV
	Remdesivir	Nucleotide analog Decreases activity of viral RdRp Decreases viral replication
Cytokines	Pegylated Type I IFN	Increases expression of MHC ^b on cells Increases T killer and NK cell activity Induces production of IFN- γ
Repurposed drugs	Chloroquine (antimalarial drug) ^c	Reduces viral entry into cell's cytoplasm Alters ACE2 structure (glycosylation) Decreases release of proinflammatory cytokines Raises pH of endosomal vesicles and lysosomes Inhibits production of ATP
	Niclosamide (treats tapeworm infections)	
	Amiodarone (treats irregular heartbeat)	Blocks sodium channels
	Glycyrrhizin (decreases liver cancer due to hepatitis B and C viruses)	Inhibits generation of ROS ^d by neutrophils
	Reserpine (treats high blood pressure)	Inhibits the ATP/Mg ⁺² pump Destruction of some neurotransmitters Slow nervous system activity Decreased heart rate and cardiac output Lowers blood pressure
Anticancer medications	Thalidomide	Inhibits production of IL-6 and TNF- α Stimulates apoptosis
	Imatinib	Abl2 kinase inhibitor Blocks viral adhesion to endosomal membrane
Immunosuppressants	Prednisone	Prevent production of inflammatory cytokines

^aRNA-dependent RNA-polymerase.^bMajor histocompatibility complex molecules.^cOriginal purpose.^dReactive oxygen species.

test detects products of the adaptive immune response, it takes 10–14 days to produce detectable levels of antibodies. “Antibody tests” in the diagnosis of SARS-CoV infection, while they may be specific for coronavirus infection, do not detect very early-stage infection.

2.8 Prevention

2.8.1 Physical means of prevention

Some of the people who were at the highest risk of developing the severe disease during the 2002–2003 SARS outbreak were those who came into close contact with SARS patients, such as medical personnel and family members. The numbers of positive cases were significantly inflated due to overdiagnosis in many patients with probable SARS who did not have close contact with an infected person.²²¹ In Singapore and Toronto, healthcare workers comprised 41% and 43% of the people known to be infected, respectively. Secondary bacterial infection from close contact with an infected person, however, occurred infrequently and only 8% of household members of SARS patients developed respiratory system bacterial coinfections. Other high-risk factors for the development of severe disease comorbidities include visiting a fever clinic, eating out, and using taxi cabs frequently.²²¹ Having visited a school or university, taking part in large social gatherings outside the home, and staying home from work or school were not factors in SARS acquisition.²²¹

The risk of contracting SARS after flying on an airplane varies.²²² Traveling with a SARS case in the crowded environment of a plane was found to generally be of very minimal risk.²²³ However, following a flight carrying an infected passenger with fever and cough plus 119 other persons, 18 people developed SARS and four others had probable cases, even though they had no known prior exposures to patients with SARS. Passengers seated up to three rows in front of the infected person had a 3% greater risk of becoming ill than those seated in other areas of the plane. The ill passenger had a fever and cough during the flight, was hospitalized immediately upon arrival in Beijing, and died of atypical pneumonia five days later. Several of the other passengers who later became ill transmitted the infection onward to additional people during a subsequent bus tour of the city. A second flight carrying 246 people included four passengers with SARS who had fever and cough. One fellow passenger later contracted an illness characterized by fever and respiratory symptoms but was not tested for SARS-CoV infection. On the third flight of 315 people, including one passenger with presymptomatic SARS, there was no evidence of transmission to the others on board. The infected passenger developed fever four days after this flight and was later hospitalized and received mechanical ventilation. In a larger study, the World Health Association reported that in 4 of 35 flights carrying a symptomatic person, transmission to another person may have occurred.²²²

2.8.1.1 Personal protective equipment

During the epidemic, Asian countries' healthcare workers were required to use **personal protection equipment (PPE)**, including N95 masks, goggles, and aprons.²²³ The number of procedures that generate aerosols was also reduced. Members of the general population were requested to use frequent hand washing with soap or application of alcohol-based disinfectants. They were also advised to avoid situations in which they might be exposed to infected individuals, especially in enclosed environments, such as restaurants, airplanes, and taxis, and to wear face masks if using public transportation, working in restaurants, or entering hospitals.²²³

While WHO did not recommend screening passengers even from areas with recent local transmission, Asian countries did use this kind of screening. The screening techniques used included filing a Health Declaration Card and thermal screening using thermometers or thermal scanners.²²³ While active monitoring helps in the early detection of people with minimal symptoms, implementation is costly and local conditions may indicate instead use of passive monitoring for managing contacts having lower-risk exposure.²⁹

To prevent viral spread, protective methods utilized in the 2002–2003 outbreak included those designed to increase the distance between people by canceling large gatherings and closing schools and theaters. Suspected or probable infected people were isolated using barrier nursing technique in healthcare facilities along with voluntary or mandatory quarantine of their suspected contacts for 10 days.²²³

To limit the international spread, travel advisories were issued to avoid travel to some regions of the world that were experiencing an outbreak. Travelers were educated about the disease using signs and videos, public address announcements, and the distribution of 31 million public health alert notices in Asia. Contact tracing was used to determine the spread and was a major factor in disease control.²³

During the SARS outbreak, disinfectants were applied to the homes and vehicles of infected people, ambulance tires, and pedestrian walking zones. It was recommended to disinfect any surfaces that may have been contaminated with sweat, saliva, mucus, vomit, stool, or urine while wearing disposable gloves and discarding the gloves afterward.²²⁴ The effectiveness of any or all of these strategies is unknown since the epidemic did eventually spread to 29 countries despite these measures. The epidemic may well have ended in 2003 due to a combination of other, unknown factors in addition to the above noted public health measures. It should be noted that the SARS epidemic ended without the benefit of full randomized clinical tests for drugs, massive specific testing procedures, or effective vaccines. Nevertheless, it is also possible that some of these public health procedures, alone or in combination, prevented much greater loss of life in the affected areas and spread to even more regions.

Regardless of potential public health safety measures, many of these procedures had negative personal and societal impacts, including infringing on personal freedom of movement, generating feelings of isolation, and leading to loss of income or employment.²⁹ In Toronto, the economy was severely impacted. Predicting the extent to which various public health measures will impact both the illness and economic loss is difficult due to a combination of factors which include the often-unexpected behaviors of people, climatic factors, and the ability of coronaviruses to mutate and recombine. Fortunately, except for several cases, SARS-CoV infection did not reappear the following winter or thereafter.

2.8.1.2 Hand hygiene and decontamination of infected surfaces

A large percentage of SARS infections are believed to have resulted from contact with contaminated surfaces, followed by touching the face. HCoV-229E, -OC43, and -NL63 human coronaviruses may survive on dry surfaces.¹⁴⁹ SARS-CoV and MERS-CoV may be detected on dry surfaces for extended periods, sometimes months. While SARS-CoV or its RNA may persist on inanimate surfaces, it is not known whether the remaining virus was infectious and could cause disease in humans.²²⁵ Nevertheless, while decontamination of surfaces, such as doorknobs, land-line telephones, and elevator buttons, may have played

a role in preventing the spread of this coronavirus, the efficacy of this protective intervention is unknown. Microbes often survive much longer when protected by a covering of external organic material. SARS-CoV persists in diarrheal stool samples for four days and respiratory tract secretions for at least seven days at room temperature.²²⁶ Again, the remaining microbes may not be infectious.

Frequent use of hand disinfectants was also encouraged. These often contain some form of alcohol, usually ethanol or isopropanol. Of the four-hand rubs tested, within 30 seconds of application, all the following were able to decontaminate surfaces to below the point at which no virus was measurable: Sterillium, SterilliumRub, SterilliumGel, and SterilliumVirugard.²²⁷ The following three surface and instrument disinfectants completely inactivated the virus within 15–30 minutes, the earliest times in which they were tested: Mikrobac forte, Kohrsolin FF, Dismozon, and Korsolex basic. SARS-CoV can therefore be easily inactivated by many commonly used disinfectants. The same may also be true for MERS-CoV and SARS-CoV-2. Disinfectants are to be used only outside of the body and must not be used internally or as a mouthwash. SARS-CoV is also inactivated by a 5-minute exposure to 1:100 hypochlorite (bleach),²²⁶ but bleach damages many surfaces and oxidizes metals, causing them to rust.

Other protective factors that may have decreased infections from environmental surfaces include increased heat, exposure of surfaces to natural or other sources of UV light, and increased humidity levels, in addition to decreasing the density of people in crowded areas. Some of these factors have been long known to aid in lowering viral levels in the environment.

Massive efforts by biomedical researchers and the international health community led to the rapid accumulation of relevant information about the virus, its relatives, and its transmission, allowing the outbreak to be swiftly brought under control using both traditional means and novel surveillance and protection strategies. A 2020 report found that 92% of hospitalized patients in China had used traditional Chinese medicine as well as Western medicine.²²⁸ Many of these strategies have been also used during the ongoing SARS-CoV-2 pandemic. It should be noted, however, that without the critical deletion in ORF8 that greatly reduced SARS-CoV replication, the 2002–2003 epidemic may have been more pathogenic and lasted longer despite the use of masks and other strategies that decrease person-to-person contact.

2.8.2 Immunization

Passive and active immunization strategies decrease SARS severity. During passive immunization, an infected recipient receives antibodies from the blood of another person (the donor) who had previously been infected and recovered (**convalescent serum**). Passive immunotherapy may also use animal sera or antibodies, in the same manner that the antibodies present in horse antivenom had been used to protect against snake venom. During the current COVID-19 pandemic, laboratory-produced monoclonal antibodies have also been used.

Convalescent sera have been used for the treatment of SARS.²²⁹ Passive immunization of SARS patients with this serum did indeed lead to shorter hospital stays and higher survival rates.²³⁰ Also, antibodies from people who survive SARS may differ from the type of

antibodies found early during infection in patients with fatal outcomes.¹⁵⁹ Convalescent sera against the typically mild human coronaviruses 229E and OC43 cross-react with virulent SARS-CoV.²³¹ The serum also stimulates both antibody production and T cell immune responses.²³² Administration of IL-1 to SARS patients also protects against severe disease.²³³ This cytokine stimulates **T cell-independent antibody** production.

Passive immunization of BALB/c mice with immune serum blocks viral replication in the lower respiratory tract after subsequent intranasal challenge with SARS-CoV. This indicates that in this mouse model system, antibodies in the serum alone suffice to stop viral replication. In the recipient mice, viral replication reaches its peak within two days of infection in the absence of observable disease. The virus is then cleared within the first week. By contrast, in nonimmunized mice, SARS-CoV typically replicates vigorously and rapidly in the respiratory tract, including the cells lining the bronchioles. In addition to passive immunization, active vaccination protects mice, which produce neutralizing antibodies that block reinfection for at least four weeks.¹⁷⁷ It should be noted that this model system does not reproduce the severe pathology that is found in humans.

Another, older approach to passive immunization, utilizes sera from horses that were previously immunized with inactivated SARS-CoV-2. The horse sera contain high levels of neutralizing antibodies. While not as safe as monoclonal antibodies, administering humans infected with COVID-19 with horse sera may be practical and affordable for emergency treatment in regions of the world that are unable to receive vaccines or medication promptly.²³⁴ Care must be taken, however, to limit the number of times in which horse sera is administered to humans to avoid causing **serum sickness**.

It is also important to note that when either convalescent human or horse sera are used for passive immunization, the recipients do not develop their own immune responses and so are vulnerable to reinfection. In the case of SARS, passive immunization strategies were tested by injecting anti-S protein antibodies into SARS-CoV-infected ferrets and mice. This approach also decreased viral replication in the lungs but was less effective in the throat. By contrast, during active immunization, the vaccine stimulates the people to produce their own immune response against the infecting microbe, complete with memory cells that protect the recipient against reinfection by the same microbe. Some anti-SARS vaccines protect experimentally-infected hamsters and African green monkeys.

2.8.3 Active immunization

2.8.3.1 *Introduction to severe acute respiratory syndrome-specific vaccines*

Several different vaccine strategies have been attempted to protect against SARS-CoV infection. These include the following: inactivated, noninfectious (“dead”) whole virus (as was used by Salk in his polio vaccine); nonpathogenic, infectious virus (as was used by Sabin in his polio vaccine); individual components of the virus that were grown in a vector in bacteria or yeast (as used in the hepatitis B vaccine); and administration of viral RNA. The latter approach was utilized in the first USDA-approved anti-SARS-CoV vaccine.²³⁵ Immunization using some regions of the coronavirus’ S protein stimulates the production of protective antibodies in monkeys, while immunization with other regions of the S protein instead increases infection in these animals. This warns that during vaccine

development, the choice of regions of the S protein (or other viral proteins) that are to be included in potential vaccines needs to be examined carefully. Adjuvants are materials added to a vaccine to increase its efficiency. Some adjuvants used in SARS-CoV vaccines include surface-linked liposomal peptide, muramyl dipeptide derivative, and CpG oligodeoxynucleotide. These increase SARS-CoV-specific T cell responses, some of which are durable.¹⁴⁹ Unfortunately, some of the most effective adjuvants cause strong side effects and may not be appropriate for use in humans.

2.8.3.2 Severe acute respiratory syndrome-coronavirus and antibodies in vaccinated people

In those vaccinated, the levels of IgG and neutralizing antibodies peak at 4 months,^{149,236} but SARS-CoV-specific IgG is still detectable for at least two years in 89% of those immunized. A higher percentage of people have detectable levels of neutralizing antibodies at this time.²³⁶ IgG is only detectable in 10% of people after 6 years after illness onset.²³⁷

2.8.3.3 Severe acute respiratory syndrome-coronavirus and T cell responses in vaccinated people

SARS-CoV-specific memory CD8⁺ T killer cells are present for up to 6 years, indicating long-term protection against this virus. The risk of a given person developing severe SARS after either vaccination or natural infection correlates with that person's types of MHC class I molecules.²³⁸ These molecules are expressed on the plasma membrane of almost all mammalian cells and differ greatly among individuals. Since CD8⁺ T killer cells can only recognize SARS-CoV-infected cells that bear the correct MHC class I molecules, differences in vaccine efficacy may arise from genetic differences in MHC class I molecules among different individuals.¹⁴⁸

One study found that SARS-specific memory T cells from three SARS survivors have been present for at least 9 to 11 years after recovery.¹⁴⁸ All the memory T cells from this small group targeted the viruses' structural proteins. The memory CD8⁺ T killer cells from two of these individuals targeted the SARS-CoV M and N proteins. Furthermore, these responses are still present in these survivors in 2021. The persistence of T cell responses makes it likely that patients who survived SARS in 2002–2003 are protected against re-infection with the same SARS-CoV strain and perhaps any additional strains that may arise over time. An absence of cross-reactivity of these T killer cell responses against MERS-CoV suggests that the SARS-CoV anti-N and -M would not protect against MERS or most other coronaviruses.¹⁴⁸ However, given the similarities between SARS-CoV and SARS-CoV-2, vaccines against one of these viruses may provide at least some protection against the other.

While multiple vaccine candidates utilize whole SARS-CoV or only their structural genes, few studies have reported the efficacy of vaccination using nonstructural SARS-CoV proteins to produce T cell immune responses. Since nsp's are more conserved than structural proteins, they make attractive vaccine candidates that may cross-protect against any SARS-CoV strains and maybe even mutated SARS-CoV-2 strains. Both nsp's and short synthetic **peptides** derived from SARS-CoV proteins generally do not have toxic effects when administered to people. While normally the viral peptides do not induce a strong

immune response by themselves, attaching the peptides to the surface of **liposomes** improves their performance. Attaching liposomes to one of these peptides was reported to induce large numbers of long-lasting memory CD8⁺ T killer cells in vivo.¹⁴⁸ On the other side of the size scale, regulatory proteins, such as the large nonstructural polypeptide 1a (pp1a), are even more conserved among SARS-CoV strains and are produced earlier after infection than are the S and N proteins.²³⁹ Given the importance of T killer cells in viral clearance, pp1a is a promising vaccine candidate because it is the largest of the SARS-CoV proteins and thus has a better chance of containing regions able to produce a strong T killer cell response than are the smaller proteins or peptides.¹⁴⁸ Out of 30 potential CD8⁺ T killer epitopes from pp1a, nine can induce IFN- γ -producing T cells after immunization in mice.¹⁵²

Vaccinating aged mice with SARS-CoV RNA stimulates not only high levels of neutralizing antibodies but also CD4⁺ T helper and CD8⁺ T killer cell activity, as well as long-lasting memory T killer cells. These T memory cells help to protect mice against subsequent exposure to infectious SARS-CoV by rapidly removing infected cells, especially from the lungs. In immunized mice, memory T killer cells specific for given regions of the S protein produce several cytokines, including IFN- γ , TNF- α , and IL-2. They also produce granzyme B, a CD8⁺ T killer and NK cell molecule, that causes apoptosis in infected cells.¹⁵⁰ These findings with aged mice may hasten the development of similar vaccines that will protect elderly people at high risk for severe to fatal illness as well as younger people.

2.8.3.4 Challenges in the development of anti-severe acute respiratory syndrome-coronavirus vaccines

Several obstacles exist to developing anti-SARS-CoV vaccines. Some vaccines based upon a variant of the SARS-CoV S protein which was isolated from the 2002–2003 outbreak increased the susceptibility of human cells to infection by SARS-CoV in vitro in a process known as an **antibody-dependent enhancement**. This considerable obstacle can be reduced by removing part of the SARS-CoV S protein from the vaccine.²⁴⁰ Also, using relatively high doses of vaccine appears to be protective, since, at higher doses, this vaccine triggers the production of neutralizing, rather than pathogenic, antibodies. Another potential problem for vaccine development is that by altering the inflammatory response, some macaques passively immunized with soluble, neutralizing IgG antibodies developed severe lung injury during acute infection.²⁴¹ Administration of some types of SARS-CoV-specific IgG antibodies to infected mice also increases the risk of severe lung disease, specifically acute DAD. In humans, patients who rapidly produce anti-S protein-neutralizing antibodies had a higher risk of death than patients who produce this particular type of antibody more slowly.

A double-inactivated vaccine (DIV) against SARS-CoV was produced by “killing” the virus twice by exposure to a weakened form of formaldehyde followed by ultraviolet irradiation. Even though this vaccine was protective in vitro, DIV causes **eosinophilic** immunopathogenic lung injury when administered to aged BALB/c mice without protecting a nonlethal **heterologous challenge** (infecting the animals with a different strain of the virus).⁵⁹ This pathology, as exemplified by increased immune infiltrates, exceeds that produced in nonimmunized animals. Increased lung injury in the DIV-immunized mice

appears to be due to higher levels of Th2 cytokines as well as chemokines that recruit eosinophils (IL-5, IL-13, and CCL11) when compared to unvaccinated aged mice. The DIV used in this study does, however, protect younger mice against lethal disease upon either challenge with **homologous** or heterologous virus. Even the mice that were protected by the DIV, however, had an influx of eosinophils in their lungs. DIV also requires an **adjuvant**, in this case, alum, to protect the young mice.⁵⁹ Alum is one of the few adjuvants approved for human use.

When BALB/c mice are immunized **intradermally** with a hybrid vaccine expressing the SARS-CoV S protein alone or a vaccine that also contains all the structural proteins, they develop severe pneumonia after **intranasal** SARS-CoV challenge.²³² The extent of disease in these vaccinated mice is similar to that seen in unvaccinated animals, although the viral load is less in the vaccinated mice.²³² Mice that receive hybrid vaccines against the N protein or the N and S proteins also develop severe pneumonia. Mice vaccinated against only the N protein increase their production of the Th1 cytokines IFN- γ and IL-2 as well as the Th2 cytokines IL-4 and IL-5. The production of the antiinflammatory Treg cytokines IL-10 and TGF- β was decreased. This vaccine-induced alteration of cytokines leads to a high degree of infiltration of neutrophils, eosinophils, and lymphocytes into the lungs and thickening of the lining of the alveoli which decreases oxygen and carbon dioxide exchange.²³² These findings imply that vaccines containing the N protein may increase, rather than decrease, SARS-CoV-associated lung injury, at least in some strains of mice, especially in older animals. Interestingly, inoculation of mice with both the N and M protein produces a favorable response with increased production of Th1 cytokines and decreased pathology and mortality rates.²⁴²

Some vaccines may result in smaller numbers of IL-2-, IL-4-, IL-10-, and IL-12-producing cells, together with abundant production of the proinflammatory cytokines IL-6 and TNF- α and the chemokines CCL2, CCL3, and CXCL10. CXCL10 may be at least partially responsible for the pathogenic influx of activated T cells and monocyte/ macrophages into the lungs.²⁴³ By contrast, another study reported increased production of IFN- γ , IL-1, IL-6, and IL-12 p70, but not other cytokines, such as IL-2, IL-4, IL-10, or TNF- α .^{209,244} The N protein increases levels of IL-6, one of the causes of severe pneumonia.²³²

Despite their promise, vaccines directed against the S protein may not provide protection for long periods of time because this protein mutates rapidly during the course of an epidemic or pandemic. Examination of many of the available SARS-CoV isolates shows that the S protein has greater sequence variation than the N protein, which is relatively conserved.⁵⁹ A potential vaccine that targeted the SARS-CoV S protein increased infection of some human cells in vitro, whereas it had been shown to be protective when tested in rodents. Unfortunately, a large amount of vaccine testing is performed in mice and rats and the findings may not always be applicable to humans. Another obstacle to SARS-CoV vaccine development is the relative lack of a clear demand for anti-SARS-CoV vaccines since the SARS epidemic in 2002–2003 only lasted for a very short time-period and some of the vaccine candidates did not protect against infection with other human coronaviruses known at that time. At least some SARS-CoV vaccines may, however, protect against SARS-CoV-2 infection or may decrease COVID-19 severity. Anti-SARS vaccines might also be useful if administered to animals being raised in captivity for sale in wet markets, perhaps preventing future zoonotic transmission of SARS-CoV or similar coronaviruses from animals into humans.

Great care must be taken when developing an effective, yet safe, vaccine. A cautionary note: when an inactivated SARS-CoV vaccine was tested in ferrets, monkeys, and mice, the immunized animals developed an inflammatory lung disease when they were later exposed to the live virus. Additionally, in humans, a massive vaccine campaign against the H1N1 “swine flu” in the United States in 1976 was halted when hundreds of people developed a dangerous neurological condition known as **Guillain-Barré syndrome**. That vaccine had been hastily produced and administered before proper safety testing was performed. As frustrating as it seems, it is literally vital to very carefully prepare and test vaccines and that may take over a year. Encouragingly, when changes were made to how a SARS-CoV virus was administered, immunized hamsters did not develop the disease after subsequent exposure to the live virus. Some types of SARS-CoV vaccine preparations, therefore, are promising and, if their production and testing are given high priority by governmental bodies and public health groups, as has been the case in the development and testing for the SARS-CoV-2 vaccines, safe and effective vaccines may be developed in less time, especially when new technologies are used in the process.

2.9 Surveillance

The world health community was watchful for clusters of cases of severe respiratory diseases, especially in Asia, from 2002–2003. After reports of the SARS outbreak, rapid international responses identified the virus and its genetic structure, traced its transmission to and among humans, and appeared to have aided in halting the spread of infection. During the outbreak, several unusual strategies were adapted to attempt to rapidly detect ill people so that they could be isolated to limit disease spread. The infrared thermal screening was one such method and was used to identify people with fever in public areas and at border crossings. People living in affected areas were advised to take their temperatures daily. Telephone hotlines and evaluation clinics were developed. Entry and exit screening of travelers included the use of questionnaires to determine a person’s possible exposure to SARS-CoV. Visual inspection was also used to detect people who were overtly ill. The extent to which these strategies were useful is unknown.

Surveillance techniques were developed to identify and interrupt otherwise undetected chains of SARS transmission, including an immune system test that detected the presence of anti-SARS-CoV antibody¹³⁹. These surveillance strategies were used to survey the numbers of infected, but healthy, Taiwanese healthcare workers who had treated patients with SARS. Surprisingly, antibodies indicating SARS-CoV infection were only found in workers from two hospitals. Some of these individuals continued to be antibody-positive for more than 100 days, suggesting long-lasting protection against that particular SARS-CoV variant. Some screening procedures were expensive and required sophisticated equipment, but the comparison of those hospitals with or without infected personnel may provide clues as to the factors affecting transmission in healthcare settings and may apply to locations with large numbers of high-risk people, such as nursing homes and rehabilitation facilities, dialysis and cancer treatment centers, maternity wards, intensive care units, and prisons.^{245,246}

In some countries, detection and isolation of infected individuals were combined with rapid identification and isolation of their contacts (**contact tracing**). Both infected people and their contacts were often placed in quarantine in their homes. Other people, such as travelers, the homeless, noncompliant people, and people who feared exposing their families to the virus, were placed within residential facilities. Isolating in hospitals was only recommended if medically necessary.²⁴⁷ Local and state authorities should also be prepared to isolate patients at home or in alternative facilities designated for this purpose. Some of these people were temporarily excused from quarantine restrictions if they wore masks, did not use public transportation, and avoided crowded public areas. Quarantine during the 2002–2003 SARS outbreak stressed separating people with symptoms from unnecessary contact with uninfected people, isolating infected people for a set minimum time period, closely monitoring them for disease symptoms, and rapidly providing medical assistance for those who did become ill. SARS was diagnosed in 0.2%–6.3% of quarantined people who had contact with a person who tested positive for infection.

SARS is believed to have entered the human population by contact with infected animals that did not result in disease in these animals, so many asymptomatic infections in humans, as well as animals sold in wet markets and people exposed to these animals, may have remained undetected. It may, therefore, be wise to routinely monitor animals and people working in wet markets to avoid future transmission to humans. This could allow for rapid responses, including quarantine if animal-to-human transmission of coronaviruses or other microbes were to occur again, but only if accurate reporting is made available soon after disease detection. Cooperation among countries and public health workers, healthcare personnel, veterinarians, and those monitoring wild animal populations is important to detect zoonotic transmission of animal viruses or other types of microbes into people in time to respond to any new disease threats rapidly. Unfortunately, since the adaptive immune response is extremely specific to one part of one type of microbe, some vaccines against RNA viruses, such as SARS-CoV, are unlikely to fully protect all people infected by a new, currently undetected, coronavirus unless the vaccine targets a conserved region of the genome and not the S protein. Otherwise, the specificity of the adaptive immune response makes it extremely difficult to develop and stockpile effective vaccines against novel coronaviruses before they have infected people, although cross-reacting antibodies may provide some critical degree of protection, especially if two closely related viruses have very similar S proteins.

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Middle Eastern respiratory syndrome

3.1 Introduction to Middle Eastern respiratory syndrome and Middle Eastern respiratory syndrome coronavirus

3.1.1 A brief introduction to Middle Eastern respiratory syndrome

MERS is a mild to life-threatening respiratory disease in humans and is associated with atypical pneumonia and other severe respiratory diseases. It also includes diseases in the urinary, digestive, cardiovascular, and nervous systems that may result in acute renal failure, **hepatic** abnormalities, gastrointestinal upset, **pericarditis**, abnormal **coagulation**, **encephalitis**, or **Guillain-Barré syndrome**.¹ Accordingly, high levels of viral RNA are present in **bronchoalveolar lavage (BAL)** fluid as well as in much lower RNA levels in urine and stool samples from some MERS patients.² BAL fluid also contains high numbers of neutrophils and macrophages, **phagocytic** cells of the **innate immune system**. The immune system acts as a double-edged sword during MERS, with major roles in both virus elimination and pathogenic inflammation.

As of the end of March 2019, at least 2442 cases and 842 deaths have been reported worldwide with a fatality rate of 35.5%.³ Most of the deaths occurred in people with **comorbidities**. It should be noted, however, that many people with asymptomatic infection were not detected or included in the above numbers, so the true fatality rate is likely to be lower. Reports of new cases in humans follow a seasonal pattern in which case numbers begin to increase in April and May. This pattern is similar to that seen in dromedary camels since their calves begin to be weaned in March and April.⁴ Dromedaries serve as intermediate hosts for transmission of MERS-coronavirus (MERS-CoV) to humans. Implementation of preventative measures, including restriction of camel movement in affected areas, enhanced surveillance, early detection of cases, and containing outbreaks in hospitals are estimated to have averted 1465 cases of MERS and 293–520 deaths from 2016 to September 2018.⁵

3.1.2 A brief Introduction to Middle Eastern respiratory syndrome-coronavirus

MERS-CoV (formerly HCoV-EMC) is similar to other betacoronaviruses. It is a positive-stranded RNA virus that encodes four structural proteins and a variety of **nonstructural proteins (nsp's)**.⁶ MERS-CoV binds to its target cells via interactions between the **receptor-**

binding domain (RBD) of its **spike (S) protein** and its host cell receptor, **dipeptidyl peptidase 4 (DPP4)**; also known as CD26).⁷ The genes, proteins, and life cycle of coronaviruses are described in Chapter 1. Briefly, after the binding of the S protein to DPP4, the virus and host cell membranes undergo fusion, followed by viral entry into the cell by one of several routes. Once within the cytoplasm of the cell, the MERS-CoV genomic RNA is translated into two large **polyproteins**. These are then cleaved by several viral and host **proteases** and processed to form the mature nsp's and structural proteins – the S, membrane (M), envelope (E), and nucleocapsid (N) proteins.

DPP4 is a cell surface **serine protease** that is present in many cell types. Its normal function is to control the activity of multiple cellular regulatory molecules, including **neuropeptides**, **vasoactive peptides**, **cytokines**, and **chemokines**. Neuropeptides modulate long-lasting **synaptic transmission** and may act as small **neurotransmitters**. Vasoactive peptides regulate heart contraction and increase the diameter of blood vessels, lower blood pressure, and relax smooth muscle in the trachea. Cytokines and chemokines are immune messenger molecules, whose functions include regulation of cellular responses to microbes and triggering the movement of **leukocytes** and other cells to the site of infection.

3.1.3 Transmission of Middle Eastern respiratory syndrome-coronavirus to humans

Phylogenetic analysis of MERS-CoV genomes from 43 patients indicates that the virus has been transmitted to humans from the one-humped dromedary camel (*Camelus dromedarius*) by multiple zoonotic transmission events.^{8–10} Almost all adult dromedaries throughout the Middle East have high levels of **neutralizing antibodies** to MERS-CoV, indicating past infection.¹¹ Since MERS-CoV infection in dromedaries is asymptomatic or results in only mild respiratory symptoms, its presence in these animals may be undetected. **Zoonotic transmission** occurs during contact with camel respiratory secretions, uncooked camel meat, or consumption of raw camel milk or urine.⁴

3.1.3.1 Zoonotic transmission via camel respiratory secretions

Transmission to humans may occur by exposure to live dromedary camels' respiratory secretions.⁴ MERS-CoV seroprevalence is greater among people having contact with camels than among the general population.^{12–14} Several of the first reported MERS cases in humans were linked to an animal market and the central slaughterhouse in Qatar. Four of the five slaughterers had anti-MERS-CoV-specific IgG antibodies. Of the camels awaiting slaughter, 59% had nasal shedding of MERS-CoV. At least five different virus strains were found in the camels at this location.¹⁵ In a 1-year study of about 10,000 people conducted in 2012–13 in Saudi Arabia, the overall seroprevalence rate was 0.15%. This rate, however, was fifteen times greater in dromedary shepherds and 23 times higher in slaughterhouse workers.^{12,16} This strongly supports the role of dromedaries in zoonotic transmission. Other people come into contact with dromedaries at festivals, races, sales, and parades.¹¹

Juvenile dromedaries carry very high virus loads in their nasal secretions and so can transmit the virus to humans. Such transmission is not common since only young dromedaries lacking maternal antibodies to MERS-CoV (beginning at 5–6 months after birth) are

susceptible to active infection and they only shed the virus for 8 days.¹⁷ The young animals have little contact with humans during that time since they are reared with their mothers for their first year of life. Additionally, only 1% of infected calves develop nasal discharge, further reducing the risk of transmission to humans.¹⁸

3.1.3.2 Zoonotic transmission via camel milk and urine

Camel milk is very popular in Saudi Arabia, where 78% of it is sold as unpasteurized fresh or fermented milk.¹⁹ Drinking raw milk or urine or having contact with raw dromedary meat may lead to infection in humans. The virus remains infectious in raw camel milk when refrigerated or if stored for as long as two days at room temperature. Additionally, Bedouins and other camel-herding populations in the Arabian Peninsula and East Africa wash their hands, face, and hair in camel urine, which is also used to treat a variety of health conditions. Fresh urine in combination with camel milk is a component of ointments and skin creams.⁴

3.1.3.3 Human-to-human transmission of Middle Eastern respiratory syndrome-coronavirus

Several reports suggest that, at least in the initial stage of MERS-CoV emergence into the human population in 2012, self-sustaining transmission between people was low.^{20,21} Using Bayesian analysis, the **basic reproduction number (R_0)** was estimated to be 0.6–0.7 while that of prepandemic SARS-CoV-2 was 0.8. These findings suggest that MERS-CoV did not at that point have pandemic potential²¹ since self-sustaining transmission requires an R_0 of greater than 1. The R_0 varies greatly over time and in different locations and settings. A 2015 study estimated a worrisome R_0 of 2.0–2.8 for Riyadh and 3.5–6.7 for Jeddah, Saudi Arabia.²² In a brief, but highly contagious, epidemic in South Korea in that year, the R_0 was reported to be 4.9 with a serial interval of 6–8 days.²³ A serial interval is the time between successive cases of infectious diseases. Nevertheless, as of 2022, person-to-person transmission is not self-sustaining and most infections occur through contact with dromedaries or their biproducts.

During human-to-human transmission, **index cases** (the initial case in an area) have been traced to living in or traveling to several areas in the Middle East in or near the Arabian Peninsula, including Saudi Arabia, Jordan, Qatar, the United Arab Emirates, Bahrain, Kuwait, Oman, Yemen, Iran, and Lebanon. Since almost all zoonotic transmission of MERS-CoV occurs from dromedaries, MERS-CoV may have major reservoirs in many regions of the Middle East, the Canary Islands, and North and Eastern Africa since dromedaries have tested antibody-positive in these regions.^{24–27}

The occurrence of multiple zoonotic transmissions of MERS suggests that multiple index cases in humans exist. In addition to South Korea, travel-associated cases have been found in an ever-expanding number of locations, such as the United Kingdom, Europe, North Africa, North America, Asia and Southeast Asia, and Eurasia.²⁸ **Secondary transmission** from one person to one or more other people has become a major means of transmission to healthy family members and healthcare workers (HCWs). The ability of MERS-CoV to be transmitted between people appears to be increasing over time and was the sole factor driving the large MERS outbreak in South Korea in 2015. A 2018 report found that up to 50% of MERS-CoV cases in Saudi Arabia were due to secondary transmission.²⁹

Nosocomial transmission is a major factor in spreading MERS-CoV in hospitals and other healthcare facilities to doctors and nurses, to other patients, and even to visitors to a hospital ward containing an undiagnosed MERS patient. However, the chains of transmission between household members and in healthcare facilities appear to be limited and not self-sustainable,³⁰ unlike the case in SARS-CoV and SARS-CoV-2.

Infected persons pass MERS-CoV to uninfected people either directly via inhalation of respiratory droplets or indirectly by contact with **fomites** (contaminated inanimate surfaces). MERS-CoV does not survive on these surfaces as well as SARS-CoV and environmental conditions alter the time during which MERS-CoV is both viable and infectious to humans,³¹ as described later in this chapter. People with subclinical MERS are also contagious.³² About 2.5 million people partake in the Hajj pilgrimage to Muslim holy sites in Saudi Arabia annually. Most of the pilgrims are foreign visitors who subsequently return to their own country.³³ Surprisingly, no large MERS outbreak has been associated with this large, yearly assemblage.

3.2 The history

MERS-CoV was first isolated in humans from a 60-year-old man in Jeddah, Saudi Arabia. The infected man developed acute pneumonia and kidney failure and died in late June 2012.⁶ Retrospective analyses, however, found that at least 80% of the dromedaries in Somalia and Sudan had antibodies against MERS-CoV in 1983 and seropositive dromedaries were present in Egypt in 1997.³⁴ Similarly, MERS-CoV appears to have been present in camels by 1992 in Saudi Arabia and, by 2003, in the United Arab Emirates. Thus MERS-CoV was widespread among dromedaries for decades before the first known cases in humans.³⁵ MERS-CoV-specific antibodies were also found in stored human serum from Jordan in April 2012.^{36,37}

Two human MERS-CoV infections were linked to a camel farm in Qatar in 2013 in which 78.6% of the dromedary camels had positive nasal swabs for MERS-CoV RNA. The virus in the camels was very similar to that present in these two people.³⁸ Lending support for dromedaries as a major source of human infection, a MERS-CoV isolate from a MERS outbreak in humans from Saudi Arabia was genetically identical to the MERS-CoV virus from the upper respiratory tract (URT) of dromedaries at that location.³⁹ This demonstrates the ability of MERS-CoV to jump directly from dromedaries into humans without prior adaptive mutations.^{11,40}

A large outbreak of imported MERS began in South Korea on May 20, 2015, from a traveler following visits to Saudi Arabia, Qatar, the United Arab Emirates, and Bahrain.⁴¹ During this outbreak, 186 cases, and 38 deaths were reported with another 16,752 suspected cases.⁴² Many of the cases in South Korea occurred in people working or visiting one of the several hospitals that the traveler visited. Over the course of the next few years, many countries in the Middle East reported cases of MERS. Since then, the disease has been reported in at least 27 countries.⁴³ Cases in the Middle East or Eurasia have been reported in Bahrain, Iran, Jordan, Kuwait, Lebanon, Oman, Qatar, Saudi Arabia, the United Arab Emirates, and Yemen. Cases in Africa have been reported in Algeria, Egypt, and Tunisia. Cases in Europe have been reported in Austria, France, Germany, Greece,

Italy, the Netherlands, Turkey, and the United Kingdom. Cases in Asia have been reported in China, South Korea, Malaysia, the Philippines, and Thailand. The United States of America is the only country in the Americas with reported cases.⁴³ MERS was introduced to these locales by travelers to the Middle East.

Most of the initial MERS cases in humans in the Middle East were associated with contact with dromedaries or their milk or urine.⁴⁴ Secondary transmission of MERS between humans, however, was responsible for a cluster of cases of acute respiratory illness in a hospital in Jordan in April 2012⁴⁵ as well as the 2015 Korean outbreak in which “hospital-shopping” (patients traveling between multiple hospitals) among MERS patients was a contributing factor.⁴⁶

Patient zero (the first known case) in South Korea was admitted to Pyeongtaek St. Mary’s Hospital in mid-May 2015. Soon afterward, an outbreak occurred that consisted of 36 cases by early June. The fatality rate was 16.7%.⁴⁷ While the total disease prevalence was 3.9%, it increased to 7.6% among inpatients, and was 18.6% among inpatients on the floor where patient zero was kept. The prevalence among caregivers and medical staff at this hospital was 3.3% and 1.1%, respectively.⁴⁷ The outbreak spread as four patients moved to other hospitals without appropriate quarantine. This epidemic revealed that in developed regions of the world, crowded wards, patient movement between care centers without data sharing, and the lack of an initial broad quarantine appear to have been important factors in this group of nosocomial infections and, as such, should be avoided to decrease the risk of similar outbreaks in the future.⁴⁷ Unfortunately, these measures may not be possible in some of the more remote regions of the Middle East and Africa since hospitals and medical care centers are scarce and it may be difficult to retain the patient or asymptomatic contacts in one location.

Clusters of cases are often found, indicative of human-to-human spread.²⁰ The time from the onset of MERS symptoms to hospital discharge during the Korean epidemic was shorter for the **second-generation**, than for the **first-generation**, cases (17 vs 26.5 days, respectively). The fatality rates were similar, however.⁴⁷ Nevertheless, a 2016 report detected only a low percentage of confirmed MERS cases among patient household or hospital contacts, indicating that at least at that time, human-to-human contact was not an effective means of transmission.⁴⁸ Nevertheless, many outbreaks in the Middle East, primarily in Saudi Arabia, have been nosocomial in origin and not due to changes in the virus itself.^{49–52} Some of the factors underlying transmission within a single hospital include the following: failure to rapidly isolate MERS patients due to large numbers of asymptomatic or mild cases; poor infection control practices; overcrowded facilities; and the use of aerosol-generating procedures.

3.3 The disease

3.3.1 Introduction to Middle Eastern respiratory syndrome in humans

MERS-CoV in humans typically has an incubation period of 2 to 14 days, similar to that of SARS-CoV. The median time between the onset of symptomatic illness to diagnosis is 7 days, with approximately 95% of patients showing symptoms within 13 days.²⁰ An unknown

number of people are asymptomatic or develop the mild disease. Those who are asymptomatic are still able to shed viruses and infect other people, sometimes resulting in severe disease. For an extreme example, one HCW released the virus for 6 weeks without any signs of disease. During the progression of an epidemic, including SARS, MERS, and COVID-19, the apparent percentage of asymptomatic people and those with mild symptoms generally increases. The increase in the proportion of asymptomatic infection leads to a corresponding decrease in the proportion of people with severe disease and this case fatality rate. This is due, at least in part, to increased surveillance and contact testing. Another potential reason for the increased number of people with minimal to no symptoms may result from mutations in the viruses that make them less pathogenic and from increased numbers of people who developed “**natural immunity**” during a prior infection.

In the initial stages of MERS, people may develop generalized symptoms, such as fever, fatigue, headache, runny nose, sore throat, and wheezing.⁴⁶ MERS-CoV appears to have broader tissue tropism than SARS-CoV and causes pathology in multiple tissues and organ types.⁵³ Later symptoms of MERS include fever with chills or **rigors** in 87%, cough in 83%, **dyspnea** (shortness of breath) in 72%, and **myalgia** (muscle ache) in 32%. Several days after the initial symptoms, patients’ health rapidly declines, characterized by abnormalities in the urinary, respiratory, and cardiovascular systems, such as **tachypnea** and **tachycardia** (rapid breathing and heart rate, respectively), **hypotension** (low blood pressure), **pericarditis** (inflammation of the tissue surrounding the heart), and heart arrhythmias.⁵⁴ The digestive system is impacted in about 25% of hospitalized patients.³⁷ The symptoms include diarrhea in 26% of those with MERS, vomiting in 21%, and abdominal pain in 17%. The liver may also be dysfunctional.⁵⁵

Blood abnormalities include elevated serum levels of lactate dehydrogenase in 49% and aspartate aminotransferase in 15% of MERS cases.³⁷ Increased levels of serum transaminases, potassium, creatine kinase, troponin, C-reactive protein, and procalcitonin may be present along with decreased levels of sodium.⁵⁶ Levels of serum albumin are low and can serve as a predictor of severe pneumonia.²⁰ Other findings during MERS include **thrombocytopenia** (decreased platelet levels) in 36% of the cases and **lymphopenia** (decreased lymphocyte levels) in 34%³⁷ as well as anemia and decreased numbers of eosinophils.⁴⁶

3.3.2 The mortality rate of Middle Eastern respiratory syndrome

Symptomatic MERS is associated with a high mortality rate. Obobo⁵⁰ reported a fatality rate of 36.5% among laboratory-confirmed MERS cases in an outbreak in Jeddah, Saudi Arabia. The median age of patients was 45 years and 68.2% were male. Approximately 25.1% of those infected were asymptomatic. Of the symptomatic patients, 20.9% of those displaying symptoms were healthcare personnel. Among the remaining symptomatic patients, 97.3% had contact with a healthcare facility or someone with a confirmed MERS-CoV infection or severe respiratory illness within the past two weeks. Many cases in this outbreak were linked to people using renal dialysis or their contacts.⁵⁰

The mortality rate in one healthcare center in Saudi Arabia in 2014 was 60%.²⁰ It should be noted that in this center, only one HCW was infected and no patient-to-patient transmission occurred. This reduction in nosocomial infection may be due to more rigorous

precautionary activities, including the placement of MERS cases in single rooms, a dedicated 1:1 nurse to patient ratio, and greater compliance with hand washing and isolation procedures.²⁰ The high case fatality rate in this center is similar to reports of hospitalized people in other facilities (58%–65%).^{45,57}

3.3.3 Middle Eastern respiratory syndrome and the respiratory system

While some people develop only mild URT symptoms, such as a runny nose and sore throat, approximately 90%–100% of MERS patients typically develop diffuse bronchopneumonia or other severe, acute lower respiratory tract (LRT) disease which requires intensive care.^{20,45} Viral RNA has been found in 93% of LRT samples collected within 3 weeks after diagnosis. This virus is also present in the URT, but at much lower levels.⁵⁸ MERS-CoV replicates in ex vivo human bronchial and lung organ cultures and causes extensive **apoptosis** in these cells.⁵⁹ Infected cells types include nonciliated bronchial **epithelium**, bronchiolar and alveolar epithelial cells, type I and type II **pneumocytes**, and **endothelial cells**, while SARS-CoV replicates primarily in the lung, but not bronchial, tissue.⁵⁹

Patients with the severe disease generally begin with a clinically mild form of pneumonia for the first week, followed by severe disease manifestations.⁶⁰ Radiographic abnormalities are almost universal among severe cases and typically progress from a mild unilateral focal lesion to wide-spread multifocal lesions or bilateral involvement of the lungs, especially the lower lobes.^{37,56} MERS-CoV can also cause **acute respiratory distress syndrome (ARDS)** and multiorgan failure.^{6,53,56}

In one study, six experimentally infected common marmosets (*Callithrix jacchus*) developed ARDS, with one death due to severe illness.⁵³ In this nonhuman primate model, multinucleated cells (**syncytia**), composed primarily of macrophages and some epithelial cells, were found in infected rhesus macaques and marmosets with bronchointerstitial pneumonia.⁶¹ In addition to early infiltration of the lungs by neutrophils, lymphocyte and macrophage infiltration is seen in the later stages of inflammation. The number of infiltrating neutrophils, B lymphocytes, and macrophages is higher in marmosets than in macaques. Experimentally infected dromedaries also have high numbers of MERS-CoV-infected cells, T cells, and macrophages within the **nasal turbinates** and trachea on day four postinfection.⁶² By day 6 postinfection in macaques, the amount of the lung infiltrated by B cells and macrophages decreases together with a reduction in pulmonary viral load that is likely due to viral clearance from the lung and decreasing inflammation. By contrast, in the marmosets, the pulmonary neutrophil influx continues along with increasing acute lung damage.⁶¹

Experimentally infected dromedaries also develop the disease in both the URT and LRT. The virus is present in both locations, indicating that MERS-CoV can survive at normal body temperature in the lungs as well as at the lower temperatures found in the nasal cavity and throat. While severe disease occurs from LRT infection, the presence of MERS-CoV in the URT may increase the risk of transferring the virus to other animals or people. While MERS-CoV is detectable for about 1 month in human LRT samples, during that time, swabs of the mouth/nasal cavity are negative. Testing only these two areas may therefore fail to detect infected people or animals.

3.3.4 Middle Eastern respiratory syndrome and the kidneys

MERS-CoV has a renal **tropism** and its genomic RNA is present in urine.⁶³ MERS is accompanied by a high rate of renal failure, beginning approximately 11 days after the onset of symptoms.⁵³ Most of these patients require **renal replacement therapy**, which involves either renal dialysis or a kidney transplant.⁶³ By contrast, only 6.7% of patients developed acute renal impairment during SARS and 5% required renal replacement therapy.⁵⁶ MERS-CoV-infected **primary cell cultures** derived from human kidneys, but not from human bronchial epithelial cells, have a cytopathogenic infection. Such infection is not seen in SARS-CoV-infected cells. Additionally, kidney epithelial cells produce nearly 1000-fold more infectious MERS-CoV than bronchial epithelial cells.⁵⁶

MERS-CoV infects and causes apoptosis in kidney cells in organ culture *ex vivo* and in common marmosets *in vivo*. One study detected viral RNA and **antigen** in 67% of the common marmosets' kidneys. Infected kidneys had symptoms associated with **acute kidney injury (AKI)** including mitochondrial shortening and fragmentation.⁵³

Expression of some renal genes is affected during MERS-CoV, but not SARS-CoV. I. Infection with MERS, but not SARS, is associated with a high rate of renal failure.^{53,64,65} Expression of the **Smad7** and **fibroblast growth factor 2 (FGF2)** genes are upregulated in MERS-CoV-infected kidney and lung cells. Over-expression of Smad7 or FGF2 or the immunoregulatory cytokine **transforming growth factor- β (TGF- β)** induces apoptosis in the kidney cells.⁵³ Smad7 mediates apoptosis through the inhibition of the cell survival factor **NF- κ B**, whereas FGF2 and TGF- β induce apoptosis by activating one of the **mitogen-activated protein kinase** pathways.

3.3.5 Middle Eastern respiratory syndrome and the cardiovascular system

Circulatory system disorders, particularly malfunctioning of the coagulation system, are a major consequence of pathogenic coronavirus diseases in humans, including MERS, SARS, and COVID-19.^{66,67} Platelets are activated by the recognition of microbes via **pathogen pattern recognition receptors** and work together with leukocytes to clear the viruses.⁶⁶ Platelets are depleted due to an excessive coagulation response that occurs in about one-third of the patients, especially during the first week of infection.^{45,46} The extent of thrombocytopenia during this stage of the infection does not differ in patients with mild or severe disease.⁶⁸ **Disseminated intravascular coagulation (DIC)**, however, is a major complication during fatal MERS-CoV infections.^{66,67}

Microthrombi are present in the pulmonary vasculature by day 4 postinfection. **Parenchymal consolidation**, **alveolar edema**, and cellular infiltration into the lungs are also seen during MERS. Microthrombi are small blood clots found in the lung vasculature. During parenchymal consolidation, areas of lung tissue are filled with liquid instead of air. This is due to inflammatory cell **exudate** in the alveoli and the associated air ducts.⁶⁹ DIC is a major finding in fatal MERS cases.⁵⁵ Respiratory coronaviruses often cause the formation of fibrin clots within the alveoli of the lungs or systemically in humans and some animals.⁶⁶ The clotting may be due to a prothrombotic response, which aids in the prevention of hemorrhaging in the alveoli, but can also result in pathogenic clotting that worsens the course of the disease and decreases survival.⁶⁶

Other cardiovascular conditions that have been linked to MERS include **hyperkalemia** (high levels of blood calcium) and **pericardial effusion** (excessive fluid in the sac-like structure surrounding the heart).⁷⁰ Hyperkalemia may decrease both muscular and nervous system activity and may be fatal. During pericardial effusion, if the fluid levels become too high, the pericardium puts pressure on the heart and prevents its chambers from being able to fill, decreasing the flow of oxygen to the tissues which is potentially fatal.

3.3.6 Middle Eastern respiratory syndrome and the nervous system

All human coronaviruses have been known to cause neurological diseases involving the central or peripheral nervous systems. The type and extent of disease differ according to the virus and the presence and location of its cellular receptor.⁷¹ Approximately 20% of MERS patients develop some type of nervous system disorder,⁷² even though MERS-CoV has not been isolated from neural tissues in humans. One study reported that headache was present in 13%, confusion in 25.7%, and seizures in 8.6% of hospitalized MERS patients in Saudi Arabia.²⁰ Some MERS patients have strokes that may be due to MERS-CoV-associated coagulopathies (pathogenic blood clotting).⁶⁶ **Intracerebral hemorrhage** has also been reported due to DIC and platelet depletion.⁷³

Other serious nervous system diseases have also been reported in patients with MERS, including **acute disseminated encephalomyelitis**, **critical illness polyneuropathy**, and **Bickerstaff's encephalitis** overlapping with Guillain-Barré syndrome.^{57,72,73} Bickerstaff's encephalitis is characterized by weakness of the eye muscles, **ataxia** (uncoordinated voluntary muscle movements), and decreased consciousness. Guillain-Barré syndrome is an autoimmune system disease that targets the nerves of the peripheral nervous system. Its symptoms include weakness and tingling in the arms and legs and an inability to walk. It may lead to paralysis. Some patients have also developed **acute sensory neuropathy** that may be due to their treatment regimen. Often the neurological disorders appear 2–3 weeks after the respiratory symptoms.⁷²

In a mouse model of CNS infection, **transgenic** mice were produced that expressed high levels of human DPP4 in the brain.⁶⁹ In these mice, **intranasal** infection with MERS-CoV results in early neuronal damage that is most significant in regions unrelated to **olfaction** (the sense of smell). The animals do, however, develop **perivascular inflammatory cell infiltrates**.⁶⁹ When similar studies are performed in transgenic mice bearing ACE2, intranasal infection with SARS-CoV yields very different results.⁷¹ In these transgenic mice, SARS-CoV travels up the **olfactory bulbs** into the brain. This is followed by rapid dissemination into brain regions with first- and second-order connections to the olfactory system. Infection of neurons leads to their loss.^{74,75} In this system, the expression of ACE2 is very low in the brain but results in extensive infection of neurons without developing inflammatory cell infiltration.⁷⁴

3.3.7 Risk factors for Middle Eastern respiratory syndrome in humans

3.3.7.1 Comorbidities as risk factors

Severe to fatal MERS is often found in people with comorbidities, including diabetes; hypertension; chronic kidney, heart, or lung disease, including asthma; obesity; smoking;

use of corticosteroids or other immunosuppressants; cancer; or recent surgery.⁵⁷ A study conducted in Saudi Arabia in 2012–13 found that 96% of the patients had underlying medical disorders: 68% had diabetes, 34% had **hypertension**, 28% had chronic cardiac disease, and 49% had chronic kidney disease.³⁷ MERS patients with comorbidities develop respiratory failure much more rapidly than patients with SARS or COVID-19. MERS patients also have a higher fatality rate than that seen during SARS or COVID-19.⁷⁶

During the South Korean epidemic in 2015, patients with comorbidities had a 64% fatality rate, while the fatality rate was 14% in patients with no other health concerns.²² In the South Korean epidemic, all patients with fatal disease had comorbidities, including at least one of the following: diabetes, **chronic obstructive pulmonary disease**, tuberculosis, **cirrhosis** of the liver, and asthma.⁷⁷ During MERS, people with comorbidities develop respiratory failure more rapidly and have higher case fatality rates than those with no other underlying diseases. Additionally, levels of patients with comorbidities are higher in patients with MERS than in patients with SARS-CoV or SARS-CoV-2 infection.⁷⁶

Even though MERS is typically linked to older adults or the elderly, life-threatening disease is also found in infected children, especially in those with underlying disease conditions, including **cystic fibrosis**, **Down's syndrome**, **congenital heart disease**, or **hypothyroidism**.⁷⁸

3.3.7.2 Coinfection with other microbes as risk factors

Concurrent infection with bacteria is common in patients with MERS and includes **bacteremia** (bacteria in the blood), bacterial pneumonia, urinary tract infection, skin and soft tissue infection, and a rare case of *Clostridium difficile* infection.²⁰ Multidrug-resistant bacteria are found in approximately one-third of the patients. Coinfection with fungi also occurs, such as candidemia (*Candida* yeast in the blood).²⁰ Concurrent infection with other viruses also occurs, including infection with HIV, influenza A virus, parainfluenza virus, herpes simplex virus, and pneumococcus.

3.3.7.3 Biological sex and occupation as risk factors

Of the initial 7000 cases of MERS, 63.5% were in males.⁵⁶ A study performed in Saudi Arabia found that males are infected at a much higher rate than females (77% males).³⁷ Interestingly, during the South Korean epidemic, males had an increased risk for a fatal disease, but this difference was almost, but not quite, significant.²² The difference in these two countries might be because infection in Saudi Arabia may occur either by zoonotic or person-to-person transmission, while no zoonotic transmission occurred in South Korea.

Person-to-person infection is less common with MERS-CoV than it is with SARS-CoV and appears to primarily result from close contact with infected people, especially during nosocomial transmission.²⁹ People over the age of 65 years have an increased mortality rate.²⁰ Older men appear to be at greater risk of **primary infection** than do women since men have much greater contact with camels. The male: female ratio in secondary cases is more balanced, as is also seen among HCWs in Saudi Arabia and the United Arab Emirates.⁴ This suggests that the great prevalence of primary infection among men than women results from differential exposure to dromedaries rather than sex-related differences in susceptibility. In support of this contention, camel rearing and racing is an exclusively male activity popular among middle-aged and retired men.⁴

Antibody-based assays found that in Saudi Arabia, 50% of the people who work with dromedaries had previously been infected with MERS-CoV, while none of the people without camel contact had signs of past or present infection. Many of those found to have anti-MERS-CoV antibodies had not developed a significant respiratory infection. The camel workers included herders, truck drivers, and handlers.¹⁴

3.3.7.4 The risk of Middle Eastern respiratory syndrome-coronavirus transmission to household contacts

The risk of transmission to household members is approximately 4%⁴⁹ and may occur from contact with asymptomatic household members.²⁹ Risk factors for acquiring MERS from an infected household member include sleeping in the patient's room and handling respiratory secretions or other bodily wastes from an infected individual.⁷⁹ A 2014 study of one extended family in Saudi Arabia found that 24% of the family members tested positive for MERS-CoV, 13.9% were hospitalized, and 0.3% died. The infected adults in this family were prone to be older, male, and to have underlying medical conditions.⁷⁹ Casual contact does not appear to be a risk factor, including eating from the same plate, sharing a cup, hugging, kissing, and shaking hands.⁷⁹

Other studies of disease in family clusters or household contacts reported that MERS occurred in <1% to 19% of the people.^{49,79–81} Interestingly, a 2013 study of a family cluster in Saudi Arabia revealed that the two family members who became infected with MERS-CoV had had less direct contact with the index case than three other uninfected family members, including the wife of the index case, none of whom used specific infection prevention precautions.⁸¹

In two clusters of infection in eastern Saudi Arabia between April 1 and May 23, 2013, human-to-human transmissions were responsible for 91.3% of the MERS-CoV infections, which resulted in 15 deaths.⁴⁵ Viral transmission, however, occurred in only 2.3% of the household contacts.⁴⁵

3.3.7.5 The risk of nosocomial transmission

A 2021 report found that nosocomial transmission was a major source of MERS-CoV and SARS-CoV infection, while SARS-CoV-2 relies predominantly on community transmission.⁷⁶ Early after the emergence of MERS-CoV into human populations, many of those infected were HCWs. Reports of the prevalence of MERS in HCWs differ. This may be due to several reasons, including the viral strain, length of time and degree of HCW interaction with the patient, whether the facility was overcrowded, and adherence to infection control practices. Among these potential reasons, the viral strain varies in different geographical regions and over time.

In a small outbreak in Jordan in April 2012, 76.9% of those infected were HCWs.⁸² Disease prevalence among the workers differed among hospital units: 11.7% of those working in intensive care units and 4.1% of those working in emergency departments became infected. Radiographers had an infection rate of 29.4%, followed by 9.4% of nurses, 3.2% of respiratory therapists, and 2.4% of physicians.^{29,83} The infection rate in these HCWs was decreased by consistent use of medical or N95 masks. A study conducted in eastern Saudi Arabia in the spring of 2013 found that nosocomial transmission occurred most frequently in the hemodialysis, intensive care, or in-patient units in three healthcare facilities and the viruses were part of a single, monophyletic **clade**.⁴⁵ Another study of

MERS-CoV-infected people from Jeddah, Saudi Arabia that was conducted in early 2014 found that 87.5% of those infected had exposure to a healthcare facility. The HCWs comprised almost 21% of the symptomatic and 64% of the asymptomatic people.⁵⁰ MERS cases in Saudi Arabia suddenly increased in March–April 2014: many of these cases also resulted from nosocomial transmission.^{50,79,84}

Nosocomial transmission is greater in healthcare settings that use central air conditioning and have overcrowded emergency departments. Nosocomial transmission is also linked to dialysis and aerosol-generating equipment and procedures, including continuous positive airway pressure devices, nebulizers, cardiopulmonary resuscitation, and intubation.^{29,45} A retrospective study in Jordanian hospital cases found that poor compliance with infection control measures and the lack of isolation rooms serve as risk factors for nosocomial outbreaks as well. HCWs can also transmit MERS-CoV to other people, including their other patients, via hand and surface contamination, by contact with bed sheets, bed rails, and intravenous fluid hangers.^{81,85} Methods of decontamination are described later in this chapter.

3.4 The causative virus

3.4.1 Middle Eastern respiratory syndrome-coronavirus classification

MERS-CoV is a member of the subgenus *Merbecovirus*, a betacoronavirus of lineage C.⁸⁶ Its closest known relatives are the bat coronaviruses *Tylonycteris* bat virus HKU4 from lesser bamboo bats (*Tylonycteris pachypus*), *Pipistrellus* bat virus HKU5 from Japanese pipistrelles (*Pipistrellus abramus*), and *Neoromicia zuluensis* bat virus PML/2011 from Zulu pipistrelles.^{86–89}

MERS-CoV has been divided into three clades.⁹⁰ Clade A contains four strains - two human isolates and two from camels, all from the Middle East. Clade B has been divided into four lineages. Lineage B1 contains human and camel strains from the United Arab Emirates, while lineage B3 contains most of the Saudi Arabian strains as well as strains from Korea and China. Clade C contains isolates from Africa, including Ethiopia, Burkina Faso, Egypt, Morocco, and Nigeria.⁹⁰

MERS-CoV or a closely related virus may have been present in African dromedaries for 35 years.³⁴ All African MERS-CoV strains are believed to have been descended from common ancestors in eastern Africa, however, MERS-CoV isolates from western and northern Africa differ from those currently present in eastern Africa. The western and northern African isolates are members of a distinct group of clade C1 viruses that contains deletions in the **open reading frame 4b**. These deletions may alter virus replication or virulence.^{91,92} Sudanese dromedaries (in east Africa) are infected with both clade C1 and C3 MERS-CoV strains, but no evidence of recombination between these clades has been reported.⁹³ Clade C viruses from the east (Egypt, Kenya, and Ethiopia), north (Morocco), and west (Nigeria and Burkina Faso) Africa have lower replication competence than clade A and B viruses in ex vivo cultures of human lung cells. This may at least partially account for the absence of MERS in humans in Africa, despite their production of anti-MERS-CoV antibodies. It should be noted that the prevalence of MERS-CoV in African dromedaries is similar to that found in the Arabian Peninsula, so the reason for the absence of human disease in Africa is not due to the lack of MERS-CoV in the camels.⁹⁴

In addition to MERS-CoV (betacoronavirus, lineage C), several other potentially pathogenic coronaviruses cocirculated in Saudi Arabia in 2014–15—camel β 1-HKU23-CoV (betacoronavirus, lineage A) and camelid α -CoV (an alphacoronavirus related to HCoV-229E of humans). Co-infection with MERS-CoV and camelid α -CoV is highest in juvenile camels, followed by calves less than 6 months of age. Despite frequent coinfection of dromedaries with these two coronaviruses, genetic recombination between them has not been detected.¹¹

In dromedaries, over time, the dominant MERS-CoV clades are replaced by other clades, producing short waves of prevailing individual viral variants,^{11,84} similar to the rise and fall of SARS-CoV-2 variants in humans. A recombinant clade of MERS-CoV, clade NRC, was determined to have emerged in 2014 in Arabian camels.¹¹ It has been the only viral variant found in regional camels ever since.⁹³ It was also found in humans in Saudi Arabia in early 2015 and soon became the dominant strain.¹¹ It, like some of the SARS-CoV-2 variants, was associated with an increased transmission rate, but not with increased virulence.

At least five major phylogenetically stable recombinant groups of MERS-CoV are known that contain both human and camel MERS-CoV sequences. Saudi Arabian camel viruses are present within each of the five groups.¹¹ Group 5 MERS-CoV strains were predominant in Saudi Arabian camels in late 2014. MERS-CoV in humans from the 2015 South Korean outbreak also belonged to group 5. These viruses appear to have arisen from a recombinant virus containing the 5' region of ORF1ab and the 3' region of the S protein gene from group four and the rest of the genome was derived from group three viruses.¹¹

3.4.2 Genetic variation in Middle Eastern respiratory syndrome-coronavirus

Coronaviruses, including MERS-CoV, have high rates of both genetic recombination and mutation that allow them to emerge in new animal host species and alter their virulence.^{89,95,96} During recombination, parts of the viral genomic RNA are exchanged among viruses of different strains or from different host species. EMC-2012 was the first MERS-CoV strain discovered. The high level of mutations in MERS-CoV arises, at least in part, from the large length of coronavirus genomic RNA, as increasing the genome size also increases the risk of error during replication. A study of genetic changes in MERS-CoV from 2012 to 2019 found that the highest mutation rate is in the S protein gene. Other mutations are also found in ORF1a/b, ORF3/4a, and ORF4b.⁹⁰ Evidence suggests that MERS-CoV evolves primarily through recombination of the S protein gene instead of by accumulation of point mutations.⁹⁰ For more information about genetic variation by recombination and mutation in coronaviruses, see Chapter 1, Introduction.

3.4.3 DPP4 and the viral S protein in Middle Eastern respiratory syndrome-coronavirus and Middle Eastern respiratory syndrome-coronavirus-like viruses of humans and animals

3.4.3.1 DPP4 and Middle Eastern respiratory syndrome-coronavirus in humans and dromedary camels

DPP4 normally binds to the human enzyme **adenosine deaminase (ADA)**. ADA's normal functions include helping to activate types of T helper cells that increase the

production of inflammatory molecules. Importantly, when ADA is added to a mixture of human cells and MERS-CoV in vitro, ADA can block the binding of the virus to the cells and thus decrease their rate of infection.⁷ ADA and similar molecules, therefore, have the potential to be used therapeutically to decrease disease severity in infected people.

While the RBD of the S1 portion of the SARS-CoV S protein uses ACE2 as its receptor on mammalian cells, the MERS-CoV S protein instead uses as its primary human host cell receptor DPP4. In humans, DPP4 is found on the surface of cells of the respiratory tract (pneumocytes and pulmonary macrophages), renal proximal tubular cells of the kidneys, small intestine, liver, parotid salivary gland, spleen, testes, prostate, some immune system cells, and macrophages within skeletal muscle. MERS-CoV does not infect most of these organs or cells, however. Nevertheless, because a larger number of cell types carry DPP4 than ACE2, MERS-CoV infects a broader range of cells than SARS-CoV does in vitro.^{7,62} MERS-CoV RNA is also present in 93% of the LRT and 47.6% of the UTR samples, 14.6% of fecal, and 2.4% of urine samples of hospitalized patients.⁵⁸ By comparison, SARS-CoV was detected in 38% of URT, 50% of urine, and very high percentages of fecal samples.^{58,97} During SARS, the virus is found in the intestines and live, infectious virus was isolated at this location.⁹⁸ By contrast, no infectious virus has been isolated from the intestines during MERS.⁵⁸ Accordingly, fecal excretions do not appear to contribute to the nosocomial spread of MERS-CoV. Additionally, MERS-CoV RNA, but not an infectious virus, has also been found in 33% of serum samples.⁵⁸

Only a limited number of the DPP4-positive cells in humans have currently been reported to be infected by MERS-CoV.⁷ Nevertheless, MERS-CoV-induced pathology is found in several organs and tissues outside of the respiratory tract, where it causes vascular disease and cardiac fibrosis, AKI, hepatitis, and **myositis**.^{62,99,100} The location of MERS-CoV-infected cells and pathology differs greatly in humans and dromedaries. In dromedaries, DPP4 is only known to be expressed in epithelial cells of the respiratory tract, including ciliated brush border cells and ciliated epithelial cells of the trachea and bronchioles. Extensive loss of cilia is seen during MERS in dromedaries in the absence of large amounts of cell death. This loss of cilia is accompanied by decreased expression of DPP4 in the infected cells and may be due to these cells downregulating the viral receptor. DPP4 is still expressed on the adjacent, uninfected cells.⁶² In alveoli, DPP4 is present primarily in the endothelial cells, with very small amounts in the alveolar epithelial cells.⁴⁸

3.4.3.2 DPP4 and Middle Eastern respiratory syndrome-coronavirus-like coronaviruses in other animals

The form of DPP4 in humans is very similar to that present in several animal species, including bats, nonhuman primates, dromedary camels, sheep, cattle, and goats.^{101,102} Accordingly, MERS-CoV infects and replicates in vitro in cell lines from humans, nonhuman primates (rhesus macaques and common marmosets), and bats as well as pigs (*Sus scrofa*).^{10,103} Without laboratory manipulation, MERS-CoV does not infect cells from mice, hamsters, and ferrets in vitro and or in vivo. This is problematic since these small animals are commonly used in in vivo respiratory models of disease in humans.^{10,101}

When DPP4 from humans, but not hamsters or ferrets, is expressed in ferret cells, they become susceptible to infection. Five amino acids participate in the binding of DPP4 to the S protein's RBD site in humans. When the hamster RBD was engineered to express these human amino acids, the hamster cells became susceptible to MERS-CoV infection, even

though the viral titer was low. This suggests that other amino acids in the DPP4 receptor may also be needed for optimal MERS-CoV binding and infection.¹⁰ Taken together, these findings indicate that differences in DPP4 play a major role in determining which animal species are permissive or nonpermissive to MERS-CoV infection.¹⁰

MERS-CoV co-localizes with DPP4 in the nasal cavity of pigs, specifically in the medial and frontal turbinate epithelial cells, while colocalization with DPP4 occurs unevenly through the nasal cavity and cervical lymph nodes in llamas.⁴⁰ When llamas (*Lama glama*), American camelids, and pigs are experimentally infected with MERS-CoV, the former two groups have a greater number of infected cells than pigs do.⁴⁰ MERS-CoV causes a sub-clinical, self-resolving infection of the URT of both llamas and pigs that manifests as mild to moderate mucus secretion. MERS-CoV N protein is primarily found in URT sites by day 4 postinfection in both pigs and llamas. As is the case in dromedaries, pigs experience severe loss of cilia on the nasal mucosal cells, followed by a moderate loss in the trachea, and no loss in the bronchial tubes. By contrast, there is little change in the ciliation of respiratory system cells in llamas.⁴⁰ Loss of cilia in the URT has also been described in other viral infections, including SARS-CoV in humans¹⁰⁴ and canine respiratory coronavirus in dogs.¹⁰⁵ Cilia play a major role in preventing the entry of microbes and particulate matter into the lungs by moving respiratory tract mucus and the entrapped material upwards and away from the lower respiratory regions.

3.4.4 Other molecules involved in Middle Eastern respiratory syndrome-coronavirus entry into its target cells

In addition to DPP4, other host molecules play a role in MERS-CoV infection of its host target cells.¹⁰⁶ **Carcinoembryonic antigen-related cell adhesion molecule 5** facilitates MERS-CoV entry into cells in vitro, as is the case with other coronaviruses, including coronaviruses of mice.¹⁰⁷ MERS-CoV also binds **sialic acids** on sialoglycans. Removal of sialic acid from the cell surface blocks entry of MERS-CoV into human epithelial human airway cells in vitro.¹⁰⁸ MERS-CoV S protein preferentially binds to α 2,3-linked rather than to α 2,6-linked sialic acids. This correlates with the preferential localization of α 2,3-linked sialic acids and the preferred sites of viral replication in the URT and LRT of dromedary camels and humans, respectively. This may at least partially explain the differences in virulence of MERS-CoV in camels and humans.¹⁰⁸

The **cellular tetraspanin scaffolding protease** CD9 is required to form a cell entry complex. CD9 normally functions as a cell-signaling and adhesion protein that is expressed in the lungs, but during MERS, it might work together with plasma membrane cholesterol to bring DPP4 into proximity to the **transmembrane protease serine 2 (TMPRSS2)**, increasing both the speed and efficiency of cell entry.¹⁰⁹ Another human coronavirus, HCoV-229E, also form a similar cell entry complex with its receptor, **aminopeptidase N (APN)**.^{109,110} The coronavirus receptors DPP4, APN, ACE2, and CEACAM all can associate with various tetraspanin molecules.¹⁰⁹

Interestingly, while virulent MERS-CoVs use a rapid TMPRSS2-mediated form of cell entry that requires CD9, at least some avirulent MERS-CoV be brought into their target cells via a slower and less efficient “late route” via endosomes that do not utilize CD9.^{109,111} The late route is approximately 90% less efficient than the early, CD9-dependent route.¹⁰⁹ In the

late entry route, following endocytosis, the MERS-CoV S protein is cleaved by **furin** proprotein convertases, **cathepsin L** and/or **cathepsin B**, in the acidic environment of the protease-rich endosome/lysosome vesicle. MERS-CoV utilizing late, but not early TMPRSS2-mediated, entry may also be subject to inactivation by **interferon-induced transmembrane proteins (IFITMs)**.^{112,113} IFITM 1, 2, and 3 inhibit in vitro host cell entry by SARS-CoV, but this inhibition is independent of the accumulation of endosomal cholesterol levels.¹¹⁴

3.5 Animal hosts of Middle Eastern respiratory syndrome-coronavirus

3.5.1 Middle Eastern respiratory syndrome-coronavirus and bats as reservoir hosts

Many studies suggest that the ancestral relatives of the currently existing bat MERS-CoV-like coronaviruses evolved in such a manner as to allow them to infect dromedaries, and then evolved into a form that infects humans. Genetic information from some camel, but not bat, coronaviruses is identical to that of human MERS-CoV. MERS-CoV-like viruses are present in many regions of the world, including areas that have no dromedaries.

In western Africa of ten tested bat species from Ghana, a lineage C betacoronavirus was detected in 25% of Gambian slit-faced bats (*Nycteris gambiensis*).¹¹⁵ It was not found in the other tested bats, including the following five members of the *Hipposideros* genera of roundleaf bats: *H. abae*, *H. gigas*, *H. fuliginosus*, *H. jonesi*, and *H. ruber* (Aba, giant, sooty, Jones, and Noack's roundleaf bats, respectively).¹¹⁵ Interestingly, although MERS-CoV is endemic in some parts of the Arabian Peninsula, the Middle East, and northern Africa, some European *Pipistrellus* bat coronaviruses are closely related to MERS-CoV as well.¹¹⁶ MERS-CoV-like viruses are found in banana pipistrelles (*Neoromicia nana*) and in *Tylonycteris* and *Nycteris* species bats (bamboo and split-faced bat species, respectively) from Ghana and Europe.^{88,89,115} The gene encoding the **RNA-dependent RNA-polymerase (RdRp)** of the MERS-CoV-like virus from *N. gambiensis* differs from that of HKU4 and HKU5 bat coronaviruses by 8.8%–9.6% and MERS-CoV by 7.5%.¹¹⁵

A study of *Pipistrellus* bat species from Europe found a total of 14.7% of the bats tested positive for a MERS-CoV-like virus.¹⁶ MERS-CoV-like viruses were present in 36.6% of Nathusius' pipistrelle (*Pipistrellus nathusii*), 2.4% of the common pipistrelle (*Pipistrellus pipistrellus*), and 6.4% of the soprano pipistrelle (*Pongo pygmaeus*) in the Netherlands in northwestern Europe, and in Romania and Ukraine in southeastern Europe, respectively.¹¹⁵ In both Europe and Ghana, while no significant differences in viral prevalence are found between male and female bats, MERS-CoV-like viruses are present in 45.4% of juvenile bats and 22.4% of adults and in 44.0% of **lactating** and 9.3% of nonlactating female bats.¹¹⁵ It should be noted that MERS-CoV infection has not been found in humans or domestic animals in these regions.¹⁶

The South African bat coronavirus, PML/2011, derived from an adult female Zulu pipistrelle appears to be closely related to MERS-CoV.¹¹⁷ It should be noted, however, that this information was not based on a complete genome sequence, but rather that of a portion of the gene for the highly conserved viral RdRp.⁸⁸ Several bat coronaviruses which have greater similarity to MERS-CoV include HKU4 and HKU5. HKU4 was isolated from lesser bamboo bats (*Tylonycteris pachypus*), while HKU5 was isolated from Japanese

pipistrelles (*Pipistrellus abramus*) in Hong Kong. This pipistrelle is widely distributed, not only in China, but also in Russia, the Korean peninsula, Japan, Vietnam, Burma, India, Saudi Arabia, and neighboring countries in the Middle East. In addition to bat DPP4, the S protein from HKU4 can bind to the DPP4 of dromedaries and humans, however with a lower binding affinity.¹¹⁸

HKU4 replicates efficiently and produces a **cytopathic effect** in some human cell lines in vitro (colorectal adenocarcinoma and hepatocarcinoma cells).¹¹⁸ Interestingly, HKU4 is not able to infect human respiratory tract epithelial cells. HKU4 can, however, infect transgenic mice bearing human DPP4 and cause inflammation in the lungs and brains of the infected animals.¹¹⁸ Since HKU4 can use the human DPP4 receptor to infect human cells, this or another MERS-CoV-like bat virus might have had or gained the potential to directly infect humans. Since this was an in vitro study, it may not reflect conditions in vivo, especially since there is no serological cross-reactivity between MERS-CoV and HKU4.¹¹⁸ Also, lesser bamboo bats are not native to the countries in which MERS-CoV is endemic.

MERS-CoV and HKU4 share amino acid identities of 89.9%, 67.3%–67.4%, and 71.6%–72.1% in the RdRp, S protein, and N protein, respectively.⁸⁹ MERS-CoV and HKU5 share amino acid identities of 92.1%, 64.3%, and 68.8%–69.5% in the RdRp, S protein, and N protein, respectively.⁸⁹ Other *Pipistrellus* bat species, such as the Arabian pipistrelle (*Phrynocephalus arabicus*), the desert pipistrelle (*P. ariel*), Kuhl's pipistrelle (*P. kuhlii*), the common pipistrelle (*P. pipistrellus*), Rüppell's pipistrelle (*P. rueppellii*), and Savi's pipistrelle (*P. savii*) are also found in the Arabian Peninsula and may host other, yet unknown, coronaviruses closely related to MERS-CoV.⁸⁹

A virus from the Cape bat (*Neoromicia capensis*), NeoCoV, is also closely related to MERS-CoV and may be a link between MERS-CoV in dromedaries and MERS-CoV-like viruses in bats. The RNA from human MERS-CoV and bat NeoCoV have 85% overall identity, with the genes for the E, M, and N structural proteins having the greatest sequence identity (89.0%, 94.5%, and 91.7%, respectively). The genes for ORF3 and ORF5 in MERS-CoV and NeoCoV are 76.5% and 88.4% identical, respectively.¹¹⁹ Less than 45% identity, however, is found in the critical S1 domain of the S protein that determines which animal species may host the virus. Because of this large difference in the S proteins of these viruses, the bat coronaviruses may not play a direct role in the development of MERS-CoV.

MERS-CoV replicates in type I and II pneumocytes in the lungs of Jamaican fruit bats (*Artibeus jamaicensis*).¹⁰² Following experimental infection of these bats by the **intranasal** and **intraperitoneal** routes, all bats shed MERS-CoV from their respiratory and intestinal tracts. Since they did not have disease symptoms, they might be able to serve as MERS-CoV reservoir hosts.^{16,102}

Bats may indirectly transmit MERS-CoV to humans. One index case lived and worked in close proximity to an abandoned date palm orchard.¹²⁰ The abandoned wells and ruins contained roosting bats and their feces. Food and water of domestic animals, such as dromedaries, in the vicinity of these palm orchards, may also be contaminated with bat feces, saliva, or urine. Consumption of contaminated food or water may have transmitted MERS-CoV to the dromedaries and, from them, into humans. While MERS-CoV-like viruses are present in at least 14 bat species from insect-eating bat families, none of these viruses is the direct ancestor of the human form of MERS-CoV due to the extent of the differences in their S proteins. [Table 3.1](#) lists the species, diet, and location of bats that carry MERS-CoV-like viruses.

TABLE 3.1 Bat hosts of MERS-CoV-like viruses.

Bat family	Common name	Bat species	Diet	Location
Nycteridae	Gambian slit-faced bat	<i>Nycteris gambiensis</i>	Insectivore	Northwestern Africa
Rhinolophidae	Egyptian fruit bat	<i>Rousettus aegyptiacus</i>	Frugivore	Northern Africa and the Middle East
Vespertilionidae	Great evening bat	<i>Ia io</i>	Insectivore	Eastern and Southeastern Asia
Vespertilionidae	Cape serotine bat	<i>Neoromicia capensis</i>	Insectivore	Sub-Saharan Africa
Vespertilionidae	Zulu serotine	<i>Neoromicia zuluensis</i>	Insectivore	Africa and the Middle East
Vespertilionidae	Banana pipistrelle	<i>Neoromicia nana</i>	Frugivore	Throughout northern Africa
Vespertilionidae	Common pipistrelle	<i>Pipistrellus pipistrellus</i>	Insectivore	Europe, North Africa, Southwestern Asia
Vespertilionidae	Soprano pipistrelle	<i>Pipistrellus pygmaeus</i>	Insectivore	Mediterranean Europe Western Asia Minor, Southern/Central Europe
Vespertilionidae	Japanese pipistrelle	<i>Pipistrellus abramus</i>	Insectivore	Asia
Vespertilionidae	Nathusius' pipistrelle	<i>Pipistrellus nathusii</i>	Insectivore	Throughout Europe
Vespertilionidae	Dusky pipistrelle	<i>Pipistrellus hesperidus</i>	Insectivore	Africa
Vespertilionidae	Lesser bamboo bat	<i>Tylonycteris pachypus</i>	Insectivore	Southeast Asia
Vespertilionidae	Greater bamboo bat	<i>Tylonycteris robustula</i>	Insectivore	Southeast Asia
Vespertilionidae	Particolored bat	<i>Vespertilio superans</i>	Insectivore	East Asia

3.5.2 Middle Eastern respiratory syndrome-coronavirus and dromedary camels

3.5.2.1 Geographical location and prevalence of Middle Eastern respiratory syndrome-coronavirus-infected dromedaries

Dromedary camels, depicted in Fig. 3.1, are present in the hot, desert regions of the Arabian Peninsula, the Middle East, Afghanistan, Central Asia, India, and several separate parts of Africa but their density is highest in and around the Greater Horn of Africa. MERS-CoV is absent from a large part of the dromedaries' range.

MERS-CoV-infected dromedaries are limited to and ubiquitous in camels from several areas of Africa (Tunisia, Morocco, the Canary Islands, Egypt, Ethiopia, Kenya, Sudan, South Sudan, Nigeria, Burkina Faso, and Mali) and in the Middle East and Asia (Saudi Arabia, United Arab Emirates, Qatar, Oman, Jordan, Kuwait, Israel, Iran, Iraq, and Pakistan).^{4,87,121–126} Many of these countries are along or near the coasts of the Mediterranean Sea; the northern Atlantic or Indian Oceans; the Gulf of Arabia, and the Red Sea, while others are land locked. Most countries are desert, while other "tropical" countries contain desert or semiarid regions, such as northern Nigeria and Burkina Faso in western Africa and Kenya in East Africa.



FIGURE 3.1 Sampling the blood of a dromedary camels for MERS. CDC/ Awadh Mohammed Ba Saleh, Yemen.

The seroprevalence of MERS-CoV in dromedaries in most of the afore-mentioned African countries is reported to be greater than 90%, although seroprevalence in dromedaries is 48.5% in Tunisia in extremely northern Africa and 46.9% in Kenya in eastern Africa.^{16,127,128} The situation in Kenya is of interest since MERS-CoV seroprevalence is much greater in the arid northeastern and eastern regions (53.4%–100%) than in the wetter northern Rift Valley region (0%–17.5%).⁸⁷ Curiously, even though MERS-CoV infection is common in camels present in or imported from African countries, camel-to-human transmission has not been reported in any African country.

MERS-COV seroprevalence is high in almost all the countries of the Arabian Peninsula. In 2016, 78% of MERS-CoV-infected dromedaries were found in Saudi Arabia, the United Arab Emirates, and Yemen.⁴ Studies from 2013 found that the MERS-CoV prevalence rate among dromedaries was 72% in Saudi Arabia, 96% in the United Arab Emirates, and 100% in Oman.^{122,129} Despite the large geographical range of MERS-CoV-infected dromedaries, zoonotic transmission appears to only occur in the Arabian Peninsula, even though most of the world's dromedaries are located in Africa.^{91,130} African MERS-CoV lineages are also not known to infect camels in Saudi Arabia, suggesting that Arabian viral strains might be able to maintain endemic circulation.⁹³

Dromedaries may have originally been infected with a MERS-CoV-like virus from bats while in Africa and then passed a slightly different form of the virus to humans in the Middle East. It should be noted, however, that the critical gene which encodes the viral S protein in bat MERS-CoV-like viruses is only 60%–70% identical to that of the corresponding region of dromedary and human MERS-CoV. Genetic studies of other animals residing in Africa are needed to determine whether they are also infected with MERS-CoV-like viruses that have greater similarity to the S protein gene in human MERS-CoV or bat MERS-CoV-like viruses.

Most camels in the Arabian Peninsula are imported from the Greater Horn of Africa (Ethiopia, Sudan, South Sudan, Somalia, and Kenya) where several *Neoromicia* bat species are found. This is important since bats have only limited contact with humans in the Arabian Peninsula. MERS-CoV strains from African and Arabian camels have been reported to be genetically distinct, with Arabian viruses possessing gene deletions in regions of the genomic RNA that may alter virus replication or virulence. Other studies, however, did not see a significant difference in viral strains from Africa and the Middle East.^{131,132}

The prevalence of MERS-CoV infection in indigenous dromedaries from Jeddah, Saudi Arabia was compared to that of imported dromedaries from Sudan and Djibouti from 2016 to 2018. Most of the imported African dromedaries enter Saudi Arabia through the Jeddah Islamic Seaport. In this study, imported animals tested before leaving the ships had higher seroprevalence compared to resident herds (93.8% and 87.6%, respectively).¹³³ The prevalence of MERS-CoV RNA in these countries was 13.3% and 35.5% in imported and native camels, respectively.

In the Arabian Peninsula, many imported dromedaries are used for their meat or milk and are sent to fattening farms.¹³³ The imported African camels come into close contact with Arabian camels in such farms as well as in holding yards and wholesale markets.^{11,134} This mixing of dromedaries from different locales could amplify camel-to-camel infection and increase genetic recombination among MERS-CoV lineages.¹³³ Camels from Burkina Faso, Ethiopia, and Morocco that are used primarily for travel have lower viral prevalence than those that are used for food or drink.¹³⁴

3.5.2.2 Bodily location and shedding of Middle Eastern respiratory syndrome-coronavirus by dromedary camels

Adult dromedary camels experimentally infected with MERS-CoV develop a transient infection that is predominantly located in the URT but is also present in the LRT and draining lymph nodes.¹³⁵ The location of MERS-CoV in the URT may at least partially explain the lack of systemic illness in experimentally or naturally infected camels as well as the means of camel-to-camel and camel-to-human transmission. Infected dromedaries shed large quantities of virus in their nasal secretions. Infectious MERS-CoV is present in nasal secretions for seven days after infection, while viral RNA is present for 35 days.¹³⁵ The time during which infectious virus is present and shed by the respiratory route is limited and is not found 28–42 days postinfection.⁴

One study of experimentally infected adult dromedaries reported that infectious virus is not detectable in organs other than the lungs, including the camels' digestive tract, liver, spleen, kidney, urinary bladder, or heart.¹³⁵ Accordingly, neither infectious viruses nor

viral RNA was found to be present in feces, urine, serum, or whole blood of these dromedaries.¹³⁵ Several other studies of naturally infected dromedary calves did detect the virus in their feces.^{11,15,136} The difference between these findings may be due to the camels' ages or to experimental versus natural infection.

3.5.2.3 Middle Eastern respiratory syndrome and dromedary calves and juveniles

In the 2013–14 season, dromedary infections in Saudi Arabia were seasonal, with viral RNA detectable in the cooler months and decreasing in warm weather since cooler temperatures allow these coronaviruses to survive longer outside of the host. Calves are first weaned from their mothers (cows) at the beginning of the hot season. Many of these calves develop diarrhea at that time. The peak of MERS infection in humans coincides with the weaning of camel calves. A 2014 study detected MERS-CoV RNA in the milk of 71.4% of infected dromedary camel cows.¹³⁷ The infected calves excrete MERS-CoV in their feces so diarrhea outbreaks in calves may contaminate milk, by direct contact with the infected feces or indirectly by the hands of the camel milkers. After the spring weaning period, the dromedary cows are milked for human consumption.⁴ Milking is usually performed manually and, if the teats are not properly cleaned, fecal material from the calves may enter the milk.

Very young dromedary calves retain their mothers' antibodies for about 4–8 months after birth and these antibodies protect them from infection by MERS-CoV. Afterward, for the short period of time before they develop their own antibodies, calves under 1 year of age have lower seroprevalence than do juvenile and adult animals,³⁶ leaving them unprotected and, during this time, many become infected with MERS-CoV. A study of dromedaries from Saudi Arabia in 2013 reported that seroprevalence was 95% in animals at least two years old, while it was 55% in juveniles.^{16,36} MERS-CoV RNA was detected in 25% of dromedary nasal swabs, indicating that they were shedding potentially infectious viruses. Interestingly, 71% of the animals that shed virus were juveniles.³⁶ By the time they reach adulthood, almost all dromedaries have been infected with MERS-CoV and have protective antibodies in their blood. The virus is not found in the nasal passages, tonsils, lungs, or mammillary lymph nodes of adults.

3.5.2.4 Risk factors for Middle Eastern respiratory syndrome-coronavirus infection in dromedaries

Several factors affect the MERS-CoV prevalence rate in dromedaries, including the age and sex of the animals, the location from which they originated, and grazing practices. Due to the rapid rate of mutation and genetic recombination, the strain of the virus circulating at any given time is also a factor. Adult camels were reported to have a greater seroprevalence in comparison to animals under the age of two years (86.6% and 57.7%, respectively).¹²⁸ A study of dromedaries in Kenya, however, found that camels older than 3 years of age were 6.9 times more likely to be seropositive than juvenile camels. Other studies of camels from Saudi Arabia also found a higher rate of seroprevalence in older dromedaries.^{36,138,139} By contrast, the younger animals have a higher prevalence rate of viral RNA than do adults.¹²⁸ Following a MERS outbreak in a dromedary herd, the antibody levels rapidly decline and the camels become re-infected in a repeating cycle that results in a high rate of seroprevalence in older animals.^{130,140} Thus MERS-CoV remains in

the herds for extended periods of time, allowing the young camels to be infected and perpetuate the cycle.

One study reported that male camels have a higher antibody and RNA prevalence than female camels, while another study¹³⁰ found no significant difference in seroprevalence rates between the sexes and that viral RNA levels were higher in females. Several other studies found that female camels from Kenya are 2.8 times more likely to have anti-MERS-CoV antibodies than males are.^{140–142} It should be noted that many of the female camels in Kenya are older since they are better able to withstand the harsh climate. The higher rate of seropositivity among female camels in this country, therefore, may be at least partially due to them having a greater lifespan than the male camels.¹⁴⁰

A study performed in Egypt in 2014–16 found that imported camels had a higher rate of seroprevalence than local camels (90% and 61%, respectively) as well as having a higher rate of MERS-CoV RNA detection (21% and 12%, respectively).¹³⁰ This demonstrates that the dromedaries' region of origin also is a factor in the prevalence of MERS-CoV antibodies and RNA.

A 2020 study of camels in Kenya reported that 69.0% of the dromedaries raised in open grazing areas were seropositive for IgG. This is much higher than the 9.9% seropositivity rate in camels raised in ranching systems.¹⁴⁰ Other countries also reported high seropositivity rates in dromedaries raised in open grazing systems in both Africa and the Middle East: 93%–97% in Ethiopia, 87.5% in Somalia, and 81.7% in Sudan, and 79.1%–84.5% in Egypt and 61.8%–71.8% in Israel.¹⁴⁰ Using the open grazing system, dromedaries are moved seasonally in search of water and pasture, forcing camel herds from different geographical regions to interact at the common watering sites during the dry season. This could increase camel-to-camel transmission of MERS-CoV among animals that come from greatly separated locales.¹⁴⁰ This positive association between seropositivity rate and open grazing practice was not found in Burkina Faso, Ethiopia, Morocco, or Saudi Arabia.^{139,140}

3.5.3 Middle Eastern respiratory syndrome and Bactrian camels

Natural infection with MERS-CoV has not been reported in the two-humped Bactrian camels (*Camelus bactrianus*).¹⁴³ Bactrian camels inhabit the colder steppes of Mongolia, Central Asia, Pakistan, and Iran. Examination of herds of Bactrian camels from southern Mongolia, West Inner Mongolia, and Kazakhstan did not reveal the presence of MERS-CoV RNA or anti-MERS-CoV antibodies, indicating the absence of present or past infection.¹³⁴ Bactrian camels can, however, be experimentally infected via the intranasal route, producing a transient, mild illness, primarily in the URT that is typified by mild to moderate nasal secretions that contain large amounts of MERS-CoV.¹⁴⁴ The ability of Bactrian camels to be infected is not surprising, since the DPP4s of Bactrian and dromedary camels are 98.3% identical.

3.5.4 Middle Eastern respiratory syndrome-coronavirus and other camelids

The largest herd of alpacas (*Vicugna pacos*) and llamas, outside of South America, is in the arid Negev region of Israel. MERS-CoV-specific antibodies were present in 29.4%–34.3%

and 31.5%–36.8% of Israeli alpacas and llamas, respectively, between 2015 and 2017.¹²⁶ However, no MERS-CoV genomic RNA was detected in the nasal swabs of the dromedary camels, alpacas, and llamas during that time period, suggesting that no active MERS-CoV circulation was occurring but that it had done so in approximately one-third of the animals previously.¹²⁶ No MERS was reported in the Israeli populace either. Imported alpacas in Qatar also had anti-MERS-CoV antibodies in their blood but no viral RNA in their nasal cavities.¹⁴⁵

Alpacas that are experimentally infected with MERS-CoV develop neutralizing antibodies. While these animals do not have nasal discharge, they can, nevertheless, transmit MERS-CoV to other alpacas housed in the same room.¹⁴⁶ It is thus possible that alpacas may be able to also act as reservoir hosts for MERS-CoV in the Americas. The number of alpacas is estimated at 3 million animals, the vast majority of which live in the high Andean regions of South America. However, they are increasingly being imported by North America, the United Kingdom, and Australia as a source of fleece. If infected, these animals could greatly expand the range of MERS-CoV.

Other camelids are also able to host MERS-CoV. After experimental infection of llamas via the intranasal route, MERS-CoV is primarily found in the nasal respiratory epithelium, including the epithelium lining the llamas' noses.¹⁴⁷ Infectious virus can be isolated from nasal swabs for 7 days,⁴⁰ although viral levels and shedding are 100-fold lower than that seen following experimental infection of the dromedary and Bactrian camel species.¹⁴⁴ Infected llamas also develop an effective immune response to MERS-CoV. In addition to producing antibodies against the virus, lymphocytes and neutrophils migrate into the nasal tract. In one study, 37.5% of the infected llamas had moderate amounts of nasal mucus secretion 4–18 days after infection,¹⁴⁸ suggesting that the infection was either very mild or absent in these animals. Further work could examine whether the MERS-CoV isolates from the llama's nasal secretions or saliva can infect other animals *in vivo* or human cells *in vitro*.

While MERS-CoV is a betacoronavirus, alphacoronavirus are also present in South American camelids with respiratory or gastrointestinal disease. One such alpaca coronavirus was isolated in California in 2007. This virus is closely related to camelid α -CoV.^{149,150} The California coronavirus is virulent in the alpacas, causing acute respiratory symptoms and a high fever. It may also lead to sudden death, especially in pregnant alpacas.¹⁴⁹ This coronavirus species is distinct from previously reported enteric coronavirus present in alpaca herds.

3.5.5 Middle Eastern respiratory syndrome-coronavirus in other agricultural animals

In addition to camelids, other agricultural animals from Qatar and other countries in the region have been tested for MERS-CoV infection. In the tested region of Qatar, MERS-CoV is hyperendemic in dromedaries due to the presence of an international camel racing track and multiple barns in which the camels are housed. Nevertheless, neither MERS-CoV antibodies nor viral RNA is detectable in the sheep (*Ovis aries*), goats (*Capra aegagrus hircus*), cattle (*Bos taurus*), or horses (*Equus ferus caballus*) in that region or elsewhere in the Arabian Peninsula.^{129,145,146,151,152}

Nevertheless, following experimental infection with MERS-CoV, pigs seroconvert and have low levels of virus replication as evidenced by the presence of viral RNA. MERS-CoV is primarily found in the nasal respiratory epithelium, similar to the findings in dromedary camels and alpacas.¹⁴⁸ Viral RNA is also present in their tracheas and bronchi. The noses of 21.4% of the pigs produced a mild excretion of white material and shed infectious virus until day 4 postinoculation.¹⁴⁸ Experimentally infected young goats also produce anti-MERS-CoV neutralizing antibodies, but neither infected goats nor sheep shed the virus.^{153,154}

Another study tested for natural transmission of MERS-CoV to several agricultural animals following their exposure to infected dromedaries. This study found neutralizing antibodies against MERS-CoV in the blood of sheep from Senegal and Tunisia, as well as from the blood of ~1% of exposed Egyptian goats. MERS-CoV RNA was also found in the noses of sheep and goats from Egypt or Senegal and ~0.5% of cattle and 7.1% of donkeys from Egypt.¹⁴⁷ It would be interesting to discover whether MERS-CoV in the nasal secretions of these animals is infectious and able to be transmitted to other animals in their proximity or to humans.

Surprisingly, several studies found that horses and mules are not susceptible to MERS-CoV infection since they express DPP4 in their respiratory tracts. In the closely related donkeys, however, MERS-CoV-specific antibodies are found in the blood and MERS-CoV RNA is present in nasal swabs.¹⁴⁷

3.5.6 Middle Eastern respiratory syndrome-coronavirus and other animals

In the Hubei Province of China, 9.8% of the tested hedgehogs (*Erinaceus amurensis*) are positive for a MERS-like coronavirus and shed virus in their feces.^{155,156} Hedgehogs are genetically related to members of Chiroptera (bats) and have a lifestyle that is like that of insectivorous bats. The RBD region of the hedgehog coronavirus is highly homologous to that of human MERS-CoV and bat MERS-CoV-like viruses. Interestingly, partial sequencing of the gene for the RdRp of the MERS-CoV-like viruses of hedgehogs from the Hubei and the Guangdong Provinces of China found that they all differ from each other.¹⁵⁶ This suggests that hedgehogs may act as “mixing vessels” for different MERS-CoV viruses and that genetic recombination may occur among them and continuously produce new coronavirus species or variants. They may thus be an important animal reservoir host of MERS-CoV-like coronaviruses and should be monitored for possible cross-species transmission to bats or humans.

3.5.7 Animal models of Middle Eastern respiratory syndrome

Whenever possible, animal models of disease are used to adequately determine drug and vaccine safety and efficacy before entering human clinical trials. Ideally, these animals should mimic some aspect of human disease, be inexpensive, reproduce rapidly, have a short life span, and not be endangered. While dromedaries cause virtually all cases of MERS-CoV spillover to humans, they lack most of the above-listed characteristics. They respond to MERS-CoV infection very differently than humans, are large and expensive to house and feed, reproduce slowly, and have a long lifespan. Dromedaries thus are poor

models of human disease. Primate models of MERS-CoV could be attractive, given their close genetic and physiological relationship with humans. The great apes (chimpanzees, bonobos, and gorillas) might make the best nonhuman primate models, but are larger than most animals used in animal model systems, expensive to house and feed, reproduce slowly, and are endangered species. Other primates, while less closely related to humans, are smaller and thus are less expensive and easier to house and feed. Additionally, some of these primates are not endangered. Rhesus macaque monkeys and common marmosets are two small primate species that differ in MERS-CoV-induced disease manifestations, severity, and immune responses.¹⁰ They can be therefore be used to mimic different degrees of MERS-CoV severity.

The levels of DPP4 expression in macaques and marmosets are similar to that in humans and, following experimental infection by multiple concurrent routes (intranasal, intratracheal, oral, and ocular), both of these primate species display respiratory symptoms.^{61,157} Rhesus macaques develop the mild disease, suggesting their potential usefulness as a model of less severe human infection. By contrast, common marmosets are smaller primates and develop moderate to severe, life-threatening diseases, including bronchointerstitial pneumonia with severe airway lesions, as well as harboring large amounts of virus in the lungs.⁶¹ The clinical signs in marmosets also persist for long periods of time and they develop a strong immune response to MERS-CoV infection in the lungs. This immune response includes increased numbers of infiltrating **neutrophils** that degranulate and release toxic compounds, including reactive oxygen species, that damage the surrounding lung cells. As is the case in humans, the intensity of this immune response in marmosets may increase the severity of lung disease, in part due to the great extent of inflammation. Together, these two primates express the range of symptoms found in infected people and may serve as complementary animal models that cover the range of SARS-induced pathology seen in humans.⁶¹ It should be noted, however, that humans develop acute renal failure or gastrointestinal disease that may be absent in these nonhuman primates.⁶¹ Despite the advantages of using small primates, they still are larger and more expensive to house and feed, reproduce relatively slowly and are long-lived in comparison to rodent models. A smaller animal model is needed to obtain sufficient numbers of animals required for studies of the mechanisms that underlie the MERS pathology as well as to conduct vaccine and drug efficacy studies.

Unlike the case with SARS-CoV, clinical strains of MERS-CoV do not replicate in mice, hamsters, or ferrets due to differences between their DPP4 and that of humans.^{101,157,158} While MERS-CoV can infect rabbits and replicate in the URT, they do not develop severe MERS symptoms. When the human DPP4 receptor is transiently expressed in mice, however, MERS-CoV can replicate in these animals.^{157,159} Chimeric mice have been produced that bear the human DPP4. Repeated passage of MERS-CoV in these genetically altered mice has produced several mouse-adapted strains of the virus, including MERS-MA and MERS-15. Both of these viruses cause severe, life-threatening diseases in the chimeric mice expressing human DPP4.^{111,160} MERS-MA numbers in mouse lungs are more than 100 times higher than that produced by the original human MERS-CoV virus. The mouse-adapted viruses cause fatal lung disease characterized by diffuse alveolar damage with pulmonary edema and infiltration of activated inflammatory macrophages and neutrophils.¹¹¹ These mice model systems may thus prove to be useful during in vivo drug and vaccine trials.¹⁵⁷ One drawback to these systems, however, is that they require both a

mouse-adapted virus and a hybrid mouse host and yield a different response than seen in wild-type MERS-CoV in naturally infected people.

3.6 The immune response

Much of the early information about infectious diseases begins with observational studies on infected and ill patients. This is followed by infection of a variety of cell lines in vitro, in vivo studies in animal models, and finally, human clinical disease or autopsy reports. MERS-CoV may be grown in vitro in human kidney and lung cancer cells, bat and goat kidney and lung cells, and dromedary **umbilical cord stem cells**. MERS-CoV infects dendritic cells (DCs) and macrophages and some immune system organs, such as the spleen and tonsils, in vivo.¹⁶¹ Unlike SARS-CoV, MERS-CoV also infects T lymphocytes.¹⁶¹ Lymphopenia is present during the acute phase of MERS.¹⁶² Gradual increases in lymphocyte numbers are a critical part of an effective immune response against MERS-CoV, while lymphocyte levels rapidly decrease in patients with the fatal disease.^{77,163}

3.6.1 Middle Eastern respiratory syndrome and T lymphocytes

T and B lymphocytes (T and B cells, respectively) and natural killer (NK) cells express variable levels of DPP4 on their cell surface. These levels are upregulated upon the cells' activation.^{70,164} DPP4 is involved in several T cell functions, including their activation and signal transduction.¹⁶¹ T cells may become infected in the lungs or by infected DCs in the draining lymph nodes during the process of **antigen presentation**. CD4⁺ T helper cells require antigen presentation to become activated. These cells produce cytokines and chemokines that coordinate the activity of other immune cells. DCs, monocytes/macrophages, and B cells are the typical antigen-presenting cells.¹⁶¹ By contrast, in llamas and pigs, few leukocytes are infected and those that are infected have the appearance of DCs.⁴⁰ In humans, MERS-CoV-infected macrophages and DCs have a decreased ability to activate CD4⁺ T helper cells. MERS-CoV infection can also induce apoptosis in primary cultures of human T cells ex vivo, which might play a role in the observed lymphopenia in vivo.¹⁶¹ As is true for SARS-CoV and other viruses, CD8⁺ T killer cells, and NK cells are the body's best cells for protection against viral diseases.

Anti-MERS-CoV T cell responses are higher in people with moderate to severe disease than those with mild infection.¹⁶² High levels of anti-MERS-CoV CD8⁺ T killer cells, but not CD4⁺ T helper cells or antibodies, were found in patients during the acute phase of moderate to severe MERS during the 2015 South Korean epidemic.¹⁶² During convalescence, MERS-CoV-specific Th1 and CD8⁺ T killer cells are both present. Th1 cells are a subset of T helper cells that secrete inflammatory and antiviral cytokines that kill microbes but often play a major role in immune-mediated pathology. Th1 cell numbers gradually increase during disease progression in all survivors but rapidly decline during the second week of infection in people with the fatal disease.⁷⁷ CD8⁺ T killer cells are primarily specific for the viral S protein, while CD4⁺ T helper cells recognize the E, M, N, and S structural proteins.¹⁶²

CD4⁺ T helper and CD8⁺ T killer cells are very important in the immune response to other mammalian coronaviruses as well, including coronaviruses found in cats, mice, and

birds (Chapters 5 and 6). The animal coronaviruses target not only the respiratory system of these animals but also cause disease in the digestive system and liver. Because of their key roles in combating viral infection, it is important that MERS-CoV vaccines also trigger appropriate levels of T cell activity. Anti-MERS antibodies only persist for a short time, especially in people who are asymptomatic or have mild illness.¹⁴ T cell responses, however, may be detected for at least a decade.^{165,166}

Th17 cells are another subset of CD4⁺ T helper cells that play a key role in the causation of the “cytokine storm” that is found during severe coronavirus disease.¹⁶⁷ Elevated Th17 activity, which includes production of the cytokine **interleukin (IL)-17**, is present during MERS, SARS, and COVID-19. MERS patients with a greater than normal level of IL-17 together with lower levels of **interferon (IFN)- γ** and **IFN- α** generally fare worse than patients with higher IFN- γ and lower IL-17 levels.¹⁶⁷

3.6.2 Middle Eastern respiratory syndrome, B lymphocytes, and Antibodies

By day 16 after symptom onset, high titers of anti-MERS-CoV neutralizing antibodies are found in the serum.^{2,56} Anti-MERS-CoV-specific IgM and IgG are present and target the S and N proteins. An overview of antibody classes is present in Chapter 1. Levels of MERS-CoV-specific IgG are at least ten times higher than those of IgM.² Early production of antibodies is associated with decreased disease severity and mortality rate, while weak and delayed responses are associated with severe or fatal disease outcome.^{168,169} Antibody responses, however, do not correlate with rapid viral clearance, not even from the lungs.^{58,162,169} During the South Korean epidemic, most patients produced MERS-CoV-specific serum IgG and secretory IgA antibodies in the mucus membranes of the respiratory tract, although one person with a fatal outcome had no detectable IgA.⁷⁷ Thus, while MERS-CoV-specific IgA is detectable in respiratory fluids of most patients,¹⁷⁰ its production appears to be too late to halt viral replication in this site.⁵⁸ Nevertheless, higher and longer-lasting levels of antibodies are present in the blood and respiratory tract of survivors compared to patients with fatal cases of the disease.⁷⁷ All patients who recovered, but approximately half of those with a fatal disease, produced IgG and neutralizing antibodies.⁵⁸ Nevertheless, viral shedding continues despite the presence of an antibody response, indicating that antibodies are only weak protective against lung infection and viral replication during primary MERS-CoV infection.^{58,77}

Antibody levels against MERS-CoV in survivors drop more rapidly than do antibodies against SARS-CoV and the timing of this drop is correlated with disease severity. Short-lived anti-MERS-CoV neutralizing antibodies are present at low levels after mild infection.⁴⁹ In dromedaries, antibody levels rapidly decline in as little as two weeks. These animals are often reinfectd as seen by later increases in anti-MERS-CoV antibody levels.¹³⁰

3.6.3 Middle Eastern respiratory syndrome, dendritic cells, monocytes/macrophages, and neutrophils

The blood of MERS patients with severe or fatal cases has increased numbers of monocytes and neutrophils.⁷⁷ Patients with severe disease have a larger increase in neutrophil

numbers than people with mild disease, beginning during the first week of infection.⁶⁰ Large numbers of DCs and monocytes are present in the respiratory tract. Primary cell cultures of both immature DCs and macrophages can be derived from blood monocytes (MDDCs and MDM, respectively). While immature MDDCs are poor stimulators of T cell immune responses, they are involved in antigen uptake and processing. Immature MDDC and mature MDM can be infected by MERS-CoV, but mature MDDCs are not.¹⁷¹

3.6.4 Middle Eastern respiratory syndrome, cytokines, and chemokines

Activated CD⁺ cells and monocytes/macrophages produce the cytokine **IL-23**. IL-23 induces the proliferation of inflammatory **Th17 cells** and their secretion of **IL-17**. The fatal disease is associated with increased levels of IL-23 and IL-17 in serum and BAL fluid early after symptom onset.⁵⁶ MERS-CoV-infected MDMs and immature MDDCs increase their production of the antiviral cytokines IFN- α 2, IFN- γ , and IL-12p40, and the proinflammatory cytokines **tumor necrosis factor- α (TNF- α)** and **IL-6**.¹⁷¹ Patients with severe disease have much higher serum levels of IL-6 and TNF- α and the proinflammatory chemokines **IL-8**, **CXCL10 (IP-10)**, and **CCL5 (RANTES)** than those with mild disease or moderate disease.⁶⁰ Later, levels of IL-6 and the chemokines CXCL10 and **CCL2 (MCP-1)** decrease as patients transition from the acute to a convalescent phase of MERS.¹⁶² CXCL10 recruits monocytes/macrophages, CD4⁺ T helper and CD8⁺ T killer cells, NK cells, and DCs to the site of infection, while CCL2 recruits monocytes, **memory T cells**, and DCs. MERS-CoV induces greater expression of **IL-12** and IFN- γ and the chemokines IL-8, CXCL10, CCL2, **CCL3 (MIP-1)**, and CCL5 than SARS-CoV does. MERS-CoV also induces greater expression of **major histocompatibility complex class I** molecules and costimulatory molecules for CD8⁺ T killer cells.¹⁷² MERS-CoV induces elevated levels of **Fractalkine** and CCL5 which also recruit T lymphocytes to inflamed tissues.⁷⁷ Lower levels of these two chemokines are found in people with fatal diseases than in patients who recovered. CCL2, CXCL10, and Fractalkine may both increase the inflammatory response and viral clearance in the lungs. The chemokines IL-8, CCL3, and **CXCL1 (GRO)** recruit neutrophils and stimulate their infiltration into infected lung tissues during MERS-CoV-related pneumonia. By contrast, CCL5 and **CCL11 (eotaxin-1)** recruit eosinophils and basophils into the inflamed lungs of MERS patients.⁷⁷

IL-6 and TNF- α , as well as the **T regulatory cell** cytokines, **IL-10** and TGF- β , down-regulate T cell activity and generally correlate with MERS severity and mortality.⁷⁷ Levels of IL-6 and IL-10 are higher in people with severe or fatal diseases than in those with mild illnesses.⁷⁷ IL-6 expression is also increased in ex vivo cultures of infected human MDM and in lung lesions of infected animals in vivo.^{60,171}

Cytokines and chemokines are also expressed in MERS-CoV-infected DCs and monocytes/macrophages.⁷⁷ Levels of the monocyte cytokines **IL-7** and **IL-15** are increased during MERS in animal models.¹⁷³ Under normal conditions, these cytokines play a role in T cell homeostasis. IL-7 is needed for the development of mature T cells in the **thymus** and helps **naïve T cells** and memory T cells to survive. IL-15 also regulates T cell responses, NK cell activation, tissue repair, and inflammation.

Levels of the growth factor **granulocyte colony-stimulating factor (G-CSF)** and **granulocyte-macrophage colony-stimulating factor (GM-CSF)** are elevated during the

second and third week of MERS, especially in fatal cases. Since G-CSF stimulates the production of **granulocytes** (neutrophils, eosinophils, and basophils) in the bone marrow, the observed increase in the numbers of neutrophils may be due to the activity of these **hematopoietic** cytokines.⁷⁷ GM-CSF stimulates the production of granulocytes and monocytes/macrophages. G-CSF levels rapidly increase in MERS survivors during antiviral treatment and relatively low levels of GM-CSF are found in people with fatal disease.⁷⁷

3.6.5 Middle Eastern respiratory syndrome and interferons

IFNs inhibit viral replication and thus play a major role in host defense against viral infections, including infection with MERS-CoV. Among a variety of IFNs (IFN- α 2a, IFN- α 2b, IFN- β , and IFN- γ), IFN- β has the most potent anti-MERS-CoV activity in vitro, being 16-times as active as IFN- α 2b.¹⁷⁴ High-dose treatment with **type I IFN** (IFN- α and IFN- β) and **type III IFN** (IFN- λ) is very effective against SARS-CoV and MERS-CoV in vitro and in animal models in vivo.¹⁷⁵ Mice lacking a functional IFN response develop MERS-associated lethal pneumonia. Levels of proinflammatory IFN- α are higher in the acute than the convalescent stage of the disease.¹⁶² CD4⁺ Th1 cell production of the strongly anti-viral **type II IFN**, IFN- γ , is high during the acute and convalescent stages of moderate MERS, but not in severe disease, while CD8⁺ T killer cell production of this cytokine is high in both the acute and convalescent stages in moderate and severe MERS.^{162,176}

A study of the APCs B cells, monocytes/macrophages, MDDCs, mDCs, and plasmacytoid dendritic cells (pDCs) found that only pDCs produce high amounts of type I and III IFNs in response to MERS-CoV. Additionally, the pDCs produce much higher levels of IFN in MERS than they did during SARS.¹⁷⁷

3.6.6 Middle Eastern respiratory syndrome-coronavirus escape mechanisms

Both SARS-CoV and MERS-CoV utilize escape mechanisms that induce very small amounts of IFN in most cells.¹⁷⁵ However, unlike SARS-CoV, MERS-CoV remains sensitive to the small amount of IFN that is produced.¹⁷⁸ MERS-CoV's nsp4a, nsp4b, and nsp5 act as IFN antagonists. Nsp4a both blocks type I IFN production and activity of the IFN-stimulated response element gene promoter.¹⁷⁹ Deletion of the MERS-CoV gene encoding nsp4a or mutation of the gene encoding the viral **phosphodiesterase** nsp4b results in increased IFN- λ expression.¹⁸⁰ Nsp4b is also an **RNase L** antagonist.¹⁸⁰ RNase L is an anti-viral enzyme used by the innate immune system to degrade both viral and cellular RNA (Thornbrough 2016).¹⁸⁰

Several other MERS-CoV proteins are involved in blocking host immune responses, including nsp1, nsp5, and PL^{Pro}, the latter of which cleaves viral polyproteins.¹⁷⁵ These viral proteins and nsp4a and nsp4b act by preventing the following: (1) the interaction between viral **double-stranded RNA** and a cofactor for the **pattern-recognition receptor**, **RIG-I-like receptor**; (2) the translocation of the transcription factor **interferon response factor (IRF) 3** to the nucleus; and (3) the binding of **TANK-binding kinase** to an **inhibitor of NF- κ B kinase epsilon**, which results in the phosphorylation of IRF3.^{175,180} The M

protein of MERS-CoV and SARS-CoV, but not that of the mildly pathogenic HKU1 coronavirus, also block IRF3 activation.¹⁸¹

Pattern-recognition receptors identify microbes: RIG-I-like receptors identify double-stranded RNA that is produced during MERS-CoV replication. Upon entering the target cell nucleus and binding to the DNA, IRF3 normally induces the expression of type I IFNs and other cytokines. MERS-CoV proteins also inhibit IFN activity by **deubiquitination** (removal of **ubiquitin** from molecules) and stimulating histone modifications that downregulate the expression of **IFN-stimulated genes**.¹⁷⁵ For a more extensive review of MERS escape mechanisms and comparison to those used during SARS, see Kindler.¹⁷⁵

3.7 Diagnosis

Diagnosis of MERS-CoV infection of humans or animals often involves *in vitro* studies which determine the ability of potentially MERS-CoV contaminated blood or other material to be tested to replicate in cells from bats of western Asia and northern Africa. MERS-CoV replication occurs in cells taken from bat embryos, fetal lungs and kidneys, and adult kidneys, but, unexpectedly, not from adult bat lung cells.⁶³ This suggests that if human or dromedary MERS-CoVs did indeed evolve from bat coronaviruses, they may have been transmitted by urine, instead of the respiratory route. These tests have the advantage of determining only infectious viruses, they involved the growth of live viruses and must be performed in special Biosafety laboratories.

Diagnosis of infection with MERS-CoV in patients may instead use genetic or immunological methods of testing for the presence of viral RNA or antigens.⁵⁶ Genetic tests amplify and detect MERS-CoV RNA present in material derived from the URT or LRT using the **reverse transcription-polymerase chain reaction (PCR)**. PCR tests are very sensitive and specific for MERS-CoV. Tests that target the N protein gene may be more sensitive than those targeting other viral genes, including the gene for the S protein.⁵⁶ These tests require specialized equipment and are performed in specialized testing facilities. Emergency use authorization was issued in mid-July 2015 for a commercial PCR to be used to detect the presence of MERS-CoV RNA in tracheal aspirates or respiratory secretions.

Immunological tests are relatively rapid and inexpensive and can be done on-site. They are 100% as specific and 80% as sensitive as the more expensive and time-consuming genetic tests. Paired testing of acute and convalescent serum samples may be useful for confirming the presence of infection and for surveillance of human population exposure and immunity to infection. See Uyeki for an excellent review of diagnostic procedures.¹⁸²

3.8 Treatment

3.8.1 Generalized, physical treatments

Generalized treatment options include supportive treatment with **extracorporeal membrane oxygenation** for those people whose heart and lungs cannot provide sufficient

oxygen and carbon dioxide gas exchange the lungs or adequate blood supply to sustain life.⁵⁶ During this process, blood is pumped to a heart-lung machine that removes carbon dioxide from the blood and returns oxygenated blood to the body. Dialysis or a kidney transplant may also be required for patients with severe kidney disease as described earlier in this chapter.

3.8.2 Introduction to Middle Eastern respiratory syndrome drug treatment options

Several anti-MERS-CoV drug candidates were tested *ex vivo* in primary cultures of leukocytes taken directly from donors' blood or tissues. MERS-CoV infects and productively replicates in several types of primary cells, including human MDMs, MDDCs, airway epithelial cells, and *ex vivo* human lung tissue.¹⁷² The use of primary cells is often far superior to using long-term laboratory cell lines *in vitro* since primary cells more accurately reflect conditions in the human body, but these tests are more expensive and time-consuming than using cell lines. Additionally, working directly with human blood may expose researchers to other human blood pathogens, including HIV, hepatitis C virus, and, in some cases, the malaria parasite. Testing using cultured cell lines, however, may not as accurately represent conditions *in vivo*, perhaps giving false data on drug efficacy and safety.¹⁷¹

Several treatment regimens have been or are being examined for efficacy against viral infection or disease. These include drugs that target replication, including RdRp activity; specific viral proteins, such as the S and helicase proteins; immunomodulators; inhibitors of glycosylation of viral proteins; and inhibitors of host proteases, including DPP4, TMPRSS2, cathepsin L, and furin proteases, in addition to the viral proteases 3CL^{pro} (also known as the main protease) and PL^{pro}.^{52,182} These proteases are necessary for the cleavage of the S protein and the viral polyproteins. While MERS-CoV with uncleaved S protein may use cathepsin L-dependent endocytosis to gain entry to the host cell via endosome vesicles, cleavage by host proteases is required for MERS-CoV protein maturation.¹⁸³ The viral PL^{pro} inhibitors, 6-mercaptopurine and 6-thioguanine, block MERS-CoV protease activity *in vitro*.⁵² Both convalescent plasma from MERS-CoV survivors that contain virus-specific antibodies and laboratory-produced monoclonal antibodies (mAbs) against various coronavirus components have been used to treat MERS.⁵²

Many of the candidate treatment strategies are based upon therapeutic approaches used to combat SARS-CoV or the H1N1 influenza virus. While both SARS-CoV and MERS-CoV are highly pathogenic betacoronaviruses, they differ in several key aspects: including using different host cell receptors (ACE2 and DPP4 for SARS-CoV and MERS-CoV, respectively), and a wider cellular tropism for MERS-CoV. While influenza viruses are single-stranded RNA viruses, their genetic information is negative-sense while that of coronaviruses is positive-sense.⁵² Influenza viruses also mutate at a much higher rate.

3.8.2.1 Nucleoside analogs

Some nucleoside analogs impair virus replication. Most of these, however, are ineffective at inhibiting coronaviruses, at least partially due to the viruses' ExoN **proofreading**

activity, which removes the nucleoside analogs. Ribavirin, a commonly used guanosine analog, has little effect upon MERS-CoV.¹⁵⁷ One cytosine analog, β -D-N4-hydroxycytidine, does inhibit MERS-CoV with minimal cytotoxicity.¹⁸⁴

Remdesivir, a **prodrug** for GS-5734, is an analog of adenosine. It has in vitro activity against not only MERS-CoV, but also against many other coronaviruses, including SARS-CoV and HCoV-NL63 of humans, mouse hepatitis virus, and various bat coronaviruses, including HKU5, HKU3, and WIV1.¹⁸⁵ Remdesivir is also active in vivo and decreases weight loss and viral load in the lungs of SARS-CoV infected mice, suggesting that it may be a **pancoronavirus drug**.¹⁵⁷ After infection with MERS-CoV, in vivo studies of mice receiving one dose of remdesivir produced decreases in MERS-CoV replication by as much as 1000-fold by 48 hours after infection. Additionally, remdesivir increases lung function in MERS-CoV infected mice, while severe lung disease decreases. In infected rhesus macaque monkeys, when remdesivir was administered 24 hours before infection, MERS-CoV did not cause disease or replicate in respiratory system cells. More importantly, when administered postinfection, remdesivir reduces virus replication as well as the number and severity of lung lesions.¹⁸⁶

3.8.2.2 Immunosuppressive drugs

Immunosuppressive drugs are other potential drug candidates since they decrease excessive, pathogenic inflammation. Anti-inflammatory corticosteroids are not successful in treating respiratory illness caused by MERS-CoV and might instead increase viral reproduction in airways due to the suppression of the antiviral adaptive immune response. Corticosteroids also increase viral replication during infection with some other coronaviruses, including SARS-CoV and porcine respiratory coronavirus in pigs.^{187,188}

A different type of immunosuppressive drug, cyclosporine, however, inhibits coronavirus replication in vitro, but its effects in vivo must be determined.¹⁸⁹ Another immunosuppressive compound, mycophenolate mofetil, blocks **inosine monophosphate dehydrogenase** activity that is required for **de novo synthesis** of guanosine nucleotides, especially in T and B lymphocytes.¹⁹⁰ Mycophenolate mofetil is the prodrug of mycophenolic acid, which is used to prevent graft rejection after tissue transplantation.⁵⁶ Depletion of guanosine nucleosides reduces amounts of tetrahydrobiopterin which is used for the production of nitric oxide by the inducible form of nitric oxide synthase as well as lowering the production of **peroxynitrite**, a powerful reactive nitrogen and oxygen species.^{52,190} Decreasing the level of peroxynitrite decreases tissue damage. Extreme caution should be used in the administration of this drug since it increases the risk of death in experimentally infected marmosets.¹⁹¹

3.8.2.3 Interferons

As is the case with SARS-CoV, several types of IFNs may protect a person from MERS-CoV infection. MERS-CoV is 50–100 times more susceptible to inactivation by IFN than is SARS-CoV.¹⁸⁹ IFN- α , alone or together with the broad-spectrum antiviral drug ribavirin or the combination of IFN with lopinavir and ritonavir, appears to be effective in vitro. MERS-CoV is sensitive to either IFN- α or ribavirin alone when used at relatively high concentrations. The combination of IFN- α and ribavirin, however, was similarly effective, but at lower concentrations, decreasing the risk of drug toxicity.¹⁹² The combination of IFN- α

and ribavirin is also effective in vivo in MERS-CoV-infected rhesus macaques and humans.^{193,194} Macaques treated with IFN- α and ribavirin have lower levels of IL-6, IFN- γ , and CCL2 in their lung tissue compared to untreated animals. Serum levels of these proinflammatory cytokines and chemokines, however, were similar in treated and untreated macaques, suggesting that this treatment had a localized effect.¹⁹⁴ Treated animals have less inflammation and neutrophil infiltration into the lungs. It should be noted that disease severity in the rhesus macaques corresponds to mild to moderate MERS in humans.¹⁹⁴ It would be interesting to see if this drug combination is effective in infected common marmosets as well since they model more severe diseases. If these drugs are not effective in marmosets, their safety and efficacy could be tested using high drug doses. Using the current dosages of IFN and ribavirin is not protective in severely ill MERS-CoV patients.¹⁹⁵

IFN- β also decreases MERS-CoV-related pathology in common marmosets.¹⁹¹ Infection of these animals produces a disease similar to the severe, disseminated form of MERS in humans. Administration of IFN- β 1b to MERS-CoV-infected marmosets decreases weight loss and infiltration of leukocytes into the lungs as well as mean viral loads in the lungs and kidneys.

3.8.2.4 Repurposed drugs

Repurposed drugs that were originally developed to treat the noncoronavirus disease have also been examined for their efficacy against MERS. These drugs have the advantage of being FDA-approved, having known toxicity in humans, their availability, and relatively low cost. Members of several major categories of drugs have been tested for anti-MERS activity. These include drugs with antimicrobial activity against viruses, bacteria, and parasites. Drugs that block various critical metabolic functions include inhibitors of neurotransmitters, estrogen, protein processing, kinase signaling, **cytoskeleton** function, lipid or sterol metabolism, **ion channel** activity, apoptosis, and cathepsin activity. Some of these drugs are effective against both MERS-CoV and SARS-CoV. A detailed list of repurposed drugs has been compiled by Dyall.¹⁹³

When used at nontoxic levels, chloroquine (an antimalarial), chlorpromazine (used to treat schizophrenia and bipolar affective disorder), loperamide (an antidiarrheal drug), and lopinavir (developed to treat HIV) safely inhibit replication of MERS-CoV, SARS-CoV, and the mildly pathogenic HCoV-229E in vitro.¹⁹¹ In MERS-CoV infected patients, the combination of lopinavir and ritonavir (antiviral drugs) improves disease outcomes, including decreasing fever, eliminating the virus from serum and sputum, and increasing survival rates.^{68,157} These drugs are also used in combination with IFN, as described earlier.

In addition to chloroquine, several antiparasite drugs have in vitro activity against MERS-CoV. These include niclosamide (which treats tapeworm infection) and nitazoxanide (used to treat diarrhea and enteritis caused by *Cryptosporidium* species and *Giardia lamblia*).¹⁹⁶ Nitazoxanide is also effective against SARS-CoV-2 infection in vitro.¹⁹⁶ It blocks the expression of the N protein and decreases levels of TNF- α , IL-2, IL-4, IL-5, IL-6, IL-8, and IL-10 in peripheral blood mononuclear cells ex vivo and production of IL-6 in mice.¹⁹⁷

It should be noted that while chlorpromazine protects against MERS-CoV infection of primary culture cells ex vivo,¹⁹¹ it is very toxic to these cells, and may be toxic against the corresponding cells in vivo as well. This finding shows that it is very important to test potential drugs in human primary cells, in addition to the established cell lines, before administering these drugs to humans. Drug dosage must also be carefully examined for

safe use against MERS-CoV and other coronaviruses. The antimalarial drug hydroxychloroquine has a well-documented safety record when used to treat malaria, however, may have neuropsychiatric side effects or serious heart disease in some individuals with prior cardiovascular disorders, especially if used at higher doses. Additionally, there is only a narrow range between therapeutic and toxic doses.^{198,199}

3.8.2.5 Passive immunotherapy

During passive immunotherapy, animals or patients are given antibodies against selected MERS proteins. These antibodies should specifically target and kill MERS-CoV. When a MERS-CoV infected mouse was injected with horse serum containing virus-specific neutralizing antibodies, viral reproduction was halted.²⁰⁰ More importantly, this type of antibody-rich serum reduces the amount of virus and speeds up its removal from the lungs of infected mice. Great care must be taken to not use horse serum repeatedly since this may cause **serum sickness**.

Instead of using serum-containing antibodies, patients are usually given laboratory-produced mAbs that recognize the MERS-CoV specific regions of the S or N protein. In animal models, mAbs against the viral S protein protect against MERS-CoV infection, however, they do not provide cross-protection against other similar coronaviruses.²⁰¹ Additionally, due to their strict target specificity, mutations in the S or N protein may allow the virus to escape neutralization by any one mAb, suggesting that a cocktail of multiple mAbs that target different regions of the protein may be more likely to be effective.¹⁵⁷ It should be noted that people receiving antibodies for therapeutic reasons that are administered after infection do not produce their antibodies and are not, therefore, protected against reinfection, unlike the case in vaccinated people who do produce their antibody and T cell responses that either protect them from reinfection or lessen the severity of the subsequent disease.

3.9 Traditional medicinal compounds

Several natural traditional medicinal compounds or extracts have proven to have activity against MERS-CoV and HCoV-229E.²⁰² Silvestrol, derived from Chinese perfume trees (*Aglaia* species), blocks MERS-CoV replication by inhibiting the activity of the host cell's **eukaryotic initiation factor 4A (eIF4A)**, an RNA **helicase** that aids in the formation of viral replication/transcription complexes.²⁰³ Silvestrol is not toxic to the liver, spleen, or leukocytes. Griffithsin is a **lectin** (sugar-binding molecule) derived from red algae (*Griffithsia* species). It binds to sugar moieties on the MERS-CoV S protein and inhibits MERS-CoV attachment to its target cells.^{204,205} Griffithsin is also active against SARS-CoV, HCoV-NL63, and HCoV-OC43.²⁰⁴

Other phytochemicals target one of the viral proteases. The antioxidants Kazinol F and Broussonchalcone A are derived from the paper mulberry (*Broussonetia papyrifera*). They serve as noncompetitive inhibitors of the MERS-CoV PL^{pro} protease.²⁰⁶ Chicoric acid, rosmarinic acid, and myricetin are promising candidates for the treatment of MERS based upon their strong interactions with the catalytic center of 3CL^{pro}, the other major MERS-CoV protease.²⁰⁷ Chicoric acid is found in several plants, including chicory (*Cichorium intybus*), dandelion leaves (*Taraxacum* species), basil (*Ocimum basilicum*), and lemon balm

(*Citrus limon*). Rosmarinic acid is present in rosemary (*Rosmarinus officinalis*), sage (*Salvia officinalis*), mint (*Mentha arvensis*), and basil. Myricetin is found in a large variety of vegetables, fruits, nuts, berries, and grape seeds (*Vitis vinifera*).

3.10 Prevention

3.10.1 Decontamination of environmental surfaces

In a report of nosocomial infection in South Korea during the 2015 epidemic, MERS-CoV RNA was detected on environmental surfaces in hospitals for at least five days after the last detection of viral RNA from the patients.⁸⁵ Viral RNA is present on bed sheets, bedrails, IV fluid hangers, and X-ray devices which are touched by patients and HCWs. If their hands are not sufficiently cleaned after exposure to MERS-CoV-contaminated surfaces, some of the HCWs may then spread the virus to other surfaces or patients and visitors in other parts of the hospital. It is noteworthy that in healthcare facilities, the viable virus may still be shed from clinically fully recovered patients.⁸⁵ This report only studied hospitalized patients who were kept in a small area for days and may not represent the situation in patients with the milder disease who shed smaller amounts of virus and who were not confined to a small area for long periods.

Hospital anterooms help to maintain the **pressure gradient** between isolation areas and the ward's corridor so that air flows into and not out of the isolation area. Anterooms also provide a space for personnel to take on and off their **personal protective equipment** and disinfect nondisposable medical devices.²⁰⁸ In some situations, however, MERS-CoV is present on the anteroom floors and desks, indicating the need for frequent disinfection to prevent spread to other areas of the hospital.⁸⁵

MERS-CoV may still be recovered after being stored for 48 hours at room temperature (20°C or 68°F) with 40% relative humidity and is even more stable at low temperatures/low humidity.³¹ On steel or plastic surfaces, MERS-CoV is viable for 8 hours at 30°C (86.0°F) and 80% relative humidity, and 24 hours at 30% relative humidity. In aerosols, most of the viruses die at 70% relative humidity, while more than 90% remain viable at 40% humidity at room temperature.³¹ Hospital equipment and procedures which generate aerosols, such as nebulizers, suction, high-flow oxygen, and intubation, may also transmit the virus to people. Since rapid and complete disinfection of SARS-CoV on surfaces can be achieved by many commonly used disinfectants, at least some of these disinfectants should also be effective on surfaces contaminated by MERS-CoV, as described in Chapter 2, Severe acute respiratory syndrome (SARS).

Other, physical means of inactivating viruses on external surfaces include exposure to ultraviolet light (UV) which mutates viral RNA nucleosides, proteins, and lipids. An automated whole room UV-C disinfection system can inactivate several coronaviruses on surfaces, including lineage C MERS-CoV and the lineage A murine hepatitis virus. This system decreases MERS-CoV present in droplets to undetectable levels after a 5-minute treatment.²⁰⁹ UV-C light exposure should be accompanied by other forms of disinfection. It should be noted that UV light damages plastics so plastic-containing materials and equipment should not be exposed to this light for long periods.

3.10.2 Vaccination

3.10.2.1 *Anti-Middle Eastern respiratory syndrome-coronavirus vaccines*

Both neutralizing antibodies and T cells are active against severe MERS in humans, especially CD8⁺ T killer cells. An effective vaccine, therefore, needs to stimulate T cells in addition to producing neutralizing antibodies.¹⁶³ Serum levels of MERS-specific antibodies are present for a far shorter amount of time after vaccination than are activated T cells. Sustained levels of T cell activity are important since patients who die do so after a rapid decrease in their T cell numbers.¹⁶³ Most vaccines target the S1 portion of the viral S protein due to its ability to induce specific T cell and neutralizing antibody responses, primarily against the RBD.²¹⁰

The route of vaccination is also important. MERS-CoV enters humans through the mucosal membranes of the respiratory tract by inhalation or digestive tract during consumption of raw dromedary meat, milk, or urine. Effective vaccines may also be given via a mucosal route to induce a mucosal immune response. Accordingly, studies performed in mice show that intranasal immunization (a mucosal route) stimulates the long-term activity of at least one type of antibody in the blood while also stimulating protective responses in the animals' lungs.

In addition to using some of the more conventional dead or live **attenuated viruses** (nonpathogenic forms of viruses) to immunize against infectious disease, other vaccine approaches against MERS-CoV infection include the use of hybrid molecules that are part antibody and part viral S protein, plasmids that contain a MERS-CoV gene, and recombinant viral vector-based vaccines.^{211,212} The latter will be described separately.

One nonconventional vaccine approach uses a hybrid molecule that contains a shortened form of the S protein's RBD and the lower portion of the human IgG antibody (the **Fc region**). This hybrid molecule binds to human DPP4 on the host cell and physically impedes MERS-CoV from binding to its receptor in vitro.²¹³ This vaccine also produces an antibody response in mice that are infected subcutaneously. Intranasal immunization of mice also induces long-term IgG responses against MERS-CoV and greater cellular immune responses, including immune responses in the lungs.²¹⁴

Another type of anti-MERS-CoV vaccine incorporates a coronavirus gene into a **plasmid**. Plasmids are small circular pieces of DNA that act in some ways like independent entities since they can "infect" the target host cell and multiply within it semiautonomously. GLS-5300 is a vaccine that incorporates the MERS-CoV S protein into a plasmid. Transcription of these plasmids and translation produces intracellular viral S proteins. The S protein stimulates robust and specific CD8⁺ T killer cells and neutralizes antibody responses in preclinical trials in mice, camels, and macaques, and protects the immunized animals against infection.^{52,215}

3.10.2.2 *Recombinant viral vectors*

Vaccines using recombinant viral vectors have been developed in which part of the genome of a nonpathogenic virus (the viral vector) is genetically modified by the insertion of a gene encoding one of the MERS-CoV proteins. During translation, the MERS-CoV protein is produced in the patient without exposing him or her to a potentially dangerous live coronavirus. Some of the viruses that have been used as vectors for the recombinant

vaccines include vaccinia virus Ankara (formerly used to prevent smallpox),²¹⁶ adenoviruses,²¹⁷ measles virus,²¹⁸ rabies virus,²¹⁹ and baculovirus,²¹¹ each containing different lengths of the MERS-CoV S protein gene.

3.10.2.3 Vaccination of camelids

Vaccination of humans is a time-tested and generally effective preventative strategy. In the case of MERS-CoV vaccines, however, testing of vaccine candidates for safety and efficacy is challenging due to the low incidence of MERS-CoV in people and the problems associated with animal models that were described earlier in this chapter.²²⁰ The One Health approach considers not only human but also animal health, particularly those animals that may serve as a reservoir or intermediate hosts of pathogens, which in the case of MERS, are bats and dromedary camels.²²¹ While vaccinating bats is impractical, vaccinating dromedaries and other camelids may decrease viral load in these animals and their secretions, reducing the risk of zoonotic transmission of MERS-CoV.

Dromedaries that have been vaccinated and then exposed to live, infectious MERS-CoV release less infectious virus than unvaccinated animals.²¹⁶ This protection is associated with the presence of MERS-CoV-specific neutralizing antibodies in the dromedaries' blood. A vaccinia virus-based vaccine has been produced that contains the MERS-CoV S protein. This recombinant viral vector vaccine induces mucosal immunity in infected dromedaries and decreases the levels of excreted virus, which has the potential of blocking further zoonotic transmission from these animals.²¹⁶

Experimental infection of llamas and alpacas suggests that these American camelids are also potential reservoirs for MERS-CoV.^{151,211,222} When experimentally infected via the intranasal route, llamas shed MERS-CoV that could infect other, healthy animals 4–5 days after infection, indicating that llamas might serve as a reservoir host that can infect other llamas upon contact²¹¹ and perhaps humans as well. Following vaccination with a baculovirus-based vector vaccine engineered to carry the MERS-CoV S1 protein, however, infected llamas do not infect healthy llamas with which they have contact. This appears to be due to the induction of strong neutralizing antibody responses by this virus vector construct.²¹¹ It should be noted that vaccination of llamas with an S protein from a clade A virus (EMC/2012 isolate) also protects against infection by a clade B virus, suggesting that a vaccine that targets one MERS-CoV strain may be effective against multiple viral variants.²¹¹

Another vaccine utilizing S1 that is administered intramuscularly provides complete protection from MERS-CoV-associated pathology in alpacas. This protection correlates with increased neutralizing antibody titers.¹³⁵ Efficacy of this vaccine differs in dromedaries. Typically, large amounts of infectious virus are shed for approximately a week in these camels,¹³⁵ however, this vaccine reduces and delays viral shedding from the URT in some vaccinated dromedaries. While this vaccine does not achieve sterilizing immunity in camels, it may nevertheless reduce viral transmission enough to prevent transmission to humans.¹⁴⁴

3.10.2.4 Vaccinate, and vaccinate, and vaccinate again

Several studies also found that while the presence of neutralizing antibodies greatly decreases viral shedding, sterile immunity, if achievable, would require very high levels of

these antibodies.^{138,223} Accordingly, dromedaries are repeatedly reinfected with MERS-CoV.^{138,182} Due to the occurrence of re-infection of adult camels, multiple dosing and booster vaccinations may be required to make an impact on the long-term transmission rate to humans.¹⁸² When a recombinant vaccinia virus vector-based vaccine expressing the MERS-CoV S protein was administered intranasally and intramuscularly to dromedaries, the intranasal challenge with MERS-CoV lowered the animals' respiratory symptoms and as well as viral titers in the URT in comparison to unvaccinated camels.²¹⁶ Thus, even if vaccines in dromedaries do not completely eliminate the virus from the body or prevent re-infection, they do lower the amount of virus in respiratory secretions and decrease the risk of zoonotic transmission.

3.10.2.5 The best made vaccines for mice and monkeys

In addition to camelids, several MERS-CoV vaccines can effectively protect other animals in vivo, including mice and rhesus monkeys. The ability to successfully vaccinate mice and small nonhuman primates is important since it allows these animals to be used in vaccine safety and efficacy tests before they enter into clinical trials in humans.

Intraperitoneal, subcutaneous, and intramuscular inoculation of a recombinant vaccinia virus construct containing the complete MERS-CoV S protein produces strong and long-lasting neutralizing antibodies and CD8⁺ T killer cell responses in mice.^{217,224} In the mouse model system, vaccination with this type of construct protects the animals from challenges with infectious MERS-CoV.²²⁴ A vaccine that incorporates the S1 sialic acid-binding domain also induces protective neutralizing antibodies against lethal MERS-CoV challenge in mice.²²⁵

Some vaccines containing either complete or fragments of the viral S protein, including the RBD, provide partial protection in nonhuman primates.^{226,227} Vaccines that use only the RBD of the S protein, however, may not yield as high levels of neutralizing antibodies as vaccines incorporating the full-length S protein gene or S1 subunit proteins. A vaccine containing the S protein gene followed by S1 subunit proteins as a booster produces strong neutralizing antibody activity against both the RBD and non-RBD portions of the S1 subunits in several MERS-CoV strains in rhesus macaques and protects them from developing severe lung disease.²²⁸

3.10.2.6 Coronavirus vaccines: agony or victory?

When creating vaccines, caution must be taken to avoid triggering antibody-dependent enhancement of disease which increases the illness and may be fatal. This has been seen in SARS-CoV and feline infectious peritonitis virus candidate vaccines following challenges with their respective viruses.^{229,230} Cats immunized with the latter vaccine died earlier than those who were not immunized. Clinical trials of vaccines against coronaviruses thus require careful attention to vaccine safety, especially if the vaccines are to be given multiple times at close intervals. Such care is warranted for us to follow the teachings of Hippocrates in *Of Epidemics* to "do good or to do no harm."²³¹

Prior infection with the other, mildly pathogenic human coronaviruses, such as HCoV-OC43 and HCoV-HKU1, might be able to provide at least partial protection against MERS-CoV or SARS-CoV. Some of the SARS-CoV-2 vaccines might also be able to cross-react and protect against MERS-CoV infection. It is thus possible that, at some time, researchers

may be able to produce vaccines that are active against all human coronaviruses, including SARS-CoV-2, and their inevitable variants. Such vaccines would certainly be a victory in our struggle with present and future highly pathogenic coronaviruses.

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COVID-19

4.1 Introduction

4.1.1 Severe acute respiratory syndrome coronavirus and other human coronaviruses

Prior to 2019, humans were known to be infected by six coronaviruses with vastly different disease severity and mortality rates. HCoV-229E, -OC43, -NL62, and -HKU1 typically cause mild, cold-like diseases, but on occasion cause more severe illnesses in immunocompromised individuals, including croup, pneumonia, and neurological disorders, as described in Chapter 1. Infection with the severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle East respiratory syndrome coronavirus (MERS-CoV) may cause mild symptoms as well, but often causes diseases (SARS and MERS, respectively) that have very high morbidity and mortality rates (see Chapters 2 and 3, respectively).

In December 2019, a new coronavirus emerged in humans. Infection with this virus and its multiple variants also may be **asymptomatic** or cause mild disease, but like SARS and MERS, often results in highly virulent disease, coronavirus disease 19 (COVID-19). Due to its great similarity to SARS-CoV, the new virus was named SARS-CoV-2. Unlike SARS-CoV, which was primarily concentrated in parts of Asia and Toronto, Canada, SARS-CoV-2 has a high prevalence worldwide and was designated as a pandemic by the World Health Organization (WHO) on March 11, 2020.¹ The SARS epidemic lasted for only a short period of time in 2003 and parts of 2004, but the COVID-19 pandemic is ongoing and continues to mutate and spread rapidly at the time of this writing (mid-January, 2022).

Children, youth, and young adults are generally spared serious disease, but the elderly and people with comorbidities are at high risk for a severe or fatal illnesses. A study performed on hospitalized COVID-19 patients in New York City or surrounding areas during March and April of 2020 found that the most common comorbidities were **hypertension** (56.6% of the patients), obesity (41.7%), and diabetes (33.8%).²

4.1.2 Number of cases, deaths, and vaccinations

Very large numbers of life-threatening or fatal cases have been and continue to be occurring as of the date of this writing (mid-January 2022). Globally, 328,532,929 cases and 5,542,359 deaths had been confirmed and 9,395,059,118 vaccine doses had been

administered (WHO, COVID Dashboard, accessed January 18, 2022).³ At that time, the United States had 66,715,937 confirmed cases, 850,575 deaths, and 529,266,561 vaccine doses administered (79.9% of the population received at least one vaccine) (CDC COVID Tracker, assessed January 18, 2022). The 15 countries with the highest reported numbers of COVID-19 cases and deaths are shown in Fig. 4.1A and B, respectively. The United States and India had by far the highest amounts of both. Most of the cases and deaths occurred in the Americas and central and northern Europe. When the number of cases and deaths are viewed as a function of population size, however, very different results are seen (Figs. 4.1C and D, respectively). The majority of cases and deaths as a function of population size are found primarily in southeastern Europe and South America.³

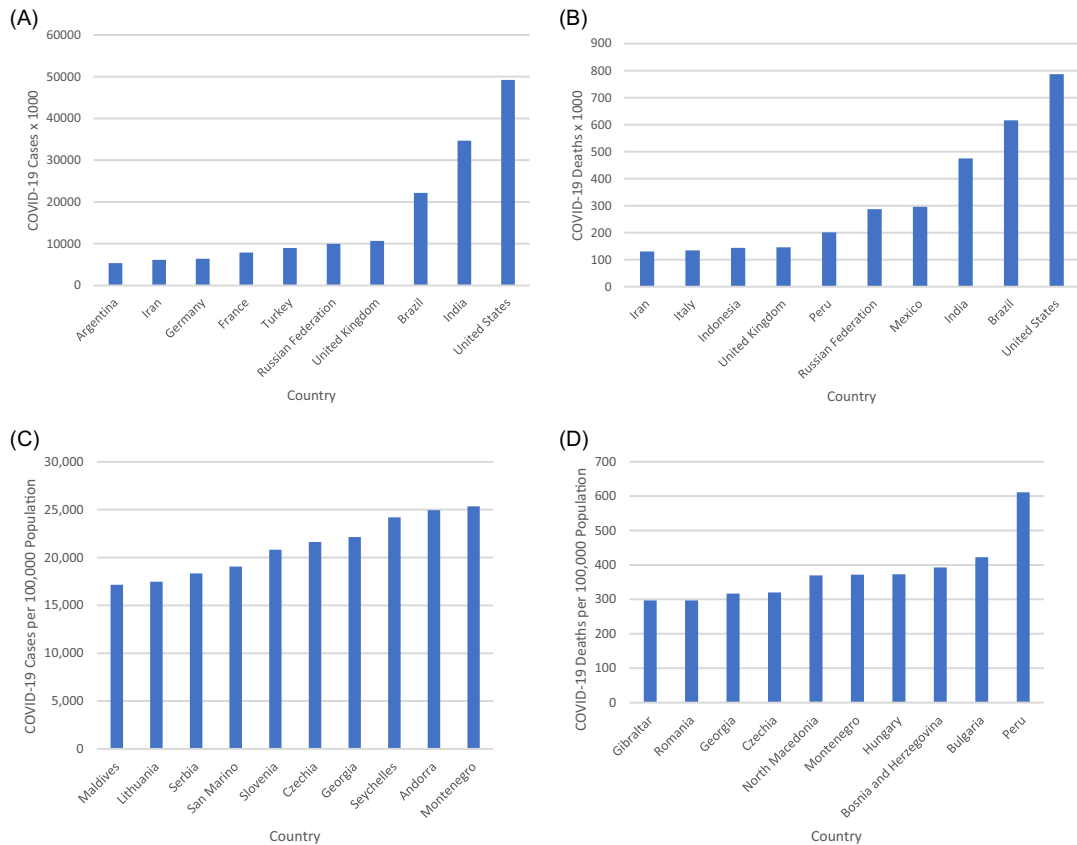


FIGURE 4.1 Countries with the Highest Cumulative Numbers of COVID-19 Cases and Deaths. a Countries with the Highest Cumulative Numbers of COVID-19 Cases. The 10 countries reporting the highest number of COVID-19 cases. b Countries with the Highest Cumulative Numbers of COVID-19 Deaths. The 10 countries reporting the highest number of COVID-19 deaths. c Countries with the Highest Cumulative Numbers of COVID-19 Cases as a Function of Population. The 10 countries reporting the highest percentage of COVID-19 cases. d Countries with the Highest Cumulative Numbers of COVID-19 Deaths as a Function of Population. The 10 countries reporting the highest percentage of COVID-19 deaths. Tables produced by the author based upon data from the World Health Organization.

4.1.3 Spread of severe acute respiratory coronavirus-2

When COVID-19 was first reported in Wuhan, China, about half of the cases were linked to the Huanan South China Seafood Market, a local live animal (“wet”) market.¹ Transmission to humans appeared to result from exposure to pangolins (spiny anteaters). As the epidemic progressed, however, the primary means of transmission to humans became, and remains, from an infected human. Very early during the pandemic, 55% of suspected cases acquired before January 1, 2020, were linked to the seafood market, which is the date on which the market was closed. Of cases reported from January 1 to January 11, this number dropped to 10%. By contrast, people having no exposure to this or another market or people with respiratory symptoms rose from 26% before January 1 to 72% reported January 1–11.⁴

The **basic reproductive number**, R_0 , is the average number of people that are anticipated to become infected by a single infected individual. An R_0 of 1 indicates that an infected person will, on average, infect one other person. An R_0 of 2 means that one infected person will infect 2 people, doubling the number of cases. The higher the R_0 , the more contagious the virus is. Using a doubling time of 6–7 days, the R_0 of SARS-CoV-2 was reported to be 2.2–2.7 early during the pandemic.⁵

The R_0 number changed with time as the virus spread to different locales and with the emergence of new viral variants. For example, another study conducted in China in early 2020 estimated the average R_0 of SARS-CoV-2 to be 3.28.⁶ By contrast, the R_0 of SARS-CoV and MERS-CoV are 1.7–1.9 and <1 , respectively.⁷ This suggests that SARS-CoV-2 is more contagious among people than SARS-CoV and MERS-CoV. The R_0 during the SARS epidemic of 2003 was driven in part by “super-spreaders,” who spread the disease much more readily than the typical SARS patient. Since the R_0 of MERS-CoV is less than 1, this indicates that, at least in part, the continuing MERS epidemic is also fueled by multiple transmissions of MERS-CoV to humans from its reservoir hosts—infected dromedary camels. It should be noted that the R_0 is altered by human interventions, including decreasing human-to-human contact by staying six or more feet from other people (**social distancing**), wearing masks in public settings, and isolating people who are infected with the virus, and increasing **herd immunity**. Herd immunity refers to a condition in which so many members of a population are immune to an infectious disease agent that sustained transmission among people is no longer possible.

Using **contact tracing**, when a person is found to be infected (first-generation of infection), the people with whom they had contact (second-generation) are monitored and perhaps quarantined. The contacts of the second-generation people are likewise identified and perhaps quarantined (third-generation). This repeated identification and quarantining of the contacts of potentially infected people is meant to prevent the spread of the disease to yet further generations of hosts. A systematic review of published articles from January 1, 2020, and April 1, 2021, found that rapid contact tracing decreased the R_0 of SARS-CoV-2 from 3.11 to 0.21. In that study, for each new case, an average of 36 people were monitored.⁸ Later during the pandemic, this number changed as new and more contagious variants arose.

COVID-19 severity and fatality rates vary. The Chinese Center for Disease Control and Prevention estimated early in 2020 that the case fatality rate of SARS-CoV-2-infected people was 2.3%.⁹ However, in the Hubei Province, where the epidemic may have begun, the

case fatality rate is sevenfold greater than that found in other Chinese provinces.⁹ Additionally, diagnostic methods differ among provinces and the early cases reported in Hubei Province were based on clinical reports rather than the now widely used tests for the presence of viral RNA or antibodies to the virus.

COVID-19 is now present in countries throughout the world. These countries differ in their quality of medical care, access to vaccines and antiviral drugs, cultural practices, socioeconomic factors, and the overall health, diet, and nutritional status of any given population. Genetic changes in SARS-CoV-2 RNA have and will continue to change transmissibility and disease manifestations as they rapidly give rise to new variants in one geographical region that appears to inevitably spread across the globe.

Given the number of the above interacting factors and the ever-changing nature of the virus itself, it is difficult to make accurate predictive models that are widely applicable over time. The importance of various biological factors in COVID-19 severity continues to change as more information is obtained due, at least in part, to a larger number of cases upon which to develop more precise and accurate disease models. Unfortunately, as new viral variants emerge, the models need to continue to be updated to reflect differences among variants. As a hypothetical example, models developed early during the pandemic that was based on data from a large urban area in one part of a country may not apply to a rural area in another country with low population density in an area that differs in temperature, humidity, elevation, and land usage. By the time this book is published, doubtless many of the factors affecting the R_0 and the severity and mortality rate of SARS-CoV-2 will have changed. Viral transmissibility, for example, is partially dependent upon the tropism of different viral variants for different portions of the respiratory tract. Infection with a viral variant which is preferentially found in the **upper respiratory tract (URT)** typically results in milder but more contagious disease. Variants which are preferentially found in the **lower respiratory tract (LRT)** (trachea, bronchi, bronchioles, and alveoli) typically are less transmissible, but cause more severe disease, as described later in this chapter.

4.1.4 Factors affecting determination of COVID-19 cases

The accuracy of the many different tests used in COVID-19 diagnosis may differ since different parts of the world use different means of diagnosis (see “Diagnosis and Surveillance”). The types of tests that are being used in any one area also change with time. The numbers and disease status of the people who are tested vary depending on the overall availability of the tests in a region and the portion of the population that is being tested at any given time. This may include differences in results of testing only individuals who have disease symptoms versus the population as a whole; testing primarily health-care providers and first-responders versus members of the public; the number of times in which a given person is tested; and whether the person being tested has comorbidities and the type and severity of the comorbidity. Genetic tests are based on the detection of viral RNA, typically from the nasal cavity, and are usually administered by healthcare workers wearing protective gear. Serological tests focus on immunological factors, especially the amount and type of viral antigen and the class of antibody that is being

produced (**IgM** or **IgG**). Some of these tests examine antibodies in the blood, while other tests utilize saliva. The saliva tests can be performed by the people themselves, reducing the amount of protective gear used and freeing up healthcare workers for other necessary tasks, including vaccine administration. Saliva tests are usually not very sensitive, however. The results from some of these tests are available in a matter of minutes, while other tests take days. Since a wide variety of tests have been developed in a short period of time, their accuracy is not always known, especially when testing the different viral variants. Some of the tests also are not able to detect low virus load (see “Diagnosis and Surveillance”).

4.1.5 Unprepared

To protect the healthcare personnel, handling of specimens that may generate infectious aerosols, droplets, or splashes should optimally be performed in a biological safety cabinet, while culturing of the virus in various types of cells should be performed under **Biosafety Level 3** conditions. It should be noted that while the appropriate testing gear and facilities may be available in some parts of the world, much of the world does not have these resources or a sufficient number of personnel to adequately care for patients with moderate or severe diseases. In the late winter and early spring of 2020, even regions of the developed world, such as large metropolitan areas (New York City, Detroit, and New Orleans), lacked the necessary amounts of testing and safety materials as well as available hospital beds.

The numbers of ventilators were also much lower than those needed to deal with the massive numbers of infected people with severe diseases. Nevertheless, given the unforeseeable scope and length of the pandemic, it would be very difficult to have the number of hospital beds, ventilators, and healthcare personnel on hand to deal with a pandemic of this magnitude. Perhaps the best way to avoid such a threat in the future is early detection of the disease and rapid reporting to agencies, such as the WHO, so that infected people may be isolated before an epidemic becomes an uncontrollable pandemic. This is particularly important for diseases that are readily transmitted among people, especially via the respiratory route.

4.1.6 Severe acute respiratory syndrome coronavirus-2 and animal hosts

Many animal species are susceptible to SARS-CoV-2 infection. Some bat families are postulated to harbor the precursors of pathogenic human and animal coronaviruses, serving as reservoir species that transmit the virus to its intermediate hosts as described in Chapters 5 and 6. Vespertilionidae and Rhinolophidae families of bats are the primary hosts of coronaviruses with **zoonotic transmission** potential in general, however, several bat coronaviruses with the potential to infect humans are also found in Hipposideridae and Pteropodidae bats.^{10,11} Bat species that are infected with SARS-CoV-2-like viruses include intermediate horseshoe bats (*Rhinolophus affinis*), Chinese rufous horseshoe bats (*Rhinolophus sinicus*), king horseshoe bats (*Rhinolophus rex*), and great roundleaf bats (*Hipposideros armiger*).^{12,13}

A wide variety of mammals infect non-domesticated animals either in the wild or in captivity (see Chapter 5).¹⁴ Captive tigers (*Panthera tigris*) and lions (*Panthera leo*), snow

leopards (*Panthera uncia*), pumas (*Puma*), grizzly bears (*Ursus arctos horribilis*), gorillas (*Gorilla* species), Angola colobus monkeys (*Colobus angolensis*), macaques (*Macaca* species), lemurs (*Lemuriformes*), treeshrews (*Scandentia*), minks (*Mustela* species), pangolins (*Manis* species), as well as a giant (*Myrmecophaga tridactyla*) and collared anteaters (*Tamandua tetradactyla*), mice (*Mus* species), hamsters (*Cricetinae* species), and European rabbits (*Oryctolagus cuniculus*) are or may be experimentally infected with SARS-CoV-2.¹³ Some of these animals, including cats (*Felis catus*), treeshrews, deer mice (*Peromyscus maniculatus*), hamsters, minks, and ferrets (*Mustela putorius furo*) also shed the virus following experimental infection. Pigs (*Sus* species) and poultry, however, appear to be resistant to SARS-CoV-2 infection and cattle (*Bos taurus*) have a low level of susceptibility.¹⁵ Pigs, chickens (*Gallus gallus domesticus*), and ducks (*Anas platyrhynchos domesticus*) have little to no susceptibility to experimental infection with SARS-CoV-2 strains present in 2020.¹⁶

In vitro assays have studied the **binding affinity** between the SARS-CoV-2 spike (S) protein and species-specific ACE2, the host cell receptor for this virus. The highest binding affinity is found between the S protein and human and rhesus monkey (*Macaca mulatta*) ACE2. Mice have the lowest S protein-to-ACE2 binding affinity of tested animals.¹⁷ Other studies have predicted that pet cats and minks are the animal species most susceptible to COVID-19 because their ACE2 molecules have a high degree of similarity to that present on human cells and have more contact with humans than do primates.¹⁸ Indeed, several massive SARS-CoV-2 outbreaks have occurred in mink farms in Utah in the northwestern United States, in addition to reports of COVID-19 cases of minks in the states of Wisconsin and Michigan.¹⁸

While infected dogs (*Canis lupus familiaris*) are generally asymptomatic, cats develop respiratory and digestive distress as demonstrated by difficulty breathing, diarrhea, and vomiting.¹⁸ Interestingly, juvenile cats are more susceptible to infection by SARS-CoV-2 than older cats, while the opposite is true in humans. Antibodies against the SARS-CoV-2 Alpha variant (B.1.1.7) have been detected in several cats and dogs 2–6 weeks after they displayed signs of cardiac disease. Alpha variant RNA is also found in rectal swabs of these animals.¹⁹ It should be noted that, in some cases, SARS-CoV-2 RNA has been found in rectal swabs of these pet animals following a negative nasopharyngeal test. The pet owners tested positive for COVID-19 and had respiratory symptoms 3–6 weeks before their pets' illness. Moreover, several people who cared for pets had COVID-associated severe myocarditis a few weeks before myocarditis was detected in these animals. While the source of viral variants in companion animals is unknown, the original strain of the virus has been demonstrated to be transmitted from humans to cats^{20,21} as described in Chapter 6.

Additional information comparing genomic similarity and binding affinity of the viral S protein of various animal coronaviruses is discussed later in this chapter. The likelihood of different animals serving as intermediate hosts for zoonotic transmission is also presented.

4.2 History

An outbreak of pneumonia and **acute respiratory disease syndrome (ARDS)** cases was first reported on December 29, 2019, in humans in Wuhan, Hubei Province.^{5,22} Evidence indicates that the virus may have been present in human populations by December 12.⁵

The delay in reporting may, in part, be due to asymptomatic or mild infections early during the outbreak, but other factors appear to have been involved as well. In addition to being a major population center with a population of 11 million people, Wuhan is the site of a major **Biosafety Levels 4** infectious disease research center that studies coronaviruses from different animal species, especially bats, and has, in the past, created chimeric bat-mouse coronaviruses that became able to infect and be highly pathogenic in mice.^{23–25} The goal of this type of research is to make microbes, including bat coronaviruses, more pathogenic, more contagious, more resistant to treatment, and with a broader host range. Such studies were partially conducted in Wuhan in collaboration with the University of North Carolina at Chapel Hill, USA, and EcoHealth Alliance. The chimeric virus produced by Menachery et al.²⁵ obtained most of its genetic information from a virulent bat coronavirus, but the gene for the S protein of a coronavirus from mice was inserted to produce a more pathogenic coronavirus that readily infects mice. If a similar tactics were used to insert a human coronavirus S protein into a pathogenic bat coronavirus, a highly dangerous human coronavirus could arise that might be very similar to SARS-CoV-2.

Wuhan is a major travel hub in central China and the reported outbreak in this city began near the time of the Spring Festival, the greatest travel period of the year.²⁶ Since coronaviruses in general, can “jump” between different animal species and adapt to new hosts, it is possible that an ancestor of SARS-CoV-2 may have jumped from bats into Malayan pangolins in a wet market and then into humans, followed by effective human-to-human transmission. By mid-January 2020, COVID-19 cases were reported throughout much of China. Other early cases also were associated with visiting such live animal markets.⁵

The human-to-human transmission was reported not long after the first reported cases. Of 227 people diagnosed from January 1 to January 22, 2020, 200 had no contact with the Wuhan market and had not been in close contact with people exhibiting respiratory symptoms. China then locked down Wuhan, prohibiting people from leaving the city in a belated and unsuccessful attempt to stop the spread of SARS-CoV-2 to other parts of the country. The lockdown strategy that China implemented works best during the early stages of an infection, which was not the case in Wuhan, as several million people had already left the city before the restrictions were imposed.²⁶

The first known SARS-CoV-2-related death occurred in early January 2020. Due to human-to-human transmission, within the initial 6 weeks of the outbreak, cases were reported in 37 countries. In mid-late January, laboratory-confirmed cases began to be detected outside of China, first in Thailand, then Japan, and South Korea. The outbreak then was detected in several continents and regions outside of Asia: North America (the United States), Australia, Europe (Germany, France, Italy, and Spain), and the Eastern Mediterranean region (the United Arab Emirates). The WHO declared COVID-19 to be a Public Health Emergency of International Concern on January 30, 2020.²⁷ In late February, laboratory-confirmed cases were found in northern Africa (Algeria), South America (Brazil), and Sub-Saharan Africa (Nigeria).

Later during the pandemic, near the end of 2020, the Alpha SARS-CoV-2 (B.1.1.7) variant was discovered in England, followed by the emergence of the Beta variant (B.1.351) in South Africa.^{28–30} Both variants are much more contagious than the original virus strain. Since then, other variants of concern have emerged, including the Delta (B.1.617.2) and Omicron (B.1.1.529) variants. The Delta variant was first reported in India in October 2021,

while the Omicron variant emerged and was reported from South Africa on November 14, 2021, although it was later detected in samples from Botswana on November 11, 2021 (WHO, 2021).³¹ Ever since the identification of Omicron, the variant appears to spread rapidly. Using genomic-sequence analysis, samples isolated in the Gauteng Province of South Africa from November 12 to 20th were all Omicron.³² Doubtless, more variants will continue to arise and we will need to carefully consider the beneficial and deleterious effects of our responses, including whether people with “**natural immunity**” and those immunized with various types and numbers of vaccine boosters are being reinfectd or infected, respectively. We need to determine whether these people are asymptomatic or become ill, the severity of their illness, and their ability to spread the virus to other people who may be at high risk of developing severe disease.

Some of the more recent and highly contagious viral variants are associated with a greater or lesser risk of developing the severe disease than their predecessors. For example, the Delta variant is associated with a higher likelihood of hospitalization and causing mild-moderate or severe-critical disease outcomes compared with persons infected with the original strain, Alpha, and Beta variant, particularly among those who are not fully vaccinated.³³

The first case of Omicron was documented in the city of Tshwane, South Africa, on November 9, 2021.³¹ The Omicron variant contains many mutations, resulting in its receptor-binding domain (RBD) of the S protein having a greater affinity for human ACE2 than does that of the Delta variant.³⁴ This may play a major factor in its enhanced transmissibility, resulting in a rapid increase in SARS-CoV-2 cases and hospitalizations in South Africa. It soon thereafter became the dominant variant in that country.³⁵ The numbers of deaths, intensive care unit (ICU) admissions, and length of hospital stay associated with the Omicron variant were 4.5%, 1%, and 4 days for Omicron, respectively, and 21.3%, 4.3%, and 8.8 days, respectively, for the previous variants.³⁵ A study from South Korea from early to mid-December, 2021, found that 47.5% of the patients infected with the Omicron variant were asymptomatic and the rest had mild symptoms, including the 15% of people with lung infiltrations. None of the latter group required supplemental oxygen.³⁶

The Omicron variant causes breakthrough cases in people with either natural immunity resulting from a previous infection or vaccine-linked immunity. Cross-reactive **neutralizing antibodies** were not able to prevent these infections. The mild symptoms associated with Omicron appear to be due to the presence and activity of anti-SARS-CoV-2 S protein-specific **CD4⁺ T helper cells** and **CD8⁺ T killer cells** that are found in Omicron variant-infected people.³⁷

4.3 The disease

4.3.1 Introduction to COVID-19

The incubation period of COVID-19 ranges from 2.1 to 11.1 days, with an average time of 6.4 days, which is somewhat shorter than those seen in SARS and MERS. SARS-CoV-2 is contagious prior to the appearance of discernable symptoms,³⁸ making infection-control measures problematic. SARS-CoV-2 also has a greater capacity than SARS-CoV to infect ciliated **epithelial cells** lining the nasal cavity and **bronchioles**.³⁹ SARS-CoV-2 produces a

strong, pathogenic inflammation response in the lungs which damages the **alveoli**. This damage is accompanied by an influx of **neutrophils** into the area. During COVID-19, the secretion products of neutrophils may cause **pulmonary embolisms** or **thrombosis**, as well as **ARDS**.⁴⁰ SARS patients were also generally more prone to gastrointestinal disease than COVID-19 patients.⁷

Many people infected by SARS-CoV-2 are asymptomatic, although they are still able to transmit the virus to other people. Depending on the variant, about 80% of those infected are either asymptomatic or only develop mild symptoms, such as fatigue, headache, fever (83%), dry cough (82%), and shortness of breath (31%). Although SARS-CoV-2 efficiently multiplies in the nose, nasal congestion, runny nose, and other URT symptoms are rare, unlike the case of SARS-CoV in which URT infection occurred more frequently.⁴¹ Interestingly, at least some variants of the SARS-CoV-2 S protein have a temperature preference of 33°C, the temperature of the URT. The human coronaviruses that typically cause the common cold share this optimal temperature. However, some SARS-CoV-2 variants also effectively replicate at 37°C in the lungs, allowing them to produce severe pathology that is like that attributable to SARS-CoV and MERS-CoV.⁴² The S proteins of SARS-CoV-2, MERS-CoV, and the mildly pathogenic 229E human coronavirus have greater stability at pH 6.3, the pH present in the nasal cavity. This pH is more acidic than that in the lungs and decreases the activity level of the SARS-CoV S protein.⁴²

COVID-19 is well known to adversely affect the respiratory system. Autopsy studies identify focal disease throughout the lungs. In addition to nonspecific or URT illness, SARS-CoV-2 causes disease in the LRT, as well as the cardiovascular, immune, nervous, endocrine, urinary, digestive, muscular, and reproductive systems. The skin and hair may also be affected.^{43–45} In addition to respiratory illness, SARS-CoV-2 infection has been linked to **cardiomyopathy** (injury to the heart) and acute viral **myocarditis** (inflammation of the heart), **hypotension** (low blood pressure), and renal **hypoperfusion** (less blood reaching the kidneys), and reduced **glomerular filtration rate** (a slowing of the rate of production of urine). Other COVID-19-related disease affects vision and causes **rhabdomyolysis** (breakdown of striated muscle), **metabolic acidosis** (acidic body fluids), and **hyperkalemia** (excessive blood potassium levels).⁴⁶ This chapter will focus primarily on diseases found outside of the respiratory system since they are less well-known.

4.3.2 COVID-19 and the respiratory system

Human ACE2 is highly expressed in lung alveolar cells, the site of oxygen and carbon dioxide gas exchange with the blood. Approximately 20% of people with COVID-19 develop a severe, life-threatening LRT disease, less than that seen in people infected with SARS-CoV or MERS-CoV. In an analysis of over 1000 Chinese COVID-19 cases, approximately 5% of the diagnosed patients developed severe pneumonia, often in both lungs, requiring intensive care treatment; 2.3% of those with respiratory symptoms required mechanical ventilation, and 1.4% of these people died. **Diffuse alveolar damage (DAD)** with **edema**, **hyaline membranes**, and inflammation are present in some COVID-19 patients, along with **hyperplasia** of type II pneumocytes.⁴⁷ Early-stage DAD was found in approximately 88% of the people who died upon postmortem examination.⁴⁸ These

respiratory symptoms are associated with ARDS.^{49,50} While the fatality rate for COVID-19 is changing over time, perhaps due to the identification of more asymptomatic people or a change in viral virulence among viral variants, far fewer people infected by SARS-CoV-2 die of respiratory disease (2.8% or less), as opposed to SARS (9.5%) or MERS (34.4%).⁷

A study of patients with SARS-CoV-2 pneumonia reported that 61.5% of these patients died within a month. Patients with fatal COVID-19-associated pneumonia tend to be older, more prone to develop ARDS, and likely to require mechanical ventilation in comparison to survivors.⁵¹ Lung damage appears to be due to the production of high levels of proinflammatory cytokines by the immune system. An abnormal form of ARDS develops in about 15% of the COVID-19 patients. This potentially fatal condition leads to multiple organs receiving an inadequate oxygen supply due to **pulmonary edema** (fluid buildup in the lungs) and lung failure. Other disease manifestations found in the pneumonia patients are acute kidney or cardiac injury and liver dysfunction.⁵¹

Elevated numbers of neutrophils in the lungs of COVID-19 patients are associated with a poor outcome. Those neutrophils remaining in the blood contain vacuoles with higher granule content, consistent with an activated state.⁵² Neutrophils compose the majority of granulocytic cells infiltrating the lungs and the numbers of **basophils** and **eosinophils** are decreased in COVID-19 patients.⁵³

While most deaths occur in older patients, severe cases may also develop in young adults, especially smokers and those with chronic diseases, including hypertension, diabetes, or hepatitis B virus infection. Young people taking corticosteroids or other immunosuppressive drugs are also at high risk for severe disease. SARS-CoV-2 also affects the respiratory system of children. Chest **computed tomography (CT or CAT scans)** findings indicate that 18% of asymptomatic children have lung abnormalities.³⁴ CT scans may be able to detect abnormalities that are not using conventional chest X-rays. These scans indicate unilateral, rather than bilateral, lung involvement is more common in children, especially in diseases involving the lower lobe of the right lung. By contrast, bilateral involvement is found in greater than 80% of adult patients with respiratory signs.⁵⁴ The most common findings in affected children are **ground-glass opacity** (40%), nonspecific patchy shadows (44%), **lung consolidation** (23%), and the **halo sign** (26%). Interstitial infiltration is the most common **ultrasound** finding.³⁴

4.3.3 COVID-19, smoking, and nicotine use

Several studies have revealed a complex relationship between smoking and COVID-19 patients.^{55–57} Current and former smokers have a greater risk of severe COVID-19 than those who have not smoked.^{58,59} Additionally, smokers were approximately 2.4 times more likely to be admitted to an ICU.⁶⁰

Unexpectedly, low smoking prevalence has been reported among hospitalized COVID-19 patients in China and the United States.^{2,56,61} The prevalence of smoking in hospitalized COVID-19 patients in the Chinese study was one-fourth that of the general population. While there is an inverse association between smoking and infection with SARS-CoV-19,⁶² once infected, smokers tend to develop more severe disease with increased rates of mechanical ventilation and death than nonsmokers.⁶³

Nicotine is one of the major active compounds in cigarette smoke. The relationship between nicotine itself and COVID-19 is also complex. The **nicotinic acetylcholine receptor (nAChR) α 7** subunit is present on the surface of **macrophages**, **B lymphocytes (B cells)**, **platelets**, bronchial epithelial cells, type II alveolar epithelial cells, and interstitial lung fibroblasts.^{56,64} By binding to this receptor, the neurotransmitter **acetylcholine** downregulates the production of the inflammatory cytokines **tumor necrosis factor- α (TNF- α)**, **interleukin-(IL)-1**, and **IL-6**.⁶⁵ **Nicotine** serves as a **cholinergic** agonist and thus also inhibits the production of the above cytokines as well as platelet activation via α 7-nAChRs.^{56,66} Additionally, pure nicotine, unlike extracts of electronic cigarettes, may decrease platelet activation and coagulation, several of the more pathogenic processes occurring during COVID-19.⁶⁷ Furthermore, the influence of smoking, vaping, or nicotine use on the expression of the ACE and ACE2 genes in the lungs is uncertain,⁵⁶ since some authors have reported that nicotine decreases ACE2 expression and increases ACE expression,^{62,68,69} while other recent studies report the opposite effects.^{64,70,71} Nicotine also increases SARS-CoV-2 replication and **cytopathic effect** in vitro.⁷²

While several reports have suggested that nicotine use may lead to a milder form of COVID-19, reducing the risk of hospitalization,^{55,73} nicotine has been linked to the development of ARDS, especially in children.⁷¹ Whether or not the overall effects of nicotine are favorable during COVID-19, it is highly addictive and the potential benefits and risks of any potential therapeutic use need to be very carefully considered.^{55,56,74}

4.3.4 COVID-19 and the cardiovascular system

4.3.4.1 COVID-19 and the heart

Pulmonary infection by SARS-CoV is linked to **myocardial** infection.⁷⁵ Myocardial injury, as evidenced by increased levels of serum **cardiac troponin I** and **creatinine kinase**, is present in 20%–40% of hospitalized cases.⁷⁶ The most common types of cardiomyopathy during COVID-19 include cardiac **arrhythmia** (**atrial** and **ventricular fibrillation**, **ventricular tachyarrhythmia**, and **heart block**), **fulminant myocarditis**, and heart failure.⁷⁶ Arrhythmia has been reported in 44% of people requiring intensive care. Additionally, examination of the cardiac muscle reveals necrosis of **myocytes** and **mononuclear leukocyte** infiltration.⁷⁶ Myocarditis usually occurs 10–14 days after COVID-19 onset.

Postural tachycardia syndrome (POTS) is present in some patients with **long COVID syndrome**, described later in this chapter.⁷⁵ POTS affects heart rate, blood pressure, and cardiac function. Disruption of the ACE2-angiotensin axis is found in patients with hypertension, **atherosclerosis**, atrial fibrillation, prolongation of the **QT interval**, and heart failure.

4.3.4.2 COVID-19 and the blood

Patients with severe or fatal diseases have small to large levels of increases in the total **leukocyte** count, respectively. This increase is primarily due to greater than normal neutrophil numbers. $CD4^+$ T helper and $CD8^+$ T killer cells, **monocytes**, and platelet counts are decreased in those with the severe disease when compared to people with mild cases and COVID-19 survivors.⁷⁷ Inflammatory damage to the heart and skeletal muscle, reduced liver and kidney functions, and elevated levels of serum liver enzymes (**alanine**

aminotransferase and **aspartate aminotransferase**) and kidney biomarkers (**blood urea nitrogen** and **creatinine**), as well as altered blood **coagulation**, are present in patients with severe to fatal COVID-19.⁷⁸

Severe disease is typically associated with excessive levels of serum IL-6, IL-10, and **ferritin** (an antimicrobial iron-binding protein).⁷⁹ Since the proinflammatory cytokine IL-6 is responsible for much of the immune-mediated damage, the production of the antiinflammatory cytokine IL-10 may be the body's attempt to mount a compensatory response. The balance between pro- and antioxidative compounds is important to avoid oxidative stress. In COVID-19 patients admitted to ICUs, blood levels of the antioxidant defense molecules **vitamin C**, **thiol proteins**, **glutathione**, **γ -tocopherol**, and **β -carotene** are decreased. The elements copper, zinc, and selenium play important roles in the body's response to toxic **reactive oxygen species (ROS)**. Copper has a strong negative correlation with γ -tocopherol, a major antioxidant that inhibits lipid peroxidation, which causes harmful alterations to plasma membranes. The copper/zinc ratio and levels of the inflammatory biomarkers **C-reactive protein** and **myeloperoxidase**, however, act as pro-oxidants at high levels, including during SARS-CoV-2 infection.⁸⁰ The roles of copper, zinc, and selenium are described later in this chapter.

4.3.5 COVID-19, endothelial dysfunction, complement, and coagulation

SARS-CoV-2 triggers activation of the coagulation and **complement** pathways in the blood. The coagulation pathway is the clotting process and involves compounds found in the blood as well as those found in activated platelets. Coagulation is critical to wound healing. In addition to their role in clot formation, activated platelets release **platelet-derived growth factor** that induces cell division during wound healing. The complement pathway is a major factor in the control of pathogen-associated disease by killing infected cells and drawing leukocytes into the infected area. Specific mutations of the genes encoding critical regulators of the coagulation and complement pathways are linked to the development of severe COVID-19 disease. Determining which mutations are involved in the viral escape of the complement system may help to determine individuals at high risk of developing adverse disease outcomes.⁸¹

4.3.5.1 COVID-19 and endothelial dysfunction

Endothelial cells line the lumens of the blood vessels. Their functions include restoring vascular integrity following injury and protecting against excessive thrombosis and blood clot formation,⁸² which drive excessive coagulation during COVID-19. Endothelial cells play a vital role in regulating vascular **homeostasis** by producing and releasing **vasoconstrictors** that decrease blood flow to the appropriate regions as well as **vasodilators** that increase blood flow by narrowing and enlarging the blood vessel's diameter, respectively.^{83,84} Angiotensin II is a vasoconstrictor that is part of the renin-angiotensin-aldosterone system described later in this chapter. Endothelial vasodilators include **nitric oxide (NO)** produced by endothelial cells and platelets via the **endothelial nitric oxide synthase-3** enzyme.⁸⁵ By contrast, localized NO produced by the **inducible nitric oxide synthase** in phagocytes acts in an antimicrobial and proinflammatory manner.⁸⁶

During COVID-19, vasodilation is decreased due to an environment that promotes coagulation disorders, such as thrombosis and **vascular leakage** during severe viral infection.^{82,85} Abnormal coagulation and thrombosis have been previously linked to pathology during SARS and MERS.⁸⁶ Some of the COVID-19-mediated disorders related to **endothelial dysfunction** include microvascular lung thrombosis, arteriole and venous **thromboembolisms** (venous, deep vein, and cerebral venous thrombosis), arterial diseases (cardiovascular and **cerebrovascular disease** and acute limb **ischemia**), and other organ-specific diseases.⁸⁴

4.3.5.2 COVID-19 and complement

Activation of the complement cascade plays a major causative role in endothelial dysfunction during COVID-19.⁸⁷ Severe COVID-19 is similar to complement-mediated **thrombotic microangiopathies**.⁸⁸ Complement fragments, as well as interalveolar endothelial deposits, are present. Activation of a complement cascade may be responsible for thrombotic complications during COVID-19.⁸⁹

The three complement pathways (classical, alternative, and lectin pathways) involve a multifunctional cascading series of enzymatic reactions that kill microbes and microbe-infected cells. They may do so by several means, including forming large pores in infected cells by complement components C5b-9, the **membrane attack complex (MAC)**. The MAC produces large pores in COVID-19-infected cells which subsequently die by **hypotonic lysis**, as excessive amounts of fluids enter the cell, which swells until it bursts. The complement cascade also is proinflammatory and **chemotactic**, attracting a variety of leukocytes to the region of infection.

Information concerning the activation of the complement system by SARS-CoV-2 is sparse. However, multiple studies have shown that infection with SARS-CoV and MERS-CoV activates the complement system component **C3**. This increases the severity of ARDS.⁹⁰ Additionally, a region of the viral nucleocapsid (N) protein that is highly conserved among SARS-CoV, MERS-CoV, and SARS-CoV-2 binds to the **mannose-binding lectin (MBL)-associated serine protease (MASP)-2**, a key activator of the **lectin pathway of complement activation**. This binding causes autoactivation of MASP-2 and results in increased cleavage of the complement component C4.⁸⁹ The SARS-CoV S protein colocalizes with both MASP-2 and deposits of **C4d** in interalveolar septa of the lungs.⁵² C4d is a breakdown component of the **classical pathway of complement activation**, implying that this pathway is also active during SARS and perhaps during COVID-19 as well.

Patients with severe COVID-19 generate the C3 cleavage protein **C3a** and C3-fragment deposition, especially in the tubules of the kidneys,⁹¹ suggesting SARS-CoV-2 activates the complement system as well as SARS-CoV. A SARS-CoV-2 infection has been linked to an **atypical hemolytic uremic syndrome** which involves the complement-derived **anaphylatoxins** C3a and C5a. **C5a** activates the syndrome within hours of infection⁹² and thus may damage the kidneys rapidly. Additionally, increased interalveolar deposits of MBL, MASP-2, C4b, C3b, and MAC are present in the lungs of patients with COVID-19.⁵² These findings suggest that SARS-CoV-2 also activates the lectin pathway in the kidneys and lungs, especially since the S protein of SARS-CoV-2 is heavily glycosylated and thus may serve as a recognition site for MBL binding.⁸⁹

4.3.5.3 COVID-19 and coagulation

Blood in the veins and arteries may become thickened into a procoagulative state, eventually resulting in clotting disorders, such as **venous thromboembolism** (blood clots that block blood flow through the veins), deep vein thrombosis, and **arteriosclerosis obliterans** (disorder in which arteries become blocked and narrowed). Arterial thrombosis, including strokes and **myocardial infarctions**, may develop in some COVID-19 patients.⁹³ Risk factors for developing deep vein thrombosis during COVID-19 include dehydration resulting from a combination of fever and diarrhea, hypotension, and bedrest for more than 3 days. Other laboratory findings during SARS-CoV-2 infection include **prolonged prothrombin time**, **thrombocytopenia**, and **disseminated intravascular coagulation (DIC)**.⁸⁶ Prolonged prothrombin time refers to slow blood clotting, while thrombocytopenia refers to low platelet count. Thrombocytopenia is an independent risk factor for COVID-related in-hospital mortality. Death is threefold more common in patients with thrombocytopenia than in those without it.⁹⁴ The role of DIC in COVID-19 will be described later in this section.

Excessive levels of **fibrin** are present during COVID-19-related ARDS. High fibrin level is one of the major factors leading to blood clot formation in the lungs. Fibrin is removed by **plasmin** during the process of **fibrinolysis** which removes clots after wound healing. **Tissue plasminogen activator (tPA)** is an enzyme that cleaves **plasminogen** and produces active plasmin. tPA is thus a key factor in clot dissolution. **Plasminogen activator inhibitor-1 (PAI-1)** blocks this cleavage and inhibits clot breakdown. Interestingly, both tPA and PAI-1 expression are increased in hospitalized COVID-19 patients.⁹³ Endothelial cells and activated platelets appear to be the major sources of tPA and PAI-1, respectively. Neutrophils infiltrating the lungs also play a role in alveolar injury.⁹⁵ Increased amounts of tPA and PAI-1 during COVID-19 correlate with neutrophil numbers and activation status.⁹³

Some COVID-19 patients have evidence of a **systemic** procoagulant state, including **retiform purpura** or **livedo racemose**, which are indicative of generalized microvascular thrombotic disorder, and greatly elevated **D-dimer** levels.⁵² Soluble D-dimers are derived from the cleavage of cross-linked fibrin during fibrinolysis. Increased D-dimer levels are present in 97% of COVID-19 patients upon hospital admission. These levels continued to increase in people with fatal outcomes. The combined detection of elevated levels of IL-6 and D-dimers is a highly specific and sensitive means for predicting disease severity in adult COVID-19 patients.⁹⁶ Elevated D-dimer levels and prolonged clotting time are associated with poor disease outcome, while their levels decrease to normal in survivors.⁹⁴ Working together, tPA and PAI-1 regulate fibrinolytic homeostasis, attempting to balance both bleeding and clotting which occurs during COVID-19.⁹³

DIC is a condition characterized by the widespread presence of small clots in blood vessels. This is often seen in cases of hemorrhagic fever and may lead to thrombocytopenia.⁹⁴ A study of people with severe COVID-19 found that they have significantly lower platelet counts and prothrombin time than people with less severe disease. In that study, DIC was present in 6.1% of those with severe diseases and was not found in people with less severe diseases.⁹⁷ In a separate study, DIC was present in 71% of patients who died as opposed to 0.6% in survivors.⁹⁸

Approximately one-third of COVID-19 patients develop thrombocytopenia. This condition is more common in patients with severe disease and the mortality rate correlates with the extent of thrombocytopenia.⁹⁴ Thrombocytopenia and prolonged clotting time result from extensive activation of the coagulation pathway, resulting in depletion of platelets. Excessive depletion of blood platelets may lead to the point at which they are unable to stop bleeding, which may become life-threatening due to extensive blood loss. The combination of disrupted coagulation and hemorrhage are among the leading causes of death from COVID-19.⁹⁴

Platelets contain cell surface pathogen-recognition receptors that detect microbial infection. In addition to their role in clotting, platelets have an important role in the induction of inflammation due to their interactions with blood monocytes, tissue macrophages, endothelial cells, and CD4⁺ T helper cells. Some of these cells respond by releasing greater amounts of the proinflammatory cytokine IL-6. Higher levels of this cytokine are associated with a procoagulant profile and increased disease severity.⁹⁴

4.3.6 COVID-19 and neurological disease

ACE2 is present on the **neuroglial cells** and neurons of the **central nervous system (CNS)** as well as on the endothelial cells lining the capillaries of the brain. Accordingly, SARS-CoV-2 RNA is present within small vesicles of the endothelial cells as well as neural cell bodies in the **frontal lobe of the cerebrum**.⁹⁹ SARS-CoV-2 has also been found in regions of the **brainstem**, **basal ganglia** of the midbrain (**substantia nigra**, **caudate nucleus**, **putamen**, and **globus pallidus**), **thalamus**, the **limbic system** (including the **hypothalamus**, **amygdala**, and **hippocampus**), **cerebral cortex**, and **cerebellum**.¹⁰⁰

SARS-CoV-2 may enter the CNS through endothelial cells in the brain through one or both of two routes. The first potential route is via the blood by either infected leukocytes crossing the **blood:brain barrier (BBB)** or by infected endothelial cells of the brain's microvasculature. However, since SARS-CoV is not found in nonneuronal cell types of the brain early after infection,¹⁰¹ the case for entry via this route by SARS-CoV-2 is relatively weak.¹⁰²

The second route of entry into the CNS is transportation by nerves, especially by the **olfactory nerve** through the **cribriform plate** of the skull and the **vagus nerve** via the lungs or nerve endings in the digestive tract.¹⁰³ ACE2 is abundantly expressed in endothelial cells of the small intestine, which interact with the **enteric nervous system**. This, in turn, communicates to the CNS via the vagus nerve, providing a more plausible pathway for SARS-CoV-2 to enter the CNS.¹⁰² Additionally, the viral S protein may bind to nAChRs⁵⁶ that are expressed at high levels in the vagus nerve terminals as well as in the **olfactory bulb**.¹⁰⁴ This is important since cholinergic neurotransmitters modulate inflammation and coagulation.⁵⁶

COVID-19 causes neurological symptoms in about one-third of the patients and 88% of those with severe disease.^{57,105} People who develop neurological diseases tend to have more severe COVID-19, be older, and have more comorbidities.¹⁰⁶ Similar neurological symptoms are present during SARS and MERS¹⁰⁶ and occasionally during infection with HCoV-OC43, which typically only produce cold-like symptoms in humans.¹⁰³

Several reports found that among hospitalized COVID-19 patients, 36% developed neurological manifestations, including stroke and **altered consciousness**.¹⁰⁷ Stroke has been reported in 2%–6% of hospitalized COVID-19 patients.¹⁰⁸ A study from Wuhan, China,

also reported **encephalopathy** and persistent alterations in consciousness in patients who died of COVID-19.¹⁰⁹ These patients, however, had severe respiratory, cardiac, and other complications and received multiple concurrent medications.

SARS-CoV-2 causes multiple alterations to the BBB.¹¹⁰ The S1 portion of the SARS-CoV-2 S protein passes through the BBB and into the brain **parenchyma**.¹¹¹ This is followed by the production of proinflammatory cytokines by activated neuroglial cells and neurons.^{112,113} These cytokines may cause several inflammatory conditions reported in the CNS of some COVID-19 patients, including encephalitis, **rhombencephalitis**, **myelitis**, **meningoencephalitis**, and **acute disseminated encephalomyelitis**.^{108,112,114,115}

Other neurological manifestations associated with COVID-19 include **spongiosis**; altered levels of smell and taste; seizures; **meningitis**; **demyelination** of the brain and spinal cord post-COVID-19; fever; headaches; **ataxia**; convulsions; **ischemic stroke**; and intracerebral hemorrhages.^{44,48,106,116–118} Examination of the brains of COVID-19 patients revealed several pathologic events,¹¹² including **hypoxic damage**¹¹⁹ and damage to the brain stem nuclei, **panencephalitis**, and meningitis.¹²⁰ COVID-19-induced alterations to the **white matter** of the CNS may be categorized in several patterns.¹²¹ One pattern results in **medial temporal lobe** signal abnormalities and the other patterns are characterized by microhemorrhages.¹²⁰ SARS-CoV-2-related acute respiratory failure has been suggested to be due, at least in part, to damage to the brainstem in addition to direct damage to the lungs.¹⁰³

As of spring of 2021, several hundred cases of **Guillain-Barré syndrome (GBS)**, a rare, postinfection neurological disorder, have been reported in people after recovery from COVID-19.^{122–124} There are several possible underlying causes: a pathogenic, postinfection immune response triggered by COVID-19 or a para-infectious process due to direct virus-mediated **radiculopathy** in which the spinal cord becomes inflamed.¹²⁵ It should be noted that while no cells or viruses were found in the **cerebrospinal fluid (CSF)** in one report,¹²⁵ at least one adult and one child have had SARS-CoV-2 RNA in their CSF.¹²⁴ There are likely more instances of SARS-CoV-2 in the CSF since this fluid is not always tested for the presence of the virus. GBS may also occur following respiratory or gastroenterological illness by bacteria, including *Campylobacter jejuni* and *Mycoplasma pneumonia*, or viruses, such as cytomegalovirus, HIV, and Zika virus.¹⁰⁶ GBS also followed immunization with an anti-influenza vaccine in the United States in 1976 which affected over 200 people.^{107,126}

GBS is associated with weakness or paralysis in the legs and arms; inability to walk; difficulty in breathing, speaking, chewing, or swallowing; and severe nerve pain. It is an acute immunopathologic disease of the peripheral nerves and the nerve roots. SARS-CoV-2 patients with GBS have a sensorimotor, demyelinating form of the disease in which **myelin**, a protective fatty material covering nerves, is removed, leading to nerve damage.¹²² One study revealed that many patients with GBS (44%) required mechanical ventilation and 11% of them died.¹²²

4.3.7 COVID and psychiatric disease

Analysis of 63 studies involving over 100,000 people from 24 different countries found an overall prevalence of 17.4% of the population at large demonstrated symptoms of

posttraumatic distress syndrome (PTDS). Among health professionals, the prevalence was 17.2% and, interestingly, it was 15.5% among COVID-19 patients.¹²⁷ Further work could address the prevalence of this disorder in the family of the patients as well as others who were quarantined or lost their employment.

Delirium is common during SARS-CoV-2 infection, especially in older patients.¹²⁸ In several studies, the prevalence of delirium in hospitalized patients ranged from 25%–33% in patients over the age of 50 years.^{129,130} Delirium was associated with poor outcomes and death.¹¹² COVID-19 patients may also have long-term neuropsychiatric syndromes.¹³¹ Additionally, people with severe psychiatric conditions, such as **schizophrenia**, are more likely to die from COVID-19 than people without these conditions.¹³²

COVID-19 is associated with other neuropsychiatric complications, such as new-onset **psychosis**, dementia-like syndrome, **affective disorders**, anxiety, confusion, and agitation.^{106,133} Confusion and agitation are primarily found in patients admitted to ICUs. Some long-term survivors of COVID-19 have depression, panic disorder, and obsessive-compulsive disorder, in addition to PTDS.¹³⁴ Patients generally have a rapid recovery from these disorders when administered low doses of antipsychotics.¹³⁵ While most symptoms are found among older patients, a disproportional number were under the age of 60 years.^{136,137} While PTDS, delirium, anxiety and depression have been previously reported in people with SARS and MERS infections, perceptual disturbances and delusions were rare in these two coronaviral diseases.^{131,138,139} COVID-19-related psychosis has been described in multiple case reports in patients without a prior history of this condition. Symptoms of psychosis include delusions, hallucinations, and disorganized thought and behavior.^{140–142} In one case study, a COVID-19 patient presented with the above symptoms and required inpatient psychiatric hospitalization. The patient had acute **hyperglycemia** attributable to diabetes as well as **hypertension**. A neurological workup did not reveal any abnormalities. His psychosis was judged to have been secondary to COVID-19 infection.¹³⁹

A study of primarily infected men from Qatar found that 50% or more presented with insomnia, anxiety, or agitation.¹³⁷ Depression, irritability, changed appetite, disorientation with or without confusion, aggression, delusions, euphoria/elation, thoughts of self-harm, and impaired concentration or memory were seen in at least 20% of the patients. It should be noted that approximately half of the men in this study had prior psychotic or **bipolar I disorder**. This suggests that people with prior mental health problems may be at risk of developing further mental health illnesses.¹³⁷

Drugs used to treat COVID-19 and other COVID-19-related diseases may play a role in psychotic illnesses in some people. In one case report, a middle-aged patient with no psychiatric history developed auditory hallucinations and attempted suicide.¹⁴² At that time, he was being treated with hydroxychloroquine, a drug with potential neuropsychiatric side effects.¹⁴³ High-dose corticosteroid use also may result in COVID-19 psychosis.¹⁴¹

Potential causes of the COVID-19-related psychiatric symptoms during COVID-19 include direct viral invasion of the CNS or severe systemic inflammation.^{139,141} Factors affecting psychiatric illnesses may vary among countries, which may be partially due to differences in lockdown measures, sociodemographic and cultural influences, and the patients' access to physical and mental healthcare.¹³⁷

4.3.7.1 COVID-19 and neurotransmitters and their receptors

In addition to the previously mentioned link between the neurotransmitter acetylcholine and its receptor, altered activity of at least two other neurotransmitter receptors is seen during COVID-19. **Molecular mimicry** is another potential cause of neuropsychiatric disease during infection with SARS-CoV-2.¹⁴⁴ For example, SARS-CoV-2 infection is occasionally associated with the development of an autoimmune response against one of the **N-methyl-D-aspartate (NMDA) receptors for glutamate**.¹⁴⁵ Glutamate is found in 90% of the neural synapses and increases the likelihood that a neural signal will be transmitted. Two subunits of the NMDA receptor have similarities to viral **nonstructural protein (nsp)** 8 and nsp9 which may result in immunological cross-reactivity.^{144,145} Anti-NMDA receptor encephalitis results from the production of IgG antibodies to the NMDA receptor GluN1 subunit. The binding of the NMDA receptors to these autoantibodies leads to the internalization of the receptors, blocking their ability to receive excitatory glutamatergic signals.¹⁴⁶ These patients have recent onset working memory defects, altered mental status, or psychiatric symptoms, including confusion, agitation, auditory hallucination, **cata-tonia**, and speech dysfunction.¹⁴⁵ Symptoms improve following high-dose steroid and immunoglobulin treatments, lending additional support to the autoimmune origin of the disease.

SARS-CoV-2 proteins interact directly with receptors for the inhibitory neurotransmitter **gamma-aminobutyric acid (GABA)**.¹⁴⁴ GABA decreases neurons' **action potential**, making the neuron less likely to transmit a signal. Viral-mediated alteration of GABA and NMDA functioning is known to be associated with multiple disease states, including memory and learning; Alzheimer's, Huntington's, and Parkinson's diseases, amyotrophic lateral sclerosis (Lou Gehrig's disease), epilepsy, depression, and anxiety disorders.¹⁴⁴

4.3.7.2 COVID-19, cytokines, and mental illness

A *metaanalysis* identified altered blood levels of proinflammatory and antiinflammatory cytokines in a variety of mental illnesses.¹⁰² Increased levels of IL-6 or its receptor are found in the blood of COVID-19 patients with depressive and bipolar disorders, schizophrenia, PTSD, and sleep disorder. Increased levels of IL-1 β are found in the blood of COVID-19 patients with depressive disorder and PTSD and the CSF of patients with schizophrenia and bipolar disorder. Increased levels of TNF- α are found in the blood of patients with depressive and bipolar disorders as well as PTSD. Increased levels of the antiinflammatory cytokine IL-10 are found in patients with depressive disorders and suicidal ideation, while another antiinflammatory cytokine, **transforming growth factor- β (TGF- β)**, is found in patients who succumbed to suicide. See Raony et al. [102] or a more complete listing of the role of cytokines in mental illnesses.

4.3.8 COVID-19 and special senses

4.3.8.1 Olfaction and gustation

Sudden-onset alterations in smell (**olfaction**) and taste (**gustation**) are very common early during COVID-19.^{112,147} Approximately 58% of tested COVID-19 patients were found

to have severe loss of olfaction that was not associated with **rhinorrhea** (runny nose), nasal obstruction, severe nasal congestion, or inflammation.¹⁴⁸ These alterations tend to occur before other COVID-19 symptoms and often last for 2–4 weeks. The loss of the gustation may be linked to loss of **tongue papillae**,¹⁴⁹ which contain taste buds, as well as decreased olfaction. Of those patients experiencing the latter, olfactory dysfunction alone was present in 65.7% of the cases, gustation alone in 25.4%, and decreases in both senses in 9% of the patients.¹⁵⁰ These decreases are even found in approximately two-thirds of patients with mild cases of the disease.¹⁵¹ Reduced olfaction is also one of the most common long-term symptoms of SARS-CoV-2 infection.¹⁵² Prevalence of altered olfaction and gustation vary by geographic region: 54% in Europe, 51% in North America, 31% in Asia, and 10% in Australia.¹⁵³ Interestingly, loss of olfaction and gustation is more common among females and younger individuals, even though males and older adults are typically more susceptible to COVID-19 overall.¹⁵⁴

In one study of 60 hospitalized, sex- and age-matched COVID-19 patients, only one person failed to have some degree of olfactory dysfunction as determined by the 40-item, quantitative, University of Pennsylvania Smell Identification Test (UPSIT).¹⁴⁸ Interestingly, only 35% of the patients were aware of olfactory deficiencies prior to the test. The olfactory test scores were not related to disease severity. The degree of olfactory loss also did not significantly differ between men and women in this study, unlike the situation in Alzheimer's and Parkinson's disease, in which the men have a greater degree of dysfunction.¹⁴⁸

While olfactory receptor cells express neither ACE2 nor **transmembrane serine protease 2 (TMPRSS2)**,¹⁴⁸ SARS-CoV-2 may enter the CNS via the olfactory neuroepithelium. Cleavage of the coronaviral S protein into S1 and S2 by TMPRSS2 is required for S protein activity. In mice, the virus is present in the olfactory bulb 60–66 hours postinfection and spreads from there to other regions of the brain.¹⁵⁰

The olfactory neuroepithelium can regenerate over time if its stem cell layer has not been damaged.¹⁴⁸ While as many as 72% of the patients recovered from olfactory dysfunction within 1 month,¹⁵⁵ other patients experienced long-term dysfunction. A study that used the 34-item, culturally adapted UPSIT, nevertheless, revealed that 11.7% of the patients still had severe loss of olfaction 6 months after COVID-19 diagnosis.¹⁵⁶ Another study revealed some degree of decreased smell perception in most patients that lasted for at least 15 months after COVID-19 onset. This study reveals that while olfactory function improved significantly, it was not completely restored.¹⁵⁷ A reduction in IL-6 levels correlates with the recovery of smell and taste.¹⁵⁰ Levels of IL-6 also correlate with the extent of olfactory and gustatory dysfunction, especially in people with reductions in both senses.¹⁵⁰

4.3.8.2 Vision

Unlike alterations in smell and taste, very few changes were reported in the eye. Only rare cases of **macular degeneration** were observed in SARS-CoV-2-infected patients (0.8%).⁸¹ A separate study conducted 3 months after recovery from COVID-19 found that all patients had normal findings in the anterior and posterior portions of both eyes with no signs of retinal damage.¹⁵⁸ Some patients with severe COVID-19 did experience changes in the thickness of the blood vessels in the walls of the eye, but not the vessels' lumens.¹⁵⁹ Most or all cases of this condition are reversed by 3–6 months.^{159,160} Although CoV-2-SARS RNA was detected in the retinas of COVID-19 patients,¹⁶¹ a separate study found no retinal lesions.¹⁵⁹

4.3.9 COVID-19 and the endocrine system

COVID-19 affects the production of various **hormones** by **endocrine** organs. Its effects on sex hormones will be discussed later in this chapter. Among other endocrine organs, the **adrenal gland**, **thyroid gland**, and **pancreas** are altered structurally as well as functionally.¹⁶² While SARS survivors had self-resolving alterations in the hypothalamic-pituitary pathway, there is no evidence of direct pituitary or hypothalamic alterations during COVID-19.¹⁶³ [Table 4.1](#) compares the characteristics of hormones that are affected during COVID-19, except for hormones of the reproductive system, which are found in [Table 4.2](#).

4.3.9.1 COVID-19 and the adrenal gland

One study found **acute adrenal insufficiency** in 23% of people with SARS-CoV-2-associated severe or critical lung tissue lesions.¹⁶⁴ This condition includes symptoms, such as high levels of potassium and low levels of sodium, due to decreased production of the adrenal hormone **aldosterone**. **Epinephrine**, **norepinephrine**, and the immunosuppressive stress hormone **cortisol** are also produced by the adrenal glands. Epinephrine and norepinephrine (the “fight-or-flight” hormones) mobilize leukocytes into the blood, while epinephrine and cortisol direct leukocytes to become more specialized and direct them to the

TABLE 4.1 The Effects of COVID-19 on Hormones.

Hormone	Organ of Origin	Functions	Effect of SARS-CoV-2
Aldosterone	Adrenal cortex	Regulates blood pressure	Decreases levels
Epinephrine Norepinephrine	Adrenal medulla	Increases heart rate Increases breathing rate Increases blood sugar levels	Decreases levels
Cortisol	Adrenal cortex	Reduces inflammation Immunosuppressant	Decreases levels
Thyroid-stimulating hormone	Anterior pituitary	Stimulates thyroid hormone release	Decreases levels
Triiodothyronine (T3)	Thyroid gland	Upregulates metabolism	Decreases levels
Insulin	β cells of pancreas (islets of Langerhans)	Decrease blood sugar levels	Decreases levels
Amylin	β cells of pancreas (islets of Langerhans)	Slows emptying of stomach Reduces food intake	Increases levels
Glucagon	α cells of pancreas (islets of Langerhans)	Increases blood sugar levels	Increases levels
Adiponectin	Adipose tissue	Decreases glucose production Decreases triglyceride levels in blood	Decreases levels
Leptin	Adipose tissue	Slows digestion Slows absorption of carbohydrates	Increases levels

This table summarizes the effects of COVID-19 on the level of hormones produced by the adrenal, anterior pituitary, and thyroid glands, as well as hormones produced by the islets of Langerhans in the pancreas and white adipose tissue. Hormones produced by the reproductive system are found in [Table 4.2](#).

TABLE 4.2 The Effects of COVID-19 on Reproductive System Hormones.

Hormone	Organ of Origin	Functions	Effects of SARS-CoV-2
Testosterone	Testes	Develops male characteristics Increases libido Decreases neutrophil numbers Increases lymphocyte numbers	Increases viral entry into cells Increases disease severity Decreases ACE2 expression Decreases hospitalization rate ^a Decreases mortality rate Aids in viral clearance in URT ^b Antiinflammatory in URT Increases lung repair in LRT ^c
Estrogen	Ovaries	Develops female characteristics Stimulates antiviral T cell activity	
Progesterone	Ovaries	Increases secretory activity of female tissues Develops new blood vessels prior to pregnancy	
Luteinizing hormone in females	Anterior pituitary	Stimulates produce estrogen and progesterone Triggers ovaries to release eggs	Increased levels during COVID-19
Luteinizing hormone in males	Anterior Pituitary	Stimulates testosterone production Sperm maturation	Increased levels during COVID-19

^aDuring pregnancy.^bUpper respiratory tract.^cLower respiratory tract.

This table summarizes the effects of COVID-19 on the level or activity of hormones related to the reproductive system, including hormones produced by the testes, ovaries, and anterior pituitary gland.

sites where they are required.¹⁶³ The adrenal cortical cells of people with fatal cases of SARS in 2003 underwent degeneration and necrosis. A similar situation might also contribute to the acute adrenal insufficiency found during COVID-19.¹⁶⁵

Multiple case reports reveal acute bilateral adrenal hemorrhage.¹⁶⁶ Additionally, in COVID-19 patients with severe or critical lung lesions, 23% have signs of acute **adrenal infarctions (AAI)**, usually bilateral.¹⁶⁴ Bilateral AAI in COVID-19 patients is associated with **antiphospholipid syndrome**.¹⁶⁶

4.3.9.2 COVID-19 and the thyroid

COVID-19 is frequently associated with thyroid abnormalities, including COVID-19-related **subacute thyroiditis** that is usually followed by complete remission.^{162,167} This condition might result from an antiviral immune response and has, in rare cases, been attributed to reactions following vaccination with the Pfizer and AstraZeneca vaccines.¹⁶⁸

While total levels of **thyroxine (T4)**, one of the two thyroid hormones that regulate metabolism are not changed during COVID-19, hospitalized patients with moderate to critical COVID-19 have decreased levels of **triiodothyronine (T3)** and **thyroid-stimulating hormone (TSH)** than those having non-COVID-related pneumonia.¹⁶³ The levels of T3 and TSH correlate with disease severity and are lower in patients with fatal disease than in survivors.¹⁰⁹ TSH is produced by the pituitary gland, but it regulates the release of thyroid hormones. Thyroid functions are restored after recovery from COVID-19.¹⁶⁹

4.3.9.3 COVID-19 and the pancreas

The pancreas is unusual in that its **islets of Langerhans** serve as endocrine organs that secrete hormones that regulate blood sugar levels, while other areas of the pancreas function as an **exocrine** organ that secretes enzymes involved in the digestion of food. The activity and viability of some pancreatic cells are altered during COVID-19.¹⁶² ACE2 is expressed by both exocrine and endocrine cells.¹⁷⁰ Accordingly, SARS-CoV-2 RNA has also been detected in pancreatic endocrine, exocrine, endothelial, and immune cells.¹⁷¹ In some individuals with a fatal disease, some of the islet β cells are infected with SARS-CoV-2.^{171–173}

Insulin is produced by β cells in the islets of Langerhans and is the primary hormone that decreases blood glucose levels upon binding to its receptor on cell surfaces. Infection of the β cells by SARS-CoV-2 in vitro decreases the numbers of insulin-secretory granules and insulin secretion.¹⁷² The hormone **amylin** is also produced by the pancreatic islet β cells and is cosecreted with insulin. This hormone reduces food intake and slows the emptying of the stomach. Higher levels of amylin are released during COVID-19.^{155,174} SARS-CoV-2 selectively damages β cells.¹⁷³ In this manner, the virus may thus aggravate prior diabetes and alter energy metabolism.¹⁷⁵ Several studies have also found new-onset hyperglycemia and **diabetic ketoacidosis** in COVID-19 patients.¹⁷³

Like ACE2, TMPRSS2 is expressed at low levels on the β cells in the islets, but to an even lower extent on islet α - and γ -cells.¹⁷² **Heparan sulfate** and **neuropilin-1**, a member of a family of signaling proteins, are highly expressed on β cells. Heparan sulfate regulates β cell survival, while neuropilin-1 serves as a viral entry factor. A study by Wu et al.¹⁷³ found that neuropilin-1 allows SARS-CoV-19 to selectively enter β cells in cultured pancreatic islets in vitro as well as β cells in vivo. The interaction between the viral S protein and neuropilin-1 might impede the insulin-secretory pathway, thus raising blood glucose levels.¹⁷⁶ High levels of blood glucose are predictors for mortality and severe morbidity in SARS patients and might therefore be similar during COVID-19.¹⁷⁷

In addition to reductions in β cell numbers and secretory ability, infection of the islet cells alters the cells' physical characteristics and the type of hormone released. Transmission electron microscopy visualizes SARS-CoV-2 particles inside the cells and near the nucleus. The infected cells' endoplasmic reticulum-Golgi intermediate compartment is enlarged and vacuolized, as is also seen in infected intestinal, kidney, and airway epithelial cells.¹⁷² SARS-CoV-2 infection induces β cell **transdifferentiation** via the **eukaryotic translation initiation factor 2 signaling pathway** both *ex vivo* and as seen upon autopsy of COVID-19 patients.¹⁷¹ As a result, transcriptional expression of islet α and exocrine cell markers are upregulated in β cells after SARS-CoV-2 infection. This process results in lower expression of the insulin gene and higher expression of the genes encoding **glucagon** and **trypsin**.¹⁷¹ Glucagon raises blood glucose levels and is a hormone typically produced and secreted by the islet α cells, while trypsin is a digestive system enzyme produced by pancreatic exocrine cells.

4.3.9.4 COVID-19 and energy homeostasis

White adipose tissue has many functions, including acting as a storage site for fats. It is also an endocrine organ that produces hormones, including **adiponectin** and **leptin**.¹⁷⁸ The small **adipocytes** present in lean individuals aid in metabolic homeostasis, while the

enlarged adipocytes found in obese individuals are proinflammatory, secreting molecules that contribute to insulin resistance.¹⁷⁸ Macrophages infiltrate and accumulate in white adipose tissue, contributing to inflammation.¹⁷⁸

Adiponectin is a member of a group of hormones that regulate blood glucose levels and glucose metabolism. It increases insulin sensitivity while decreasing levels of serum fatty acids, glucose, and **triacylglycerol**.¹⁷⁸ SARS-CoV-2 can infect adipocytes and reduce levels of adiponectin, decrease the adiponectin/leptin ratio, and increase circulating leptin levels in patients with severe disease.¹⁷⁴ Leptin is produced predominantly by adipose cells and the intestine. It works with the hypothalamus to decrease hunger and reduce fat storage in the adipocytes. The imbalance in the adiponectin/leptin ratio promotes insulin resistance and increases blood levels of glucose and fatty acids. In addition to its effects on hormonal production and secretion, infection with SARS-CoV-2 promotes strong antiviral activity in white adipose tissue.^{174,178}

Not all energy-regulating hormone levels are altered during COVID-19. The following hormones are not known to be affected: **pancreatic polypeptide**; the intestinal hormones **gastric inhibitory polypeptide**, **peptide YY**, and **glucagon-like peptide-1**; and **ghrelin** from the stomach.¹⁷⁴ The activity of these hormones varies and includes increasing or decreasing appetite and **satiety** (a feeling of fullness); the emptying of the stomach; the rate at which food travels along the digestive tract; and the secretion of hydrochloric acid into the stomach.

4.3.10 COVID-19 and the urinary system

4.3.10.1 COVID-19 and the kidneys

Kidneys are large, complex biological filtration systems that produce urine and determine which materials are to be removed from the body and which are to be resorbed into the blood as well as the quantity of each. The kidneys thus play a major role in blood homeostasis and waste removal. The **nephron** is the functional unit of urine production in the kidney. The majority of the nephron is composed of a series of tubules that return materials from the **glomerular fluid** to the capillaries surrounding them. Material that is normally released in the urine includes water, salt ions, **uric acid**, and other metabolic waste products at levels appropriate to the person's condition at that time. Proteins, sugar, and cells are not normally found in the urine.

SARS-CoV-2 directly infects kidney cells.⁴⁷ In one study, **proteinuria** (the presence of protein in the urine) was seen in 43.9% of COVID-19 patients and **hematuria** (bloody urine) in 26.7% of the patients, along with high levels of glucose, leading to the **electrolyte**.¹⁷⁹ Elevated levels of serum **creatinine** and **urea nitrogen** were present in approximately 14% of COVID-19 patients. **Acute kidney injury (AKI)** was present in 5.1% of the patients. Patients with kidney disease are at higher risk for in-hospital death.¹⁸⁰ Additionally, the immunosuppressive agents used to treat diseases of the **glomerulus** and kidney transplant recipients cause decreased lymphocyte numbers and function¹⁸⁰ that may impede the elimination of the virus.

SARS-CoV-2-induced **renal** pathology includes loss of the **brush border** of the nephrons' **proximal convoluted tubules** and the presence of **hemosiderin granules** and **pigmented casts**.⁴⁷ These casts are associated with the breakdown of skeletal muscle and

are one of the main causes of acute renal failure. Renal failure is at least partially due to increased levels of **myoglobin** that are released following SARS-CoV-2-induced damage to muscle cells. High levels of myoglobin damage the kidneys by constricting the kidney's blood vessels, killing the cells in the proximal convoluted tubules and obstructing the distal convoluted tubules.¹⁸¹

SARS-CoV-2 RNA accumulates in the tubules along with viral N and S protein deposits, both of which are only present in tubules expressing ACE2. Additionally, aggregated red blood cells obstruct those renal capillaries which resorb the water, sugar, and ions from the glomerular filtrate.⁷⁴ The reported incidence of COVID-19 patients developing AKI varies widely, from 0.9% to 29% in different treatment centers.⁴⁷ Diagnosis of AKI is based upon estimated glomerular filtration rate and levels of serum creatinine and urea nitrogen.^{91,180} As many as 50% of patients with COVID-19-associated ARDS develop AKI.^{182,183} and COVID-19 patients with AKI are more likely to require mechanical ventilation. The in-hospital mortality rate for COVID-19 patients with AKI is 50%, as opposed to 8% in those without AKI.¹⁸⁴ Although AKI also occurs during SARS and MERS, it is less common than during COVID-19. Nevertheless, AKI was still a major risk factor for mortality during SARS.^{185,186}

Patients surviving COVID-19-associated AKI are more susceptible to developing longer-term adverse kidney outcomes than patients without COVID-19.¹⁸⁷ The primary histological finding upon kidney autopsy is an **acute tubular injury** in which the cells of the renal tubules are injured or die, which may lead to renal failure.¹⁸³ **Collapsing glomerulopathy** is also found in COVID-19 patients, especially those of African descent.¹⁸⁸ This disease manifestation involves the collapse of the **glomerular capillaries**, hypertrophy, hyperplasia of the **podocytes** (increased size and number of these cells), and proteinuria. Podocytes are found in the **Bowman's capsule** that wraps around glomerular capillaries and are involved in blood filtration during one of the earliest steps in the production of urine.

The mechanism by which SARS-CoV-2 induces AKI may involve the excessive systemic inflammatory response that occurs during **cytokine release syndrome** ("**cytokine storm**") in which the kidney tubules are so greatly damaged that dialysis is necessary.¹⁸⁹

The cytokine storm is characteristic of infection with SARS-CoV-2 and SARS-CoV in patients with AKI.³⁸ IL-6 is the most important causative agent for cytokine storms and the IL-6 titer correlates with serum creatinine levels.¹⁸⁹ Among patients with COVID-19-associated AKI, 15%–20% require **kidney replacement therapy (KRT)**, which includes renal dialysis and kidney transplantation.^{183,184,190} Almost half of the patients with AKI who require KRT die during hospitalization. The remainder eventually recover enough of their kidney function to discontinue KRT and 62.2% of them experienced full renal recovery.¹⁹¹

AKI is common in critically ill adults with COVID-19.^{51,78,179,189} Children may develop AKI but are at far lesser risk than adults. One very small study found that among COVID-19 cases in children, 1 of 3 children was critically ill and admitted to an ICU with AKI.¹⁸⁹ Several of the infants developed the gastrointestinal disease, thrombocytopenia, anemia, abnormal complement activation, or high levels of serum IL-6. While one infant died, the other two infants either completely or partially recovered after treatment involving plasma exchange and continuous KRT to remove proinflammatory cytokines and restore complement regulation.¹⁸⁹ Dehydration due to intestinal involvement and hypoperfusion of kidneys significantly increased the risk of developing AKI.¹⁸⁹

The kidneys of COVID-19 patients experience **hypoxia**, which is partially due to an increase in the levels of the **prostaglandin D synthase** enzyme.⁹¹ SARS-CoV-2 infection of the kidneys triggers the expression of hypoxic damage-associated molecules by the infected tubules. Infection also increases infiltration of macrophages into the area surrounding the renal tubules as well as deposition of the complement system's MAC on the tubules, followed by hypotonic cell death.⁹¹ Release of inflammatory compounds by macrophages and the complement cascade may be responsible for the tubular damage to the kidneys. Interestingly, while the kidney tubules experience moderate to severe damage accompanied by abundant leukocyte infiltration, the nephrons' **glomeruli** are not affected.⁹¹

4.3.10.2 COVID-19 and the renin-angiotensin-aldosterone system

Angiotensin-converting enzyme (ACE) is a crucial part of the **renin-angiotensin-aldosterone system**. The operation of this system begins with **renin**, a molecule produced by the kidneys, inducing the cleavage of **angiotensinogen** to form angiotensin I. ACE cleaves angiotensin I to generate angiotensin II, while ACE2, the cellular receptor for both SARS-CoV and SARS-CoV-2, acts in an opposing manner, decreasing angiotensin II levels.⁴⁷ Angiotensin II triggers the release of the hormone aldosterone from the adrenal cortex. Aldosterone increases the amounts of sodium and potassium ions and water that are brought back into the blood during urine production in the kidney tubules, increasing blood pressure. Proper levels of these ions are critical to the activity of the nervous and muscular systems, including the **cardiac muscles** of the heart.

Host cell vitamin D3 described later, stimulates the expression of ACE2 while reducing that of renin.¹⁹² Binding of large amounts of SARS-CoV-2 to ACE2 on the kidneys, lungs, intestines, and brain cells reduces ACE2 expression, which increases levels of angiotensin II and raises blood pressure. Angiotensin I and II are also linked to inflammation and fibrosis as well as increased production of ROS that oxidize DNA, RNA, proteins, and phospholipids.^{52,193} ACE2 may thus protect infected kidneys and lungs from an acute injury, despite binding to SARS-CoV as it enters its target cells.¹⁹⁴

While large differences in plasma ACE levels exist among individuals, ACE plasma levels tend to be similar within families, indicating a genetic component.¹⁹⁵ Some COVID-19 patients have a large deletion of a 287-bp repeat in the ACE gene. SARS-CoV patients that developed ARDS were more likely to be **homozygous** for this deletion than patients who do not develop ARDS. The presence of the deletion correlated with the mortality rate in ARDS patients.¹⁹⁴ Since ACE2 actions are **antagonistic** to those of ACE, ACE2 protects against the development of ARDS.¹⁹⁴ The SARS-CoV S protein downregulates ACE2, increasing the likelihood of infected people developing ARDS.¹⁹⁶

4.3.10.3 COVID-19, the kidneys, lungs, and heart

The kidneys and lungs cooperate in the **lung-kidney axis**. Injury to the kidney tubules leads to increased production of IL-6, resulting in high levels of this cytokine in the blood. This, in turn, promotes higher permeability between the alveoli of the lungs and the capillaries surrounding them, which increases the exchange of oxygen and carbon dioxide between the lungs and the circulatory system. IL-6 may contribute to pulmonary hemorrhaging in the lungs of COVID-19 patients, accompanied by a large drop in blood volume and blood pressure that can result in life-threatening shock.⁴⁶

In addition to triggering immune-mediated damage to the kidneys, SARS-CoV-2 may also kill kidney cells directly. Affected people may also have **albuminuria** (albumin in the urine). Viral RNA is also present in the urine. Removal of IL-6 from the blood might decrease damage to the kidneys and other organs.⁴⁶

Crosstalk between the heart and kidneys may play a role in the development of AKI in patients with COVID-19 as well. Cardiomyopathy and acute **viral myocarditis** may cause congestion in the renal vein which carries blood away from the kidneys, hypotension, and renal hypoperfusion which reduces the rate of urine production.⁴⁶ SARS-CoV and MERS-CoV infection can also contribute to the causation of myocarditis.⁷⁵

4.3.11 COVID-19 and the digestive system

ACE2 is abundant on the surface of cells lining the stomach, small intestines, and rectum, thus providing an entry molecule for SARS-CoV-2 throughout most of the digestive tract. Infectious virus is present in human feces and blood. SARS-CoV-2 can be detected in the stool even after it is no longer detectable in respiratory secretions.¹⁹⁷ Many COVID-19 patients have diarrhea and vomiting. A small amount of SARS-CoV-2 is found in some patients' feces.¹⁹⁷ Digestive symptoms are also commonly caused by some coronavirus of animals, while other animal coronaviruses primarily attack the respiratory or nervous systems (see Chapters 5 and 6).

A metaanalysis found that many children infected with SARS-CoV-19 have viral RNA in their feces, contact with which may transmit the virus to other people.¹⁹⁸ Rectal swabs of 80% of infected children, including those who are asymptomatic, contain virus RNA even after it was no longer detectable in the nasopharyngeal swabs.¹⁹⁹ Interestingly, even though COVID-19 is generally less severe in children, virus levels are higher in rectal swabs of children than in adults.²⁰⁰ SARS-CoV-2 with solely gastrointestinal symptoms is sometimes seen in both adults and children.¹⁹⁷ Infants and young children are at higher risk of COVID-19-associated gastrointestinal involvement and dehydration than older patients. One study found that infants with AKI stage 3 have gastrointestinal symptoms with dehydration very early after infection.¹⁸⁹

Given the similarities between SARS-CoV and SARS-CoV-2, it should be noted that as many as 60% of people with SARS reported liver abnormalities.²⁰¹ The liver sometimes becomes dysfunctional during COVID-19 due to **steatosis** and other liver injuries caused directly by the virus or indirectly by side effects of medications. Abnormally high serum levels of the liver enzymes alanine aminotransferase and aspartate aminotransferase are found during COVID-19 progression and are indicative of liver damage.²⁰² Elevated levels of the latter enzyme have been reported in 62% of COVID-19 cases admitted to ICUs, as opposed to 25% of those who were not admitted to an ICU. Additionally, patients with severe COVID-19 have higher liver aminotransferase levels than patients with less severe disease.²⁰³

The liver chemically alters and detoxifies material in the blood and then removes the resulting waste products. SARS-CoV-2-related liver damage affects not only waste removal but also decreases the normal chemical changes to drugs by the liver and delays their removal. This increases the levels of drugs in the body and may lead to excessive and pathogenic drug concentrations. This liver dysfunction may be caused by SARS-CoV-2 or

by antiviral drugs. Both liver cells and **bile duct** cells express ACE2, especially the latter. This is of importance since bile duct cells are important for liver regeneration and the immune response.²⁰⁴

4.3.12 COVID-19 and the integumentary system

SARS-CoV-2 infection results in a variety of disorders of the **integumentary system**, some of which are mild and others that are very serious. Often COVID-19 severity correlates with the severity of integumentary diseases. The integumentary system consists of skin, hair, a variety of glands, nervous system receptors, and other associated tissues including white adipose and other connective tissues. Several types of cells in these structures are infected by SARS-CoV-2, even though ACE2 is only found on a small number (0.2%–0.5%) of skin cells. The highest levels of ACE2 in the integumentary system are found on **keratinocytes** of the skin's epidermis, which comprise 97.4% of the infected cells, but ACE2 is also expressed on sweat glands, and skin **fibroblasts**, and **melanocytes** that are partially responsible for skin color.⁴⁵

4.3.12.1 COVID-19 and the skin

Dermatological lesions are present in 1%–20% of patients with COVID-19 and usually are benign and self-limiting.^{149,205} Biopsy samples only detect low levels of SARS-CoV-2 RNA.²⁰⁶ While the virus is rarely found in the **epidermis** or **sebaceous glands**, viral S protein is detected in sweat glands and ducts.²⁰⁷ ACE2 and TMPRSS2 are found in abundance in the glands' luminal secretory cells and their location corresponds to that of the S protein.²⁰⁷ See Agnihothri¹⁴⁹ for an excellent review of the many types of rashes and papules associated with COVID-19, including oral and genital ulcerations, blisters, and **macules**. A transient rash may also be present in infected newborns.²⁰⁸

Early during the pandemic, only 2 of 1099 COVID-19 patients were reported to have a rash.²⁰³ Soon afterward, however, cutaneous disorders were found in small groups of patients.²⁰⁹ At the end of 2021, however, approximately one-third of COVID-19 patients have rash or **mucositis** (inflammation of the mucus membranes), including lip mucositis; **erythema** (reddening of the skin); **urticaria** (hives), and **maculopapular** or **vesicular** rash.¹⁴⁹ COVID-associated urticaria and fever may be signs of infection in otherwise asymptomatic people.²¹⁰ Manifestations of COVID-19 in the skin are linked to disease severity: **pernio-like lesions** are present in cases of mild disease, vesicular/urticarial/maculular erythema/morbilliform eruption in people with intermediate disease severity, and **retiform purpura** in critically ill patients.^{149,211} Patients with pernio-like lesions and maculopapular rash have the highest survival rates (approximately 98%).²¹²

Erythema multiforme-like lesions are self-limiting allergies of the skin and mucous membranes with concentric three-ring target-like plaques. They may be found during COVID-19 in adults and children.¹⁴⁹ Children with COVID-19 who develop this rash usually have mild respiratory/gastrointestinal symptoms or are otherwise asymptomatic.²¹³ Temporal studies indicate that vesicular and urticarial manifestations generally occur earlier than other COVID-19 symptoms. Maculopapular, **papulosquamous**, and vascular lesions are present in symptomatic patients.^{149,214}

Pernio-like lesions may be present in COVID-19 patients with mild symptoms, especially children and young adults, and are usually present on the digits.^{215,216} This condition is characterized by **chilblain lupus**, a cutaneous form of systemic lupus erythematosus ("**lupus**") and is manifested as bluish-red lesions of the skin that may be itchy or painful.^{215,216} Patients with "**COVID toes**" rarely develop systemic disease symptoms or are hospitalized. Their age is generally between 32 and 35 years.²¹¹ During pernio-like lesions, **T lymphocytes (T cells)** and monocyte-derived **dendritic cells (DCs)** infiltrate the area, especially in the region adjacent to the epidermis and dermal glands. These cells include CD8⁺ T killer cells and macrophages, but not CD4⁺ T helper cells, B cells, or neutrophils.²⁰⁷ COVID-19-related pernio-like lesions are associated with high-level expression of **myxovirus resistance protein A**, a marker of **type I IFN** signaling. The type I IFNs include **IFN- α** and **IFN- β** and are important for SARS-CoV-2 eradication. Only low levels of SARS-CoV-2 RNA, IL-6, and **caspase 3** are present.²¹⁶ Caspase 3 is part of an enzymatic cascade that is active during apoptosis.

A much more serious skin manifestation of SARS-CoV-19 infection is thrombotic retiform purpura which is found only in critically ill adults.²¹⁶ This skin disorder is characterized by branching purpuric lesions due to blood clots completely blocking blood flow to **dermal** and **subcutaneous** blood vessels and is associated with necrosis. Significant levels of IL-6 and caspase 3 are present, but the type I IFN response is minimal.²¹⁶ The lack of adequate IFN responses allows large-scale viral replication. Viral proteins are present in the skin vessels' endothelium, where they trigger extensive complement activity. A small study of the skin of five individuals with severe COVID-19 found evidence of complement activation in the vicinity of virus' S protein in the microvasculature of both diseased and normal-appearing skin.⁵² The S protein colocalizes with the complement components C4d and MAC in this microvasculature.⁵²

In young patients with **multisystem inflammatory syndrome in children (MIS-C)**, described later in this chapter, the incidence of skin rash is approximately 74%. Interestingly, patients with rash tend to have fewer respiratory symptoms, ICU visits, use of ventilators, and shorter hospital stays than those without rash.²¹⁷

Several antimalaria drugs used to treat COVID-19 may cause cutaneous disorders, including rashes, dry skin, urticaria, acute generalized **exanthematous pustulosis**, **psoriasis**, and **mucocutaneous dyspigmentation**.²¹⁸ Other drugs used to treat COVID-19 also have the potential to cause cutaneous disease.²¹⁸ The side effects of azithromycin include cutaneous severe skin reaction-associated fever, **angioedema**, skin pain, red or purple skin rashes, blistering, and skin peeling. Remdesivir may also cause rashes. Ribavirin is associated with localized **scleroderma**, maculopapular and eczematous lesions, skin dryness, **pruritus** (itchy skin), and rash. Lopinavir/ritonavir may cause **exfoliative erythroderma**, photosensitivity, areas of skin **hyperpigmentations**, pruritus, and urticaria. Nitazoxanide may cause pruritus, urticaria, rash, and redness. Camostat mesylate is associated with rash, itching, yellow discoloration of the skin, and purpura. Tocilizumab may cause pruritus and allergic reactions and increased risk for other skin infections. Anakinra has been linked to rashes, wound infection, and **cellulitis**. Additionally, vaccination may lead to urticaria, scleroderma, and maculopapular rashes. For a more detailed list of the dermal manifestations of other treatment or preventative measures, see Türsen.²¹⁸

4.3.12.2 COVID-19 and hair

Alopecia (hair loss) is a common occurrence following COVID-19. Several types of alopecia are found in different groups of people.²¹⁹ These types include **acute telogen effluvium** and **alopecia areata**, found in both children and adults, and **androgenetic alopecia (AGA)**, generally found in men in their 30s to 40s. Alopecia or bleaching of the hair may be a side-effect of several COVID-19 treatment options, including chloroquine and hydroxychloroquine, intravenous antibodies, IFN, colchicine, and several general antiviral drugs, such as ribavirin.^{22,218} For an excellent review of COVID-19 drugs and hair, see Türsen.²¹⁸

Acute telogen effluvium (TE) is a large but temporary loss of hair during the **telogen** (resting) phase of the hair cycle. This form of hair shedding appears approximately 3 months after trauma, including childbirth, starvation, high fever, and bacterial and viral infections, including SARS-CoV-2.²²⁰ This condition may last for as long as 6 months. In a cohort of nonhospitalized COVID-19 patients, excessive hair loss was present in 38.5% of the individuals, 61.5% of which had an only moderate disease.²²⁰ The members of the cohort were all adults whose ages ranged from 22 to 67 years. While these results are of interest, no children were included in this study and 92.3% were females, while COVID-19 is much more common in males.

Hair loss disorders differ among racial groups both before and during the COVID-19 pandemic. The percentage of cells in telogen, hair density, and growth rate typically varies among ethnic groups.²²¹ In whites and Asians, the hair that is lost during grooming is more likely to be full-length with an attached root. By contrast, in those of African descent, the root is likely to be absent and the shaft of the hair that is lost has longitudinal fissuring, which suggests hair breakage.²²² Prior to the COVID-19 pandemic, TE was most common among whites and least common among blacks. During the pandemic, however, TE was most common in Latinos. Blacks had no demonstrable change in TE before or during the COVID-19 pandemic (Cline et al., 2021).²²³

In a case study of the effects of COVID-19 on the hair of children,²¹⁹ one 10-year-old boy with COVID-19 was diagnosed with TE. He displayed diffuse hair loss in the absence of **alopecic patches**, erythema, scaling, crusts, and erosions. The other patient was a 13-year-old boy who had MIS-C with hepatic and gastrointestinal involvement. He had an alopecic patch and was diagnosed with alopecia areata. Alopecia areata is a chronic autoimmune disorder targeting the epithelial cells of the hair follicle.²²⁴ Taking supplemental zinc may increase hair regrowth in children with nutritional deficiencies and may do so after recovery from COVID-19 as well.²²⁵ Both forms of hair loss occur in adults with COVID-19 as well.^{226,227}

IL-6 is known to inhibit hair shaft elongation and proliferation of the hair-producing cells in the hair follicles.²²⁸ Elevated levels of IL-6 are present during COVID-19 and androgens (male sex hormones) induce its production.²²⁹ Females also produce androgens but to a far lesser extent. The presence and extent of AGA are associated with individual susceptibility to SARS-CoV-2 infection. AGA is often found in men with severe COVID-19 that requires ventilation or results in death. These men are typically 35–45 years of age.²³⁰ AGA may accompany bilateral pneumonia in SARS-CoV-2-infected white males.²³¹ Androgen sensitivity is increased in postpubescent men and appears to be a factor in the

greater prevalence of AGA in men than in women.²³² Androgen sensitivity will be described in greater detail below.

4.3.13 COVID-19 and biological sex

Multiple studies have reported that significant differences in COVID-2 severity and mortality exist between the sexes of adults, with men being much more vulnerable than women to severe disease.²³³ Although the percentages of affected males and females differ among these studies, men appear to be 7%–10% more prone to fatal disease than women, and that 57%–73% of the total deaths occur in men. It should be noted, however, that in addition to differential expression of male and female sex hormones, men also have a higher rate of comorbidities, including hypertension, smoking, and coronary artery disease.²³³

4.3.13.1 COVID-19 and sex hormones

Sex hormones are best known for their ability to stimulate sexual activity and the growth of sex-related organs. **Androgens** are “male” sex hormones that include **testosterone** and its derivatives. “Female” sex hormones include **estrogen**, **estradiol**, and **progesterone**. Both men and women make both sets of hormones, however, females make more estrogens than males and males make more androgens than females. Prior to puberty, children of both sexes make little to no sex hormones and older adults produce less of these hormones than young adults. Sex hormones have multiple effects on the body, including increased bone density and regulating muscle, nervous, and immune system activity.

The effects of sex hormones on the immune system vary in men and women. Estrogen strengthens some aspects of the immune system by stimulating antiviral T cell activity.²³⁴ The reports of the levels and activity of testosterone during COVID-19 are conflicting. Some studies report that androgens increase the numbers and activity of neutrophils, thus contributing to the cytokine storm present in severe cases of COVID-19.^{40,235} Low baseline amounts of total testosterone in men, however, have been reported to be associated with more COVID-19-related severe respiratory disease and a higher mortality rate than in men with normal testosterone levels.²³⁶ In that study, testosterone levels negatively correlated with neutrophil numbers, but positively correlated with protective lymphocyte count.

Just as the characteristics of sex hormones have differing effects on antiviral immunity, a sexual dimorphism exists in COVID-19 susceptibility. Being a biological male and having hypertension are the most important risk factors for developing COVID-19 complications.²³⁷ A possible explanation for the higher mortality rate and disease severity among male patients and the extremely low mortality rate among prepubescent boys may be due to the action of the androgens testosterone and dihydrotestosterone on target tissues and organs that bear the **androgen receptor (AR)**, such as the lungs.²³¹ Since androgens begin to be produced during puberty, postpubescent males may be more likely to develop severe COVID-19 symptoms than boys.²³² [Table 4.2](#) compares the characteristics of reproductive hormones that are affected during COVID-19.

4.3.13.2 COVID-19, ACE2, and TMPRSS2 in the reproductive system

Androgens aid SARS-CoV-2 entry into host cells and their levels decrease ACE2 activity.²³⁸ ACE2 and TMPRSS2-bearing cells are prime targets for SARS-CoV-2 infection. TMPRSS2 and ACE2 levels differ in males and females, with cells in the testes expressing higher levels of ACE2 than cells in the ovaries.²³⁹ ACE2 is widely expressed in the female reproductive system and is found in the ovary, uterus, vagina, placenta, and breasts. In the ovaries, ACE2 is present on the **ovarian stroma, granulosa cells, ovarian follicles, and oocytes**.^{162,240} ACE2 is present throughout the process of follicle and oocyte maturation. It is also expressed in the **endometrium** and its expression changes during the menstrual cycle.²⁴¹

While TMPRSS2 in males is found only in **spermatogonia** and **spermatids**, ACE2 is found in the testes (spermatogonia, **seminiferous tubules, Leydig** and **Sertoli** cells) as well as the prostate epithelium.^{239,242,243} Leydig cells produce testosterone, while Sertoli cells assist in regulating sperm cell differentiation. The differences in COVID-19 severity and mortality rate between the sexes may be at least partially due to androgen-mediated expression of ACE2 and TMPRSS2 in the sex organs.²⁴⁴

4.3.13.3 COVID-19 and the reproductive system in males

Human spermatozoa express ACE and ACE2 as well as other cellular factors required for coronaviral cell entry. Different stages of the sperm production in the testes, maturation in the epididymis, movement of the sperm through the seminiferous tubules, and the addition of the components of the semen prior to ejaculation may react differently to viral-induced alterations in angiotensin II activity.²⁴⁵ Inflamed testes (**orchitis**) are present in 19% of male COVID-19 patients, even in the absence of SARS-CoV-2 in the testes.²⁴⁶

COVID-19 patients with moderately severe disease have reduced sperm concentration and total numbers of sperm per ejaculate, progressive sperm motility, and complete motility than men who recovered from the mild disease.¹⁶³ Drugs used in COVID-19 treatment may also affect fertility in males.²⁴² The broad-spectrum antiviral drug ribavirin together with IFN decreases sperm count,²⁴⁷ while ribavirin may also cause sperm DNA fragmentation for up to 8 months.²⁴⁸ Combination treatment with lopinavir/ritonavir or chloroquine impairs spermatogenesis in vivo, possibly by oxidative damage caused by ROS.²⁴²

The reproductive system of men with fatal cases of COVID-19 incurs damage to the organs whose cells express ACE2. COVID-19 damages these cells, including the seminiferous tubules, vacuolation of Sertoli cells, reduced numbers of Leydig cells, and lymphocytic infiltration into the testes.^{162,249} While the presence of the virus in the semen is controversial, several studies report that SARS-CoV-2 is often present in the semen of men with either acute COVID-19 or during the **convalescent** phase.^{162,250}

Androgen insensitivity is a condition in which men are resistant to androgen activity. These men, therefore, have some of the physical traits of women. It is important to note that the AR gene is located on the X chromosome.²³² This means that since normal males only have one X chromosome, they are twice as likely to develop androgen sensitivity as women since women have two X chromosomes. COVID-19-related deaths in males are greater than six times that of females in the 40–49 age range and two times more than that of females aged 30–49 years.² While females generally have lower levels of testosterone than males, females with conditions such as **polycystic ovary syndrome** have larger than normal

amounts of testosterone and dihydrotestosterone and lower levels of estrogen. The resulting hyperinflammation, **hyperandrogenism**, and increased androgen sensitivity renders these women more susceptible to severe COVID-19 than normal women.^{232,238}

Androgen sensitivity is linked to a shorter CAG repeat length in the AR gene. Men with this shorter form of the receptor are more prone to androgenetic alopecia, acne, and oily skin. Androgen sensitivity is believed to increase severity and mortality in men with COVID-19.²³² Men of African descent tend to have the shorter form of this gene.²⁵¹ Blacks, Latinos, and Native Americans are hospitalized and die at a higher rate from COVID-19 than do whites.^{211,244,252} Inuits (Alaskan Natives) and Native Hawaiians have the highest number of SARS-CoV-2 infections and deaths per 100,000 people in the United States.²⁵² The length of the AR may have a major role in this ethnical vulnerability to severe COVID-19.²³²

4.3.13.4 COVID-19 effects on pregnant women and fetuses

Studies have not reported a large degree of COVID-related damage to the female reproductive systems under normal conditions. Pregnancy, however, alters levels of ACE2 expression, female sex hormones, and the type of immune response. During pregnancy, women double their expression of ACE2 in the placenta, uterus, and kidneys.^{239,253} Normally, pregnant women and their fetuses are at high-risk for many infectious diseases due to a hormonal-induced switch in the immune response from an antiviral, proinflammatory type of T helper cell response (**Th1** response) to an antiinflammatory T helper cell response that promotes antibody production (**Th2** response). This switch generally increases the mother's susceptibility to severe infection, especially viral infections.

Both estrogen and progesterone levels are higher than normal during pregnancy. This affects the immune response since estrogen receptors are expressed on T and B cells, **natural killer (NK) cells**, macrophages, DCs, and neutrophils.²³⁹ Th1 responses are associated with low levels of estrogen. At higher levels, estrogen stimulates a Th2 response.²³⁹ Women's COVID-19-related case-fatality rate increases at approximately 50 years of age, about the time in which women go through menopause. At that time, levels of estrogen and progesterone decreased and continue to do so over the ensuing decades.²³⁹

Counterintuitively, a decrease in the Th1 response during pregnancy appears to decrease some disease manifestations in the SARS-CoV-2-infected mother, perhaps due to lowering the extent of inflammatory damage that is associated with COVID-19. A study of infected women during the third trimester of pregnancy from 16 hospitals in Spain found that almost all these women only developed a cough and fever, although one woman did die from thromboembolism.²⁵⁴ Pregnant women infected by SARS-CoV-2 have decreased rates of hospitalization, admission to ICUs, and need for mechanical ventilation, but not a decreased risk of death.^{239,255} Another study, however, reported that the case fatality rate for pregnant women was nearly 0% for COVID-19. This is much less than the 18% fatality rate for SARS and the 25% rate for MERS.²⁵⁶

Full-term infants that are born when their mothers have active COVID-19 are typically healthy in most respects and have normal weight and **Apgar scores** of neonatal health. Nevertheless, abnormalities have been reported that include miscarriage, intrauterine growth restriction, small gestational size, increases in numbers of preterm births, and neonatal death.^{257,258} In one study, five of thirteen pregnant women required emergency

cesarean sections due to fetal distress, premature rupture of the membrane, or stillbirth.²⁵⁹ Infection with SARS-CoV-2 thus may put neonates at a higher-than-normal risk of complications during delivery. In one study, while 67% of newborn infants are sent to intermediate care for virus-related illness, none died or were SARS-CoV-2-seropositive for at least 1 month after birth.²⁵⁴ Newborns and infants are unable to produce their own antibodies for 6 months after birth, thus seropositivity before that time results from the mother's IgG antibodies passing through the placenta during fetal development.

As of mid-2020, confirmed cases of vertical transmission of SARS-CoV-2 *in utero* were rare.²⁵⁸ Infected women do not have viral RNA in the amniotic fluid, cord blood, or breast-milk.²⁶⁰ Nevertheless, SARS-CoV-2 can be transmitted **postpartum** from mother to child.²⁵⁸ It should also be noted that some maternal treatment options may be harmful to fetuses, especially ribavirin, which has the potential to be **teratogenic** (causing craniofacial and limb defects) to the embryos of pregnant mice.²⁶¹ However, no reports of **teratogenic** effects or embryonic pathology directly attributable to SARS-CoV-2 have been published.¹⁶²

4.3.14 COVID-19 case number and severity in children and adults

Studies conducted in China early during the pandemic focused on patients who had developed pneumonia. In this population, the mortality rate was high but young to middle-aged adults appeared to be much less likely to develop the severe disease than older adults.¹⁵⁴ Adults between the ages of 20–40 years were found to have the highest rate of hospitalizations, but most recovered.

A metaanalysis found that 17% of SARS-CoV-2-positive children are asymptomatic. Fever is present in 51% of the children, cough in 37%, and 29% had low levels of either total leukocytes or lymphocytes.⁴³ Almost none of the children developed severe illness, except for those children with comorbidities. Most of the infections in children in this study (80%) were part of family clusters and the children were the last to become SARS-CoV-2-positive.^{43,200} Despite developing less severe diseases, children are more likely to have been infected by adults than vice versa.²⁶² Infected children are more prone than adults to develop URT symptoms, including sore throat, congestion, and runny nose.⁴³ Of greater than 45,000 total cases tested, only 0.9% were under the age of 10 years and 1.2% were 10–20 years old.²⁰⁰ COVID-19 patients under the age of 18 years typically have a good prognosis and only require supportive care. They usually recover within 2 weeks.²⁶³ However, asymptomatic children may be able to transmit SARS-CoV-2 for 3 weeks, 2 weeks longer than infected adults.²⁶⁴

In a study of household contacts of 198 SARS-CoV-2-infected people, 24% tested positive for viral RNA over a 14-day followup period. Of the nonhospitalized contacts of SARS-CoV-2-positive children, 68% developed URT, and 64% developed neurologic symptoms, while a few reported having a fever or appeared to be asymptomatic.²⁶⁵ Infected contacts generally had fewer symptoms and a shorter duration of illness. The most common symptoms were URT symptoms in children younger than 18 years and adults over the age of 50, while neurologic symptoms were most common in patients aged 18–49 years. The percentage of these household contacts reporting LRT symptoms is a function of age: 21% for people under the age of 18 years, 60% for those aged 18–49 years, and 69% for patients over the age of 50 years.

One of the more important means of preventing microbes from reaching the LRT is the beating of **cilia** (short, hair-like projections found on the outer surface of cells lining much of the respiratory tract). Cilia move mucus containing microbes upward and away from the lungs. In mice, this removal system is less effective in older animals than in young mice, perhaps playing a role in the greater infection rate and development of severe disease in older people.²⁰⁰

As one ages, the cytokines become increasingly able to assume an unbalanced proinflammatory type of response named “inflamm-aging,”²⁶⁶ in which a person is in a low-grade, chronic inflammatory state. This causes a predisposition to illness and greater susceptibility to chronic diseases. It also increases the likelihood of developing more severe viral diseases in adults, especially in the elderly population.²⁰⁰ In cells lining the human nasal region, older adults have increased levels of inflammatory cytokines as well as a decreased ability to clear viral infections. This may also increase the severity of respiratory system diseases in the elderly.²⁰⁰ The decrease in immune functioning in this population also results in decreases in the efficacy of vaccines.

An interesting connection occurs during coinfection with rhinoviruses, SARS-CoV-2, and age. This connection was previously found to occur between rhinoviruses and influenza A viruses.²⁶⁷ While rhinoviruses and four species of human coronaviruses typically cause the common cold, rhinoviruses are the predominant respiratory viruses. Concurrent in vitro infection of primary human bronchial epithelial cells with human rhinoviruses and SARS-CoV-2 triggers an **interferon (IFN)** immune response that inhibits SARS-CoV-2 replication even when rhinoviruses are added to the culture 24 hours after the addition of SARS-CoV-2.²⁶⁸ Rhinovirus infection may also be a factor in the age-related differences in disease severity between children and adults since school-aged children have a higher prevalence of rhinovirus infections than adults.²⁶⁸ Mathematical models predict that interactions between these viruses may affect whole populations of people. The increasing prevalence of rhinovirus infections might affect COVID-19 epidemiology at the individual host level as well.²⁶⁸ It will be of great interest to determine whether a significant level of rhinovirus-related COVID-19 inhibition also occurs in vivo and, if so, whether this decreases COVID-19 severity and mortality rates.

4.3.15 Multisystem inflammatory syndrome in children

Even though serious SARS-CoV infections are very infrequent in children, some do develop MIS-C; formerly known as a pediatric inflammatory multisystem syndrome. The case definition for this condition includes the following: SARS-CoV-2 infection, hospitalization, age under 21 years, fever for at least 24 hours, laboratory evidence of inflammation, and multisystem organ involvement. MIS-C is characterized by fever, stomachache, abdominal pain, vomiting, diarrhea, red eyes, rashes on the trunk, and **mucocutaneous lesions**.^{269,270} Infected children are more apt to develop mild disease symptoms, such as fever, vomiting, and diarrhea, than adults.²⁶⁹ Since SARS-CoV-2 infects and replicates in the gastrointestinal tract, diarrhea may be the first symptom observed in half of the COVID-19 cases in children.²⁷¹ Some children only develop digestive system symptoms.

MIS-C affects the dermatologic, cardiac, gastrointestinal, renal, hematologic, and neurologic systems, and is due to inappropriate immune responses.^{43,272} In one study,

92% of the children with MIS-C developed symptoms of the gastrointestinal, 80% of the cardiovascular, and 70% the respiratory systems. Additionally, 76% of children with MIS-C had hematologic and 74% had mucocutaneous involvement.²¹⁵ Of these MIS-C patients, 80% were placed in ICUs, 20% received mechanical ventilation, and 48% received vasoactive support.²⁷³ Even though most children with MIS-C require intensive care, their mortality rate is approximately 2%.¹⁴⁹ Neurological symptoms of MIS-C include altered mental status, encephalopathy, **cranial nerve palsies**, stroke, seizures, and decreased reflexes.²⁷⁰

MIS-C has been reported in children in the United States, several European countries,²⁷⁴ and Asia.^{275,276} MIS-C has several similarities to **Kawasaki disease (KD)** and **toxic shock syndrome**, including fever and alterations of the skin, mucous membranes, and feet and hands.¹⁴⁹ While coronaviruses may trigger KD, they represent less than 10% of the viruses associated with this disease. Despite their similarities, MIS-C and KD are distinct disease entities, and less than half of the MIS-C affected children meet the formal criteria for KD.^{215,277} While MIS-C is typically found in older children and adolescents and Hispanic and non-Hispanic black children, KD is more common in children younger than 5 years of age who are of East Asian descent and is more frequent in males.^{149,278} Children with MIS-C also have more gastrointestinal symptoms.

In a study of 98 MIS-C patients in New York, a disproportionate number were black (43%) or Latinos (36%). Thirty-one percent of the children were 0–5 years old, 42% were 6–12 years old, and 26% were aged 13–20 years.²⁷⁹ All children had subjective fever or chills, 97% had **tachycardia** (rapid heart rate), 80% had gastrointestinal symptoms, 60% had a rash, 56% had eye conditions, and 27% had mucosal changes. Vasopressor support was required for 62% of the patients, 53% had myocarditis, 80% were taken to an ICU, and two deaths occurred in this group.²⁷⁹

MIS-C is also associated with abnormal heart muscle functioning and shock. As many as 73% of the patients in one study required aid to maintain their blood pressure and heart muscle contractions.²⁷⁸ MIS-C is often accompanied by unusual hyperimmune responses.¹²³ While the pathogenesis of MIS-C is multifactorial, the robust immune system of children, the ability of the S protein to act as a **superantigen**, and production of immune complexes play major roles in the systemic inflammation observed during MIS-C.^{149,280}

MIS-C is associated with COVID-19 in children both timewise and geographically (**temporospatial-linked**).²⁷⁸ Since the incidence of MIS-C in an area follows COVID-19 incidence with a lag time of 4–5 weeks, MIS-C appears to be a postinfectious manifestation of COVID-19 in children. MIS-C cases were detected almost simultaneously in Italy, the United Kingdom, and New York City.²⁷⁸ The incidence of this post-COVID inflammatory syndrome is 1 out of 1,000 infected children.²⁷²

4.3.16 Long COVID syndrome (chronic or post-COVID-19 syndrome)

Long COVID syndrome includes long-term symptoms or abnormalities lasting at least 12 weeks after the onset of acute COVID-19.^{183,270} Some of the common long-term pulmonary conditions are **dyspnea** (difficulty in breathing), and reduced exercise capacity, and hypoxia. In the cardiovascular system, chronic symptoms include **palpitations**, chest pain, scarring of myocardial tissue, arrhythmias, and tachycardia (rapid heart rate).

Persistent neuropsychiatric symptoms include chronic fatigue, migraine-like headaches, cognitive abnormalities, anxiety, depression, insomnia or nonrestorative sleep, anxiety, obsessive-compulsive disease, and dementia in persons aged 65 years and older.²⁷⁰ Other symptoms include new or worsening diabetes; new-onset autoimmune thyroid diseases similar **Hashimoto's thyroiditis** or **Graves' disease**; bone pain, demineralization of the bones, femoral neck fractures in the elderly, and osteonecrosis; muscle pain, weakness, and atrophy; and alterations in the gut **microbiome**.^{270,281–283}

These disorders are not only present in hospitalized COVID-19 survivors but may also be present in younger and healthier patients at low risk of mortality.²⁸⁴ A study found that 70% of these low-risk people have decreased functionality in at least one organ that lasted for 4 months or more after recovery from acute COVID-19. It should be noted that only 19% of this population had required hospitalization.²⁸⁴ Symptoms of Long COVID Syndrome include mild impairment of the pancreas (40%), liver (28%), heart (26%), lungs (11%), and kidneys and spleen (4% each). Multiorgan impairment was found in 29% of the Long COVID Syndrome patients.²⁸⁴ Due to long-term effects of COVID-19 that continue after hospital discharge, it has been suggested that interdisciplinary cooperation is indicated to provide comprehensive care for Long COVID Syndrome patients in outpatient settings.²⁷⁰

Possible mechanisms involved in the pathophysiology of Long COVID Syndrome include virus-induced pathophysiology, immunologic alteration and inflammatory damage during acute infection, and other disorders present following critical disease. The latter disorders may be due to microvascular ischemia, immobility, and metabolic alterations found after recovery from life-threatening illnesses.²⁷⁰

4.3.17 The role of genetic factors in COVID-19

Genetic differences among people and populations affect their susceptibility to SARS-CoV-2 infection and disease severity. Among the most important factors are differences in the **alleles** of the six genes encoding the **major histocompatibility complex class I and II molecules** (MHC class I and class II, respectively). MHC I and II molecules are critical for the activation of CD8⁺ T killer cells and CD4⁺ T helper cells, respectively. Since there are over a hundred alleles for each of the six genes, there are innumerable combinations of the MHC class I and II molecules. This means that, excluding identical twins, everyone has a unique MHC I and II profile. Some of these alleles are linked to better or worse immune responses to microbes, including coronaviruses.

Differences in the gene encoding the ACE2 protein play an important role in disease severity among human populations. Three common variants of the *ACE2* gene are present in European populations but are rarely found in Asians. One of these variants is believed to alter the entry of the viruses into their target cells. The other two variants change the conformation of the virus' binding site.²⁸⁵ After the appearance of COVID-19 in Italy, the number of infected people increased very rapidly and the fatality rate was approximately 10% higher than that found in China (4%) and South Korea (1.2%).²⁸⁶ Genetic differences in the *ACE2* gene include the presence or absence of a large nucleoside deletion, as described earlier in this chapter.

Animal models, including nonhuman primates, are needed to test how genetic differences affect the course and severity of infection in addition to testing the safety and efficacy of drugs and vaccines against SARS-CoV-2. This virus causes respiratory disease in infected rhesus monkeys that is similar to that seen in humans and lasts for 8–16 days. Additionally, high levels of the virus are found in swabs from the nose and throat of all tested monkeys. Monkeys also shed SARS-CoV-2 in the feces. This suggests that rhesus monkeys may serve as an animal in which to perform preclinical testing prior to clinical testing in humans.

One of the advantages of using these monkeys is that they are **outbred**, like humans, and therefore differ genetically, while many strains of mice and rats are **inbred** so that all members of these rodent strains are genetically identical. Inbred animals cannot model the complex interactions among genetic alleles that are found in natural animal populations. A large difficulty in using rhesus monkeys is their slow reproductive rate, their size, and the expense of housing and caring for these animals. Additionally, these monkeys have much longer life spans than rodents so it is difficult to study age-related changes in the vulnerability and disease severity in individual animals. Great apes, especially chimpanzees and bonobos, are much more closely related to humans and might be better models than monkeys for studies of coronavirus-related diseases. Their use has the same difficulties as rhesus monkeys and, additionally, they are endangered species.

4.4 The causative virus

4.4.1 Introduction to severe acute respiratory syndrome coronavirus-2

The RNA genome of coronaviruses is very large (27,000 to 31,000 nucleosides). Within an almost incredibly short time period from the first detection of a novel human coronavirus, the complete genetic information of one strain of SARS-CoV-2 was available in early January 2020. This original SARS-CoV-2 strain had been isolated from a person with acute respiratory infection on December 26, 2019. This information, as well as genetic information from other COVID-19 patients, led to the placement of the new virus into the subgenus *Sarbecovirus* of the *Betacoronavirus* genus, which also contains SARS-CoV.²⁸⁷ MERS-CoV, by contrast, is placed into the *Merbecovirus* subgenus. The structure and function of coronavirus proteins, as well as genomic and mRNA, are described in Chapter 1.

The large size of coronaviruses' genomes makes them very susceptible to mutations. The viral polymerase used in their replication is also very error-prone. In addition, coronaviruses' RNA frequently undergoes **recombination** (exchange of genomic RNA) with that of coronaviruses of other host species,²⁸⁸ allowing some of the viral variants to change animal hosts and undergo zoonotic transmission. See Chapter 1 for a more detailed explanation of the cause of mutations in coronaviruses.

Both SARS-CoV and SARS-CoV-2 use human ACE2 as their receptor. Its expression is upregulated in COVID-19 patients.⁴⁷ ACE2 is expressed on many cell types, including type II alveolar cells and bronchial transient secretory cells of the lungs, microglial cells, and neurons of the nervous system, heart (myocardial cells), liver and bile duct cells, renal tubular cells, epithelial cells of the small intestine, and oral epithelial cells.⁴⁵

This potentially allows the virus to infect and damage these cells and organs.²⁰⁴ The greatest amounts of ACE2 in the respiratory system are found in the ciliated cells of the nostrils and decrease as one proceeds into the lower regions of the respiratory tract.²⁸⁹ Viral infectivity and replication efficiency vary among people and are less variable in the nostrils than in the more distal portions of the airway.

4.4.2 The question of the reservoir and intermediate hosts of severe acute respiratory syndrome coronavirus-2

4.4.2.1 *Bats as reservoir hosts*

Bats and pangolins have been postulated to be the reservoir and intermediate hosts of SARS-CoV-2, respectively. The overall genetic identity of SARS-CoV-2 RNA with that of several bat coronaviruses derived from horseshoe bats, especially bat-89 SL-CoVZC45 and bat-SL-CoVZXC21, is 90%, however, they have much less similarity in the S protein gene and differ in five of six critical parts of the protein's RBD.²⁹⁰ The viral RBD of SARS-CoV-2 also contains 5 substitutions when compared to that of the Bat-CoV RaTG13 virus, while the RBD of the pangolin coronavirus hCoV-9/pangolin/Guangdong/1/2019 has only one substitution compared to SARS-CoV-2.

Interestingly, bats were hibernating during the winter when the SARS-CoV-2 epidemic began. Bats were also not sold in the live animal markets in Wuhan from which the pandemic has been proposed to have originated. This suggests that while bats may act as reservoir hosts for SARS-CoV and SARS-CoV-2, other mammals are more likely to be the intermediate hosts.²⁹⁰ Looking at domestic animals as potential intermediate hosts, pigs and dogs have relatively low levels of ACE2 in their respiratory tract and so are unlikely to act as reservoir hosts.²⁹⁰ Cats, however, are susceptible to SARS-CoV-2 infection,¹⁶ but human-to-cat transmission is much more likely than cat-to-human transmission as discussed in Chapter 6.

4.4.2.2 *Pangolins as intermediate hosts*

Pangolins from live animal markets are considered by many to be the intermediate host of SARS-CoV-2 from which zoonotic transmission occurred. Pangolins harbor viruses that are closely related to SARS-CoV-2 in their lungs, intestines, and blood. A coronavirus isolated from a Malayan pangolin has 90.7%-100% amino acid identity with human SARS-CoV-2 isolates. Importantly, the RBD of the S protein is almost identical in these two coronaviruses.²⁹¹ The pangolin coronavirus was present in 68% of 25 Malayan pangolins at a wildlife rescue center in March 2019 but was not found in 4 Chinese pangolins.

Pangolin coronaviruses can be placed into two sublineages of SARS-CoV-2-like coronaviruses, one of which has an RBD that is very similar to the RBD of SARS-CoV-2.²⁹² Analysis of the SARS-CoV-2 receptor found that the similarity of the ACE2 sequence is slightly higher between humans and pangolins (84.8%) than between that of humans and bats (80.8%–81.4%). Additionally, SARS-CoV-2 and pangolin coronaviruses share all six parts of the RBD.²⁹² Infected pangolins, however, have shortness of breath, lack of appetite, wasting, inactivity, and crying. The lungs showed DAD, reducing the size of the alveoli.²⁹¹ This coronavirus is also found in dead Malayan pangolins. Since Malayan pangolins are

susceptible to severe disease from this coronavirus, they may not serve as the immediate predecessor to SARS-CoV-2²⁹³ or maintain the transmission chain in nature.

Pangolins are often unwilling participants in the illegal wildlife trade. Two SARS-CoV-2-like coronaviruses were present in Sunda pangolins (*Manis javanica*) obtained from wildlife traffickers.²⁹² These pangolins are not **endemic** in most of China, except for the Yunnan province far to the south of Wuhan. All the pangolins from Wuhan were likely brought from Southeast Asia, the home of most of these animals.²⁹⁴ The SARS-CoV-2-like Sunda pangolin viruses are only 86%–92% identical to SARS-CoV-2.²⁹⁵ Additionally, the genomic RNA of all tested pangolin coronaviruses do not contain the insertion of the furin-like S1/S2 cleavage site that differentiates SARS-CoV-2 from other related beta-coronaviruses, including the bat RaTG13 coronavirus.²⁹⁶

Approximately 40% of pangolins rescued from illegal wildlife traders are infected by at least one SARS-CoV-2-like virus. This rate of viral prevalence demonstrates that pangolins are highly susceptible to infection by SARS-CoV-2-like viruses.²⁹⁵ Rescued pangolins are often unhealthy, with skin eruptions, acute interstitial pneumonia, and pulmonary fibrosis. More than 85% of the rescue pangolins die. Moreover, pangolin species in the wild are rare to endangered and typically live a solitary lifestyle,²⁹⁷ again making maintaining sustained pangolin-to-pangolin transmission unlikely.²⁹⁵ The pangolins may have been infected by SARS-CoV-2-like bat coronaviruses when these bats, pangolins, and other wildlife were brought into close contact during transport from Southeast Asia or while in Chinese wildlife markets.²⁹⁵

4.4.2.3 Other animals as intermediate hosts

The SARS-CoV-19 receptor is the human form of ACE2. Multiple forms of animal ACE2s are present in rhesus monkeys, Mexican free-tailed bats, Himalayan palm civets, raccoon dogs (*Nyctereutes procyonoides*), Chinese ferret-badgers (*Melogale moschata*), hog badgers (*Arctonyx collaris*), dogs, cats, rabbits, and Sunda pangolins.¹³ Based upon the avidity of binding between the viral S protein and ACE2 from various mammalian species, pangolins, cats, ferrets, pigs, cattle and other bovines, rodents, and nonhuman primates might serve as the intermediate hosts that transmit SARS-CoV-2 to humans.^{17,298,299} Human and rhesus monkey forms of ACE2 have the strongest receptor avidity for the SARS-CoV-2 S protein and 94.7% of other nonhuman **catarrhine primate** species have very high binding avidity scores.¹² Catarrhine primates include some Old World monkeys, apes, and hominids. Several species of pangolins, deer, and rabbits have high avidity scores, while many agricultural animal species found in Hubei Province have medium scores, including cattle, sheep, and goats. Camels and pigs, both of which are infected by other coronaviruses, as described in Chapter 6, score low, and rats and mice have the lowest receptor-binding activity.¹² Of note, of 37 tested bat species, 8 have low binding avidity scores and 29 bat species score very low. Importantly, the tested bats include three *Rhinolophus* bat species, believed to serve as the primary reservoir hosts for SARS-CoV-2.¹² More work needs to be done to determine whether the avidity testing was performed in a region of the S protein that is conserved among multiple coronaviruses, thus leading to false-positive results.

Cats and ferrets are highly susceptible to SARS-CoV-2 and most become ill to severely ill.^{237,299} Since some of the above animal species are agricultural animals or house pets,

these animals should be monitored for possible zoonotic infection.²⁹⁸ Our pets may be infected by their owners since SARS-CoV-19 RNA has been found in two dogs and two cats residing in homes with SARS-CoV-2 infected people.²⁸⁸ It is not known whether the pets were infected by their owners or if the cats and dogs infected their owners. In the Bronx Zoo, African lions and several species of tigers have been reported to have a dry cough that may be due to SARS-CoV-19 transmission from humans.²⁸⁸ SARS-CoV-2 replicates poorly in dogs, pigs, chickens, and ducks, while it is currently unknown whether SARS-CoV-2 infects horses and camelids.³⁰⁰

4.4.3 Comparison of severe acute respiratory syndrome coronavirus and severe acute respiratory syndrome coronavirus-2

SARS-CoV and SARS-CoV-2 have many similarities as well as some differences. Although bats may have acted as a viral reservoir for both viruses, early during the COVID-19 pandemic, both viruses are believed to have undergone zoonotic transmission from animals in wet markets of China. However, pangolins may have been the intermediate hosts for SARS-CoV-2, while palm civets and raccoon dogs appear to be the intermediate hosts for SARS-CoV (see Chapter 2).

Unlike some human coronaviruses, SARS-CoV and SARS-CoV-2 both use human ACE2 as their cellular receptor, rather than aminopeptidase N or dipeptidyl peptidase 4, which are used by some other coronaviruses. SARS-CoV-2 has a greater capacity than SARS-CoV to infect ciliated epithelial cells lining the nasal cavity and bronchioles. Both viruses infect type 1 and 2 pneumocytes,³⁹ but SARS-CoV-2 damages these cells. SARS-CoV-2 also produces a strong, pathogenic inflammation in the lungs which is accompanied by an influx of neutrophils into the area. During COVID-19, the secretion products of neutrophils may cause pulmonary embolisms or thrombosis and ARDS.⁴⁰ SARS-CoV-2 infection is more prone to result in gastrointestinal disease, including diarrhea, than SARS infection.⁷

At the whole genomic level, the RNA of SARS-CoV-2 strains is approximately 79% similar to SARS-CoV but is less stable.³⁰¹ SARS-CoV-2 RNA is also approximately 50% similar to MERS-CoV.^{302–304} Genomic sequencing also revealed that SARS-CoV-2 RNA has 96.2% similarity to RaTG13 from *R. affinis* bats, which appear to be its closest relative.³⁰² The S genes of these two viruses are also longer than those of other SARS-like-CoVs.³⁰² These human and bat coronaviruses form a distinct lineage.

SARS-CoV-2 is likely to contain genomic RNA of another, related coronavirus that was obtained by genetic recombination. While the majority of its genomic RNA is most closely related to bat coronavirus RaTG13, the SARS-CoV-2 RBD is almost identical to that of Pangolin-CoV.³⁰⁵ The complete genomic RNA of SARS-CoV-2 shares 91.0% homology with Pangolin-CoV and 97.5% amino acid identity in the S protein.³⁰⁶ The furin cleavage site portion of S protein regulates transmission between coronaviruses of other potential host species. The S protein of SARS-CoV-2 might have been the product of recombination between the furin cleavage site of RaTG13 and another coronavirus.³⁰⁵

The immune responses to SARS-CoV and SARS-CoV-2 are similar but differ in some respects. Sera from convalescent SARS patients contain neutralizing antibodies that prevent both SARS-CoV and SARS-CoV-2 from entering their target cells by blocking the viral

S proteins from binding to ACE2. These neutralizing antibodies are present in the blood for at least 2 years after a person has recovered from SARS. High levels of the **T regulatory cell (Treg)** cytokine IL-10 are also present during COVID-19, but not during SARS.³⁰⁴ Taken together, the above evidence demonstrates that SARS-CoV and SARS-CoV-2 are similar, but distinct viruses.

4.4.4 Transmission of severe acute respiratory syndrome coronavirus-2

The ability to become infected through contact with an infected surface is a major health concern and varies among viruses. The length of time that a virus remains infective is influenced by the type of surface, the susceptibility of the virus to being **desiccated** (dried out), and whether the virus is surrounded by organic material, such as blood or material in nasal secretions.³⁰⁷ SARS-CoV-2 is more stable on plastic and stainless-steel surfaces than on copper or cardboard. On plastic and stainless steel, small levels of active SARS-CoV-2 and SARS-CoV remain for up to 72 hours. By contrast, no active SARS-CoV-2 was measured on copper surfaces after 4 hours and no active SARS-CoV was detected after 8 hours.^{307,308} On cardboard, no active SARS-CoV-2 or SARS-CoV is found after 24 hours and no SARS-CoV, after 8 hours.

In aerosols, infectious SARS-CoV-2 can persist for three hours.³⁰⁷ Air conditioning systems may disperse infectious viruses unless the systems are designed to maintain negative pressure in areas containing infectious aerosols. In rooms with negative pressure, the air pressure inside the area is at a lower pressure than that outside of the area. This draws air into the area and prevents aerosolized viruses from leaving except through **high-efficiency particulate air filters** that can trap viruses.

Contact with SARS-CoV-2-contaminated wastewater is another potential transmission route. Several studies have reported the presence of viral RNA in untreated wastewater in Europe, Australia, and India.²⁰¹ While useful information, the presence of SARS-CoV-2 RNA does not necessarily indicate that the virus is still infectious to humans. However, **wastewater-based epidemiology (WBE)** is being explored as a potential tool for large-scale assessment and management of infectious disease agents.³⁰⁹ WBE might aid in identifying areas in which people shed microbes, including human coronaviruses, in their feces and urine.³¹⁰ WBE may thus be used in surveillance as an “early warning” system to ascertain whether SARS-CoV-2 has been introduced or reintroduced into a population as well as to test for the successful elimination of the virus from a region.^{309,311}

The temperature has a great influence on the decay rates of SARS-CoV-2 RNA in wastewater, which ranges from 8.0 to 27.8 days in untreated wastewater. SARS-CoV-2 RNA is likely to remain stable for a long enough time period in untreated wastewater to allow detection for WBE application.³¹² Additionally, these water samples can be stored long-term at 4°C without significant degradation of the viral RNA.³¹²

4.4.5 Severe acute respiratory syndrome coronavirus-2 mutations

Comparison of the genomic RNA from multiple human SARS-CoV-2 isolates with the very closely related bat coronavirus RaTG13 found that, unlike most other coronaviruses,

the genomic RNA of SARS-CoV-2 and RaTG13 contain unusually small amounts of **CpG** and relatively high levels of **C-to-U** conversion.³¹³ This conversion may lead to the incorporation of different types of amino acids into SARS-CoV-2 and RaTG13 proteins. C > U transitions lead to the introduction of **proline**, which breaks protein helices, into the proteins of these coronaviruses. C > U transitions also increase the levels of leucine, isoleucine, and phenylalanine, shifting to the use of more hydrophobic amino acids, which may greatly alter protein functioning.³¹³

A member of the B.1 group of SARS-CoV-2, a variant carrying a D614G mutation (aspartic acid at position 614 of the S protein is substituted by glycine) is more stable at 37°C (human internal body temperature) than other types of viral variants.⁴² The D614G variant, however, replicates better in the URT than the original strain. D614G appeared early during the pandemic and, within 4 months, became the globally predominant viral variant.³¹⁴ Due to its increased presence in the upper airways, it is more contagious than the original.^{315,316} The greater stability of the variant may have been a major factor in it becoming the dominant variant.³¹⁴ Infection with the D614G variant does not increase either disease severity, as judged by the need for oxygenation or mechanical ventilation, or mortality rates but is associated with a higher viral load and infects younger patients than variants without this substitution.³¹⁷

Another group of viral variants has an N439K mutation in its RBD. Variants with this mutation have emerged in humans at least nine times. As of January 6, 2021, viruses with the N439K mutation were reported in 34 countries. This mutation increases its RBD binding affinity for ACE2 and infection results in a slight increase in viral load in vivo. Viruses with the N439K mutation can escape inactivation by convalescent sera from some people who recovered from infection by viruses without this mutation. Nevertheless, the N439K-bearing variants do not appear to have increased virulence.³¹⁸ T cell responses to the variant were not measured in this study and may be responsible for its lack of increased pathogenicity. The fact that SARS-CoV-2 can easily and rapidly accommodate mutations in the viral genome's key region for host cell targeting suggests the potential for continuing emergence of variants that can escape at least neutralizing antibodies that protect against other variants. It should be noted that SARS-CoV-2 is preferentially spread via direct cell-to-cell transmission, rather than via an extracellular pathway, decreasing the effectiveness of neutralizing antibodies that prevent viral entry via ACE2.³¹⁹ This decreased role of neutralizing antibodies in viral variants may be important against potential reinfection as well as in the design of wide-spectrum vaccines and therapeutic modalities, many of which target **intercellular** viral transmission.³¹⁸ One approach to overcome the difficulties in vaccine and therapeutic monoclonal antibody design is to base them upon immunogenic regions of SARS-CoV-2 that cross-react with SARS-CoV³¹⁸ since these are likely to be conserved and critical to viral survival or reproduction.

4.5 The immune response

SARS-CoV-2 alters leukocytes and **erythrocytes** both physically and functionally. The virus decreases lymphocyte stiffness, increases monocyte and neutrophil size, and deforms neutrophils.³²⁰ The membranes of erythrocytes are altered, their size decreases, and the

cells are less able to stretch and relax as they pass through capillaries, inhibiting the delivery of oxygen to tissues. Additionally, these asymmetrically-shaped erythrocytes are more likely to be removed and destroyed by macrophages in the spleen. Some of the changes to erythrocytes are present for months and might be permanent.³²⁰

4.5.1 COVID-19 and the adaptive immune response

While no decrease is found in B cells levels or antibody production, numbers of T cells, NK cells, and **NKT cells** are very low during severe COVID-19.³¹⁹ Numbers of total T cells, CD8⁺ T killer cells, and CD4⁺ T helper cells are less than 800, 300, or 400/ μ L, respectively, and negatively correlate with survival. Greater than 70% of hospitalized patients have decreased numbers of these T cell types as opposed to 95% in patients in ICUs.³²¹ Low T cell numbers also negatively correlated with levels of serum IL-6, IL-10, and TNF- α .³²¹

The remaining T cells are less activated, produce low levels of IFN- γ , and have functional exhaustion as evidenced by their sustained expression of **programmed cell death 1** and **T cell immunoglobulin and mucin domain-containing-3**.^{319,321} The Treg cytokine IL-10 can trigger T cell exhaustion. The very high levels of serum IL-10 during COVID-19 may, therefore, may have an important role in the development of functional exhaustion.³²¹ In this condition, cell surface expression of the MHC II molecule HLA-DR is decreased on **professional antigen-presenting cells** (DC, monocytes, and B cells). Other molecules which are needed for T cell activation are also decreased in COVID-19 patients. These molecules include **myeloid differentiation factor 88**, the transcription factor **nuclear factor kappa-light-chain-enhancer of activated B cells** (NF- κ B), and **receptor activator of nuclear factor-kappa- β ligand**.³¹⁹

Functional exhaustion is followed by reduced levels of **interferon responsive factor (IRF)-8** and **autophagy**-related gene expression.³¹⁹ Autophagy is a process by which the cell's cytoplasm and damaged or unneeded organelles are engulfed within specialized membranes and transported to the **lysosome**, where they undergo enzymatic degradation. Calcium levels are also decreased in severe COVID-19 patients. This decrease negatively correlates with the expression of **monocyte chemoattractant protein 1 (MCP-1)**, IL-18, IL-8, IL-6, and IL-10.³¹⁹ Calcium is also a major regulator of intracellular signaling pathways, including those in T cells.

While the viral S protein is highly immunogenic and is usually used in anticoronavirus vaccines, dominant T cell **epitopes** are present in the viral **matrix protein** (M) and **nucleocapsid protein** (N) as well.³²² In patients with mild disease, the proportion of multicytokine-producing M- or N-specific CD8⁺ T killer cells is high in the blood in comparison with that of S protein-specific T killer cells. The numbers and activity levels of CD4⁺ T helper and CD8⁺ T killer cells in the variety of organs infected by SARS-CoV-2 are unknown.³²²

T cells respond to S, N, and M proteins to a much greater extent than to viral nsp's.³²³ By contrast, regions of the viral S protein contain by far the most widely recognized T cell epitopes in SARS-CoV and MERS-CoV.³²⁴ SARS-CoV-2-specific CD8⁺ T killer and CD4⁺ T helper cells have been reported in 70% and 100% of convalescent patients, respectively.³²³ Of note: anti-SARS-CoV-2 CD4⁺ T helper cell responses are also detected in 40%–60% of people who were not exposed to SARS-CoV-2. This suggests the presence of a cross-

reactive immune response following infection with one of the four less pathogenic human coronaviruses.³²³

Anti-SARS-CoV-2 IgM and IgG antibodies are present in the blood of people with mild COVID-19. Coordinated responses from CD4⁺ T helper and CD8⁺ T killer cells together with virus-specific neutralizing antibody responses are associated with milder disease. In people over the age of 65 years, virus-specific T cell responses are not well-coordinated. This may be partially due to the low levels of **naïve T cells**³²⁵ resulting from **thymic involution**, a process in which true thymus tissue decreases during aging. Since the thymus is the site in which both T helper and T killer cells mature, the elderly have lowered numbers of functional T cells even under normal conditions.

CD8⁺ T killer cells and NK cells are the two most important immune cells during viral infections. Both cell types kill virus-infected cells by releasing **perforin** and **granzymes**, which produce large pores in cells and induce apoptosis, respectively. These two cell types also release **IFN- γ** which directly interferes with viral replication and activates Th1-mediated production of primarily proinflammatory, antiviral cytokines.^{53,204} NK cells are more important in viral control during acute viral infection, while CD8⁺ T killer cells are vital for long-term protection.⁵³ Deletions in the gene encoding the **NKG2C activating receptor** decrease NK cell activation and are linked to severe COVID-19.³²⁶ NK cells from patients with severe COVID-19 may also have greater expression of the inhibitory **NKG2A receptor**. These two events are linked to functional exhaustion of NK cells.³²⁶

Some people produce feeble NK cells and CD8⁺ T killer cell responses against SARS-CoV-2. In these cases, antibodies, particularly IgM, become the principal antiviral defense.³²⁷ Large **immune complexes** are formed that consist of SARS-CoV-2 and virus-specific antibodies. The complexes are normally quickly eliminated by neutrophils and monocytes/macrophages. However, if the complexes are not rapidly removed, they can induce several **type III hypersensitivity** symptoms, including fever, inflammation, microvascular thrombosis, **glomerulonephritis**, vasculitis, rashes, and joint pain.³²⁷

Several cytokines, especially the IFNs, are also critical to an effective host immune response to viruses. Type I IFNs are produced by both immune and nonimmune cells in response to viral infections. IFN- γ , the type II IFN, is produced by activated immune system cells, especially NK and NKT cells, CD4⁺ Th1 cells, CD8⁺ T killer cells, and, to a lesser extent, by some types of activated macrophages. **Type III IFNs** are composed of the various forms of **IFN- λ** . While receptors for type I IFN are widely expressed, the type III IFN receptor is only present on a small group of cells, particularly on epithelial cells and differentiated DCs. Moreover, some subsets of type III, but not type I, IFN are expressed by SARS-CoV-infected human primary intestinal epithelial cells in vitro. Furthermore, loss of functional type III, but not type I, IFN receptors increases SARS-CoV-2 replication in human intestinal epithelial cells.³²⁸ SARS-CoV-2 has also been reported to induce an inappropriate inflammatory response that is characterized by low levels of both type I and III IFNs in the lung epithelial cells, increased levels of the monocyte-derived **chemokine (C-C motif) ligand 2 (CCL2)** (chemotactic for monocytes, **T memory cells**, and DCs) and **CCL8** (chemotactic for monocytes, T cells, NK cells, **mast cells**, eosinophils, and basophils).³²⁹

Memory cells may be divided into **effector memory** and **central memory** types. During mild COVID-19 cases, levels of effector memory CD4⁺ T cells decrease and are linked to a relative reduction in central memory CD4⁺ T subsets. It should be noted that 20%–40% of

people who never were exposed to SARS-CoV-2 have virus-specific memory $CD4^+$ T helper cells, but not memory $CD8^+$ T killer cells. This difference may be due to cross-reactivity with other, milder human coronaviruses.³³⁰ Children have “trained immunity” due to their greater exposure to viral and bacterial infections than adults and the elderly. This stimulates a memory-like response that provides a nonspecific, antimicrobial state.²⁰⁰

IFNs function in part by activating hundreds of **interferon-stimulated genes (ISGs)**. The product of one such gene is an enzyme that produces **25-hydroxycholesterol (25HC)** from cholesterol. 25HC inhibits the entry of potential host cells by a wide range of human and animal coronaviruses, including several pig coronaviruses (**porcine epidemic diarrhea virus** and **transmissible gastroenteritis coronavirus**) as well as SARS-CoV and MERS-CoV (see Chapters 2, 3, and 6). The production of this enzyme is induced by SARS-CoV-2 infection of cultured lung epithelial cells in vitro and in COVID-19 patients in vivo.³³¹ 25HC inhibits the fusion of SARS-CoV-2 to the potential host cell's plasma membrane by activating an endoplasmic reticulum enzyme (**acyl-CoA:cholesterol acyltransferase**) that depletes levels of accessible cholesterol at that site, but not in other cellular membranes. SARS-CoV-2 binding to ACE2 and S protein cleavage by TMPRSS2 is not affected by 25HC.³³¹

$CD4^+$ T helper cells are divided into several categories. Two of the major groups are Th1 and Th2 cells which often act in an antagonistic manner. In general, Th1 cells produce antiviral, proinflammatory cytokines such as $IFN-\gamma$ and $TNF-\alpha$, while Th2 cells produce antiinflammatory cytokines whose antimicrobial action is directed more against bacteria than viruses. COVID-19 patients express lower levels of mRNA for $IFN-\gamma$ and $TNF-\alpha$ than uninfected people, while IL-6 expression is upregulated in patients with severe disease.³³² Hospitalized patients with elevated levels of serum IL-6 upon admission have an increased risk of death.³³³

Another group of T helper cells is the Tregs which are characterized by their expression of **forkhead box P3 (FOXP3)**. Tregs are vital to maintaining homeostatic immune responses by decreasing the activity of other leukocytes, including their cytokine production. Expression of FOXP3 mRNA, and thus Treg activity, is generally decreased in COVID-19 patients with mild or moderate disease. By contrast, FOXP3 expression is increased in severe COVID-19 cases and this increase correlates with the severity of hypoxia and death).³³² Increased proliferation of lung epithelial cells during recovery from acute lung injury is associated with greater numbers of $FOXP3^+$ Tregs, thus greater levels of $FOXP3^+$ cells in COVID-19 patients with severe hypoxia may reflect an attempt by the host immune system to repair lung damage.³³² Further information about the various types of leukocytes, cytokines, and the coronavirus molecules that inhibit their activity is described in much greater detail in Chapter 1. The roles of **Th17** cells and **IL-17** in COVID-19 are described later in this chapter.

Severe cases of COVID-19 are more likely than moderate cases to have lower absolute numbers of $CD4^+$ T helper cells and $CD8^+$ T killer cells. B cell numbers, however, are not reduced in severe cases.³³⁴ Levels of the IL-2 receptor, IL-6, IL-10, and $TNF-\alpha$ are also lower in severe than in moderate COVID-19.³³⁴ Functionality of $CD4^+$ T helper and $CD8^+$ T killer cells, NK cells, and NKT cells in the blood is reduced. Additionally, the T killer cell population is slanted toward a terminally differentiated or **senescent** phenotype.⁵³ The ability of the four above cell types to produce antiviral cytokines, such as $IFN-\gamma$ and $TNF-\alpha$, is also decreased as is the intracellular expression of granzyme in NK cells.⁵³

IL-6 plays a major role in COVID-19 pathology. The reduction of granzyme-expressing NK cells correlates with increased serum levels of IL-6. High levels of IL-6 are linked to downregulated expression of perforin and granzyme. After treatment to reduce IL-6 levels, perforin and granzyme expression in NK cells increases.⁵³ Patients admitted to an ICU typically have higher levels of IL-6, IL-10, and TNF- α . The levels of these three cytokines are inversely correlated with CD4⁺ T helper and CD8⁺ T killer cell counts.³²¹

Levels of antiinflammatory IL-4 and IL-10 are increased during COVID-19, perhaps in an effort by the immune system to block pathogenic inflammation. Levels of the Treg cytokine TGF- β are also lower in patients with severe but not mild disease.²⁰⁴ IL-10, TGF- β , and **growth and differentiation factor 15 (GDF15)** are the key cytokines that regulate the immune system by decreasing excessive activity, thus helping to prevent a cytokine storm. GDF15 is a member of the TGF- β superfamily of proteins whose activity increases during COVID-19. High levels of GDF15 strongly predict a poor outcome.³³⁵

Immunological cross-reactivity exists between the various pathogenic and nonpathogenic coronaviruses of humans. Antibodies to the SARS-CoV-2 N protein are present in some people who had not been exposed to SARS-CoV-2 but had a prior infection with a different human coronavirus. These antibodies are believed to be cross-reactive and target antigens that are common among human coronaviruses.^{324,336} Some people who were unexposed to SARS-CoV-2 also have anti-SARS-CoV-2 T cells that cross-react with the mildly pathogenic HCoV-OC43, HCoV-HKU1, HCoV-NL63, and HCoV-229E. Most of the cross-reactive T cells are CD4⁺ T helper cells, but some are CD8⁺ T killer cells.³³⁷ In the United States, 50% of the tested, stored blood samples from 2015 to 2018 cross-reacted against SARS-CoV-2, years before the virus emerged in humans.³²³ While some T cell reactivity is directed against the S protein, the highest amount of T cell reactivity is against the N and M coronavirus proteins. High levels of cross-reactivity among human coronaviruses were also reported in Germany, the United Kingdom, and Singapore.³³⁷ Differences in cross-reactivity may vary geographically or genetically or be age-related.

If these cross-reactive cells are memory CD4⁺ T helper cells, they might be at least partially protective against SARS-CoV-2 and respond more quickly and powerfully when exposed to this virus, decreasing disease severity. Such memory CD4⁺ T helper cells could also stimulate more rapid and stronger neutralizing antibody production by B cells. The potential benefits of preexisting T cell memory responses may have previously played a role in the H1N1 influenza pandemic of 2009–2010 since disease severity was greater in young adults than in older people.³³⁷ A major outbreak of a different strain of H1N1 virus had occurred in 1976 and may have produced cross-reactive memory T cells that later protected against the 2009–2010 H1N1 outbreak. In the same manner, people previously infected with HCoV-OC43, HCoV-HKU1, HCoV-NL63, or HCoV-229E might be partially protected against severe COVID-19.

People who produced T memory cells during a previous infection with SARS-CoV have good cross-reactive immunity against SARS-CoV-2.³³⁸ Multiple regions of SARS-CoV-2 are closely related to those of SARS-CoV. Immune responses against two such regions in the S protein may protect a person against both SARS and COVID-19. These shared regions may be useful in the development of vaccines that both stimulate B and T cell activity. Importantly, these two regions rarely mutate, so they should protect against multiple variants of SARS-CoV and SARS-CoV-2 and potentially protect against other highly

pathogenic coronaviruses of animals, should they spillover into humans. Since antibodies are in the serum for a much shorter time than active T memory cells, it may be helpful to use anti-SARS-CoV-2 T cell responses as the prime markers of adaptive immunity to COVID-19 rather than the presence of neutralizing antibodies.³²⁴

4.5.2 COVID-19 immunopathology—IL-17 and the cytokine storm

The immune response may act as a double-edged sword. At moderate concentrations, various immune cells and secreted molecules protect against infection with viruses, bacteria, fungi, and other single-cell organisms as well as against cancerous cells. High concentrations of inflammatory cytokines are pathogenic and produce a cytokine storm which can result in severe inflammatory or autoimmune diseases and death. These cytokines include TNF- α and the IL cytokines IL-1 β , -2, -6, -7, -17, and -18 as well as IFN- γ , MCP-1, MCP3, and **macrophage-inflammatory protein (MIP)-1 α** . These cytokines and chemokines are associated with COVID-19 severity.³³⁹ ICU patients have even greater levels of some cytokines than patients who were not in ICUs, including IL-2, IL-7, IL-10, **granulocyte colony-stimulating factor**, **IFN- γ -induced protein 10 (IP10)**, MCP-1, MIP1A, and TNF- α . IL-1 β and TNF- α from Th1 and Th17 cells produce large amounts of TNF- α and prompt Th17 activity.³⁴⁰

Th17 cells, the major source of IL-17, are important in the induction of the cytokine storm. High numbers of Th17 cells are found in the blood during COVID-19 and their activity increases in patients with very severe disease.³⁴⁰ Increased numbers of Th1 and Th17 cells are present in SARS and MERS patients as well.³⁴⁰ Chemokines, such as IL-8, IP10, MCP-1, and MIP-1 α , are present in the blood of COVID-19 patients as well and draw other immune cells into the infected region.²⁰⁴ They are associated with disease severity due to an excessive inflammatory response in many parts of the body, including the kidneys, heart, and some regions of the brain and nerves.

IL-17 prompts the production and recruitment of neutrophils into the affected area. It also induces the production of chemokines **C-X-C motif chemokine ligand 1**, MIP2A, IL-8, IP10, MIP3A, and **matrix metalloproteinases**, the latter of which plays a role in the repair of tissue damage.³⁴⁰ ROS, **arginase-1 (Arg-1)**, and NO increase Th17 induction in vitro. The toxic effects of ROS have been described previously. Arg-1 is a Th1 cell suppressive compound. In this setting, NO is produced by **inducible nitric oxide synthetase (iNOS)** in macrophages in response to a variety of stimuli. The increased activity of iNOS in neutrophils during COVID-19 correlates with disease severity.⁸⁶ Agents that inhibit Arg-1, NOS, and ROS activity reduce Th17 numbers.⁸⁶

4.5.3 COVID-19 and the innate immune response

4.5.3.1 Noncellular components of the innate immune response

Double-stranded RNA is produced during the replication of positive-stranded RNA viruses, including coronaviruses. This form of RNA is recognized by the cytoplasmic **retinoic acid-inducible gene I (RIG-I)** and **melanoma differentiation-associated protein 5 (MDA5)** and triggers several innate immune system pathways that directly inhibit vital

viral activities. These host cell responses include production of type I IFNs, inhibition of translation via **protein kinase R (PKR)**, and degradation of RNA by the **oligoadenylate synthetase/RNase L pathway**.³⁰⁴

The IFNs are the most effective noncellular component of the immune response against viruses. IFN- β significantly shortens the time to viral clearance as assessed by reverse transcription-polymerase chain reaction. Further, patients receiving IFN- β therapeutically had a significantly lesser increase in the levels of IL-6.³⁴¹ IFN- β also reduces the numbers of patients admitted to ICUs as well as those requiring invasive ventilation, while improving survival rate. It does so without any severe adverse events. IFN- β is most effective when administered early after infection.³⁴¹

4.5.3.2 Evasion of the host immune response

To survive and replicate, SARS-CoV-2 must develop strategies to evade both the innate and **adaptive immune responses**. Coronavirus nsp1 blocks translation of host mRNAs while permitting replication of viral RNA.³⁴² Viral nsp15 inhibits IFN production and viral sensing by the MDA5, PKR, and **OAS-RNase L** pathways.³⁴³ The N protein also interferes with IFN production by inhibiting IRF3.³⁴⁴ Other SARS-CoV components that decrease IFN production are the products of **open reading frames (ORFs)** 3b, 6, 8a, and 8ab.³⁰⁴ Moreover, the SARS-CoV-2 nsp16/nsp10 complex adds a methyl group to the viral **RNA cap** that aids in evading recognition by MDA5.^{304,345}

PL^{pro} is a coronavirus enzyme that is required for cleaving viral replicase **polyproteins** (see Chapter 1). PL^{pro} also serves as an IFN antagonist and additionally modifies transcription and blocks **ubiquitination** of RIG-I, **TNF receptor-associated factor 3, stimulator of interferon genes** protein, and IRF3.³⁴⁶ Inhibition of the latter block's production of type 1 IFN.³⁴⁷ Comparing PL^{pro} from SARS-CoV with that from SARS-CoV-2 reveals that despite having 83% sequence identity, they have different host substrate preferences. PL^{pro} from SARS-CoV preferentially cleaves ubiquitin chains, while that from SARS-CoV-2 typically cleaves the ubiquitin-like protein ISG15.³⁴⁸

4.5.3.3 COVID-19 and cells of the innate immune system

The balance between inflammatory responses and cell maturity in neutrophils and monocytes/macrophages differs between mild and severe SARS-CoV-2. Decreases in monocyte activation together with dysregulated neutrophil production may lead to a dangerous loop of tissue inflammation and ineffective host defense responses.³⁴⁹ Absolute numbers and types of DCs are also altered during COVID-19.³⁵⁰ Some alterations in the monocyte/macrophage, DC, and neutrophil components of the innate immune response are present in all COVID-19 patients, while other alterations vary throughout the disease. In addition to changes in numbers and activity levels, **inflammatory transitional CD14⁺CD16⁺ monocytes** and **nonclassical (CD14^{neg}CD16⁺) monocytes, myeloid DC (mDC),** and neutrophils preferentially leave the blood and migrate to the lungs during severe COVID-19.^{351,352}

4.5.3.4 COVID-19 and monocytes/macrophages

The activity of cells of the immune system differs according to their location. This is particularly true of mononuclear phagocytes whose immature, blood form (monocytes)

differs from their larger, mature, and more active tissue forms (macrophages). Macrophages differentiate into cells that vary according to the tissues they occupy. Examples of these mature tissue macrophages include microglia, macrophages of the nervous system that often remove extracellular debris; osteocytes, macrophages that are located in the bones and tear them down; and splenic macrophages that remove old and defective red blood cells from circulation while retaining their iron. To gain a better understanding of the antiviral role of macrophages and their role in immunopathology in the lungs, it is important to examine the activity of both circulating and alveolar (lung) macrophages during COVID-19.^{353,354}

Chemokines attract macrophages and neutrophils to the sites of infection, thus contributing to localized inflammation. While neutrophil responses tend to be excessive during COVID-19, some other cells of the innate immune system, including DCs, monocytes, and NK cells, tend to be less responsive. In general, these monocytes are immature, with a lesser expression of maturation markers after stimulation.³⁵⁵ The normal resident alveolar macrophages of the lungs are replaced by an inflammatory type of macrophage in patients with severe COVID-19.³⁵⁶ The release of IL-1 β from these inflammatory macrophages is also impaired in patients with severe COVID-19.³⁴⁹ This combination of abnormal monocyte phenotype and activity together with abnormal neutrophil activity may promote a more severe disease course which leads to the development of ARDS.³⁵⁷

Inflammatory transitional monocytes expressing high levels of the MHC II molecule HLA-DR are present during mild COVID-19.³⁴⁹ During the progression of COVID-19, cell surface expression of HLA-DR decreases. Severe disease is marked by the presence of monocytes having only low levels of HLA-R.^{349,350} Since HLA-DR is a member of a group of molecules required for the activation of T cells, the CD4⁺ T helper cell response is impaired. Downregulation of HLA-DR occurs immediately before COVID-19-induced severe respiratory failure.³⁵⁸ By contrast, macrophages and DCs produce higher levels of **programmed death-ligand (PD-L1)**, a part of a pathway that suppresses T cells. This process is activated in response to SARS-CoV-induced production of the immunomodulatory cytokine IL-10 in COVID-19 patients.³⁵⁵ PD-L1 expression only occurs during the latter stages of severe COVID-19.

SARS-CoV-2 stimulates **NADPH oxidase (NOX2)** activity by macrophages. Activated NOX2 stimulates an **oxidative burst** that intentionally produces high levels of ROS to kill intracellular pathogens, including viruses.³⁵⁹ SARS-CoV infection also leads to hyperglycemia. Since glucose also triggers NOX2 enzymatic activity,³⁶⁰ diabetics are at higher risk for severe COVID-19 than normal people. Hyperglycemia is usually transient after infection. The exception is in patients with type 2 diabetes and insulin resistance associated with **metabolic syndrome**. Metabolic syndrome is characterized by the presence of three or more of the following: increased waist circumference (obesity), high levels of **triglycerides** or glucose in the blood (diabetes), hypertension, and low levels of **high-density lipoprotein** cholesterol (the “good cholesterol”). Obesity is linked to a greater risk of COVID-19-associated hospitalization, severe pneumonia, invasive ventilation, and death. Hypertension is another factor that contributes to COVID-19-related disease severity and death.³⁶¹

Glucose-6-phosphate dehydrogenase deficiency (G6PDd) also induces NOX2 activity and is present in patients with metabolic syndrome.³⁵⁹ Since **congenital** G6PDd protects

against malaria, this genetic disorder is primarily found among people whose ancestors were of Mediterranean, Asian, or African descent, putting these populations at higher risk for severe COVID-19 as well.³⁵⁹

4.5.3.5 COVID-19 and myeloid-derived suppressor cells

An unusual group of cells is the immunosuppressive **myeloid-derived suppressor cells (MDSCs)**. MDSCs interfere with T and NK cell activity during COVID-19,³⁶² including reducing the levels of granzyme A, an apoptotic enzyme found in CD8⁺ T killer cells and NK cells.³⁶³ MDSCs are divided into two groups: those that are more related to monocytes (**monocytic myeloid-derived suppressor cells; M-MDSCs**) and those that are more closely related to neutrophils (**polymorphonuclear myeloid-derived suppressor cells; PMN-MDSCs**).

M-MDSCs inhibit T cell responses by directly binding to T cell inhibitory and apoptotic receptors and increasing the production of IL-10 and TGF- β . Numbers of M-MDSCs increase in response to inflammation and, during severe COVID-19, they become more plentiful in the blood, but not nasopharyngeal or endotracheal aspirates.³⁵⁴ M-MDSC are important producers of IL-6 and IL-10 and correlate with increased inflammation and increased levels of regulatory B and T cell subsets during severe COVID-19.³¹⁹ IL-6, in turn, is required for the production of M-MDSC from peripheral monocytes.

M-MDSCs from patients inhibit T cell growth and IFN- γ production at least partially by an Arg-1-dependent mechanism. Arg-1 plasma levels are increased during COVID-19. Arg-1 is an enzyme that converts the amino acid arginine to ornithine and urea as well as suppresses T cell responses during cancer.³⁶⁴ M-MDSC numbers and activity are strongly associated with disease severity by dysregulating the antiviral immune response.^{354,365} The following immunosuppressive molecules are also increased during severe COVID-19: **indoleamine-pyrrole 2,3-dioxygenase**, **immunoglobulin-like transcript 3** downregulates myeloid cell activation, and **cyclooxygenase 2**.³¹⁹

Another, closely related group of suppressor cells, PMN-MDSCs, are more closely related to neutrophils than to monocytes. PMN-MDSC numbers increase during COVID-19, especially in patients receiving intensive care treatments. The numbers of these cells correlate with plasma levels of IL-1 β , IL-6, IL-8, and TNF- α .³⁶³ When exposed to viral peptides, PMN-MDSC inhibits IFN- γ production by Th1 cells via Arg-1; TGF- β ; and iNOS. In this situation, iNOS is associated with neutrophils and monocytes/macrophages and produces NO upon stimulation.³⁶³ Upon admission to ICUs, PMN-MDSC numbers are greater in patients with fatal disease outcomes than in survivors.³⁶³

4.5.3.6 COVID-19 and dendritic cells

Peripheral blood DCs are composed of **mDC** and the relatively rare **plasmacytoid DC (pDCs)**. The former group's antiviral activity lies in their ability to stimulate CD4⁺ T helper cells, while the latter group's antiviral activity is primarily due to their rapid production of high levels of types I and III IFN.³⁵⁰ Absolute numbers of pDC and some mDC are decreased during COVID-19. Additionally, serum levels of pDCs are even more greatly decreased in patients with more severe diseases.^{355,366}

4.5.3.7 COVID-19 and neutrophils

During COVID-19, SARS, and MERS, the numbers of neutrophils are increased (**neutrophilia**) as immature neutrophils are recruited from the bone marrow during severe disease in a process known as “**emergency myelopoiesis**” that generally promotes immunosuppressive reactions.^{349,355} Increased neutrophil numbers are accompanied by reduced numbers of blood lymphocytes (**lymphopenia**).³⁵⁵ The shifts in immune cell numbers are greater in patients in ICUs than those not in these units and the extent of the shifts correlates with the risk of death. The ratio of neutrophils to CD4⁺ T helper cells is higher in patients with severe disease. Disturbance in the neutrophil:lymphocyte ratio may lead to immune-mediated damage.^{77,355}

During COVID-19, neutrophils are hyperresponsive, particularly when immature, with increased degranulation and production of proinflammatory cytokines. Levels of immature neutrophils are greater in severe COVID-19 cases than in healthy individuals³⁵⁵ and may be linked to excessive levels of IL-17. By the excessive release of cytokines, neutrophils may be responsible for the progression of a cytokine storm to a “**proteolytic storm**” which is due to an imbalance between neutrophil serine cascade activator proteases and their inhibitors.³⁵⁵ Increases in the levels of serum **myeloperoxidase** and **neutrophil elastase** may be due to increased degranulation of peripheral blood neutrophils. The deleterious effects of these enzymes are enhanced by the depletion of protease inhibitors. Neutrophils from COVID-19 patients also decrease IFN- γ production by T cells, particularly the CD8⁺ T killer cells, to a greater degree than neutrophils from normal individuals.⁸⁶

In addition to increased numbers of neutrophils in the circulatory system, neutrophils infiltrate the lungs in response to the presence of several neutrophil chemoattractants.³⁵¹ The cells localize in pulmonary capillaries and alveolar spaces.⁴⁰ While neutrophils from patients with severe disease have normal phagocytic activity,³⁴⁹ are abnormally activated. This aberrant state of activation is responsible for some of the virus-associated lung damage.^{367,368} Neutrophils produce excessive levels of **neutrophil extracellular traps (NETs)**. NETs are networks of extracellular fibers containing material released from neutrophils to kill extracellular microbes. The material in the NETs includes DNA and **histones**, the enzymes myeloperoxidase and elastase, and ROS.^{351,367,368} NET components are cytotoxic and damage the alveoli. COVID-19 patients in critical condition produce the highest levels of NETs.^{367,368}

Neutrophils from COVID-19 patients express high levels of **tissue factor (TF)**.³⁶⁹ High levels of TF are also found in platelets. TF works together with the coagulation component **VII/VIIa** to form a complex that initiates coagulation as part of its essential role in wound healing. Exposure of normal neutrophils to platelet-rich plasma from COVID-19 patients ex vivo also induces the formation of TF-containing NETs that stimulate excessive and pathogenic thrombosis.³⁶⁹ The antimalarial agent hydroxychloroquine is protective against NET-associated thromboinflammatory diseases.³⁶⁹

4.5.4 COVID-19 and autoimmune disorders

SARS-CoV-2 infection is associated with several autoimmune diseases.³⁷⁰ These autoimmune compounds and the related diseases include the following: **antinuclear**

autoantibodies (in GBS), MDA5 (**amyopathic dermatomyositis** and **immune thrombocytopenic purpura**), anti- β 2 glycoprotein 1 antibody (**antiphospholipid syndrome**), antierythrocyte antibodies (systemic lupus erythematosus), lupus anticoagulant (**KD**), antiphosphatidylserine IgM or IgG (**neuromyelitis optica**), antiannexin V-specific IgM or IgG (**NMDA-receptor encephalitis**), **anti-GD1b antibodies** (**myasthenia gravis**), antiheparin PF4 complex antibody (type 1 diabetes), **pANCA** and **cANCA** (**large vessel vasculitis and thrombosis**), and **anti-CCP antibodies** (**psoriasis**). Other autoimmune diseases associated with SARS-CoV-2 infection include subacute **thyroiditis**, Graves' disease, **sarcoidosis**, and inflammatory arthritis.³⁷⁰

Since diabetes is a topic of general interest, it will be given special attention here. Type I diabetes is an autoimmune disease characterized by hyperglycemia due to the death of the insulin-producing β cells. Hyperglycemia lowers the patients' innate immune response.³⁵⁶ COVID-19 contributes to further pancreatic β cell injury which furthers the patient's dysfunctional **glycemic control** (control of blood sugar levels) in a pathogenic feedback loop. Hyperglycemia in patients with COVID-19 and SARS is associated with a poor outcome.^{356,371} ACE2 and the viral S protein are highly glycosylated during COVID-19 and this molecular modification enhances their binding.³⁷² Expression of ACE2 is doubled in the kidneys of diabetics,³⁷³ increasing the risk and severity of kidney infection by SARS-CoV-2. Interestingly, type 1 diabetes is associated with a lower COVID-19-related mortality rate than type 2 diabetes.³⁵⁶

4.6 Diagnosis and surveillance

An ever-growing number of diagnostic tests are being developed and brought into use around the world. These tests vary in accuracy, expense, and availability. Some tests require trained personnel, expensive equipment and reagents, and a waiting period of days before results are available. A former gold-standard diagnostic test must be performed under Biosafety Level 3 conditions. Due to increased demand in some parts of the world for testing before traveling, entering the workplace or classroom, or being released from quarantine or isolation, tests are often in short supply, even in urban centers in developed countries. If testing is to be performed weekly or even monthly, the availability of testing materials may never be adequate, even in the most developed regions of the world. Added to the above difficulties, the antibody-based (serological) tests, particularly the rapid home tests, may not have adequate sensitivity to detect low levels of virus for all present and future viral variants, even those which are the most common. It is very important to note that most developing nations are unable to afford mass-testing programs, even if accurate and inexpensive tests and testing facilities were available.

A variety of sample materials can be used for diagnosis, including blood, sputum, feces, and material obtained by nasal or mouth swabbing or **bronchoalveolar lavage**.^{198,374} Collection of some types of samples may be problematic: nasal and bronchoalveolar lavage sampling is uncomfortable, while mouth swabs may generate aerosols due to the cough reflex. Many tests must be conducted in special facilities and be stored at appropriate temperatures within an appropriate time frame until tested to yield reliable results with high percentages of **sensitivity** and **specificity**. Sensitivity measures the ability of the test to detect the virus: low

sensitivity percentages create a high number of false-negative results. Specificity measures the ability of the assay to measure only the virus being accessed, not other, closely related viruses. Low specificity produces a high number of false-positive results.

Since saliva is simple to collect, a study compared the accuracy of properly identifying SARS-CoV-2 in saliva and nasopharyngeal samples of hospitalized patients by RT-PCR and two rapid antigen detection tests).³⁷⁵ Approximately half of the tested patients were in an ICU. RT-PCR of nasopharyngeal material and saliva had sensitivities of 98% and 69%, respectively, and 100% specificity. The nasopharyngeal antigen tests had sensitivities of 35%–41% when wet swabs were used to collect the samples and 47% when dry swabs were used.³⁷⁵ Detection of the virus was negligible with salivary swab samples. Due to the high number of false-negative results, almost all diagnostic techniques use nasopharyngeal samples and not salivary material. One exception to this rule is one form of CRISPR-Dx, as described below. It has also been shown that assays have better sensitivity when RNA-based SARS-CoV-2 detection targets at least two genes and when samples are derived from multiple parts of the respiratory tract.³⁷⁶

The time of collection is also critical since some tests are only accurate early or late after symptoms are apparent. One study from 2020 found that the sensitivity of RT-PCR tests is greater than 90% for the initial 5 days after symptom onset and drops thereafter: 70%–71% sensitivity if tested on 9–11 days and 30% for testing on day 21.³⁷⁴ Sensitivity for the antibody-based tests, however, increases over time. The detection rate is very low soon after symptom onset, is greater than 50% positive by day 7, greater than 80% by day 12, and 100% by day 21. RT-PCR and antibody-based testing are thus complimentary methods of detecting SARS-CoV-2 infection.³⁷⁴

Many false-negative results from serological tests are due to the tests being performed less than seven days after symptoms were apparent when antibodies were not present in sufficient amounts for the test to detect.³⁷⁴ Other people with false-negative results have severe disease and possibly are not able to generate an antibody response. The genetic test gives negative results after people stop shedding viruses, but IgG antibodies should be present for much longer periods, including those in people with asymptomatic infections.³⁷⁴ Tests for the more protective and longer-lasting CD8⁺ T killer memory cells are lacking. Table 4.3 compares the various tests used to diagnose SARS-CoV-2 infection.

4.6.1 RNA-based (genetic) tests

Viral RNA may be detected by genetic tests using an RT-PCR. As of mid-January, 2022, the gold standard for diagnosing SARS-CoV-19 infection is a **real-time quantitative reverse transcription-polymerase chain reaction**.³⁷⁸ It should be noted that the RT-PCR diagnosis methods may be time-consuming, expensive, laborious, and require trained personnel and specialized equipment.³⁷⁹ Other assays that are used to detect the presence of viral RNA include digital reverse transcription-polymerase chain reaction (dRT-PCR), reverse transcription loop-mediated isothermal amplification (RT-LAMP), and clustered regularly interspaced short palindromic repeats (CRISPR).³⁷⁸

Since RT-PCR detects viral RNA, these data do not necessarily mean that the viruses are infective or are present in sufficient numbers to infect another person. Additionally,

TABLE 4.3 Accuracy of RNA and Serologic Means of SARS-CoV-2 Detection.

Assay	Detects	Sensitivity	Specificity	Time Required
qRT-PCR ^a	Viral RNA	100%	100%	>4 hours
dRT-PCR ^b	Viral RNA	95.0%	90.1%	Hours
LAMP ^c	Viral RNA	~100%	97.6%	1 hour
RT-LAMP ^d	ORF1ab and S genes	~100%	~100%	30–60 min
CRISPR-Dx (Bio-SCAN) ^e	Subgenomic RNA	96%	~100%	1 hour
Rapid antigen test	Viral S and N proteins	68.9%– 75.1%	99.6%	5 min
MARK-B COVID-19 antigen test ^f	Viral R protein	95%	99.0%	<15 min
FIA ^g	Viral N protein	86.7%– 93.8%	~100%	3–30 min
ELISA ^h	Antibodies to N and S	81%–98%	N/A	1–5 hours
LFA ⁱ	Antibodies to N or S1	96.8%– 100%	93.3%	15 min
CG-FP ^j	Antibodies to N, spike and S1 in dried blood spots	86.7%	~100%	15 min
CLIA ^k	Antibodies to N	82.3%	97.4%	23 min
GICA ^l	IgG and IgM	86.9%– 95.1%	91.3%– 99.4%	10 min
VNT ^m	Infectious virus	~100%	~100%	2–4 days

^aReal-Time Quantitative Reverse Transcription-Polymerase Chain Reaction.

^bDigital Reverse Transcription-Polymerase Chain Reaction.

^cLoop-Mediated Isothermal Amplification.

^dReverse Transcription Loop-Mediated Isothermal Amplification.

^eClustered Regularly Interspaced Short Palindromic Repeats.

^fMagnetic Force-Assisted Electrochemical Immunoassay-Based.

^gFluorescence Immunochromatographic Assay.

^hEnzyme-Linked Immunosorbent Assay.

ⁱLateral-Flow Assays.

^jMultiplexed Grafting-Coupled Fluorescent Plasmonics.

^kChemiluminescence Immunoassay.

^lColloidal Gold Immunochromatographic Assay.

^mViral Neutralization Tests.

This table presents the sensitivity and specificity of SARS-CoV diagnostic tests as well as the time required to complete each. They detect viral RNA (genes), antigens, antibodies, or infectious virus. The current “gold-standard” test against which all other tests are measured is the qRT-PCR; the former gold-standard tests were the VNTs. VNTs have the disadvantage of requiring BioSafety Level 3 conditions and taking days to complete. The tests’ sensitivity indicates the degree to which the virus is identified, with 100% indicating the lack of false-negative results. The antigen-based tests are the least sensitive and the RNA-based tests and the VNTs are the most sensitive. The tests’ specificity indicates the degree to which the virus is correctly identified, with 100% indicating the lack of false-positive results.

Table produced by the author; some of this material was modified from Roberts,³⁷⁷ while the remainder is cited in the text.



FIGURE 4.2 ID # 24477 CDC/James Gathany, 2020 Public Domain. This Centers for Disease Control and Prevention (CDC) scientist is preparing samples for Real-Time Reverse Transcriptase (RT)–PCR analysis of SARS-CoV-2 specimens. Millions of these test kits have been processed in the United States since the beginning of the COVID-19 pandemic.

the number of viruses required to sustain infection in people is not known and varies widely among viruses. For a genetic method of diagnosis to give a positive result, the viral RNA must be at sufficient levels at the site of sample collection (the rear of the nasal cavity, throat, or sputum).

Fig. 4.2 illustrates an RT-PCR test.

4.6.1.1 Real-time quantitative reverse transcription-polymerase chain reaction

The highly sensitive and specific qRT-PCR assay is presently the test against which nucleic acid and serological tests are compared (the “gold standard”). As such, it has 100% sensitivity and 100% specificity. It uses materials collected by nasopharyngeal swab or oropharyngeal swab.³⁷⁸ Its weaknesses include a requirement for specialized equipment and highly trained operators which limit its large-scale use.^{379,380}

4.6.1.2 Digital reverse transcription-polymerase chain reaction

When used in combination with qRT-PCR, dRT-PCR reduces the amount of false-negative reports, particularly in samples with low levels of SARS-CoV-2,³⁸¹ since dRT-PCR has a lower detection limit than qRT-PCR.³⁸² Most diagnostic tests use nasopharyngeal swabs and yield inadequate results using sputum. However, while dRT-PCR has a sensitivity of 95.0% and a specificity of 90.1% using nasopharyngeal samples, these numbers are decreased but still acceptable using saliva or sputum. When using saliva, sensitivity and specificity are 89.7% and 77.0%, respectively, while these percentages using sputum are 100% and 88.6%.³⁸²

4.6.1.3 Loop-mediated isothermal amplification and reverse transcription loop-mediated isothermal amplification

RT-LAMP uses isothermal nucleic acid amplification technology. Results are expressed by changes in turbidity, color, or fluorescence of the tested material. It is performed in a laboratory setting and can only test a single sample at a time.^{377,383} This test amplifies viral RNA by 10^9 – 10^{10} times in less than an hour and does not require a thermocycler since it is performed at a constant temperature of 60–65°C.³⁷⁸ Its sensitivity and specificity are both approximately 100%.³⁷⁷ This test can detect a combination of the N and E protein and ORF-1ab genes with 92.3%, 98.5%, and 99% accuracy, respectively.³⁷⁷

4.6.1.4 Clustered regularly interspaced short palindromic repeats diagnostic

CRISPR-associated proteins (Cas) are of microbial origin and serve as part of the microbe's immune system. The CRISPR-Cas system uses a guide RNA and Cas endonuclease. The binding of the guide RNA to a complementary foreign nucleic acid sequence triggers the Cas enzyme to cleave either microbial RNA or DNA and produce single- or double-strand breaks.³⁸⁴

Several forms of CRISPR-Dx have been developed. They use Cas mutants that lack cleavage activity but still have DNA-binding ability.³⁸⁵ The Cas mutant is linked to a variety of reporter systems, often using a fluorescent tag. The reporter system is only activated in the presence of microbes in the sample to be tested.³⁸⁴ Using the enzymes Cas12 and Cas13a, CRISPR assays are rapid, inexpensive, highly sensitive, and do not require specially trained operators. Their results are expressed as fluorescent signals that are visible under blue light.³⁸⁴

Instead of using fluorescence, CRISPR-Dx may instead use an electrokinetic microfluidic chip as the reporter system.³⁸⁴ This version is faster and uses 100-fold fewer reagents than other forms with similar specificity. It may thus be used for mass screening of large numbers of samples.

Biotin-coupled specific CRISPR-based assay for nucleic acid detection (Bio-SCAN) can be completed in less than 1 hour from nasopharyngeal swab material.³⁸⁶ Results are detected on commercially available lateral flow strips and are visible to the naked eye in the absence of additional probes, reagents, specially trained personnel, or elaborate analysis equipment. Bio-SCAN has 100% sensitivity and 96% specificity compared to RT-qPCR. This test can distinguish the α , β , and δ SARS-CoV-2 variants³⁸⁶ and might be able to be modified to identify the omicron variant as well.

4.6.2 Antibody-based (serological) tests for severe acute respiratory syndrome coronavirus-2 infection

4.6.2.1 Introduction to antibody-based tests

Multiple serological tests detect antibodies against SARS-CoV-2, typically either IgM or IgG.^{378,387} The presence of IgM generally indicates recent infection or exposure to SARS-CoV-2, while IgG antibodies indicate a virus infection that occurred a long time ago. Some of these tests can detect blood antibodies within 15 minutes and can differentiate patients at different stages of infection, including symptomatic and asymptomatic carriers. Some of

the tests are “user-friendly” for home use since they employ blood derived from finger sticks, rather than a blood draw by a phlebotomist. Some tests also discriminate between the presence of neutralizing antibodies and other types of antibodies that have less antiviral activity.

Most serological tests may not need to be performed in special laboratories and may be used in hospitals, clinics, businesses, schools, and public travel locations. Unfortunately, an increasing number of antibody-testing systems are being used which differ in their specificity and sensitivity. Studies are necessary to determine whether each of these kinds of antibody tests cross-react with other nonpathogenic human or animal coronaviruses.

Some of the more commonly used antibody/antigen-based assays include **rapid antigen detection** tests, **enzyme-linked immunosorbent assays**, **viral neutralization** tests (VNTs), and **nanoparticle-based lateral-flow** assays.³⁷⁸

A cautionary note: antibody detection tests, but not antigen tests, not only detect infection, but also significant exposure to SARS-CoV-2 components and so may register as “positive” in people who have not been infected. These tests may also cross-react with similar coronaviruses, including the human coronaviruses HCoV-OC43 and HCoV-HKU1 that generally cause the common cold, again running the risk of false-positive results.³⁸⁸ One study reported that 57% of people with laboratory-confirmed infection with human coronaviruses that cause the common cold had false-positive antibody test results for SARS-CoV-2.³⁸⁹ These findings might unnecessarily lead to individuals and their contacts being quarantined as well as producing overestimates of the number of people in a population who are infected. False-positive results may lead to the patient receiving the wrong type of treatment modality.

4.6.2.2 Rapid antigen detection tests

In antigen tests, monoclonal antibodies are used to detect the SARS-CoV-2 S and N proteins through antigen-antibody interactions.³⁹⁰ These tests yield results within five minutes and are simple enough for home use. Antigen tests are 1000 times less sensitive than viral culturing assays and 100,000 times less sensitive than qRT-PCR.³⁹¹ In a *metaanalysis*, results from 84 studies from 16 commercially produced assays were compared to qRT-PCR. Their average sensitivity was 68.9% (range = 61.8–75.1) and the average specificity was 99.6% (range = 99.0–99.8°).³⁹² The detection rate is better for symptomatic than asymptomatic cases that have an average sensitivity rate of 58.1% (range of 40.2–74.1) compared to symptomatic cases with an average sensitivity rate of 72.0% (range = 63.7%–79.0%).³⁹² Since these tests have poor sensitivity, they have been recommended only for use in conjunction with other testing procedures.^{378,391} Additionally, no studies have evaluated the accuracy of serial screening strategies. This is important given recent proposals for repeated antigen testing in asymptomatic groups, including children and school staff and hospital and healthcare home workers.³⁹²

The time of sample collection after symptom onset is very important. Sensitivity was 78.3% (range = 71.1%–84.1%) the first week after symptom onset and dropped to 51.0% (range of 40.8%–61.0%) in the second week.³⁹² There were no significant changes in specificity between these two times of collection. Approximately half of the antigen tests were not conducted in manners that followed the manufacturer’s instructions or were not used at the **point of care**.³⁹²

Large differences in test sensitivity are due in large part to differences in settings, sample type and collection methods, and sample storage and preparation. Sensitivity rates in tests conducted on-site by trained nonhealthcare workers were 57.5% (range of 52.3%–62.6%), 70.0% (range of 63.5–75.9) when tests were conducted on-site by healthcare workers, and 78.8% (range = 72.4%–84.3%) when conducted by laboratory scientists.³⁹²

A much more sensitive antigen detection test has been developed that uses material collected by nasopharyngeal swabs. This test is magnetic force-assisted electrochemical immunoassay-based (MARK-B COVID-19 Ag test) and detects viral N protein. Its sensitivity is 95% (range = 79.4%–96.2%) and specificity is 99.0% (range = 95.0%–99.9%).³⁹³ Instead of a test strip, this assay utilizes a fully automated portable device that is easy to read and the results are available in 15 minutes. Additionally, these tests are semiquantitative.³⁹³

Another study of rapid antigen tests compared two assay kits that use the COVID-19 antigen fluorescence immunoassay, a **qualitative test** that detects the presence of the viral N protein. In this test, the detection reagent is a fluorescent compound that absorbs light at a specific wavelength and emits light using a different wavelength. One of the two assays was designed to evaluate their usefulness as a screening tool in a large reference hospital.³⁹⁴ Using nasopharyngeal swab material, these tests have an average sensitivity of 86.7%–93.3% (range = 75.4%–98.2%) and specificity of 100% (range = 92.9%–100%). They give results in 3–30 minutes and do not require specialized equipment or trained personnel.³⁹⁴

Different results are obtained when rapid antigen tests were used to detect viable, infectious SARS-CoV-2 in cell culture-positive material. Several studies using different commercial rapid antigen tests found sensitivity rates of 78.6%–100%^{395,396} and specificity of 100%.³⁹⁶ They also have a 66.7% rate of differentiating material containing viable viruses from subgenomic RNA.³⁹⁶ This means that rapid antigen tests have significantly better results for the task of identifying viable and infectious SARS-CoV-2 rather than viral RNA.³⁹⁵ This could be important in determining whether an individual is capable of not just shedding viral RNA, but whether the material detected might be infectious and transmissible to other people.

4.6.2.3 Enzyme-linked immunosorbent assays

The various types of ELISAs detect serum antibodies against specific viral proteins using the test material, capture and detection antibodies, an enzyme, and its substrate. Results are presented as a change in enzymatic activity that alters the color, fluorescence, or luminescence of its substrate. Fig. 4.3 depicts an ELISA which measures the color change. Results are available in 1–5 hours. Since these assays are performed in microplates, ELISAs can test over 300 samples at one time and can be fully automated.^{378,397}

Several of the more commonly used ELISAs detect the SARS-CoV-2 N protein or the RBD of the S protein.^{398,399} When tested in combination, about 10% of the tests yield false-negative and false-positive results.³⁹⁹ These tests are highly time-sensitive: when an automated RBD-based test was performed 9 or more days after the patient's initial symptoms, the sensitivity ranged from 81%–98%, depending upon the class of antibody being detected, but dropped to 43%–57% when tested at 7 or 8 days.³⁸⁸

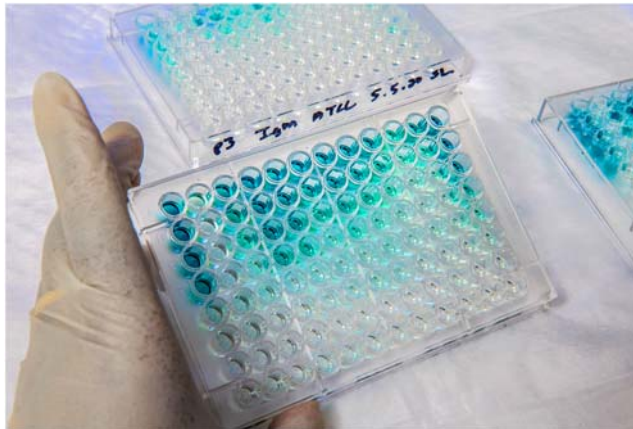


FIGURE 4.3 ID# 2448 CDC/James Gathany, 2020 Public Domain. This is a close view of a SARS-CoV-2 serological test, used for the detection of IgM. Serological tests are used to identify the presence of antibodies, which can be found in persons who have previously been infected with SARS-CoV-2.

4.6.2.4 Lateral-flow assays

LFAs are simple, rapid, and highly accurate in the detection of SARS-CoV-2.³⁷⁸ These assays can detect serum levels of either IgM, IgG, or both anti-SARS-CoV-2 IgG and IgM. In LFAs, the detection reagent consists of recombinant SARS-CoV-2 N or S1 antigens conjugated to colloidal gold or magnetic nanoparticles, quantum dots, or fluorescein isothiocyanates on rapid diagnosis strips. This type of diagnosis strip has previously been used in point-of-care testing applications, such as pregnancy testing.³⁸⁷ The gold nanoparticles are inexpensive and have long-term stability that does not require a **cold chain**, allowing their use in developing or impoverished regions that lack refrigeration or freezing equipment.³⁸⁷

Results are seen in the form of a color change. The process takes 10–15 minutes and requires 10–20 μL of serum, an amount that can be easily obtained using a finger stick.³⁷⁹ LFA has a sensitivity of 100% and a specificity of 93.3%.^{379,387} One study reported that the sensitivity of colloidal gold nanoparticle LFA for detection of IgG, IgM, or both antibody classes is 11.1% during the first 7 days, 92.9% at 8–14 days, and 96.8% after 14 days of infection.^{377,400} The sensitivity of IgM assays is 75% in intermediate to late-stage cases, while the IgG sensitivity rate was 96.8% in late-stage cases. Dual detection of IgM and IgG is recommended for maximal testing efficacy, especially during the intermediate stage of infection.³⁷⁷

4.6.2.5 Nanoparticle-based assays

Nanoparticles may be used in some of the above diagnostic tests, such as gold nanoparticles can be used in LFAs. Several basic types of assays may utilize nanoparticles. These include tests based upon the following: (a) electrochemical biosensors, (b) volumetric tests, (c) microarray-based tests, and (d) immune-based.⁴⁰¹ These types of assays have their strengths and weakness, but of particular interest is that some tests may be used multiple times or can identify asymptomatic infections.

4.6.2.6 Other antibody-antigen-based immunoassays

An important technique is **multiplexed grafting-coupled fluorescent plasmonics** which detect antibodies in dried blood as well as serum. It has $\sim 100\%$ sensitivity and specificity.^{377,402} Other antibody-based assays range in sensitivity from 82.3%–91.5% and in specificity from 91.3%–99.4% and take under 30 minutes to perform.³⁷⁷ These assays include the following: the **chemiluminescence immunoassay**,⁴⁰³ the **colloidal gold immuno-chromatographic assay**,⁴⁰⁴ and the **fluorescence immunochromatographic assay**.⁴⁰⁵

4.6.3 Viral neutralization tests

Several forms of VNTs constitute another approach that is being used to detect the presence of antibodies in a donor's serum. VNTs assess the ability of various dilutions of serum from a potentially infected person to kill mammalian cells in vitro. Results rely upon multiple factors, including the type of tissue and species of animal from which the cells were derived and which viral isolate and antibody type is being.³⁹⁸ The forms of the test are the live VNT, surrogate VNT (sVNT), and lentivirus-based SARS-CoV-2 pseudo-virus neutralization test (pVNT).³⁹⁸ Live virus VNT was previously considered to be the "gold standard" test but must be performed under Biosafety Level 3 conditions and so is not able to be used in wide-scale detection or surveillance efforts.³⁹⁸ The VNT tests are sensitive and specific alone or in combination with ELISA tests.³⁹⁸ A major problem with using the VNT, sVNT, or pVNT assays is that they take 2–4 days to yield results.

4.6.4 Surveillance

The use of Dorfman pooled testing makes rapid, mass-screening of samples from symptomatic or asymptomatic people feasible.^{406,407} This pooling strategy divides all of the samples to be tested into pools that contain the same number of samples. If a pool is found to be positive, then all samples within it are then tested individually.⁴⁰⁸ Pooling may also permit large-scale multiple repeat testing to be performed in a manner that does not rapidly deplete available resources.

Mass-screening using serological tests can be used for surveillance to ascertain the viral presence and spread on a population-level scale in asymptomatic as well as symptomatic infections. Additionally, some tests are **quantitative** and can measure the levels of antibodies present in infections of various severity as well as during convalescence,³⁹⁸ allowing epidemiologists to track the time course of the pandemic in a local region. Serological tests might be able to be modified for use in detecting whether different animal species are infected with SARS-CoV-2 or a SARS-CoV-2-like virus using a One Health approach. These tests can then be used to monitor the potential for zoonotic transmission or the emergence and spread of new viral variants among humans or susceptible animal species.

In the fall of 2020, almost 1200 asymptomatic people at a university were tested for the presence of SARS-CoV-2. Material collected by oral-nasopharyngeal swabs was tested for the presence of the genes encoding the N and polymerase proteins using a multiplex qRT-PCR.⁴⁰⁹ The samples were first combined into 400 distinct pools. Five of the pools were deemed to be positive within 24 hours and the results of four of these pools were

confirmed using a commercially available *in vitro* diagnostic kit. The positive pools were subsequently tested individually and 4 people were judged to be infected. This multiplex assay had a sensitivity of $\sim 100\%$ and a specificity of 99.9% when compared to a reference *in vitro* test. Since this study conducted 463 tests rather than 1195, it decreased human and material testing resources by greater than 60% without a significant loss of time or sensitivity.⁴⁰⁹ In this study, each pool contained material from three individuals, however, increasing this number to five or seven samples per pool reduced sensitivity. It should be noted that the subjects in this study were asymptomatic, which may also reduce sensitivity since the tested people had a lower viral load than ill people.

A second study used RT-PCR with five-sample pooling to test ~ 1400 samples for the SARS-CoV-2-specific ORF1 gene and the conserved E gene.⁴⁰⁶ This study found that 9.5% of the pools were positive and further individual testing revealed a 2.9% positivity rate. Both the sensitivity and specificity were 100%, even though the samples were diluted 1:64. The total throughput was increased threefold in one-third of the daily cost. One potential problem is that populations with a higher positivity rate may need lower pool sizes, which could raise the turn-around time considerably.⁴⁰⁶ Prevalence rate, therefore, is a factor that needs to be taken into consideration for each population to be studied.

4.7 Treatment

4.7.1 Medications and monoclonal antibodies

Many types of therapy for COVID-19 are currently under development, undergoing clinical trials, or are in use. Some of the treatment options under consideration include blocking maturation of the S protein (camostat mesylate), binding the viral S protein to ACE2 (neutralizing antibodies), viral fusion with the host cell membrane (Arbidol), and activity of receptors for IL-1 or IL-6 (anakinra, tocilizumab). Other antibodies or drugs serve as anti-TNF- α agents (alimumab) or prevent viral entry via endocytosis (hydroxychloroquine).^{410,411}

As more and more drug candidates and other therapeutic measures are becoming available, ideal efficacy studies would be prospective, case-controlled, multicenter, and multinational. Since such studies take time, healthcare personnel need to weigh safety profiles, when available, against potential efficacy in groups of patients representing a wide range of ages and those with preexisting medical conditions. The best drug candidates would be not only safe and efficacious but would be active against most or all human coronaviruses, including SARS-CoV, MERS-CoV, HCoV-NL63, HCoV-229E, HCoV-OC43, and HCoV-HKU1, and animal coronaviruses, whether these viruses use ACE2, APN, or DPP4 as their host cell receptor. Findings from previous studies focusing on SARS and MERS may be useful as well, especially in the identification of safety issues.

Due to the continuing increase in the number of medications and therapeutic antibodies that act against SARS-CoV-2, this section will only mention some of the major categories of drug treatment measures and briefly address several drugs which have shown to be particularly effective or have been the subject of much attention. The reader is referred to some of the many reviews to obtain a fuller scope of drugs, drug combinations, and antibody options against SARS-CoV-2 and COVID-19.^{410,412,413}

4.7.1.1 Nucleic acid analogs

Remdesivir, favipiravir, ribavirin, tenofovir, sofosbuvir, and galidesivir are examples of nucleoside analogs or **prodrugs** with potential anti-SARS-CoV-2 activity.⁴¹⁴ Many of these drugs halt viral replication by mimicking normal nucleosides and are substituted into the newly produced RNA molecule, halting its replication.^{415,416} Several of these nucleoside analogs are undergoing human clinical trials for their activity against SARS-CoV-19.⁴¹⁵

Remdesivir is an adenosine analog of particular interest since it has previously been shown to be active against other human coronaviruses (SARS-CoV, MERS-CoV, HCoV-NL63, HCoV-OC43, and HCoV-229E) and several animal coronaviruses (mouse hepatitis virus, several bat coronaviruses, and porcine deltacoronavirus).⁴¹⁷ Remdesivir is active against SARS-CoV-2 in vitro and in in vivo settings in which it improves clinical outcomes, such as viral load in the lungs, recovery rate, and development of serious events in infected patients.⁴¹⁸ Remdesivir is inexpensive and has a good safety record in humans.

Ribavirin is a commonly used wide-spectrum antiviral drug that acts as a guanosine analog. When administered to patients with severe COVID-19, ribavirin therapy does not improve the mortality rate. It has also been used in combination with other drugs,⁴¹³ as described below.

4.7.1.2 Anticoagulation agents

Heparin is one of the major **anticoagulants** (blood-thinners) used to decrease the risk of developing thromboembolisms in people experiencing coagulation disorders, including those associated with COVID-19. Guidelines of The International Society of Thrombosis and Hemostasis suggest that a prophylactic dose of low-molecular-weight heparin should be administered to all hospitalized COVID-19 patients who are not actively bleeding or have thrombocytopenia.⁴¹⁹

The presence of kidney or liver disease or gastrointestinal tract dysfunction may counter-indicate the use of some of the drugs that are used to prevent or treat coagulation disorders in COVID-19 patients. Therapeutic-dose low molecular weight heparin improves the prognosis of patients with elevated D-dimer levels. At this dose, heparin decreases the amounts of thromboembolisms and death in comparison with standard heparin therapy without increasing major bleeding. It is not effective in patients in ICUs, however.⁴²⁰

Bivalirudin and nafamostat mesylate are anticoagulants currently used for DIC during COVID-19. A case study found bivalirudin to be beneficial in a COVID-19 patient with severe **hypoxemic** and **hypercarbic respiratory failure** requiring **extracorporeal membrane oxygenation**. Bivalirudin directly attaches to and inhibits freely circulating and fibrin-bound thrombin. It is an option for maintaining systemic anticoagulation in COVID-19 patients.⁴²¹ Nafamostat mesylate is an anticoagulant that has been used for over 30 years to treat DIC and to prevent coagulation of perfused blood during dialysis as well as having potent antifibrinolytic activity and, unlike heparin, does not lead to hemorrhaging.⁴²² Nafamostat mesylate is also a serine protease that blocks activation of the SARS-CoV-2 S protein and viral entry into cells.⁴²² It should be considered as a potential drug for use during COVID-19, particularly in patients who are heparin-resistant.⁴²³

4.7.1.3 Chloroquine and its derivatives

Chloroquine is more effective at decreasing SARS-CoV-2 infection than some other antiviral drugs, including favipiravir, penciclovir, nitazoxanide, and ribavirin.⁴²⁴ Patients treated with chloroquine have decreased fever and regain normal pulmonary functioning more rapidly than patients treated with many other antiviral medications.⁴¹¹ Hydroxychloroquine has greater antiviral action and a better safety profile than chloroquine.^{411,425,426} When used at the correct dosage, it decreases pneumonia severity and shortens the disease course in the absence of apparent side effects.⁴²⁷ In critically ill patients, low dose hydroxychloroquine decreases the mortality rate.⁴²⁸

Hydroxychloroquine acts by increasing the pH in some intracellular organelles, particularly the endosomes and lysosomes. Since SARS-CoV-2 requires an acidic environment to enter the cell by endocytosis, hydroxychloroquine blocks viral entry by this route. The action of this drug also is linked to iron homeostasis.⁴²⁹ Previous studies using a mouse model system reveal that hydroxychloroquine also inhibits infection by other viruses such as HCoV-OC43, enteroviruses, Zika virus, and influenza A H5N1.⁴²⁹

The advantages and disadvantages of treating COVID-19 patients with chloroquine or hydroxychloroquine have been the subject of much debate even though both drugs are considered to be safe for use in most people and generally have only mild side effects.^{430,431} Since chloroquine has been used for decades to treat malaria,⁴³² it has a well-established clinical safety profile, unlike most antiviral drugs. It should be noted, however, that there is only a narrow range between therapeutic and toxic doses. Additionally, life-threatening disorders may develop in people with preexisting cardiovascular diseases.⁴³³ It is, thus, very important to monitor drug recipients for the development of life-threatening conditions, such as thrombocytopenia and leukopenia, changes in liver or kidney function, as well as slow heart rate and elongation of some key parameters of heart beats (longer QT-intervals as detected by electrocardiograms).

4.7.1.4 Repurposed antimicrobial drugs

Niclosamide, used to treat tapeworm infections, has been previously shown to be active at low concentrations against SARS-CoV and MERS-CoV infections.⁴³⁴ Nitazoxanide, a drug that is used against microscopic blood parasites, is structurally similar to niclosamide. Nitazoxanide inhibits both SARS-CoV-2 and MERS-CoV reproduction at low concentrations in vitro as well as decreases the time of hospitalization and inflammation in vivo.^{435,436} Teicoplanin, an antibiotic used to treat staphylococcal bacterial infections, is active against SARS-CoV and MERS-CoV.⁴³⁷ Since these drugs have a record of usefulness against some human coronavirus infections, they may now be revisited in the search for compounds that are active against SARS-CoV-2.

Other repurposed drugs include the antiviral compounds prulifloxacin, tegobuvir, bictegravir, and nelfinavir which have been used in the treatment of hepatitis C or AIDS.⁴³⁷ They block the active sites of enzymes or hinder the dimer formation of some viral proteins. Lopinavir and ritonavir are drugs that have previously been used to treat AIDS and have activity against SARS and MERS as well. They are commonly administered together.⁴³⁷ Lopinavir/ritonavir inhibits the functioning of the viral protease CL3^{Pro}.

4.7.1.5 Immunosuppressants

Since much of the pathology occurring during COVID-19 is due to immune-mediated inflammation, corticosteroids have been administered to patients. While they inhibit inflammation, they also downregulate multiple other aspects of the immune response. This is particularly true of dexamethasone, which may greatly decrease antiviral activity. These drugs have been used previously to treat patients with SARS-CoV or MERS-CoV and were found to decrease the rate of virus removal.^{438,439} The usefulness of corticosteroids in treating patients with COVID-19 is therefore questionable.

Some monoclonal antibodies target and suppress more specific aspects of the immune response. Tocilizumab and sarilumab inhibit the proper functioning of the IL-6 receptor, while anakinra antagonizes the IL-1 receptor. These antibodies thus target two critical components of the cytokine storm. Administration of tocilizumab or anakinra to COVID-19 patients decreases the COVID-19-induced mortality rate, but not the risk of secondary bacterial infection. Moreover, tocilizumab increases the risk of fungal co-infection.⁴⁴⁰ The risk of fungal infections in patients treated with anakinra is unknown.

4.7.1.6 Severe acute respiratory syndrome coronavirus-specific antibodies

Several anti-SARS-CoV-2 monoclonal antibodies have been used to treat COVID-19 with various degrees of success. Most of these antibodies target the RBD portion of the viral S protein, however, other antibodies target the N protein. Antiviral antibodies include bamlanivimab, etesevimab, casirivimab, imdevimab, cilgavimab, tixagevimab, regdanvimab, and sotrovimab.⁴⁴¹ Due to the ever-increasing numbers of new antibodies, antibody combinations, and different dosages of these monoclonal antibodies, the reader is referred to several reviews of the subject for more up-to-date information.^{441–443}

4.7.1.7 Combination therapy

Combination therapy may be more effective than the use of any of the drugs alone. Several examples of combined anticoronavirus therapeutic drugs include remdesivir/IFN, lopinavir/ritonavir together with IFN- β , lopinavir/ritonavir together with both IFN- β and ribavirin, and hydroxychloroquine/azithromycin.^{413,444–446} While some of these combinations reduce disease severity and hospitalization and mortality rates, the testing of these combinations is not standardized, making comparisons difficult.

4.7.1.8 To use or not to use, that is the question

A metaanalysis reports that administration of corticosteroids, particularly dexamethasone, hydrocortisone, and methylprednisolone, with tocilizumab and sarilumab reduces mortality rate in hospitalized COVID-19 patients.⁴⁴⁷ This study also recommends that the following treatment options not be used based on their lack of effect on mortality rates: hydroxychloroquine, azithromycin, hydroxychloroquine/azithromycin, remdesivir, colchicine, lopinavir/ritonavir, and IFN- β .⁴⁴⁷ However, since the study focused on mortality rate in hospitalized patients, these drugs, either alone or combination, may still be effective in decreasing other parameters of COVID-19 severity in hospitalized or nonhospitalized patients.

4.7.2 Traditional medicinal compounds

Unlike synthetic antiviral drugs, traditional medicinal compounds are widely used in many cultures. A major advantage to the use of these alternative medications is that they are recognized as beneficial in societies that may be distrustful of synthetic drugs⁴⁴⁸ and, therefore, members of these populations may be more likely to afford, have access to, and use the traditional medicinal compounds. Nevertheless, case-controlled, scientific studies of these compounds' properties are needed to ensure their efficacy compared to placebos and to assess the range of dosages over which these compounds are safe. It should also be noted that the levels of protective and toxic chemicals in these plants or their extracts may be considered variable and thus differ greatly among batches. This complicates scientific studies as well as their use by traditional healers.

The traditional Chinese herbal medicine Lianhuaqingwen, used to treat influenza, inhibits SARS-CoV-2 replication as well as reduces the production of inflammatory cytokines in vitro. Lianhuaqingwen treatment also alters the structure of the remaining viruses. Preliminary results suggest that it decreases symptoms in infected humans as well.⁴⁴⁸

Many other traditional medicines are known to protect against SARS, MERS, and other coronavirus-associated diseases. These compounds include the high blood pressure medicine reserpine (from the Indian snakeroot *Rauwolfia serpentina*), and escin (from the horse chestnut *Aesculus hippocastanum*) that have been or are being used to prevent and treat SARS and MERS.⁴⁴⁹ Such compounds are prime candidates for potential anti-COVID-19 treatment as well. Lycorine from lilies (*Lillium* species) and daffodils (*Narcissus* species) and *Allium porrum* **agglutinin** from American flag leeks prevent SARS-CoV-2 from killing infected cells in vitro.⁴⁴⁹ Silvestrol, which is derived from the bark of *Aglaia foveolata*, a tropical evergreen found in Southeast Asia, inhibits coronaviral mRNA translation by blocking the activity of the host RNA **helicase** enzyme **eIF4A** that is needed to bind mRNA to **ribosomes** during translation.⁴⁵⁰ Two **flavonoids**, scutellarein, and myricetin, strongly inhibit coronaviruses helicases as well.⁴⁵¹ Scutellarein comes from the Baikal skullcap (*Scutellaria baicalensis*), while myricetin is found in many vegetables, fruits, nuts, and berries.

The following natural products target the coronavirus 3CL^{pro}: the **triterpenes** iguesterin, pristimerin, tingenone, and celastrol from *Catha cassinoides* and the flavonoids hesperetin from citrus, amentoflavone from *Ginkgo biloba* and St. John's wort (*Hypericum perforatum*), and luteolin from wild mignonette (*Reseda luteola*).⁴⁵⁰ Two compounds derived from rosemary (*Salvia rosmarinus*), carnosol, and rosmanol, also decrease the activity of this protease.⁴⁵²

Wang et al.⁴⁵³ have reviewed multiple other natural compounds that have been found to have anticoronavirus activity. A drug that is used to treat cancer, homoharringtonine, is derived from the Japanese pine-yew (*Podocarpus macrophyllus*). It is also active against some viruses, including the porcine epidemic diarrhea virus.⁴⁵⁴ Treatment with either homoharringtonine or hydroxychloroquine decreases virus levels by 30- and 3.5-fold, respectively, but, in combination, reduces virus levels by 200-fold. Emetine from *Psychotria ipecacuanha*, also known as the "roadside sick-making plant," is used to induce vomiting. It is also active against SARS-CoV and MERS-CoV.⁴⁵³ It is, however, potentially cardiotoxic, so caution is advised when considering whether to use this compound. Cepharanthine is derived from *Stephania cephalantha*, a tall vine found in Asia. Its antiviral,

antimalarial, and anticancer activity includes blocking replication of the human coronavirus SARS-CoV, SARS-CoV-2, and HCoV-OC43 in vitro.⁴⁵³

4.8 COVID-19, micronutrients, and vitamin D

Small amounts of **micronutrients**, some of which are known as **trace elements**, are critical for life, partially because they may bind to enzymes and are required for enzymatic activity.⁴⁵⁵ These enzymes are vital for protection against damage caused by ROS.⁴⁵⁶ Micronutrients that are important in defense against coronavirus-associated disease include zinc, copper, selenium, and iron. Patients in ICUs have an especially great need for these micronutrients since they are undergoing severe stress. Stress increases the rate at which the trace elements are used and depleted, particularly zinc.⁴⁵⁷

4.8.1 COVID-19 and zinc

Zinc is one of the most common micronutrients in the body and is an essential part of **zinc finger motifs** which bind specific DNA sequences and regulate the cell's genomic replication. It also serves as a **cofactor** for critical enzymes, including RNA and DNA polymerases. Zinc is especially important for the proper functioning of the immune and nervous systems.¹¹² It regulates immunological activity by controlling leukocyte replication, transcription, proliferation, and activation.⁴⁵⁸ Zinc is directly involved in immunity against viral and bacterial infections.^{459,460} Low zinc levels are associated with COVID-19 severity.⁴⁶¹ Zinc is also directly active against other coronaviruses,¹¹² including SARS-CoV,^{174,462,463} mouse hepatitis virus,⁴⁶⁴ transmissible gastroenteritis virus,⁴⁶⁵ and feline infectious peritonitis virus.¹⁸⁹ For the first three of the above viruses, zinc is known to inhibit viral polyprotein cleavage. Zinc also inhibits SARS-CoV by inhibiting the viral **RNA-dependent RNA-polymerase (RdRp)** activity and feline peritonitis virus replication.^{112,463}

Zinc has a wide range of effects on the immune system. It plays a role in the production and functional activity of neutrophils, NK cells, macrophages, and T and B cells. It stimulates NK cells to kill virus-infected cells by upregulating perforin expression and increases the production of IFN- α .^{458,466} Additionally, zinc is important for the maturation of T cells in the thymus, cytotoxicity by CD8⁺ T killer cells, and IL-2 production by Th1 cells.⁴⁵⁸ Moreover, zinc increases the development of Tregs, which, in turn, lowers the release of the proinflammatory cytokine IL-17.⁴⁶⁷ Zinc deficiency decreases neutrophil, monocyte, and NK, T, and B cell activity, while increasing the production of IL-1 β , IL-6, and TNF- α .^{458,466} This is particularly important in the elderly, who have a greater risk for zinc deficiency than younger people.

Zinc supplementation during COVID-19 reduces the infiltration of neutrophils into the airways and the release of TNF- α .⁴⁶⁸ In hospitalized patients, serum zinc levels inversely correlate with the length of total hospital stay, but not with the mortality rate.⁴⁵⁶ Zinc also inhibits the SARS-CoV-induced downregulation of ACE2, which is a zinc-containing **metalloenzyme**.^{466,469} Supplemental zinc, therefore, may upregulate the expression of

functional ACE2.⁴⁶⁶ It is important to note, however, that zinc supplementation may be harmful to people who have normal levels of this micronutrient since very high zinc concentrations can decrease T and B lymphocyte functions, lead to excessive levels of Tregs, and lower IFN- γ production.^{470,471}

Zinc **ionophores**, such as chloroquine and pyridoxine, increase the cellular uptake of zinc.⁴⁷² During treatment for COVID-19, zinc plus chloroquine or hydroxychloroquine increases intracellular zinc concentrations, particularly in the lysosomes.⁴⁷³ In a case study, all patients who received supplemental zinc together with hydroxychloroquine showed improvement in COVID-19 symptoms after only 1 day of treatment.^{471,474} Several studies found treatment with zinc and hydroxychloroquine significantly increases the rate of patients being discharged to their homes while decreasing in-hospital mortality rates or transfer to hospice facilities.^{112,475} Low levels of zinc and pyridoxine together inhibit replication of SARS-CoV in vitro by blocking RdRp elongation activity⁴⁶³ and may do so to SARS-CoV-2 as well.

Complexes of zinc oxide and berberine, an **alkaloid** with antioxidant and antimicrobial activities, inhibit binding of the S protein to ACE2 and PL^{pro} activity as well as decreasing expression of the viral E protein and RdRp at concentrations lower than that achieved by either component alone.⁴⁷⁶ These complexes decrease the risk of secondary respiratory bacterial infections, including *Klebsiella*-related pneumonia. They also greatly reduce the toxic effects of long-term hydroxychloroquine use.⁴⁷⁶

4.8.2 COVID-19 and copper

Copper is an essential trace element that, in proper amounts, acts as an antioxidant, but may, in excessive concentrations, instead act as an oxidant.⁴⁵⁸ Copper and zinc are necessary components of **copper/zinc superoxide dismutase**, an enzyme that converts the ROS **superoxide** into **hydrogen peroxide**. Copper also serves as a **scavenger** of other ROS, removing them from circulation.⁴⁵⁸

Like zinc, copper is a component of several important enzymes, including **cytochrome c-oxidase** and **ceruloplasmin (CP)**. Copper ions also play a vital role during aerobic respiration, iron absorption, and the production of several neurotransmitters.⁴⁵⁸ Copper deficiency is associated with multiple and diverse symptoms, including dysfunctional neuronal signaling, muscle weakness, hematological symptoms, and cardiomyopathy.⁴⁷⁷ CP binds to most of the copper circulating in the blood and serves as the copper transport protein. Both copper and CP behave as positive acute phase reactants, whereas serum selenium and its transporter **selenoprotein P (SELENOP)** behave oppositely.

In vitro, **copper gluconate** decreases SARS-CoV-2 infection of cells by greater than 70%.⁴⁷⁸ COVID-19 survivors have higher mean serum copper and CP concentrations compared to nonsurvivors.⁴⁷⁷ Higher copper levels coincide with the recovery of normal selenium levels in COVID-19 survivors as well.⁴⁷⁹

Copper exerts anti-SARS-CoV-2 activity on external substances.⁴⁵⁸ SARS-CoV-2 and SARS-CoV survive on copper surfaces for 4 and 8 hours, respectively.³⁰⁷ Copper and cuprous oxide inactivate over 99% of SARS-CoV-2 in 1–2 hours.³⁰⁸ Furthermore, masks containing copper oxide microparticles reduce levels of infectious SARS-CoV-2 by greater than 99.9% within 1 minute.⁴⁸⁰

4.8.3 COVID-19 and selenium

Selenium-containing enzymes (**selenoenzymes**) contain **selenocysteine** in their active sites and require abundant amounts of selenium for their enzymatic activities.⁴⁸¹ Selenoenzymes play a role in protection against viral infections, oxidative stress, protein folding, and mitochondrial health. The activity of the selenoenzyme **glutathione peroxidase-3** is low in COVID-19 patients.⁴⁷⁹ This enzyme is an antioxidant that protects cells from oxidative damage by chemical **reduction** of the ROS hydrogen peroxide.

COVID-19 patients have large deficits in total levels of serum selenium and its transporter SELENOP. One study reported that upon admission to ICUs, patients' serum selenium and SELENOP levels were 50% and 69% below normal, respectively. Levels of IL-6 and IL-10 inversely correlate with SELENOP levels as well.⁴⁸² Selenium levels during COVID-19 are below the 2.5th percentile of the population.⁴⁷⁹ Selenium deficiency correlates with COVID-19 severity and mortality risk.^{477,479,483} Throughout the disease, survivors restore their selenium levels, unlike nonsurvivors.⁴⁷⁹

Selenoenzymes are essential for proper immune responses, including macrophage signaling, NK cell activity, and T cell differentiation and proliferation.⁴⁸⁴ In COVID-19 patients, NK and CD8⁺ T killer cell numbers are low. However, following intravenous supplementation with selenium and zinc, inflammation levels decrease and lymphocyte counts increase.⁴⁸²

A study of critically ill COVID-19 patients with ARDS found that most patients have low selenium and zinc serum levels upon admission to an ICU.⁴⁶¹ In a separate study, a combined deficit of zinc and SELENOP was seen in 0.15% of healthy people, 19.7% of COVID-19 survivors, and 50.0% of nonsurvivors.⁴⁸³ While zinc deficiency decreases in response to medical care, selenium deficiencies are reversed only in survivors.⁴⁸³

4.8.4 COVID-19 and iron

Iron has an important but complex role in microbial viability as well as in the host's immune response and health in general. Some molecules that are vital to life contain iron, including hemoglobin and myoglobin, both of which require the proper concentrations of iron to bind and transport oxygen in the blood and muscle tissue, respectively. Production of erythrocytes also requires an adequate iron supply.⁴⁸⁵ **Catalase** is an enzyme that requires iron for its activity. Catalase is an antioxidant that degrades hydrogen peroxide into water and oxygen. Iron deficiency thus impedes the removal of hydrogen peroxide. The European countries whose populations have low dietary iron uptake are most affected by COVID-19.⁴⁸⁶

Iron is not only needed for the survival of the host animal but is also necessary for viral replication, adhesion, and entry into host cells.^{487,488} Serum **transferrin** and **lactoferrin** in milk bind up free iron, making it unavailable for viral use. **Hepcidin** from the liver inhibits iron transport. Inflammation can increase hepcidin levels which decrease serum iron concentrations. The immunomodulator GDF15 suppresses hepcidin expression. Levels of GDF15 are increased during COVID-19, thus the immune system plays a role in iron availability during infection.³³⁵ Iron, however, acts as a double-edged sword since excessive levels are toxic to the host, in part due to the **Fenton reaction**, an iron-dependent reaction

that converts ROS to more toxic forms. Low iron availability is associated with the progression of ARDS in COVID-19 patients.⁴⁸⁵

Patients with severe COVID-19 have **hypoferremia** (low iron levels).^{485,489} Most COVID-19 patients have hypoferremia. Furthermore, multivariate analysis found that levels of serum iron and ferritin, but not transferrin, can predict COVID-19 severity.⁴⁸⁵ Following treatment, serum iron levels differ between survivors and nonsurvivors. Serum iron deficiency is also an independent risk factor for death.⁴⁹⁰ Additionally, patients with high oxygen demand have low serum iron levels.⁴⁸⁵

4.8.5 COVID-19 and vitamin D

Vitamin D is very important to human health in multiple ways, including proper immune, skeletal, muscular, and nervous system activity. People with vitamin D deficiency are at risk of developing severe COVID-19.^{491,492} The most serious complications of low vitamin D levels in COVID-19 patients are bilateral pneumonia (76%), ARDS in patients admitted to ICUs (29%), and multiorgan failure (8.5%).⁴⁹³ Additionally, levels of vitamin D3 are lower in the milk of COVID-19-infected women than in uninfected women.⁴⁹⁴

Currently, Vitamin D deficiency is common. The reasons for this deficiency are multifactorial and involve decreased exposure to UV light from sunlight, skin pigmentation, occupation, and age. The initial step in the production of the active form of vitamin D3, the “sunshine vitamin,” relies upon low levels of UV light, often from the sun, penetrating the epidermis of the skin. Vitamin D is required to absorb calcium from the digestive tract, thus decreased vitamin D levels also reduce calcium levels.

After Vitamin D is chemically modified in the liver and kidneys, the hormone **calcitriol** is formed. Calcitriol subsequently binds to the vitamin D receptor and this complex acts as a **transcription factor**. One of the most important actions of calcitriol is to regulate blood calcium levels.¹⁹² Calcium is essential for maintaining bone density, regulating nervous and muscular system activity, and intracellular signaling pathways.⁴⁹⁵

Melanin, the dark pigment in the skin, decreases the amount of UV light passing through the epidermis. The amounts of an individual’s melanin and UV light exposure need to be balanced for optimum protection from sunburns and skin cancer (too much UV light exposure) and low production of vitamin D3 (too little UV light).⁴⁹⁶ Descendants of people from the more northern or southern regions of the earth tend to have lighter skin since they are exposed to less UV light. Descendants of people from the tropical regions typically have darker skin since they had high levels of UV exposure.

When darker-skinned people leave the equatorial region, they may no longer receive the levels of UV light necessary to penetrate the epidermis and produce adequate vitamin D levels for a proper antiviral immune response to SARS-CoV. This may at least partially explain why dark-skinned populations have a greater risk for severe COVID-19.⁴⁹⁷ One study in Chicago in the northern part of the United States found that 48.7% of the COVID-19 deaths are among African Americans and 26.2% among are Latinos, even those these two groups represent only 31% of the city’s population.⁴⁹⁸ Another study found that the COVID-19-related mortality rate in people of African descent living in England and Wales

is greater than four times that in people of European descent.⁴⁹⁹ The reasons for these disparities are complex and may include differences in socioeconomic status, nutrition, and healthcare access. The relatively low levels of Vitamin D3 produced by the skin by these populations, however, may also be a major factor since their immune systems are less functional in the absence of proper levels of this vitamin.^{498,500}

Currently, due to changing lifestyles and occupations, many people throughout the world are spending less time outside in both summer and winter.¹⁹² During the winter, exposure to sunlight is particularly low since in addition to less direct sunlight, people spend less time outdoors and, when they do, most of their skin surface is covered. Vitamin D deficiency is thus widespread among most people living in temperate regions, especially among nonwhites. Ingesting greater levels of dairy products fortified with vitamin D or taking supplemental vitamin D may help to reduce the risk of severe COVID-19-related illness.

Serum calcitriol levels generally decrease with age.⁵⁰¹ The calcitriol levels of elderly people in European countries are severely low. In this population, a negative correlation exists between vitamin D levels and COVID-19 case numbers and mortality.⁵⁰² The elderly not only have a reduced immune response than younger people but also spend less time outdoors due to their cold sensitivity.¹⁹² It should be noted that COVID-19 is more common in the winter and that SARS-CoV-2 is first known to have emerged and spread in the Northern hemisphere during the winter of 2019 when vitamin D levels are at their lowest.⁴⁹⁹

Supplemental vitamin D boosts immune responses during viral infections.⁵⁰³ This is due in part to the presence of surface vitamin D receptors on most leukocytes, including monocytes/macrophages, T and B cells, NK cells, and DCs.¹⁹² Vitamin D decreases the production of the antiviral Th1 cells and their proinflammatory cytokines while inducing Treg activity.⁵⁰¹ Thus, vitamin D deficiency may stimulate the cytokine storm.⁵⁰⁴ Vitamin D also stimulates the production of **cathelicidin** by macrophages and lung epithelial cells. This antimicrobial peptide disrupts viral envelopes and alters the viability of host target cells.¹⁹²

In an Egyptian study, low vitamin D levels were present in 97.6% of COVID-19 patients. Low levels of this vitamin and blood calcium are associated with greater disease severity and higher blood levels of inflammatory markers (D-dimers, C-reactive protein, and ferritin), in addition to longer disease duration.^{505,506} After 10 days of hospitalization, COVID-19 patients with severe vitamin D deficiency had a 50% probability of death as opposed to 5% in people with vitamin D levels 10 ng/mL or more.⁵⁰⁴

The importance of this vitamin during COVID-19 has been the subject of debate,^{492,507,508} but the detrimental effects of vitamin D deficiency are supported by several studies. For example, Treg levels are low in many COVID-19 patients but can be increased by the administration of supplemental vitamin D.⁴⁹² Vitamin D deficiency is also associated with increased episodes of thrombosis, which is a frequent occurrence in COVID-19 patients.⁴⁹² Supplemental vitamin D also reduces this and other coagulation abnormalities in critically ill COVID-19 patients.^{509,510} Furthermore, administration of supplemental 25(OH)D3 (**calcifediol**) to COVID-19 patients with low serum levels raises the percentage of blood lymphocytes and lowers that of blood neutrophils. This change in the neutrophil-to-lymphocyte ratio is associated with decreased length of stay in ICUs and reduced mortality rates.⁵¹¹ Other studies also found that supplemental calcifediol decreases the mortality rate by 70% or more.^{512,513} Supplemental vitamin D3 also increased

survival rates twofold.⁵¹⁴ Care must be taken, however, during the administration of supplemental vitamin D since excessive levels of this vitamin are toxic.

4.9 Prevention

4.9.1 Rapid, mass scanning measures

Before entering many venues, people must have their forehead scanned to detect fever, which could be indicative of COVID-19. While this type of scan is rapid and inexpensive, 56% of those infected have no fever, even at the time of admission to a hospital.²⁰³ Also, thermal scanning of exposed skin upon entry into a building in the winter may not detect elevated body temperature. Questionnaires concerning possible exposure to SARS-CoV-infected people have also been used extensively. In areas experiencing large numbers of cases of severely ill people, emergency room triage measures may include testing the potentially severely ill patient's respiratory rate and using **pulse oximetry** to determine whether the patient's blood contains adequate amounts of oxygen.^{203,334}

4.9.2 Personal protective equipment and social distancing

Different regions of the world have relied on different methods to slow the infection rate. One of the most important measures is to avoid contact with infected people, especially those who have signs of infection. Uninfected as well as infected people have been advised or required to wear one or two layers of masks that securely cover the nose and mouth and some people also wear face shields as well, as depicted in [Fig. 4.4](#). During parts of 2020 and 2021, the Center for Disease Control and Prevention in the United States recommended that when venturing outside of the home, asymptomatic people should allow 6 feet between them, especially while indoors, in addition to face masking.⁵¹⁵

Masks and respirators restrict the release of infected droplets and particles during breathing and coughing or sneezing. They provide some protection against SARS-CoV-2 infection if they fit snugly to the face. Reusable masks may be made of cloth. Disposable masks may be made of layered finely woven products. The cloth products provide the least protection, woven products offer more protection, well-fitting disposable surgical masks are even better, and well-fitting respirators (including N95s) offer the highest level of protection.⁵¹⁶ Healthcare providers require greater protection against infection, which includes N95 masks, goggles, and gowns. [Fig. 4.5](#) shows several types of N95 masks.

4.9.3 Hand hygiene

Infection against enveloped viruses, including coronaviruses, can also be decreased by washing hands with soap and water for at least 20 seconds and is more effective than hand sanitizers. The public is recommended to wash their hands often, especially when dealing with potentially contaminated material.⁵¹⁷ Touching eyes, nose, and mouth should be avoided since SARS-CoV-2 can remain in an infectious state for hours to days.



FIGURE 4.4 ID# 24613 CDC Public Domain. Personal protective equipment (PPE). PPE is used to prevent the spread of SARS-CoV-2. This worker is used a face shield, mask, and gown.



FIGURE 4.5 ID #15484 CDC/Debora Cartagena, 2013 Public Domain. Two N95-type and a N1000-type face masks. N95 masks are air-purifying respirator certified by the National Institute for Occupational Safety and Health. They protect against particulate matter and biological particles, including viruses.

4.9.4 Decontamination of infected surfaces

Since rapid and complete disinfection of SARS-CoV and MERS-CoV on surfaces can be achieved by many commonly available disinfectants, these disinfectants might also be

TABLE 4.4 Comparison of Measures to Prevent Against SARS-CoV and SARS-CoV-2 Infection.

Protective Measure	SARS-CoV	SARS-CoV-2
Hand hygiene	Soap Alcohol-based hand sanitizers	Soap Alcohol-based hand sanitizers
Masks	Disposable or cloth Single mask Mandated in some areas Mandated in some situations	Disposable, cloth, N95 Single or double Mandated in most areas Mandated in many situations
Goggles and face shields	Goggles in some situations	Face shields in some situations
Identification cards	Health declaration card in some areas	Proof of vaccination in some situations
Fever detection	In some regions and situations	Widespread and many situations
Quarantine	In some regions for patients and contacts	In many regions for patients
Closure of businesses	In some areas and some situations	Widespread in areas and many situations
Contact tracing	In some areas	In many areas
Vaccines	Not available	Multiple types. Mandated in some areas and situations

This table lists and compares methods that have been or are used to decrease the risk of infection by SARS-CoV and SARS-CoV-2. These methods include physical methods of reducing contact with these viruses, identification and sequestration of infected people, and vaccine availability.

active on surfaces contaminated by SARS-CoV-2, as described in Chapter 2. [Table 4.4](#) compares the various methods of avoiding contamination with SARS-CoV and SARS-CoV-2.

4.9.5 COVID-19, quarantine, and closure of businesses, schools, and recreational areas

In order to decrease SARS-CoV-2's geographical spread and protect high-risk people from exposure to the virus, strict movement restrictions ("lock-downs") of nonessential personnel have been imposed to varying degrees in different countries and areas within these countries, including travel restrictions between countries. These strict measures change with time and have produced variable levels of success, most likely due to very different conditions and demographics in different locations, so that "one-size fits all" regulations are highly unlikely to be effective in almost any given region. Restrictions and other preventive measures have negatively affected people's physical and mental health as well as their monetary conditions. This has also impacted local, country-wide, and global economies, especially in developing countries and impoverished areas of developed countries as well. A greater understanding of how and where viral transmission and severe disease are most and least likely to occur is critical to making informed decisions about the extent of restrictions needed to protect any given population against moderate to life-threatening disease. The continuing emergence of SARS-CoV-2 variants makes this chore

much more challenging since the efficacy of protective measures, including vaccines, differ among variants.

Ongoing studies search for patterns and settings in which clusters of cases are most likely to occur may permit the implementation of proper precautionary measures. A 2020 study of eight countries from North America, Europe, and Asia, excluding China, reported that mass accumulations of people, especially in indoor settings, have the highest number of both case clusters and infected people. Social events and residential settings, including workers' dormitories and nursing facilities, are also associated with high numbers of cases. Workplaces have been and might continue to be major sources of infection in the United States, including facilities involved with food production and delivery.⁵¹⁸ Large outdoor sporting events, such as football/soccer, and other types of events in which people are brought into close contact, such as outdoor weddings and burials, might also be opportunities to transmit disease on a large scale.

A **geospatial study** using **geographic information systems** in Bangladesh found that population density and case frequency were related.⁵¹⁹ This type of analysis can locate COVID-19 clusters and hotspots and may be very useful in planning policies to prepare for and possibly prevent localized epidemics of SARS-CoV-2 or other infectious diseases based upon the geological, climatic, demographics, socioeconomic, and social characteristics present in small to large regions as well as the proportion of immune persons (natural or vaccine-induced immune responses). As an example, in southern Asia, the extended family includes the elderly, young people, and children living in the same household, thus transmission is more likely to occur in homes in that region. In the developed world, however, infection among the elderly occurs rapidly in eldercare facilities.⁵¹⁹

Whether or not to close businesses, churches, schools, outdoor recreational areas, and other areas in which people congregate is a topic of much concern and consternation. A thorough review of these ever-changing situations and their sociological, psychological, developmental, and economic effects is beyond the scope of this book.

4.9.6 Natural immunity

4.9.6.1 *T lymphocyte responses*

During the acute phase of COVID-19, SARS-CoV-2-specific T cells have a highly activated cytotoxic profile, while the SARS-CoV-2-reactive T cells during convalescence are polyfunctional memory cells.⁵²⁰ Virus-specific T cells are also found in antibody-negative family members and convalescing patients who were either asymptomatic or have mild disease. Natural infection by SARS-CoV-2 produces functional and active long-lasting memory T cell responses that may prevent or mitigate future SARS-CoV-2 infections.⁵²⁰ Reinfection of people who recovered from documented COVID-19 disease is uncommon for most viral variants, with the exception of Omicron.

Anti-SARS-CoV memory T cell responses against the N protein and nsp7 or nsp13 may be present for many years and protect mice and rhesus monkeys against a lethal challenge with SARS-CoV-2.^{338,517,521} SARS survivors as well as uninfected people among the general population bear cross-reactive or preexisting memory CD4⁺ T helper cells and CD8⁺ T killer cells against the N protein of SARS-CoV-2. The memory T cells formed during the

2003 SARS-CoV epidemic have been functional for at least 17 year³³⁸ so the T memory cells formed following infection with SARS-CoV-2 should also be functional for decades. Nsp7-specific anti-SARS-CoV T cells react against epitopes conserved among animal beta-coronaviruses, but not to the mildly pathogenic common cold human coronaviruses.³³⁸

4.9.6.2 *B lymphocyte and neutralizing antibody responses*

Since people are often reinfected by seasonal, common cold coronaviruses 6–12 months after their prior infection, protective immunity against at least some human coronaviruses appears to be short-lived. It has also been reported that levels of anti-SARS-CoV-2 neutralizing antibodies in the blood also decrease rapidly, especially antibodies to the viral N protein.^{522,523} This strongly suggests that antibody-based immunity to SARS-CoV-2 could also be short-term, especially in those vaccinated with the mRNA vaccines (Pfizer and Moderna vaccines). Levels of anti-SARS-CoV-2 antibodies began to decrease within 5 months after infection.⁵²⁴

A more recent, 6-month study, however, found that multiple factors influence the rate of decline of SARS-CoV-2-specific neutralizing antibodies, leading to great amounts of variation among patients.⁵²⁵ A predictive algorithm anticipates that the length of time in which neutralizing antibodies are functional ranges from 40 days to many decades. Accordingly, an accurate prediction of the rate of antibody decline should be determined.⁵²⁵ Some of the differences between this report and prior reports are an increase in cohort size and more regular sampling intervals. In addition, the more recent report also determined changes in the avidity of SARS-CoV-2-specific antibodies over time. Rapid IgG avidity maturation is a major determining factor in both the level of neutralizing antibodies and the rate at which neutralizing antibody activity is lost.⁵²⁵ Longevity of neutralizing antibody levels also correlates with persistent levels of inflammatory cytokines for at least 180 days. Proinflammatory T cell responses do not correlate with the differences in rates of neutralizing antibody loss.⁵²⁵ These findings may not only apply to those people who acquired natural immunity but to those receiving vaccination with a variable number of boosters.⁵²⁵ It should also be noted that the extent of disease severity is independently associated with longer persistence of neutralizing antibody levels. Patients with more severe diseases have increased antibody longevity.⁵²⁵

Considering the pace at which levels of neutralizing antibodies drop, reinfection with the original viral strain or infection by its variants may occur. This may force a reexamination of herd immunity strategies. If a similar loss of immunity is seen following vaccination, annual administration of additional vaccines or booster shots against the more pathogenic SARS-CoV-2 variants present at the time may be a key factor in preventing large outbreaks as population immunity declines.⁵²⁵ However, since T memory cells persist for decades, those with natural immunity may still be protected against reinfection.

Examination of a type of bone marrow B cells paints a different picture for the rate of loss of protective B cells and antibody responses. The antibody-secreting **plasma cells** that are produced from B memory cells release large amounts of virus-specific antibodies upon re-exposure to the same virus. Long-lived **bone marrow plasma cells (BMPCs)** in COVID-19 patients are a vital and long-term supplier of protective antibodies specific for SARS-CoV-2.⁵²⁶ People who have recovered from COVID-19 are thus unlikely to be reinfected

with SARS-CoV-2 again,⁵²⁷ even though their anti-SARS-CoV-2 serum antibodies decrease rapidly over several months.⁵²⁸

In people who recovered from mild SARS-CoV-2 infections, levels of anti-SARS-CoV-2 serum antibodies against the S protein dropped rapidly for 4 months, then more gradually for the next 7 months.⁵²⁸ Their BMPCs were **quiescent**, suggesting that they are in a stable condition. Resting SARS-CoV-19-specific memory B cells continue to be found in the serum of convalescent people, indicating that mild infection with SARS-CoV-2 might induce virus-specific, long-lived B memory cells⁵²⁸ that may provide long-term protection against reinfection. This study did not address whether these individuals are protected against SARS-CoV-2 variants.

4.9.6.3 The anti-severe acute respiratory syndrome coronavirus-2 immune response and viral variants

A Brazilian study found that people who had acquired natural immunity following infection by a **B lineage** of SARS-CoV-2 had an 8.6-fold lower neutralizing antibody response against the **P lineage virus**, such as the P.1 Gamma variant, which originated in that country. The Gamma viral variant might therefore also be able to escape an antibody response and infect people who have natural immunity against lineage B viruses.³⁰ The antibody response against the Beta variant (B.1.351) was lower in people receiving one or more doses of the chemically inactivated SARS-CoV-2 vaccine CoronaVac in comparison with people with natural immunity against B lineage variants. However, in Phase 3 clinical trials, CoronaVac protected against severe disease and death⁵²⁹ and it appears that in addition to neutralizing antibodies, memory T cell responses might reduce disease severity.³⁰

4.9.7 Vaccines against severe acute respiratory syndrome coronavirus-2 infection

Multiple anti-SARS-CoV-2 vaccines have been developed using different tactics and incorporating different viral components. Some vaccine formulations require one initial vaccine dose and others require two. Efficacy against emerged and newly emerging SARS-CoV-2 variants differ among the formulations, resulting in changing numbers of booster immunizations recommended for each vaccine to become “fully vaccinated.”

Anti-SARS-CoV-2 vaccines use several general types of vaccine strategies. Sinovac (CoronaVac) and Sinopharm/BBIBP-CorV use inactivated viruses. AstraZeneca (AZD1222), Johnson & Johnson (JNJ78436735), and Sputnik V use **adenovirus vectors**. Pfizer (BNT162b2) and Moderna (mRNA-1273) use **lipid nanoparticles** to deliver S protein mRNA. Noravax (NVX-CoV2373) is a nanoparticle protein-based vaccine.

Examination of sera from COVID-19 survivors or people vaccinated with 1–2 doses of the Pfizer or Moderna mRNA vaccines found high levels of cross-neutralization against most of the SARS-CoV-2 variants, but only limited neutralization against the Beta and Gamma variants, especially in those receiving a single recent dose of vaccine.⁵³⁰ Both Beta and Gamma variants contain an E484K mutation in the RBD of the S protein in addition to other mutations. As they spread, Gamma and P.1 variants have been reported to reinfect or infect people with previous natural or vaccine-derived immunity.⁵³⁰ A study from May

2021 reported that two SARS-CoV-2 variants having S protein sequences identical to Beta variants 1 and 2 have nearly complete neutralization resistance to the antibodies found in convalescent plasma.⁵³¹ The ability of these strains to avoid CD8⁺ T killer and NK cells has yet to be shown, however, so T cells may still protect people exposed to the Beta variants.

The Pfizer, Moderna, and Sputnik V anti-SARS-CoV-2 vaccines have an efficiency of 90% against symptomatic infection and induce neutralizing antibody levels that are much higher than those in convalescent patients.^{532,533} CoronaVac, by contrast, has approximately 50% efficacy against SARS-CoV-2 and neutralizing efficacy several-fold less than that present in convalescent patients.⁵³⁴ The Sinopharm/BBIBP-CorV and AstraZeneca vaccines have intermediate values of clinical efficacy against symptomatic infection and inducing the production of neutralizing antibodies.^{535,536} Adenovirus-vectored vaccines induce the production of high levels of SARS-CoV-2 CD4⁺ and CD8⁺ T cell activity.⁵³⁴ It should be noted that except for inactivated virus vaccines, all vaccines have only targeted S or N protein **epitopes** while immunogenic regions of the other proteins in the virus envelop are not targeted. These epitopes from other viral proteins are present in people who had been previously infected with SARS-CoV-2 and thus are very likely to provide anti-SARS-CoV-2 T cell protection that is not detected by antibody neutralization assays.⁵³⁰

The effectiveness of one dose of either the Pfizer or AstraZeneca vaccines is lower among people infected with the Delta, rather than the Alpha, variant.⁵³⁷ The effectiveness of the Pfizer vaccine after two doses is 93.7% and 88.0% for the Alpha and Delta variants, respectively. The effectiveness of the AstraZeneca vaccine is 74.5% and 67.0% after two vaccinations for the Alpha and Delta variants, respectively.⁵³⁷ Receiving a booster shot may decrease the risk of infection in comparison to that seen in people receiving only the originally recommended two doses of the vaccines. People receiving 2 or 3 doses of the Pfizer vaccine had a 6.6% and 1.8% rate of subsequent positive test results and hospitalizations, respectively.⁵³⁸

While the efficacy and length of protective responses of vaccines are judged by the presence of SARS-CoV-2-specific neutralizing antibodies, the extent of the immune response involves the coordination of not only antibody activity but also CD4⁺ T helper, CD8⁺ T killer, and NK cell functioning. While neutralizing antibody titers are important in host defense against COVID-19, by themselves, they are not predictive of reduced COVID-19 severity. SARS-CoV-2-specific CD4⁺ T helper and CD8⁺ T killer cells play a significantly greater role than antibodies during the infection. Given the importance of these three arms of the adaptive immune response together with NK cell activity, it may be wise to investigate the interactions among all aspects of the host antiviral immune response over time before recommending booster shots for the population as a whole.

A study was performed on healthcare workers who had large amounts of exposure to SARS-CoV-2 but remained seronegative despite having a COVID-19-like illness.⁵³⁹ Their T cell responses were suggestive of SARS-CoV-2 infection. This study also reported that greater than 90% of convalescent people or those who were not knowingly exposed to the virus had immune responses that are indicative of the presence of preexisting, cross-reactive T cells. Evidence indicates that T cell responses against the viral N protein may be longer-lasting than neutralizing antibody levels.⁵³⁸ All convalescent patients had CD4⁺ T

helper cell responses to SARS-CoV-2 and 70% had CD8⁺ T killer cell responses.³²³ Interestingly, 50% of people in that study had T cell reactivity to SARS-CoV-2 in serum samples predating the pandemic, suggesting that they had preexisting immunity, perhaps from prior infection with a different human coronavirus. Using ELISpot assays, survivors of SARS-CoV infection in 2003 still react against the SARS-CoV-2 S protein, suggesting the presence of long-term, cross-reactive T cell memory reactions.⁵³⁹ Other T cell assays can differentiate between present and prior infection with SARS-CoV-2 and cross-reacting T cell immunity to SARS-CoV or other human coronaviruses.⁵³⁹

High levels of both CD4⁺ T helper and CD8⁺ T killer cell responses are produced after two doses of the Pfizer vaccine, with most of the T cell responses directed against epitopes that are conserved between the original isolate and the Alpha and Beta variants.⁵³⁴ In people who received one dose of the Pfizer vaccine and 50% of previously infected people, however, neutralizing antibody activity in vitro was almost undetectable, especially against the Beta strain.

Clinical trials for Novavax revealed 60% and 85.6% protection against infection by the Alpha and Beta variants, however, none of the vaccinated people were hospitalized.⁵⁴⁰ Furthermore, preliminary work indicates that the two-dose regimen of the AstraZeneca vaccine is not protective against mild-to-moderate disease caused by the Beta variant.⁵⁴¹ AstraZeneca produces several single-dose adenovirus vector-based vaccines that express various components of the SARS-CoV-2 S protein. Of these, the Ad26-S.PP vaccine is the most promising. This vaccine induces vigorous neutralizing antibody responses in rhesus monkeys and almost total protection following challenge with the original viral strain as assessed by the absence of viral subgenomic RNA in bronchoalveolar lavage material and only a low amount of virus in the nasal swabs of one of the six animals tested.⁵⁴² A second dose of the Ac26-S.PP vaccine increases its efficacy 10-fold. The T cell responses are primarily of the antiviral, Th1 type.⁵⁴²

Several words of caution concerning repeated vaccine boosters: the possibilities of developing (1) myocarditis in young males and (2) **antibody-dependent enhancement (ADE)**. Of almost 2 million people receiving mRNA vaccines, approximately 1600 were determined to acquire myocarditis, well above the normal rate for their sex and age.⁵⁴³ The majority (82%) were males and the median age was 21 years. The prevalence of myocarditis was highest following the second vaccination: in males aged 12–15 years (70.7 per million doses of the Pfizer vaccine), in males aged 16–17 years (105.9 per million doses of the Pfizer vaccine), and in men aged 18–24 years (52.4 and 56.3 per million doses of the Pfizer and the Moderna vaccines, respectively).⁵⁴³ Symptoms include elevated troponin levels (98%), abnormal electrocardiogram results (72%), and abnormal cardiac magnetic resonance imaging results (72%). Approximately 96% of those with myocarditis were hospitalized.⁵⁴³

Repeated vaccination might also result in ADE, a condition in which, over several months, the waning levels of antibodies to one viral variant, upon infection or exposure to an analogous virus, form large antibody-virus complexes. These complexes are ingested by macrophages and replicate, increasing viral load and pathogenicity. This is more likely to occur if the vaccines were from different manufacturers since the viral components used in the vaccines would not be identical. ADE has been seen during infection with coronaviruses, including SARS-CoV, MERS-CoV, and feline coronavirus.^{544,545} This condition may greatly increase disease severity. During SARS, ADE appears to be associated with

antibodies against the S protein.⁵⁴⁴ To avoid ADE and other serious or life-threatening responses to vaccines, proper safety studies need to be conducted to study the potential beneficial and harmful effects of repeated boosters within a short period of time. N protein-based vaccines do not appear to trigger ADE in coronaviruses and may perhaps prove to be better for use in vaccines.

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Further reading

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Coronaviruses of wild and semidomesticated animals with the potential for zoonotic transmission

5.1 Introduction

Coronaviruses of animals, including wild, semidomesticated, agricultural, and companion animals, have the potential for zoonotic transmission. This chapter focuses on wild and semidomesticated animals. These include the following alphacoronaviruses: bat coronavirus HKU8, the rat Lucheng RN coronavirus, ferret enteric coronavirus (FRECv), and ferret systemic coronavirus as well as the following betacoronaviruses: Bat-CoV HKU4 and HKU5, WIV1 and WIV16 of bats, and murine hepatitis virus of mice. See [Fig. 5.1](#) for an overview of coronaviruses of wild and semidomesticated animals.

5.2 Transmission of coronaviruses

Some of the above coronaviruses have or may serve as the ancestral or reservoir hosts of both mildly and highly pathogenic human coronaviruses. Bats are believed to have housed coronaviruses that infected and adapted to other animals before undergoing zoonotic transmission. People living in low-income regions are particularly vulnerable to zoonotic transmission since they live in close proximity to either urban or rural wild animals, including bats and rats, which are known to directly or indirectly introduce their viruses to humans.¹

Cross-species transmission among animal hosts is believed to be an important factor in zoonotic transmission (“spill-over”) from animals into humans,² especially from bats to other mammalian hosts before infecting humans. For example, members of the alphacoronavirus Ghana bat coronavirus group I and human HCoV-229E appear to share a common ancestor.^{3,4} The human betacoronaviruses, the respiratory HCoV-OC43 and the human enteric coronavirus HCoV-4408, appear to have originated from a bovine coronavirus (BCoV).⁵ Despite their similarities, HCoV-OC43, BCoV and canine respiratory coronavirus

Bat-CoV α HKU2 HKU6 HKU8 HKU10	Bat-CoV β Lineage B HKU3 LYRa11 Rf1 Rs3367 RsSHC014 WIV1 WIV16	Bat-CoV β Lineage C HKU4 HKU5 HKU25 NeoCoV PML/2011 SC2013	Bat-CoV β Lineage D HKU9

Mouse-CoV HKU24 MHV Longquan Aa mouse-CoV	Rat-CoV HKU24 Lucheng Rn CoV Longquan Rl rat-CoV RCV	Other Rodent-CoV AcCoV-JC34 Myodes CoV 2JL14 PLMg1 UKMa1 UKMa2 UKRn1

Rabbit-CoV HKU14 KU739072 Rabbit coronavirus Rabbit enteric coronavirus	Ferret-CoV Ferret enteric coronavirus Ferret systemic coronavirus	Mink-CoV Mink-CoV

Hedgehog-CoV HKU31

FIGURE 5.1 Coronaviruses of wild and semi-domestic animals. An overview of the best studied and named coronaviruses of bats, rodents, ferrets, minks, rabbits, and hedgehogs. Bat and rodent coronaviruses are believed to have hosted the ancestors of the great majority of coronaviruses currently found in other mammalian host species. *Figure created by Lisa Beltz (author).*

(CRCoV) differ in species specificity. While they are all believed to bind to sialic acids of heparin sulfate present on proteins within target cell plasma membranes, they bind to different extents.⁶ Additionally, these molecules permit entry for only the clinical strain of HCoV-OC43 while other molecules function as attachment receptors. CRCoV and BCoV use human leukocyte antigen class I (HLA-1) as their entry receptor. Severe acute respiratory syndrome virus-2 (SARS-CoV-2) infection and replication may occur in ferrets and cats, but not in dogs or pigs.⁷

In addition to preventing the zoonotic transfer of coronaviruses from animals to people, it is important to prevent the transmission of coronaviruses from people to animals, including human-to-wildlife coronavirus transmission, not only from an ecological and humanitarian standpoint but also to prevent the establishment of novel coronavirus reservoirs in wild animals.⁸ Accordingly, it may be useful to produce lists of procedures that minimize the risk of both zoonotic and human-to-animal transmission of coronaviruses, with different lists for each type of illness, subdivided into procedures for different groups of animals. Also, cross-species transmission has shown that even if a coronavirus-induced disease is mild in one animal species, it may become more pathogenic in the recipient species, such as the mild form of MERS-CoV found in dromedaries in comparison to the potentially fatal form of infection in humans.

It should be noted that there is strong evidence that SARS-CoV-2 and several other human coronaviruses may also be passed from humans to animals, including threatened species of wild animals, such as nonhuman primates and wild felines. For example, “human” SARS-CoV-2 infects captive tigers (*Panthera tigris*) and lions (*Panthera leo*), snow leopards (*Panthera uncia*), pumas (*Puma* species), gorillas (*Gorilla* species), macaques (*Macaca* species), minks (*Neogale* species), and pangolins (spiny anteaters; *Echidnas* species),⁹ while bats (*Chiroptera* species), mice (*Mus* species), ferrets (*Mustela putorius furo*), hamsters (*Cricetinae* species), and other nonhuman primates, such as tree shrews (*Scandentia* species), can be experimentally infected with SARS-CoV-2.^{7,10}

5.2.1 Genetic recombination between coronavirus animal hosts

Coronaviruses primarily cause respiratory and gastrointestinal (enteric) diseases in humans, while they are associated with respiratory, enteric, or neurological diseases in animals.¹¹ Many species of coronaviruses from different host species have large regions of RNA that share genetic identity or similarity in their proteins. The reason for the large degree of similarity among some of the genes of coronaviruses inhabiting different host species is due to a large degree to the importance of the encoded proteins in the viruses’ life-cycle. If these genes were to change, coronaviruses of the host species, whether humans, cats, bats, palm civets, camels, ferrets, and many other coronaviruses, would not be able to function. These are the **conserved genes**. The similarities in the conserved genes might also indicate that one of the viruses served as an ancestor of another coronavirus in the past with the degree of similarity corresponding to the time period from the evolutionary split. The extent of the identity of the total genome among coronaviruses of humans, cats, bats, camels, and other mammals is very high, often greater than 95%.¹² Coronaviruses may “jump” between animals as well. For example, MERS-CoV might be able to spread among llamas (*Lama glama*), pigs, and young sheep and horses via the intranasal route. By contrast, signs of MERS-CoV replication and virus-specific antibodies are not detected in adult sheep or horses.¹³

Other regions of the RNA, however, contain genes that can mutate without harming the virus, although mutations of these genes could lead to substantial changes in the viruses’ life cycle. This is particularly true of the S protein since it binds to a specific receptor on its target cells and allows the virus to infect that specific cell type from a specific species of animal. Genetic analysis indicates that gene recombination may be a major factor in the transmission of coronaviruses between host species.² For example, canine coronavirus (CCoV) II is believed to have originated from the recombination between CCoV I and another coronavirus. Feline coronavirus (FCoV) II appears to be a recombinant virus that contains the CCoV-II S protein gene and the backbone of FCoV-I based upon their accessory protein open reading frame 3 (ORF3).¹⁴ Genetic studies suggest that transmissible gastroenteritis coronavirus of pigs (TGEV) may have originated from CCoV-II as well.^{14,15} Due to some unusual features in their 5’ untranslated region, recombination also appears to have occurred between *Scotophilus* bat CoV-512 and porcine epidemic diarrhea virus (PEDV) strains recently found in the United States.¹⁶ BCoV-like coronaviruses have been found in captive wild ruminants as well as members of the **camelid** group.²

Deltacoronaviruses are genetically very diverse and undergo frequent shifts between their bird hosts. They are believed to have originated in birds and evidence suggests that they are transmitted from birds to swine as well.^{17,18}

Mutations in the coronavirus genomes were described in much greater detail in Chapter 1.

5.2.2 The viral spike protein and host coronavirus receptors

The angiotensin-converting enzyme 2 (ACE2) enzyme serves as a receptor for a variety of coronaviruses, including SARS-CoV and SARS-CoV-2. Both viruses and some other human coronaviruses can infect human cells with ACE2 on their surface, including type II pneumocytes in the lungs and cells found in the heart, kidneys, small intestine, and liver. The genes encoding the ACE2 receptor differ between host species so that a coronavirus of pigs is usually unable to bind to the ACE2 receptor of sheep. Accordingly, pig coronaviruses typically only infect pigs, unless the pig S protein mutates to a form that allows it to bind to an ACE2 from a different host species. This is called **species restriction**. In the same manner, a bat coronavirus typically cannot bind to or directly infect human cells without undergoing a specific mutation that would allow the bat coronavirus to bind to the human ACE2 receptor. The extent of the similarities in ACE2 among various animal species also, to a large degree, determines if a given animal is a suitable host for the various species of coronaviruses. ACE2 RNA sequences from the domestic cat have an 85% overall identity with that of human ACE2, while the ACE2 gene from Malayan pangolins, European rabbits, raccoon dogs (*Nyctereutes procyonoides*), masked palm civets (*Paguma larvata*), dromedary camels, and domestic dogs have 83%–85% sequence identity.¹⁹ Interestingly, the ACE2 from the proposed reservoir hosts for SARS-CoV and SARS-CoV-2, Chinese rufous horseshoe bats (*Rhinolophus sinicus*) and great roundleaf bats (*Hipposideros armiger*), respectively, is only 80.7% and 80.5% similar to that from humans.

The gene that is most important in determining which host species a coronavirus can infect encodes the S protein. Unlike the high degree of identity or similarity among the coronaviruses' total RNA, the S protein genes from coronaviruses of various host species differ substantially, with identity between human and bat coronaviruses being only 70%–80%. On rare occasions, a mutation in the S protein gene occurs that allows it to infect another host species. Such a rare genic alteration appears to have occurred in certain bat coronaviruses S protein genes, either naturally or artificially in a laboratory, so that a bat coronavirus was then able to infect palm civets or raccoon dogs. A similar, rare S protein gene mutation may then have occurred that allowed the now civet-specific coronavirus to infect humans, as in the case of SARS-CoV. This type of mutation is very uncommon, but when it does occur, the mutant coronavirus may produce many asymptomatic infections or mild cold-like diseases. Some of these novel coronaviruses might, however, cause life-threatening diseases in some infected humans, especially if this particular new strain of coronavirus is able to spread from human to human, as is the case for SARS-CoV, SARS-CoV-2, and MERS-CoV. Such viruses may cause pandemics that infect and kill hundreds of thousands of people. Fortunately, once a person who has been infected recovers and has cleared the virus, as is the case for the majority of people infected with SARS-CoV-2,

the person is immune to that particular variant of the virus and will not be infected again the next time that the same viral variant appears in the population. This process may be seen as a natural form of immunization and, usually, those who were infected and recovered are better protected against reinfection than those who received a man-made vaccine. Recent data suggest that many people have been infected with SARS-CoV-2 and were either asymptomatic or had only a mild form of the disease. These people are now immune to reinfection, making it much less likely that this and perhaps other coronavirus variants will cause massive infections later.

5.2.3 Introduction to coronaviruses and intracellular signaling pathways

Protein kinases and **phosphatases** add or remove phosphate ions from proteins and thereby control many cellular processes, including cell proliferation and differentiation and extracellular and intracellular signaling pathways. These compounds link these pathways to other signaling pathways, including the **Ras/mitogen-activated protein kinase** (MAPK), **PI3K/Akt**, and **JAK/STAT** pathways.²⁰ Pharmaceutical alteration of these pathways may strongly affect viral replication and disease severity. Receptor tyrosine kinase inhibitors, such as A9, strongly inhibit replication of various coronaviruses, including mouse hepatitis virus (MHV), TGEV and PEDV of pigs, and feline infectious peritonitis virus (FIPV) of cats.²⁰ In TGEV-infected pigs, this inhibition occurs via the MAPK **p38** signaling pathway,²⁰ one of the three MAPK pathways is used during MHV RNA synthesis.²¹ The phosphorylation state of the **epidermal growth factor receptor** regulates TGEV entry into its target cell.²² Imatinib, an inhibitor of **Abelson kinase**, inhibits SARS-CoV and MERS-CoV in vitro.²³ The effects of various coronaviruses on intracellular signaling will be described more fully later, in the sections for each type of coronavirus.

5.2.4 Coronavirus vaccines

Vaccines are available against some animal coronaviruses discussed in this chapter. Much useful information can be obtained from the examination of problems that have been encountered with these vaccines and may aid in the development of vaccines against human coronaviruses. For example, PEDV, a coronavirus of pigs, undergoes frequent genetic changes, as does infectious bronchitis coronavirus of chickens. CCoV vaccines, while protective in puppies, are not of great importance since the associated disease is mild and self-limiting. Pathology induced by FCoV vaccines ranges from mild to fatal immune-mediated disease. This means that care must be taken in the administration of vaccines to domestic cats and depends upon the species of virus and its mechanisms of action. Vaccines against BCoV protect against intestinal and respiratory disease in young calves. These vaccines are frequently used in cattle farms. Pigs, by contrast, are subject to infection with multiple coronaviruses.²⁴ PEDV is difficult to control due to its frequent genetic changes, even though several vaccines have been developed against it. Some of the vaccines only have a short period of time in which they are protective.²⁴ More importantly, sometimes antibodies produced following vaccination are pathogenic due to their triggering of antibody-dependent enhancement, as described later in this chapter.

5.2.5 Severe acute respiratory syndrome virus-2 and its animal hosts

Zoonotic transmission of SARS-CoV-2 has been reported in mink farms and perhaps from pangolins in live animal markets (“wet markets”).²⁵ Of note: a new mink-associated SARS-CoV-2 variant has emerged and is found in both humans and minks.²⁵ The severity of SARS-CoV-2 diseases of the respiratory and gastrointestinal tracts and the central nervous system (CNS) varies by host animal species. For example, SARS-CoV-2-associated disease is typically mild to moderate in felines but may be severe to fatal in minks.²⁶ Several instances of SARS-CoV-2 outbreaks have resulted in acute interstitial pneumonia in minks that are in mink farms in the Netherlands as well as Denmark and Spain.⁹

SARS-CoV-2 binds to ACE2 on host target cells via the S protein’s receptor-binding domain (RBD). ACE2 from 18 species of Old World primates have a very high likelihood of binding to the SARS-CoV S protein. The ACE2 RBD of all tested primates is 100% similar to that found in human cells. Other animal species whose cells have a high susceptibility to infection by SARS-CoV-2 include cetaceans (whale, dolphin, or porpoise), rodents, cervids (deer), lemuriform primates (lemur-like animals), giant and collared anteaters, and Angola colobus monkeys.²⁷

Studies of molecular docking of host ACE2 to the SARS-CoV-2 S protein indicate that the greater horseshoe bat (*Rhinolophus ferrumequinum*), shown in Fig. 5.2, is potentially the virus’ primary reservoir. Wild animals, such as European rabbits (*Oryctolagus cuniculus*) and grizzly bears (*Ursus arctos horribilis*) are potential SARS-CoV-2 secondary reservoirs.²⁸ Docking studies offer a way to examine many types of animals for the potential to act as reservoirs for many viral species, however, both host receptor and viral binding proteins must be first known. Viral RNA has been detected in organs or tissues of animals infected with SARS-CoV-2 via the nasal route. Ferrets and domestic cats (*Felis catus*) are susceptible to infection and may



FIGURE 5.2 Greater horseshoe bat. The greater horseshoe bat (*Rhinolophus ferrumequinum*) has a large range throughout southern Europe and Asia and Northern Africa. They are believed to have hosted the ancestor to many pathogenic animal and human coronaviruses and continue to serve as a reservoir for many other coronaviruses of unknown pathogenicity. By Marie Jullion - Own work, CC BY-SA 3.0, <https://commons.wikimedia.org/w/index.php?curid=3359176>.

serve as animal models of COVID-19, but dogs (*Canis canis*), pigs (*Sus*), chickens (*Gallus gallus domesticus*), and ducks (*Anas platyrhynchos domesticus*) are not.⁷

ACE2 proteins from a wide range of mammals allow SARS-CoV-2 to enter cells. These animals include rabbits (*Oryctolagus cuniculus domesticus*) and pangolins, as well as domestic animals, such as dromedary camels (*Camelus dromedarius*), cattle (*Bos taurus*), horses (*Equus caballus*), goats (*Capra aegagrus hircus*), sheep (*Ovis aries*), and cats.²⁹ Furthermore, the pangolin and bat coronaviruses, Pangolin-CoV-2020 and Bat-CoV RaTG13, respectively, can bind to human ACE2 and ACE2's from other animals, suggesting that these two viruses have the potential for transmission into both humans and other animals.

Pangolins are believed to be the intermediate host for zoonotic transmission of SARS-CoV-2. The pangolin-CoV MP789 isolate has the strongest genetic resemblance to SARS-CoV-2 found among pangolins.^{30,31} However, using whole-genome analysis, this pangolin coronavirus has only 86% nucleoside identity with the SARS-CoV-2 genome,^{30,31} making it unlikely to be the direct precursor to SARS-CoV-2. It has been suggested that SARS-CoV-2 may have arisen by recombination of a pangolin coronavirus with another coronavirus species.³¹

Pangolins host several other species of SARS-CoV-2-related coronaviruses. Pangolin-CoV-2020 and GD Pangolin CoV have 90.23 and 92.4% nucleoside sequence similarity to SARS-CoV-2, respectively, and have only a single amino acid difference from SARS-CoV-2 within the ACE2 binding portion of S protein.^{29,32}

5.3 Coronaviruses of bats

5.3.1 Introduction to bat coronaviruses

Coronaviruses have been detected in the following bat families: primarily Vespertilionidae and Rhinolophidae, however several bat coronaviruses are also present in Hipposideridae and Pteropodidae. *Miniopterus* and *Myotis* genera bats harbor alphacoronaviruses, while *Pipistrellus*, *Tylonycteris*, and *Rhinolophus* genera bats harbor both alpha- and betacoronaviruses.³³ *Rhinolophus* species are the primary hosts of SARS-like CoVs in China, while *Chaerephon* and *Hipposideros* bat species host these viruses in China and Africa.³³

A great deal of work has focused on the potential link between coronaviruses of bats and those of humans. Bats, as well as some other mammals, harbor a great variety of coronaviruses that only very rarely spill over into human populations. Of the seven known human coronaviruses, five (HCoV-229E, HCoV-NL63, SARS-CoV, MERS-CoV, and SARS-CoV-2) are believed to have been transmitted to humans from bats, especially rhinolophid (horseshoe) and vespertilionid (common or vesper) bats.³⁴ The viruses are then transmitted to intermediate hosts, including wild carnivores (palm civets and raccoon dogs), dromedary camels, alpacas, and perhaps pangolins.³⁵ Domestic cats may also serve as vehicles by which coronaviruses are passed back and forth between bats and cats due to bat predation by cats.³⁶ This is a well-established phenomenon in the acquisition of rabies by bats.

A study of zoonotic transmission of coronaviruses from various animals was conducted between 2017–2019 in rural regions of Southern China (Yunnan, Guangxi, and Guangdong Provinces). Bat infection with SARS-related coronaviruses is common in this area. However,

one study indicated that only 0.6% of the greater than 1500 people tested were seropositive for bat coronaviruses, indicating that a bat coronavirus spillover is a rare event.³⁷

A study of coronaviruses in wildlife was conducted from 2009–2015 throughout Gabon in Central Africa. The wildlife species included over 1800 samples from cave-dwelling bats, rodents, nonhuman primates, and other wild animals.³⁸ Bat feces and organs were tested for coronavirus RNA. In this study, coronaviruses were only detected in the following cave-dwelling bats from the northeast part of the country: the giant roundleaf bat (*Hipposideros gigas*), Noack's roundleaf bat (*Hipposideros* cf. *ruber*), and the greater long-fingered bat (*Miniopterus infatus*). No coronaviruses were found in other bats in caves housing infected *Hipposideros* species, which may be attributed to the latter bat species roosting in a separate part of the cave. All alphacoronaviruses were detected only in *H. gigas* and *H. cf. Ruberg*. The viruses are grouped with human coronavirus 229E, with a nucleoside identity of 91%–93% with bat coronaviruses as well as with a coronavirus from alpacas. The inability to detect coronaviruses in other Gabonese wild animals may be due to the sample size.³⁸ It should also be noted that the tested rodents came primarily from urban areas, not from rural areas that are home to many rodents and bat species. A separate study conducted in Nigeria in 2008 detected Zarian bat coronavirus in *Hipposideros commersoni*.³⁹

Most bat coronaviruses do not cause disease in bats, just as most coronaviruses in humans do not cause disease or, if they do, it is usually mild and cold-like. Many coronaviruses that are pathogenic to humans belong to betacoronavirus lineages A and B. In addition to lineage A, B, and C viruses, a lineage D betacoronavirus has been reported in Leschenault's rousettes (*Rousettus leschenaulti*). These bats are also infected with the betacoronavirus Ro-BatCoV HKU9 and did not develop clinical disease and thus may serve as a reservoir for Ro-BatCoV HKU9 in rodents.⁴⁰ In this study, complete genome sequencing indicated the coexistence of at least two distinct coronavirus genomes in both of the two bats tested.⁴⁰ In one case, the two genomes isolated from the same digestive tract sample had greater than 20% nucleoside substitutions that were evenly distributed throughout the genome. Finding multiple genotypes of a single bat coronavirus appears to be unique to *R. leschenaulti* or very rare among other bat species, perhaps due to the unusual aspects of *Rousettus* species bats, such as roosting in extremely densely packed colonies of up to 6,800 individuals.⁴⁰

5.3.2 WIV1, WIV16, SARS-CoV, and adaptation to different host species

Some bat coronaviruses may be able to directly infect humans. The SARS-like bat coronavirus, WIV1, is one such virus. Its primary host appears to be *R. sinicus* bats. It has been found in the fecal material of *R. sinicus* but neither causes clinical disease nor replicates in the related Egyptian fruit bat (*Rousettus aegyptiacus*).⁴¹ WIV1 has 99.9% sequence identity to the bat Rs3367 coronavirus, described later. A closely-related coronavirus, WIV16, also infects *R. sinicus* bats in Yunnan, China. Both WIV1 and WIV16 use ACE2 from these bats, as well as ACE2 orthologs from civets and humans, as their host receptors,^{42–44} implying that these coronaviruses may have the potential to make the species jump from bats to humans with or without an intermediate host.⁴⁵

WIV1 and WIV16 are among the closest relatives to SARS-CoV among animals, especially in the RBD region of the S protein.⁴¹ Nevertheless, WIV1-CoV only replicates to a low degree in primary human airway epithelial cells in vitro.⁴¹ In in vivo studies performed in chimeric mice engineered to express human ACE2, the titers of WIV1-CoV in the lungs of experimentally infected animals are 100-fold greater than those in wild-type mice. WIV1-CoV remains, however, significantly **attenuated** (nonpathogenic live virus) in mice bearing human ACE2.⁴⁶

5.3.3 Chimeric bat coronaviruses and severe acute respiratory syndrome virus

SARS-CoV replicates effectively in the respiratory tract of inexpensive, abundant, young BALB/c mice, but the mice do not develop clinical disease following experimental infection, unlike older, less available, adult mice, which develop pneumonitis. To be able to use young mice in coronavirus studies, a **chimeric** coronavirus was engineered in which the SARS S protein from a mouse-adapted SARS-CoV Urbani strain variant (SARS-MA15) was produced by serial passages in mice.⁴⁷ Following intranasal infection with SARS-MA15, young mice develop a rapid, high viral titer and pathological damage to the lungs, and infection of bronchial epithelial cells and alveolar pneumocytes. This is followed by the production of necrotic cellular debris and death.⁴⁷ Viremia is also evident, leading to the dissemination of the virus to extrapulmonary sites.

Another example of a study using chimeras involved the replacement of the RBD of the bat coronavirus Rp3 with that of SARS-CoV, which conferred the ability of bat SARSr-CoV Rp3 to bind to the human ACE2 receptor, facilitating viral entry into human cells.^{48,49} Additionally, amino acid substitutions in either the RBD of Y442S or L472F of the SARS-CoV BJ01 variant increase their affinity to human ACE2 in vitro.⁵⁰ Such gain-of-function studies in which pathogenic viruses are created that have even greater pathogenicity or are given the ability to cause disease in a previously resistant host were soon banned in the United States.

Another chimeric virus, WIV1-MA15, is the product of WIV1 in which the S protein of the mouse-adapted SARS-MA15 virus replaces that of WIV1. WIV1-MA15 has the same cellular binding and entry abilities as WIV1-CoV. When young mice were infected with SARS-CoV MA15, they rapidly lost weight and died by day 4 postinfection. WIV1-MA15-infected mice, however, do not die or experience significant changes in body weight.⁴⁶ Mice infected with WIV1-CoV or SARS-CoV Urbani did not have significant weight loss in comparison with animals infected with SARS-MA15. Viral replication was nearly 10,000- and 1000-fold less in mice infected with WIV1-CoV than those infected with SARS-CoV Urbani,⁴⁶ indicating the importance of the coronavirus's S protein in viral replication, weight loss, and lethality. It should be noted that results in mice need to be repeated in nonhuman primates to aid in determining the situation in humans.⁴⁶

While WIV1 and WIV16 may have the ability to undergo limited human-to-human transmission, additional adaptations, however, would be required for them to cause epidemic disease in humans.⁴⁶ These alterations could be present in regions of the S protein that do not affect receptor binding activity, such as other portions of the S1 or the S2 regions, as is the case for the MHV variant V51.⁵¹ The potential for either WIV1 or WIV16

to adapt to humans indicates the need for continuing study and surveillance of these two viruses and related bat coronaviruses.⁴⁶

The RBD of bat WIV1 differs from that of human SARS-CoV in critical three amino acids. A gain-of-function study in which these three residues in SARS-CoV were replaced with their counterparts in WIV1 indicates that one particular RBD alteration (Y442S) increases the ability of WIV1 to bind to bat ACE2, potentially allowing the chimeric SARS/WIV1 to infect bats as well as humans.⁵⁰

Entry of WIV1 into host target cells is decreased by interferon (IFN)-induced transmembrane proteins (IFITMs), which are also active against all human coronaviruses, except HCoV-OC4 in humans.⁵² The airway **protease** TMPRSS2, however, allows WIV1 to partially escape the effects of IFITM3. The antifungal agent amphotericin B also increases cell entry of SARS-CoV and similar viruses, including WIV1, by escaping IFITM3-mediated restriction.⁴⁵

An important collaborative study from researchers at the University of North Carolina, Chapel Hill, in the United State, and a BioSafety Level 4 laboratory in Wuhan, China, produced a chimeric coronavirus using a SARS-like virus, SHC014-CoV, from *R. sinicus* bat populations and a mouse S protein.⁴⁴ The S protein from this engineered chimeric virus can use human ACE2 to enter and replicate efficiently in primary human airway cells, reaching in vitro titers similar to those of epidemic strains of SARS-CoV. In vivo experiments demonstrate that the chimeric virus not only replicates in mouse lungs, but it is notably pathogenic. Furthermore, currently available SARS-based therapeutic and prophylactic modalities are not effective in preventing or treating the severe disease, thus both the novel viruses' host species range and pathogenicity are increased.⁴⁴ Since the chimeric virus produced by this study can infect human airway-derived cells in vitro, it may also have gained the ability to do so in humans in vivo with potentially devastating effects.

Several naturally occurring species of bat SARS-like-CoV that have greater or lesser similarity to SARS-CoV continue to be found. These bat viruses are known to be present in Japan, China, and Thailand.⁵³ The bat coronaviruses ZXC21 and ZC45, were reported in a 2018 study of *Rhinolophus* species bats from the Zhejiang province of China, an eastern coastal region that does not border the Yunnan province in which many of the SARS-like-CoV have been reported. These two new species are only 81% similar to SARS-CoV from humans or palm civets and are thus unlikely to undergo zoonotic transmission.⁵⁴

Many different SARS-related-CoVs, including WIV1, are present in bats from a single cave in Yunnan Province, China, which houses *Rhinophilus*, hipposiderid, and myotis bats. *Rhinophilus* species have highly diverse genes for the S protein RBD, ORF3, and ORF8. Some of these genes are highly similar to those present in SARS-CoV and are also very closely related to SARS-CoV's ORF1a and 1b.⁵⁵ Some bat coronaviruses have over 98% amino acid identity to civet and human SARS-CoV in their E, M, and N genes, in addition to ORF1a and ORF1b.⁵⁵ It has been hypothesized that even if WIV1 can infect humans, it may cause mild symptoms. A study using the BALB/c mice model demonstrated that WIV1 virus titer and symptoms are significantly less for WIV1 compared to those produced by the severe human SARS-CoV isolates Urbani.⁵⁶

The bat coronavirus Rs4231 is closely related to another bat coronavirus, WIVI6, but has a distinct RBD. WIVI6 may have arisen from recombination between Rs4231 and WIV1.⁵⁵ *R. ferrumequinum* appear to host bat coronaviruses that are closely related to SARS-CoV in

the ORF1a/1b region. Similar or identical viral strains are also present in bats from another location that is close to the Yunnan cave.⁵⁷

WIV16 present in fecal material from *R. sinicus* in the Yunnan Province, China, is one of the closest relatives to SARS-CoV. Its RBD is almost identical to that found in the WIV1 coronavirus as well. Like WIV1, WIV16 has an additional ORF (ORFX) between ORF6 and ORF7, making it slightly different from the genomes of civet and human SARS-CoV.⁵⁸ ORFX is expressed during WIV1 infection and replicates efficiently in vitro. This gene blocks the production of the antiviral host immune molecule IFN and activates the host cell's transcription factor **nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B)**, but is not required for replication.⁵⁹ Interestingly, several SARS-CoV ORFs (ORFs 3a, 3b, and 6) also inhibit the IFN response, while ORF3a and ORF7a activate NF- κ B.⁵⁹

Other similarities between WIV16 and SARS-CoV have been reported. The WIV16 and SARS-CoV S protein genes share 95% nucleoside and 97% amino acid sequence identity. Additionally, the critical WIV16 S protein's RBD and that of SARS-CoV share 95% sequence identity.⁵⁸ Another bat coronavirus, Rs4874, also has an S protein gene that is almost identical to that of WIV16.

The SARS-CoVs initially present in patients during the 2003 SARS epidemic all contained a single full-length ORF8, as do some of the bat SARS-related-CoVs from the above Yunnan bat cave. During the latter parts of the human SARS epidemic, however, ORF8 split into the overlapping ORF8a and ORF8b RNA regions, resulting in a 29-nucleotide deletion. The above cave also contained a bat SARS-related-CoV with split ORF8a and ORF8b, although this split appears to be independent of that present in human SARS-CoV.⁶⁰ Four more split SARS-CoV isolates from later in the 2003 epidemic contain a 415-nt deletion that entirely removes ORF8. These may have occurred well after zoonotic transfer.

Other potential recombination sites are found around the ORF8 gene region in both SARSr-Rs-BatCoVs and SARSr-Rf-BatCoVs, derived from *R. sinicus* and *R. ferrumequinum*, respectively. These are believed to be involved in the production of civet SARSr-CoV SZ3, whose ORF8 may have been acquired from SARSr-Rf-BatCoVs.⁵⁷ Since these horseshoe bat species have overlapping geographical ranges, similar diets, and can roost in man-made structures, they may cohabit in similar environments in Yunnan Province and might be able to exchange their coronaviruses.⁵⁷

Two novel coronaviruses, RmYN02 and RaTG13,^{61,62} have been reported in Yunnan Province. These coronavirus species are similar to SARS-CoV-2. RmYN02 from the acuminate horseshoe bat (*Rhinolophus acuminatus*) of southern China and Thailand shares a genome-wide 93.3% nucleoside identity with SARS-CoV-2. RnYN02 is also present in the Malayan horseshoe bat (*Rhinolophus malayanus*)⁶¹. RmYN02 contains an insertion at the S1/S2 cleavage site in the S protein, leading to only 61.3% nucleoside identity in other RBD nucleosides.⁶¹ Whole genomic sequencing demonstrates that nucleosides of RaTG13 from *R. affinis* bats have 96.1% identity to SARS-CoV-2. RmYN02 is also closely related to RacCS203 present in *R. malayanus*. Highly specific SARS-CoV-2 **neutralizing antibodies** are present in other bats from the same colony as well as in a Malayan pangolin from southern Thailand.⁵³

Bat SARS-like-CoVs can be placed into two lineages that either do or do not use ACE2 as their receptor. Interestingly, of the tested bat and pangolin coronaviruses, GX-P5L pangolin coronavirus bound to human ACE2 the best, while RaTG13 bound the worst.⁵³

Additionally, highly specific neutralizing antibodies to SARS-CoV in 2003 and SARS-CoV-2 in 2020 have been detected in pangolins. Bat SARS-related CoVs with loop deletions in the RBDs of ZXC21, ZC45, RMYN02, and RaCS203 do not bind human ACE2 and may therefore use a currently unknown receptor. In contrast, RaTG1, pangolin/MP789/2019, and pangolin/P5L/2017 have no deletions in the RBD and their external domains have similar conformations to that of SARS-CoV-2, indicating that they may also use ACE2 as their cell receptor.

Analysis of coronavirus proteins found that SARS-CoV-2 is most closely related to viruses sampled from Malayan pangolins, the intermediate horseshoe bat (*Rhinolophus affinis*), and *R. malayanus*.^{31,62–65} Pangolin GD/2019 has 90.7% and 97.4% amino acid identity to SARS-CoV-2 in the S protein and its RBD, respectively, pangolin GX/P5L/2017 has 92.4% and 86.8% identity; bat coronavirus RMYN02 has 72.9% and 62.4%; bat RaTG13 from *R. affinis* has 97.4% and 89.3% identity; bat ZC45 has 80.2% and 63.5% identity, bat ZXC21 has 79.6% and 62.9% identity, while SARS-CoV GZ01 has only 76.2% and 74.6% identity (Andersen et al., 2020).⁶⁶ A table from Zhou et al. reports the degree of identity in other proteins and genes from pangolins and bats. Very recently, the betacoronavirus *Myodes* CoV 2JL14 was found in the Yunnan Province of China.^{67,68}

New coronaviruses of bats continue to be discovered. Coronavirus RNA was present in 22% of fecal samples from the insectivorous lesser Asiatic yellow bat (*Scotophilus kuhlii*) from bat farms in Viet Nam as well as in 4.4% of rat fecal samples from wet markets.⁶⁹ These novel viruses are related to coronaviruses in bat and rodent populations throughout the world, including bat coronaviruses in China and the Philippines. Zoonotic transmission of the new coronaviruses from bats or rats has not yet been directly observed.

A 2016–2017 study of bat coronaviruses in the Yunnan, Guangxi, and Sichuan Provinces of southern China found 15 species of coronaviruses.⁷⁰ *S. kuhlii* is host to four alphacoronaviruses that are closely related to a known bat coronavirus and PEDV of pigs. BtCoV/512 is another bat coronavirus that infects *S. kuhlii* and is closely related to PEDV.^{71,72} Taken together, these findings suggest a possible bat-swine lineage.⁷⁰

An alphacoronavirus, α -YN2018, is present in *R. sinicus* and appears to have resulted from multiple recombinations between bat coronaviruses over long periods of time. Five SARS-related coronaviruses are also found in *Rhinolophus* bats. The betacoronavirus strain β -GX2018 from the greater short-nosed fruit bat (*Cynopterus sphinx*) appears to have independently evolved from other coronaviruses found in flying foxes (*Rousettus* species) and dawn bats (*Eonycteris* species).⁷⁰ The S1 region of β -GX2018 is more closely related to that of PEDV than to other bat coronaviruses.

5.3.4 The spike protein of bat and human coronaviruses and angiotensin-converting enzyme 2

Critical residues in bat ACE2 receptors restrict viral entry into some types of host species and host cells, leading to the suggestion by Yan et al. (2021)⁷³ that many bat species are not potential hosts for SARS-CoV or SARS-CoV-2. When testing the ability of SARS-CoV and SARS-CoV-2 to utilize ACE2 orthologues from 46 diverse bat species representing eleven bat families to enter bat cells in vitro, 24 and 21 bat species are unable to permit

infection by SARS-CoV and SARS-CoV-2, respectively.⁷³ Bat coronaviruses often are unable to enter ACE2-positive human cells either. Another study that included 252 mammals estimated the ability of ACE2 to bind to the SARS-CoV-2 S protein based on sequence similarities. It predicted that all tested 37 bat species fell into the low or very low binding categories.²⁷ Interestingly, even closely related bat species differ in their binding categories. *R. sinicus* allows SARS-CoV entry into its target cells, while *R. ferrumequinum* and Pearson's horseshoe bat (*Rhinolophus pearsonii*) do not. This suggests that many bat species are not potential hosts for SARS-CoV or SARS-CoV-2 and that it might be important to pay more attention to those bat species that are currently potential hosts of pathogenic human coronaviruses. This study also found no correlation between bat proximity to humans and the ability to serve as natural hosts of SARS-CoV or SARS-CoV-2.⁷³

Whether or not an animal species can act as a host for a given species of coronavirus relies upon the ability of the viruses' S protein to recognize a potential host species' form of the receptor, such as human vs bat vs pig ACE2. Many studies have suggested that the ancestors of human SARS-CoV, MERS-CoV, COVID-19, HCoV-229E and NL63 coronaviruses may have originated in bats and rodents.⁶¹ Studies of the entire viruses' RNA genome found 88%–92% identity between SARS-like coronaviruses in bats and SARS-CoV in humans and civets, due to a large degree of the sizeable amounts of conserved genes among these coronavirus species. When only the gene for the coronavirus S protein is considered, however, the genetic identity between coronaviruses from bats and humans or civets falls to 76%–78%. This difference is important since the S protein is crucial to the binding of coronaviruses to either human or civet cells. The difference between the bat and civet viruses' S protein genes is especially high in the RBD, the region most directly involved in binding to the form of the ACE2 found on civet or human cells. Without a specific mutation in that portion of the S protein gene, the bat coronavirus is not able to infect civet or human cells.

A rather unusual alphacoronavirus, bat-CoV HKU2 from *R. sinicus*, has the smallest coronavirus genome. It is similar to Bt/CoV/512/05 from *S. kuhlii* in that these viruses share a small ORF following the N protein gene that is not present in similar coronaviruses.¹⁸ Its S protein is odd in that it has deletions similar to those found in betacoronaviruses as well as a 15-amino acid peptide homologous to that in the RBD of SARS-CoV.¹⁸ The S protein of bat-CoV HKU2 also contains multiple deletions and has only 27% amino acid identity to S proteins of other known alphacoronaviruses. This indicates that part or all of its S protein may have been obtained by recombination with a currently unidentified coronavirus or that this protein may be the product of many mutations in response to selective pressure.¹⁸ Both *R. sinicus* and *R. ferrumequinum* in China harbor both alpha- and betacoronaviruses.⁷¹ Bat-CoV HKU2 has been found in alimentary specimens of 8.3%–10.9% *R. sinicus* from Hong Kong and Guangdong, China, respectively, suggesting a possible enteric tropism for HKU2 in this area of Asia.¹⁸ It is also possible that the virus may be merely passing through the alimentary canal of the bats rather than infecting them. An HKU2-related bat coronavirus from pigs, swine acute diarrhea syndrome coronavirus (SADS-CoV), caused a large-scale outbreak of the fatal disease in swine in China. This coronavirus has 96%–98% sequence identity to HKU2 and they are categorized as variants of the same coronavirus species that are found in two different mammalian hosts.¹⁹

A 2006 study of Chinese bat coronaviruses found a high degree of genetic diversity. Many bat coronaviruses are alphacoronaviruses, other species are viruses that are only

present in bats, and the remainder are related to coronaviruses from different domestic animals. In a SARS-CoV and SARS-like coronavirus group, the genes, including that encoding the S protein, fall into two subgroups, one composed of bat coronaviruses and the other of SARS viruses from humans and other mammalian hosts. The similarity of the S protein genes between these subgroups is only approximately 80%.^{20,71} Almost all bat coronaviruses that were sequenced in this study were found in a single bat species, indicating a high degree of host restriction among bat coronaviruses.

Two SARS-related coronaviruses, RsSHC014 and Rs3367 from *Rhinolophus* bat species, can use the ACE2 of bats, humans, and civets to infect cell lines derived from human and many other animal species *in vitro*.⁴¹ These two bat coronaviruses are closely related to SARS-CoV, having an amino acid sequence identity of 85% and 96% for RsSHC014 and Rs3367, respectively. These coronaviruses are particularly closely related to the S protein's RBD. The ORF3 from Rs7327 only differs from that of the SARS-CoV GZ02 strain in only one amino acid. The protein encoded by ORF3 interferes with proper IFN functioning.⁷⁴

LYRa11 is another SARS-like CoV from China's Yunnan Province. It has 91% nucleoside identity with human and civet SARS-CoVs.³⁴ LYRa11, and perhaps other pathogenic human SARS-like coronaviruses from bats, is likely a recombinant arising naturally or through gain-of-function research. The S1 domain of LYRa11 and Rs3367 share high sequence identity with that of SARS-CoV, including an RBD sequence that only differs by a single amino acid in the two key sites which determine host tropism.³³ By contrast, the S1 domain of bat SARS-like CoVs reported before 2013 has very little nucleoside similarity to that of SARS-CoV, including key deletions and other mutations in the RBD and are most likely to be incapable of infecting humans and civets using human ACE2.³³

Chinese bat SARS-related-CoVs have been placed into two clades based on their size and similarity of their S protein to that of humans.⁷⁵ Members of clade-1 are restricted to the Yunnan province in southeastern China. They have an S protein that has the identical size to that present in SARS-CoV and only use ACE2 as their host cell receptor. Clade-2 bat coronaviruses are found throughout China. They do not use ACE2 as their receptor due to deletions of 5, 12, or 13 amino acids in their S protein.⁷⁶ The ACE2 molecule on the surface of *R. sinicus* from four Chinese provinces contains highly **polymorphic** sites (regions whose structure varies) in the region that interacts with the SARS-CoV S protein.⁷⁵ Regardless of the differences in this critical binding region, most bat ACE2s allow entry of clade-1 SARS-related-CoVs into bat cells, however, their binding affinity differs.⁷⁵

While ACE2 serves as the cellular receptor for both the human coronaviruses SARS-CoV and HCoV-NL63, these two viruses bind to different areas of the receptor.⁷⁷ Phylogenetic analysis indicates that SARS-CoV-2 has a high degree of similarity to HKU4-related virus from lesser bamboo bats (*Tylonycteris pachypus*) and HKU5-related virus from Japanese pipistrels (*Pipistrellus abramus*).⁷⁸

5.3.5 Bat Coronaviruses, MERS-CoV, and dipeptidyl peptidase IV

Several bat coronaviruses have close phylogenetic similarities to MERS-CoV: CoV-HKU4, CoV-HKU5, the Chinese pipistrelle (*Hypsugo pulveratus*) bat CoV-HKU25, SC2013, and NeoCoV from the Asian particolored bat (*Vespertilio superans*), and PML/2011 from

Pipistrellus species.^{77,79–81} NeoCoV from a cape serotine bat (*Neoromicia capensis*) from South Africa is one of the bat coronaviruses with the greatest similarity to MERS-CoV over most of its genome.⁸¹ The S protein from isolates from different host species, however, shows a high degree of diversity.⁸² The S1 region of NeoCoV is closely related to that of the betacoronavirus Eri-CoV from the European hedgehog (*Erinaceus europaeus*), suggesting that NeoCoV may have been produced by recombination of African bat and hedgehog coronaviruses.⁸⁰ Hedgehogs belong to the order Eulipotyphla, which is closely related to Chiroptera, whose members include bats.⁸³

Several bat coronaviruses, such as bat HKU4, and human MERS-CoV use dipeptidyl peptidase IV (DPP4) to infect cell lines derived from camels and humans.⁸⁴ After several rounds of replication in vitro cell cultures, the MERS-CoV S protein also rapidly adapted to the DPP4 receptor of vampire bats (*Desmodus rotundus*), changing it from being semipermissive to being permissive.⁸⁵

The distribution of DPP4 varies among host species.⁷⁷ Similar to MERS-CoV, the frugivorous Gambian epauletted fruit bat (*Epomophorus gambianus*) and *R. aegyptiacus* express DPP4 in the respiratory and GI tracts, thus these bat coronaviruses may be able to transmit coronaviruses via the respiratory and fecal-oral route.⁸⁶ MERS-CoV also replicates in the lungs of Jamaican fruit bats (*Artibeus jamaicensis*) and its RNA is detectable in oral and rectal swabs, although it does not cause clinical disease in these bats.⁸⁷ This implies that bats with only limited clinical signs may nevertheless shed pathogenic human coronavirus.⁴¹

While most of the genome of HKU25 is closely related to MERS-CoV, its S protein occupies an evolutionary position between that of HKU4 and HKU5. HKU4, but not HKU5, uses hDPP4 as its host receptor. While the HKU25 RBD also binds to the human DPP4 protein on DPP4-expressing cells, it binds with less efficiency than that of MERS-CoV.⁸⁰

Even though several insectivorous bat species, such as the common pipistrelle (*Pipistrellus pipistrellus*) and the serotine bat (*Eptesicus serotinus*), do not express DPP4 in the cells lining the respiratory tract, they do express it in the gastrointestinal tract, suggesting that transmission of MERS-like-CoVs from at least some insectivorous bats may be primarily infected by the fecal-oral route.⁸⁶ DPP4 is also found in the nasal tissue of dromedary camels, llamas, and pigs, potentially allowing upper respiratory tract infection by MERS-CoV in these domestic animals.^{13,88} DPP4 is not present in sheep, however.⁸⁶ By contrast, in humans, DPP4 is found exclusively in the lower respiratory tract⁸⁹ and, accordingly, acute pneumonia is the primary outcome of MERS-CoV infection in humans.⁸⁸ Since humans do not express DPP4 in the upper respiratory tract, the likelihood of human-to-human transmission is decreased, while its chances of causing more severe disease in its human hosts are increased.

A study of 5000 insectivorous bats from Ghana, Ukraine, Romania, Germany, and the Netherlands detected MERS-CoV-like coronaviruses in 24.9% of slit-faced bats (*Nycteris* species) and 14.7% in *Pipistrellus* bats. Camels express DPP4 in the upper respiratory tract⁸⁸ which increases its likelihood to be transmitted to humans via aerosolized droplets while decreasing its pathogenicity to camels. Camels also express DPP4 in their intestines.⁸⁶ These differences may affect the susceptibility of different tissues to coronaviruses from the different animal species as well as the extent of disease severity.⁷⁷

Eleven of the eighteen bat families harbor alpha- or betacoronaviruses that undergo host switching between bat species. This is particularly true for alphacoronaviruses in which interfamilial host switching events are five times greater than that found in

betacoronaviruses in China.⁹ The majority of bat coronaviruses have been reported in **insectivorous** (insect-eating) bats and only four species in **frugivorous** (fruit-eating) bats. The straw-colored fruit bat (*Eidolon helvum*) is infected by several unclassified alpha- and one unclassified betacoronavirus. Malagasy fruit bats (*Pteropus rufus*) also serve as hosts to coronaviruses. This bat species is found only in Madagascar. Five members of the insectivorous group of horseshoe bats are infected with coronaviruses in Asia or Southeast Asia, while the bats whose coronaviruses are most closely linked to human MERS-CoV are found in Africa and the Middle East. While bats infected with SARS-CoV-like and MERS-CoV-like coronaviruses are present in South Korea, they are not responsible for the large MERS outbreak in that country since the index case had just traveled to the Middle East and MERS-CoV was spread through South Korea by person-to-person contact.

5.3.6 Characteristics of coronavirus species of bats

Most of the well-known coronaviruses of bats infect members of the Rhinolophidae or Vespertilionidae bat families. Interestingly, Rhinolophidae bats host α -CoV or β -CoV lineage B viruses and are primarily insectivorous. Both SARS-CoV and SARS-CoV-2 belong to β lineage B. Vespertilionidae bats host α -CoV or β -CoV lineage C viruses and are also insectivorous (Table 5.1). MERS-CoV is a β lineage C virus. None of the named bat coronaviruses are from the insectivorous families Emballonuroidea, Miniopteridae, Molossidae, Mormoopidae, and Natalidae; the carnivorous family Megadermatidae; the frugivorous, hematophagous (blood-feeding), nectarivorous, insectivorous, and omnivorous Phyllostomidae family; or from the frugivorous or nectarivorous family Phyllostomidae (LA Beltz, unpublished data).

Examination of whole-genome nucleoside sequences of fecal samples from 11 African bat species detected HCoV-229E-related viruses in **hipposiderid** (roundleaf) bats,⁴ even though HCoV-229E is associated with respiratory disease in humans. Despite their similarities, HCoV-229E contains a deletion in the S protein gene that is not present in bat 229E-related CoVs. This deletion may play a role in differences in tissue tropisms in bat coronaviruses.⁴ The bat form of this coronavirus was reported in 7.8% of Aba roundleaf bats (*Hipposideros abae*; $n = 242$) and 3.8% of Noack's round-leaf bats (*Hipposideros* cf. *ruber*; $n = 11611$) and none in the two *Rhinolophus* species tested ($n = 13$).⁴ This may reflect the significant difference in the number of individual animals tested in each bat species or genera.

The bat form of the 229E coronavirus is present in *Hipposideros* species in Ghana and Gabon, two widely separated regions of Africa. This bat-virus association is also seen in closely related coronaviruses in different *Hipposideros* species from Nigeria and Thailand.⁴ The human coronavirus HCoV-229E and coronaviruses from roundleaf bats appear to share a common ancestry. Nevertheless, all reported Ghanaian patients with respiratory disease are infected with the globally circulating HCoV-229E and not bat 229E-related coronaviruses.⁸⁹ A 229E-related alpaca virus appears to occupy an intermediate position between bat and human viruses. Coronaviruses from humans, bats, and alpacas may be members of a single coronavirus species which has undergone multiple recombination events.⁴ The bat and alpaca forms of HCoV-229E contain eight ORFs, while HCoV-229E does not. The human form of the virus does have a conserved transcription regulatory sequence upstream of this ORF.⁴

TABLE 5.1 Characteristics of specific coronaviruses and the infected bats.

Bat species	Bat name	Bat family	Coronavirus	Coronavirus group
<i>Hipposideros pomona</i>	Pomona roundleaf bat	Hipposideridae	HKU10	Alpha
<i>Rousettus leschenaulti</i>	Leschenault's rousette	Pteropodinae	HKU9	Beta Lineage D
<i>Rhinolophus sinicus</i>	Chinese rufous horseshoe bat	Rhinolophidae	HKU2	Alpha
<i>Rhinolophus sinicus</i>	Chinese rufous horseshoe bat	Rhinolophidae	HKU3	Beta Lineage B
<i>Rhinolophus affinis</i>	Least horseshoe bat	Rhinolophidae	LYRa11	Beta Lineage B
<i>Rhinolophus ferrumequinum</i>	Greater horseshoe bat	Rhinolophidae	Rf1	Beta Lineage B
<i>Rhinolophus</i> species	Horseshoe bats	Rhinolophidae	Rs3367	Beta Lineage B
<i>Rhinolophus</i> species	Horseshoe bats	Rhinolophidae	RsSHC014	Beta Lineage B
<i>Rhinolophus sinicus</i>	Chinese rufous horseshoe bat	Rhinolophidae	WIV1	Beta Lineage B
<i>Rhinolophus sinicus</i>	Chinese rufous horseshoe bat	Rhinolophidae	WIV16	Beta Lineage B
<i>Miniopterus pusillus</i>	Small bent-winged bat	Vespertilionidae	HKU8	Alpha
<i>Tylonycteris pachypus</i>	Lesser bamboo bat	Vespertilionidae	HKU4	Beta Lineage C
<i>Pipistrellus abramus</i>	Japanese pipistrelle	Vespertilionidae	HKU5	Beta Lineage C
<i>Myotis ricketti</i>	Rickett's big-footed bat	Vespertilionidae	HKU6	Alpha
<i>Hypsugo pulveratus</i>	Chinese pipistrelle	Vespertilionidae	HKU25	Beta Lineage C
<i>Neoromicia capensis</i>	Cape serotine bat	Vespertilionidae	NeoCoV	Beta Lineage C
<i>Vespertilio superans</i>	Asian parti-colored bat	Vespertilionidae	NeoCoV	Beta Lineage C
<i>Neoromicia zuluensis</i>	Zulu serotine bat	Vespertilionidae	PML/2011	Beta Lineage C
<i>Vespertilio superans</i>	Asian parti-colored bat	Vespertilionidae	SC2013	Beta Lineage C

Four bat families host the majority of well-studied and named bat coronaviruses: Rhinolophidae and Vespertilionidae. While both families host a one alphacoronavirus, the two families host different lineages of betacoronaviruses. Although not shown in the Table, all of these bats are insectivorous.

Further studies are needed to examine the extent of diversity of coronaviruses in other groups of mammals, especially in Asia and the Arabian Peninsula. Particular attention should be paid to coronavirus infections in rodents that are commonly found in or around human habitations since mice and rats are infected by a variety of coronaviruses. In a study in Viet Nam, coronavirus RNA was present in 22% of 248 fecal samples from bats and in 4.4% of 270 rat fecal samples. The bat and rat coronaviruses (RCV) do not appear to jump between mammalian hosts and thus cannot exchange genes. It should be noted that care needs to be taken when interpreting the discovery of microbes in fecal samples of bats, rodents, or other animals since the presence of infectious viruses in feces does not necessarily mean that the animals are infected. Instead, infectious microbes may have simply passed through the animals' digestive systems without entering the suspected "host" animals' tissues.

5.3.7 Prevention against bat coronavirus infection

Antibody therapy and vaccination may help to mitigate or prevent coronavirus disease. Relatively low amounts of four broadly neutralizing SARS-CoV antibodies effectively control WIV1 bat coronavirus. While a double-inactivated SARS-CoV vaccine (“killed” by both formaldehyde and ultraviolet light) neutralizes and protects young mice, in older mice, it causes immunopathology and **eosinophilia** (excessive numbers of eosinophils which may exacerbate disease).^{90,91}

5.4 Coronaviruses of rodents

5.4.1 Introduction to coronaviruses of rodents

Rodents contain the largest number of mammalian species and are the most common group of mammals in the world, composing 42% of all mammalian species. Many of these animals live in close proximity to human habitations and other buildings as well as in crop fields. They may live in high densities, allowing them to serve as reservoir species for a wide range of microbes.⁹² In addition, some rodent species live near or in human residences or workplaces, including agricultural fields and food storage facilities, where their urine and feces may enter food and their dried secretions may become aerosolized during activities such as plowing or sweeping floors in which the viruses are ingested or inhaled. As such, indirect rodent-human interaction makes rodent-borne diseases a threat for zoonotic infection. This was the case for zoonotic transmission of Sin Nombre virus, a member of the Bunyaviridae family, from inhalation of aerosolized mouse urine or saliva. Infected people subsequently develop the severe respiratory disease with a high fatality rate. Several American hemorrhagic fever viruses, such as the Machupo virus, a member of the Arenaviridae family, are also transmitted from rodents to humans. Machupo virus is the causative agent of Bolivian hemorrhagic fever that has a fatality rate of 5%–30%.⁹³

The *Betacoronavirus* 1 species, which includes BCoV as well as the human coronaviruses HCoV-OC43 and HCoV-HKU1, likely has its origin in rodents since no bat coronaviruses belonging to this lineage have been reported.⁹⁴ All known rodent alphacoronaviruses from West Europe and East Asia are members of a monophyletic group that has a similar genomic structure and a recombinant S protein gene. This suggests that rodents have been associated with the above viruses for long periods and that they share a common ancestor.⁹⁵

Rodents may also serve as important reservoirs for ancestors of lineage A betacoronaviruses.⁹⁶ HKU24, found in some Chinese rats, contains unique cleavage sites in nsp1/nsp2 and the S protein. HCoV-NL63 may share ancestry with alphacoronaviruses from the North American tricolored bat (*Pipistrellus subflavus*). Lineage A human coronaviruses HCoV-OC43 and HCoV-HKU1 may have emerged in humans directly from bovine viruses.⁹⁶

In mouse cell lines, almost all MHV strains use murine carcinoembryonic antigen (CEA) gene family receptors, particularly **CEACAM1**, as well as several related murine biliary glycoprotein (Bgp) that also belong to the CEA group of the **immunoglobulin superfamily**, a group of molecules with structural similarity to antibodies.⁹⁷ The sole

reported exception to this rule is the highly neurotropic JHM strain.⁹⁸ MHV is present in the small intestine and liver, both of which have high levels of CEACAM1a. This coronavirus is not found in the kidneys, however, despite them possessing high levels of the receptor.^{99,100} The lowest levels of CAECAM1a mRNA are present in the brain, muscles, and lungs.¹⁰¹ Taken together, an alternate MVH receptor may also be present in these organs.

At least one MHV-resistant mouse strain uses a different allele of Bgp1.¹⁰² Bgp1a is expressed on **hepatocytes** and the MHV-A59 strain infects the liver in vivo. Bgp1a is present on intestinal, endocrine, and respiratory epithelial cells, kidney tubules, and **endothelial cells**. Some of these cells, however, are not infected by this MHV strain, including exocrine cells, intestinal or kidney cells, respiratory cells, and neurons or glial cells.¹⁰²

The addition of human CEA (hCEA) and human Bgp1 into a cell line that normally lacks these molecules allows infection by MHV-A59 and MHV-2 coronaviruses, with the immunoglobulin-like loop I of hCEA determining virus-binding specificity.¹⁰³ Accordingly, experimental inoculation of several species of monkeys by the intracerebral or peripheral route results in CNS **demyelination** that is similar to that seen in mice.^{104,105} **Myelin** is a fatty material that surrounds some of the **axons** of neurons, protecting them from damage and aiding in nerve repair.

After the viral S protein binds to CEACAM1, MHV fuses with this receptor either at the cell surface or the surface of endosomes. After attaching and fusion with these membranes, MHV enters its target cells and then replicates in the cytoplasm. The viral nucleocapsids obtain their lipid envelopes and surface proteins during budding through the membranes of the rough endoplasmic reticulum and Golgi apparatus.¹⁰⁶

MHV increases its genetic diversity by exchanging genes with other MHV strains. These genes are located in the last one-third of their genomes in a region that encodes the viral structural genes.¹⁰⁶ When the S protein gene is exchanged between the MHV JHM, SD and A59 strains, a high level of neurovirulence occurs in A59 viruses. Mutations in the RBD are especially important in host cell tropism and the severity of the disease.¹⁰⁶ The JHM.WU strain replicates robustly in the liver and induces hepatitis, while the JHM.SD strain fails to replicate or induce liver disease.

While the MHV receptor in the liver is typically CEACAM1a, which mediates viral entry through the plasma membrane, viral entry may also occur via an endosomal route that may be dependent upon the host cell type¹⁰⁷ in which the S protein is cleaved by **cathepsin** in the low pH environment of endosomes.¹⁰⁸ While liver cells, but not brain cells, express CEACAM, the brain remains a major target of MHV infection.¹⁰⁹ Change in just one of the RBDs in the S protein permits MHV to replicate in the absence of CEACAM1a, with the most neurovirulent strains spreading in a cell-to-cell manner.^{110,111} MHV is eliminated from its animal host primarily by CD8⁺ T killer cells with help from CD4⁺ T helper cells.¹¹²

All coronaviruses encode a macrodomain in nonstructural protein 3 (nsp3) whose function is unknown. A specific mutation in the **hepatotropic** MHV-A59's nsp3 gene results in less weight loss, viral titers, proinflammatory cytokine and chemokine levels, and mortality rate. The mutated virus is also unable to replicate in the liver and or cause acute hepatitis in mice.¹¹³ Similarly, a mutation in **neurotropic** JHNV has reduced replication and pathogenesis in the CNS.¹¹⁴ This mutation decreases immune cell infiltration into the brain, particularly by macrophages.¹¹⁴

Another nonstructural protein, nsp2, is a phosphodiesterase that degrades **interferon-inducible 2,5-oligoadenylate synthetases** and **ribonuclease L** (RNase L), components of a potent host antiviral pathway that is critical for viral replication in macrophages and the production of hepatitis.¹¹⁵ Mutations in this protein halt nsp2's phosphodiesterase activity, attenuates viral reproduction in the liver, and decrease the occurrence of hepatitis. It does not, however, affect CNS infection by MHV-SD.¹¹⁶

Coronavirus nsp15 has **endonuclease** activity. Mutant MHV without this enzyme has greater levels of dsRNA.¹¹⁷ It is believed that the primary function of nsp15 is not to correct errors during replication, but that its major role is immune evasion by degrading dsRNA.¹¹⁷ Mutants lacking IFN- γ gene expression or RNase L-mediated rRNA degradation are much more susceptible to MHV infection.¹¹⁷

5.4.2 Mouse hepatitis virus

5.4.2.1 Introduction to mouse hepatitis virus

MHV is a betacoronavirus lineage A whose S protein typically binds to the 9-O-Ac-Sia region of CEACAM1a. There are, however, exceptions to MHV receptor usage. One viral variant with an insertion in its **hemagglutinin-esterase** protein gene binds to the 4-O-Ac-Sia portion of the receptor.^{118,119}

MHV causes hepatitis and demyelinating encephalitis in mice, damaging the liver and the brain, respectively. Some MHV variants may also attack the gastrointestinal and respiratory tracts (Fig. 5.3). The primary receptor in vivo, CEACAM1a, also regulates cell growth and signaling pathways¹⁰¹ and is the main receptor for other groups of coronaviruses as well. The location of MHV in the body and the severity of infection depends upon the viral variant and route of infection.

MHV, the first identified pathogenic rodent coronavirus, was reported in 1949.¹²⁰ In 1970, a variant MHV strain was discovered in brown rats (*Rattus norvegicus*).^{92,121} Alphacoronavirus and betacoronavirus are present in several rodent species, including Chevrier's field mouse and Yunnan red-backed vole (*Eothenomys fidelis*).¹²²

Different strains of MHV that differ only in their S protein also differ greatly in their tropism and their virulence. MHV-A59 infects both the liver and the CNS. It causes hepatitis, mild encephalitis, and subacute demyelination that removes the protective coating of the white matter nerves in the CNS, but not from peripheral nerves. This suggests that MHV-A59-induced acute demyelination may be due to the direct viral killing of oligodendrocytes.¹²³ In MHV-JHM-infected mice, however, microglia (brain macrophages) play an important role in **remyelination** (adding myelin back to demyelinated neuronal axons). Depletion of microglia during viral clearance interferes with myelin repair and lengthens clinical disease but does not alter the time required for virus clearance.¹²⁴ (Fig. 5.4)

MHV-JHM is primarily neurotropic and produces more CNS damage than MHV-A59 by causing acute, fatal encephalitis in many animals and leading to acute infection and chronic demyelination in the survivors. The S protein of MHV-JHM is a very important determinant of the extent of neurovirulence.¹²⁵ Using a chimeric MHV in which the S gene of MHV-JHM and MHV-A59 were exchanged, the resulting viruses bearing the MHV-JHM S protein are highly neurotropic and have pathology similar to that of JHM.¹²⁶



FIGURE 5.3 Intestines of a mouse infected with mouse hepatitis virus. # 16467 Public Health Image Library (PHIL). This infant mouse was infected with the mouse hepatitis coronavirus which causes lethal enteritis. Only the intestines showed any histopathologic changes. Intestines of healthy infant mice have a pink coloration.

Another MHV variant, JHM-WU, causes severe CNS disease as well as replicating in the liver and inducing hepatitis. Polymorphisms in the JHM-WU M structural protein and in the nonstructural replicase proteins nsp1 and nsp13 are necessary for these effects on the liver.¹²⁷

MHV appears to have resulted from genetic recombination with other rodent coronaviruses. As much as 25% of the genome of this coronavirus was produced by genetic recombination with other mouse coronaviruses. A different study of 267 wild house mice in Australia found that 95% of the animals had antibodies to murine coronaviruses without any apparent illness. A study conducted in West, East, and northern Africa suggests that viral recombination may also have occurred between a rabbit coronavirus (RbCV) and a rodent coronavirus. Interestingly, the recombinant mouse coronavirus also contains genes from an influenza C virus, suggesting that coronaviruses are also able to trade genetic information with members of other families of viruses.

Infection of mice with the hepatotropic MHV-3 or recombinant viruses expressing the S protein of the dual neuro- and hepatotropic MHV2 strain replicates to high levels in the liver and result in severe hepatitis. MHV containing the A59 S protein, however, have a lower viral titer in the liver and moderate to severe hepatitis, while virus with the

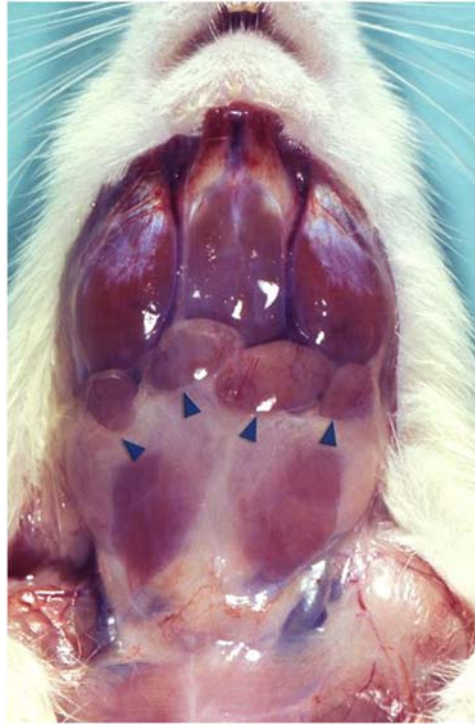


FIGURE 5.4 Enlarged salivary glands caused with sialodacryoadenitis virus. #18683 Public Health Image Library (PHIL). This image depicts swelling of the salivary glands in the neck of a rat infected with sialodacryoadenitis virus.

neurotrophic MHV-JHM S protein has little, if any, viral replication or disease in the liver.¹²⁶ *In vitro*, MHV-3 triggers **apoptosis** in primary macrophage cultures, while infection with MHV-A59 or MHV-2 causes apoptosis in hepatocytes.¹²⁸ The extent of liver pathology induced by MHV-3 depends upon the mouse strain. DBA/2, BALB/c, and C57BL/6 mice are prone to developing the lethal disease while A/J mice are highly resistant.¹²⁹ In susceptible mice strains, severe virulence appears to be due in part to the lack of robust T cell responses.¹⁰⁹ In the primarily pneumovirulent MHV-1 strain, pneumonitis also varies among mouse strains, with A/J mice being the most susceptible of the tested mice and BALB/c and C57Bl/6 mice being resistant due to a less effective **type I IFN** response in A/J mice.¹⁰⁹

The MHV N protein complexed with genomic RNA enhances the efficiency of transcription. The N protein also associates with microtubules of the cells' cytoskeleton and may thus play a role in trafficking and axonal transport of MHV within neurons,¹³⁰ antagonizing type I IFN activity by blocking RNase L,¹³¹ and inducing **fibrinogen-like protein 2 (FGL2)**, which has procoagulant and immunosuppressive activities.¹³² When the N protein genes are exchanged between the MHV-JHM and MHV-A59 strains, expression of the JHM N protein in the A59 genome increases the spread of viral antigen throughout the mouse CNS and decreases the **lethal dose₅₀** by 1000-fold despite the lack of a change in the

amount of type I IFN levels in the brain. The small effect is seen in the severity of hepatitis in these chimeric mice.¹³³ The increase in neurovirulence appears to be partially due to a lesser T cell response since MHV-A59 infection is cleared from the CNS and liver by powerful CD8⁺ T killer cell responses.¹³²

The gene for the N protein of MHV contains a large, embedded ORF in the +1 position that codes for the I protein, a 23 kilodalton structural protein that is expressed in infected cells both in vitro and in vivo in the brain and liver of mice following intracerebral inoculation.¹³⁴ While not required for replication, MHV lacking this protein produce smaller plaques in vitro in comparison to MHV possessing the I protein, suggesting that this protein gives the virus a minor growth advantage.¹³⁴ The I protein is also found in the RCV sialodacryoadenitis virus¹³⁵ and BCoV.¹³⁶ The small membrane (5b) protein is also a minor structural protein that is located in the MHV membrane.¹³⁷

In addition to the CNS, MHV resides in the liver, spleen, lungs, and kidneys. Several species of rats and hamsters, but not rabbits or guinea pigs, are susceptible to this virus following intracerebral inoculation. These animals develop a disease that is similar to that seen in mice. Infected mice are not able to regain their footing after being rolled onto their backs, apparently due to incoordination and weakness of the hind limbs.^{120,138} These disease manifestations may result from the activation of **microglia** by MHV-JHM.

5.4.2.2 Mouse hepatitis virus and adaptive immunity in the central nervous system

CD4⁺ T helper and CD8⁺ killer cells have a complex, interacting role in MHV infection. The **adaptive immune response** does not eliminate MHV and it remains in a noninfectious form that contributes to a chronic, ongoing state of demyelination that is similar to multiple sclerosis in humans.¹³⁹ Viral persistence in the CNS also occurs in a variety of both RNA and DNA viruses.¹⁴⁰

Intranasal infection of suckling mice with the highly neurotropic MHV-JHM strain produces some form of **encephalomyelitis**. When the dams of the infected sucklings are not immune to MHV-JHM, within days MHV-JHM RNA is found throughout the brain in those mice dying of acute encephalomyelitis. JHM enters the CNS via an interneuronal route via the **trigeminal** and **olfactory** nerves, both of which connect to the nasal region, and the virus then spreads throughout the brain during the next several days.¹⁴¹ Virus in these sucklings also appears to enter the CNS via the blood-borne spread. Infected regions of the CNS include the olfactory and **limbic systems**, **thalamus**, **basal ganglia**, **cerebral gray matter**, and **white matter** tracts near the **optic chiasm**.¹⁴² However, if MHV-JHM produces a state of low-level persistence in the nerves or their connections, the mice do not develop acute encephalomyelitis.¹⁴¹

By contrast, suckling mice nursed by immunized dams remain asymptomatic for several weeks. However, 40%–90% of these sucklings then develop hindlimb paralysis, but not encephalitis, at this time. A maternal antibody present in the mouse dam's milk prevents the dissemination of the virus via extracellular fluid. Nevertheless, in late-onset disease, MHV-JHM RNA can be detected in the olfactory bulb, parts of the trigeminal nerve, the **brainstem**, and the spinal cord, but not in other parts of the CNS.¹⁴²

In the CNS, MHV-JHM-infected cells include several types of **glia**, including **astrocytes** and oligodendrocytes. Oligodendrocytes are the cells responsible for producing the **myelin sheath** in the CNS. Infection of these cells is associated with demyelination.

While activated CD8⁺ T killer cells do not directly affect MHV replication in oligodendrocytes, they do secrete IFN- γ .¹⁴³ IFN- γ controls the replication of neurotropic JHMV oligodendrocytes which express the IFN- γ receptor, but not **major histocompatibility complex** (MHC) molecules, and reduces MHV-JHM replication in oligodendrocytes.¹⁴⁴ While IFN- γ contributes to viral clearance, it does not directly influence macrophage-mediated demyelination. **Natural killer (NK) cells** and neutralizing antibodies have little or no role in this infectious process.^{112,144}

Astrocytes are also infected by MHV-JHM. CD8⁺ T killer cell responses limit acute infection of astrocytes and microglia in a **perforin**-dependent manner in which infected cells are killed by immune-mediated production of large pores in their plasma membranes that allows great amounts of fluid to enter and burst the cells.^{143,144} In asymptomatic mice, 20% of the MHV-JHM-infected cells are astrocytes, with higher levels in mice with late-onset hindlimb paralysis.¹⁴⁵ Astrocytes may therefore be a potential cellular reservoir for MHV-JHM in asymptomatic as well as symptomatic mice.¹⁴⁰ Astrocytes, however, also secrete **tumor necrosis factor- α** (TNF- α), IL-1 β , and IL-6 and stimulate the production of nitric oxide, which enhances dysregulation of oligodendrocyte function and may contribute to myelin loss. MHV-4 variant V5A13.1 induces expression of CXC and CC chemokines in vitro in primary mouse astrocyte cultures as well as in vivo in the CNS of infected mice during acute and chronic phases of CNS.¹⁴⁶

Chronic phase viral persistence is due, at least in part, to the **blood:brain barrier (BBB)** and to the restricted expression of MHC class I molecules in the CNS. MHC class I molecules are necessary for CD8⁺ T killer cell activity. Together, these factors hinder viral clearance and lead to persistent viral infections in the CNS.¹⁴⁷ While a strong CD8⁺ T killer cell response, working together with IFN- γ , initially curbs MHV infection,¹⁴⁸ nevertheless, by killing virus-infected cells, CD8⁺ T killer cells play a major role in MHV-induced liver damage. A relative absence of activated CD8⁺ T killer cell activity is found in lymphoid organs, such as the spleen and lymph nodes, in comparison to cells from the CNS.¹⁴⁹ The sequestration of these T cells within the CNS may be at least partially responsible for the decreased splenic CD8⁺ T killer cell activity in MHV-infected mice.¹⁴⁰

CD4⁺ T helper responses have a strong association with viral clearance and blockage of demyelination during the early, acute phase of hepatitis. At later times after infection, these cells no longer require the presence of IFN- γ to function and may enhance, rather than decrease, demyelination. At this time, CD4⁺ T helper cell activity correlates with decreased numbers of infected oligodendrocytes.¹⁵⁰

Later in the course of infection, neutralizing antibodies from B cells help to prevent the reemergence of the disease.¹⁴⁸ Thus, after initially controlling infection by the neurotropic MHV-JHM strain, the viruses reemerge in the CNS of mice lacking antibodies, despite the continuing presence of CD4⁺ T helper cells or CD8⁺ T killer cells in the CNS.¹⁴⁰ B cells additionally affect cellular tropism during the reemergence stage. Reemerging viruses reside in oligodendrocytes in mice possessing B cells but lacking antibodies. In mice lacking both antibodies and B cells, the virus replicates in astrocytes in a manner that coincided with the regulation of CNS MHV-specific CD4⁺ T helper cells. Reactivation is not associated with increased inflammation or virus-specific T cells.¹⁴⁸ Unlike T cells, NK cells do not remain in the CNS after viral clearance.¹⁵¹

Interestingly, in perforin-deficient mice which lack fully functional CD8⁺ T killer cells, **naïve B cells** may trigger **cytolysis** of MHV-JHM-infected cells as part of a B cell-mediated, antibody-independent, innate immune mechanism that aids in the clearance of reemerging virus from astrocytes and microglia/macrophages only after T cell responses decline.¹⁵² Interaction between the cellular virus receptor and the viral S glycoprotein, but not other viral components, is necessary for this process to occur.¹⁵¹ It has been postulated that B cell-mediated lysis results in the death of both the B cell and the infected cell.¹⁵³

During chronic infection of MHV in mice, some inflammatory cytokines, chemokines, and reactive nitrogen species are present. They include interleukin-1 β (IL-1 β), TNF- α , IL-6, **nitric oxide**, **CCL5** (RANTES), and **CCL4** (macrophage-inflammatory protein 1b).^{146,154} Several chemokines either lessen or worsen MHV-induced pathology in mice. Chemokine CC chemokine ligand 2 (CCL2) supports the development of antiinflammatory Th2 responses, while the C-C chemokine receptor 2 (CCR2) supports proinflammatory, antiviral Th1 responses. CCL2 also regulates leukocyte migration and accumulation at the site of infection. CCR2 is the receptor for CCL2 and several other chemokines. It is expressed by some cells of the CNS in addition to inflammatory T cells and macrophages. When mice possessing or lacking functional CCL2 are infected with MHV by the intracranial route, there is no difference in morbidity or mortality and replicating viruses are partially cleared from the brain. Mice lacking the CCR2 receptor, however, are not able to clear the virus from this site.¹⁵⁵ The CCR2-deficient mice also have decreased numbers of MHV-specific T cells in the CNS in comparison to wild-type mice despite the production of normal or elevated numbers of MHV-specific CD4⁺ or CD8⁺ T cells. Decreased macrophage infiltration into the CNS of MHV-infected mice is seen in animals lacking either CCL2 or CCR2,¹⁵⁵ demonstrating differences in T cell and macrophage responses to CCL2. Given this difference, it appears that CCR2 ligands other than CCL2 protect against MHV pathology.¹⁵⁵ The discovery of these ligand(s) will give us insight into the relative roles of Th1 and Th2 cells in MHV infection. The CCR2 ligand CCL7 (monocyte chemoattractant protein-3) is also expressed within the CNS during MHV infection and may affect T cell trafficking.¹⁴⁶

CCL3 enhances dendritic cell (DC) migration and activation. Mice lacking this chemokine have decreased DC trafficking to the draining lymph node. DCs play a very important role in CD4⁺ T helper cell activation by their production of the CD4⁺ T helper cell activation factors CD40 and MHC class II. Accordingly, the absence of CCL3 during MHV infection correlates with altered T cell activity.¹⁵⁵

CCR5 is a β -chemokine receptor that binds to CCL3, CCL4, and CCL5. It recruits macrophages and immature DCs into areas of infection. CCR5 directs T cell infiltration into the CNS early, but not later, during the disease.¹⁵⁶ The ability of proinflammatory CD4⁺ T helper cells and macrophages to infiltrate and function in the brains of MHV-infected mice is decreased in mice lacking CCL3 or CCR5, correlating with a decrease in the severity of demyelination.^{156–158} CD8⁺ T killer cells have an active role in demyelination as well. CCR5 regulates IFN- γ production by T killer cells¹⁵⁹ but does not affect their migration and infiltration into the CNS.¹⁶⁰ CCR5 additionally recruits inflammatory macrophages and microglia into the white matter of the spinal cord.¹⁵⁶

Other chemokines, **CXCL10** (IP-10) and **CCL5**, also induce Th1 cell migration into the CNS during MHV infection. The lack of these functional chemokines increases the viral

load in the brain in addition to increasing the MHV-induced mortality rate.^{160,161} CXCL10 is expressed by day 1 after experimental infection with MHV. It stimulates the production of the strongly antiviral cytokine IFN- γ , which then stimulates the expression of CXCL9, which, in turn, increases CD4⁺ T helper and CD8⁺ T killer cell infiltration into the brain.^{156,161,162} Levels of CXCL9 mRNA molecules are decreased in MHV-infected mice lacking CCR2 at day 7 postinfection. Th1 cell production of IFN- γ is decreased in the brain due to the above-mentioned reduction of T cell infiltration into the CNS. The lack of sufficient levels of IFN- γ may be involved in lowering CXCL9 gene expression since IFN- γ plays a role in CXCL9 transcription.¹⁵⁵ MHV infection thus disrupts the balance between pro- and antiviral chemokines, cytokines, and leukocytes infiltrating the brain.

5.4.2.3 Mouse hepatitis coronavirus, interferon, and innate immunity

Some of the cytokines of the innate immune system, particularly IFN- α and IFN- β , provide the first protection against coronavirus infection, even though these viruses are generally poor IFN α/β inducers. Strains of MHV whose M proteins contain N-linked sugars stimulate the production of type I IFN to higher levels than those with O-linked sugars. Viruses whose M proteins lack glycosylation are poor IFN inducers.¹⁶³ M protein glycosylation is associated with differing abilities to replicate in the liver, but not in the brain. Replication in the liver of animals differing in glycosylation status correlates with the ability to induce type I IFN.¹⁶³ Several proinflammatory mediators are also pathogenic and upregulated during MHV infection, including CCL2, IFN- γ -induced protein 10 (IP-10), C-X-C motif ligand 9 (CXCL9; MIG), IFN- γ , IL-8, and IL-6.¹⁶⁴

Several intracellular receptors recognize various regions or characteristics of pathogens and upregulate IFN responses. These include Toll-like receptors (TLRs) 3 and 7 in endosomes, a **retinoic acid-inducible gene I** (RIG-I), and **melanoma differentiation-associated protein 5** in the cytoplasm which activates the **interferon regulatory factors (IRFs) transcription factors**. Stimulation of endosomal TLR3 by viruses leads to IFN- β production by macrophages that either diminishes (MHV-A59) or suppresses (MHV-JHM, MHV-3) virus replication in macrophages, depending on the MHV strain.¹⁶⁵ MHV-A59 infection induces only very low levels of type I IFNs, thus allowing high viral replication and a high mortality rate.¹⁶⁶

High levels of interferon regulatory factor 7 (IRF7) are constitutively expressed by plasmacytoid DCs (pDCs).¹⁶⁷ These cells are the principal source of type 1 IFNs and play a critical role in the early control of virus infection. After TLR7 recognizes either MHV or SARS-CoV, pDCs rapidly stimulate IFN- α expression without the typical IFN- β -mediated feedback loop.¹⁶⁸ The production of IFN- β is inhibited by both SARS-Co-V and MHV's N protein by interfering with the host cell's RNase L activity.⁶⁰

Upon activation, IRF3 dimerizes and enters the nucleus, where it stimulates transcription of the IFN- β gene. IFN- β is then secreted from the originally infected cell and induces an antiviral state in neighboring ("by-stander") cells to decrease viral spread.¹⁶⁹ For most coronaviruses, when bound to its receptor, IFN- β activates a complex signaling cascade that ultimately leads to the transcription of **IFN-stimulated genes** (ISGs) with their antiviral, antiproliferative, and immunomodulatory properties.¹⁷⁰ In addition, IFN- β primes cells to produce IFN- α after viral infections.¹⁶⁹

Host cells maintain elaborate **ubiquitination** and **deubiquitination** pathways that also influence the type I IFN induction pathway. Perturbation of these pathways often alters type I IFN production.¹⁷¹ Interestingly, nsp3 of some coronaviruses, including murine MHV-A59, porcine PEDV and TGEV, and human SARS-CoV, contain the conserved **deubiquitinase** enzyme **papain-like protease-2** (PLP2).⁶⁰ PLP2 inhibits the ubiquitination of RIG-I and **stimulator of interferon genes** (STING), another antiviral pattern recognition receptor that informs cells of viral infection. Deubiquitination of RIG-I and STING are required for type I IFN signaling. PLP2 also causes the deubiquitination of IRF3, inhibiting its translocation into the nucleus.¹⁷¹ Viral nsp7, nsp14, and nsp16 are also type I IFN **antagonists**, while nsp8 blocks **type III IFN (IFN- λ)** responses. Nsp10 enhances nsp16's negative effect on IFN production.¹⁷²

ISG15 is one of the most highly expressed ISGs. ISG15 is only active when conjugated to target proteins, a process termed ISGylation, through consecutive interactions with an **E2-conjugating enzyme**.¹⁷³ The role of ISGylation in viral infections depends upon the virus since the process may have antiviral activity against some viruses, including MHV while stimulating replication in other viruses.¹⁷⁴ ISG15 has multiple targets, some of which regulate IFN's antiviral activities. These targets include RIG-1, STAT-1, **Janus kinase-1** (JAK-1), and **myxovirus resistance protein 1**.¹⁷⁴ The protease PL1 of TGEV, a pig coronavirus, also plays a major role in suppressing IFN- β expression and inhibiting nuclear translocation of IRF3.

In a mouse fibroblast cell line, MHV-A59 induces IFN- β gene transcription, however, MHV-JHM does not, but may inhibit IFN- β posttranscriptionally.¹⁶⁹ In the brains of infected animals, MHV induces the expression of IFN- β mRNA and protein production. Other human or animal coronaviruses, such as HCoV-229E and BCoV, also stimulate IFN- α /IFN- β production by human monocyte-derived macrophages.^{169,175} SARS-CoV also modulates the IFN- α /IFN- β response by multiple mechanisms. In addition, the nsp1 protein of SARS-CoV promotes host mRNA degradation¹⁷⁶. Other studies have found that coinfection with MHV reduces IFN- β protein by heterologous viruses.

One of the methods that coronaviruses use to evade destruction by IFN is **selective packaging**.¹⁷⁷ During this process, single-strand positive-sense genomic RNA is preferentially incorporated into nascent virions, while the incorporation of other types of cellular or viral RNA is excluded. Selective packaging serves as a unique method of shielding its RNA products to avoid activating the host's innate immune response. This process is conserved among coronaviruses, suggesting that this is important for coronavirus fitness and survival.¹⁷⁷ In lineage A betacoronavirus, including MHV, selective packaging depends on the packaging signal (PS) and its interaction with viral structural proteins. The MHV PS lies within the nsp15 locus and interacts with viral N and M proteins.¹⁷⁸ The hemagglutinin/esterase glycoprotein is only found in certain MHV strains and is not required for virus entry,¹⁷⁹ while the M and E proteins are embedded in the envelope and are essential for virion assembly.

Several MHV mutants have been produced with that package with higher than normal levels of **subgenomic RNA**, **negative-sense genomic RNA**, and cellular RNAs into virions at the expense of genomic RNA packaging. These mutants replicate normally in vitro but are attenuated in vivo, resulting in decreased weight loss and a lower mortality rate in infected mice.¹⁷⁷ Hepatitis and encephalitis are reduced in hepatotropic MHV-A59 and neurotropic MHV-JHM mutants, respectively. Since wild-type PS plays a role in inhibiting

type I IFN activity in MHV-infected bone marrow-derived macrophages, infection of such macrophages by the packaging mutants also increases IFN production.¹⁷⁷

5.4.2.4 Mouse hepatitis virus, the innate immune response, and the central nervous system

Type I IFNs play a protective role against MHV-induced CNS pathology since infected mice that lack the IFN α/β receptor have uncontrolled viral replication, widespread viral dissemination in the CNS, and a greater degree of tropism for neurons, followed by rapid death.¹⁸⁰ The MHV-A59 strain produces delayed but significant upregulation of IFN α/β pathway genes within infected astrocytes.¹⁸¹ Later, IFN α and ISGs are expressed at higher levels in astrocytes than in microglia, suggesting that astrocytes are important to the anti-viral innate response.

The S protein of both A59 and JHM MHV strains use murine CEACAM1a as the receptor for murine cells. Interestingly, even though MHV-JHM is primarily neurotropic, the expression of this receptor is much lower in the CNS than in other tissues. It is noteworthy that in the brain, CEACAM1a is only found on the surface of the endothelial cells lining the blood vessels, however, MHV-JHM infects neurons, astrocytes, microglia, ependymal cells, and oligodendrocytes.¹³⁸ The latter cell type is responsible for producing **myelin** that surrounds axons in the white matter of the CNS. Oligodendrocyte numbers decrease in MHV infected mice, perhaps directly by the virus or indirectly by the secretion of cytokines or nitric oxide by infected microglia.¹³⁸ **Fas-mediated apoptosis** is involved in the death of MHV-infected rat astrocytes in vitro and is triggered by MHV-mediated oligodendrocyte fusion between the viral envelope and cell membranes.¹⁸²

Neither T cells nor B cells are necessary for CNS demyelination in mice infected with MHV-A59.¹⁸³ Demyelination due to MHV-JHM infection, however, relies upon the presence of T lymphocytes since mice without these cells do not undergo this process, even though they do have high levels of infectious virus in the CNS and subsequently die from encephalitis.¹²⁵ In normal conditions, the BBB prohibits the passage of lymphocytes into the CNS. If CD8⁺ T killer cells do enter the brain, downregulation of MHC class I molecules is decreased, limiting the killing of infected cells. Another means of downregulating immune-mediated CNS damage is via antiinflammatory molecules, such as TGF- β and IL-10 by **T regulatory cells** (Tregs).¹²⁵ These cells and their cytokines balance the immune-mediated antiviral activity of CD8⁺ T killer cells with their autoimmune and inflammatory functions. Mice infected with a neurovirulent strain have a lengthened innate response in which IFN- β production remains in the CNS longer than five days after infection. Levels of the proinflammatory cytokines IL-1, IL-6, CCL3, and CCL4 (aka MIP-1a and MIP-1b, respectively), and CXCL-2 (MIP-2) are also increased in the CNS of mice infected with neurovirulent strains.¹⁸⁴

A mutation in the S protein of highly virulent strains is linked to increased macrophage infiltration along with increased CCL3 and CCL4 transcription.¹²⁵ In a less CNS-virulent strain, macrophages, and microglia are still recruited into the white matter of the CNS, and plaques of demyelination are present due to phagocytic macrophage activity even without the presence of the infectious virus. When the S protein of a highly virulent MHV variant is compared with that of a less virulent CNS strain, the former produced higher viral titers and greater spread throughout the CNS. The S protein of the former is also

associated with a receptor-independent spread in vitro infection of tissue culture cells.¹²⁵ Demyelination and entry of CD8⁺ T killer, but not CD4⁺ T helper, cells into the CNS are decreased in mice that do not produce IFN- γ .¹⁸⁵ Macrophages exposed to exogenous IFN- γ increase the production of nitric oxide and phagocytosis.¹⁴⁴ $\gamma\delta$ T cells also enhance demyelination in a process that relies upon IFN- γ and **NKG2D**, an activating molecule from NK cells.¹⁸⁶ Additionally, antibodies and **complement** play a role in demyelination.¹²⁵

Infection of the CNS by MHV-JHM produces acute encephalomyelitis and acute and chronic demyelination.¹⁸⁷ MHV-JHM infects CNS macrophages and **ependymal cells** and replicates in other glial cells, such as oligodendrocytes, astrocytes, and microglia, but rarely in neurons.^{144,165} Viral replication in most CNS cell types is controlled by activated CD8⁺ T killer cells utilizing perforin. By contrast, viral replication in oligodendrocytes is controlled by a perforin-independent mechanism that involves IFN- γ .¹⁴⁴ Neutrophils are also important in controlling pathology due to MHV-JHM infection in mice. In mice lacking neutrophils, viral titers increase in the brain, and the time to death decreases.¹⁸⁸

A highly neurotropic Mu-3 strain was cloned from JMH-JHM variants cl-2 and srr7 virus and is resistant to soluble receptor treatment. Infection with Mu-3 produced apoptotic lesions in the pyramidal neurons of the **hippocampus** of the brains of all infected mice. By contrast, infection with the viral cl-2 variant only caused apoptosis in 10%–20% of the hippocampus and **pyramidal neurons**. The apoptotic cells in these mice included infected, as well as high numbers of uninfected, neurons.¹⁸⁹ This suggests that at least some of the apoptotic cells were killed indirectly by infected monocytes or brain microglia mediated by cytokines since all CNS cell types, including glia and neurons, can produce cytokines as part of the innate immune response. Cultures of primary rat oligodendrocytes allow MHV infection, but not virus replication.¹²³ Exogenous agents that block acidification of endosomes or compounds that block the **caspase apoptotic pathway** prevent oligodendrocytes from MHV-induced apoptosis fusion.¹²³

The MHV-HM.SD variant is highly virulent in the CNS and is transmitted more rapidly between neurons than are other neurovirulent MHVs. The neurotropic MHV-JHM.WU strain also replicates robustly and induces hepatitis, while JHM.SD fails to replicate or cause pathology in the liver.¹²⁷ The M protein, nsp13, and nsp1, but not the S protein, are necessary for efficient replication in the liver in vivo.¹¹⁶ A MHV-A59 nsp2 mutant replicates in the brain while remaining highly attenuated for replication in the liver and hepatitis. The effects of nsp1 differ between coronaviruses with that of SARS-CoV promoting mRNA degradation while inhibiting translation, while an nps1 mutant is not able to degrade mRNA but still inhibits protein synthesis.¹⁹⁰ The basal levels of IFN in MHV are much higher in the liver than in the brain. This may be responsible for a more vigorous innate immune response in the liver.¹¹⁶

Interestingly, JHM.SD operates in the CNS in the absence of CEACAM1a. While JHM.SD infects a greater number of cells in the CNS, it produces less infectious virus per cell than the hepatotropic A59 strain. In this system, efficient replication may not correlate with high neurovirulence.¹⁹¹ The effect of the immune system on JHM.SD also differs from some of the other MHV strains in that it induces primarily protective immunity, but the strong neutrophil response that it also stimulates may be more pathogenic than

protective. JHM.SD also stimulates minimal T-cell responses, in comparison with the protective, strong T cell response and IFN- γ induced by MHV-A59.¹⁹¹

MHV strain dv2.2–1 is a sublethal glia-tropic viral strain that attacks both the liver and CNS, resulting in acute **encephalomyelitis** (inflammation of the brain and spinal cord) in the latter. Persistence of the dv2.2–1 strain is associated with continuing demyelination. RNA from this viral strain is detectable even in the absence of an infectious virus.¹⁹² When administered intracranially, MHV dv2.2–1 infects **ependymal cells** (glia that line the **ventricles** of the brain), and then spreads to microglia, astrocytes, and oligodendrocytes.¹⁹² On day 5 postinfection, virus replication peaks during the activation of astrocytes and microglia. The BBB is disrupted and neutrophils, NK cells, and monocytes enter the CNS tissue. The monocytes and neutrophils cause further BBB disruption, permitting T and B cells to infiltrate the CNS as well. CD8⁺ T killer and CD4⁺ T helper cells are vital in decreasing the amount of infectious virus to undetectable levels over the next two weeks. The T cells' antiviral activity correlates with the start of axon demyelination.¹⁹² The chemokines CXCL10 and CCL5 recruit T cells and macrophages, respectively, and the level of these cells increases late during disease. Depletion of either of these chemokines decreases the extent of demyelination, indicating an important protective role for CXCL10 and CCL5 in MHV-induced CNS pathology.¹⁰⁶

During acute CNS infection, neutrophils, NK cells, macrophages, B cells, and CD4⁺ T helper and CD8⁺ T killer cells infiltrate the brain. While neutrophils contribute to brain pathology, they also serve a vital role in protection against MHV by increasing BBB permeability and allowing T cell entry into the CNS.¹⁹³ CD8⁺ T killer cells are vital for viral clearance during the acute stage of the disease and remain in the CNS during the chronic stage, while B cells prevent reactivation of virus after viral clearance during acute infection.¹⁰⁶ Inhibition of T cell functions together with antiinflammatory factors, such as IL-10 and **transforming growth factor- β** decrease excessive T cell functions but also promote the formation of persistent coronavirus infection.¹⁹⁴ The inability of the CNS to fully remove coronavirus may result from several “checkpoint” molecules that reduce demyelination. One such checkpoint molecule is the T cell inhibitory molecule B7-H1 which is strongly increased on infected oligodendrocytes. The absence of these cells correlates with increased death, although viral control was accelerated.¹⁹⁵ The antiinflammatory cytokine IL-10 also downregulates T cell responses and neurodegeneration in the brain.¹⁹⁶ Some of this IL-10 is released by Tregs in cervical lymph nodes. Increased IL-10 levels also correlate with decreased levels of BBB permeability.¹⁹²

Cytokines and other immune molecules also play a role both in mitigating and enhancing pathology in the CNS. The “**cytokine storm**” caused by many coronaviruses must be carefully balanced to reduce immune damage to the CNS tissues while still holding the viruses in check. This is especially important in the CNS since it controls critical and unique body functions along with the inability of mature neurons to reproduce to replace those lost either directly by the viruses or via bystander damage to cells in close proximity.¹⁹² Levels of IL-33 are increased in mice infected with the viral MHV-3 strain and correlate with increased levels of FGL2 in the liver.¹⁹⁷

Type I IFNs inhibit MHV-JHM strain spread that is associated with early mortality. Perforin and IFN- γ work together to clear viruses in astrocytes, microglia, and oligodendrocytes. Mice lacking perforin-mediated cytotoxicity do not halt viral replication in

microglia and astrocytes, while mice without the type IFN receptor cannot control viral reproduction in oligodendrocytes.^{144,198} Importantly, memory CD8⁺ T killer cells from either perforin-deficient or IFN- γ -deficient animals enter the infected CNS without help from CD4⁺ T helper cells.¹⁹⁹ T killer cells' cytolytic activity blocks MHV replication in all neuron and glial cell types. Cytolytic activity without IFN- γ decreases infection of astrocytes, but not oligodendroglia. Moreover, cells secreting IFN- γ in the absence of cytolytic activity block viral replication in oligodendrocytes, but not in astrocytes.¹⁹⁹ CD8⁺ T killer cells utilize several nonspecific cytolytic antiviral mechanisms, including the perforin, TNF- α , and Fas/FasL pathways.^{200,201} Moreover, T killer cells are a major producer of IFN- γ ²⁰² and upregulate MHC I and MHC II expression required for activation of T killer cells and T helper cells, respectively. MHC II expression on microglia is completely dependent on IFN- γ .¹⁹⁹

In the CNS of MHV-infected mice, T killer cells use only perforin-dependent antiviral activity during the acute stage of infections and then remain and continue to secrete IFN- γ during chronic infection.¹⁹⁹ Nucleocapsid-specific memory CD8⁺ T killer cells produce much stronger antiviral activity than those directed against the S protein in immunodeficient mice,^{185,203} however, activated anti-S protein T killer cells still play a role in demyelination.¹⁹⁹ In the case of neurotropic strains of MHV, decreased viral replication in astrocytes, microglia, and infiltrating macrophages relies upon perforin-dependent, but Fas/FasL-TNF- α -independent mechanisms.^{204,205} It should also be noted that both highly activated effector and memory T killer cells can gain entry into the CNS in the absence of CD4⁺ T helper cells.¹⁹⁹ The antiinflammatory cytokine IL-10 decreases the extent of CNS lesions during chronic infection without decreasing viral persistence. The actions of both pro- and antiinflammatory cytokines therefore must be balanced to protect the host against viral infection while limiting damage to the CNS.¹⁹⁴

Oligodendrocytes and astrocytes have lower levels of ISG levels than microglia. However, in astrocytes, the MHV-A59 strain induces delayed but substantial type I IFN pathway genes during infection, with levels of some of the ISGs and IFN α expressing higher levels in astrocytes by day 5 postinfection when compared to microglia.¹⁹⁴

Decreasing titers of neurotropic MHV requires both CD4⁺ T helper and CD8⁺ T killer cells. CD8⁺ T killer cells, aided by CD4⁺ T helper cells, are the primary effector T cells within the CNS. High levels of both T killer and T helper cells are found in the CNS during acute encephalitis and remain there during the chronic stage of infection. T helper cells play a protective role in viral control by decreasing apoptosis of T killer cells while increasing T killer cell infiltration of the CNS **parenchyma** (brain tissue proper). CD8⁺ T killer cells lower MHV-JHM replication in astrocytes and microglia via perforin-induced creation of large pores in the infected cell's plasma membrane. Furthermore, mice that are deficient in perforin-mediated cytolysis do not control viral replication within microglia and astrocytes.¹⁹⁴ Viral replication in oligodendrocytes is blocked by IFN- γ produced by CD4⁺ T helper cells.¹⁵⁰ Neutrophils also play an important role during MHV-JHM virus infection since their absence results in increased viral numbers in the brain and more rapid death but does not affect disease severity in mice infected with MHV-A59.^{188,206}

INF- γ plays an important role in long-term virus control at least partially by increasing the expression of MHC I and MHC class II molecules on microglia. MHC class II molecules are necessary for CD4⁺ T helper cells to recognize infected cells and secrete

cytokines, while MHC class I molecules are needed for CD8⁺ T killer cells to identify and kill virally infected cells. Expression of MHC I on the many infected cell surfaces coincides with type I IFN production. Oligodendrocytes need IFN- γ to upregulate MHC I.²⁰⁷ Delaying MHC I expression oligodendrocytes may protect them from being killed by CD8⁺ T killer cells.¹⁹⁴ While IFN- γ signaling by oligodendrocytes does not directly result in demyelination, increased levels of infected oligodendrocytes increase IFN- γ -induced neutrophil entry into the CNS and activate inflammatory Th17 cells.²⁰⁸

IFN- γ triggers innate and adaptive immune system components to infiltrate into the brain. It also promotes demyelination by stimulating extensive macrophage and microglia activation.¹⁹⁴ These activated macrophages produce complement system components, proteases, and **reactive oxygen species (ROS)**, including the superoxide anion, hydrogen peroxide, and hypochlorous acid. ROS are linked to neuronal demyelination and death during MHV-associated **optic neuritis**, inflammation of the optic nerve that may cause temporary vision loss.¹⁹⁴ Molecules that decrease ROS levels also reduce optic neuritis pathology.²⁰⁹

5.4.2.5 Mouse hepatitis virus and the liver

Fulminant viral hepatitis (FH) is a rapidly progressive disease with a high mortality rate. The MHV-3 strain of MHV causes FH in susceptible mouse strains. These mice may have increased blood levels of the neurotoxic protein **bilirubin**, a hemoglobin by-product normally degraded in healthy livers. Additionally, susceptible mouse strains have large fibrin deposits in the liver as well as large-scale **necrosis** of hepatocytes. The percentage of a rare subset of T cells that has neither CD4 nor CD8 on its surface (double-negative T cells) rises in the blood, liver, and spleen of MHV-3 infected mice.²¹⁰ Double-negative T cells cause apoptotic death of anti-MVH CD8⁺ T killer cells and additionally produce the Th1 cytokines IFN- γ and IL-2. Together, these activities increase survival and viral persistence of infected mice.²¹¹ Blood and liver levels of proinflammatory IL-1 β also increase during FH. MHV-3-infected mice with dysfunctional IL-1 receptor activity have decreased production of the prothrombinase enzyme FGL2, which regulates both innate and adaptive immune responses and decreased levels reduce viral replication and mortality.^{210,212}

Experimental infection of the susceptible BALB/cJ and C57BL/6J mouse strains with MHV-3 results in fatal hepatitis with neutrophil and mononuclear cell infiltrates and deposition of fibrin in the liver sinuses as well as coagulative hepatocellular necrosis.^{129,213,214} MHV-3 infection of the resistant A/J mice, however, does not result in liver injury and the virus is cleared from the liver in 10–14 days.²¹⁵

While MHV-3 induces the production of *flg* 2 RNA by macrophages from both resistant and susceptible mice, it is produced within 3 hours postinfection in susceptible mice and peaks at six hours. Production of *Fgl*-2 RNA in resistant mice is delayed and is 120-fold lower than that found in susceptible mice.²¹⁶

Abnormal microcirculatory blood flow and localized avascular foci (areas without blood vessels) appear early during the acute phase of disease in the semisusceptible C3HeBFeJ mice.²¹⁴ Afterwards, 80% of these mice develop **chronic granulomatous hepatitis** and 20% have severe chronic aggressive hepatitis with hepatocellular necrosis and infiltration by macrophages and lymphocytes. Microcirculatory abnormalities are present in mice with either form of hepatitis and are concentrated in the vicinity of visible lesions. This corresponds to elevated monocyte-related **procoagulant activity (PCA)**, which is

elevated throughout the chronic phase and is higher in animals with severe disease. Resistant mice strains continue to have normal blood flow without an increase in PCA. Interestingly, equal levels of active MHV-3 replication are found in resistant and susceptible/semi-susceptible mice.²¹⁴ The disturbances in the microcirculatory occur before viral replication.

Th1 immune responses play an important role in resistance to MHV-3-associated disease by inhibiting the production of FGL2 by infected monocytes/macrophages in vitro and protecting susceptible mice from fatal disease in vivo.²¹⁷ Corticosteroids, such as methylprednisolone, direct CD4⁺ T helper cell responses from Th1 to Th2. Resistant mice treated with methylprednisolone die of acute hepatitis by day ten postinfection. Additionally, Th2 lymphocytes from susceptible mice increase FGL2 production by macrophages.²¹⁵ Taken together, these studies demonstrate the importance of macrophages and FGL2 in liver pathology and how the balance of Th1 and Th2 cells help to determine the severity of disease by their effects on macrophage activity.

MHV-3 infects liver sinusoidal endothelial cells, hepatocytes, **Kupffer cells** (resident liver macrophages), and **Ito cells** (mesenchymal cells that produce collagens and other extracellular matrix proteins). All of these cells express CEACAM1a.²¹⁶ Endothelial cells constitutively produce antiinflammatory compounds under normal conditions and encourage immune tolerance toward food and microbial antigens by the production of TGF- β and IL-10 by Treg cells.

TLR2, but not TLR3, TLR4, and TLR5 are induced by infection of **liver sinusoidal endothelial cells** (LSECs) which normally help to maintain liver homeostasis. Typically, intracellular endosomal receptors recognize viruses inside liver cells.²¹⁸ Wild-type C57BL/6 mice infected with MHV-3 produce FH accompanied by high blood levels of the liver enzymes alanine and aspartate transaminase levels and die soon after infection. Disease in this mice strain correlates with higher liver levels of the cytokines IFN- β , IL-6, and TNF- α ; the chemokines CXCL1, CCL2, and CXCL10; and the **alarmin** IL-33.²¹⁸ Infection of mice lacking TLR2 have a comparably mild disease.²¹⁸ Type I IFNs, however, do not appear to play a major role in controlling MHV replication in the liver or the severity of resulting hepatitis.²¹⁸ Type I IFN responses during MHV infection are primarily triggered by TLR3 and other endosomal pathogen recognition receptors.²¹⁸

CXCL1, CCL2, and CXCL10 rapidly recruit neutrophils, macrophages, and NK or CD8⁺ T killer cells, respectively, into the livers of MHV-3-infected mice. Recruitment of these cells is delayed and reduced in mice lacking functional TLR2. In wild-type mice, numbers of neutrophils, NK cells, NK-T cells, and macrophages rapidly decrease during infection with MHV-3 but do not do so in TLR2-deficient mice.²¹⁸ Viral infection triggers macrophages, LSECs, NK cells, and NK-T cells to release cytokines and chemokines into the liver.²¹⁸ During acute MHV-3 infection, infected macrophages produce proinflammatory mediators, including TNF- α , IL-1, TGF- β , and the **leukotriene** B₄, as well as FGL2.²¹⁹ Viral replication is lower in macrophages infected with an attenuated MHV-3 strain compared to levels in macrophages infected by a pathogenic strain.²²⁰ Additionally, decreasing macrophage numbers in mice infected with a mildly hepatotropic MHV strain results in lethal FH soon after infection.²²¹

When activated by TLR2, MHV-infected LSECs both produce the proinflammatory factors IL-6 and TNF- α and inhibit the release of IL-10. By contrast, levels of

antiinflammatory and immunosuppressive compounds, such as **prostaglandin E₂** (PGE₂) TGF- β , IL-10, and nitric oxide, are reduced during MHV infection.²²² Susceptible BALB/cJ mice infected with MHV-3 develop FH with massive hepatic necrosis, **hypoglycemia**, **metabolic acidosis**, and increased serum levels of the liver enzyme alanine aminotransferase (ALT). By contrast, administration of the immunosuppressive compound 16,16 dimethyl prostaglandin E₂ (dmPGE₂) before and up to two days after infection reduces liver damage and blood glucose levels, total CO₂, and ALT remain normal, despite the absence of decreased viral titers in the liver²²³. PGEs relax circular smooth muscles, resulting in **vasodilatation**, leading to improved liver microcirculatory flow. Additionally, splenic macrophages from MGV3-infected mice typically have increased PCA. This is not seen in mice treated with dmPGE₂.²²⁴ Levels of macrophage PCA correlate with susceptibility to MHV-3 and disease severity. Interestingly, treatment with dmPGE₂ did not alter the mortality rate,²²³ but perhaps could do so if used in combination with another treatment modality.

In addition to the contribution of macrophages to -induced liver disease, LSEC also secretes proinflammatory and coagulation mediators and chemokines. MHV-3 also activates hepatic **coagulation** (formation of blood clots in the liver) by increasing macrophage/monocyte PCA which leads to **intravascular thrombosis**.^{214–217} Levels of procoagulant vascular factors produced by LSECs correlate with susceptibility to MHV-3 infection and inversely correlate with levels of hepatic IL-10 and nitric oxide. The latter regulates intrahepatic sinusoidal blood flow by dilating blood vessels. These changes in the cytokine profile contribute to an increased rate of viral replication.²¹⁸ The attenuated YAC-MHV-3 strain, which replicates to a lesser extent in LSECs than the parent strain, only produces small, transient hepatic lesions and increased production of the Treg cytokine IL-10.²¹⁸ The livers of mice infected by attenuated MHV-3 strains contain low levels of the chemokines CXCL1 and CCL2, which may play a part in the decreased numbers of infiltrating neutrophils and macrophages. They are perhaps responsible for producing smaller inflammatory foci without the extensive necrosis areas found in mice infected by virulent MHV-3.²¹⁸ In the later mice, however, neutrophil recruitment is transient and the numbers of T and B lymphocytes decrease throughout infection.

LSECs and hepatocytes infected by virulent, but not attenuated, MHV increases the production of the alarmin IL-33.^{218,219} Alarmins are molecules released by a damaged or diseased cell to stimulate an immune response. IL-33 belongs to the IL-1 family of cytokines and has several roles in regulating inflammation and infection. It activates Th2 cells, mast cells, Treg, CD8⁺ T cells, and NK cells.²²⁰

In mice infected with an attenuated MHV-3 strain, the percentage of CD4⁺ T helper cells in the liver decreases, while in mice infected with a pathogenic MHV-3 strain, the percentage of CD4⁺ cells and the CD4/CD8 T cell ratio increases during acute hepatitis.²²¹ Many of the CD4⁺ T helper cells, however, are **anergic** (nonresponsive to stimuli), while the intrahepatic CD8⁺ T killer cells are not. The latter cells may play an important role in viral elimination.²²¹ Viral persistence in the liver may result from the dual roles of the CD4⁺ T helper cells which weaken the antiviral immune response and inhibit the mouse host from eradicating the virus from the liver.²¹¹

The **NLRP3 inflammasome** is a cytosolic protein complex that may be at least partially responsible for resistance to the development of FH. It regulates the processing and

secretion of proinflammatory cytokines, including IL-1 α and - β as well as IL-18, which may play a role in both host protection and pathology, dependent upon the circumstances at any given time.²²² The rapid release of ROS by infected macrophages may result in inflammasome activation. MHV-infected mice that lack inflammasome signaling produce less IL-1 β , are more susceptible to infection, have poor survival rates, and increased levels of viral replication.²²³ IL-18 is produced by both CD4⁺ T helper cells and NK cells and the loss of this cytokine increases viral replication but decreases survival since it also signals IFN- γ production by T cells.²²³ Additionally, although IL-18 levels increase during MHV-3 infection, IL-18 is not completely required for the production of hepatitis.²²² The loss of IL-1 signaling increases viral replication but not survival when compared to wild-type mice. IL-1 α and IL-1 β recruit neutrophils, encourage CD4⁺ T helper cells to develop into Th17 phenotype and activate DC to prime T cell responses.²²³

The high mortality rate in MHV-3-induced FH is accompanied by increased levels of IL1 β in the serum and liver. Interference with the IL-1 β receptor decreases virus replication, progression of hepatitis, and mortality. Mice that lack functional IL-1R1 (an IL-1 receptor), have less neutrophil infiltration of the liver as well as decreased FGL2 production by macrophages. ROS produced by infected macrophages may be important for coronavirus stimulation of NLRP3 inflammasomes. While IL-1 β acts synergistically with TNF- α to induce the NF- κ B transcription factor and the extracellular signal-regulated kinase 1/2 (ERK) and p38 MAPK phosphorylation pathways, NF- κ B appears to play the major role in reducing FGL2 production.²²² Although NLRP3 inflammasomes, IL-1 β , and TNF- α help to stop viral infection, their hyperactivation is associated with pathogenic inflammatory syndromes. Mitochondrial production of ROS is also of great importance in FH.²²²

MHV-3 triggers the production of TNF- α in vivo and *ex vivo*. High levels of TNF- α , in turn, increase FH pathogenesis²²⁴ by upregulating the expression of the TNF- α receptor TNFR2 on Tregs, promoting the proliferation and activity of these cells. TNFR2 is also vital for in vitro immunosuppression.²²⁵ Mice lacking functional TNF- α are resistant to MHV-3-mediated FH.²²⁶ In addition to the production of TNFR2, MHV-3 also enhances the production of another TNF- α receptor, TNFR1, in the liver. These TNF- α receptors have a high degree of homology in their extracellular domains, but their cytoplasmic signaling regions differ. TNFR1, but not TNFR2, contains a **death domain** that promotes cell death signals.²²⁷ Accordingly, infected mice lacking TNFR1, but not TNFR2, are resistant to FH in comparison to their wild-type littermates. Lack of TNFR1, but not TNFR2, also allows fibrinogen deposition in the liver and well as decreasing serum and tissue levels of FGL2. Expression of apoptosis-associated molecules, **Fas** and **Fas ligand**, in infected organs from TNFR1-deficient mice is also decreased. Furthermore, infiltration of neutrophils, rather than Treg cells, into the liver increases proinflammatory factors and FGL2 in the liver and spleen.²²⁶

The viral N protein induces expression of the *fgl2* gene and is thus indirectly responsible for the induction of FH in MHV-3-infected mice.²²⁸ Two deletions in the N protein gene may be responsible for differences among MHV strains.²²⁸ While infection of macrophages with MHV-A59 and MHV-3 in vitro induces *fgl2* expression, infection with MHV-JHM and MHV-2 do not do so.²²⁸

Infection with MHV-3 triggers a macrophage-dependent cytokine storm during which proinflammatory cytokines IL-1 and TNF- α , the Treg cytokine TGF- β , the proinflammatory

lipid **leukotriene** B4, and FGL2 are secreted, resulting in acute necrosis of the liver and death.²¹⁹ MHV-3-activated macrophages aid in the production of FH. This process is regulated by the **V-set immunoglobulin-domain-containing 4** (VSIG4), which is expressed by resting macrophages, including Kupffer cells (liver macrophages).²²⁹ VSIG4 suppresses the secretion of ROS by the mitochondria and reduces proinflammatory **M1 macrophage** activity. Macrophages and neutrophils intentionally produce mitochondrial ROS in response to microbial infection. The ROS are detected by NLRP3 inflammasomes, resulting in IL-1 β maturation. The balance of protective vs pathogenic responses depends upon ROS concentration and timing. At the proper concentration, these toxic molecules kill invading microbes, including MHV-3, but at higher levels or when produced long-term, they cause pathogenic inflammation. By suppressing the production of high levels of ROS, VSIG4 reduces inflammation. Lack of VSIG4 enhances M1 macrophage activity, leading to the development of FH.²²⁹

5.4.2.6 Treatment of mouse hepatitis virus infection

Nucleoside analogs, including ribavirin and 5-fluorouracil, do not typically inhibit coronaviruses due to the proofreading activity of the viral 3'-5' **exoribonuclease**.²³⁰ β -D-N4-hydroxycytidine (NHC), however, inhibits replication of MHV, MERS-CoV, HCoV-NL63, and SARS-CoV^{231,232} with minimal toxicity to the host cells. Resistance to NHC is modest in coronaviruses, although approximately twofold resistance was achieved after 30 passages of MHV in vitro. Establishing resistance to NHC appears to be based upon a delicate balance of resistance-promoting mutations, viral fitness, and accumulation of deleterious mutations.²³³ Importantly, NHC equally inhibits MHV with or without this exoribonuclease's proofreading activity, making it a potential therapeutic agent for at least some coronaviruses.²³³

A hemoglobin–ribavirin conjugate bound to **haptoglobin** has greater antiviral activity on hepatocytes and Kupffer cells than ribavirin alone in mice infected with MHV-3.²³⁴ Both hepatocytes and Kupffer cells express a hemoglobin-haptoglobin receptor. Viral replication in macrophages is required for MHV-3 to infect hepatocytes. Infected macrophages produce an ineffective Th2 immune response as well as nonneutralizing antibodies that fail to control viral replication.²³⁵ Both ribavirin and the conjugate decrease fibrin deposition and necrosis in the liver as well as the production of IFN- γ and TNF- α by infected macrophages. Additionally, only the conjugate reduces the **cytopathic effect** (formation of large groups of dying cells) in vitro.²³⁴ In vivo, untreated MHV-3–infected mice develop acute viral hepatitis and die within four days, with their livers containing greater than 90% necrotic cells. Mice treated with the conjugate had less than 10% liver necrosis. Ribavirin, but not conjugate, treatment may produce side effects, such as decreased **hematocrit** (percentage of red blood cells in the blood), lethargy, and abnormal fur.²³⁴

Administration of the Clara cell 10 kDa (CC10) protein has antiinflammatory activity in the nose and lungs of patients during allergic rhinitis and asthma. Administration of CC10 to mice with FH increases their survival from 0 to 12.5%. Levels of the liver enzymes ALT and AST in serum and liver damage also decrease. Additionally, the amount of TNF- α , IL-1 β , and Th17; fibrin deposition in the lungs; area of lung lesions; and hepatocyte apoptosis are reduced by CC10, demonstrating promising possibilities for its use in treating MHV-induced FH.²¹² The mechanism of CCL10's action relies at least in part upon reduced

amounts of FGL2 produced by macrophages in response to viral infection. In the THP-1 monocytic cell line, CC10 directly inhibits IFN- γ -induced FGL2 level.²¹²

Another potential therapeutic target is the Na⁺, K⁺-ATPase ion pump.²³⁶ In addition to its ion pump activities, this compound transmits cardiotonic steroid-binding-induced signals into cells.²³⁶ Lack of the functional α 1 ATP1A1 subunit does not affect virus binding to target cells but does block entry of MHV. Nanomolar concentrations of **cardiotonic steroids**, however, inhibit infection of cells with several coronaviruses, including MHV, FIPV, and MERS-CoV, when added at the time of experimental infection, but not at later times, decreasing their usefulness against natural infection. Importantly, both alpha- and betacoronaviruses are inhibited by these compounds.²³⁶ Cardiotonic steroids block MHV at an early stage, resulting in the accumulation of virions close to the cell surface and preventing viral fusion and cell entry. Such steroids, including ouabain, are presently FDA-approved and may be useful in preventing coronavirus entry into target cells. Inhibitors of Src kinases can reverse the activity of this ion pump, suggesting that Src signaling mediated by the ATP1A1 subunit is necessary to inhibit infection with at least some coronaviruses,²³⁶ while other coronaviruses gain entry by **caveolar-mediated endocytosis**. While ATP1A1-mediated Src signaling induces phosphorylation during this type of endocytosis, it is not currently known how ATP1A1-mediated Src signaling interferes with **clathrin-mediated endocytosis** of coronaviruses.²³⁶

Glycyrrhetic acid (GA) is a major component of the aqueous extract from licorice root that functions as an antiinflammatory agent. It is used in Chinese medicine to treat several inflammatory diseases, including hepatitis.⁷ MHV-A59 infection triggers the release of IL-17A, IL-6, and IL-22 cytokines into the serum. This release is inhibited by GA both in vivo and in vitro.⁷ GA also inhibits MHV replication indirectly by activating CD8⁺ T killer lymphocytes and directly, resulting in decreased liver pathology and the mortality rate of infected mice.⁷

Host proteasomes are important in the replication of some coronaviruses, including MHV. Pretreatment of **peritoneal macrophages** with several proteasome inhibitors in vitro blocked transcription of MHV-1 RNA, cytotoxicity in the cultured macrophages, and global production of cytokines, particularly proinflammatory mediators, such as CXCL10, IFN- γ , and CCL (monocyte chemoattractant protein-1; MCP-1). Intranasal inoculation of A/J mice with lung-tropic MHV-1 normally results in lethal pneumonitis, however, at least three proteasome inhibitors increase the survival rate to 40%.¹⁶⁴ Proteasome inhibitors block the release of some coronaviruses into the cytoplasm, resulting in the accumulation of viruses in late endosomes and **lysosomes**.²³⁷ By contrast, in another study, viral titers and pathology increased in MHV-A59-infected C57BL/6 mice.^{238,239} These differences may be due in part to the use of a different mouse as well as viral strains that differ greatly in their organ tropism. This suggests the need for caution in this mode of therapy if used to treat outbred humans and should also take into consideration the tropism of the given virus.

The long pentraxin PTX3 mediates innate immunity and inflammation and has antiviral effects. In MHV-1-induced acute lung injury, the virus rapidly induced PTX3 expression in the lungs and serum. PTX3 binds to MHV-1 and decreases its infectivity in vitro. Exogenous PTX3 accelerates viral clearance from the lungs, reduces lung injury, decreases the influx of neutrophils and inflammatory mediators into the lungs, and lengthens the

period of macrophage presence in that site. Mice that lack PTX3 activity have more severe lung injury.²⁴⁰

5.5 Rat coronavirus

5.5.1 Introduction to rat coronavirus

Several strains of RCV exist. Two strains of RCV isolated from the United States differ in their receptor usage. The sialodacryoadenitis virus (RCV-SDAV) strain binds to cellular receptors using its S protein while the RCV-P (Parker's RCV) strain may bind to cells using either its S protein or its hemagglutinin-esterase.¹²¹ Anti-MHV antibodies do not block infection of murine cell lines by either RCV-P or RCV-SDAV.⁹⁷ Much less is known about the CARS strain of RCV from Japan. RCV-SDAV can replicate in **Clara cells** of the lower respiratory tract, ciliated cells in the bronchial airway, and alveolar type I and II cells.²⁴¹

5.5.2 Rat coronavirus and disease

Infection with RCV-SDAV typically results in diseases of the respiratory tract, especially the upper respiratory tract, salivary and lacrimal glands, and eyes, as well as causing mild interstitial pneumonia in young rats (Table 5.4).

RCV-P is associated with pulmonary lesions and fatal pneumonia in experimentally infected suckling rats.²⁴² RCV-P infection of adult rats results in interstitial pneumonia and focal edema of the **alveoli** which self-resolves approximately a week postinfection.²⁴³ RCV also causes focal, transient inflammatory lung lesions and mild weight loss in adult rats.²⁴¹ The SDAV strain also increases lower respiratory tract illness in Wistar rats coinfecting with *Mycoplasma pulmonis*, a common respiratory tract bacterium.²⁴⁴

5.5.3 Rat coronavirus and the immune response

Type I-like alveolar epithelial (AT1) cells serve as the primary target for RCV in the alveoli. These cells compose 95% of the alveolar surface area and function in gas exchange and fluid **homeostasis**.²⁴⁵ RCV induces the expression of proinflammatory cytokines and chemokines by rat AT1-like cells in vitro. Both RCV-SDAV and RCV-P and UV (ultraviolet light)-inactivated virus induce expression of the following neutrophil chemokines by uninfected cells: cytokine-induced neutrophil chemoattractant 2 and 3 (CINC-2 and CINC-3, respectively) and lipopolysaccharide-induced CXC chemokine (LIX).²⁴⁶ RCV-infected cells produce IL-1 which, in turn, induces the production of the chemokine receptor C-X-C motif chemokine receptor 2 (CXCR2; interleukin 8 receptor, beta), macrophage inflammatory protein-3 α (MIP-3 α), and fractalkine.²⁴⁶ CXCR2 regulates cellular **proliferation** and **morphogenesis**, **angiogenesis**, and wound healing. MIP-3 α is chemotactic for DC, B cells, and memory T cells. It is produced by bronchial epithelial cells in response to IL-1 β and TNF- α . Fractalkine is found in neural cells in the brain and induces microglial cell migration.

Increased levels of CXC chemokines are also present in **bronchoalveolar lavage fluid** of rats infected with RCoV via the trachea.²⁴¹ Multiple CXC chemokines bind redundantly to CXCR2 and cause neutrophil chemotaxis in an RCV-infected AT1 cell line *in vitro*.²⁴⁷ The recruited, infected neutrophils then produce the chemokines CXCL10; C-X-C motif ligand (CXCL)-1 and -3; and CCL2 in the airways. Binding of CXCR2 to its ligands blocks spontaneous neutrophil apoptosis via the **caspase 8—dependent extrinsic pathway**.²⁴⁷ Apoptotic neutrophils are removed by macrophages, preventing the release of ROS and proteolytic enzymes. Cytokines that affect neutrophil apoptosis include granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), IL-1 β , IL-6, IFN- γ , TNF- α , and IL-15, while TNF- α and IL-6 trigger neutrophil apoptosis in a manner that depends upon cytokine concentration, cellular activation state, and time postinfection. At early times, TNF- α is proapoptotic while, after 18 hours, it decreases apoptosis.²⁴⁷

While RCV typically causes mild respiratory infections that self-resolve, in the absence of lung neutrophils, infection leads to more severe disease with weight loss, prolonged pulmonary viral replication, and, in some cases, death.¹⁹³ Neutrophil-mediated lung inflammation correlates with the production of hemorrhagic lesions, epithelial barrier permeability, and cellular inflammation in the lungs. Infiltration of neutrophils into the respiratory tract early after RCV infection is thus a double-edged sword since these cells stimulate antiviral activity by other immune cells yet can themselves be pathogenic. To avoid severe clinical disease, respiratory tract neutrophil activity needs to be carefully balanced throughout RCV-induced disease.¹⁹³

CXC cytokines are primarily produced by uninfected type I pulmonary endothelial cells. Virus-induced chemokine expression is reduced by the IL-1 receptor antagonist, suggesting that the IL-1 produced by RCV-infected cells induces chemokine expression by the uninfected cells. CXC chemokines recruit CD8⁺ T killer cells, CD4⁺ T helper cells, monocytes/macrophages, and additional neutrophils to the site of infection. In their absence, the disease in RCV-infected mice is worsened, with prolonged viral replication in the lungs and increased morbidity and mortality rates.¹⁹³

5.5.4 Other coronaviruses of rodents

Coronaviruses of rodents make up a major portion of known genetic diversity in betacoronaviruses, lineage A.⁹² Since rodents and rodent excretions often come into close contact with people, coronaviruses of rodents might be able to spill over into humans. Rodents harbor several alphacoronaviruses and betacoronaviruses.

Coronaviruses have been found in bank voles, common voles (*Microtus arvalis*), field voles, brown rats (*Rattus norvegicus*), and *Apodemus* species mice in Europe.²⁴⁸ Alphacoronavirus and betacoronavirus are also present in the feces of the common Chevrier's field mouse, Oriental voles (*Eothenomys* species), and *Apodemus ilex* field voles in the Yunnan Province of China.²⁴⁸ The prevalence of both alphacoronaviruses and betacoronaviruses in Chevrier's field mouse was 21.4% in this study as compared to less than 5% in other tested Chinese rodents.

The AcCoV-JC34 alphacoronavirus has little amino acid sequence similarity to other alphacoronaviruses. Its S protein has less than 20% identity with those of other

alphacoronaviruses, while the typically conserved N protein has only 25% sequence identity.⁶⁷ Several groups of rodent alphacoronaviruses have only been reported in liver samples of Norway rats (*Rattus norvegicus*), bank voles, wood mice (*Apodemus sylvaticus*), and noncyclic field voles (*Microtus agrestis*)¹²² as well as a novel coronavirus of the *Embecovirus* subgenus, China Rattus coronavirus (ChRCoV) HKU24 in Norway rats.⁹⁶ Myodes CoV 2JL14 is found in Norway rats, field voles, the common shrew (*Sorex araneus*), and bank voles.¹²² These rodents were captured in the same geographical area and share a common predator, domestic cats (*Felis catus*). The coronaviruses are also greater than 90% similar to the corresponding amino acids from Lucheng Rn CoV but do not belong in the same phylogenetic cluster as those present in bats.¹²² Alphacoronaviruses are present in Chinese rodents, including Chevrier's field mouse, gray red-backed voles (*Myodes rufocanus*), and lesser rice field rats (*Rattus losea*). A rabbit alphacoronavirus also clusters with rodent alphacoronaviruses.²⁴⁸

A novel betacoronavirus, *Apodemus peninsulae* coronavirus, from Korean field mice shares about 99% amino acid identity with coronavirus HKU24. Its pathogenic potential is yet unknown. An alphacoronavirus, AcCoV-JC34, has high levels of structural similarity to HKU24 bat coronavirus but is most closely related Norway RCV LNRV. Nevertheless, the S and N proteins have only 66.5 and 77.4% identities with LNRV, respectively.⁶⁷ A full genome sequence was produced from the UK RCV, along with partial genome sequences of coronaviruses from field voles in the United Kingdom and bank voles in Poland, and has a short conserved ORF1b fragment from the French RbCV. Genomic and phylogenetic analyses indicate that despite their diverse geographic origins, all rodent alphacoronaviruses form a single monophyletic group and share similar features, such as the same gene constellations, a recombinant betacoronavirus S protein gene, and similar core transcriptional regulatory sequences. These data suggest that all tested rodent alphacoronaviruses sampled originate from a single common ancestor.

Other rodent betacoronavirus lineage A include Longquan Aa mouse coronavirus (LAMV) and Longquan RI RCV (LRNV), which are members of *Betacoronavirus 1* and mouse coronavirus (MCoV) species, respectively.⁹² LRNV is a member of the subgenus *Luchacovirus* in China and Poland.¹²³ LRNV appears to have resulted from genetic recombination.⁹² Intergenotype recombination among coronaviruses is rare but has been seen between coronaviruses of bats and cats.⁹² MCoV are betacoronaviruses of the *Embecovirus* subgenus, first isolated during the late 1940s. They infect the liver, gastrointestinal tract, and CNS of mice and cause hepatitis, gastroenteritis, and acute and chronic encephalomyelitis.⁹ Some strains also cause respiratory illness in rats.

China Rattus coronavirus HKU24 is another member of the *Embecovirus* subgenus that was discovered in 2015. Its genome is similar to both *Betacoronavirus-1* and MCoV.⁹ HKU24 infects several Chinese rodent species: Chevrier's field mouse (21.4% of 98 mice), a species of *Eothenomys* vole (1.6% of 62 voles), and a species of *Apodemus* mice (19% of 17 mice).⁹⁶ Chevrier's field mouse is the dominant rodent species in southwest China and is the only known rodent species that are infected by alpha- and betacoronaviruses.

The European rodent alphacoronaviruses PLMg1, UKMa2, UKMa1, and UKRn1 have been found only in liver cells of mice and voles. By contrast, LAMV, and LRNV are found in a variety of cell types. In Maryland, about 92% of the wild-caught Norway rats were infected with the nonpathogenic RCV-SDAV.²⁴² This RCV is in a sister group that also

TABLE 5.2 Rodent Coronaviruses.

Rodent	Coronavirus	Coronavirus group
Mouse	Murine Hepatitis Virus	Beta-CoV
Mouse	Longquan Aa mouse coronavirus	Beta-CoV
Mouse	<i>Apodemus peninsulae</i> CoV	Beta-CoV
Mouse	AcCoV-JC34	Alpha-CoV
Rat	Sialodacryoadenitis virus	Beta-CoV
Rat	Parker's rat coronavirus	Beta-CoV
Rat	<i>Rattus</i> coronavirus HKU24	Beta-CoV
Rat	Longquan Rl rat coronavirus	Alpha-CoV
Vole	Myodes CoV 2JL14	Alpha-CoV
Mouse and Vole	PLMg1	Alpha-CoV
Mouse and Vole	UKMa1	Alpha-CoV
Mouse and Vole	UKMa2	Alpha-CoV
Mouse and Vole	UKRn1	Alpha-CoV

The majority of well-studied and named rodent coronaviruses are found in mice and rats. The four coronaviruses shared by mice and voles are alphacoronaviruses and have only been reported to inhabit liver cells.

contains HCoV-HKU1 and BetaCoV1 coronaviruses. These viruses differ from another beta- and alphacoronaviruses in that they may be descendants of rodent, rather than bats, viruses. Due to several shared features, it has been suggested that all rodent alphacoronaviruses, including those from West Europe and East Asia, except HKU24, may have originated from a single common ancestor and that alphaviruses frequently practice “host-jumping” among rodent species. An overview of rodent coronaviruses is found in [Table 5.2](#).

5.6 Coronaviruses of nonhuman primates

5.6.1 Introduction to coronaviruses of nonhuman primates

Due to the similarities between humans and nonhuman primates, the nonhuman primates are often experimentally infected with various microbes to observe the extent of pathology and to test a variety of drugs or vaccines before performing clinical tests on humans. It should be noted that MHV has not been found to infect any primate cell line in vitro,¹⁰³ although several species of nonhuman primates can be infected by MHV. At least one human coronavirus, HCoV-OC43, caused an outbreak among wild chimpanzees.²⁴⁹

In the Kingdom of Saudi Arabia, 22% of 50 free-living hamadryas baboons (*Papio hamadryas hamadryas*) had antibodies against human coronaviruses and 10% had anti-CCoV antibodies. Surprisingly, none were positive for MERS-CoV, which is endemic in that

country. These baboons live in close contact with feral dogs and human communities where they mingle with people and aggregate in large groups.²⁵⁰ At least some nonhuman primates, the rhesus macaque (*Macaca mulatta*) and the common marmoset (*Callithrix jacchus*), are susceptible to MERS-induced lung pathology after experimental infection, as described below.²⁵¹ Vaccination of the former primates with a recombinant RBD protein vaccine lessens the severity of pneumonia and decreases viral load in the lungs.²⁵²

Some endangered captive nonhuman primates are susceptible to SARS-CoV-2 infection. Western lowland gorillas at the San Diego Zoo Safari Park were accidentally infected by SARS-CoV-2, likely from an asymptomatic member of the park's wildlife team. All gorillas in the troop had mild respiratory symptoms. An older gorilla with comorbidities developed pneumonia and heart disease but, after treatment with antibiotics, heart medications, and monoclonal antibodies, completely recovered.²⁵³

When night monkeys (*Aotus* species), cynomolgus macaques (*Macaca fascicularis*), African green monkeys (*Chlorocebus aethiops*), or rhesus macaques are infected intracerebrally with neurotrophic MHV-JHM, the viruses replicate and disseminate in the CNS where they may cause acute **panencephalitis** with or without demyelination of the nerves.^{105,254} These nonhuman primates represent outbred species of both Old and New World primates. It should be noted that this mouse coronavirus was passaged in mouse cells and had not been conditioned to replicate in primate cells. It remains to be seen if MHV can enter the primate CNS naturally.¹⁰⁵

5.6.2 Pathology of coronaviruses of nonhuman primates

Rhesus macaques are often used as role models for the human disease since, unlike chimpanzees (*Pan troglodytes*), they are not endangered species. The ability of coronaviruses to cause neurological disease was shown by 1992 study¹⁰⁵ of *Aotus* species monkeys and African green monkeys that were infected with the mouse coronavirus MHV. The monkeys develop acute to subacute panencephalitis that affects both gray and white matter in the presence or absence of demyelination. Similar results were obtained when the monkeys were infected by the ocular, nasal, or peripheral blood routes.¹⁰⁴ HCoV-229E has also been found in the CNS of infected people and may be associated with multiple sclerosis.²⁵⁵

Despite the usefulness of nonhuman primates in infectious disease research, differences between humans and other primates do exist and affect susceptibility to infection and disease. Experimentally infected macaques often recover spontaneously and do not die from some human coronavirus diseases, including SARS. Additionally, clinical signs of other primate species are mild, typically including loss of appetite, agitation, and aggression. Examination of the primates' lungs, however, found various degrees of pathology. Some monkeys develop severe lung damage, while others only had mild interstitial pneumonia. The SARS fatality rate is high in humans, but not in the closely-related rhesus macaques, even in those macaques with severe lung disease. It is important to determine the extent of lung damage and fatality rates in chimpanzees since they are more closely related to humans. The results seen in monkeys and chimpanzees might allow us to develop new treatments that decrease fatality rates in humans.

When rhesus macaques and common marmosets are experimentally infected with MERS-CoV, both animal species develop bronchointerstitial pneumonia. While disease in the macaques is generally mild, it is moderate to life-threatening in marmosets. In the latter, infection is accompanied by relatively higher levels of pulmonary viral antigen and neutrophil infiltration into the lungs even though these animals have a similar pulmonary expression of the DPP4 MERS-CoV receptor.²⁵⁶ MERS-CoV antigen is found in the cytoplasm of lung macrophages in both of these primate species as well. Early after infection, the levels of T and B lymphocytes and macrophages in the lungs of marmosets and macaques are similar. The expression of proinflammatory genes and RNA transcripts is increased at this time as is the number of pulmonary lesions.^{251,257} Later, marmosets had relatively higher levels of infiltrating neutrophils, B lymphocytes, and macrophages.²⁵⁶

When the effects of SARS-CoV infection of cynomolgus macaques were compared to that found in the green monkey (*Chlorocebus sabaeus*), numbers of viruses in the upper and lower respiratory tract were similar. Green monkeys develop more severe lung diseases than do macaques. Interestingly, the types of inflammatory immune-system molecules differed between infected macaques and green monkeys. The type of inflammatory molecules produced during SARS infection may thus determine, to some degree, the extent of disease in these two groups of monkeys. Since several coronavirus proteins inhibit IFN- β production, administration of type I interferon to infected macaques and marmosets improves the outcome of both SARS and MERS in these animals.^{258,259} SARS lung disease severity is greater in aged macaques than that seen in young adult animals. It should be noted that when rodents are used as the animal models of SARS, unlike normal adult mice, aged mice do develop clinical disease and severe lung damage with different types of inflammatory molecules being produced. The differences in responses to SARS-CoV between monkey species and the animals' age may also suggest that caution be used when interpreting and extrapolating these findings to humans and suggest that future work may consider the type of inflammatory responses present in humans.

Common marmosets are small primates that, in most ways, have less similarity to humans than do chimpanzees and monkeys, but still develop a disease that is similar to SARS in humans. Using marmosets has the advantage of being able to test greater numbers of animals than is practical for most other primates since marmosets may be raised in large breeding facilities. In one study, 6 juvenile and 6 young adult marmosets were infected via the trachea with SARS-CoV.²⁶⁰ All animals developed a form of interstitial pneumonitis in which the inflammatory macrophages and the type-1 pneumocyte lung cells were infected. In marmosets, virus is also found in the nearby lymph node, **skeletal muscles**, **cardiac muscles** of the heart, and **smooth muscles** of the intestine. Most animals also develop hepatitis.

When marmosets are infected with two common strains of MERS by the same route, they only develop mild to moderate, nonlethal respiratory disease.¹⁹⁷ Infection with inactivated ("dead") MERS-CoV produces symptoms that were similar to those seen in marmosets receiving the live virus, suggesting that the small amount of lung disease did not result from infection *per se*, but may instead be the result of an excessive immune response. In an earlier study, however, some of the MERS-CoV-infected marmosets did die.²⁵¹ The earlier study, however, infected marmosets by multiple routes, such as oral, intranasal, ocular (via the eyes), and intratracheally and used a larger dose of the virus. The use of two different

amounts of virus in these studies, in addition to other differences in their study design, makes it difficult to compare the results. However, they do call into question the usefulness of marmosets to study MERS pathology. Further studies that use the same route of infection and the same amount of virus could, perhaps, resolve the matter.

5.7 Coronaviruses of ferrets and minks

5.7.1 Introduction to coronaviruses of ferrets and minks

Ferrets, mink, otters, martins, badgers, fishers, weasels, and wolverines are members of Musteloidea. Ferret and mink coronaviruses (MCoV) are alphacoronaviruses of the subgenus *Minacovirus*.²⁶¹ Some Musteloidea species are susceptible to infection by betacoronaviruses, including SARS-CoV and SARS-CoV-2. SARS-CoV-2 RNA is present in domestic ferrets and farmed and wild minks (*Neogale vison*) in the United States. The latter is present in at least two rivers in Spain as well.^{262,263} Mink infections are asymptomatic. Feral minks may be infected by human SARS-CoV present in leaks from sewage facilities.²⁶³

Ferrets are naturally infected by two types of ferret coronaviruses, FRECV with a low mortality rate, and ferret systemic coronavirus (FRSCV) which produces a fatal disease. It has been postulated that FRSCV is a mutant form of FRECV, a cocirculating distinct strain, or arose by recombination between FRECV and another alphacoronavirus.²⁶⁴ FRECV and FRSCV ferret coronaviruses are related to MCoV, FCoV, and canine enteric coronavirus. FRSCV and FRECV strains have 89% nucleoside identity.²⁶⁵ The receptor for both viruses is unknown, as well as the mechanism driving pathogenesis, but macrophages appear to be important to the pathogenic inflammatory response.²⁶⁶

Ferrets and mink are also susceptible to experimental infection by SARS-CoV and SARS-CoV-2, but not MERS-CoV, even though ferret and MCoVs are alphacoronaviruses, while SARS-CoV and SARS-CoV-2 are betacoronaviruses.²⁶¹ SARS-CoV-2-infected ferrets can infect other ferrets in their proximity by direct contact or by the air-borne route.^{25,267} SARS-CoV-2 is shed in ferrets' saliva, nasal washes, feces, and urine for up to 8 days post-infection.²⁶⁸ Large numbers of ferrets are used for rabbit hunting and rabbit control in some areas, especially in Spain. Some of these ferrets escape and establish feral populations that may serve as viral reservoirs.²⁶⁹

SARS-CoV-2 has been detected in mink farms in the Americas and across Europe. It rapidly spreads throughout mink farms with a 100% prevalence on many farms, however, has not been detected in rabbits, chickens, or horses from the same farm.²⁷⁰ SARS-CoV-2 infection of minks can result in either subclinical or clinical illness, including acute severe interstitial pneumonia or diffuse alveolar damage and death.²⁷¹ SARS-CoV-2 RNA is present in the lung and throat and rectal swabs of infected minks as well as the liver and intestines, but not the spleen, in nearly 50% of the ill animals.²⁷² Human-to-mink transmission has been reported in farmed American minks in the Netherlands.^{271,273,274} Incidental spill-over of SARS-CoV-2 from humans to animals has also been seen in dogs, cats, and tigers.

Several cases of mink-to-human transmission of the Y453F variant form of SARS-CoV-2 have been reported in mink farms in Denmark and the Netherlands. Workers in these farms

may have acquired infection by the inhalation of contaminated dust from SARS-CoV-2-infected feces collected from the farms, indicating a possible exposure of workers to viruses excreted by minks.²⁷² The Y453F variant has an altered S protein that increases its ability to bind to human ACE2.^{275,276} While it can partially escape from commercially-available neutralizing antibodies, this variant does not decrease established immunity in previously infected people.²⁵ Of note: since minks and ferrets are members of Mustelidae, other members of the family may also be susceptible to SARS-CoV-2,²⁵ including weasels, badgers, otters, martens, and wolverines. No evidence of infection has been reported for other small mammals such as rock squirrels (*Otospermophilus variegatus*), deer mice (*Peromyscus maniculatus*), house mice (*Mus musculus*), striped skunks (*Mephitis mephitis*), and raccoons.²⁷⁷

5.7.2 Ferret enteric coronavirus

FRECV is an alphacoronavirus that was first reported in the Eastern coastal region of the United States in 1993.²⁷⁸ Juvenile ferrets develop mild to subclinical disease, but this coronavirus can cause more severe disease in older animals.²⁷⁹ In many ways, FRECV is similar to FIPV in cats, however, FIPV primarily targets young cats.²⁸⁰

Infection typically causes **epizootic catarrhal enteritis** (ECE) in ferrets that has a mortality rate of less than 5%.²⁷⁸ It is characterized by foul-smelling bright green diarrhea with large amounts of mucus. During the chronic stage of the disease, the feces often contain grainy material that resembles birdseed. FRECV's primary target cell type is the enterocytes of the gastrointestinal tract. No lesions have been found outside of this tract in infected ferrets. The virus appears to only replicate in the ileum, colon, and rectum. **Hyperproteinemia** resulting from hyperglobulinemia is found in the blood of animals infected by either FRECV or FIPV. Proteins and blood are also present in the greenish urine of FRSCV-infected ferrets.²⁸⁰

FRECV is highly contagious and is transmitted between animals by the fecal-oral route and, accordingly, has been detected in saliva and feces, but not in serum, spleen, or lymph nodes.^{266,279,280} FRECV is closely related to the other major ferret coronavirus, FRSCV, as well as FCoV, which causes relatively mild disease in cats.²⁷⁹ While ECE may affect ferrets of any age, it is most severe in aged animals. FRSCV-induced disease, however, affects primarily young animals.^{280,281} ECE is also found in minks infected by a MCoV.

FRECV causes lethargy, anorexia, and vomiting in infected ferrets. Clinical findings are confined to the intestine and include **atrophy**, thin walls, fusion, and blunting of the intestinal villi as well as vacuolar degeneration and necrosis of the "top" end (apical region) of the intestinal epithelium.^{266,282} Whitish nodules are present in many locations, including mesenteric adipose tissue and lymph nodes, the visceral peritoneum, liver, kidneys, spleen, and lungs.²⁸⁰ FRECV also causes **pyogranulomatous inflammation** that is similar to that caused by FIPV and includes macrophage, lymphocyte, and neutrophil infiltration of the visceral peritoneum, mesenteric adipose tissue, liver, lungs, kidneys, lymph nodes, spleen, pancreas, adrenal glands, and blood vessels.²⁸⁰ Older ferrets are typically more likely to develop severe disease, while juveniles tend to develop a mild or subclinical illness.²⁸³

5.7.3 Ferret systemic coronavirus

The ferret alphacoronavirus, FRSCV, was first reported in Spain in 2004. Since then, it has been reported elsewhere in Europe, Asia, and North and South America.²⁸⁴ Many of the infected ferrets are pets and the international pet trade has played an important role in the geographical spread of the virus. One study reported that a ferret exported from Spain was asymptomatic for about 150 days, indicating a long incubation period that may exceed the quarantine period.²⁸⁴ While FRSCV causes disease features that are similar to the highly pathogenic FIP in cats, the causative viruses are distinct entities.^{280,285} FRSCV is also significantly different from FRECV in the S protein gene.²⁸²

A study from 2014 found FRECV RNA in 95% of fecal samples from healthy pets and laboratory ferrets.²⁶⁵ Only FRECV RNA was found in 60% of these samples, only FRSCV in 11%, and both viruses were found in 23% of the samples.²⁸⁶ Other studies have detected smaller percentages of approximately 50% of RNA-positive animals.²⁶¹ FRSCV is typically transmitted between ferrets by contact with infected body fluids or by people holding an infected animal before touching healthy ferrets but has also been detected in ferrets living indoors in the presence or absence of dogs, cats, or other ferrets. The average age of disease onset is 11 months. Male and female ferrets are equally affected by this virus.²⁸⁷

FRSCV causes a wide spectrum of pathology that affects multiple organs and organ systems, including the intestines, liver, and pancreas (digestive system), brain (nervous system), kidneys (urinary system), lungs (respiratory system), adrenal glands (endocrine system), blood vessels (circulatory system), and spleen and abdominal lymph nodes (immune system). Symptoms include fever, diarrhea, vomiting, loss of appetite, excessive weight loss, nerve damage that leads to weakened hind limb movement, **dyspnea** (breathing problems), enlarged spleen and kidneys, sneezing, heart murmur, greenish discoloration and the presence of protein and blood in the urine, **suppurative pancreatitis** in which pus-like fluid is released from inflamed pancreases, and muscle spasms that cause **opisthotonos** (backward arching of the head, neck, and spine).^{264,266,280,284,288} Unusual blood conditions include mild anemia, **thrombocytopenia** (low levels of plates), **hypergammaglobulinemia** (elevated blood levels of antibodies and related proteins) and elevated serum lipase, blood urea nitrogen, serum alanine transferase, alkaline phosphatase, and serum gamma-glutamyltransferase.^{280,282}

The pathology resulting from FRSCV infection is similar to those seen in the “dry” form of FIP. Like FIPV in cats, FRSCV is invariably fatal.²⁸⁰ Both viruses cause **pyogranulomatous inflammation** in which whitish, tan, or slightly pink nodules are present in the liver, kidneys, spleen, lungs, and other locations. Other conditions associated with these two viruses include pneumonia and inflammation of the kidneys, pancreas, adrenal glands, and muscles of the heart, as well as peritonitis.^{280,282,284} In ferrets infected by FRSCV, large **palpable** masses are found in the abdomen where these nodules are present on **serosal surfaces** (the outermost layer of **serosal membranes** that covers organs and lines several body cavities), abdominal organs, **mesenteric adipose tissue** (fatty material in the abdominal regions), lymph nodes, and **visceral peritoneum** (a serosal membrane that covers the organs of the abdominal cavity).

Masses in the CNS are characteristic of **pyogranulomatous meningitis** in which multiple nodules are present on the surface of **meninges** (three layers of tissue that cover the

brain).²⁸⁰ In ferrets with neurologic disease, primary lesions may be found only within the brain and include severe **pyogranulomatous leptomeningitis, choroiditis, epididymitis**, and encephalomyelitis. Inflammation is present, to a large degree, around the venules along the inner and outer surfaces of the brain.²⁸² Eyes may also be infected and affected by FRSCV, including the development of ocular lesions, anterior **uveitis** (inflammation of the middle layer of the eye), and corneal vascularization and opacity (formation of blood vessels or cloudiness in the cornea, respectively).²⁶⁴ This is also similar to ocular manifestations present during FIP. Ferrets with CNS involvement may also develop acute or progressive hind limb paresis, **ataxia** (lack of muscle control or coordination), seizures, abnormal gait, and **proprioceptive** defects (loss of the sense of body positioning).²⁶⁴

Coronaviruses are present in the cytoplasm of macrophages with FRSCV, but not in macrophages from ferrets with FRECV.^{283,287} Key differences are found in the S protein genes of FRSCV MSU-1 and FRECV MSU-2 and may partially explain the differences in macrophage infectivity between the two ferret coronaviruses. The ability to infect macrophages is important since these leukocytes are mobile and may disseminate the viruses throughout the body, leading to more severe disease, as is the case for the mild and severe diseases found between two cat coronaviruses.²⁸³ Additionally, preliminary studies indicate that the 3c-like protein from FRSCV MSU-1 is truncated in comparison to that from FRECV MSU. This is also comparable to FIPV in cats, which have truncations in their 3c-like protein compared to the intact gene found in all analyzed FECV strains.²⁸³ Nevertheless, complete genome sequencing determined that FRSCV is more closely related to FRECV than to any other alphacoronavirus (89% identity vs 49.9%–68.9% identity, respectively).²⁶⁵

FRSCV causes four types of granulomas.²⁸⁹ In naturally infected ferrets, granulomas without necrosis compose 30% of the lesions and are characterized by macrophages primarily located in the center of the granuloma, in addition to a moderate amount of T cells scattered among macrophages, B cells, and **plasma cells** (activated B cells that are releasing antibodies). Granulomas with necrosis comprise 15% of the lesions. They have a necrotic center surrounded by macrophages with some T cells, plasma cells, and a few B cells. The necrotic lesions contain few, if any, multinucleated giant cells or fibrosis. Additionally, granulomas with necrosis have higher levels of the virus than granulomas without necrosis. Granulomas with neutrophils comprise 20% of the lesions and have a central area primarily populated by neutrophils and lower amounts of macrophages, plasma cells, and T and B cells. Diffuse granulomatous inflammation composes 11% of the lesions and while they have similar cell proportions to that present in other types of granulomas, the cells are more evenly distributed throughout the lesions.²⁸⁹ While all these lesions contain abundant numbers of phagocytic cells, macrophages predominate in most of them, except in granulomas with neutrophils, which contain more neutrophils than macrophages, and many cells, including macrophages, are necrotic. While phagocytes are most common in all types of lesions, T cells are more numerous than resting B cells, except in diffuse granulomatous inflammation, in which both types of lymphocytes are much less common and plasma cells outnumber T cells.²⁸⁹ In ferrets infected by FRSCV, the inflammatory, antimicrobial M1 macrophage response is most common.²⁸⁹

5.7.4 Treatment options and protection against ferret coronavirus-induced diseases

Currently, there are no FDA-approved, specific treatments for FRSCV. Fluid and nutritional support, antimicrobials, sucralfate, and steroids, such as prednisolone, do not resolve the disease.²⁶¹ In a 2018 study, however, ferrets treated with prednisolone significantly improved.²⁸⁸ Prednisolone downregulates T and B cell activity as well as the movement of macrophages into infected areas and reduces their phagocytotic ability. This drug may play a role in decreasing the dissemination of coronavirus by circulating macrophages and monocytes. Prednisolone may also increase the ferret's appetite.²⁸² Caution is advised in determining the dosage of such drugs, however, since the use of immunosuppressants, including prednisolone and other corticosteroids, may decrease protective responses of the antiviral CD8⁺ T killer cells and NK cells, the most important antiviral cell types. Ferrets, however, are relatively resistant to corticosteroid use and rarely develop side effects during chronic use.

By contrast, instead of dampening inflammatory responses during coronavirus infection, a polyprenyl immunostimulant is also used to treat ferret infection by coronaviruses by increasing the production of Th1 antiviral cytokines, which may also induce pathogenic inflammation.²⁸² This highlights the delicate balance between pathogenic inflammation and inflammation that facilitates viral clearance. This balance may be dependent on circumstances, such as the person's particular immune response at that time and well the individual's age, the infecting viral strain, the person's medication usage, and nutritional status.

Other treatment options include doxycycline, a tetracycline antibacterial drug, that has antiinflammatory properties, such as inhibiting TNF- α production and decreasing fibrosis. Doxycycline may also decrease damage to blood vessels.²⁹⁰ Broad-spectrum antibiotic treatment is recommended for use during immunosuppressive therapy to prevent secondary bacterial infections.

Protease inhibitors against viral 3CLpro have been tested for use against SARS-CoV, MERS-CoV, and coronaviruses of mice and cats.²⁹¹ Although some regions of coronaviruses have a high rate of mutation, the overall structure and function of 3CLpro are conserved among coronaviruses. It may, therefore, be possible to develop protease inhibitors that are active against multiple coronaviruses. A reversible inhibitor of 3CLpro is effective in treating cats with either experimental or naturally occurring FIP as well as human coronaviruses.^{291–293} A structure-activity relationship study of protease inhibitors demonstrates that the tested inhibitors have similar activities against the 3CLpro of cat, ferret, and MCoV, despite an 83.44%–86.09% amino acid similarity between ferret and MCoV 3CLpros and only 71.52%–77.81% between ferret (or mink) and FCoV 3CLpros.²⁹¹

Symptomatic treatment may reduce nausea and loss of appetite. Antacids may be administered to reduce gastric acid. Several drugs, maropitant citrate or metoclopramide, reduce vomiting. Fluid replacement therapy may offset dehydration caused by water loss resulting from vomiting and diarrhea.²⁸² Treatment for coronavirus-induced malnutrition may include the use of soft foods, including human baby food, especially those containing turkey or chicken. Supplemental vitamins and minerals may also be beneficial. Iron supplementation reduces anemia and may be used together with the kidney cytokine **erythropoietin** to increase red blood cell production, while vitamin B12 may reduce chronic diarrhea.²⁸²

Prevention of ferret coronavirus diseases may include avoiding exposure to infected ferrets. However, FRECV is ubiquitous in most multiple-ferret homes, shelters, and breeding farms. Infection may be decreased by reducing fecal contamination by disinfecting litter boxes, cages, and bowls with bleach weekly and keeping litter boxes away from food and water bowls.²⁸²

5.7.5 Ferrets and feline infectious peritonitis virus of cats

Ferrets may also be infected with FIPV from cats. FIPV produces granulomas in the liver, spleen, bone marrow, and lymph nodes of ferrets. Nodules contain several types of leukocytes, as is seen during FIPV infection. The macrophages react with anti-FIPV IgG antibodies. **Glomerulonephritis** of the kidneys may also present and is associated with the deposition of immune complexes in the kidney tubules, impeding the production of urine.²⁹⁴ Degeneration of **afferent** or **efferent arterioles** which bring blood into or away from the kidneys is also found in FIPV-infected ferrets. As is the case for FIPV-infected cats, ferret coronaviruses may damage multiple eye structures, including intrusion of blood vessels into the cornea which is usually avascular.²⁶⁴

5.7.6 Coronaviruses of minks

There are two types of minks: European minks and American minks. The former is a critically endangered species, while American minks are either wild or are raised on farms, primarily for their fur.²⁹¹ **Epizootic catarrhal gastroenteritis** (ECG) was first reported in minks in the United States in 1975²⁹⁵ and has infected several million of these animals in North America, Scandinavia, and other parts of Europe, the former USSR, and China. Upon necropsy, a study found ECG to be present in 4.1% of farmed mink in North America and is most common in juvenile minks.²⁹⁶ Mink ECG and its symptoms resemble those of ECE, including anorexia, mucoid diarrhea, and decreased pelt quality. Minks over four months of age are most susceptible.²⁹¹

A 1990 report found coronavirus-like particles in fecal samples of mink with ECG as well as some healthy animals, indicating that asymptomatic infections also occur.²⁹⁷ Pathology in ECG in minks is similar to that occurring in ferrets.²⁸⁰ Ill minks produce very large amounts of green mucus-covered diarrhea, poor pelt quality, vomiting, anorexia, dehydration, ulcers, and, in severe cases, starvation. MCoV is widespread among domestic and wild minks and almost 100% of minks have antibodies to it.²⁹⁸ In one study, all experimentally-infected mink become symptomatic within 2–3 days with 100% **morbidity**, but without fatalities.²⁹⁷ Overall, ECG has a mortality rate of less than 5% in the absence of concurrent infection with bacteria or Aleutian disease virus. The latter is a highly contagious parvovirus that causes progressive wasting and weight loss, **splenomegaly** (enlargement of the spleen), hypergammaglobulinemia, various kidney disorders, spontaneous abortion, and death in minks and ferrets.²⁸¹ Healthy animals survive given supportive treatment, including rehydration and force-feeding.²⁹⁷

The complete genomes of two strains of MCoVs are highly variable and their nucleoside sequence identities are only 91.7%. The genes for both ferret and MCoVs lack ORF3a and

TABLE 5.3 Ferret enteric coronavirus, ferret systemic coronavirus, and mink coronavirus.

Coronavirus	Classification	Pathology
Ferret enteric coronavirus	Alpha-CoV	Epizootic catarrhal enteritis
Ferret systemic coronavirus	Alpha-CoV	Systemic pyogranulomatous inflammation
Mink coronavirus	Alpha-CoV	Epizootic catarrhal gastroenteritis

The three best-studied and named coronaviruses of ferrets and minks are all alphacoronaviruses. They cause either enteric or systemic disease.

ORF3b due to mutations or large deletions. Lack of functional 3c appears to correlate with the acquisition of systemic tropism by FRSCV as well as increased FIPV virulence. ORF3 is necessary for replication in gut tissues but is dispensable for systemic replication.²⁹⁹ Table 5.3 compares coronaviruses of ferrets and minks.

In general, coronaviruses from carnivores tend to have higher intraspecies genomic diversity than those from herbivores and omnivores.²⁹⁹ They are distantly related to HCoV-229E and HCoV-NL63 human coronaviruses and are not members of *Alphacoronavirus-1*. Genetically, MCoV appears to be very closely related to FRECV but is also closely related to swine TGEV, canine CCoV, and feline FIPV.²⁹⁹ MCoV, however, has unique numbers and arrangements of small ORFs. ECG in mink resembles that in ferrets with high levels of morbidity, but low mortality. The MCoV-associated disease is increased by coinfection with other gut viruses, particularly rotaviruses, parvoviruses, and caliciviruses.²⁹⁹

5.7.7 Coronaviruse of other musteloidea

In 1976, a possible FIP-like disease was found in two captive short-clawed otters (*Aonyx cinereus*). Both animals had excessive levels of abdominal fluid as well as pathology in the liver, kidneys, lungs, and mesenteric lymph nodes.³⁰⁰ In 1995, RNA from Eurasian otters (*Lutra lutra*) was reported in Portugal. Coronaviruses are also present in Chinese ferret badgers (*Melogale moschata*).³⁰¹ Additionally, in 1996, a fisher (*Pekania pennanti*) was reported in British Columbia that reacted with CCoV antibodies.^{261,302,303}

5.8 Coronaviruses of rabbits

Domestic rabbits, a subset of the wild European rabbit, are infected by two coronaviruses. These viruses are associated with different pathological manifestations, causing an enteric disease or **myocarditis**.⁹

5.8.1 Rabbit enteric coronavirus

Rabbit enteric coronavirus (RECV) belongs to the species *Betacoronavirus 1* of the subgenus *Embecovirus*. This virus was first reported in Canada in 1980 in young rabbits with

diarrhea but is also present in some healthy rabbits.³⁰⁴ Some rabbits experimentally infected with RECV by the oral route develop enteritis. Lesions are detectable in the small intestine within six hours postinfection and the severity of damage increases with time. The **brush border** of the enterocytes at the tips of the intestinal villi is lost and these cells undergo necrotic death, followed by the appearance of shortened intestinal villi.³⁰⁵ The **M cells** of the gut innate immune response also undergo necrosis. Some, but not all, infected young rabbits have diarrhea by three days postinfection. RECV replicates in the small intestine and coronavirus particles are present in fecal material from young rabbits with enteritis as well as in some clinically normal adult rabbits who may serve as viral carriers. Transmission of viruses between rabbits is via the fecal-oral route. Interestingly, antibodies to RECV cross-react with HCoV-229-E in humans.³⁰⁵

5.8.2 Rabbit coronavirus

RbCV is an alphacoronavirus, formerly known as the Stockholm agent, that was first reported in 1979, although the disease was described in Scandinavia in 1963.³⁰⁶ Experimental infections of rabbits produce an acute phase that is characterized by fever, anorexia, and weakness, followed by death or recovery within 11 days. A virus that is morphologically similar to coronaviruses is found in normal animals, but not in normal rabbit serum, and antibodies to this virus cross-react with HCoV-229E and OC43 of humans. Antigens cross-reacting with HCoV-229E are also present in ill, but not from healthy, animals. Later studies determined that this virus is an alphacoronavirus.^{9,306}

RbCV causes myocarditis and **congestive heart failure** in infected rabbits.³⁰⁷ **Myocyte** damage occurs before the entry of inflammatory leukocytes into the region and correlates with high numbers of viruses in the heart. During the acute phase of infection (days 2–5), myocytes degenerate and may undergo **necrotic death**.³⁰⁷ **Interstitial edema** and hemorrhage are also present in the heart during this stage of infection. During the subacute phase (days 6–12), the pathology becomes more severe.³⁰⁷ Lesions of necrotic fibers that are surrounded by macrophages and lymphocytes are found throughout the ventricles. **Pleural effusion** fills the lung cavities with fluid. **Congestion of the lungs and liver** also occurs as the blood vessels of the liver and lungs distend. Alveoli of the lungs fill with blood and blood flow slows. Myocarditis may be present by day 9 of infection, followed by dilation of the heart's **left ventricle**.³⁰⁷ The mortality rate is 60% and, of the survivors, 41% have increased heart weight, biventricular dilation, **myocyte hypertrophy** (thickening of the ventricle walls), **myocardial fibrosis**, and myocarditis that are associated with **dilated cardiomyopathy**, a condition in which the heart's ability to pump blood is impaired due to the enlargement and weakening of the left ventricle.³⁰⁸ All regions of the myocardium are affected as well as the atrial and ventricular muscles, but not the heart valves or blood vessels. Mitochondria in myocardial cells are also swollen.³⁰⁶ Electrocardiograms reveal sinus **tachycardia** (increased heart rate) along with disruption of the electrical conduction system of the heart, resulting in abnormal heart rhythms and **repolarization** of cardiac muscles. These conditions return to normal functioning in most surviving animals during the chronic phase of RbCoV infection.³⁰⁹ Low levels of infectious RbCoV persist in heart tissue in about half of the rabbits for 1–4 months, and in some cases may be found for at least two years.³⁰⁸

TABLE 5.4 Rabbit coronavirus and rabbit enteric coronavirus.

Coronavirus	Category	Organs affected	Disease	Mortality rate
RbCV ^a	α	Heart, lungs, liver	Myocarditis and congestive heart failure Congestion in lungs and liver	60%
RECV ^b	β lineage A	Small intestine	Enteritis and diarrhea	3%–40%

^aRabbit Coronavirus.

^bRabbit Enteric Coronavirus.

Two rabbit coronaviruses cause diseases that differ greatly in viral group, organs infected, disease manifestations, and mortality rate.

Lesions are present in the diaphragm, but not in other skeletal muscles, as well as the lungs, thymus, lymph nodes, and spleen. Pink, proteinaceous fluid is also seen in the lungs in addition to a swelling of the cells lining the alveoli together with large numbers of alveolar macrophages.³⁰⁶ Only low numbers of lymphocytes were found in most **thymuses**. Several changes are found in lymph nodes, including increased numbers of medium and large basophilic lymphocytes in the absence of secondary follicles. Lymphoid follicles were not typically present in the spleen.³⁰⁶

In 2012, RbCV HKU14 (RbCoV HKU14) was first reported from healthy rabbits in live food animal markets in Guangzhou Province in southern China bordering the South China Sea.³¹⁰ This virus represents a distinct branch of *Betacoronavirus subgroup A* coronaviruses. It is most closely related to the species *Betacoronavirus 1* but is not a member of that species since they share less than 90% amino acid identities in two of seven conserved domains of the replicase gene used for coronavirus demarcation.³¹⁰ Additionally, the lengths of five nonstructural proteins in ORF1 differ from those found in at least some members of *Betacoronavirus 1*. More important is the presence of cross-reacting antibodies against the RbCoV HKU14 and HCoV-OC43 N protein. These RbCoV HKU14 neutralizing antibodies are also present in significant numbers of healthy blood donors and SARS patients, perhaps resulting from prior infection with another human betacoronaviruses.³¹⁰ Unlike many other coronaviruses, except SARS-CoV, RbCoV HKU14 grows and produces a cytopathic effect in human cell cultures, including a human colorectal cell line. Variants of HCoV-OC43 from humans, BCoV in cattle, and MHV-H2 in mice can also replicate in these cells in vitro, indicating that at least some *Betacoronavirus* lineage A coronaviruses can infect the same tissues, at least under laboratory conditions.³¹⁰ The two species of RbCVs are compared in Table 5.4.

5.8.3 Other rabbit coronaviruses

Several other betacoronaviruses and alphacoronaviruses are also found in wild European rabbits. A study of the prevalence of these viruses in almost 300 wild rabbits found that 7.6% had coronaviruses in their intestines.²⁴⁸ Most of these viruses were very similar to other coronaviruses, including the betacoronavirus RbCoV HKU14 (94%–98% identity) and an alpha-CoV from hares (*Lepus* species) and rabbits from Spain (97%–98% identity). Other wild RbCVs are closely related to the KU739072 alphacoronavirus of rodents (96%–97%). Both rabbit beta- and alphacoronaviruses inhabit the same geographical location during the same year.²⁴⁸

5.9 Coronaviruses of other wild or semidomesticated mammals

Hedgehogs also host coronaviruses, including *Erinaceus* coronavirus HKU31, a member of the betacoronavirus lineage C, subgenus *Merbecovirus*. HKU31 was found in Western European hedgehogs in 2013.⁸³ It is a member of a clade that contains HKU4, HKU5, and *Nycteris* bat coronaviruses as well as MERS-CoV.⁸³ While HKU13 is currently not linked to disease, hedgehogs may serve as an asymptomatic reservoir of coronaviruses. Interestingly, hedgehogs are members of the order Eulipotyphla which also contains moles, solenodons, and shrews. This order is closely related to the order Chiroptera, to which bats belong. Bat and hedgehog coronaviruses have 78% nucleoside identity to MERS-CoV.^{83,311} They share 57.9%–58.2% identity in the S protein, but only 36.7% in the RBD. Higher levels of amino acid identity ranging from 71.9% to 79.4 are found in the E, M, and N proteins.⁸³

A study of fecal samples in hedgehogs in France found that the prevalence of RNA in hedgehogs in animal shelters was 50% and 58.9% in Germany.^{83,248} In a wide area of Great Britain and Wales, but not Scotland, 10.8% of hedgehogs' feces or distal large intestinal tract contents were positive for this coronavirus's RNA. The highest HKU13 concentration is found in the lower gastrointestinal tract, suggesting fecal-oral transmission.⁸³

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Coronaviruses of agricultural and companion animals with the potential for zoonotic transmission

6.1 Introduction

6.1.1 Coronavirus genera and species

Coronaviruses have been divided into four genera: *Alpha-*, *Beta-*, *Gamma-*, and *Deltacoronaviruses*. Almost all coronaviruses of mammals are members of the alpha- and betacoronaviruses genera, including agricultural and companion mammals. The exceptions to this division are the porcine deltacoronavirus (PDCoV) of pigs and a gammacoronavirus of beluga whales.¹ Alphacoronaviruses (ACoV) of domesticated animals belong to the *Pedacovirus*, *Rhinacovirus*, *Tegacovirus*, and *Duviacovirus* subgenera, while betacoronaviruses belong to the *Embecovirus* and *Merbecovirus* subgenera as shown in Table 6.1. These animal coronaviruses primarily infect and damage tissues and organs of either the respiratory or digestive system or both. Several coronaviruses of domesticated animals primarily infect the **central nervous system (CNS)**.

The nomenclature of coronavirus species is complicated due to the multitude and impact of mutations within a viral species in addition to **genetic recombination** within and between coronaviruses of different host species. The literature also is fraught with instances in which two viral “species” are grouped as a single species even if the members of this combined species infect the same or different hosts or target different organ systems. An example of this is bovine coronavirus (BCoV) which has been divided into viruses causing an **enteric** form of the disease that targets the intestines (bovine enteric coronavirus; BECV) and a respiratory form (bovine respiratory coronavirus; BRCV). Furthermore, BCoV is a member of the *Betacoronavirus-1* species. Members of *Betacoronavirus-1* use several different host species. Other members include equine coronavirus of horses, canine respiratory coronavirus of dogs, porcine hemagglutinating (HI) encephalomyelitis virus of pigs, and HCoV-OC43 in humans.² Some of these viruses cause only mild illness in healthy animals, while others cause severe to fatal disease. Most

TABLE 6.1 Alpha- and beta-coronaviruses of agricultural and companion animals.

Animal species	Virus	α - or β -CoV	Subgenus	Affected system
Cattle	Bovine-CoV (Bovine enteric and bovine respiratory coronaviruses)	β	<i>Embecovirus</i>	Digestive respiratory
Pigs	Porcine epidemic diarrhea virus (PEDV)	α	<i>Pedacovirus</i>	Digestive
	Porcine hemagglutinating encephalomyelitis virus (PHEV)	β	<i>Embecovirus</i>	CNS
	Porcine deltacoronavirus (PDCoV)	δ	<i>Buldecovirus</i>	Digestive
	Swine acute diarrhea syndrome coronavirus (SADS-CoV)	α	<i>Rhinacovirus</i>	Digestive
	Transmissible gastroenteritis virus (TGEV) ^a	α	<i>Tegacovirus</i>	Digestive
	Porcine respiratory coronavirus (PRCV) ^a	α	<i>Tegacovirus</i>	Respiratory
Horses	Equine coronavirus (ECoV)	β	<i>Embecovirus</i>	Digestive
Dromedary Camels	Dromedary camel Alpha-CoV	α	<i>Duviacovirus</i>	Respiratory
	DcCoV-HKU23	β	<i>Embecovirus</i>	Digestive
	Middle East respiratory syndrome coronavirus (MERS-CoV)	β	<i>Merbecovirus</i>	Respiratory
Alpacas	MERS-CoV	β	<i>Merbecovirus</i>	Respiratory
	Alpaca enteric-CoV	β	<i>Embecovirus</i>	Digestive
	Alpaca alpha-CoV	α	<i>Duviacovirus</i>	Respiratory
Cats	Feline enteric coronavirus (FECV) ^b	α	<i>Tegacovirus</i>	Digestive
	Feline infectious peritonitis virus (FIPV) ^b	α	<i>Tegacovirus</i>	CNS
Dogs	Canine coronavirus (CCoV) Genotypes I and II	α	<i>Tegacovirus</i>	Digestive Systemic
	Canine respiratory coronavirus (CRCoV)	β	<i>Embecovirus</i>	Respiratory

^aTGEV and PRCV are members of the same species.

^bFECV and FIPV are members of the same species.

Notes: This table compares characteristics of coronaviruses of cattle, pigs, horses, camelids (dromedaries camels and alpacas), cats, and dogs. Almost all of these viruses belong to the α - or β -coronavirus groups, except for bovine deltacoronavirus. This is noteworthy since members of γ - and δ - coronaviruses almost always infect birds. Also, several of the listed coronaviruses are members of the same species that infect different tissues or differ in disease severity. This Table also shows that most of the coronaviruses of domesticated animals cause respiratory or digestive disease, but several of the viruses infect and damage the central nervous system.

members of *Betacoronavirus-1* infect the respiratory or digestive tract while the porcine HI encephalomyelitis virus infects the CNS.

ACoV-1 is a species that includes feline coronaviruses of cats, canine coronavirus of dogs, transmissible gastroenteritis virus (TGEV), and porcine respiratory coronavirus

(PRCV) of pigs.³ Additionally, feline coronaviruses have been divided into two biotypes. Feline enteric coronavirus is ubiquitous in multi-cat environments and causes mild digestive illness. Feline infectious peritonitis virus (FIPV) arises from mutations of the enteric form of feline coronavirus and causes severe, life-threatening disease of the CNS of cats. Both feline and canine coronaviruses are divided into genotypes I and II. Some of these coronaviruses are produced by genetic recombination and can infect either cats or dogs.

Gamma- and deltacoronaviruses primarily infect bird species. Since members of these latter two coronavirus genera are rarely found in mammals, these viruses appear to pose little risk of **zoonotic transmission** and will only be briefly described at the end of this chapter.

6.1.2 Severe acute respiratory syndrome coronaviruses, severe acute respiratory syndrome coronaviruses-2, and domesticated animals

Evidence suggests that several “human” coronaviruses are also present in our agricultural and companion animals. These coronaviruses include those causing either mild or severe disease in humans. Even though SARS-CoV and SARS-CoV-2 originated in bats (**reservoir hosts**), they infect wild animals sold in live animal markets in China. From these **intermediate hosts**, SARS-CoV and SARS-CoV-2 underwent zoonotic transmission that resulted in a human epidemic and a pandemic, respectively.

The spike (S) protein of many coronaviruses uses the **angiotensin-converting enzyme-2 (ACE2)** as its host cell receptor during viral entry into its target cells. In addition to humans, the SARS-CoV-2 S protein’s **receptor-binding domain (RBD)** can bind ACE2s from a wide variety of domesticated animals, including cats, pigs, cattle, goats, and sheep in vitro.^{4,5} Cats, especially, are highly susceptible to infection by SARS-CoV-2 and most become ill.^{5,6} Infected domestic cats also shed SARS-CoV and transmit the viable virus to other cats⁷ and possibly to humans. By contrast, experimentally infected pigs, dogs, chickens, and ducks are resistant to this virus in vivo.^{8–10} Since SARS-CoV and SARS-CoV-2 infect agricultural and companion animals, domestic animals should be monitored for the potential to act as a reservoir or intermediate host for zoonotic transmission. Additional information about SARS-CoV and SARS-CoV-2 in animals may be found in Chapters 2 and 4 of this book.

6.1.3 MERS-CoV and domesticated animals

Bats are typically considered to be the reservoir hosts of Middle East respiratory syndrome coronavirus (MERS-CoV) that transmit the virus to dromedary camels (intermediate hosts), primarily in the Middle East. [Fig. 6.1](#) depicts testing of a dromedary for MERS-CoV.

MERS-CoV is believed to have then undergone multiple zoonotic transmission events, resulting in severe disease in humans. One study reported that other domestic animals, including sheep in North Africa (Senegal, Tunisia, and Egypt) and Egyptian goats and donkeys, are also infected by MERS-CoV, suggesting that placing noncamelid livestock in prolonged close proximity to infected dromedaries may result in interspecies transmission



FIGURE 6.1 This photograph shows collection of blood from a dromedary camel from Yemen being tested for anti-MERS antibodies. Image number 19622 Content provider: CDC/Awadh Mohammed Ba Saleh.

to other agricultural animals.¹¹ It is noteworthy that in this study, all genetic sequences of MERS-CoV from the latter animals have the same specific mutation in the RBD of the S protein gene.¹¹ While MERS-CoV has undergone multiple zoonotic transmission events from dromedaries, this virus does not appear to jump the species barrier from sheep, goats, or donkeys to humans.

A 2019 study in Ghana in western Africa did not find any viral RNA or antibodies against either MERS-CoV or a *Nycteris* species bat coronavirus in cattle, sheep, goats, donkeys, or swine despite the significant amount of contact that occurs among livestock and bats in this part of Africa.¹² A previous study conducted from 2009 to 2011, however, reported that 24.9% of *Nycteris* bats in Ghana were infected with the bat coronavirus.¹³ Several other, smaller studies had not detected MERS-CoV in sheep, goats, or cattle from Europe, Northern Africa, or the Middle East. These livestock are, therefore, unlikely to serve as hosts for zoonotic transmission of MERS-CoV.¹³ Additionally, following intranasal infection of MERS-CoV into llamas, pigs, sheep, and horses, MERS-CoV was found in the nasal cavities of pigs and llamas, but not in sheep or horses,¹⁴ suggesting that llamas and pigs could perhaps serve as reservoir hosts for the zoonotic transfer of MERS-CoV. Additional information about MERS-CoV in animals may be found in Chapter 3.

6.1.4 Diagnosis of coronaviruses of domesticated animals

Serological diagnosis, as well as molecular analysis, are used to determine infection by various viruses, including most coronaviruses. Serological testing uses **enzyme-linked immunosorbent analysis** to detect specific antiviral antibodies and molecular tests use the **polymerase chain reaction** to detect specific viral RNA.¹⁵

6.2 Bovine coronavirus and its enteric and respiratory forms

6.2.1 Introduction to bovine coronaviruses

BCoV is a **pneumoenteric virus** member of the *Betacoronavirus-1* species, subgenus *Embecovirus*. HCoV-OC43 is believed to have resulted from the zoonotic transmission of BCoV¹⁵ following a 290-nucleoside deletion in the genomic RNA downstream of the S protein gene.¹⁶ More than 90% of adult cattle have anti-BCoV antibodies, indicating infection or exposure to the virus.

BCoV enters its hosts via the respiratory system and then spreads to the gastrointestinal tract.¹⁷ These viruses are found in the respiratory and intestinal tracts of both healthy and diseased cattle.¹⁸ Accordingly, BCoV has been divided into two groups, **BECV** and **BRCV**. BECV and BRCV tend to genetically group according to their geographical region rather than their clinical manifestations. The genomic RNAs of BECV and BRCV are very similar,¹⁹ yet these viral groups infect different organ systems and cause different disease manifestations. The nucleocapsid (N) proteins of BECV and BRCV are antigenically and genetically similar. This suggests that they are members of a single viral **serotype** with 2–3 subtypes.^{20,21} Examination of the S protein, however, indicates that it differs significantly among isolates. It should be noted that sequencing of the genes for the S1 portion of the S protein, hemagglutinin esterase (HE), and the **open reading frames** (ORFs) 4 and 5 indicates that neither insertions nor deletions are responsible for the differences in BECV's and BRCV's tropisms,²² even though the S protein is the driving factor of coronaviruses' cellular tropism.

BECV infects and causes disease in the small and large intestines of both dairy and beef cattle before being shed in feces. BRCV infects and causes disease in the upper and lower respiratory tracts before being shed in upper respiratory tract secretions. This dual organ system tropism is also seen in SARS-CoV and SARS-CoV-2, both of which infect the respiratory system and the intestines and are released via respiratory secretions and in stools.^{20,23} Even asymptomatic carrier animals release BECV in their feces, especially in autumn and winter as well as during **parturition** (the birthing process). The cows act as viral reservoirs and infect their **neonatal** calves. BCoV is shed in the feces of up to 70% of adult cows, even by those with anti-BCoV antibodies in both their feces and serum (Carman 1992).²⁴ While the major routes of transmission appear to be from cow to calf or between calves, infected dogs may serve as viral carriers as well.²⁵

BCoV is found in cattle farms on all inhabited continents. BCoV has been classified into European and American lineages with periodic introductions of the North American lineage into Asian countries, including its entry into Japan during the 1990s.²⁶ The European and American lineages often differ in the S protein gene. The continuing evolution of current BCoV strains may result from genetic recombination events. Interestingly, there is a larger genetic difference between older and newer BCoV isolates than among isolates expressing different clinical syndromes.¹⁷

BCoVs and bovine-like coronaviruses are also present in domestic and wild ruminants, including water buffaloes, sheep, goats, dromedary camels, llamas, alpacas, deer, antelopes, giraffes, and wild cattle and goats, as well as dogs and humans.²⁷ In the latter case, a child presenting with acute diarrhea was found to be infected with a coronavirus that

was genetically and antigenically similar to BCoV.²⁸ Ancestral forms of BCoV may have given rise to HCoV-OC43 and porcine HI encephalomyelitis virus in the past.²⁹

Disease outbreaks usually occur during autumn and winter when the cattle are housed in close proximity. BCoV survives in cool, moist environments and is more stable at both low temperatures and in the presence of low levels of ultraviolet (UV) light. During the winter, cattle are less exposed to UV-containing sunlight than cattle that are kept in a pasture during other times of the year. Taken together, these factors may be at least partially responsible for BCoV being most frequently detected in winter.^{30,31} Nevertheless, this is not universally true since winter dysentery, an important BCoV-associated disease also occurs in warmer seasons, such as the summer in Korea, and tropical countries, such as Thailand, Brazil, and Cuba.

The **morbidity** rate in BCoV-infected adult cattle may be high, causing an economic loss through decreases in milk production on dairy farms and meat production on beef farms.² Treatment of infected calves may include substituting a milk diet with a diet that replaces fluids and electrolytes to prevent dehydration.³⁰ BCoV infection of calves may become chronic, with the disease reoccurring in adult cattle.³² Agricultural animals are extremely important for food and as a source of income in many developing and developed countries. They provide meat and milk as well as serving as a means of transportation, draught power, fuel, and clothing. In addition to cattle, a 2020 study reported that anti-BCoV antibodies were also found in 25.8% of the tested sheep, 43.1% of goats, and 55.8% of cattle in rural Ghana.³³ Infection was much higher in cattle from large vs small farms in this report (82.2% vs 17.8%, respectively). An Iranian study of BCoV detected a virus in 28.5% of calves that were less than a week old, while the virus was detected in 71.4% of older calves.³⁴ All Iranian strains are independent clusters and do not belong to the clusters present in other parts of the world.

6.2.2 Pathology of bovine coronavirus diseases and their underlying causes

BCoV causes three types of disease in cattle as depicted in [Table 6.2](#). **Calf diarrhea** in young animals and **winter dysentery** with hemorrhagic diarrhea in adult cattle are caused

TABLE 6.2 Comparison of calf diarrhea, winter dysentery, and shipping fever.

Disease name	Virus	Animal age	Disease description
Calf diarrhea	Bovine enteric coronavirus	Calf	Severe malabsorptive diarrhea Dehydration Hypothermia Metabolic acidosis Hypoglycemia
Winter dysentery	Bovine enteric coronavirus	Adult	Hemorrhagic diarrhea
Shipping fever	Bovine respiratory coronavirus	Calves Young adults	Fever Coughing and runny nose Difficulty breathing Bronchopneumonia Bloody diarrhea Weight loss

Notes: This table compares the diseases caused by bovine enteric coronavirus and bovine respiratory coronavirus. The former causes enteric disease in calves and adult cattle, while the latter produces respiratory disease in younger animals.

by BECV, while BRCV is responsible for respiratory infections, particularly **shipping fever** of feedlot cattle. These conditions will be discussed separately later in the chapter.

BECV and BRCV isolates may be simultaneously present in an individual animal.^{19,35} In one study, BCoV isolates from the respiratory tract and the intestine of a single cow with winter dysentery were classified as members of different BCoV antigenic groups based upon differing antibody reactivity to their respective S proteins.³⁶ Numerous genetic differences are present among isolates of the S protein gene from BECV and BRCV, including several deletions and point mutations in this gene. Genetic differences are also present within BCoV ORFs in an area of genomic RNA located between the S and E genes in cattle and bovine-like coronaviruses isolated from wild ruminants and humans.²⁷

ORF5 and ORF6 have the highest degree of genetic variation, although a high number of variations are also present in the genes for the S, N, and HE proteins. The composition of the S1 region of the S protein gene is variable and mutations may lead to differences in pathogenicity. The S2 domain, however, has little variance among the various BCoV strains.³⁷ Interestingly, the S1 domain found in a group of Brazilian BCoV strains contains a deletion of 6 amino acids. These deletions are also present in HCoV-OC43, suggesting the possibility of zoonotic transmission, especially since the deletion is absent from other species of human coronaviruses.³⁸ Short deletions are also present in the N protein gene from different BCoV strains. However, genetic differences do not appear to result in the separation of BECV and BRCV groups or BCoV strains in the upper and lower respiratory tracts. The differing clinical diseases associated with BCoV infections may result from a variety of other interacting factors, including stress, temperature, overall host health, immune responses during the period of infection, an infectious dose of the virus, and route of inoculation (fecal-oral or aerosolized respiratory secretions).^{37,39} Interactions among these factors may have a major role in determining the clinical manifestation and outcome, rather than the disease being solely dependent upon genetic differences among BCoV groups.³⁷

6.2.3 Bovine coronaviruses—the viruses

6.2.3.1 *The location and shedding of bovine coronaviruses*

The major sites of BRCV and BECV infection are the **epithelial cells** in the respiratory tract or **enterocytes** in the distal small intestine and colon, respectively. Initial replication of BCoV in the respiratory tract is followed by the movement of some viruses to the intestines after the animal has swallowed virus-containing mucus.³⁴ Generalized depression and a dry, barking cough correlate better with the peak period of shedding of BRCV and BECV RNA than with peak respiratory rate and peak rectal temperature, respectively, which appear more than a week later.⁴⁰

Most infected calves have BCoV in epithelial cells lining both the intestines and upper respiratory tract. Fecal shedding occurs after the onset of nasal shedding in animals experimentally infected via the intranasal route, whereas in calves infected orally, BCoV is detected first in fecal material and later, in nasal secretions. Nasal and rectal shedding of the virus often occurs concurrently as well.⁴¹ This suggests that the route of infection may play a major role in the sequence of infection of the respiratory and intestinal tracts.

Although viral RNA is not detected in the blood of infected calves six weeks after infection, it is still detected in intestinal and lymphatic tissue.⁴⁰ Importantly, BCoV is not transmitted by exposure of naïve calves to calves infected three weeks previously even though the latter calves are still shedding BCoV RNA. This implies that prolonged shedding of BCoV RNA may not indicate a potential for transmission to other calves.⁴⁰

6.2.3.2 The spike and hemagglutinin esterase proteins of bovine coronaviruses

There were 45–56 nucleoside differences between the total genomic RNA of virulent and avirulent BCoV strains. Nine amino acid substitutions appear to correlate with the severity of the ensuing disease. It should be noted that the S protein gene sequences of virulent and avirulent strains have greater than 98% nucleoside identity.⁴² This is important since the S protein is the primary viral molecule that determines host species and cellular tropism. The primary host cell receptor for the BCoV S protein is **N-acetyl-9-O-acetylneuraminic acid**. Both isolated BCoV S protein and virions **agglutinate** mouse, rat, and adult chicken red blood cells by binding to this molecule.³⁰ The S protein also mediates cell-to-cell fusion, helping to spread the virus between cells.

In addition to genes that are common to all coronaviruses, BCoVs contain the HE enzyme that binds to **sialic acids** found on the end of viral glycoproteins. This enzyme is required for **hemagglutination** (formation of large clumps) of red blood cells and **hemadsorption**, the process by which red blood cells are adsorbed to the plasma membranes of infected cells.³⁰ HE acts as a secondary viral receptor during the process of BCoV binding to host cells. HE also contains a receptor-destroying cleavage enzyme that releases 9-O-acetyl sialic acids from glycoproteins, thus inactivating the receptor for red blood cells.⁴³ The BCoV HE aids in shifting host specificity and tissue tropism.⁴⁴ HE from BCoV and other similar coronaviruses appears to have been obtained from a relatively recent genetic recombination with influenza C virus.⁴³

6.2.3.3 Bovine coronaviruses quasispecies and genetic recombination

BCoV and other coronaviruses are composed of intra-host and intra-isolate **quasispecies**⁴¹ that might have adapted to survive and replicate in specific tissues or organs within the host. The quasispecies also may have resulted from in vitro tissue culture adaptation if the viruses were grown for numerous passages in tissue culture cell lines derived from different organs or host species. Quasispecies may select for viral virulence and production of new viral species or subspecies. The production of quasispecies may be driven by genetic recombination. SARS-CoV appears to also exist as quasispecies within individual patients.⁴⁵

In a 2017 BCoV outbreak in Liaoning Province, the northern-most province of coastal China 2018, diarrhea from calves under the age of 3 months all contained a BCoV recombination variant.⁴⁶ Some of these variants also contain a 12-nucleoside insertion in their recombinant HE gene.⁴⁷ Recombination also occurs in the genes for the M and N protein as well as ORF1.⁴⁶

6.2.3.4 The immune response against bovine coronaviruses and vaccines

CD8⁺ T killer cells and natural killer (NK) cells are the major, longest-lasting, and most powerful immune system components against viral infections. **Neutralizing antibodies**

also play a role in defense against viral diseases.⁴⁸ Both the viral S protein and HE induce the formation of anti-BCoV neutralizing antibodies.^{20,30} The IgA class of antibodies protects the entry of enteric or respiratory pathogens through mucus membrane-covered surfaces. The IgG class of antibodies primarily protects against blood-borne infectious diseases. Functional T cells' responses are also needed to confer significant protection against these viruses. Various types of **interferons (IFN)** are produced by activated T cells and NK cells. One of the functions of BCoV **nonstructural proteins (nsp's)** is to inhibit IFN responses. Differing degrees of immunosuppression may contribute to differences in virulence seen in various BCoV isolates.⁴⁹

Effective vaccines against BCoVs are currently licensed and produce virus-specific neutralizing and **HI** antibodies.¹⁷ Since disease occurs in calves within a few days after birth, vaccines are usually given to cows before calving, thus protecting the newborn calves by passive immunity via antibodies from the mother's **colostrum** and milk. Alternatively, calves may be administered an **attenuated**, live vaccine by the intranasal route at or slightly after 1 day of age. This vaccine induces an immediate innate immune response that produces type I IFNs which protect the young calves against both calf diarrhea and winter dysentery.⁴⁸

6.2.4 Bovine enteric coronavirus

BECV is present in both dairy and beef cattle and causes calf diarrhea in young animals and winter dysentery in older cattle. Symptoms of these diseases include dehydration, metabolic acidosis, and electrolyte imbalance caused by the loss of sodium, chloride, potassium, and bicarbonate ions.⁵⁰ Calves inoculated orally with this type of BCoV develop loose or diarrhetic stools a little more than a week postinfection. Viral RNA is detected intermittently from plasma, nasal discharge, and feces for 1085, 700, and 280 days, respectively.⁵¹

BCoV replication occurs in the epithelial cells lining the small intestine and colon, particularly in regions containing **villi** in the distal small intestine. Infection of these intestinal cells results in loss of intestinal villi and **crypt hyperplasia** (changes in the shape of the villi) that decrease absorption of food. The infected cells in the small intestine die and are replaced by immature cells with less surface volume, leading to stunting and fusion of adjacent villi and decreasing nutrient uptake. In the large intestine, the **colonic ridges** undergo atrophy. The tall, elongated **columnar epithelial cells** normally lining the small intestinal villi and colonic ridges are replaced by **cuboidal epithelial** and **squamous epithelial** cells. These immature cells are flatter and have less surface area for absorption of nutrients and fluids than the mature columnar cells, resulting in **malabsorptive diarrhea**.³⁰ The immature cells also produce lower levels of digestive enzymes. Malabsorptive diarrhea leads to dehydration, acidosis, high levels of blood potassium, and low levels of glucose that may result in circulatory failure and death.²⁰

6.2.4.1 Calf diarrhea

BECV may be detected in both healthy and sick calves. In sick animals, calf diarrhea is usually a self-limiting illness, but it is associated with a high morbidity rate. Calf age is

one of the factors that influence the degree of dehydration and risk of severe disease.²⁰ Infected calves develop severe, malabsorptive diarrhea that may last for 8 days. The yellowish fluid diarrhea contains mucus and blood clots.²⁰ The majority of the calves recover and are protected from BCoV-associated diarrhea after subsequent exposure to the same or different strains of BCoV although a later group of calves may develop subclinical BCoV infection.⁵² Calf diarrhea is typically found in the winter, perhaps due to enhanced viral survival in the cold.²⁰

6.2.4.2 Winter dysentery

Winter dysentery in adult dairy cows leads to a great reduction in milk production, resulting in significant economic losses.²⁷ Continued feeding of infected cattle may lead to excessive levels of nutrients in the intestine than cannot be absorbed due to damage to the small intestine's epithelial cells. These excess nutrients undergo fermentation in the large intestine. Other alterations in the large intestine include fluid accumulation, increased levels of bacteria, and production of organic acids, which worsens diarrhea.²⁷

6.2.4.3 Colostrum and prevention of bovine enteric coronavirus

Newborn calves are protected against disease for as long as maternal IgA antibodies are present in the calves' intestines. The maternal antibodies in the colostrum are secreted by the mammary glands soon after birth. This may help to explain why calves often develop diarrhea between 1 and 2 weeks of age when the secretion of colostrum is replaced by that of milk.^{20,34} In Uganda, BCoV frequency in calves born to vaccinated cows is 3.3%, much less than the 12.2% frequency detected in calves born to unvaccinated cows.³¹

6.2.5 Bovine respiratory coronavirus

6.2.5.1 Introduction to bovine respiratory coronavirus

BRCV is present in the nasal and pharyngeal regions and the lungs of infected calves. It may additionally be shed in feces. Virus RNA is found in nasal discharges for over 900 days postinfection and for over 1000 days in feces.⁵¹ The presence of BRCV in these nasal discharges and feces, however, does not necessarily mean that these viruses are infectious. The virus is also found in low levels in **ocular** secretions of BRCV as is also the case for patients with COVID-19.^{20,53} The main transmission route of BRCV, however, is via inhalation or ingestion of the virus from nasal discharges into the nasal cavity or mouth.¹⁸ Of note: BRCV antigens are not detected in feces of healthy adult cattle and vertical infection from cow to calf has not been reported.⁵⁴ The release of immunosuppressive stress hormones, such as corticosteroids, may increase the severity of the disease.¹⁷ This should be kept in mind when designing treatment regimens, some of which contain immunosuppressants to control excessive inflammation. This type of antiinflammatory and immunosuppressive treatment is currently being used to treat COVID-19⁵⁵ and will be described in detail in Chapter 4.

BRCVs, like other coronaviruses, evolve fairly rapidly. Older and newer isolates of BRCV differ substantially, even to a greater extent than that found between BRCV and contemporary BECV strains.^{17,41} Moreover, the same clinical symptoms are produced in

calves experimentally infected with BCoV strains isolated from animals with either the enteric or respiratory forms of BCoV.⁵³

6.2.5.2 Pathology due to bovine respiratory coronavirus

BRCV may cause subclinical respiratory tract infections in calves, particularly in cells lining the nasal cavity and trachea. Infection may also cause mild upper respiratory signs, including runny nose, sneezing, and coughing. BRCV may also infect cells in the lower respiratory tract. Infection in the lower respiratory tract is typically asymptomatic and causes only minor lung lesions. Nevertheless, severe lower respiratory tract disease has been reported,⁵⁶ which may arise from BRCV increasing the cattle's risk of acquiring secondary bacterial infection in this location.³⁰ Calves that are coinfecting by respiratory system bacteria may be more susceptible to severe disease and death than those animals infected with either pathogen alone. Coinfection of calves with BRCV and the bacteria *Mannheimia hemolytica*, *Pasteurella multocida*, or *Mycoplasma* species may develop fatal pneumonia. **Necrotizing lobar pneumonia** may involve 50%–80% of the lung volume.¹⁷ **Lipopolysaccharide** on the surface of **Gram-negative bacteria** stimulates an intense inflammatory reaction in the lungs which not only kills bacteria but may also result in extensive lung damage when the inflammation is excessive.¹⁷

Shipping fever may result from infection with BRCV. This is a severe multifactorial disease with many interacting underlying causes, including BCoV and/or bovine respiratory syncytial virus, parainfluenza-3 virus, and bovine herpesvirus. Calves 2–6-month-old develop fever, cough, runny nose, pneumonia, and bloody diarrhea. Introduction of calves and young adult feedlot cattle 6–10 months of age into open feedlots containing animals from multiple farms may additionally lead to difficulty breathing due to severe inflammatory lung disease and may result in bronchopneumonia, weight loss, and death.²⁰

BRCV-induced lung lesions are consistent with mild-to-moderate interstitial pneumonia. Immunohistochemistry confirms the presence of BRCV antigen in the affected area of the lung. Dual infection with bovine viral diarrhea virus (BVDV) and BRCV increases BRCV-induced lung pathogenesis compared to calves infected solely with BRCV. The timing between infections with these two viruses is critical to the severity of lesions.⁵⁷ Also, while BVDV is not typically found in nasal swabs of infected calves, during dual infection with BRCV, BVDV is also isolated from nasal swabs.

6.2.5.3 The immune response to bovine respiratory coronavirus

Antibodies play an important role in the severity of BRCV-associated lung disease. Cattle with low levels of antibodies become ill and shed BRCV via the respiratory route, whereas cattle with high levels of antibodies against the HE and S viral proteins do not develop respiratory BRCV illness. Serum levels of neutralizing and **hemagglutinin-inhibition (HI) antibodies** negatively correlate with severe respiratory disease. Moreover, BRCV IgM, IgG1, and IgG2 antibodies significantly correlate with the levels of neutralizing and HI antibodies. In cattle with fatal respiratory BRCV infections, only IgM antibody responses are detected.¹⁷

Levels of HI and neutralization antibody titers increase for the first several days after disease onset.⁵¹ The presence of anti-BRCV IgG1, IgG2, IgA, and neutralizing antibodies in

the serum of naturally infected calves or cattle arriving in feedlots is associated with protection against developing the respiratory disease, including pneumonia, and BCoV shedding via the respiratory route. This indicates that these classes of antibodies have a protective role against developing disease or transmitting this coronavirus.^{20,58} Antibodies from acutely infected calves may persist for long periods of time, perhaps even years.⁴⁰

Calves previously infected with BRCV retain their susceptibility to infection with a different viral strain as characterized by transient increases in serum anti-BRCV antibodies together with intermittent nasal shedding. Nevertheless, these reinfected calves do not become ill.⁵² Similar findings of asymptomatic reinfection have been reported during MERS-CoV infection in dromedary camels⁵⁹ and humans infected by the common cold coronaviruses.⁶⁰ The antibody arm of the adaptive mucosal immune response in the upper respiratory tract of BRCV-infected calves is not long-lived,²⁰ but the more effective CD8⁺ T killer and NK cells may be.

During the first 18 hours of infection, there is no evidence of an innate mucosal immune response as evidenced by the lack of expression of IFNs, cytokines, and **Toll-like receptor (TLR)** genes in newborn calves.³² TLRs serve as early warning systems that detect microbial infection by recognizing features that are unique to that group of organisms. By contrast, bovine rotavirus activates TLR3 and IL-6 genes in infected host tissues early after infection.³² The lack of an early innate immune response may allow BRCV to establish a foothold in mucosal tissues. Nevertheless, during later stages of infection, some elements of the adaptive immune responses, such as CD8⁺ T killer cells activity, together with NK cells, may be able to partially protect the calves against severe disease.

When uninfected calves are brought into contact with infected calves that are actively shedding BRCV, all introduced healthy calves become infected. They develop a mild respiratory disease and shed BRCV RNA in their nasal secretions through day 28 and in their feces through day 35. Nevertheless, no infectious virus could be isolated from their nasal swabs after 13 days and they are unable to transmit the infection to naïve calves on day 21. This strongly suggests that prolonged shedding of viral RNA via the nasal and fecal routes might not accurately indicate viral infectivity.^{20,40} BRCV does not induce proinflammatory responses in calf intestines and transcription of the proinflammatory IL-6 and TNF- α genes are downregulated following infection.³²

6.2.5.4 Prevention of bovine respiratory coronavirus infection

Since there is only one serotype of BRCV, a single broadly cross-reactive strain of BRCV may suffice for a vaccine. Alternatively, because of variations among field strains, including strains from animals displaying different clinical syndromes, a single broad-spectrum vaccine composed of respiratory BRCV and enteric BECV isolates may cross-protect strains associated with different clinical syndromes.¹⁷ However, vaccines against mucosal pathogens, including not only BRCV but also SARS-CoV, SARS-CoV-2, and MERS-CoV, often do not completely halt infection nor do they always prevent reinfection. It has been suggested that the major function of BRCV and BECV vaccines should be to prevent severe disease, weight loss, and reduced milk production.¹⁷

6.2.6 Bovine coronaviruses-like coronaviruses of other animals

BCoV-like coronaviruses are betacoronaviruses of the subgenus *Embecovirus* that infect sheep, goats, alpacas, and llamas. They cause enteritis and neonatal mortality in domestic sheep worldwide.⁶¹ In Sweden, 19% of sheep flocks have antibodies to BCoV, especially in animals at least four years old, present in large flocks containing at least 100 sheep, and those in contact with cattle.⁶² Similarly, studies of sheep in Germany found that 16%–22% were positive. In addition to contact with infected cattle feces, sheep-to-sheep transmission may also occur.

A 2018 study found that in South Korea 1%–3% of the goats had anticoronavirus antibodies.⁶¹ The BCoV-like coronaviruses may be BCoV variants that have adapted to different ruminant hosts or different species of coronaviruses. This group of viruses causes gastroenteritis and/or respiratory disease in at least some of these ruminants.²⁰ Less than 10% of the goats from Ghana had diarrhea, respiratory distress, and fever.³³

Other agricultural animals are also infected by BCoV or one of its close relatives, demonstrating interspecies transmission. BCoV relatives include swine HI encephalomyelitis virus in pigs, canine respiratory coronavirus in dogs, and the mildly pathogenic HCoV-OC43 and HCoV-HKU1 in humans. Additionally, the HI intestinal human coronavirus, HCoV-44, was isolated from a child with acute diarrhea.²⁷ It is very closely related to BCoV since it has 99% nucleoside identity in the genes for the S protein and HE.²¹ This viral strain also infects the upper respiratory tract and intestines of germ-free calves, resulting in diarrhea and intestinal lesions.⁶³

Following experimental inoculation, BECV can also infect species that are as divergent as turkeys and dogs. Young pups inoculated intranasally with BCoV produce antibodies to the virus and infect other pups. Infected pups do not, however, develop a respiratory illness or diarrhea⁶⁴ as further described below. In nature, one may wonder whether pathogenic coronaviruses of dogs, wild birds, cattle, or other ruminants are transmitted between these animals and perhaps lead to zoonotic transmission. Feedlots are ideal grounds for this type of interspecies transmission since in such areas, several different species of animals are brought into proximity to each other and humans.¹⁷

Genetic recombination reveals a close relationship among the dromedary DcCoV-HKU23/362F and DcCoV-HKU23/CAC1019, rabbit HKU14, rodent coronavirus M2014, HCoV-OC43 and BCoV. Additionally, two rodent coronaviruses, distinct from other rodent coronaviruses, appear to have arisen from a common ancestor of rodent and BCoVs in France.²⁷ Similarly, viral RNA from a pig coronavirus and HCoV-OC43 appear to have evolved from ancestral BCoV.¹⁶ Adaptation to a new host species may have also occurred between another pig coronavirus responsible for swine acute diarrhea syndrome and a bat virus, with a possibility to further adapt to humans.

In addition to cattle and sheep, BCoV or BCoV-like coronaviruses infect many domestic or wild ruminants in North and South America, the Caribbean, the Middle East, Eastern Asia (China, South Korea, and Vietnam), and Africa. The RNA of BCoV infecting white-tailed deer (*Odocoileus virginianus*), sambar deer (*Rusa unicolor*), giraffes (*Giraffa camelopardalis*), waterbucks (*Kobus ellipsiprymnus*), sable antelopes (*Hippotragus niger*), and wild sheep (*Ovis* species) have 99.3%–99.6% amino acid identity with both enteric and respiratory BCoV.¹⁷ BCoV RNA was also detected in 69.1% of fecal samples from

diarrhetic yaks (*Bos grunniens*) from all 29 of the tested farms in the high-altitude Qinghai-Tibet Plateau in southwestern China.⁶⁵ This strain of BCoV contains unique amino acid differences in its HE gene when compared to more than 100 known BCoV HE genes.

Some coronaviruses of cattle and domestic camelids are identical or closely related to human coronaviruses, including MERS-CoV, or coronaviruses infecting other agricultural animals. Some coronaviruses are important pathogens in captive or wild ruminants. Bovine-like coronaviruses have been isolated from captive giraffes, sambar deer, white-tailed deer, waterbuck, and elk (*Cervus* species) as well as from ruminants in the wild, including mule deer (*Odocoileus hemionus*), sika deer (*Cervus nippon*), water deer (*Hydropotes inermis*), and caribou (*Rangifer tarandus*). Some of these animals had mild to severe diarrhea.^{61,66,67} In very young elk, infection with a bovine-like coronavirus is often linked to enteritis. Bovine-like coronavirus has also been reported in sambar deer during an outbreak of diarrhea in a wild animal park in the United States during the winter of 1993/1994. Infection in these deer was characterized by severe bloody diarrhea with a 30% mortality rate. This disease resembled winter dysentery in adult cattle. In 1994, another wild animal park in the same region reported watery diarrhea in white-tailed deer. Coronavirus particles were present in the fecal samples from sambar and white-tailed deer.⁶¹ BCoV-like coronaviruses were also detected in the feces of musk oxen (*Ovibos moschatus*) in the United Kingdom. Coronaviruses are common pathogens in calves and adult European bison (*Bison bonasus*) and cause severe diarrhea.⁶¹ BCoVs are also present in over half of the tested water buffaloes (*Bubalus bubalis*) of various ages in Bulgaria.⁶¹ A bovine-like coronavirus was also present in nasal samples in 2016 and 2017 from water deer in South Korea. Full genome sequencing revealed that the coronaviruses from water deer had greater than 98% nucleoside identity to BCoV despite causing respiratory, rather than enteric, disease.⁶⁸

Antelopes are a group of similar ruminants that are composed of waterbucks, sitatunga (*Tragelaphus spekii*), naylas (*Tragelaphus angasii*), and sable antelopes. An outbreak of watery diarrhea occurred in a herd of nine waterbucks in a wildlife park in the United Kingdom. Several of the infected animals died.⁶¹ Sitatunga from several zoos are susceptible to both BCoV-like coronavirus-induced enteric and respiratory symptoms, including outbreaks of diarrhea in two zoos in the United Kingdom and South Korea.^{69,70} Several naylas were also infected at that time in a zoo in the Republic of Korea.⁷⁰ Virus particles were present in rectal swabs from both antelope species.⁶¹

A sable antelope developed diarrhea in a wild animal park in the United States. The virus later spread to giraffes located 0.5 miles away from these antelopes. Coronavirus particles were also present in fecal samples of the sable antelopes. The coronavirus particles were closely related genetically to coronaviruses from the infected giraffes, BCoVs, and a lesser degree to coronaviruses from sable deer, white-tailed deer, and waterbucks.⁶¹ The concurrent outbreak of bovine-like-CoV in giraffes caused mild to severe diarrhea.⁶⁶ The isolated giraffe coronaviruses are very similar to not only viruses from sable antelopes but also to several respiratory and enteric BCoV strains. Nevertheless, one giraffe coronavirus S protein gene had a small deletion that is not present in the corresponding sable antelope gene. Several point mutations in viral structural proteins are shared in strains of both giraffe and BCoVs.⁶⁶ Possible transmission between cattle and giraffes is a matter of

concern since the grazing regions of these animals overlap and giraffes are vulnerable to extinction.⁶¹

The Himalayan tahr (*Hemitragus jemlahicus*) is a species of wild goat in Central Asia. During the 2010 BCoV outbreak in the zoo in South Korea mentioned previously, three Himalayan tahrs became weak and experienced depression, **anorexia**, bloody diarrhea, and dehydration. Their feces contained bovine-like coronaviruses as well.^{61,70}

6.3 Coronaviruses of dromedaries, llamas, and alpacas

As depicted in Table 6.3, camelids of Africa and the Middle East (1-humped dromedary camels), Asia (2-humped Bactrian camels), and Latin America (llamas and alpacas) are infected with several coronaviruses that cause mild to severe disease. MERS-CoV from dromedaries (*Camelus dromedarius*) also infects humans, often leading to severe to fatal outcomes. There is also evidence that suggests that Bactrian camels (*Camelus bactrianus*) may be infected with MERS-CoV or a similar coronavirus,⁷¹ but this is a matter of controversy that is discussed later in this chapter.

6.3.1 Coronaviruses of dromedary camels

Dromedary camels harbor at least three species of coronaviruses: dromedary camel ACoV, DcCoV-HKU23, and MERS-CoV.⁷²

TABLE 6.3 Comparison of coronaviruses of camels and alpacas.

Animal	Virus	Organ system	Symptoms
Dromedary	Dromedary camel alphacoronavirus (dromedary camel CoV-229E)	Respiratory	Usually asymptomatic
Dromedary	DcCoV-HKU23	Digestive	Diarrhea in calves
Bactrian			Gastroenteritis
Dromedary (Bactrians?)	Middle East respiratory syndrome coronavirus	Respiratory	Nasal discharge
			Inflamed trachea
			Bronchitis
Alpaca	Middle East respiratory syndrome coronavirus	Respiratory	Nasal discharge
			Inflamed trachea
			Bronchitis
Alpaca	Alpaca enteric coronavirus	Digestive	Severe to fatal diarrhea
Llama			
Alpaca	Alpaca alphacoronavirus	Respiratory	Respiratory distress
			High fever
			Severe pulmonary congestion
			Edema, pleural effusion
			Pneumonia

Notes: This table compares coronaviruses of Old World dromedary and Bactrian camels and New World alpacas. Both types of these camelids may be infected with MERS-CoV and cause only a mild respiratory disease. The two coronaviruses of alpacas, alpaca enteric coronavirus and alpaca alphacoronavirus, cause severe disease of the digestive or respiratory tract, respectively.

6.3.1.1 *Dromedary camel alphacoronavirus*

Dromedary camel ACoV (also known as dromedary camel CoV-229E; DCoV-229E) is an alphaCoV of the *Duvinacovirus* subgenome. It is very similar to an alphaCoV of humans, HCoV-229E, and to a coronavirus from alpacas in California^{73,74} Like HCoV-229E, it uses aminopeptidase N (APN) as its cellular receptor and may cause a respiratory illness in dromedary camels.

6.3.1.2 *Dromedary camel coronavirus DCoV-HKU23*

DCoV-HKU23 is a BCoV-like dromedary camel betacoronavirus of the *Embecovirus* subgenus that causes gastroenteritis.²⁰ It was isolated from dromedaries in Dubai in 2013 and is related to HCoV-OC43 in humans.⁷⁵ DCoV-HKU23 is found in the intestines of dromedaries in Sudan, the Middle East, and Pakistan and may cause diarrhea in calves. A viable and infectious virus isolated from camel feces produces a **cytopathic effect** in a human colon cancer cell line in vitro.⁷⁶ Antibodies against camel DCoV-HKU23 were found in various parts of Africa, including 92% of Nigerian dromedaries, 91% of those from Ethiopia, and 79% of those in Morocco. Immunization with the DCoV-HKU23 N protein produces negligible antigenic cross-reactivity to MERS. Since the fourteen strains of DCoV-HKU23 form a cluster that is distinct from those of other members of *Betacoronavirus-1*, including alpaca coronavirus, it is classified as an independent species.⁷⁶ Interestingly, the S protein of DCoV-HKU23 has a potential N-glycosylation site that is present in canine respiratory coronavirus and HCoV-OC43, but not in S proteins of wild ruminant coronaviruses.⁷⁶

Further examination of the genomic RNA of dromedary DCoV-HKU23 from Morocco in Northern Africa, Ethiopia in East Africa, and Nigeria in West Africa detected regions that suggest that genetic recombination had occurred among DCoV-HKU23 and similar betacoronaviruses of lineage A. One such recombination appears to have transpired between the genes for HE and the S protein from DCoV-HKU23 and the corresponding region of RNA of rabbit coronavirus (HKU14). Similar recombination may have occurred in the S protein gene of a rodent coronavirus. This type of gene exchange between camelid coronaviruses and coronaviruses from different species of mammals may have contributed to the emergence and continuing evolution of MERS-CoV.⁷⁷

6.3.1.3 *Middle East respiratory syndrome coronavirus in dromedaries*

MERS-CoV is a betacoronavirus of the *Merbecovirus* subgenus.²⁰ It has undergone multiple zoonotic transmission events and causes an often-deadly disease in humans. The cellular receptor of MERS-CoV, dipeptidyl peptidase 4 (DPP4), is confined to the upper respiratory tract epithelium of dromedaries and might potentially cause mild respiratory disease in these camels. In humans, however, DPP4 is found in the epithelium of the lower respiratory tract, which most likely explains why the virus causes more severe diseases in humans.⁷⁸ MERS-CoV has also been isolated from fecal samples of a naturally infected camel, which suggests MERS-CoV replicates in the intestinal tract of dromedaries as well as in the respiratory system.⁵⁹ The importance of dromedary camels as an intermediate host of MERS-CoV is further described in Chapter 3.

6.3.1.4 Middle East respiratory syndrome coronavirus and Bactrian camels

There are conflicting reports concerning whether MERS-CoV infects Bactrian camels and if so, the relevance of this to humans. A 2015 report examined the prevalence of MERS-CoV in Bactrian camels in the Umnogovi and Dundgovi Provinces of southern Mongolia, regions that contain a large portion of the camel population in the country. This report did not detect MERS-CoV RNA in any of the serum or nasal samples of 200 tested Bactrian camels, although BCoV RNA was detected.⁷⁹ A 2015 report of Bactrian camels in the West Inner Mongolia Autonomous Region of China also did not detect either anti-MERS-CoV antibodies or RNA.⁸⁰

Other evidence suggests that Bactrian camels may be infected with MERS-CoV experimentally and naturally. As mentioned previously, DPP4 serves as the cellular receptor for MERS-CoV. DPP4 from Bactrian camels is 98.3% identical to that of dromedary camels, and the region that binds to the viral S protein is 100% identical. Following experimental infection with MERS-CoV via the intranasal route, Bactrian camels have a transient upper respiratory tract infection and shed large amounts of the virus through nasal secretions.⁷¹ These results are similar to those found in dromedary camel infections. It is important to determine whether the virus that is being shed by Bactrian camels is infectious to either humans or other members of its species since this could greatly expand the range of human exposure to MERS-CoV.

In a 2020 report on the presence of MERS-CoV in Bactrian camels, captive camels from two locations were tested for anti-MERS-CoV antibodies. The first tested location contained both Bactrian and dromedary camels in a private collection in the United Arab Emirates, while the second tested location contained only Bactrian camels in a camel farm in Xinjiang, China. In the private collection, anti-MERS-CoV neutralizing antibodies were detected in 41% of 29 Bactrian camels.⁸¹ Seroprevalence was even higher in hybrid camels from this collection (82%). Hybrids are the offspring of a dromedary and a Bactrian camel. By contrast, all 92 Bactrian camel serum samples from the camel farm were negative for MERS-CoV-specific antibodies.⁸¹ These data suggest that high MERS-CoV seropositivity may be found in Bactrian camels in environments containing both camel species. In the absence of dromedaries, however, none of the Bactrian camels had detectable levels of anti-MERS-CoV antibodies, suggesting that Bactrians are infected by contact with dromedaries but cannot sustain the infection alone. Nevertheless, Bactrian and hybrid camels are potential sources of MERS-CoV infection.⁸¹ It should be noted that the presence of MERS-CoV RNA and viral shedding by Bactrian camels were not examined in this study.

In natural settings, dromedaries primarily inhabit hot desert terrains of the Arabian Peninsula, the Middle East, Afghanistan, Central Asia, India, and parts of Africa. By contrast, Bactrian camels typically reside in the cold desert steppes of Mongolia, northern China, Central Asia, Pakistan, and Iran.⁷⁹ There are areas, however, in which these camels have overlapping ranges. It would be interesting to examine if Bactrian camels in these regions are infected under natural conditions rather than when placed together in restricted areas. If so, it is possible that a variant of MERS-CoV could, at some point, establish itself in the Bactrian camel populations of Asia.⁸² MERS-CoV presence should be monitored in both species of camels and the people of the area, especially in the regions where the camels have overlapping ranges. If either the animals or the people are found to be

seropositive, the presence of MERS-CoV RNA should be examined in all of these populations as well as whether an infectious virus is being shed in sufficient amounts to infect camels or humans. This approach is very challenging given the remoteness of these desert steppes and the poverty of the inhabitants. It is also possible that people in these regions are currently being infected with MERS-CoV, but these infections are misdiagnosed even if the ill people are willing and able to access medical facilities.

Importantly, in a 2021 report, anti-MERS-CoV antibodies were detected in approximately 10% of the tested Bactrian camels from Mongolia, but none had detectable levels of neutralizing antibodies or MERS-CoV RNA.⁸³ This suggests that infected Bactrian camels either produce nonneutralizing antibodies or that they are infected by a MERS-like coronavirus.⁸³ Alternatively, the camels may be producing antibodies in response to exposure to, but not infection with, MERS-CoV or a similar coronavirus.

6.3.2 Coronaviruses of alpacas and llamas

6.3.2.1 Middle East respiratory system coronavirus in alpacas and llamas

MERS-CoV has also been detected in experimentally infected alpacas or animals infected by contact with infected animals.⁸² All animals seroconverted and infectious virus was present in five or six of these animals. Upon rechallenge 70 days later, the three experimental animals were protected and the contact-infected animals were partially protected.⁸² Following experimental infection, MERS-CoV resides primarily in the nasal respiratory epithelium of alpacas and llamas as well as pigs. MERS-CoV RNA is found in the nose, trachea, and bronchi of these animals as well as in lung tissue from pigs euthanized on day 2 postinfection. Horses are not susceptible to infection even though they have high levels of expression and wide distribution of the virus' receptor along their respiratory tract.¹⁴

6.3.2.2 Alpaca enteric coronavirus in alpacas and llamas

In 1998, a novel betacoronavirus, alpaca enteric coronavirus, was discovered in the digestive tract of llamas (*Llama glama*) and alpacas (*Vicugna pacos*), members of the camelid family in the Americas.⁸⁴ Infection of these camelids is associated with severe diarrhea. About 42% of the juvenile animals are infected, apparently by the fecal-oral route.^{10,62} This coronavirus infects the enterocytes lining the lumen of the gastrointestinal tract.¹⁰ It may cause severe to fatal diarrhea in juvenile alpacas that is similar to that seen in young cattle infected with BCoV.⁶¹ It has also caused outbreaks not only in unweaned **crias** but also in the adults.⁸⁵

One infected adult alpaca had diffuse thickening of the wall of the third gastric compartment, enlarged dark red **mesenteric lymph nodes**, and watery, mucus-containing intestinal substances. The small intestine had multiple disease alterations of the **lamina propria**, **submucosa**, and **mucosa** along with moderate **autolysis** and small regions of **necrotic** debris in the intestinal crypts. The mesenteric lymph node sinusoids were hemorrhagic. The observed deficiencies in copper and other minerals may have worsened the disease in these animals.^{10,84}

Alpaca enteric coronavirus is most closely related to BCoV (>99.5% nucleoside identity), HCoV-OC43 (>96% identity), and porcine HI encephalomyelitis virus (>93% identity).¹⁰ Examination of the S protein gene sequences reveals that this virus is closely related to strains of HCoV-229 in humans.⁶¹ The genetic material from this isolate of North American camelid coronavirus has greater than 99.5% similarity to the RNA of two BCoV strains that cause shipping fever pneumonia and intestinal disease in feedlot calves.⁶¹

6.3.2.3 *Alpaca alphacoronavirus in alpacas and llamas*

Another pathogenic coronavirus of alpacas was isolated from the lungs of an animal with **alpaca respiratory syndrome (ARS)** in the United States in 2007.⁷⁴ It was named alpaca ACoV, subgenus *Duvinacovirus*. Infection with this coronavirus results in a wide range of symptoms, from mild upper respiratory disease to a life-threatening lower respiratory tract disease. Pathology associated with this virus includes severe pulmonary congestion and edema with substantial pleural effusion. Diffuse interstitial to bronchointerstitial pneumonia may be found in the terminal airways.⁷³ This alpaca virus infects the cells lining the respiratory tract and is believed to be transmitted by aerosolized respiratory secretions. It causes mild to severe respiratory system disease, including ARS.¹⁰ The symptoms of ARS range from mild to severe and include respiratory distress, high fever, and death.⁷³ It is typically found in pregnant alpacas and may result in abortion. Full-length genomic sequencing of the ACoV found that it is most similar to HCoV-229E (92.2% nucleotide identity).⁷⁴

6.4 Coronaviruses of swine

6.4.1 Introduction to swine coronaviruses

There are six coronaviruses known to infect pigs. They include alpha-, beta-, and one deltacoronavirus. These viruses cause mild to severe, potentially fatal diseases. [Table 6.4](#) gives an overview of some of the properties of swine coronaviruses.

While none of these viruses have been reported to infect or cause disease in humans, they still pose a great threat to human health due to the death of pigs. These animals are a vital part of the diet in some parts of the world and, additionally, are a source of income. Massive death of pigs due to coronavirus-induced disease has a disproportionate negative economic impact on some developing countries that threaten the entire health system and may also lead to malnutrition and its associated effects.

The rate of infection for some pig coronaviruses is very high as is the rate of coinfection by one or more coronaviruses or other microbial pathogens. As an example, a retrospective study of the prevalence of swine coronaviruses in the Guangdong Province of south-eastern China found an infection rate of 78.3% for porcine epidemic diarrhea virus (PEDV), 43.5% for swine acute diarrhea syndrome coronavirus (SADS-CoV), and 9.0% for PDCoV, but no TGEV. In one study, the prevalence of coinfection of SADS-CoV and one to four other viruses was 62.2%. In all samples positive for SADS-CoV, PEDV was also present.⁸⁶ The effect of concurrent infection with multiple infectious disease agents may be

TABLE 6.4 Comparison of the coronaviruses of swine.

Name	Cell receptor	Organ system	Symptoms
Porcine deltacoronavirus (PDCoV)	Aminopeptidase N	Digestive	Severe to fatal diarrhea Vomiting Severe dehydration Anorexia, malnutrition
Porcine hemagglutinating encephalomyelitis virus (PHEV)	Neural cell adhesion molecule (CD56)	Digestive Central nervous system	Vomiting Wasting Encephalomyelitis Polioencephalomyelitis Gliosis Neuronal degeneration
Porcine epidemic diarrhea virus (PEDV)	Aminopeptidase N Cholesterol Sialic acid Occludin	Digestive	Severe watery diarrhea Vomiting Dehydration
Swine acute diarrhea syndrome coronavirus (SADS-CoV)	Unknown	Digestive	Acute diarrhea
Transmissible gastroenteritis virus (TGEV) ^a	Aminopeptidase N Sialic acid	Digestive	Vomiting, Profuse diarrhea Severe dehydration
Porcine respiratory coronavirus (PRCV) ^a	Aminopeptidase N Sialic acid	Respiratory	Lung consolidation Necrosis of respiratory tract cells

^aMembers of the same species.

Notes: This table compares the coronaviruses of swine. Most of the swine coronaviruses cause digestive system disease, but other coronaviruses may infect the respiratory system or the central nervous system. Two of the viruses belong to the same species but infect and damage different tissues and organ systems. All of these viruses are capable of producing severe illnesses.

TABLE 6.5 Comparison of feline enteric coronavirus and feline infectious peritonitis virus.

Virus	Prevalence	Origin	Cells infected	Pathology	Symptoms
FECV ^a	~100% in multi-cat environments	Cat-to-cat Fecal-oral route	Enterocytes	Mild	Diarrhea
FIPV ^b	5% of FECV-infected cats	Internal mutations ^c	Macrophages	Life-threatening	Encephalitis

^aFeline enteric coronavirus.

^bFeline infectious peritonitis virus.

^cPost-infection mutations of FECV.

Notes: These "species" are in actuality members of the same viral species; however, they are acquired by different means, infect different organ systems, and differ in pathogenicity. Feline infectious peritonitis virus is a much more deadly mutated form of feline enteric coronavirus.

synergistic since some microbes either decrease the host animal's immune system or cause wasting, both of which magnify the risk of severe disease in these hosts.

Pigs are known to act as mixing vessels and intermediate hosts of some respiratory viruses, particularly pandemic influenza viruses. Coronaviruses of pigs are known to

recombine sections of their genomic RNA with that of other pig coronaviruses as well as coronaviruses of other animals, including humans. Since pigs can be infected by SARS-CoV and MERS-CoV, it is possible that if a pig enterocyte were infected by either of these two human coronaviruses and a pig respiratory coronavirus, they could form a recombinant pig-human hybrid coronavirus. Since the pig form of the DPP4 receptor used by some pig coronaviruses and the human form of DPP4 used by MERS-CoV are 94.5% similar⁸⁷, such dual infection events could occur. The hybrid viruses could undergo zoonotic transmission and have the potential to produce a highly lethal pandemic.⁸⁷ It should be noted, however, that even though pigs and humans have extensive interactions in some parts of the developing world, no such pathogenic or nonpathogenic hybrid viruses have been found in either pigs or humans despite the intensive scrutiny of potential coronavirus threats during the current pandemic.

6.4.2 Pathology due to swine coronaviruses in general

Swine coronaviruses affect the gastrointestinal and respiratory tracts or the peripheral and central nervous systems. SADS-CoV, PEDV, and TGEV are examples of ACoVs that infect pigs and cause diarrhea, vomiting, and dehydration. SADS-CoV belongs to the *Rhinacovirus* subgenus. Genetically, isolates of SADS-CoV from piglets are almost identical to isolates of the bat coronavirus HKU2. PEDV and PDCoV, a deltacoronavirus of the *Buldecovirus* subgenus, are the major causative agents of watery diarrhea in piglets. The death rate may be high, especially in very young piglets in which the mortality rate may approach 100%. TGEV belongs to the *Tegacovirus* subgenus. It causes gastroenteritis which was responsible for many deaths of pigs worldwide during the 1990s. Its presence has sharply decreased in much of the world since then.⁸⁸ Since the symptoms of enteric pig coronavirus diseases are indistinguishable, laboratory diagnosis is needed to distinguish between the above listed enteric coronaviruses.

Two other pig coronaviruses cause disease in other organ systems. PRCV infects the respiratory tract but belongs to the same species as TGEV. Porcine hemagglutinating encephalomyelitis virus (PHEV) is a betacoronavirus of the *Embecovirus* subgenus that infects the CNS in addition to the digestive system.

The high rate of infection and death resulting from infection of suckling piglets by these pig viruses causes substantial economic loss. Weaned pigs and sows often experience serious gastrointestinal disease symptoms as well, including lethargy, anorexia, diarrhea and vomiting, dehydration, weight loss, and sometimes death. The clinical disease typically lasts up to 10 days in animals belonging to this age group, however, very few older pigs die.⁸⁹

PEDV, PDCoV, and SADS-CoV are considered emerging coronaviruses that originated in China. They all cause **necrosis** of infected cells lining the intestinal tract that often results in fatal malabsorptive diarrhea in piglets.⁸⁹ Despite their similarities in pathology, immunologic cross-reactivity among the various pig coronaviruses is very limited or absent. Polyclonal hyperimmune antisera against PDCoV does not cross-react with PEDV and monoclonal antibodies against the SADS-CoV's N protein do not cross-react with PEDV, TGEV, or PDCoV.⁹⁰

The genomes of coronaviruses contain scattered **point mutations** that affect potential coronavirus host selection without any major alterations in their basic biological functions. Major modifications, such as deletions or insertions, in the genes encoding accessory proteins or the S protein, may change host or tissue **tropism**.⁸⁹ The size and location of such deletions affect potential host species and tissue tropism as well as viral virulence.⁹¹ In PEDV, however, a large deletion in the S protein led to a partial lessening of the viral pathology without changing its tissue tropism.^{41,92} Also, a large deletion in the S protein of PRCV changes it from a primarily gastrointestinal to a respiratory virus.⁸⁹

The number of ORFs encoding accessory proteins varies among pig coronaviruses.^{93,94} PEDV has only ORF3. TGEV has three accessory proteins that are encoded by ORF3a, ORF3b, and ORF7. PDCoV has the smallest pig coronavirus genome and has two ORFs, ORF1a and ORF1b. PDCoV has no gene for nsp1 and so has 15, rather than 16, nsp's. Since several nsp's are involved in immune evasion, the presence or absence of an nsp can greatly alter the efficiency of the host immune response and subsequent pathology.

6.4.3 The immune response to swine coronaviruses in general

Several cells and cytokines of the innate immune system are important in controlling viral activity and dissemination early during infection with porcine coronaviruses. Monocytes and DCs are the first immune cells to encounter these viruses and are the major sources of type I IFN.⁸⁸ NK cells are also important during porcine coronavirus infections and may affect pathogenesis and disease outcome. They are a major source of IFN- γ , the type II interferon.⁹⁵ Numbers and activity levels of NK cells change with age. Suckling piglets experimentally infected with PEDV have lower numbers of NK cells than weaned piglets and the cells that are present in the sucklings have substantially less lytic activity. Lower levels of IFN- γ producing NK cells are present in the blood and small intestine of the suckling pigs.⁹⁵

Increased serum levels of the proinflammatory cytokine IL-17 and the **T helper cell 1** (Th1)-associated cytokine IL-12 are seen earlier in suckling vs weaned pigs. The levels of proinflammatory cytokines in suckling and weaned pigs correlate with disease severity and viremia. Uninfected human neonates also have low levels of cytokine secretion and NK cell lytic activity. These are decreased even more during infection.⁹⁵ While the inflammatory response to coronaviruses is important in viral elimination, excessive or chronic inflammation is pathogenic. Weaned pigs have delayed production of proinflammatory cytokine production in comparison with suckling pigs. The delayed onset of inflammation coincides with the delayed disease.⁹⁵

There are a variety of IFN- λ s, the **type III IFNs**, that have antiviral actions against mucosal infections, including infection by enteric viruses. The various types of IFN- λ only perform their antiviral activity in epithelial cells that line or cover surfaces of portions of the intestinal tract.⁹⁴ In mucus membranes, epithelial cells are the predominant source of IFN- λ .⁸⁸ It has been demonstrated that during other viral infections of the intestines, the major effects of IFN- α and IFN- β are on intestinal lymphocytes, while the effects of IFN- λ are on the epithelial cells.⁹⁴

The adaptive immune response to enteric coronaviruses of pigs includes antibodies and CD4⁺ T helper and CD8⁺ T killer cells. Secretory IgA antibodies are produced by B cells found in **mucosal** tissues and the **systemic** antibodies IgG and IgM are found in blood and **interstitial fluid** found between cells.^{96,97} The CD4⁺ T helper cells direct antibody production while the CD8⁺ T killer cells kill virus-infected cells. T helper, but not T killer, cell numbers are also higher in the small intestine of suckling pigs compared to weaned animals.⁹⁵ Many of the T cells present belong to a small, specialized group, the $\gamma\delta$ T cells, found in the intraepithelial layer.⁹⁶

As is the case in many viral infections, T cells and NK cells have vital roles in protection against pig coronaviruses. CD4⁺ T helper cells produce antiviral cytokines, such as IFN- γ , and CD8⁺ T killer cells and NK cells directly kill infected cells. PEDV-infected suckling pigs have lower numbers of NK cells and IFN- γ production than weaned animals. This situation is associated with the more severe disease during PEDV and TGEV infection of sucklings in comparison with weaned pigs.^{89,95} Between 30–120 days postinfection, both T helper and T killer cells, as well as monocytes, eosinophils, and neutrophils, are present in the small intestine.⁹⁷

Coronaviral E protein is present in the endoplasmic reticulum (ER) and the nucleus, albeit at much smaller levels. The E and N proteins trigger stress in the ER. The N and M proteins as well as the product of ORF3 retard the growth of infected intestinal epithelial cells by arresting cells in the S-phase of the **cell cycle**.^{98–100} That allots more of the cellular resources for viral, rather than cellular, reproduction. The E and M proteins also induce high levels of the antiapoptotic protein **Bcl-2**, which increase the time for the viruses to complete their lifecycles and produce progeny viruses before the host cell dies. The E protein, however, does not affect cell proliferation or the cell cycle.⁹⁸ The protein encoded by ORF3 is a potassium **ion channel** and affects virus production, infectivity, pathogenicity, and release of the mature virus from the host cell.¹⁰¹ Ion channels are also found in the E proteins of MHV, SARS-CoV, and HCoV-229E. Of note: the ORF3 gene from an attenuated virus contains a 49-nucleotide deletion and, therefore, lacks this ion channel. A complete ORF3 is also present in SARS-CoV, HCoV-NL63, and PEDV.¹⁰²

Interestingly, the highly pathogenic PEDV strain (CN/Liaoning25/2018), isolated during an outbreak, also contains a 49-bp deletion in the ORF3 gene that is typically associated with attenuated viral strains used for vaccination. The CN/Liaoning25/2018 strain was produced by the recombination of a viral strain containing the S gene from the highly pathogenic CN/GDZQ/2014 strain and a low pathogenic PEDV SQ2014 strain in the field by natural means.¹⁰¹ Recombination which increases genetic diversity is the bane of attempts to produce effective vaccines, immune responses, and antiswine coronaviral drugs. While several recombinant PEDV strains have been reported, they are the product of two different highly pathogenic strains.¹⁰¹ This new CN/GDZQ/2014 strain, however, appears to have arisen from the recombination of a highly pathogenic strain and a vaccine strain. This warns of the possibility of similar recombination between human coronaviruses if live attenuated viruses are used in vaccines. Generally, live attenuated vaccines administered orally yield better and longer protection than vaccines using inactivated viruses since they are more likely to activate virus-specific CD8⁺ T killer cells and IgA antibody activity. Their disadvantages include the possible gain of virulence by either mutation or recombination, as seen in the case of PEDV.¹⁰¹ These factors should be

carefully considered when using live attenuated viruses or chimeric vaccines which incorporate the viral S protein for vaccination. As of October 2020, other anticoronavirus vaccines are either in use or in development, including vaccines that target TGEV, SADS-CoV, and PDCoV in addition to the human coronaviruses MERS-CoV, SARS-CoV, and SARS-CoV-2.¹⁰¹ Care must be used in the production of anticoronaviral vaccines since the possibility exists for recombination between these highly pathogenic human viruses and the common human coronaviruses that cause cold-like symptoms.

6.4.4 Viral inhibition of the immune response to swine coronaviruses in general

Several nsp's aids in the escape of coronaviruses from the host immune response. Nsp1 is only present in alpha- and betacoronaviruses and, therefore, is not found in the PDCoV deltacoronavirus. This viral protein varies greatly among swine ACoVs. Similar to the SARS-CoV nsp1, the PEDV nsp1 variant potentially blocks the production and signaling activity of type I and III IFNs.^{103,104} by either triggering the degradation of the IFN proteins in proteosomes or by inhibiting the translocation of IFN signaling proteins to the nucleus. This decreases activation of the genes for IFN- β and the proinflammatory cytokines TNF- α , IL-1 β , IL-6, IL-15, and IL-17 at least in vitro.⁸⁸ Acutely infected neonatal piglets, however, instead increase their levels of systemic innate and proinflammatory cytokines, including IFN- α , TNF- α , IL-6, IL-8, IL-12, and IL-17.^{95,97} These cytokines are beneficial during the early stage, acute PEDV infection since they activate both CD4⁺ T helper and CD8⁺ T killer cells, which remove infected cells and increase production of antiviral antibodies.¹⁰⁵ When high levels of these cytokines are prolonged, however, they are detrimental.¹⁰⁶

Several gut coronaviruses, such as PDCoV, PEDV, TGEV, and SADS-CoV, block the production of type I IFNs by intestinal cells.¹⁰³ The intestinal epithelial cells differ from that of epithelial cells in other sites, perhaps due to the unique environment of the gut mucosal surface, taken together with the presence of normal gut microbes. PDCoV's accessory protein nsp6 inhibits IFN- β production while the product of ORF7 from TGEV interferes with type I IFN signaling.¹⁰⁷ PDCoV and SADS-CoV block the activity of several host cell molecules, **IFN- β promoter stimulator (IPS-1)** and **retinoic acid-inducible gene I (RIG-I)**. IPS-1 and RIG-I normally play a role in IFN- β production by inhibiting the ability of two **transcription factors**, **IFN-response factor 3 (IRF3)** and **nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B)**, to enter the nucleus and trigger transcription of the gene for IFN- β . PEDV also affects the ability of RIG-I to produce IFN- β .¹⁰⁸ RIG-I and **melanoma differentiation-associated gene 5** recognize double-stranded RNA that is formed during the replication of some viruses, including coronaviruses. While TGEV does not inhibit IFN- β induction, it delays its activation, allowing the virus to get a "head-start" on the immune response. The human coronavirus SARS-CoV also interferes with IRF3 activity.¹⁰⁹ MHV blocks IFN- α/β response by inhibiting the **posttranscriptional** maturation of IRF3.¹¹⁰ Coronavirus nsp1, nsp15, and the viral N potentially block the production and signaling activity of IFN- λ as well.¹⁰⁴ IFN- λ 1 and IFN- λ 3 are vital to protect the mucosal epithelial cells lining the gut from an enteric viral infection, including PEDV.⁹⁴ In response, PEDV and other pig coronaviruses have evolved the means to evade IFN

activity.¹⁰⁴ The following PEDV components inhibit the IFN- λ antiviral responses: nsp1, nsp3, nsp5, nsp8, nsp14, nsp15, nsp16, ORF3, and viral E, M, and N proteins. Nsp7, nsp14, and nsp16 are type I IFN antagonists, while nsp8 blocks IFN- λ responses. Nsp10 enhances nsp16's negative effect on IFN production.¹⁰⁰

IFN- λ production takes place in the **peroxisome**, a cellular organelle that also regulates levels of hydrogen peroxide and other reactive oxygen species as well as the metabolism of fatty compounds. PEDV decreases the numbers of peroxisomes in a pig intestinal epithelial cell line in vitro and thus partially evades this critical antiviral cytokine.¹⁰⁴ The **mitochondrial antiviral signaling (MAVS) protein** is found in the mitochondria and peroxisomes and is involved in regulating IFN pathways. While mitochondrial MAVS stimulates type I IFN responses, peroxisomal MAVS activates type III-dependent antiviral responses, especially in intestinal epithelial cells. Since type III IFN receptors are only present on mucosal epithelial cells, type III IFN responses are primarily confined to this cell type. By contrast, mucosal epithelial cells only bear low levels of type I IFN receptors, so type I IFN does not protect these cells against viruses. During viral infections, the cells of the gut mucosa respond to type III IFNs responses, while the other cells in the intestines respond to type I IFNs.¹⁰⁴

Nsp3's PEDV **papain-like protease 2 (PL^{pro})** and the **3-chymotrypsin-like cysteine protease (3CL^{pro})** of PEDV and PDCoV block IFN- β activation by cleaving members of the host IFN signaling pathway. The PDCoV nsp5 is unique among coronaviruses in that it cleaves **signal transducer and activator of transcription (STAT)2**, a host cell signaling molecule activated by type I IFN.⁸⁸ PEDV reduces STAT1 levels in vitro by degrading them in the proteasome. Addition or removal of the host **ubiquitin** molecules to proteins either "tags" them for degradation by the proteasome or prevents proteasomal degradation, depending on which type of ubiquitin is involved. PEDV's nsp3 contains a conserved **deubiquitinase** region, as is also the case for MHV and SARS-CoV. The PEDV PL^{pro} protease, the catalytic domain of nsp3, significantly inhibits the ubiquitination of RIG-I and STING. Ubiquitin is required for type I IFN signaling as well as for signaling by **interferon-stimulating gene 15**.¹¹¹ PL^{pro} deubiquitinates IRF3, inhibiting its translocation into the nucleus.¹¹²

The PEDV nsp15 accessory protein is an **endoribonuclease** (an enzyme that cuts and inactivates RNA). It cuts and downregulates host **TANK-binding kinase 1 (TBK1)** and IRF3 RNA, molecules that are vital components of the IFN signaling in vitro. This is not the case for SARS-CoV, however.¹¹³ The typical protein degradation systems, the ubiquitin-proteasome system, and **autophagy**, are not involved in the reduction of TBK1 or IRF3 levels by nsp15.¹¹³ The nsp15 of other coronaviruses, including SARS-CoV and MHV, also block antiviral innate immune responses, but do so using different mechanisms.¹¹³

PEDV's structural proteins also alter host immune responses. The PEDV S protein directly interacts with and activates the host cell's **epidermal growth factor receptor (EGFR)**, a molecule that typically induces cell division when necessary, but, in excess, is involved in tumor formation and cancer. In the case of PEDV, binding of this growth factor receptor to the viral S protein from active or inactivated coronavirus drives viral replication in the cells via the **Janus activated kinase2-STAT3** pathway. This binding also decreases type I IFN activity.^{88,114} Specific inhibition of EGFR or STAT3 activity reduces PEDV production while raising that of type I IFN.¹¹⁴ EGFR signaling also regulates lung damage during SARS.¹¹⁵

The PEDV N protein also inhibits IFN- β production but does so in a manner that is not used by other coronaviruses, including SARS-CoV and MHV. PEDV outcompetes host IFN for binding to TBK1, an enzyme that regulates the antiviral innate immune response and cell division as well as apoptotic cell death.¹¹⁶

6.4.5 Porcine epidemic diarrhea virus

6.4.5.1 Introduction to porcine epidemic diarrhea virus

PEDV was first reported in swine populations in 1971 in the United Kingdom. It then caused a large outbreak in China in 1973, followed by an outbreak in Belgium in 1977. Sporadic outbreaks of PED were subsequently found in the Czech Republic, Hungary, Italy, South Korea, China, Japan, and Thailand in the 1980s and 1990s. Afterward, the prevalence of PEDV decreased throughout Europe, only causing periodic outbreaks, until its reemergence in Italy in 2005–2006, in Thailand in 2007–2008, and in China in 2010.⁸⁹

PEDV spread to the Western Hemisphere where it was first reported in the United States in 2013–2014.^{89,117–119} It dealt a major blow to the pig industry, killing about 10% of the total United States swine, 7 million piglets, in less than 1 year.¹²⁰ Performance of the surviving pigs was also impaired.¹²¹ The strains of the 2013 outbreak in the United States were closely related genetically to the 2011–2012 Chinese strains. A different PEDV variant containing a 197-amino acid deletion emerged in the United States only one year after the 2013 outbreak.¹¹⁷ The prevalent isolates in a recent outbreak in Korea are more closely related to Chinese strains and are significantly different from the vaccine strains used in that country. These differences may be at least partially responsible for the reduction of efficacy of these vaccine strains.¹¹⁷ In addition to the emergence of a highly virulent PEDV in the United States, the virulent strain also emerged in Japan, South Korea, Taiwan, and Vietnam in 2013 and the Philippines in 2014. In 2014, a highly virulent PEDV strain was isolated from a piglet with severe diarrhea in Vietnam. This strain contains a 72-nucleoside deletion in ORF1a.¹¹⁹ Except for Ukraine, no highly virulent PED outbreaks have been reported in Europe, Africa, or Australia since 2013.¹¹⁹

In 1994, a vaccine was developed and widely used in Chinese pig populations. This vaccine may have played an important role in controlling PEDV infections in China temporarily.¹¹⁹ A highly virulent PEDV strain emerged and became a major swine pathogen in over 10 provinces in China in 2010. The mortality rate was 50%–100% and more than one million piglets died, even those which were immunized against an inactivated PEDV or TGEV. In this outbreak, all infected piglets died, primarily in the first week of infection, but occasionally in a matter of hours.¹²² While pigs of all ages had diarrhea and loss of appetite, disease severity was age-dependent. Vaccinated herds also had lower morbidity and mortality rates, which may indicate the emergence of new PEDV strains against which the vaccine was only partially protective.¹²³ Examination of herds in 12 Chinese provinces revealed the presence of several variant strains whose S proteins contain insertions and other mutations and have increased pathogenicity.¹²³ Since 2010, China has had several major outbreaks that may be due to the emergence of new and more pathogenic strains.¹²⁴ Of note, the coinfection rate of PEDV and PDCoV increased to 51% in some parts of China.¹²⁵

PEDV, an ACoV belonging to the *Pedacovirus* subgenus, has been divided into two genetic groups: genogroup 1 (G1; classical PEDV) and genogroup 2 (G2; field epidemic or pandemic PEDV). G1 is found in Europe and is further divided into subgroups G1a and G1b. G1a variants include vaccine strains and those grown for multiple generations in cultured cell lines in vitro and have adapted to growth in those cells, often becoming less virulent. However, when the mothers of piglets are ill due to poor immune, nutritional, or health conditions, the virulence of the G1a strains increases in experimentally infected PEDV suckling piglets with a mortality rate increases from 0% to 75%.⁹⁰ G1b strains (also known as S-INDEL) consist of new PEDV variants whose spike protein contains deletions and insertions. G1b strains typically are found in a high number of piglets on farms that fail to properly adhere to biosecurity guidelines and have low herd immunity.⁹⁰ The highly pathogenic PEDV G2 strains are present in America and Asia and are also divided into two genetic groups: genotype G2a and G2b. G2 strains have a deletion or insertion in the S protein gene and are variants from the originally isolated G1 CV777 strain.¹²⁶ The PEDV genogroups will be further described later.

6.4.5.2 Pathology caused by porcine epidemic diarrhea virus

Within two days of infection, pigs begin to release PEDV into their feces and continue to do so for up to four weeks. PEDV infection in piglets under 8 days of age produces watery diarrhea and vomiting for several days before they become extensively dehydrated due to electrolyte imbalance before their death.¹²⁷ Suckling pigs develop a more severe disease than weaned pigs.

Lesions are present in and confined to the gastrointestinal tract from the ileum to the jejunum of the small intestine in young piglets. PEDV infects villus enterocytes, **intestinal crypt cells**, and macrophages.⁹⁰ The stomach of these animals is distended and full of undigested milk curd and the intestine walls are thin. The intestines also contain yellowish fluid.¹²⁸ The length of the finger-like intestinal villi that are vital for absorption of nutrients is greatly reduced.¹²⁹ The villous enterocytes of pigs' colons are often infected but do not undergo necrotic death due to PEDV.¹¹⁷ However, the colon of piglets is not physiologically mature in comparison with that of older pigs. Water reabsorption is lesser in immature pigs and contributes to extensive diarrhea in piglets.¹¹⁷ Transmission of PEDV to young pigs is primarily via the fecal-oral route.

In addition to reducing nutrient absorption, the activity of digestive enzymes is reduced.¹²⁷ PEDV-infected piglets also develop **hyperkalemia** (high levels of blood potassium) and **acidosis** (acidic blood) because of the loss of **bicarbonate**, important in the regulation of blood components and pH.¹¹⁷ The combination of acidosis and dehydration decreases the ability of the heart to contract. In older animals, the disease self-resolves within a week but may interfere with the growth of previously infected animals.¹²⁷

Viral RNA is present in feces and blood as early as one day after infection, whereas weaned pigs develop milder and delayed disease symptoms as well as delayed shedding of fecal PEDV RNA. The serum levels of viral RNA are approximately 100-fold less than that found in suckling pigs.⁹⁵ With the advent of several highly pathogenic strains, PEDV produces a morbidity rate of 80%–100% and a mortality rate of 50%–90% in suckling piglets 1–3 days old¹²² which decreases to 1%–3% in weaned pigs, and 0% in fattening pigs.

G1b strains from the Americas appear to have less damage to the intestinal villi, reduced intestinal infection and lower mortality than members of the G2b subgroup.^{90,129} Inoculation of piglets with these US G1b PEDVs provides partial protection against subsequent infection with original US PEDV strains, which lack the deletions found in G2 strains, even though all piglets had diarrhea.¹²⁹ However, other reports found that European S-V1a PEDV strains have a high mortality rate in suckling piglets in Germany and Portugal.¹²⁹ In the above studies, substantial variations in disease severity were found between litters as well as among members of the same litter, partially due to the size and age of the individual piglets. The health of infected sows and their ability to produce adequate amounts of milk containing protective IgA antibodies to their offspring also play a role in the piglets' susceptibility to severe disease.¹²⁹

One of the ways by which the intestines are damaged is due to the destruction and disorganization of the proteins that form seals between infected intestinal cells (tight junction and adherens proteins) and the loss of the structural integrity of the intestinal wall.^{130,131} **E-cadherin** and **ZO-1** are two of these cell surface proteins that play major roles in the formation of adherens and tight junctions, respectively, helping to form barriers between adjacent cells. Their surface expression is decreased during infection with TGEV alone or with PEDV/TGEV dual infection in vitro in cultured pig **jejunum** cells.¹³¹ This reduction is seen during early infection, but their levels return to normal levels within 24 hours.¹³¹ The rapid reduction of E-cadherin and ZO-1 may be due to virus entry into their target cells. The reformation of the actin-containing microfilament structures is vital for viral attachment, entry, replication, and release.¹³¹ PEDV and TGEV move along **filopodia** towards their target cells. These structures are produced by microfilaments. In the cell's cytoplasm, multiple microfilaments surround PEDV and aid in the formation of membrane vesicles.¹³¹ Changes in tight junction and microfilaments of cells are stimulated by TGEV and PEDV, perhaps due to activation of the **extracellular signal-regulated kinase (ERK)**, **p38**, and **Janus kinase** MAPK signaling pathways. Inhibition of ERK, p38, and JNK decreases PEDV and TGEV infection of target cells.¹³¹

Signals from the EGFR play a major role in PEDV-mediated pathogenic processes.¹¹⁴ Infection of pigs with PEDV leads to activation of the EGFR, even if the virus has been rendered unable to replicate. Chemical stimulation of this receptor increases viral replication. Inhibition of the EGFR, by contrast, decreases virus titers by increasing the expression of type I IFN genes.¹¹⁴ Other viruses also use EGFR activation to help circumvent the host's immune response. One of these viruses is SARS-CoV, in which the EGFR is involved in lung damage.¹¹⁵

PEDV replicates in the cytoplasm of cells throughout the small intestine, killing these cells by necrosis or apoptosis. Apoptosis is like a double-edged sword, a carefully regulated process by which a cell dies in a self-imposed, controlled manner that is unlikely to harm surrounding cells and tissue. It is essential to normal development and homeostasis by removing unnecessary, infected, or cancerous cells. When unchecked, however, as is the case in several viral infections, it is pathogenic, killing healthy, sometimes vital, cells, including those in the CNS or T helper cells during AIDS. By contrast, necrosis is premature cell death due to cell injury caused by external factors, including microbes or trauma. It is a chaotic process that often damages surrounding cells or tissues.

During viral infections, apoptosis may be protective or harmful. CD8⁺ T killer cells and NK cells often trigger the death of infected cells by apoptosis, thus depriving the virus of a place to replicate, but, in some cases, also kill cells that are important for the health or survival of the host organism. In addition, some viruses induce apoptosis to allow the release of the newly produced viruses, permitting them to infect new host cells and continue the viral lifecycle. Several apoptotic pathways exist and involve either the **caspase** cascade or **cytochrome c** and the loss of mitochondrial integrity. Coronaviruses, including SADS-CoV, PEDV, TGEV, PDCoV, and MHV often trigger pathogenic apoptosis.^{132–134} SADS-CoV, TGEV, and CCoV activate several key members of the different caspase cascades, caspase-8, -9, and -3 both in vitro and in vivo.¹³⁵ For several coronaviruses, including TGEV, apoptosis involves activation of **Fas/FasL** and caspase-8.^{136,137} Upon activation, caspase-8 cleaves **Bid** which subsequently moves into mitochondria where it begins releasing cytochrome *c*, a proapoptotic molecule, into the cytoplasm. This results in apoptosis via caspase-9 activation.¹³⁵

6.4.5.3 Porcine epidemic diarrhea virus and the microbiome

The gut **microbiome** (the total number of microbes in an organism) is less diverse in young piglets than in adult pigs. Piglets have a smaller *Firmicutes* to *Bacteroidetes* bacteria ratio that contributes to immature gut innate immune response¹³⁸ and is linked to several diseases, including **systemic lupus erythematosus** (lupus), **celiac disease**, and **Crohn's disease**. Unlike some epithelial cells, the intestinal epithelium juggles the necessity to tolerate the presence of **commensal** microbes while ensuring an effective immune response against pathogens. PEDV reduces the number of the commensal microbes that are present in healthy intestinal tracts, causing unbalanced shifts of the gut microbiome¹³⁹ which may alter the IFN- λ response.⁹⁴

Alteration of the gut microbiome is seen in both nursing pigs and their mothers,⁹⁰ blocking the transfer of healthy gut microbes to their offspring.¹⁴⁰ The number of beneficial bacteria decreases and that of harmful bacteria increases during PEDV infection.¹⁴¹ Feeding the beneficial bacteria *Bacillus subtilis* to 2-week-old piglets infected with PEDV reduces damage to the intestines. Additionally, some strains of *Lactobacillus plantarum* produce anti-PEDV responses in vitro.^{90,142}

6.4.5.4 Porcine epidemic diarrhea virus—viral genogroups

As stated previously, PEDV has been divided into G1a, G1b, G2a, and the highly pathogenic G2b types. After ingestion, G2 PEDVs replicate in the jejunum and iliac enterocytes of the small intestine. Approximately 3 days after infection, the virus attacks and shortens the intestinal villi, impairing nutrient absorption and causing an electrolyte imbalance.¹⁵ Members of the G2a subgroup cause local epidemic outbreaks in Asia. The G2b subgroup (also known as non-S-INDEL) consists of recent pandemic outbreak strains in both North America and Asia but is most genetically related to a 2010 Chinese strain.¹²⁷ G2b is extremely virulent and causes PED outbreaks throughout the world.¹¹⁹ A lack of protective immunity in the milk of sows in the United States swine population may contribute to the virulence of G2b, at least in North America.¹⁴³

Airborne transmission of PEDV is higher in pigs infected with G2b viruses than in pigs infected with members of the viral G1b group. When uninfected pigs are exposed to

aerosolized virus strains, even though aerosolized G1b is present, none of the pigs in contact with the aerosol became infected, while all the pigs exposed to aerosols containing G2b were infected.¹⁴⁴

The reproductive organs of experimentally infected boars, including the **Cowper's glands**, do not contain PEDV RNA. However, viral RNA is found transiently (day 7 post-infection) in the sperm-rich portion of semen (containing sperm, spermatozoa, white blood cells, and immature germ cells) from boars infected with G2b PEDV.¹⁴⁵ By contrast, PEDV RNA is detectable for up to 16 days in the sperm-rich fraction of semen of boars infected with a G1b strain.¹⁴⁴ G1b RNA is also present in the seminal fraction of semen of these boars. No G2b PEDV RNA is detectable in semen after the boars recover and fecal shedding has ended, in contrast to the findings of semen from G1b PEDV.¹⁴⁴ PEDV is shed in semen before shedding in feces as well as before symptoms occur.¹⁴⁵ Viral transmission via artificial insemination, however, is very unlikely due to the low levels of virus present in semen.¹⁴⁵ This is very important since 90% of sows in Europe, North America, and Latin American and 70% of sows in Thailand and Taiwan are inseminated artificially.¹⁴⁶

When six weaned pigs were infected with PEDV-positive semen, they did not develop an antibody response or clinical symptoms and only one piglet shed the virus. These data suggest that while sexual transmission of this virus may occur, further research needs to focus on the prevalence of viral shedding by the sexual route as well as whether these viruses are infective.¹⁴⁵ It should be noted that this study could not rule out contamination of the semen by feces during its collection and preparation of the semen. Future studies should perhaps also be performed using suckling piglets since weaned piglets are much less likely to develop the disease by the fecal-oral route.

Virulent global G2b strains seem to have been produced by point mutations in local virulent field G1a populations. New members of the G1b subgroup, however, have diverged genetically from both the G1a and G2 strains. Genetic analysis of the S protein and the entire viral RNA genome suggests that these novel G1b variants may be the product of recombination between G1a and epidemic G2a viruses.^{90,127,133} In vivo, sows exposed to G1b PEDV seven months before **farrowing** and then re-exposed to a G2b strain at day 109 of gestation produced protective passive immunity in piglets challenged with the same G2b strain.¹⁴⁷ None of the passively immunized piglets died compared to 33% mortality in infected control piglets. Also, the incidence of diarrhea in the passively immunized group decreased by 57%.⁹⁰

6.4.5.5 Porcine epidemic diarrhea virus transmission

PEDV is stable over a large temperature (4°C–50°C) and pH range (pH 4–9), enabling it to withstand temperature-related challenges.¹²⁴ The virus is usually transmitted by the fecal-oral route, vomitus via contaminated fomites (manure, contaminated cloths or footwear of swine workers or trailer drivers, pig pens), and by contaminated feed or feed totes.¹²⁴ Remarkably, PEDV on feed totes was still infectious after 35 days at room temperature.¹⁴⁸ PEDV is also present in sow milk, but to a much lesser degree than in the sows' fecal samples (40.8% and 82.0%), respectively. Additionally, field observations reveal a decrease in piglet death rates in fostered piglets.¹²² Taken together, it appears that PEDV may also be transmitted vertically from sows to their piglets.

PEDV transmission may also occur by aerosolized nasal secretions. The nasal cavities of pigs housed at a distance from experimentally infected pigs frequently carry PEDV RNA.¹⁴⁹ Furthermore, PEDV RNA is often found in the nasal cavity. It infects the cells lining the cavity and may be responsible for the transient infection found there. PEDV does not infect lung cells, however.⁹⁰ Dendritic cells in the mucus region of the nasal cavity or lymphoid tissue, such as the tonsils, can transfer the virus to T cells in the blood. The infected cells then carry the virus to the cells lining the small intestines.^{88,90} The presence of virus in the nasal cavity suggests that limited airborne transmission via the fecal-nasal route may also contribute to pig-to-pig and farm-to-farm transmission.^{90,150} Nevertheless, PEDV is primarily transmitted via the fecal-oral route or by contaminated fomites,¹⁵¹ as stated previously.

6.4.5.6 The spike protein and host cell receptor of porcine epidemic diarrhea virus

As described in Chapter 1, coronaviruses' S proteins must be cleaved into S1 and S2 proteins to become active and bind to their receptors. PEDV uses the lysosomal cysteine proteases **cathepsin L** and **cathepsin B** to cleave its S proteins. Extracellular **trypsin**, a protease that is produced in the pancreas and activated in the small intestine, can take part in the entry of PEDV into host cells in vitro.¹⁵² PEDV binds to several molecules protruding from the plasma membrane of the host cells.¹⁵³ Plasma membrane **cholesterol**, sialic acid, **occludin**, and APN have been implicated as receptors or coreceptors for PEDV.⁹⁰ Cholesterol is a fatty molecule found in the plasma membrane of eukaryotes, where it plays a vital role in the horizontal movement of materials in the membrane. Sialic acid is a sugar that is found attached to the terminal ends of some glycoproteins in humans and is used as a primary receptor for the lineage A betacoronaviruses HCoV-OC43, BCoV, and PHEV.¹⁵⁴ The ability of the PEDV S protein to bind to sialic acid may depend on the viral strain. Among other locations, sialic acid is found on the surface of **goblet cells** that secrete mucus in the digestive and respiratory systems and may serve as a secondary receptor for TGEV.⁸⁹ PEDV molecules have been detected in goblet cells of infected pigs. Occludin is found on those enterocytes that absorb nutrients from the gut.⁹⁰

There are conflicting views about whether APNs serve as the sole PEDV receptor as well as if there is a need for a minimum number of APN molecules to bind to the virus to allow entry into host cells, at least in cell lines in vitro.^{155,156} PEDV can infect cell lines derived from humans, monkeys, and Mexican free-tailed bats (*Tadarida brasiliensis*),¹⁵³ even though not all these cell lines express APN. These findings suggest that pig APN may not be a functional receptor for PEDV or may not be this virus' sole receptor since pigs lacking APN are still susceptible to PEDV, but not TGEV, infection.^{139,157,158} Nevertheless, the levels of APN correlate with the degree of disease severity. As the levels of APN on the target cells' surface increase, the extent of disease severity increases as well.¹⁵⁹ Accordingly, piglets born with lower levels of APN in the intestines may have a natural resistance to PEDV infection compared to normal piglets.⁹⁶

PEDV has not been reported to infect animals other than pigs, including bats, although bats have been proposed to house an ancestral form of PEDV as well as other bat coronaviruses.⁸⁹ It should be noted that *Scotophilus* bat CoV-512 may enter target cells using receptors other than APN.¹⁵⁵ This bat coronavirus can infect multiple animal species,¹⁵⁵ but it is more closely related to PEDV than to other ACoVs of humans or pigs, including

TGEV.¹¹⁸ Despite similarities between *Scotophilus* bat CoV-512 and PEDV, the amino acid similarity is only 48% in the S1 subunit that contains the RBD critical for binding to host target cells.¹⁵³

6.4.5.7 The immune response to porcine epidemic diarrhea virus

The TLR signaling pathway is triggered by PEDV invasion. It stimulates the production of inflammatory cytokines, chemokines, and antimicrobial peptides. This pathway involves TLRs 2, 3, 4, 7, 8, and 9 as well as the host proteins **myeloid differentiation primary response 88**, **Toll/interleukin-1 receptor domain-containing adapter-inducing IFN- β** , IRF3, and NF- κ B. Detailed information about these signaling pathways is reviewed by Du.¹⁶⁰

Antibodies play an important role in preventing and eliminating PEDV infection. IgG antibodies against PEDV S protein are present by 7 days after infection and antibodies against the N and M proteins are present by day 10, including some neutralizing anti-N protein antibodies. The PEDV M protein is highly cross-reactive with TGEV- and PRCV-specific antisera.¹⁶¹ Anti-S protein IgG antibodies directed against PEDV do not cross-react with TGEV, PRCV, or PDCoV and are, therefore, good candidates for differentially diagnosing the species of pig enterovirus in a patient.¹⁶¹ Anti-PEDV E protein-specific antibodies are not present. Experimentally infected pigs produce IgG and serum IgA antibody response to PEDV 7–21 days postinfection. Lower serum antibody levels are induced by G1b strains than by G2b PEDV strains.⁹⁰ The levels of IgA and IgG antibody-secreting cells in the duodenum, ileum, mesenteric lymph nodes, and blood correlate with protection against PEDV challenge.⁸⁹ Anti-PEDV IgA antibodies are also present in oral fluids.⁸⁹ Moreover, antibodies against PEDV are found in the milk of previously infected sows. These antibodies help to protect the suckling piglets until they are weaned and begin to produce their own protective antibodies.¹⁶²

NK cells specialize in killing virus-infected cells and are believed to be important in the clearance of PEDV by producing the cytokines IFN- γ , TNF- α , and granulocyte-macrophage colony-stimulating factor.⁸⁸ Infected neonatal and nursing piglets that have diminished NK numbers develop more severe symptoms.⁹⁵ Additionally, uninfected suckling pigs typically have much lower NK cell numbers and undetectable levels of NK cell activity, including production of IFN- γ , in their blood and ileum than do weaned pigs, which are significantly more resistant to PEDV disease. Following infection, weaned animals produce more IFN- γ than suckling pigs. Interestingly, the frequency of NK cells in the blood is higher than that found in the ileum, and this difference increases throughout infection.⁸⁸ Furthermore, serum levels of the Th1-associated cytokines IFN- α , IL-12, and TNF- α peak earlier in infected suckling than in weaned piglets, suggesting that sucklings have a more rapid disease progression.

IFNs play an important role in halting viral replication, including that of coronaviruses. PEDV, however, is partially resistant to type I IFNs even though STAT1 expression is significantly reduced in PEDV-infected cells.¹⁶³ This reduction does not arise from alteration of STAT1 transcription but appears to result from PEDV-CoV-induced STAT1 ubiquitination and subsequent degradation in the proteasomes.¹⁶³ Removal of STAT1 inhibits both types I and type II IFN responses.

PEDV-infected suckling pigs have higher and earlier increases in the levels of serum IFN- α than do weaned pigs. They have lower levels of TNF- α and the proinflammatory **chemokine** IL-8, which attracts neutrophils to the infected area. Neutrophils are protective early during infection, but their prolonged presence is pathogenic.⁹⁵ These findings are similar to those in TGEV infection of suckling.¹⁶⁴

6.4.5.8 Vaccination against porcine epidemic diarrhea virus

One **attenuated** (weakened, nonpathogenic) form of PEDV contains a deletion of 9–26 amino acids in ORF1a/b and the S protein in addition to mutations in other proteins. The deletion of one amino acid of the S protein may be a marker for attenuated PEDV.¹⁶⁵ Some of the vaccines provide adequate protection against the traditional strains of PEDV, but not against new PEDV epidemic strains.¹⁶⁶ Inactivated and subunit PEDV vaccines for pregnant sows fail to protect neonatal piglets. Additionally, many highly attenuated PEDV/TGEV strains replicate poorly in vivo and provide only low levels of immunity in weaned pigs and sows.¹⁶⁷ A safe and efficacious live, attenuated vaccine may provide adequate protection for the young piglets.¹⁶⁷ While inoculation with one such live, attenuated vaccine strain did not cause diarrhea in pigs, it did not protect against challenges with a different, virulent viral strain. PEDV RNA was present in the feces of almost all inoculated piglets. However, one of the attenuated vaccines did produce higher levels of PEDV-specific IgG and viral neutralizing antibodies by 14 days postinfection. Importantly, vaccinated piglets rapidly reduced the levels of viral RNA. One of the attenuated vaccines also produced higher serum levels of PEDV-specific plasma IgG, IgA, and neutralizing antibodies in addition to increased levels of intestinal IgA.¹²⁹ In pregnant sows that recovered from an infection, gut-derived IgA lymphocytes travel to the mammary glands and produce high titers of IgG and secretory IgA antibodies in the colostrum and milk, respectively, which protects suckling piglets against infections.¹²⁹ Unfortunately, the available PEDV vaccines do not completely protect piglets against infection by highly pathogenic viral strains. An ideal vaccine would produce IgG antibodies in the sows that are transmitted to her offspring in utero¹⁰⁰ and IgA in milk that would protect suckling piglets from PEDV.

Since PED attacks neonatal piglets, there is insufficient time before disease onset for an infected piglet to develop its adaptive immune response. It may be protected by passive immunity by its mother's IgA in her colostrum if the sow is vaccinated before birth.⁹⁷ Three-to-four-day old calves also may receive a live attenuated intranasal vaccine that rapidly results in IFN production and protection.⁴⁸

Immunity against pig coronaviruses is conferred by IgA against enteric or respiratory disease or by IgG against viremic disease. It is also clear that for many of these coronaviruses, a T cell-mediated response is required for significant protection. Livestock producers need to minimize loss as well as expenses accrued by unnecessary vaccination. This may be conducted by revaccination only when herd immunity is decreased significantly.⁴⁸

Multiple inactivated PEDV vaccines are available and often combined with anti-TGEV and anti-rotavirus vaccines and are administered as a single dose.⁴⁸ Another anti-PEDV vaccine was constructed to simultaneously immunize sows against PEDV, TGEV, and the enteric bacterium *Escherichia coli*.¹⁵ Another approach to vaccination against PEDV is administered in a series of live-killed-killed or live-live-killed-killed vaccines. Other

vaccine approaches include the insertion of the PEDV S protein gene into viral vectors; subunit vaccines expressed in Baculovirus, yeast, or plant cells; and insertion of PEDV S, N, or M protein genes into **plasmid**-vectored DNA vaccines. One such vaccine against TGEV induces IgG, IgA, IL-4, and interferon- γ and a similar vaccine may be effective against PEDV infection.¹⁶⁸

6.4.6 Porcine deltacoronavirus

6.4.6.1 Introduction to porcine deltacoronavirus

PDCoV (formerly PorCoV HKU15) is an emerging deltacoronavirus that readily infects human and chicken cell lines in vitro. Wild and domestic birds are believed to have been the hosts of the ancestors of deltacoronaviruses, including PDCoV. PDCoV is a member of the same species as the bird deltacoronavirus Sp-CoV HKU17 from sparrows and QuaCoV UAE-HKU30 from quails,^{169,170} demonstrating that at least some deltacoronaviruses can be transmitted from birds to mammals. The ability of this virus to infect human cells in vitro suggests that it may also be able to infect humans. This is a matter of concern, given the high mortality rate of PDCoV in pigs. Nevertheless, since none of the swine coronaviruses has yet to be proven to cause disease in humans, the harmful effects of PDCoV on humans are currently economical rather than medical.

PDCoV was first reported in pigs in China in 2007 and Hong Kong in 2012. In 2014, PDCoV was detected in suckling piglets in North America, where 10.1% of the animals tested positive for this virus. By 2014, PDCoV was also reported in East to Southeast Asia^{171–173} in mainland China, Thailand, Laos, Vietnam, and South Korea.¹⁶⁸ Viral strains causing outbreaks in the United States have highly similar genomic RNA and are closely related to those from Hong Kong, while other Asian strains were more diverse and **para-phyletic**, belonging to a group that contains some, but not all, descendants of a common ancestor.¹⁷³ Most of the members of the deltacoronavirus group are found in multiple bird species.

PDCoV is found in wild animals as well. It was detected in 1%–2.5% of rectal swabs of Asian leopard cats (*Prionailurus bengalensis*) and Chinese ferret badgers (*Melogale moschata*) in live animal markets and is more closely related to these coronavirus strains than to those from birds.¹⁷⁴ The infection rate in pigs from mainland China exceeds 30% and often is found in pigs concurrently infected with PEDV. The overall global identity among PDCoV isolates is 98.9%,¹⁷⁵ although multiple mutations and deletions are found in the S protein and nsp genes in Chinese strains, but not those from the United States.

6.4.6.2 Pathology due to porcine deltacoronavirus

PDCoV can cause profuse watery diarrhea, vomiting, dehydration, anorexia, wasting, and may result in death, depending upon the age of the pig. In older pigs, PDCoV infection may be asymptomatic or may lead to severe disease.¹⁵ In neonatal piglets, however, the mortality rate is high and is at least partially dependent upon the viral strain. Infection with American strains of PDCoV results in a mortality rate of up to 40% in suckling pigs, while an outbreak in China reported a mortality rate of greater than 80%.^{175,176} The S protein of Chinese viral variants contains a deletion of three nucleosides which is absent from

American and Korean PDCoV strains. This mutation may contribute to the difference in mortality rates among the stains.¹⁷⁵ While high, the mortality rate in PDCoV-infected piglets is less than that associated with PEDV or TGEV infections, which have mortality rates of 90%–100%.^{174–176} Due to differing degrees of pathology and mortality, correct differential diagnosis of these three porcine coronaviruses is very important in areas, including the United States, where all these coronaviruses circulate.

Diarrhea begins 1–2 days after the detection of PDCoV RNA in the feces, with viral RNA levels peaking 1–3 days later.¹⁷⁶ Diarrhea persists for up to 5 days postinfection and is accompanied by prolonged shedding of the virus in feces, even after recovery in surviving animals. The enteric symptoms of PDCoV infection are indistinguishable from those caused by PEDV or TGEV. Infected piglets have gross lesions in the gastrointestinal tract, with thin, transparent intestinal walls containing large amounts of yellow fluid.

Unlike TGEV and PEDV, PDCoV infection also produces severe lesions in the stomach of very young animals.¹⁷⁴ In nursing pigs, the stomach contains curdled milk. Compared with grower pigs (gastric pH 2–3), the stomachs of neonatal pigs have a higher pH (4–6). The pH in the neonatal animals is thus much closer to neutral and therefore allows PEDV to survive in this locale.¹⁷⁷

While PDCoV replicates only in the small intestine, moderate levels of viral RNA are also present in the blood, saliva, enteric lymph nodes, lungs, liver, and kidneys, indicating multisystemic dissemination.^{173,174} Unlike TGEV and PEDV, PDCoV may also cause mild interstitial pneumonia in infected piglets. Furthermore, pigs are commonly coinfecting with PEDV or TGEV as well as with rotavirus C, a member of the Reoviridae family of double-stranded RNA viruses. Rotavirus C is found in 58% of PDCoV-infected piglets.¹⁷⁶

6.4.6.3 Porcine deltacoronavirus—the virus

PDCoV uses APN as its cell surface receptor as does HCoV-229E from humans, pig PEDV and TGEV, feline coronavirus type II, and canine coronavirus type II.¹⁵⁰ In humans, APN is primarily expressed on the epithelial cells of the kidneys, intestines, respiratory tract, monocytes, the endothelial cells that line blood vessels, cerebral cells at the **blood-brain barrier**, and **synaptic** membranes on cells of the CNS. Of these, the cells of the small intestine and renal tubular epithelium have the highest level of APN expression.¹⁵⁵

Genetic recombination in the area of the S protein is common among deltacoronaviruses. Since the S protein plays a major role in determining potential host species, this recombination may lead to cross-species transmission of coronaviruses. One method of bird-to-bird transmission of deltacoronaviruses has been shown to involve the food chain, in which a predatory bird (falcons) is infected by its prey (houbara bustards and pigeons). These birds vary greatly in both behavior and habitat. Falcons (*Falco* species) are medium-size birds of prey in Arabian areas, houbara bustards (*Chlamydotis undulata*) are large birds that are restricted to arid areas, and pigeons (*Columbidae* species) are smaller and are found worldwide.¹⁶⁹ PDCoV is the only deltacoronavirus known to infect mammals.¹⁷⁸ It is believed to have arisen by recombination between HKU17 and coronavirus HKU11 of bulbuls (*Pycnonotidae* species), songbirds found in most of Africa, the Middle East, Asia, and Indonesia.^{169,172} Despite the zoonotic transmission of several pandemic influenza strains from birds to pigs to humans as well as a rare jump of avian influenza from birds to humans, gammacoronaviruses and deltacoronavirus are not known to infect humans.

PDCoV has dual tropism and is present in fecal/intestinal samples as well as in nasopharyngeal samples, especially during winter. The locations of PDCoV imply that the virus can cause enteric and respiratory infections in pigs. This also suggests that, in addition to fecal-oral transmission, the virus could spread among pigs via the respiratory route.¹⁷³ Interestingly, the RNA of PDCoV samples from the respiratory tract and feces do not have significant differences in the genes encoding the S protein.¹⁷³ Based on genetic analysis, nasopharyngeal samples contain at least two viral quasispecies. The viral outbreak quasispecies in the United States are very closely related to each other. The Asian PDCoV quasispecies are more diverse and, very rarely, have been found outside the digestive tract in blood, livers, lungs, kidneys, mesenteric lymph nodes, and saliva.¹⁷³

The United States PDCoV strains infect the cells lining the entire length of the small and large intestinal tracts, especially those of **jejunum** and **ileum** areas of the small intestines, which contain villi that increase the intestinal surface area that is needed for optimal absorption of nutrients. Like that used by other pig **enteroviruses**, PDCoV-infected gut cells are rapidly killed by acute necrosis, leading to the loss of villi in the small intestine. PDCoV does not induce **apoptosis** in the intestinal lining cells.¹⁷⁶ Malabsorption due to loss of villi or abnormal functioning of the enterocytes may be at least partially involved in PDCoV-induced diarrhea. Infection of cells lining the colon may also decrease their ability to absorb water and electrolytes.¹¹⁷ Macrophages, lymphocytes, eosinophils, and neutrophils infiltrate the mucus lining of the small intestine.¹⁷⁶

While PDCoV is typically transmitted by the fecal-oral route and may spread between farms on the wheels of contaminated transport trailers and in pig feed,¹⁷⁶ it was found in 9.6% of nasopharyngeal samples in Hong Kong, usually during the winter. Accordingly, PDCoV might be transmitted by the respiratory route as well even though lung tissues of orally infected pigs did not have detectable levels of virus **antigens**.^{173,176} A separate study, however, did not find the virus on nasal swabs or **bronchoalveolar lavage** fluids 3–10 days after infection, nevertheless the animals had developed a clinical disease and were shedding the virus in their feces at this time.¹⁷⁶ The ACoV TGEV and the betacoronavirus BCoV are also present in fecal and respiratory samples.²³

6.4.6.4 Treatment of porcine deltacoronavirus

Since no effective PDCoV vaccine has been developed, preventive measures attempt to maintain effective biosecurity rules. Treatment of infected animals includes hydration and eliminating of secondary infection with pathogenic bacteria, including *Actinobacillus pleuropneumoniae* and *Mycoplasma hyopneumoniae*.⁸⁹

6.4.7 Porcine hemagglutinating encephalomyelitis virus

6.4.7.1 Introduction to porcine hemagglutinating encephalomyelitis virus

PHEV is a betacoronavirus that cause **HI encephalomyelitis**. It was first reported in Canada in the fall of 1957 and in Western Europe in the 1970s. The virus is also found in commercial farms in Western Poland.¹⁵ In some farms, this disease is present in 80% of the piglets.

6.4.7.2 Pathology due to porcine hemagglutinating encephalomyelitis virus

HI encephalomyelitis in suckling piglets is initially asymptomatic but the disease is found in the CNS a week after infection when replication occurs in the stomach and ganglia of the digestive and nervous systems, respectively. Initial symptoms are vomiting and the presence of undigested milk clots in the stomach. While a lack of locomotor coordination is sometimes seen, HI encephalomyelitis may cause severe neurological disease with a mortality rate approaching 100%.¹⁵

In young piglets, the infection may be characterized by vomiting, severe weight loss, shivering, huddling, squealing, and anorexia, also known as “vomiting and wasting disease.”¹⁷⁹ PHEV also may cause serious infection of the neural system which includes polioencephalomyelitis with **perivascular cuffing** of mononuclear cells (a dense mass of lymphocytes clustering around a blood vessel), degeneration of neurons, and **gliosis** (formation of a dense fibrous network of glia in the CNS that may lead to scarring).¹⁸⁰ PHEV has also been associated with an influenza-like respiratory illness.¹⁸¹

PHEV is presently the only known neurotropic pig coronavirus. While some rodents may be experimentally infected with PHEV, only pigs are reported to be infected with this virus naturally. While it primarily replicates in the respiratory tract, PHEV enters peripheral axons and moves from the peripheral nervous system to the CNS across **neural synapses**.¹⁸² In the CNS, the virus only replicates in the cytoplasm of sensory neurons,¹⁸² while the brainstem, horns of the spinal cord, and trigeminal ganglia are affected. The virus may also be found in the cerebrum and cerebellum.

While PHEV infects pigs of any age, clinical disease is typically found only in young piglets.¹⁷⁹ These young animals commonly develop muscle tremors and **hyperesthesia** (extreme pain sensitivity). This may be accompanied by a jerky gait; walking backward; a backward arching of the head, neck, and spine; and weakness and loss of ability to stand. Blindness and **nystagmus** (rapid uncontrollable eye movements), and coma may also be found.¹⁸³ PHEV is prevalent in swine herds throughout the world, with subclinical manifestations in older animals. Previously infected dams may pass on protective antibodies to their offspring.¹⁸⁴ High levels of IgA and IgG are present by 10 days of infection, peaking at day 28, and slowly declining afterward. The amounts of IL- α , TNF- α , and IL-8 also increase.

6.4.7.3 Porcine hemagglutinating encephalomyelitis virus—the virus

Unlike the host cell receptors of most coronaviruses, PHEV's S protein binds to the **neural cell adhesion molecule (NCAM; CD56)**, a coronavirus receptor that bears sialic acid at the terminus of its glycoproteins.^{185,186} NCAM is found on neurons, glial cells, skeletal muscle, and NK cells. Its functions include cell-cell adhesion, **synaptic plasticity** (the strengthening or weakening of neural synapses over time), learning, memory, and the production and growth of **axons**, especially in very young animals whose brains are developing. The binding of NCAM to PHEV's S protein may disrupt NCAM activity and affect a large range of normal neural activity.

In addition to the S, M, E, and N proteins, PHEV also has a bifunctional **hemagglutinin-esterase enzyme** that has both hemagglutinin and esterase activities. The hemagglutinin activity promotes binding to sialic acids, including those present on

NCAM, and aids in attachment to red blood cells.¹⁸⁵ Its acetylcholinesterase activity acts as a receptor-destroying enzyme.¹⁸⁷ A hemagglutinin-esterase enzyme is also present in BCoV and has similar functions.

6.4.7.4 Protection against porcine hemagglutinating encephalomyelitis virus

The only method of PHEV prophylaxis currently in use is the early contact of piglets with sows. Infected sows passively immunize their piglets with virus-specific antibodies via their colostrum, which protects the piglets for about four weeks.¹⁸⁸

6.4.8 Swine acute diarrhea syndrome coronavirus

6.4.8.1 Introduction to swine acute diarrhea syndrome coronavirus

The ACoV SADS-CoV was first reported in 2017 in the Guangdong Province in southeastern China where it is currently a regional epizootic virus.^{189–192} A retrospective study, however, found evidence that SADS-CoV has infected pigs as early as August 2016 and about 17% of the pigs were coinfecting with PEDV.⁸⁶ Another outbreak of SADS-CoV-related diarrhea occurred in Southern China in February 2019.⁸⁶ SADS-CoV is also known as swine enteric ACoV (SeACoV) and porcine enteric ACoV (PEAV).

6.4.8.2 Pathology due to swine acute diarrhea syndrome coronavirus

SADS-CoV causes severe diarrhea in neonatal piglets and was responsible for a large, sometimes fatal, outbreak in Chinese piglets in 2016–17. Among pigs less than 5 days old, the mortality rate is 90%–100%, but only 5% in pigs older than 8 days.¹⁹³ Afterwards, no new SADS cases were reported from May 2017 to January 2019, but the disease reemerged in Southern China on February 2019, in a large outbreak that killed 2000 piglets.⁸⁶ The S protein of the SADS-CoV strain responsible for the 2019 outbreak had two amino acid changes when compared to the previous SADS-CoV S protein.

Lower relative levels of **serotonin**-secreting enteric, hormone-producing cells are found in the mid-jejunum and ileum or the colon of pigs that vomit than in control pigs, indicating levels of serotonin (5-HT) may directly or indirectly induce vomiting.¹⁰⁶ Serotonin is both an immune system modulator and a neurotransmitter. SADS-CoV causes mild-moderate intestinal damage in three-day-old piglets in which the colon contains large amounts of yellow liquid causing the intestine to become inflated with thin and transparent walls.¹⁹¹ Experimentally infected **immunocompetent** mice only develop a subclinical infection that does not damage intestinal tissues. At least part of SADS-CoV pathology is due to the apoptotic death of infected cells both in vitro and in vivo in animal models. SADS-CoV induces apoptosis via both caspases and mitochondria-mediated pathways.¹³⁵

6.4.8.3 Swine acute diarrhea syndrome coronavirus—the virus

SADS-CoV currently has the largest species range in vitro among known coronaviruses, infecting 21 of 24 cell lines from different tissue types and a variety of species: humans

and nonhuman primates, bats, mice, rats, gerbils, hamsters, pigs, and chickens.¹⁹¹ In vivo studies are needed to confirm that this wide host species extends to animal models. The virus infects primarily splenic dendritic cells in vivo in mice.¹⁹⁴

The SADS-CoV host cell receptor is currently unknown but is not ACE2, porcine APN, or DPP4.¹⁹³ Due to its wide host and tissue range in cell culture, the SADS-CoV receptor is likely to be a common molecule whose genes are conserved among the hosts.¹⁹⁴ Besides pig intestinal cells, SADS-CoV RNA is present in the heart, liver, spleen, kidneys, stomach, and lungs, but not in the serum, of piglets **euthanized** on day 7 postinfection, a time at which the pigs still had severe diarrhea. SADS-CoV leads to prolonged infection of the spleen, especially in the DC of that immune organ.^{89,194}

SADS-CoV RNA is very closely related to that of the bat ACoV HKU2 from the bat *Rhinolophus sinicus*. The RNA of the GDS04 strain of SADS-CoV has a 95% overall nucleoside identity with that of HKU2.¹⁸⁹ However, they share only 80% and 87% identity with the RNA and proteins of the S protein, respectively, and the S protein of SADS-CoV is six nucleosides longer than that present in HKU2.¹⁸⁹ Given that the closeness of their genes, these coronaviruses appear to have shared a common ancestor. A comparison of the nucleosides of the terminal domains of the S1 subunit of the S protein gene of HKU2 and SADS-CoV found them to be closely related to each other and some β -coronaviruses. The differences are primarily located in the RBD of S1.^{190,195} The HKU2 and SADS-CoV S proteins are also much smaller than that present in other closely related coronaviruses and their amino acid identities with these other coronaviruses are less than 28%.¹⁹⁵ Except for the 5' region of S1, the remainder of the SADS-CoV S protein is similar to that found in betacoronavirus. This region of the SADS-CoV genetic RNA is associated with intestinal tropism. It contains 75 amino acid substitutions and a 2 amino acid insertion which are not present in HKU2. These alterations may be responsible for SADS-CoV having a greater host range and transmission.¹⁹⁰ Taken together, the similarities of SADS-CoV and HKU2 to betacoronaviruses, Yu et al.¹⁹⁶ suggest that they should be placed into a subgroup of betacoronavirus and these viruses may have formed by the recombination of the ACoV TGEV backbone with an S protein gene from a currently unknown betacoronavirus S gene.¹⁹⁶ Additionally, the S2 subunit of SADS-CoV and HKU2 share a unique conformation.¹⁹⁶ The genomes of these two viruses place them in a group that also contains a rat coronavirus, Lucheng Rn rat coronavirus.¹⁹⁶

Bat-to-pig transmission may have occurred since a few HKU2-like CoV RNA sequences are present in several species of horseshoe bats, including the intermediate horseshoe bat (*Rhinolophus affinis*), Chinese rufous horseshoe bat (*R. sinicus*), and king horseshoe bat (*Rhinolophus rex*) from the coastal Guangdong Province of China from 2013 to and 2016.¹⁹³ Importantly, the S proteins of two of these bat viruses have ~98% identity, strongly suggesting that horseshoe bats may function as viral reservoirs. The route by which these viruses were transmitted from bats to pigs is not yet known.¹⁹¹ One hypothetical route of transmission is that infected bats could release their guano and contaminate the pig feed which would then infect the pigs. Alternatively, mice, rats, or other rodents could eat the contaminated pig feed and become infected. Pigs could subsequently become infected by consuming rodent feces in their food. In support of the latter postulate, bat HKU2-like coronaviruses are grouped with rat coronaviruses in the phylogenetic tree.¹⁹¹ After being

introduced into the pig population, pig-to-pig transmission could occur and the HKU2-like bat coronaviruses, with their high mutation rate, could evolve and adapt to their new pig hosts.

An additional recombinant strain, swine enteric coronavirus, was reported in Slovakia in early 2015. It has also been found in the Czech Republic, Italy, and Germany.^{86,197} This virus has a mortality rate of 30%–35% and is preceded by yellow watery diarrhea and dehydration. Since no vaccine is currently available, pregnant sows are dosed orally with a 10% suspension of material from the intestine and feces of infected piglets in warm water. After 3 weeks, newborn piglets are healthy.¹⁵

6.4.9 Transmissible gastroenteritis virus and porcine respiratory coronavirus

6.4.9.1 *Introduction to transmissible gastroenteritis virus and porcine respiratory coronavirus*

TGEV was reported in the United States in 1945.¹⁸³ It was a major cause of endemic and epidemic viral **enteritis** in neonatal and older pigs.⁹⁴ After multiple passages in cell culture, the TGEV strains become **attenuated** (less virulent) and may infect the respiratory system, rather than the intestines. Several pairs of virulent and attenuated TGEV strains differ by a point mutation in nucleoside 1753 of the S protein.¹⁹⁸ While inactivated (“killed”) TGEV suspensions induce early and strong production of IFN- α by leukocytes, some viral strains having mutations in the gene encoding the M protein have 30–300-fold lower levels of IFN- α transcription and translation than the typical virulent strains.¹⁹⁹

A much less pathogenic form of TGEV, PRCV, was discovered in 1984 in Belgium and, later, in the United States, Canada, Japan, Korea, Croatia, and Slovenia.²⁰² It is a natural mutant of TGEV containing deletions in the genes for its S protein (621–681 nucleosides) and ORF3. The overall identity of genomic RNA between TGEV and PRCV is almost 98%, suggesting a common origin.¹⁵ While European strains of PRCV had a 672-nucleoside deletion in the S protein gene, the initial PRCV strains from the United States had a 681-nucleoside deletion. The size of the deletion varies in strains isolated later.¹⁹⁸

After the emergence of PRCV, fewer TGEV infections have been reported.¹⁸³ In fact, while TGEV killed many piglets in the 1990s and subsequently lead to great economic loss, fewer vaccines are available against TGEV are available in North America and Europe due to the virtual disappearance of this virus throughout much of the world. Outbreaks of TGEV still occur in parts of eastern Asia.⁹⁶

6.4.9.2 *Pathology due to transmissible gastroenteritis virus and porcine respiratory coronavirus*

TGEV is a form of BCoV that is typically present in enterocytes of the small intestine. It causes severe diarrhea, vomiting, and dehydration that is almost always fatal in **seronegative** neonatal piglets.¹⁹⁸ It is also found, to a lesser extent, in the respiratory tract where it is shed transiently via nasal secretions.²⁰⁰

PRCV is a form of BCoV/TGEV. The above-mentioned deletion in TGEV’s S protein is of great importance since the S protein plays a major role in target cell **tropism**.²⁸ The great majority of PRCV is found in the upper and lower portions of the respiratory tract,

including the nasal mucosa, tracheal, bronchial, and **bronchiolar epithelium**; type 1 and 2 pneumocytes; **alveolar macrophages**; and tonsils.²⁰¹ It typically causes mild respiratory disease, such as coughing, that is accompanied by **bronchoalveolitis** and airway plugging, moderate to marked **consolidation** of the lungs, and necrosis of cells lining the upper and lower respiratory tract.²⁰² PRCV replicates at a moderate to a high degree in the lungs 4–8 days after infection, but only replicates to a small degree in **villous enterocytes** of the intestines. Accordingly, unlike TGEV, PRCV does not cause intestinal disease.²⁰

6.4.9.3 Transmissible gastroenteritis virus and porcine respiratory coronavirus—the viruses

Severe disease is also found in pigs that are coinfecting with PRCV and a mild strain of porcine reproductive and respiratory syndrome virus (PRRSV), which is not a coronavirus.²⁰³ PRRSV increases the number of abortions and severe respiratory tract disease in young pigs. PRRSV infection typically decreases innate immune responses while PRCV increases innate immunity. Dual-infected pigs have more severe pneumonia. Both the innate and adaptive immune responses are suppressed. Levels of IFN- α in the lungs and activity of blood NK cells are reduced, while PRRSV replication increases. Levels of the proinflammatory Th1 cytokine IFN- γ increase while those of the T helper 2 (Th2) cytokine IL-4 decrease.²⁰³ Apoptosis of pulmonary alveolar macrophages also increases in coinfecting pigs. Coinfecting animals have higher levels of IgA antibodies against PRCV in their respiratory tract. This might at least partially contribute to the reduced PRCV nasal shedding and replication in the lungs of the dual-infected pigs.²⁰³

Suppression of an innate immune response due to prior infection with another respiratory pig virus, such as PRRSV and swine influenza H1N1, can exacerbate the pathogenesis of respiratory coronavirus infections, such as that caused by the typically mild disease agent PRCV.²⁰⁰⁶ Additionally, PRCV-infected animals exposed to certain key components of Gram-negative or Gram-positive bacteria (**lipopolysaccharide** and **lipoteichoic acids**, respectively) have a higher degree of respiratory disease compared with pigs infected with PRCV alone.²⁰⁴

TGEV uses APN as its host cell receptor, but still binds to sialic acid, the loss of which prohibits it from infecting gut cells.¹⁵⁴ One region of TGEV's S protein recognizes porcine APN while another region of the virus' S protein recognizes the sugar coreceptors, N-acetylneuraminic acid, and N-glycolylneuraminic acid.

HCoV-229E of humans and TGEV belong to the same coronavirus group and HCoV-229E also uses APN as its receptor for the viral S protein. In this case, binding to APN is host-specific since HCoV-229E binds to human, but not porcine APN, while TGEV binds to its porcine counterpart, but not to human APN, as stated above. Since coronaviruses are prone to mutations and genetic recombination, TGEV may at some point be able to bind to human APN and gain entry into cells of the human digestive system and cause mild to severe diarrhea. APN is also present on the surface of cells in the kidney, respiratory tract, several immune system cells, and parts of the nervous system, suggesting possible infection of these cells as well. TGEV, while typically associated with the intestines has also been found in the nasopharynx of pigs.²³ TGEV thus infects and replicates in enterocytes of the small intestine but can also infect cells of the respiratory tract.²⁰⁵

Infection by the TGEV mutant, PRCV, only produces a very mild and transient disease in swine, regardless of their age. It has moderate immunological cross-reactivity with pig TGEV and human SARS-CoV, suggesting that these coronaviruses may have a common ancestor.¹⁸³ PRCV is closely related to feline enteric coronavirus and canine coronavirus as well.²⁰⁰² PRCV has been divided into European and American types which appear to have arisen independently.

PRCV is spread via aerosol and pig-pig contact, typically postweaning after transplacentally-acquired maternal IgG and IgA from milk is lost and before the piglet itself produces adequate levels of antibody. Swine in the United States also lack PEDV-specific protective immunity in their milk.¹⁴³ Viral transmission is at least partially dependent on season in some herds and is not present during the summer months. Other risk factors include pig population density and distance to neighboring swine farms since infectious PRCV may travel several kilometers by aerosol distribution.²⁰² It also remains in air samples of experimentally infected pigs for 6 days.

A TGEV isolate from pigs coinfecting with TGEV and PRCV retained its virulence even though in the field outbreak, the diarrheal disease was of reduced severity. The gene deletion in the S protein region of the PRCV isolates from these pigs differed from those previously reported. Variable sequence changes in ORF3 also affect its size and amino acid sequence. Mutations or deletions in ORF3a play a role in the reduced ability of PRCV to multiply in the intestine. The reduced severity of TGE in this herd could be due to the presence of PRCV, which induce antibodies that cross-react with TGEV and decrease disease severity.²⁰⁵ PRCV may also infect pigs by contact and all pig age groups are susceptible. Experiments showed that the susceptible pigs experimentally infected with PRCV shed virus from nasal secretions for less than 2 weeks. Levels of TGEV in feces are small and there are no reports of virus transmission via saliva.¹⁵

TGEV and PEDV target differentiated enterocytes covering the villi of the pig small intestine, disrupting the actin microfilaments of the cytoskeleton.^{206,207} These microfilaments act as girders of the cell, controlling the movement of the cell and movement of material around the interior of the cell as well as playing an important role in endocytosis and vesicle transport. Since TGEV and other coronaviruses enter the cells via vesicles during endocytosis, altering actin structure allows these viruses to invade gut epithelial cells and, through them, enter the intestinal lumen. TGEV infection triggers F-actin to gather at the cell's plasma membrane, polymerize, and form ruffles and protrusions. Disrupting the F-actin organization inhibits TGEV from entering its target cells. When the TGEV spike protein binds to EGFR, it sets in motion the microfilaments' polymerization by activating an intracellular signaling pathway that involves phosphoinositide-3 kinase and Rac1/Cdc42 GTPases as well as the ERK MAPK pathway. Inhibition of EGFR and phosphoinositide-3 kinase activity decreases the entry of TGEV.²⁰⁸

Soon after infection of the cells, TGEV disrupts the barrier integrity of intestinal epithelial cells *in vitro* by downregulating some proteins that form the tight and adherens junctions between cells that prevent all but selected material in or out of the intestinal lumen.¹³¹ PEDV also causes some damage to the integrity of the epithelial barrier. Coinfection with PEDV and TGEV creates a greater amount of damage to tight junctions and remodeling of microfilaments than either virus alone.¹³¹

6.4.9.4 The immune response to transmissible gastroenteritis virus and porcine respiratory coronavirus

Macrophages and lymphocytes infiltrate the infected regions along with inflammatory cytokines, including IFN- α , TNF- α , IL-6, IFN- γ , and IL-12,^{203,209} similar to the events induced by SARS-CoV or SARS-CoV-2 in humans, although the extent of proinflammatory conditions is much lower in the PRCV-infected pigs and is not **systemic**.²⁰ The early increases in the levels of innate immune system cytokines may block viral replication early after infection and modulate the Th1/Th2 (IFN- γ /IL-4) responses in favor of a Th2 response that stimulates B lymphocytes to secrete protective neutralizing antibodies²⁰ rather than an antiviral inflammatory condition. In addition to fever, anorexia, and delayed growth, PRCV typically causes mild-to-moderate self-limiting respiratory illness. The virus can be isolated readily from nasal swabs for 6–10 days postinfection.^{205,210} By themselves, respiratory coronavirus infections of pigs generally result in a very mild, short-lived disease characterized by coughing and respiratory distress, regardless of their age.

6.4.9.5 Vaccines against transmissible gastroenteritis virus and porcine respiratory coronavirus

Modified live and inactivated TGEV vaccines are licensed for use in the United States. The former is administered to pregnant sows or orally to nursing or weaned piglets to induce active immunity, but fails to induce a strong IgA response. By contrast, the inactivated vaccines are given to nursing or weaned piglets intramuscularly. They are not effective against acute infection but can help to control low-level enzootic infections.⁴⁸

Due to the lack of an effective commercial vaccine for TGEV and the virtual absence of mortality in PRCV-infected animals, live PRCV is a potential vaccine candidate against TGEV. Piglets born to sows previously exposed to PRCV have a decrease in TGEV-induced mortality of 11%–67%.²⁰⁰² Large pig nurseries in the United States that are infected by PRCV do not have high piglet mortality or morbidity and less spread to neighboring farms.²⁰⁵ Some PRCV isolates, however, cause pathogenic respiratory disease.

6.4.9.6 Treatment of transmissible gastroenteritis virus and porcine respiratory coronavirus

Some of the drugs that are developed for use against one coronavirus are active against other coronaviruses as well since they use similar enzymes or signaling pathways. One such drug is A9, a receptor tyrosine kinase inhibitor that works by blocking the addition of a phosphate molecule onto a receptor. Since phosphorylation is necessary for the action of the receptor and blocking this process changes the activity of the infected cell. Some viral proteins are also phosphorylated, including the N protein of SARS-CoV.²¹¹ When added to cells infected with TGEV in vitro, A9 potently blocks TGEV's replication. A9 also has strong antiviral activity against other coronaviruses, including MHV in mice, PEDV in pigs, and FIPV in cats. The antiviral activity of A9, however, needs to be tested in vivo to show its usefulness in decreasing disease severity.

A9 inhibits the activity of p38, one of the mitogen-activated protein kinase (MAPK) signaling pathways that are required for TGEV replication.²¹¹ TGEV increases

phosphorylation of p38 by greater than 10-fold when compared to untreated control cells.²¹¹ Since many potential host cell types use the p38 pathway, this drug may be widely applicable against other coronaviruses of animals and humans. For example, infection of a macrophage cell line by this coronavirus results in the activation of two MAPKs, p38, and c-Jun N-terminal kinase (JNK) pathways, but not of the ERK, the other MAPK pathway. p38 phosphorylates eIF4E, a protein involved in the translation of proteins and, by doing so, aids in virus protein synthesis.²¹² Activation of p38 also increases the production of the proinflammatory cytokine IL-6, despite the general inhibition of host protein synthesis.²¹²

6.5 Coronavirus of horses

6.5.1 Introduction to coronaviruses of horses

In 1975, equine coronavirus (ECoV) was reported in the feces of foals and adult horses with enteritis.²¹³ This virus is also present and causes disease in donkeys.²¹⁴ Infection of horses is associated with fever and depression as well as digestive system pathology in adult horses. Case numbers of ECoV have been rising since 2010 in Japan, the United States, and Europe.²¹⁵ Draft horses in the United States and Japan have the highest rate of infection and outbreaks are often found in riding, racing, and show horses and less frequently among breeding horses.²¹⁶ This may be due to fecal-oral transmission between asymptomatic young animals and adult horses in breeding farms and the ensuing production of protection against symptomatic infection.²¹⁵ Although the rate of morbidity ranges from 10%–to 80%, most infections are self-limiting but with intensive supportive care, the mortality rate in adult horses is low.²¹⁴

6.5.2 Pathology due to coronavirus of horses

In 2000, a novel coronavirus was reported in feces and intestinal tissue of a neonatal foal with a protracted case of neonatal enterocolitis. Symptoms of ECoV infection include fever, depression, anorexia, watery diarrhea, low levels of protein in the blood, **hyperlipidemia** (increased levels of fats in the blood), anemia, thrombocytopenia, dehydration, and imbalance of electrolytes in the absence of enteric bacterial pathogens.^{215,217,218} Anorexia may partially be responsible for decreased electrolyte intake. The disease is typically self-limiting and the animals recover with supportive care.²¹⁹ Foal and adult horses of some breeds of horses are more susceptible than others.

In some cases, naturally infected horses may develop severe diffuse necrotizing enteritis that is similar to that present in BCoV. The lumen of the small intestine has shortened villi containing necrotic enterocytes, the presence of a **pseudomembrane** composed of a membranous mass of cells and fluid, hemorrhage, and **microthrombosis**. Necrotic cells were also found in the crypts between intestinal villi.¹⁰

Euthanization of a foal with the severe disease found mucosal ulceration of the intestines and mucosal to submucosal edema as well as moderate accumulation of lymphocytes and **plasma cells** (antibody-producing B lymphocytes). The peritoneal cavity contained large amounts of transparent, watery fluid. Submucosal or mucosal edema was present in

the small intestine and the colon contained a green mucoid material. Raised tan nodules with caseous (cheese-like) centers were present in the lung.²¹⁷ Infection of intestinal cells may result in severe **diffuse necrotizing enteritis** during which regions of the intestines become inflamed, causing a hole in the intestinal walls that allows bacteria to leave the intestines and enter blood vessels. This may lead to secondary **endotoxemia** and septicemia in which bacterial toxins and other toxins are present in the blood.

The injury to the intestine may result in malabsorption and maldigestion, in addition to the damage to the epithelial cells lining the intestinal lumen, which may lead to dehydration and metabolic dysfunction. ECoV may also disseminate from the intestine and produce nodules in the liver, spleen, and lungs.²¹⁴ Adaptive and innate immune system activities are also decreased since both lymphopenia and neutropenia are often present in infected horses.

ECoV may also cause neurologic disease in horses that are responsible for most of the ECoV-associated deaths. The neurological disease includes **hyperammonemia encephalopathy**, in which often fatally high levels of ammonia in the blood damage the nervous system.^{214,219} This may be accompanied by **astrocytosis** throughout the **cerebral cortex** of the brain. Astrocytosis is characterized by abnormally high numbers of **astrocytes** resulting from the destruction of surrounding neurons.²¹⁴ Encephalopathy occurs in 3% of infected horses and is characterized by circling behavior, **ataxia** (loss of motor control, including walking), abnormal **proprioception** (loss of the sense of position and movement of body parts), nystagmus, **recumbency** (laying down), head tilt, and seizures.²¹⁹ By contrast, miniature ECoV-positive horses develop encephalopathy with a 27% fatality rate, especially in younger animals. Fecal viral numbers are also higher in these horses than in surviving animals.²¹⁶

ECoV infections of foals with gastrointestinal disease only occur in the presence of coinfections, including infection with rotavirus or the bacterium *Clostridium perfringens*. ECoV infection in the dually infected foals may put the animal at higher risk for secondary infections with other intestinal pathogens as well.^{211,219} This is not the case for adult horses. ECoV is not frequently found in nasal secretions from either healthy horses or those with respiratory infections.²¹²

6.5.3 Coronaviruses of horses—the virus

ECoV belongs to the *Betacoronavirus-1* species of the subgenus *Embecovirus*. The first complete sequencing of the ECoV strain NC99 genome found that most genes in ECoV are conserved with the corresponding genes in other members of the *Betacoronavirus-1* species.¹⁹⁸ However, the ECoV nsp3 protein contains 3 amino acid deletions and 55 insertions when compared to the nsp3 proteins of BCoV, HCoV-OC43, and PHEV, the three coronaviruses most closely related to ECoV.¹⁹⁸ The nsp2 of ECoV also has 67%, 67%, and 45% amino acid identity to those present in BCoV, HCoV-OC43, and PHEV, respectively.¹⁹⁸

Viruses from Asia, North America, and Europe are genetically similar, although at least one Japanese isolate has a 185-nucleoside deletion in the region following the S protein gene, causing the loss of an ORF that encodes an nsp in the United States strain NC99. The Japanese strain appears to have originated from a different lineage than that of the

North American strains.²²⁰ At least one French strain, however, is more closely related to this American strain.²²¹ Their cellular receptors have not been identified.¹⁰

In a 2017 report of greater than 5000 adult horses throughout the United States, 9.6% of the horses produced anti-ECoV antibodies. Geographic region, breed, and usage were risk factors.²²² Draft horses and horses used in ranching/farming and breeding from the American Midwest are at the highest risk. Infection is more common in older adult animals as seen in a 2014 study that found that 20.5% of the infected horses were foals (0–6 months of age), 25.3% were aged 6 months to 5 years, and 54.2% were greater than 5 years of age.²²³

ECoV transmission is via the fecal-oral route.^{220,221} ECoV is shed between 3 and 25 days of infection, especially in cold weather,²²⁴ however, the length of time in which viruses may remain infectious is unknown. Rectal swabs and sera of healthy horses in Saudi Arabia and Oman contain ECoV RNA. MERS-CoV RNA has not been detected in horses, even though the equine DPP4 host cell receptor is closely related to that found in humans and the binding affinity of the MERS-CoV S protein for equine DPP4 is similar to that of humans and dromedaries.^{225,226} RNA from both equine ECoV and the dromedary DCoV-HKU23, however, has been reported in horses from Saudi Arabia.²²⁷

6.6 Coronaviruses of sheep

A coronavirus was isolated from sheep in Australia, while a study in Algeria detected a coronavirus in 3.6% of neonatal lambs. Coronaviruses are among the microbes causing neonatal diarrhea in lambs, especially in the presence of rotaviruses.^{228,229} Sheep, however, do not appear to be susceptible to MERS-CoV infection.¹⁴

6.7 Coronaviruses of companion animals

6.7.1 Coronaviruses of cats

6.7.1.1 *Introduction to feline coronaviruses*

Feline coronaviruses (FCoVs) are common throughout the world in both domestic and some wild cats. In addition to FCoVs, canine coronavirus, TGEV, and PRCV are classified as members of the ACoV-1 species based on whole-genome sequences or sequences of the conserved ORF1ab gene.^{3,230,231} FCoVs have been placed into two biotypes, feline enteric coronavirus (FECV) and FIPV, based on differences in pathology. Each of these FCoV biotypes contains two serotypes, serotype I and serotype II. Some of these undergo genetic recombination with canine coronavirus serotypes. The FECV serotype I biotype is transmitted primarily by the fecal-oral route and causes mild disease.²³² If, however, the infection becomes more severe and systemic, the virus is classified as belonging to the FIPV biotype, thus FIPV is a mutated form of FECV.

Subtype I and type II viruses differ in their susceptibility to IFNs and several antiviral drugs.²³³ FCoV type II also lacks a cleavage site between the S1 and S2 domains of the S protein. This cleavage site is present in type I FCoV.²³¹ Type I FCoV viruses are

responsible for 70%–98% of FCoV infections worldwide, including in China.²³⁴ While type I FCoV is prevalent in China, Europe, Australia, Korea, and the United States, FIPV type II infections are more common in Japan and Taiwan.²³⁵ Both type II FECV and FIPV use the feline APN as their host cell receptor.²³⁶ This glycoprotein is found on the cell surface and is a metalloproteinase. It is present in several cell types, including cells of the respiratory, digestive, and nervous systems; neutrophils and monocytes of the immune system; fibroblasts; and endothelial cells of the kidneys but is undetected in the blood.²³⁷ Type I and II FCoV differ in their methods of entering their host cells, utilizing late and early endosomes, respectively.²³⁸ Since feline C-type lectin **dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin** (feline **DC-SIGN**) is involved in attachment to host cells, it may be a coreceptor for type I and II FCoV serotypes, at least in vitro.²³⁹ The actions of cellular **furin**-like proteases cleave and activate the FCoV S protein at the junction of S1 and S2 domains of S proteins during entry via the early route, while cathepsins play a role in an entry via the late, endosomal route. The fusion of FCoV to the host cell membranes usually also involves a decrease in the endosomal pH.²⁴⁰

Infection with type I FECV biotype is typically asymptomatic in cats but may cause enteritis in kittens.⁹¹ This biotype replicates only in the lower part of the small intestine to the **cecum** of the large intestine even though its RNA is present throughout the digestive tract, the blood, and several other locations.²³⁹ By contrast, infection with the FIPV biotype results in severe neurological disease in cats that is often fatal. FIPV is a mutated form of FECV that infects the nervous, rather than the digestive, system.²⁴¹ Infection with FIPV is more common among young and male cats.^{242,243} It is present in **effusions** and leads to the formation of lesions in many cat tissues and organs.

Due to the lack of serological tests that differentiate the biotypes, diagnosis includes consideration of the cat's age, sex, history, clinical signs, and clinicopathological abnormalities.²⁴⁴ Both biotypes are controllable by IgG responses in the blood and IgA responses in mucosal tissues. Cats are also infected by SARS-CoV, SARS-CoV-2, pig TGEV, canine coronavirus, and the mildly pathogenic HCoV-229E.^{7,48}

6.7.1.2 *Feline enteric coronavirus*

The FECV biotype is almost ubiquitous in multi-cat settings. It is highly contagious and is transmitted via the mucosal route by contact with a virus from feces, but not by the oral-nasal route.^{245,246} FECV is shed in the feces of infected cats, including subclinical carriers, for 2 days to 2 weeks postinfection, followed by decreased viral loads with intermittent viral shedding for up to 20 weeks.¹⁰ FECV infection is either asymptomatic or results in minor digestive symptoms, such as diarrhea. The virus persists in both the colon and in macrophages within lymphatic organs.²⁴⁷ If the macrophages are unable to eliminate the virus, FECV replicates in the cytoplasm of these cells. The infected macrophages transport the virus to the intestines via the circulatory system, from where it infects the epithelium of the colon.²⁴⁸ Some cats with FECV clear the infection, while other animals continue to shed the virus intermittently or persistently.²⁴⁹ Since protective immunity to FECV is not lifelong, cats are often reinfected. This allows FECV to retain a presence in cat populations, especially among animals in shelters or breeding colonies where shedding increases by up to one million-fold, in part due to high levels of stress.²⁴⁵ Stress activates stress hormones, such as immunosuppressive corticosteroids. The high levels of virus shedding, together

with the high mutation rate in coronaviruses, further encourage mutations to arise, with some mutations leading to the development of the much more severe FIP.²⁵⁰

6.7.1.3 Introduction to feline infectious peritonitis virus

Experimental infection of cats with FIPV produces different patterns, dependent upon the FIPV strain and the route of inoculation.²³⁸ FIP incidence following intraperitoneal inoculation of cats with the type I FIPV UCD4 strain is 37.5% while following oronasal inoculation, it approaches 0%.²⁵¹ FIPV incidence following oral inoculation with type I FIPV KU-2 strain does not result in disease, while subcutaneous and intraperitoneal inoculation with this viral strain may produce FIP.²⁵²

Pathology is especially severe in kittens and is universally fatal for those cats in which clinical signs develop.^{10,244} Cat breeds with a higher-than-normal risk include Abyssinian, Bengal, Birman, ragdoll, and rex cat breeds.¹⁰ A FIP-like disease is also present in the following species of wild felids: African lions (*Panthera leo*), cheetahs (*Acinonyx jubatus*), mountain lions (*Puma concolor*), leopards (*Panthera pardus*), jaguars (*Panthera onca*), lynx (*Lynx* species), servals (*Leptailurus serval*), caracals (*Caracal caracal*), European wild cats (*Felis silvestris*), sand cats (*Felis margarita*), and Pallas cats (*Otocolobus manu*).¹⁰

Generally, the FIPV biotype is not transmitted among cats but rather arises from postinfection mutations in approximately 5% of cats chronically infected with FECV.²⁵³ These “internal mutations” change the virus’s tropism from enterocytes to **peritoneal** macrophages as well as transform FECV into a highly virulent, but nontransmissible, FIPV.⁴⁸ In support of these findings, individual FIPV strains isolated from the same environment shared 97% genetic homology, which is higher than that found in FECVs from that location in cats or that found in FECV strains from animals from other catteries.²⁵⁴ In cats from the same cattery, animals with FIP shed 100-fold higher levels of virus in their feces than their siblings without this disease as well as having longer durations of shedding.

Unlike FECV, FIPV is readily able to infect and replicate in monocytes/macrophages in the **peritoneal cavity** in the abdomen, a key step in the advancement of illness from the relatively harmless FECV into the highly virulent FIPV.^{10,239,255} Usually, the mutations occur independently within each cat so that each FIPV strain is in some ways unique.²⁵⁵ Mutations in the genes of the S protein, nsp3c, and nsp7b are relatively common and may contribute to the differences in tropism and virulence between FECV and FIPV.^{255–258}

6.7.1.4 Mutations and feline infectious peritonitis virus

Pathogenic FIPVs appear to have originated as mutants of otherwise relatively nonpathogenic feline coronaviruses. Mutations of two of the feline coronavirus genes, the 3c and S protein genes, are of particular importance since they could cause these relatively nonpathogenic cat viruses to become more pathogenic in cats and may perhaps allow zoonotic transmission as well. No such mutations of either of these genes were present in cat feces. Drugs, such as GC376, have been identified that inhibit symptoms of feline, ferret, and mink coronaviruses and, perhaps could also protect against disease in humans if they ever were infected with cat FIPV.

Most ORF 3c mutations result in a **truncated protein** product,^{255,259} while the remaining one-third of the mutations have increased numbers of nucleoside changes in the gene’s 3’ terminus.²⁶⁰ Only the truncating mutations affect the mutated virus’s host cell tropism

from intestinal epithelium to macrophages.^{10,257,260,261} An intact 3c gene is required for FIPV replication in the small intestine but not for systemic replication.²³⁹ FIPVs with non-truncating mutations replicate in the intestines but are not infectious in vitro.²⁶⁰

Two single nucleotide changes present within the region of the S protein gene encoding the fusion peptide are found in almost all FIPVs, but not in FECVs.^{255,257} This trait has been used for differential diagnosis.²³⁹ A substitution in this portion of the fusion peptide allows for the spread of FECV from the intestines, but not the conversion to FIPV.²⁶² Another important group of S protein gene mutations is present in the S1/S2 furin cleavage site.²⁶³ These mutations are present in all FIPVs and are unique to each FIPV. They usually increase the furin-mediated S protein cleavage that permits coronaviruses to enter via the “early” pathway and, by doing so, evades an important component of the host immune response. (See Chapter 1 for details of these pathways).

The 7ab gene in FECV is intact, however, FIPV contains deletions in this gene, some of which may involve almost the entire nsp7b gene.²⁶⁴ Small mutations, including those of only two nucleotide changes, are also present in this gene.²⁶⁵ FIPV containing deletions in the 7b or 7ab genes are less virulent in vivo.²⁵⁸ Some FIPV strains contain a 735-nucleoside deletion in their S protein gene that may greatly increase their virulence. Feline infectious peritonitis viruses are compared in Table 6.5.⁹¹

6.7.1.5 Pathology caused by feline coronaviruses serotypes I and II

FCoV incidence in field cats varies greatly in different areas of the world. It is less than 15% in Japan and greater than 70% in Austrian cats.²⁶⁶ Cats may be infected by two serotypes of these ACoVs: type I and type II FCoVs based upon differences in antibody reactivity to the S protein as well as genetic information.^{231,264,267} Type I FECVs/FIPVs are unique to cats.²⁵⁵ High levels of antibodies are found against the FCoV S proteins. Serotype I and serotype II FCoVs appear to be distinct entities. One study conducted in Switzerland found that among FCoV-infected feral cats, 68% were seropositive for serotype I FCoV, while only 9% were seropositive for serotype II. Interestingly, 23% of the cats had similar levels of antibodies for both serotypes I and II.²⁶⁶ In China and Europe, infection with FCoV serotype II is uncommon, however, serotype II viruses are more frequently used during in vitro research since it is difficult to culture FCoV serotype I in cultured cells. Interestingly, despite the proximity of China, Japan, and Taiwan, the incidence of FCoV type II is more common in the latter two countries.²⁶⁸

In addition to being more common, serotype I FCoVs are more virulent in cat populations and are the leading cause of FIP. This disease was first reported in the United States in the 1960s and rapidly spread to other countries from the late 1960s to the early 1970s.^{269,270} Serotype II FCoVs also caused FIP occasionally.²⁶⁶ FIP is the single most important lethal infection of young cats, with a fatality rate of 10% of infected kittens under the age of one year.²⁶⁶ as well as being the most common infectious disease agent of cats' CNS.²⁷¹ Clinical symptoms include ataxia, seizures, nystagmus, **hyperesthesia** (excessive sensitivity to sensory information), and damage to **cranial nerves**.¹⁰ Damage to the CNS includes **ventricular dilation** with the accumulation of inflammatory cells, flattening of **cerebral gyri** (ridges of the outer cerebrum), and **meningeal congestion** (excessive accumulation of fluid in the linings covering the brain). FIPV is present in the **cerebrospinal fluid** and the levels of proteins and cells in this fluid are increased.^{10,272} The

leptomeninges (outer two membranous layers that cover the spinal cord and brain) are infiltrated by neutrophilic and lymphocytic cells.²⁷³

Several of the less common diseases associated with FIP include **skin fragility syndrome**, **nodular dermatitis** (coin-shaped spots on the skin), **orchitis** (inflamed testicles), **syringomyelia** (cavities in the upper region of the spinal cord), **glomerulonephritis** (inflammation of the glomeruli of the kidneys) and **myocarditis** (inflammation of the heart muscle cells).^{10,274,275} Cats' eyes frequently sustain damage including a detachment of the retina and severe and massive inflammation of multiple eye structures, such as the **optic nerve**, **sclera** (whites of the eyes), and **conjunctiva** (membrane lining the inside of the eyelids).²⁷⁶ Granulomatous **phlebitis** and **periphlebitis** cause inflammation of the wall and an outer coat of a vein in the iris, choroid, retina, and sclera and around the optic nerve.²⁴⁷

Since the eye is an immunologically privileged site, the entry of macrophages, but not neutrophils, is associated with increased permeability of the **blood–ocular barrier**. In the eye, B cells and plasma cell numbers are more numerous than T cells or macrophages. Some FIPV-infected, activated ocular macrophages are detected.²⁴⁷ Increased levels of **glial fibrillary acidic protein** are characteristic of activation of **Müller cells** and **astrocytes** in the retina. The breakdown of the **blood-retinal barrier** is also present during FIV and allows the migration of macrophages and lymphocytes into the retina.²⁴⁷

Vasculitis is one of the most important pathogenic processes occurring in FIPV-infected cats. It is driven by the host inflammatory agents TNF- α , IFN-1 β , and **matrix metalloproteinase-9**.²⁷⁷ While CD4⁺ T helper and CD8⁺ T killer cells as well as resting B cells and plasma cells are present and play roles in vasculitis, activated macrophages are the key inflammatory cell type. These macrophages may be activated locally since the proinflammatory Th1 cytokine IFN- γ is also present in the lesions.²⁷⁸ Some anti-FIPV vaccines enhance disease progression. Rather than protecting the animals, antibodies that are produced increase viral uptake by macrophages during antibody-dependent enhancement (ADE), as described later.²⁷⁸ Production of toxic reactive oxygen species by activated macrophages increases during FIP and leads to a hyperactive, inflammatory immune response. Vitamins A, C, and E and **melatonin**, a typically sleep-inducing hormone, are potent antioxidants that have been used to decrease inflammation associated with FIP-induced pathology.²⁷⁷

6.7.1.6 Feline coronaviruses, canine coronaviruses, and recombination

Coronaviruses have a high rate of mutation which leads to the production of a diverse group of similar individual viruses which differ genetically by point mutations and small or large insertions and deletions. Such FCoV quasispecies form a collection of viruses with non-identical but closely related genomes in the intestine of cats.²⁷⁹ Both coronaviruses and influenza A virus adapt quickly in response to their environment due to their high rates of mutation and ability to swap their genes with similar genes from other, closely related viruses that normally infect different species of animals. Coronaviruses from cats, dogs, pigs, and humans are all able to bind to the feline APN receptor. It is possible that, on very rare occasions, cats may also act as mixing vessels for type I coronaviruses from different host species to produce a highly severe or deadly hybrid coronavirus that could infect large numbers of humans and result in another coronavirus pandemic. It is therefore important to watch for abrupt changes in the characteristics of a cat, dog, pig, and human coronaviruses that infect

cats. The human HCoV-229E could conceivably be altered into a very dangerous or deadly human virus upon leaving the cat mixing vessel and making its way into humans.

Type II feline coronaviruses appear to have arisen by genetic recombination between a type I FCoV and type II canine coronavirus. Such variants appear to be continuously produced. Production of type II recombinant strains is often highly complex. Additionally, the type II FCoVs that have been studied have large differences in their critical S protein genes. This suggests that the production of at least some type II feline coronaviruses occurred by independent genetic recombination events. Type I FECVs and FIPVs are the predominant FCoVs in Europe and the Americas, however, type II viruses compose at least 25% of FCoVs in Asia.^{268,280,281} Unfortunately, few Japanese cats have protective antibodies against type II FCoVs, however, this type of feline coronavirus is unable to readily transfer among cats.

6.7.1.7 Feline aminopeptidase N and feline coronavirus host range

As stated previously, HCoV-229E and TGEV use either human or pig APN as their host cell receptor.¹⁵⁰ Binding to APN is host-specific for human and pig coronaviruses, so HCoV-229E does not bind to pig APN and TGEV does not bind to human APN. Feline APNs are only 77%–78% identical to human and pig APNs. Several digestive system FCoVs bind to feline APN and successfully infect and replicate in the intestinal cells that express this cell surface receptor. Surprisingly, other type I coronaviruses, including two canine coronaviruses, TGEV, and HCoV-229E, are also able to use the feline APN as a host cell receptor.²³⁶ This may partially explain why some strains of canine coronavirus or pig TGEV can infect cats without causing disease and why cat FIPV also can infect pig cells in vitro.

Ferrets from Europe and the United States may also be infected by FIP, producing granulomas in the liver, spleen, bone marrow, and lymph nodes that are similar to the dry type of FIP lesions.²⁷⁴ In vivo, while dogs produce anti-FIP antibodies, no FCoV has been detected. Dogs kept near the infected dogs were not infected.^{6,282} Taken together, these results suggest that FIPV does not productively infect dogs.

6.7.1.8 The immune response to feline coronaviruses

FIP lesions have been characterized as effusive (“wet”) or noneffusive (“dry”) types.²⁷⁴ Effusive FIP produces **pyogranulomatous masses**, tumor-like groups of leukocytes, with a central core of degenerative neutrophils and cell debris surrounded by epithelioid macrophages with additional layers of lymphocytes and plasma cells. Masses on the serosal surfaces of the abdomen and thorax produce large amounts of exudate. Widespread immune-mediated **vasculitis** may be present as well. The dry type of FIP is characterized by the formation of classic granulomas (aggravated masses of macrophages) in the lymph nodes, kidneys, liver, eyes, and brain. The dry form is generally less aggressive than the wet form of the disease and is more likely to appear in the eyes than the wet form.²⁸³

Perivascular granulomatous inflammation may be at least partially due to type III and type IV hypersensitivity reactions.¹⁰ **Type III hypersensitivity reactions** result from the formation of large immune complexes consisting of antibodies and antigens that are deposited in vessel walls. Injury and vascular permeability may instead be caused by a **type IV hypersensitivity reaction** in which hyperstimulated CD4⁺ T helper cells activate

monocytes/macrophages to produce **vascular endothelial growth factor (VEGF)** and MMP-9. VEGF induces vascular permeability in cats with FIP,²⁶⁵ while MMP-9 degrades **type IV collagen** and the **basal lamina** of blood vessels during FIP vasculitis, allowing fluid to leak out of the bloodstream and enter the tissues.^{10,265,284}

The immune response to FECV/FIPV is complex. On one hand, type III hypersensitivity appears to be active due to the presence of immune complexes or complement fractions in cats presenting with either effusive or noneffusive lesions. On the other hand, a type IV reaction is suggested by the distribution of FIPV and immune cells in advanced lesions. These lesions contain activated macrophages, a small number of scattered IgG- or IgM-positive B cells, and CD8⁺ T killer cells around the periphery of the lesions.^{251,285}

Virulence of FIP in cats is associated with severe suppression of NK cell and T regulatory cell activity.²⁸⁶ Cats with a strong **cell-mediated immune (CMI) response** appear to be less likely to develop FIP. By contrast, cats with a weak CMI coupled with a strong antibody response are more prone to develop the pathogenic noneffusive form of FIP, and cats with no CMI tend to develop the effusive, highly pathogenic form of FIP.²⁷⁸

FCoV-infected cats develop **follicular hyperplasia** in their peripheral lymph nodes.²⁸⁷ FCoV-infected cats that remain healthy have a transient increase in T cell subsets early after infection. A reduction of T cell subsets, especially CD8⁺ T killer cells, occurs in cats with mild cases of FIP, but cell numbers are transiently increased if a cat with FIP is introduced into a cattery. Cats that develop full-blown cases of FIP experience very severe declines in lymphocytes expressing CD5, CD4, CD8, and CD21 (T cells and B-1a B cells, T helper cells, T killer cells, and mature B cells, respectively).²⁸⁷ Additionally, infected cats with advanced FIP lesions have lower levels of CD4⁺ T cells than cats with early stage lesions.²⁸⁵ Rises in the incidence of FIP occur in cats coinfecting with FECV and either feline leukemia or feline immunodeficiency viruses, both of which suppress cell-mediated immunity.²⁸⁸ The importance of cell-mediated immunity is also supported by the finding that more severe disease is found in **thymectomized** kittens infected with FIPV than in uninfected cats since T cell maturation occurs in the thymus.²⁸⁹

An increased rate of apoptosis may be responsible for lymphopenia and lymphoid depletion in the spleen and lymph nodes, especially among CD4⁺ T helper cells. During FIP, lymphocytes undergo **chromatin condensation**, a morphological trait of apoptotic cells. FIPV does not infect CD4⁺ T helper cells, so apoptosis may result from the release of soluble mediators from infected macrophages or neutrophils.²⁸⁸

Levels of the proinflammatory cytokines IL-1 β and IFN- γ are typically increased in whole blood samples from asymptomatic FIPV-infected cats compared to pathogen-free animals.²⁹⁰ Asymptomatic FCoV-infected cats also have high numbers of CD8⁺ T killer cells that may produce high levels of IFN- γ .²⁸⁷ Cats with FIP, however, have little, if any, IFN- γ expression in the blood, but it is upregulated within lesions.^{278,291} Cats producing a high TNF- α ; low IFN- γ mRNA ratio after challenge are more likely to develop the disease, while cats producing a low TNF- α :high IFN- γ mRNA ratio are protected. High levels of proinflammatory IL-6 are present in tissues and **ascites**, but not blood, of FCoV-infected cats.^{292,293}

IFN- γ plays an important role in the severity of FIP. The development of FIP is associated with certain feline interferon-gamma gene (*IFNG*) genotype variants in non-pedigree cats.²⁴² The blood leukocytes of cats that remain healthy following infection with FCoV

have higher levels of *fIFNG* transcription and production of IFN- γ than cats that develop FIP. The serum IFN- γ concentration, however, is similar between cats with effusive and noneffusive FIP.²⁹⁴ However, IFN- γ is 40-times more plentiful in lesions of FIPV-infected cats than in their blood.²⁷⁸ The protective vs pathogenic activity of IFN- γ appears to depend upon its location since it is produced in the mesenteric lymph nodes and ascites fluid of cats with FIP.^{242,295}

A strong systemic IgG and mucosal IgA presence are found in cats with active FECV infection, but it is rapidly lost after the infection ends.²⁴⁵ Mucosal T cell IFN- γ was not evident in infected animals although the IL17:FoxP3 expression ratio increased.²⁴⁵ IL-17 is associated with an inflammatory immune response while FoxP3 is a marker for T regulatory cells which downregulate inflammation. Infection of cats with Type I FCoV, including either FECV or FIPV, fails to trigger a robust T cell response.²⁴⁵

6.7.1.9 *Feline coronavirus and vaccination*

When cats are immunized with a Type I avirulent strain of FIPV and later challenged with a Type I highly virulent form of the virus, some cats are protected against developing FIP and others are not. In one case, immunization resulted in accelerated, severe disease which was likely due to ADE.²⁹¹ This highly dangerous condition is seen in dengue virus infection, in which it appears after infection with a different dengue serotype, but during FIPV infection, occurs following reinfection with viruses of the same serotype.²⁵² During ADE, the antibody response to the viral S protein forms pathogenic immune complexes with the virus that are readily consumed by macrophages. This serves to increase levels of infectious virus in these cells, leading to more severe disease characterized by **disseminated intravascular coagulation** and fibrinoid necrosis of the walls of blood vessels.²⁸⁵ Some of the macrophages are deposited in various tissues, causing **pleuritis** in the lungs, **peritonitis** in the gut, or **glomerulonephritis** in the kidneys. Cats with preexisting high levels of anti-FCoV antibody develop effusive FIP rapidly upon infection.⁴⁸

An alternative, less risky, vaccine strategy uses a modified live, temperature-sensitive vaccine which is administered by the intranasal route to stimulate a local, protective IgA response. Since it is temperature-sensitive, it is not able to infect the lungs due to their higher temperature in comparison with the nasal cavity. This vaccine is licensed in the USA but is not recommended by the American Association of Feline Practitioners.⁴⁸

It appears that vaccines against FCoV may need to induce a strong T cell response to be effective in producing B cell memory cells as well as the longer-lived **plasma cells** as well as cell-mediated immunity to prevent the development of FIP. Effective vaccines against microbes that are transmitted via mucosal areas, such as FECV, perhaps would best be delivered via a mucosal route, such as orally, to produce a mucosal IgA immune response.²⁴⁵

6.7.1.10 *Treatment of feline coronavirus diseases*

Until recently, FIP-infected cats were given only supportive care or antiinflammatory treatment that often improved their quality of life. Several drug regimens are currently under development or are in clinical trials. One promising therapeutic drug candidate is the active form of the nucleoside analog Remdesivir, which also is effective against COVID-19.²⁹⁶ GC-364 and tripeptidyl protease inhibitors, including those that target

3CL^{Pro}, are also promising. The latter group of inhibitors is effective in the nanomolar or low micromolar range against FCoV in vitro.²⁹⁷

Several forms of immunotherapy have been investigated for efficacy against FCoV. In a small study of three cats with the dry form of FIP, the animals received long-term treatment with Polyprenyl Immunostimulant, a mixture of phosphorylated, linear polyisoprenols, compounds that upregulate transcription of the mRNA of Th1 cytokines. The two cats who received continuing treatment remained well for at least two years after diagnosis. The third cat was treated for a shorter time but still survived for over a year.²⁹⁸ This treatment was not effective against the wet form of FIP. In a non-placebo-controlled study using glucocorticoids and recombinant feline interferon- ω (another type I IFN), a third of the cats which had primarily wet FIP underwent a 2-year remission.²⁹⁹ However, in a placebo-controlled report of cats also presenting primarily with wet FIP and treated with steroids, amoxicillin/clavulanic acid, and recombinant feline IFN- ω , no significant differences in survival time were seen between the treated and placebo groups, although the treated group had lower lymphocyte counts.³⁰⁰ Additionally, administration of type I IFNs either or before infection with FIP did not increase survival rates. However, when given before infection, IFN- α did increase survival time from five to fourteen days in untreated and treated cats, respectively.³⁰¹

Differing results between cats treated with different drug regimens may be due at least in part to differences in the form of FIP since cats with the wet form of the disease usually die in approximately 9 days while those with the dry form may live weeks to months.²⁹⁸ Differences in the serotypes may also be an important factor in treatment efficacy. It has also been suggested that FIP was misdiagnosed in some of the studies, especially the study in which the cats survived for over two years following treatment.³⁰⁰

Hydroxychloroquine, an antimalarial compound, used together with IFN- ω is also promising.³⁰² Hydroxychloroquine is effective against COVID-19 but has yet to be tested in FIPV-infected cats. Inotodiol, a fungal antiinflammatory compound, has also shown favorable responses, completely and rapidly removing FIPV from the intestines of FCoV-infected cats.²⁹⁶

6.7.1.11 SARS-CoV, SARS-CoV-2, and cats

SARS-CoV and SARS-CoV-2 infect and reproduce in experimentally infected cats, despite belonging to betacoronavirus lineage B, while feline coronaviruses are ACoVs. Additionally, cats are also highly susceptible to experimental infection with SARS-CoV-2.²⁹⁶ In experimentally infected cats, viral RNA was found in the respiratory tract and the small intestines. More importantly, cats have also been found to be naturally infected by SARS-CoV-2. Transmission is via the respiratory route, although SARS-CoV-2 is also shed in cat feces. Some infected cats are asymptomatic while others develop a mild disease in the upper respiratory tract or gastrointestinal systems, including lesions in the mouth or ulcerations on the tongue. While adult infected cats do not have overt symptoms of disease, they may develop mild lesions in their lungs.²⁸² Infection of juvenile cats, however, may be severe to fatal.⁶

Human-to-pet transmission of SARS-CoV has been demonstrated. In a French study of SARS-CoV-2 in pet cats and dogs from households having a COVID-19 human resident, antibody prevalence in cats ranged from 21% to 53%.³⁰³ Another study reported that oral,

nasal, and rectal swabs from 40% of the cats and 31% of the dogs from households with a SARS-CoV-2-infected human had either RNA or antibodies to SARS-CoV-2. The presence of anti-SARS-CoV-2 antibodies, however, may only indicate exposure to the virus rather than infection. Cats are more readily infected with SARS-CoV and more likely to develop symptoms than dogs, perhaps because cat ACE2 is more closely related to human ACE2 than is dog ACE2. The affected cats and dogs were found to be infected 11–51 days after the infected human developed symptoms.³⁰⁴ Of these infected cats, six of thirteen animals developed mild disease symptoms. The B.1.1.7 SARS-CoV-2 variant was also found in a domestic cat and dog in Texas in the southwestern United States, preceded by infection of the owner. Both the cat and the dog began to sneeze several weeks later.³⁰⁵ No further pathology was noted in these animals. Other studies have also detected SARS-CoV-2 in animals residing in homes of infected people in Asia, Europe, and, possibly, the United States. Some of the cats developed the severe or fatal respiratory diseases.^{304,306–308} Sharing beds with their owner is the highest risk factor for human-to-cat transmission of SARS-CoV-2.³⁰⁴ Ducks, chickens, and pigs are not susceptible to natural or experimental infection with SARS-CoV-2.³⁰⁸

Cats can transmit SARS-CoV or SARS-CoV-2 to other cats or other animals through direct contact or aerosols.^{308,309} SARS-CoV-2 can be transmitted from infected to sentinel cats via respiratory droplets.³¹⁰ It should be noted that while SARS-CoV-2 can be transmitted from humans to cats or dogs, several studies failed to find the cat-to-human transmission of SARS-CoV.^{7,311}

6.7.2 Canine coronaviruses

6.7.2.1 Introduction to canine coronavirus

First reported in 1971 in dogs in Germany, CCoV is one of two species of coronaviruses that infect dogs. CCoV generally causes mild, self-limiting diarrhea, especially in young puppies. CCoV is found in dogs worldwide.^{10,312} Approximately 50% of the dogs in North America have antibodies to this virus.⁴⁸ Many CCoVs themselves cause only mild digestive system disease in dogs, especially adult animals. Severe gastroenteritis and hemorrhagic disease with a high mortality rate, however, may occur in dogs that are coinfecting with other viruses, such as parvoviruses.²⁸² Additionally, some CCoV strains cause a fatal systemic disease involving lethargy, vomiting, hemorrhagic diarrhea, ataxia, and seizures.³¹³ CCoV can also infect wild canids, including foxes (*Vulpes* species), raccoon dogs, and wolves (*Canis lupus*).^{10,314} Interestingly, CCoV sequences from European wolves (*Canis lupus lupus*) are 98%–99% identical to those from domestic dogs.³¹⁵ By contrast, CCoV and canine respiratory coronavirus have a nucleoside identity of 68.5% overall, but only 21.1% in the spike protein gene.³¹⁶

CCoVs are currently divided into genotypes I and II which share almost 96% nucleoside identity, however, the S protein genes have only 56% sequence identity.¹⁰ CCoV-I is found in about 20% of infected dogs, CCoV-II in 44%, and the remaining 36% of dogs are infected with both genotypes. Infection with the type I virus does not protect dogs against infection by CCoV-II.³¹⁷ CCoV-I is closely related to FCoV-I. CCoV type II is further divided into two subtypes: CCoV-IIa (**pantropic**) and CCoV-IIb. CCoV-IIa is pantropic since it causes both

enteric and systemic symptoms. It is present in Europe, Brazil, Columbia, and at least one Caribbean island. CCoV-IIb is related to FCoV-II via multiple recombination events. CCoV subtypes IIa and IIb were found to cause fatal enteritis in puppies in the United States as well.³¹⁸

In one case study, moderate, diffuse, and mild enteritis were present in one animal as well as enteric and splenic lymphoid depletion.¹⁰ The other case animal had lymphoid depletion of the intestines and spleen along with diffuse villous blunting and intestinal crypt necrosis.³¹⁹ In a separate case report of an animal with pantropic CCoV, lesions were present in multiple organs, resulting in **fibrinopurulent bronchopneumonia** in the lungs, and **renal cortical infarcts** in the kidneys, severe coalescing centrilobular hepatic fatty change in the liver, and multifocal hemorrhage in the spleen. It should be noted that the latter animal was coinfecting with adult **ascaris** (parasitic worms of the digestive tract).³²⁰

6.7.2.2 Pathology due to canine coronavirus

Virulent strains of CCoV cause serious enteric disease in dogs in the absence of other microbes.^{10,319} Pups, especially those younger than 3 months, that are infected by a type II canine coronavirus are lethargic, have a high fever, vomit, and produce hemorrhagic diarrhea. The pantropic, highly virulent variant of CCoV type IIa (strain CB/05) may cause systemic disease due to its widespread dissemination via infected macrophages, similar to FIPV in cats and the more pathogenic form of ferret coronavirus. CB/05 has a 38-nucleoside deletion in ORF3b that produces a shortened 3b protein,³²¹ but no deletions in the genes for other proteins, including the S protein gene.³¹³ This strain has been found in multiple internal organs, such as the lungs, kidneys, and brain.³²¹

Pantropic CCoV was isolated from severe lesions in the lungs, spleen, liver, and kidneys and is associated with bronchopneumonia, hemorrhagic diarrhea, hemorrhaging, and loss of lymphocytes in the spleen, fatty changes in the liver, and dead areas in the kidneys.^{320,322} Neurologic symptoms include ataxia and seizures, followed by death after 2 days. Necropsy revealed that infected dogs had hemorrhagic enteritis, excessive levels of fluid in the abdominal cavity, and severe lesions in the abdominal organs. The lungs had multiple, patchy, red areas and excessive fluid. The livers are yellow-brown with surface hemorrhages, and the spleens are enlarged and hemorrhagic. The kidneys also have multifocal hemorrhagic dead areas.³¹³

6.7.2.3 Canine coronavirus—the viruses

Type II CCoV RNA is present in intestinal cells and is released in dogs' feces. Type II CCoV also infects cats. Due to the large number of RNA mutations and recombination of genomic RNA, many coronaviruses, including SARS-CoV, jump into new host species. SARS-CoV-2 rarely if ever infects dogs, even in situations involving repeated contact with humans with confirmed infection. Additionally, SARS-CoV-2 transmission to humans from these pets is extremely low.¹⁵ Nevertheless, since people have close contact with their cats and dogs, including dogs licking people's faces, care should be taken to rapidly identify any zoonotic transmission of dog coronaviruses into humans.

CCoV of dogs and TGEV of pigs are closely related to FCoV of cats and may exchange genetic information amongst themselves. Several such single or double **homologous recombination** events appear to have occurred in several locations between the genomes

of FCoV serotype I and CCoV type II and appear to have produced new chimeric FCoV serotype II strains whose S protein appears to have originated in CCoV while the M, N, 7a, and 7b proteins originated in FCoV type I.²⁶⁴ In support of this contention, the S proteins of two CCoV strains have 93.3% amino acid identity. S proteins of FIPV and FECV have 91.4% and 93.2% identity to those of CCoV, while PRCV and TGEV have 81.9% and 89.6% identity, respectively.³²³ Additionally, cats can be experimentally infected with CCoV.²⁶⁵ Cats may be simultaneously infected with both feline and canine coronaviruses.

6.7.2.4 Prevention of canine coronavirus infection

Both inactivated and attenuated CCoV vaccines are available, however, the disease usually affects puppies younger than 6 weeks old and lasts for only a few days. These vaccines do not prevent infection but do protect vaccinated dogs from disease.⁴⁸ Protection against CCoV infection relies upon the presence of IgA antibodies in the intestine. When dogs are given the vaccine intravenously, they do not stimulate IgA release. Antibody production resulting from vaccination may not provide adequate protection against infection with another CCoV strain. Immunity due to natural infection fails to produce complete protection against infection against the highly virulent CB/05 pantropic strain even in dogs with high serum antibody titers from their prior infection with CCoV. These naturally infected dogs, however, have a much milder disease that was typified by vomiting, diarrhea, and lymphopenia, as well as a reduction in the length of time during which viral shedding in the feces occurs. The lymphopenia resulting from infection may be important to disease severity since it could decrease the immune response to other enteric canine viruses, such as canine distemper and canine parvovirus. Interestingly, CB/05 RNA was not detected in the blood, despite its presence in lymphoid organs and tissues.³²²

Several modified live, attenuated vaccine strains of CCoV have also been produced. Following vaccination with the attenuated strain, the dogs did not develop clinical signs or shed the detectable virus. Dogs immunized with another attenuated vaccine were asymptomatic and the virus was not found in their feces after the challenge. This latter vaccine, however, produces adverse side effects in the lining of the chest and abdominal cavities as well as in the kidneys.⁴⁸

6.7.3 Canine respiratory coronavirus

6.7.3.1 Introduction to canine respiratory coronavirus

CRCoV is a member of the species *Betacoronavirus-1*, whose members include BCoV, ECoV, HCoV-OC43, and PHEV. It is distinctly different from CCoV as depicted in Table 6.6.

CRCoV and CCoV share 69% nucleoside identity in the highly conserved polymerase region of their RNAs and only 21% amino acid identity in the S protein.³¹² CRCoV was first reported in 2003 in the United Kingdom in dogs with acute respiratory infection³²⁴ but is present in many areas of the world. Seroprevalence in dogs varies among countries: 54.7% in the United States, 63.2% in the United Kingdom, 30.3% in the Republic of Ireland, 32.1% in Italy, 17.8% in Japan, and 12.8% in Korea.³²⁵ A Korean study from 2010 found that in addition to the 12.8% of dogs with antibodies against CRCoV, 4.9% had

TABLE 6.6 Comparison between canine coronavirus and canine respiratory coronavirus.

Name	Species	Close relatives	Receptor	Disease	Severity	Location	Route of infection
Canine coronavirus	<i>Alpha-coronavirus-1</i>	FCoV ^a TGEV ^b	APN th SA ⁱ	Gastrointestinal Hemorrhagic Systemic	Mild	Intestines Brain Kidneys Lungs Liver, Spleen URT ^e	Fecal-oral
Canine respiratory coronavirus	<i>Beta-coronavirus-1</i>	BCoV ^f HCoV-OC43 ^g	HLA-1 ^c	CIRD ^d	Severe	URT ^e	Inhalation

^aFeline coronavirus of cats.^bTransmissible gastrointestinal enteric virus of pigs.^cHuman leukocyte antigen-1.^dCanine infectious respiratory disease.^eUpper respiratory tract.^fBovine coronavirus of cattle.^gHuman coronavirus-OC43.^hAminopeptidase N.ⁱSialic acid.

antibodies against canine influenza virus and 1.2% against both viruses.³²⁵ In a study in New Zealand published in 2009, 29% of the tested dogs had antibodies against CRCoV, while in a study conducted in 2014, 53.0% tested positive.³²⁶ It would be interesting to perform such studies regularly to detect trends in coronavirus prevalence and virulence.

CRCoV is readily transmitted among dogs. One study found that 30% of the dogs were infected upon entry into a shelter, while three weeks later, almost 100% of dogs had antibodies to CRCoV under these types of crowded conditions.³¹² Moreover, dogs having anti-CRCoV antibodies when entering a shelter had a decreased risk of developing the **canine infectious respiratory disease (CIRD)** as described below.³²⁴ This indicates an important role for antibodies in host defense.

CRCoV from widely separated areas of the world appears to be antigenically similar since viral strains isolated from infected dogs in the United States and the United Kingdom produce cross-reacting anti-CRCoV neutralizing antibodies.³²⁷ This antigenic similarity suggests that a single vaccine may protect dogs against CRCoV strains throughout much of the world, although additional work needs to be performed to support this assertion. More importantly, no detectable levels of anti-CRCoV IgM are present in adult humans with intense occupational contact with dogs, strongly suggesting that zoonotic transmission does not occur despite dog-to-dog infection via inhalation of aerosolized droplets.³²⁸

A study of kenneled dogs in the United States detected CRCoV RNA in 26.9% and 16.9% of tracheal and lung samples, respectively.³²⁴ Rural dogs in western Canada are significantly less likely to harbor CRCoV than dogs from a shelter in the area, however, seronegative animals commonly developed antibodies after admission to the shelter, but this was not correlated with respiratory disease.³²⁹ An Italian study of adult domestic dogs reported antibodies against CRCoV and CCoV in 23.3% and 86.1% of the animals, respectively. Interestingly, 4.0% and 97.0% of kenneled pups had antibodies against CRCoV and

CCoV, respectively.³¹⁶ This surprising finding may result from healthy pet dogs being less likely than kennelled dogs to be tested for infection by these canine coronaviruses during a typical veterinary investigation, while such testing of apparently healthy kennelled animals is often part of standard outbreak investigations.³³⁰ While CRCoV is found in dogs of all ages, dogs over the age of one year are more likely to be seropositive than young dogs, while CCoV is often detected in younger dogs.^{316,331,332}

6.7.3.2 Pathology due to canine respiratory coronavirus

In the absence of coinfection with other respiratory disease agents, CRCoV generally causes a mild, self-limiting illness, **kennel cough**, characterized by a dry, hacking cough.³²⁶ More commonly, the cause of kennel cough is multifactorial and is best described as the CIRD complex.³²⁴ CIRD is highly contagious and has high morbidity but low mortality rate. It is typically found in densely housed dogs.³²⁷ CIRD may be at least partially due to CRCoV limiting the ability of the cilia to remove microbes from the upper respiratory tract and allowing their entry into the lungs.³³³

CRCoV was first isolated from dogs with respiratory disease in a shelter in the United Kingdom in 2003 and is now known to be present worldwide.³²⁴ Transmission is probably primarily via inhalation rather than through contact with fecal material since CRCoV is released by the oropharynx but not by the rectum.³²⁷ Other conditions in which many dogs are in relatively close contact include breeding facilities, dog shows, and dog racing facilities, in which greyhounds are kept in close quarters. In addition to close contact between dogs, crowded animals are more likely to be frequently exposed to high concentrations of pathogens as well as develop physiological stresses.³²⁹ Environmental temperature, age, and sex, however, do not appear to play a role in virus prevalence.

While CRCoV infection typically results in mild symptoms of upper respiratory disease, such as nasal discharge, sneezing, and coughing,¹⁰ its presence increases the severity of secondary infections. Coinfection with other microbes increases the risk of developing CIRD. Some of these other microbes include the following: canine adenovirus types 1 and 2, canine parainfluenza virus, canine herpesvirus, canine pneumovirus, canine influenza virus, reoviruses, and the bacteria *Bordetella bronchiseptica*, *Streptococcus equi* subspecies *zooepidemicus*, and *Mycoplasma* species.^{10,324,325,334,335}

6.7.3.3 Canine respiratory coronavirus—the virus

CRCoV replicates in cells lining the respiratory tract, including the ciliated epithelial cells and mucus-secreting goblet cells. Harmful foreign material, such as viruses, bacteria, and particulate matter, are trapped within a coating of mucus in the respiratory tract. Cilia sweep the mucus and the material trapped within upwards and away from the lungs, decreasing the incidence of secondary infection. Cytokines may alter mucus secretion or the beating of cilia. By altering cytokine production in inflammatory sites in the respiratory system, CRCoV may indirectly disturb the tract's epithelial lining, resulting in loss and damage to the cilia.³²⁷ Examination of the tissues of the respiratory tract of infected dogs found it to be devoid of cilia and goblet cells, but it did contain inflammatory cell infiltrates.³²⁷ CRCoV is most commonly found in and at the highest levels in the nasal cavity, nasal tonsil, and trachea, although it is also present in the lungs, bronchial lymph

nodes, and palatine tonsil. It can also infect elements of the immune system, such as the spleen and mesenteric lymph nodes, and the colon of the digestive system.^{10,312}

CRCoV has a 98.8% and a 98.4% identity with the polymerase gene of BCoV and HCoV-OC43. HLA-1, a cell surface immune recognition receptor, serves as the receptor for in vitro infection of human airway epithelial cells by CRCoV and BCoV. Interestingly, HLA-I belongs to the same molecular superfamily as the receptor for mouse hepatitis virus, another coronavirus of animals.³³⁶ CRCoV, BCoV, and HCoV-OC43 all produce the HE structural protein that binds to sialic acid on the cells' plasma membrane as well. Various types of sialic acid serve as attachment factors for these viruses.³³⁶ Neuromeric acid, a molecule that blocks binds to sialic acid, decreases these coronaviruses from binding to their target cells competitively.³³⁶

As stated previously, CRCoV is closely related to BCoV and HCoV-OC43.^{282,336} The genes for the S proteins of CRCoV and BCoV have 97.3% nucleoside identity, while CRCoV and HCoV-OC43 of humans have 96.9%.³²⁴ This strongly supports the hypothesis of recent host-species shifts between CRCoV and BCoV and/or HCoV-OC43.³¹³ BCoV can infect pups that subsequently transmit the virus to other pups with which they are housed. When young pups are inoculated with BCoV by the oronasal route, the inoculated dogs and their kennel mates produce neutralizing antibodies to BCoV and BCoV RNA is found in oral and rectal swabs of both groups of animals. Other than a transient loss of appetite, none of the BCoV-infected dogs develop fever or observable respiratory or digestive symptoms.^{64,324}

CRCoV enters its target cells via **caveolin-dependent endocytosis**, one of the processes by which the cell brings materials, including viruses, into the cell within small membrane-enclosed vacuoles. This process in CRCoV entry requires **dynamin**, an important **GTPase** that is also active in other forms of endocytosis.³³³ Other coronaviruses use different forms of endocytosis to enter cells. SARS-CoV uses a **clathrin-dependent, lipid raft-mediated, caveolin-independent** pathway.³²⁶ FIPV uses clathrin- and caveolin-independent endocytosis, while HCoV-229E uses caveolin-dependent endocytosis.

6.7.3.4 The immune response to canine respiratory coronavirus

CRCoV decreases mRNA levels of the proinflammatory cytokines TNF- α and IL-6 as well as the chemokine IL-8 during the initial 72 hours of experimental infection of canine tracheal epithelium cells in vitro. By 96 hours, however, cytokine and chemokine levels are raised. When viral numbers are low soon after infection, cytokine mRNA levels in infected cells are lower than those present in uninfected control cells. Once the virus numbers increase by 96 hours postinfection, however, cytokine mRNA levels increase. CRCoV decreases mRNA levels of TNF- α and stimulates mucus secretion.³³¹

CRCoV infection also impacts immune cell numbers. The blood of many CRCoV-infected dogs has decreased neutrophil concentrations and mild **left shift** (increased levels of newly produced, immature cells). This has been suggested to be the result of acute inflammatory reactions in which there is a high demand for neutrophils, leading to increased production of these cells in the red bone marrow.³²⁷ Neutrophil levels in cows infected by the closely related BCoV have significantly lower neutrophil concentrations early after infection, followed by high levels of these cells later. In contrast, in SARS-CoV infected patients, blood neutrophil levels may be higher or lower than normal, but high blood neutrophil concentrations are often associated with poorer prognosis.³²⁷

Dogs infected by CRCoV that have antibodies against this virus are less likely to develop severe disease. Young dogs are at greater risk for CIRD and develop more severe disease since they are less likely to have anti-CRCoV antibodies.³³⁷

6.8 Brief overview of domestic avian coronaviruses

Avian infectious bronchitis virus (IBV) of chickens (*Gallus gallus*), pheasant coronavirus (PhCoV) of commercially raised pheasants (*Phasianus colchicus*), and turkey coronaviruses are among the most important agricultural coronaviruses of birds.^{338,339} Whether these and other gammacoronaviruses are members of a single species or different but very closely related viral species is a matter of debate. Some researchers consider all avian gammacoronaviruses to be members of a single species which is designated avian coronavirus,³⁴⁰ while other researchers consider gammacoronaviruses from different hosts to be distinct, but closely related, species. In whatever manner they may be classified, these coronaviruses have a negative economic effect that is particularly problematic for people in developing countries who rely on domestic birds for food and money.

IBV infects the upper respiratory, digestive, and reproductive tracts, as well as the kidneys³⁴¹ and is associated with high morbidity and variable levels of mortality.³⁴² PhCoV frequently causes either respiratory or kidney disease.³³⁹ Turkeys (*Meleagris gallopavo*) may be infected by turkey coronavirus which causes enteric disease, unlike IBV and PhCoV, which attack the respiratory system.³³⁹ **Poults** infected by turkey coronavirus have a greater risk of death than older birds. Adult turkeys, nevertheless, have a more debilitating illness which results in decreased meat and egg production.³⁴³

Turkey and PhCoVs are very closely related to, or are variants of, IBV and have a high overall genetic identity.^{343,344} Nevertheless, there is only 34% identity between the S protein of IBV and turkey coronavirus. The S proteins of IBV and PhCoV are approximately 90% identical.³³⁹ Coronaviruses of peafowl (*Pavo* species), guinea fowl (*Numida meleagris*), partridges (*Alectoris* species), and teals (*Anas* species) are also very closely related to IBV.³³⁹

Quails (*Coturnix japonica*) host a coronavirus that is associated with enteritis.³⁴⁵ Other bird coronaviruses that may be of economic importance infect greylag geese (*Anser anser*), mallard ducks (*Anas platyrhynchos*), and pigeons (*Columbia livia*). Goose coronaviruses contain a large insert in the 3' untranslated region of the genomic RNA. An additional one or two ORFs are present in pigeon and goose coronaviruses, respectively.³³⁹ These findings may suggest that these bird coronaviruses are sufficiently genetically different from IBV to be considered novel coronavirus species. Interspecies transmission of avian coronaviruses is likely to continue or increase due to greater amounts of free-range practices in which different avian species may interact.³³⁹

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Pulling it all together: where do we go from here?

7.1 Coronaviruses—friends and family

Viruses, like living organisms, have been assigned to various families whose members share important traits. The viruses, like the animals that serve as their hosts, have close and remote acquaintances that may act in ways that are beneficial, detrimental, or inconsequential, depending on existing and changing circumstances. This book deals primarily with the pathogenic coronaviruses of humans or other mammals with which we come into contact. On one hand, infections with even these “pathogenic” coronaviruses are often asymptomatic or result in mild disease in at least a portion of the viruses’ host species, just as SARS-CoV-2 rarely results in serious disease in young children.¹ On the other hand, some of the generally “nonpathogenic” human coronaviruses (HCoV) can cause serious disease in some of their hosts. These coronaviruses include HCoV-NK63 and HCoV-OC43, which normally cause cold-like symptoms. HCoV-NK63 may cause croup, and HCoV-OC43, in some rare cases, causes neurological disorders.^{2,3} To better understand the risk of a given coronavirus causing disease in a particular host at a particular time and under particular circumstances, it helps to glean information about the normal activity of its friends (other RNA viruses) and family members as well as their “quirky” behavior and what triggers it.

Coronaviruses are members of the Coronaviridae family of **Baltimore Class IV viruses**.⁴ These (+) single-stranded RNA viruses include both nonpathogenic and pathogenic viruses of humans and animals and may cause mild to life-threatening disease, depending upon the host’s species, general health, immune status, and age. The severity of the disease also depends upon the viral species, the variant, and which **nucleosides** occupy key positions in the genomic RNA. These positions include those in the gene encoding the spike (S) protein since these nucleosides determine host and cell tropism.

7.1.1 Baltimore class IV viruses (coronaviruses’ friends)

Class IV viruses share important traits, including their manner of replication.⁵ During this process, their **positive-sense**, single-stranded RNA genome is copied into a complementary **negative-sense** RNA. The two strands briefly are part of a double-stranded RNA

intermediate. The negative-sense strand is then copied to form a new positive-sense RNA strand that may function as either the genome of a progeny virus or, after modifications, serve as a messenger RNA that is translated into viral polyproteins which are enzymatically cleaved to form several smaller, functional proteins. The enzymes involved in this process are **RNA-dependent RNA-polymerase (RdRp)** and several viral and cellular proteases. As RNA viruses, Class IV viruses tend to have a high mutation rate since RdRp makes many mistakes during the copying process. This high mutation rate enables the production of high numbers of mutated viral variants, which differ in replication rate, pathogenicity, and host species.

Class IV viruses, however, are also very diverse, differing in many aspects. Some of these viruses are primarily transmitted between humans by the fecal-oral route, including poliovirus and hepatitis A virus (**picornaviruses**) and norovirus (a **calicivirus**); while others travel between people by respiratory secretions, including coronaviruses and rhinoviruses (other picornaviruses)⁶. The **flaviviruses** are transmitted by mosquitoes or ticks and include yellow fever, dengue, West Nile, and Zika viruses.⁷

Age plays a major role in disease severity in many Class IV viruses. Some Class IV viruses, such as hepatitis A and West Nile viruses, cause more severe diseases in older patients.^{8,9} This is also true for HCoVs, such as SARS-CoV-2, and animal coronaviruses, such as bovine coronavirus.^{1,10} The latter causes diarrhea in calves that is self-limiting, but has a high degree of **morbidity**, and **winter dysentery** with hemorrhagic diarrhea in adult cattle.¹⁰ Zika virus causes extreme, life-threatening, neurological disease if acquired during fetal development,¹¹ while poliovirus infection early in life is protective against the life-threatening paralytic disease that occurs during infection of older children and adults.¹²

In addition to coronaviruses, Class IV viruses cause disease in a wide range of organ systems. Norovirus causes diarrhea or dysentery.¹³ Hepatitis A virus causes cirrhosis of the liver and liver cancer.¹⁴ Rhinoviruses are associated with the common cold.¹⁵ Flaviviruses may cause high fever or hemorrhagic fever (yellow fever and dengue viruses), encephalitis (West Nile and tick-borne encephalitis viruses), or severe neurological disease during fetal development (Zika virus).⁷ Coronaviruses, including SARS-CoV-2, affect even more organ systems, as described later.

7.1.2 Coronaviridae (coronaviruses family)

7.1.2.1 *Similarities of coronaviridae members*

Coronaviruses share common features, including the size of their genomes, which, at 27.6–31 kilobases, are the largest among RNA viruses.¹⁶ They also possess common structural proteins: the spike (S) protein which binds to the viral receptor and is vital for binding and entering target cells and the envelope, membrane, and nucleocapsid proteins, as well as haemagglutinin esterase in betacoronaviruses.^{17,18} They also possess multiple non-structural proteins with a wide range of functions, including replication, cleavage of viral polyproteins, and suppression of the host immune response.¹⁷

RNA viruses, in general, have high mutation rates since RdRp makes many errors during viral replication.¹⁹ The large size of coronaviruses amplifies the mutation rate, enabling them to undergo rapid changes to adapt to new environments and hosts.²⁰

During epidemics and pandemics of some coronaviruses, the mutation rate decreases and leads to viruses that may be more contagious but are less likely to cause severe disease requiring hospitalization or resulting in death.²¹

Unlike many other RNA viruses, however, most coronaviruses contain the **exonuclease N (ExoN)** proofreading enzyme that corrects the errors made by RdRp during replication.²² The importance of ExoN activity is exemplified by the increased numbers of mutations present in the variants of mouse hepatitis virus and SARS-CoV lacking a functional form of this proof-reading enzyme.²³

Coronaviruses also increase their genetic variability by **recombination**. In this process, sections of the genomic RNA in one virus are exchanged with that of another virus of a different viral or host species. Bovine coronavirus of cattle and some other coronaviruses, including SARS-CoV, may take the form of a **quasispecies** that is composed of numerous viral variants. Genetic recombination may play a major role in the formation of quasispecies.^{24,25} Porcine deltacoronavirus is believed to be the product of recombination between two bird coronaviruses: the sparrow coronavirus HKU17 and the bulbul coronavirus HKU11.²⁶ Feline CoV-II of cats is believed to be a recombinant formed by feline CoV-I and canine coronavirus-II.²⁷

Recombination between a pathogenic strain of the porcine epidemic diarrhea virus and a vaccine strain of this virus produced the highly pathogenic CN/GDZQ/2014 strain.²⁸ This might similarly lead to the creation of pathogenic recombinant HCoVs produced by recombination between a mildly pathogenic virus, such as HCoV-NL63, and a vaccine strain of SARS-CoV-2. These coronaviruses are closely related and share a common host cell receptor. The possibility of producing such a pathogenic coronavirus is greater if live, **attenuated** viruses are used in the vaccine. The commercially available CoronaVac and Sinopharm/BBIBP-CorV vaccines both use live, attenuated strains of SARS-CoV-2^{29,30} and, while they are protective and stimulate both antibody and T lymphocyte (T cell) responses, they need to be monitored for production of highly pathogenic recombinant progeny.

7.1.2.2 Differences among coronaviridae members

Members of Coronaviridae differ significantly in several important ways. Almost all pathogenic coronaviruses of mammals are members of the *Alphacoronavirus* or *Betacoronavirus* genera Lineages A, B, or C, while one pathogenic pig coronavirus is a member of the *Deltacoronavirus* genus (Appendix I). While SARS-CoV and SARS-CoV-2 are members of the subgenus *Sarbecovirus*, MERS belongs to the *Merbecovirus* subgenus.³¹ The less pathogenic HCoVs belong to the subgenera *Duvinacovirus*, *Embecovirus*, and *Setracovirus*. Other pathogenic mammal coronaviruses also belong to the following other subgenera: the alphacoronavirus subgenera *Pedacovirus*, *Tegacovirus*, *Rhinacovirus*, and *Luchacovirus* and the deltacoronavirus subgenus *Buldecovirus* (Appendix 1).

HCoVs use a wide variety of cell receptors and coreceptors, including the following: **aminopeptidase N**, **angiotensin-converting enzyme 2**, **dipeptidyl peptidase 4**, and N-acetyl-9-O-acetylneuraminic acid together with DC-SIGN (**dendritic cell-specific intercellular adhesion molecule-3 grabbing nonintegrin**), L-SIGN (**liver/lymph node-specific intercellular adhesion molecule-3-grabbing integrin**), vimentin, HLA-1 (**human leukocyte antigen class I**), and **sialic acid** (Appendix I). Other receptors used by mammalian coronaviruses include **neural**

cell adhesion molecule (CD56) and CEACAM1 (**carcinoembryonic antigen-related cell adhesion molecule 1**) which work in concert with cholesterol and occludin.

A common trait among coronaviruses is their tropism to various tissues and organs, the cells of which they infect and may cause disease. Usually, coronaviruses cause disease in the respiratory tract, the digestive system, the central or peripheral nervous systems, and, in many cases, more than one other organ system. Examples of coronaviruses that cause primarily respiratory system damage are the four common HCoVs (HCoV-229E, -OC43, -NL63, and -HKU1), which are some of the causative agents of the common cold in people, Middle Eastern respiratory system coronavirus (MERS-CoV) and dromedary alphacoronavirus in dromedary camels, porcine respiratory coronavirus in pigs, and canine respiratory virus in dogs (Appendix I).^{32–36} While SARS-CoV, MERS-CoV, and SARS-CoV-2 often cause cold-like symptoms in humans, they also cause severe, life-threatening pneumonia and other respiratory illnesses as well as severe disease in other organ systems (Chapters 2–4).

Other coronaviruses generally cause digestive system pathology which may result in mild diarrhea or severe dysentery. Coronaviruses in this group include porcine epidemic diarrhea virus and porcine deltacoronavirus in pigs, bovine enteric coronavirus in cattle, and alpaca enteric coronavirus.^{16,18,37,38} Another group of coronaviruses causes severe neurological pathology, in addition to attacking other organs. These viruses cause a large array of diseases. Porcine hemagglutinating encephalomyelitis virus of pigs causes severe **wasting** disease of the digestive system as well as encephalitis.^{18,39} While equine coronavirus of horses causes severe digestive system pathology, it also causes a neurological disease that is associated with loss of coordination of voluntary movement, including difficulty walking, involuntary rapid eye movements, and a tendency to lay down as well as seizures.^{16,40} Mouse hepatitis virus attacks both the liver and the nervous system, resulting in severe **hepatitis**, mild encephalitis, and life-threatening **demyelination** of some nerves (loss of the nerves' fatty covering).^{41,42}

The disease manifestations of an individual coronavirus may also vary among geographical locations during the same time period. During the SARS epidemic, the prevalence of gastrointestinal symptoms differed greatly, ranging from 23% in Toronto, Canada, to 70% in Hong Kong.^{43,44} This may be due to several, perhaps nonexclusive reasons, including the **founder's effect** and differences in culture, climate, income level, and access to and quality of healthcare facilities. It should be noted that both locations are part of the developed world, thus some of the suggested differences may not be applicable.

Other coronaviruses infect and damage multiple organ systems. The feline infectious peritonitis virus biotype of feline coronavirus attacks the nervous, urinary, and cardiovascular systems.⁴⁵ MERS-CoV not only causes severe respiratory disease, but also causes severe damage to the kidneys that may require kidney transplantation and causes disease in the liver, intestines, heart, and encephalitis and Guillain-Barré syndrome in the nervous system (Chapter 3). COVID-19 results in multisystem diseases that include the respiratory, cardiovascular, neurological, **endocrine**, digestive, **integumentary**, and reproductive systems (Chapter 4).

The **basic reproductive rate** (R_0) of the three highly pathogenic HCoVs differ greatly from each other and among each virus species in a geospatiotemporal manner. The R_0 for SARS is estimated to range from 2 to 5.⁴⁶ The R_0 for MERS-CoV appears to be a place- and situation-dependent since it was 8.59 during the **nosocomial** outbreak in South Korea and 1.15 in Saudi Arabia.⁴⁷ A literature review from January to February 2020 found that the R_0 for SARS-CoV-2 ranged from 1.4 to 6.49, with a mean of 3.28.⁴⁶ The wide range may be

due to the use of different methodologies to measure R_0 among the reviewed studies (stochastic, mathematical, and statistical methods, including exponential growth). Of these, the mathematical estimates had the greatest extent of variation and the highest scores, ranging from 1.5 to 6.49, with an average R_0 of 4.2. The stochastic and statistical studies had little variation and much less of an average R_0 (2.44 and 2.67, respectively).⁴⁶

The mortality rate for the three highly pathogenic HCoVs varies greatly (9.5% for SARS, 34.9% for MERS, and 2.3% for COVID-19 early in 2020, respectively).⁴⁸ It should be noted that much of the information concerning the MERS mortality rate was based upon hospitalized cases, including nosocomial transmission. When the cases were community-acquired, the mortality rate was approximately 10%.⁴⁸ The mortality rate for COVID-19 has been and continues to change over time. As of mid-February 2022, the cumulative mortality rate was 1.4%, while the rate for cases reported in the past seven days was only 0.5%.⁴⁹ The reasons for this drop are unclear, but are likely to be multifactorial, including the current circulation of the less virulent Omicron subvariants and the increasing numbers of people with prior immunity, whether naturally acquired or due to vaccination. Income levels also affect the cumulative mortality rate since the rates are 1.0%, 1.4%, and 2.3% in areas with high, middle-to-low, and low incomes.⁴⁹

Coronaviruses differ in their climatic preferences. In general, coronavirus outbreaks have a winter peak: 90% of the infections occur when the daily mean temperatures are under 10°C (50°F), sunshine lasts only five hours per day, and the relative humidity is greater than 80%.⁵⁰ This differs greatly among the viral species, however. SARS-CoV infection was not strongly influenced by climate, although the risk of daily disease prevalence in Hong Kong was 18.2-fold greater on days with lower, compared to higher, temperatures.⁵¹ MERS-CoV infection is also not strongly influenced by meteorological conditions, although infection is associated with higher temperatures and low relative humidity.⁵² SARS-CoV-2 infection appears to be associated with low temperature, mild daytime temperatures, and low humidity.⁵³ The animal coronaviruses porcine transmissible gastroenteritis virus, bovine coronavirus, canine coronavirus, and murine hepatitis virus survive longest under conditions of low (20%) relative humidity.⁵⁴ These differences are of great importance since the above animal coronaviruses are often used to model SARS-CoV, MERS-CoV, and SARS-CoV-2.

7.2 Zoonotic transmission of coronaviruses

As a result of the current COVID-19 pandemic, much attention has recently shown how easily some coronaviruses of animal origin may be transmitted and adapted to new hosts, including humans (transmission of MERS-CoV from dromedary camels to humans)⁵⁵ and our companion animals (transmission of SARS-CoV-2 from humans to cats).⁵⁶ In the case of MERS-CoV, while humans have a fatality rate of ~35%, dromedaries typically have, at most, **rhinorrhea** (runny noses).⁵⁷

7.2.1 Coronaviruses proposed reservoir and intermediate hosts

While bats and rodents appear to be the reservoir hosts of mammalian coronaviruses, the differences in the critical S protein, especially the **receptor-binding domain (RBD)**,

between bats with the proposed intermediate hosts, are uncomfortably high to support this hypothesis. The RBD is the region of the viral S protein that binds to its receptor on the surface of the host target cells. The S protein determines the range of animals that may be infected by a given coronavirus.

Bats harbor a very large and diverse number of coronavirus species. In eastern Thailand alone, at least 10% of the members of bat species from four families were infected by coronaviruses.⁵⁸ From the 5 tested bat species of the Pteropodidae family of flying foxes, these are lesser short-nosed fruit bats (*Cynopterus brachyotis*) and greater short-nosed fruit bats (*Cynopterus sphinx*) (28.6% of 14 bats); from the 1 tested bat species of false vampire bats (Megadermatidae), Indian false vampire bats (*Megaderma lyra*) (50% of 2 bats); from 2 tested bat species of Rhinolophidae horseshoe bats, Shamel's horseshoe bats (10.0% of 20 bats); from 6 tested bat species of Vespertilionidae vesper bats, western bent-winged bats (*Miniopterus magnate*) (20.0% of 30 bats), small bent-winged bats (*Miniopterus pusillus*) (100%, but only 1 bat was tested), common bent-winged bats (*Miniopterus schreibersii*) (22.6% of 53 bats), lesser Asiatic yellow house bats (*Scotophilus kuhlii*) (66.7% of 3 bats), and greater Asiatic yellow house bats (*Scotophilus heathii*) (37.5% of 8 bats).⁵⁸

In some roosting sites, many different bat species from different families are present and harbor the same bat coronavirus species. Corroosting in an enclosed environment may enable the exchange of viruses between bat species. Some individual bats are coinfecting with more than one species of coronavirus, which may facilitate genetic recombination.⁵⁸ Their ability to fly allows long-distance migration of some bat species that may spread different coronavirus species and variants over great distances.⁵⁹

An example of the ability of a foreign gene or gene segment within a coronavirus to spread among different bats and bat genera and families is the finding of insertion of the double-stranded RNA orthoreovirus P10 gene into the Rousettus bat coronavirus GCCDC1. This recombinant coronavirus was initially reported in 2016 in Leschenault's rousette bats (*Rousettus leschenaultia*) in China and then in 2020 in lesser dawn bats (*Eonycteris spelaea*) from Singapore.^{60,61} Both of these bats are members of the Pteropodidae family. A 2022 study then reported GCCDC1 in lesser dawn and Indian short-nosed fruit bats (*Cynopterus sphinx*) of the Pteropodidae family and Shamel's horseshoe bat of the Rhinolophidae family in Cambodia.⁶²

7.2.1.1 Severe acute respiratory system coronavirus—putative reservoir and intermediate hosts

SARS-CoV-like viruses are found in at least eight *Rhinolophus* species bats (Table 7.1; Chapter 2). Six viral species have been identified in greater horseshoe bats (*R. ferrequinum*) from Europe and parts of Africa and Asia; five in Chinese rufous horseshoe bats (*R. sinicus*) from China and parts of Southern Asia, including India; and two from lesser brown horseshoe bats (*R. stheno*) from Southeast Asia.^{63–67} Several other *Rhinolophus* species bats that are known to host one species of SARS-CoV-like viruses include four from China and Southeast Asia and one from Southeast Asia.

Two pipistrelles of the *Hypsugo* and *Pipistrellus* genera of the Vespertilionidae family of bats host SARS-CoV-like viruses. These bats are from China and other parts of eastern Asia and Southeast and eastern Asia, including Japan and China. Other bats that are known to host one virus species are wrinkle-nosed free-tailed bats (*Chaerephon plicatus*)

TABLE 7.1 Bats Infected with SARS-CoV-Like Viruses.

Bat Family	Common Name	Scientific name	Bat Location	Virus
Rhinolophidae	Intermediate horseshoe bat	<i>Rhinolophus affinis</i>	China, Southeast Asia	LYRa11
Rhinolophidae	Greater horseshoe bat	<i>Rhinolophus ferrumequinum</i>	Europe, Northern Africa, Central and Eastern Asia	16BO133
Rhinolophidae	Greater horseshoe bat	<i>Rhinolophus ferrumequinum</i>	Europe, Northern Africa, Central and Eastern Asia	EPI1
Rhinolophidae	Greater horseshoe bat	<i>Rhinolophus ferrumequinum</i>	Europe, Northern Africa, Central and Eastern Asia	JTMC15
Rhinolophidae	Greater horseshoe bat	<i>Rhinolophus ferrumequinum</i>	Europe, Northern Africa, Central and Eastern Asia	Rf1
Rhinolophidae	Greater horseshoe bat	<i>Rhinolophus ferrumequinum</i>	Europe, Northern Africa, Central and Eastern Asia	RfYNLF_31C
Rhinolophidae	Greater horseshoe bat	<i>Rhinolophus ferrumequinum</i>	Europe, Northern Africa, Central and Eastern Asia	RfYNLF_31F
Rhinolophidae	Malayan horseshoe bat	<i>Rhinolophus mayalanus</i>	Southeast Asia	RmYN07
Rhinolophidae	Chinese rufous horseshoe bat	<i>Rhinolophus sinicus</i>	China, India, Nepal, Vietnam	RsSHC014
Rhinolophidae	Chinese rufous horseshoe bat	<i>Rhinolophus sinicus</i>	China, India, Nepal, Vietnam	RS672
Rhinolophidae	Chinese rufous horseshoe bat	<i>Rhinolophus sinicus</i>	China, India, Nepal, Vietnam	HKU3
Rhinolophidae	Chinese rufous horseshoe bat	<i>Rhinolophus sinicus</i>	China, India, Nepal, Vietnam	Rs3307
Rhinolophidae	Chinese rufous horseshoe bat	<i>Rhinolophus sinicus</i>	China, India, Nepal, Vietnam	WIV16
Rhinolophidae	Lesser brown horseshoe bat	<i>Rhinolophus steno</i>	Southeast Asia	RsYN03
Rhinolophidae	Lesser brown horseshoe bat	<i>Rhinolophus steno</i>	Southeast Asia	RsYN09
Pteropodidae	Egyptian fruit bat	<i>Rousettus aegyptiacus</i>	Africa, Middle East, Mediterranean, India	WIV1
Rhinolophidae	Big-eared horseshoe bat	<i>Rhinolophus macrotis</i>	China, Southeast Asia	SARS-CoV- like virus
Rhinolophidae	Pearson's horseshoe bat	<i>Rhinolophus pearsoni</i>	China, Southeast Asia	SARS-CoV- like virus
Rhinolophidae	Least horseshoe bat	<i>Rhinolophus pusillus</i>	China, Southeast Asia	SARS-CoV- like virus
Molossidae	Wrinkle-lipped free-tailed bat	<i>Chaerephon plicatus</i>	South and east Asia	SARS-CoV- like virus

(Continued)

TABLE 7.1 (Continued)

Bat Family	Common Name	Scientific name	Bat Location	Virus
Vespertilionidea	Alashanian pipistrelle	<i>Hypsugo alaschanicus</i>	China, Korea, Mongolia, Japan, Russia	SARS-CoV- like virus
Vespertilionidea	Japanese pipistrelle	<i>Pipistrellus abramus</i>	East and Southeast Asia	SARS-CoV-like virus

All of these bats are insectivores, except for the Egyptian fruit bat.

This table presents the family, scientific and common names, and locations of the bats reported to host SARS-CoV-like bat viruses, as well as the names of the specific viruses. The Rhinolophidae family of bats host the majority of these viruses, although some are also present in a small number of Pterodidae, Molossidae, and Vespertilionidae family bats. Many of these bats reside in China, Southeast Asia, or both.

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from Southeast and eastern Asia, including China, and the Egyptian fruit bat (*Rousettus aegyptiacus*) from Africa, the Middle East, and India.^{65,68}

Bat hunters may help to bridge the gap in the transmission of coronaviruses between bats and their intermediate hosts. The hunters not only capture hibernating bats but also a diverse range of mammal species, including small carnivores, such as civets (Viverridae family) and raccoon dogs (*Nyctereutes procyonoides*), intermediate hosts of SARS-CoV (Chapter 2).^{68–70} Interspecies transmission of SARS-CoV might then occur if the captured bats are kept in close proximity to live carnivores. Ferret badgers (*Melogale* species), another intermediate host of SARS-CoV, sometimes act as scavengers and might thus feed on carcasses of dead bats on the cave floors.⁶⁸

7.2.1.2 Middle Eastern respiratory system coronavirus—putative reservoir and intermediate hosts

Bats and dromedary camels are believed to serve as the reservoir and intermediate hosts for MERS-CoV, respectively. The species of bat coronaviruses that most closely resemble MERS-CoV are HKU4, HKU5, and NeoCoV.⁷¹ Chapter 3 of this book compares the potential bat reservoir hosts. HKU4 is from two species of bamboo bats in Southeast Asia. HKU5 is from two species of pipistrelles: one found in India and the other found in the British Isles, Europe, and North Africa. NeoCoV is found in two very closely related serotine bats from sub-Saharan Africa and a pipistrelle from East and Southeast Asia. All these bats are members of the Vespertilionidae family. MERS-CoV-like viruses are present in two species of insectivorous bats: one of which is present in North America, and the other, in Southeast Asia. A MERS-CoV-like virus is also found in a frugivorous bat of the Rhinolophidae family and is found in Africa, the Middle East, the Mediterranean region, and the Indian subcontinent. Many dromedaries in the Arabian Peninsula are imported from the Greater Horn of Africa (Ethiopia, Sudan, South Sudan, Somalia, and Kenya).⁷² Interestingly, Egyptian fruit bats (*Rousettus aegyptiacus*), and the two African serotine bats are the only bats whose range overlaps with that of the MERS-CoV's dromedary camel intermediate host.

MERS-CoV and HKU4 bat coronavirus have an amino acid identity of only 67.3%–67.4% in the S protein, while that of MERS-CoV and HKU5 is 64.3%.⁷³ While the overall identity of genomic RNA from MERS-CoV and NeoCoV is 85%, less than 45% identity is present in the S1 domain of the S protein.^{71,74} The low levels of amino acid identity and RNA in the S

protein or genes of HKU4, HKU5, and NeoCoV compared to MERS-CoV in humans and dromedaries cast doubt about bats being major hosts for a recent ancestor of MERS-CoV, although bats may still be regarded as reservoir hosts for a distant ancestor of MERS-CoV and other mammalian coronaviruses.

7.2.1.3 Severe acute respiratory system coronavirus-2—putative reservoir hosts

The origin of SARS-CoV-2 is a matter of great concern since we need to do all that we can to avoid another pandemic as devastating as COVID-19 has been. SARS-CoV-2 is more closely related to coronaviruses of horseshoe bats (*Rhinolophus* species), especially the RaTG13 virus from the proposed reservoir hosts, intermediate horseshoe bats (*R. affinis*). Both virus and bat appear to be excellent candidates for the viral ancestor and reservoir host, respectively, of SARS-CoV-2. Unexpectedly, the S protein gene from this bat coronavirus differs in five of the six critical nucleosides encoding the protein's RBD.⁷⁵ Furthermore, the similarity of amino acids between the ACE2 of bats and humans is only approximately 81%,⁷⁶ making it unlikely that the same virus would bind to target cells from both bats and humans without undergoing extensive mutations. RpYN06 from least horseshoe bats (*R. pusillus*) is also a close relative to SARS-CoV-2, having an overall nucleoside identity of 94.5%, but only 76.3% to the S protein gene and 60.9% in the RBD.⁶⁷ Table 7.2 lists the SARS-CoV-2-like viruses found in bats.

A 2022 study reported the presence of three coronaviruses from northern Laos bats that appear to be the closest known relatives of SARS-CoV-2.⁷⁷ Phylogenetic analyses of the RBDs place *R. malayanus* BANAL-52, *R. pusillus* BANAL-103, and Marshall's horseshoe bat (*Rhinolophus marshalli*) BANAL-236 coronaviruses near the 2019 SARS-CoV-2 isolates.⁷⁷ These three species of Southeast Asian horseshoe bats may share caves while roosting as well as in their foraging habitats. Their SARS-CoV-2-like viruses strongly bind to human ACE2 and their RBDs only differ from that of SARS-CoV-2 by 1–2 amino acids. BANAL-52 has a greater degree of nucleoside conservation than RaTG13 in the S protein's N-terminal domain and the RBD. **Pseudoviruses** bearing their S protein are also able to enter and replicate in human cells in vitro. Moreover, cell entry is blocked by anti-SARS-CoV-2-specific neutralizing antibodies.

RaTG13 and the SARS-CoV-2-like BANAL viruses, however, lack the furin cleavage site at the S1/S2 junction of the S protein that is absent in most reported bat coronaviruses but is present in all known SARS-CoV-2 variants.^{77,78} The furin cleavage site is rarely present in alphacoronaviruses as well, except for feline coronavirus and canine coronavirus 23/03.⁷⁸ This site appears to be vital to SARS-CoV-2 replication and pathogenesis. Of 41 screened rodent coronavirus species, 78% have potential S1/S2 furin cleavage sites while 6% of 179 screened bat coronavirus sequences have predicted sites.⁷⁸ Since the furin cleavage site is so common in rodent-associated coronaviruses, rodents may play an important role in the zoonotic transfer of an ancestor of SARS-CoV-2.⁷⁸ The role of rodents in the transmission of coronaviruses to humans is described later in this chapter.

Open reading frame (ORF) 8, a major immune system target, is highly divergent among SARS-CoV-2-like virus genomic RNA. ORF8s from bat BANAL-52, -103, -236, and RaTG13 viruses are more closely related to SARS-CoV-2 than to that from pangolins.⁷⁷ The encoded protein is absent from approximately one-fourth of the SARS-CoV-2 strains isolated from China after late March 2020 due to a large mutation in ORF7b and ORF8.⁷⁹

TABLE 7.2 Bats Infected with SARS-CoV-2-Like Viruses.

Bat Family	Common Name	Scientific name	Bat Location	Virus
Rhinolophidae	Acuminate horseshoe bat	<i>Rhinolophus acuminatus</i>	Southeast Asia	RacCS203
Rhinolophidae	Acuminate horseshoe bat	<i>Rhinolophus acuminatus</i>	Southeast Asia	RacCS224
Rhinolophidae	Acuminate horseshoe bat	<i>Rhinolophus acuminatus</i>	Southeast Asia	RacCS253
Rhinolophidae	Acuminate horseshoe bat	<i>Rhinolophus acuminatus</i>	Southeast Asia	RacCS264
Rhinolophidae	Acuminate horseshoe bat	<i>Rhinolophus acuminatus</i>	Southeast Asia	RacCS271
Rhinolophidae	Intermediate horseshoe bat	<i>Rhinolophus affinis</i>	Southeast Asia, China	RATG13
Rhinolophidae	Japanese horseshoe bat	<i>Rhinolophus cornutus</i>	Japan, China	Rc-o319
Rhinolophidae	Malayan horseshoe bat	<i>Rhinolophus malayanus</i>	Southeast Asia	BANAL-52
Rhinolophidae	Malayan horseshoe bat	<i>Rhinolophus malayanus</i>	Southeast Asia	RmYN01
Rhinolophidae	Malayan horseshoe bat	<i>Rhinolophus malayanus</i>	Southeast Asia	RmYN02
Rhinolophidae	Malayan horseshoe bat	<i>Rhinolophus malayanus</i>	Southeast Asia	RmYN05
Rhinolophidae	Malayan horseshoe bat	<i>Rhinolophus malayanus</i>	Southeast Asia	RmYN08
Rhinolophidae	Marshall's horseshoe bat	<i>Rhinolophus marshalli</i>	Southeast Asia	BANAL-236
Rhinolophidae	Least horseshoe bat	<i>Rhinolophus pusillus</i>	Southeast Asia, China	BANAL-103
Rhinolophidae	Least horseshoe bat	<i>Rhinolophus pusillus</i>	Southeast Asia, China	RpYN06
Rhinolophidae	Shamel's horseshoe bat	<i>Rhinolophus shameli</i>	Southeast Asia	RshTT200
Rhinolophidae	Shamel's horseshoe bat	<i>Rhinolophus shameli</i>	Southeast Asia	RshTT182
Rhinolophidae	Lesser brown Horseshoe bat	<i>Rhinolophus stheno</i>	Southeast Asia	RsYN04

All of these bats are insectivores.

This table presents the family, scientific and common names, and locations of the bats reported to host SARS-CoV-2-like bat viruses, as well as the names of the specific viruses. The Rhinolophidae family of bats hosts all of these viruses. Most of these bats reside in Southeast Asia.

Table produced by author.

SARS-CoV-2-like viruses with this deletion are also now present in Taiwan. While these viruses have an improved ability to replicate *in vitro* when compared to the original isolates, the patient viral load was unchanged.⁷⁷ Major deletions in ORF8 of SARS-CoV were also present during the SARS epidemic of 2003 in which the earliest isolates were similar to those found in palm civet SARS-CoV-like viruses.⁸⁰ Near the end of the SARS epidemic, however, complete or nearly complete ORF8 deletions were detected.⁸¹ These deletions in SARS-CoV were linked to decreased virus replication and disease severity.⁷⁹ Whether similar ORF8 deletions will eventually be associated with decreased virulence later during the COVID-19 pandemic is unknown.

Other SARS-CoV-2-like viruses include RsYN04 from *R. stheno* and RmYN05 and RmYN08 from Malayan horseshoe bats (*R. malayanus*) in China.⁶⁷ That study did not report the presence of SARS-CoV-2 -like viruses in 34 Chinese rufous horseshoe bats (*R. sinicus*), 12 Tai horseshoe bats (*R. siamensis*), or 59 intermediate roundleaf bats

(*Hipposideros larvatus*). RshSTT182 and RshSTT200 are from Shamel's horseshoe bats in Cambodia and have an overall 92.6% nucleoside identity with SARS-CoV-2 in addition to five of six critical RBD sites.⁸²

RacCS203 from acuminate horseshoe bats (*R. acuminatus*) is in Thailand and is closely related to RmYN02 from Malayan horseshoe bats.⁸³ Antibodies against the RBD of RmYN02 cross-neutralize SARS-CoV-2 even though the RBD of RmYN02 and RacCS203 do not bind ACE2.⁸³ RmYN01 is also present in Malayan horseshoe bats.⁸⁴ Japanese horseshoe bats

(*R. cornutus*) also harbor a SARS-CoV-2-like virus, Rc-o319.⁸⁵ These bats often cohabit with greater horseshoe bats, known to host several SARS-CoV-like viruses. Bat coronaviruses often undergo host switching.⁸⁶ The receptor-binding area of Rc-o319 S is unique and contains nine amino acid deletions in region 2.⁸⁵

7.2.1.4 Severe acute respiratory system coronavirus-2—putative intermediate hosts

Pangolins, especially Sunda pangolins (*Manis javanica*), have been suggested to serve as the major intermediate host of SARS-CoV-2.⁸⁷ They inhabit Southeast Asia and are brought into China by illicit wildlife traders. The pangolins eat primarily ants and termites. Many ant species feed on carrion and pangolins might become infected by licking ants on the carcasses of dead bats on cave floors.^{68,88} The coronavirus from these pangolins has 90.7%–100% amino acid identity with SARS-CoV-2 and their RBDs are almost identical.^{76,89} However, the similarity of amino acids between the ACE2 of pangolins and humans is only 84.8%⁷⁶, suggesting that it may be difficult for the S protein of pangolin coronavirus to bind to human cells. However, the RBD of the Sunda pangolin coronavirus GX-P5L binds better to human ACE2 than that of both SARS-CoV-2, SARS-CoV-2, and RaTG13.⁸³

Infected pangolins develop severe diseases, including wasting, shortness of breath, **diffuse alveolar damage**, and death.⁸⁹ This decreases the likelihood that zoonotic transmission involved contact between humans and pangolins in live animal markets since these markets are unlikely to have seriously ill animals for sale. It would also be difficult for a deadly virus, such as the pangolin coronavirus, to maintain its chain of transmission in the wild^{68,90} without obvious and observable massive die-offs of these animals, especially since pangolins have a solitary lifestyle and are Critically Endangered. It is still possible that **intraspecies** transmission occurs during illicit transport to the markets in China.⁶⁸ Even more importantly, the vital SARS-CoV-2 furin protein cleavage site is also absent from both bat RaTG13 and pangolin coronaviruses.⁹¹ Taken together, the above information suggests that pangolins may not be the intermediate hosts for zoonotic transmission and that another mammal may instead act in this capacity or that the introduction of SARS-CoV-2 into humans occurred by a different route.

Other proposed intermediate hosts for SARS-CoV-2 are felids such as domestic cats, tigers (*Panthera tigris*), and lions (*Panthera leo*); common goats (*Capra hircus*); spotted hyenas (*Crocuta Crocuta*); and civets (the proposed intermediate hosts for SARS-CoV).^{92,93} While tigers and lions develop mild to moderate upper respiratory tract (URT) infection and transient viral shedding, infection of domestic cats is typically asymptomatic. However, given their close contact with their owners (from which these cats are typically infected), domestic cats may serve as intermediate hosts, especially since outdoor cats

often contact rodents.⁹³ Goats originate in Central Asia, near the range of some of the proposed bat reservoir hosts, while the hyenas inhabit Sub-Saharan Africa, far from these bats.

Rabbits (lagomorphs) are readily susceptible to SARS-CoV in vitro.⁹³ Based on the interactions between the virus RBD and the ACE2 protein of dogs, ferrets, and hamsters, however, they are not predicted to serve as intermediate hosts.⁹⁴ However, experimentally infected rabbits, ferrets, and hamsters do develop a **systemic** infection with severe lung disease and viral shedding from the URT. It is unknown whether they can transmit SARS-CoV-2 to humans.⁹³ This may be an excellent situation for the application of the One Health approach.

7.2.2 Comparison between the hosts and geographical locations of severe acute respiratory syndrome coronavirus- and severe acute respiratory syndrome coronavirus-2-like viruses

While bat viruses similar to SARS-CoV and SARS-CoV-2 are most often associated with horseshoe bats (*Rhinolophus* species) as their reservoir hosts and their intermediate hosts have been linked to live animal markets in China, they have some very significant genetic differences. The bat viruses' genomes only have 80% nucleoside identity and the viruses have been placed in two different *Betacoronavirus* lineages.^{68,82,84,95} It should be noted that other SARS-CoV-like and SARS-CoV-2-like viruses may also be present in bats but have not yet been detected due to a lack of sampling in Southeast Asia, especially in the Greater Mekong Subregion (Myanmar, Laos, Thailand, Cambodia, and Vietnam), and the Yunnan and Guanxi Provinces of southern China.⁸²

The bat hosts typically dwell in differing locations with different ecologies. The *Rhinolophus* bat species hosts of SARS-CoV-2-like viruses are almost exclusively found in mainland Southeast Asia (including Laos, Vietnam, Cambodia, and Thailand), while those of SARS-CoV-like viruses are primarily found in China, but also include some other regions of the world outside of Asia, such as found in the wide-ranging greater horseshoe bats and Egyptian fruit bats (Table 7.1).⁶⁸ SARS-CoV-like bat viruses, unlike known SARS-CoV-2-like bat viruses, are present in bats of the Molossidae and Vespertilionidae families, in addition to Rhinolophidae bats, as seen in Tables 7.1 and 7.2.

People living in mainland Southeast Asia seem to be less susceptible to severe COVID-19 than people from other countries in Southeast Asia, including Indonesia, Malaysia, Myanmar, and the Philippines. This may be because the rural populations of the former countries are likely to have developed cross-reactive immunity since they are regularly exposed to bats or infected intermediate hosts.⁶⁸

SARS-CoV-like viruses inhabit areas with latitudes of 18°–43°N and hibernate in the winter due to the scarcity of insects. SARS-CoV-2-like viruses inhabit areas with latitudes of 10°–24°N. The *Rhinolophus* species that host SARS-CoV- or SARS-CoV-2-like viruses are cave bats that live in groups or colonies. Coronaviruses can be transmitted among bats of the same or different *Rhinolophus* species inhabiting the same cave.⁶⁸ Genomic recombination is more likely to occur between the highly diverse, specialized coronaviruses inhabiting the transitional zone between these ecological niches (southern Yunnan Province of China, northern Laos, and northern Vietnam), which is at the northern edge of the tropical

monsoon climate.⁶⁸ Recombination makes the bat coronaviruses in this region prime candidates for causing another coronavirus epidemic. Support for this hypothesis comes from the discovery of both SARS-CoV- and SARS-CoV-2-like viruses in horseshoe bats from southern Yunnan Province.^{83,96}

7.2.3 Other animals as potential coronavirus reservoir hosts

7.2.3.1 *Coronaviruses with zoonotic potential in birds*

Host species population density and distribution and short- and long-distance migration patterns are important to microbial host and geographical range.⁹⁷ Because of their capacity to fly, some species of wild birds and bats may carry infectious disease agents to wider-spread locations than large animals. Birds also have a specialized **adaptive immune** system.⁹⁸ Birds are known to spread severe infectious diseases that have or may undergo zoonotic transmission, including the avian influenza H5N1 which began in Hong Kong in 1997, and spread from wild birds to domesticated birds, such as chickens, ducks, and geese, before undergoing zoonotic transmission to humans, in which it is highly pathogenic.⁹⁹ Fortunately, human-to-human transmission is absent or very rare.¹⁰⁰ This is not the 2021 influenza of birds.

In contrast to almost all known mammalian coronaviruses, except several coronaviruses of pigs, birds belong to the *Gammacoronavirus* and *Deltacoronavirus* genera.^{99,101} Porcine deltacoronavirus infects both calves and chickens.¹⁰² Deltacoronaviruses frequently switch their bird hosts and sparrows may be especially important in avian to mammal transmission. The porcine deltacoronavirus cluster appears to have a common ancestor with SpaCoV HKU17 from sparrows.¹⁰² They are also closely related to deltacoronaviruses in vinaceous parrots (*Amazona vinacea*) and plain parakeets (*Brotogeris tirica*) in Brazil.^{103,104}

7.2.3.2 *Coronaviruses with zoonotic potential in rodents*

Besides bats, rodents may serve as reservoir hosts for current and preemergent HCoVs. SARS-CoV-2-like viruses from bats fall within the *Sarbecovirus* subgenus of betacoronaviruses, while those of rodents are in the *Embecovirus* subgenus.¹⁰⁵ Members of *Embecovirus* subgenera had formerly been categorized as belonging to lineage 2a, which includes human coronavirus HKU-1, equine coronavirus, and dromedary camel coronavirus HKU23,¹⁰⁶ but lacks bat-associated coronaviruses.¹⁰⁷ Rodent coronaviruses, however, are now believed to be ancestors of *Embecovirus* coronavirus.¹⁰⁸

Rodents are present in two families: Cricetidae (New World mice and rats and hamsters) and Muridae (Old World mice, rats, and gerbils). The human “common cold” coronavirus HCoV-OC23 is present in some members of Muridae in China and red-toothed shrews of the family Soricidae in China in 2020. The prevalence rate varied greatly, being 23.3% in Guangzhou and 0.7% in Guilin Provinces, and is especially high in animals from urban areas, such as passenger stations and hotels.¹⁰⁹ These rodents are found in Asia, including provinces of southern China, while shrews are found in most of the inhabited world except for Australia. Though somewhat similar in appearance, shrews are not rodents. Among their differences, rodents, which have an herbivorous diet, have flattened, gnawing teeth, while shrews have sharp, spike-like teeth that aid them in their omnivorous diet. Both rodents and

shrews also harbor hantaviruses.¹¹⁰ Some of these (–) single-stranded RNA viruses cause severe respiratory disease in humans with an approximately 38% mortality rate,¹¹¹ similar to that caused by MERS-CoV (Chapter 3).¹¹² Brown rats (*Rattus norvegicus*) are candidates for such transmission since they are found in residential and indoor areas.¹⁰⁹

The genomes of the RCoV-GCCDC3 and RCoV-GCCDC5 coronaviruses from rodents are highly similar to other known coronaviruses.¹⁰⁹ The entire genome sequence of RCoV-GCCDC3 is 96% similar to that of China Rattus HKU24, while the sequence of RCoV-GCCDC5 is 97% identical to Lucheng Rn rat CoV, a recombinant CoV from the Zhejiang Province, China. Another rodent coronavirus detected in this study, RCoV-GCCDC4, appears to belong to a novel lineage A *Betacoronavirus* genus.¹⁰⁹

HCoV-OC43 and HCoV-HKU1 of humans might have initially had their origins in rodent coronaviruses, although HCoV-OC43 may have arisen through a cattle intermediate virus.^{78,113,114} Swine acute diarrhea syndrome coronavirus may have had rodent or bat coronavirus ancestors.^{78,115,116}

In addition to Asia, SARS-CoV-2 might become endemic in rodent reservoir hosts in other continents.¹¹⁷ When deer mice (*Peromyscus maniculatus*) of the North American Muridae subfamily Neotominae are experimentally infected with SARS-CoV-2, the virus replicates in the URT, lungs, and intestines, as well as entering the brain.¹¹⁷ While infection of deer mice is asymptomatic, they might nevertheless either act as intermediate hosts or transmit the virus to other animal species in the Western Hemisphere. They are especially noteworthy since deer mice are abundant in regions containing mink farms and the mice may feed upon contaminated mink food.¹¹⁷ In addition to deer mice, the ACE2 protein of other members of Neotominae possesses significant amino acid identity with that of humans (17–18 of 20 amino acids) that they may also be susceptible to SARS-CoV-2 infection. These are the northern grasshopper mouse (*Onychomys torridus*) and the desert woodrat (*Neotoma lepida*).¹¹⁷

7.2.3.3 Genetic recombination between alphacoronaviruses and betacoronaviruses in rodents

Genetic recombination has occurred in rodent coronaviruses in many regions of the world. These recombinant alphacoronaviruses are present within the *Luchacoviruses* subgenus.⁷⁸ Alphacoronaviruses of at least two Cricetidae rodents, bank voles (*Myodes glareolus*) from Poland and field voles (*Microtus agrestis*) from the United Kingdom, contain S protein genes of betacoronaviruses.¹¹⁸ For **peridomestic** rodents or those living in close-in close contact with agricultural animals, cross-species transmission of alphacoronaviruses might occur via contact with rodent carcasses, feces, or urine.^{119,120} European rats (*Rattus norvegicus*) are such rodents, as well as rodents from the United Kingdom and China, including field and bank voles, respectively. Field mice (*Apodemus chevrieri*), gray red-backed voles (*Myodes rufocanus*), and lesser rice field rats (*Rattus losea*) also harbor alphacoronaviruses, but none of the 394 tested house mice from the United Kingdom were positive (*Mus musculus*).^{118,119,121} It appears as if all rodent alphacoronavirus from Europe, the United Kingdom, and East Asia are part of a single group with similar genomic structure and the recombinant viral S gene. Rodent alphacoronaviruses thus might have evolved from a single common ancestor.¹¹⁹

Other mammalian coronaviruses may also recombine with rodent coronaviruses. Dromedary camel coronavirus HKU23 from Nigeria, Morocco, and Ethiopia, for example,

may also have resulted from recombination, based on similarities of parts of its genome with that of RodentCoV-IM2014 as well as with rabbit coronavirus HKU14.¹²² Moreover, the patterns of recombination differ across the studied areas of Africa.

7.2.3.4 *Coronaviruses with zoonotic potential in carnivores*

Pathogenic HCoV are known to have arisen in Asia, the Middle East, and the northern arid part of Africa. They may also have undergone zoonotic transmission and established themselves in other animal reservoir species in Europe, Australia, and the Americas. In addition to pangolins and rodents, members of the following other families of carnivores should perhaps be screened for coronaviruses with the potential for zoonotic transmission since they are readily infected by SARS-CoV-2: Viverridae (civets and genets throughout southeast Asia, India, and Africa), Mustelidae (weasels, badgers, otters, ferrets, martens, minks and wolverines found throughout most of the inhabited world except Australia), and Felidae (wild-spread wild and domestic cat species).⁸²

SARS-CoV-2 was discovered in mink (*Neovison vison*) farms in the northwestern United States in mid-2020, presumably due to their exposure to infected farms.¹²³ Examination of minks, cats, rodents, raccoons, and skunks in mink farms revealed that 72% of these animals are infected by coronaviruses, and 10% test positive for more than one coronavirus species.¹²⁴ Both alpha- and betacoronaviruses were detected, with SARS-CoV-2 comprising 84.1% of the betacoronaviruses in minks.¹²⁴ The colorectal area and the lungs were the only tested tissues that were positive for the presence of SARS-CoV-2 RNA. Nervous system tissue, however, was not tested in this study. Mink, and perhaps other animals in the vicinity of these farms, could serve as living mixing vessels for genetic recombination between coronaviruses of one species or between coronaviruses of different host species. The resulting recombinant coronavirus could then undergo zoonotic transmission.

7.3 Possible ways to predict and prevent future epidemics and pandemics

7.3.1 The One Health approach

A multitude of interacting and constantly changing forms and activity of living organisms (human, animal, plant, fungal, protist, and bacterial) and viruses, acting together with environmental factors, are involved in the evolution, transmission, and virulence of potential pathogens of humans and animals. The environmental factors and their interactions with organisms need to be defined before appropriate measures can be produced to provide optimal surveillance, detection, and management strategies.¹²⁵

Chiroptera (bats) and Rodentia (rodents) are the two largest orders of mammals, containing approximately 1400 and 2500 living species, respectively.¹²⁶ They inhabit numerous, varied ecological niches, many of which overlap with human residences, agricultural buildings and fields, and other occupational sites. Transmission of coronaviruses from these species into humans, agricultural animals, companion animals, and other animal species, particularly those that are endangered, is a major risk for mammals and birds that are primarily threatened by members of *Alpha-* and *Betacoronavirus* and *Gamma-* and *Deltacoronavirus*, respectively. Some cave-dwelling bat coronaviruses are most closely

related to the viruses responsible for severe diseases in humans or animals. Accordingly, guano collectors, tourists visiting caves, and members of some religious societies who spend time near caves, especially in regions known to be home to pathogenic coronaviruses, should be vigilant for signs of coronavirus disease.⁷⁷ These areas may be particularly useful for the study of the potential reservoir or intermediate pathogenic coronavirus hosts using the One Health approach.

The One Health approach to overall, rather than just human, health focuses on interconnections between humans, animals, and the environment, and recognizes that the health of each is connected to the health of all.¹²⁵ Such an approach requires the cooperation and coordination of a multidisciplinary team consisting of at least researchers, medical and public health personnel; veterinarians; specialists in **animal husbandry**, agriculture, and ecology; and microbial **phylogeneticists**.¹²⁵ Beyond the One Health approach, other important personnel might include experts in **mammalogy**, **ornithology**, **herpetology**, **entomology**, microbiology, and immunology as well as governmental and regulatory personnel and constitutional or other appropriate legal experts. This diverse group of people should have the expertise and ability to respond to and prevent major outbreaks in a manner consistent with the needs and values of all components of our ecosystems and the laws of diverse populations.

The One Health approach attempts to prevent or minimize future pandemics by focusing funds and research on the following areas: (1) wildlife surveillance that can rapidly identify high-risk pathogens that they harbor, (2) surveillance of people with direct or indirect contact with wildlife to rapidly detect zoonotic transmission of pathogenic microbes, and (3) improvement of biosecurity measures regulating the legal wildlife trade and decreasing illicit trade, such as the capture and sale of the critically endangered *Sudra pangolins*.¹²⁷ The pangolin trafficking may play a major causal role in multiple exportations of SARS-CoV-2-like viruses to China.¹²⁸ While bats are the only mammals capable of true flight,⁵⁹ legal and illicit wildlife trafficking can also move other types of animals far from their origins, spreading infectious diseases over long distances.

7.3.2 SpillOver

In the World Health Organization's 2018 Blueprint of Priority Diseases, Disease X, a putative unknown pathogen that may lead to a serious human epidemic, is a target for research.^{129,130} SpillOver is open access, web-based, risk assessment tool that may help to reach Blueprint's goals. It is designed to evaluate the likelihood of zoonotic transmission of viruses to enable disease prioritization, prevention, and control.¹³⁰ SpillOver examined data from 509,721 samples of 74,635 animals to rank the potential of zoonotic transmission of 887 wildlife viruses.¹³⁰ Its analysis included information about the viruses; their hosts; and environmental factors, including the host's geographical location and ecology; in addition to related human behaviors.^{131,132}

Using this tool, Lassa virus, the causative agent of Lassa hemorrhagic fever that is transmitted by multimammate rats (*Mastomys natalensis*),¹³³ is ranked as having the highest risk.^{78,130} SARS-CoV-2 is ranked as #2 and SARS-CoV, as #8. The bat coronaviruses that were among the top 30 include the following: 229E, Rousettus bat coronavirus HKU9, and SARS-related betacoronavirus Rp3, are ranked #13–15. Chaerephon bat coronavirus/Kenya/KY22/2006

and Chaerephon bat coronavirus/Kenya/KY22/2006 are ranked as #20–21 and Eidolon bat coronavirus/Kenya/KY24/2006 and Coronavirus PREDICT CoV-24 are ranked #27 and #29, respectively. Several rodent coronaviruses are ranked among the top 30 as well: murine coronavirus (#18), Longquan Aa coronavirus (#23), and rodent coronavirus (#27).¹³⁰

7.3.3 Museums and emerging pathogens in the Americas (MEPA)

MEPA is another approach to attempt to prevent the emergence of highly pathogenic viruses into human or domesticated animal populations.¹³⁴ MEPA could serve as a publicly available source of biological samples that include spatial, temporal, and taxonomic diversity. It would allow researchers to speedily obtain accurate identification of emerging infectious disease agents and their reservoir and intermediate hosts.¹³⁴ MEPA could provide a virtual network for communicating and coordinating rapid responses to emerging infectious disease agents. This would serve as a joint problem-solving program that involves the interaction of pathogen researchers, public health officials, and biorepositories in the Americas and then perhaps expanding to other areas of the world.¹³⁴ This virtual system increases biodiversity infrastructure and training as well as provides means of communicating among the biorepositories and biomedical communities, especially in low-income regions that may lack the means of in-person interaction.¹³⁴ This virtual program can also operate in real-time, speeding our responses to halt potential epidemics or pandemics before they transition from local outbreaks to regional or wider-spread health emergencies.

MEPA would enable the exploration of ecological, evolutionary, and environmental relationships that may factor into the enzootic or zoonotic transmission. Natural history museum biorepositories could be a critical component of such a decentralized, global network of potential pathogen surveillance. To function optimally, they require increased biodiversity infrastructure and training programs. This is especially important in the biologically diverse developing and lower-income regions.¹³⁴

The groundwork for MEPA presently exists as the “Global Museum,” an international community of natural history museums that are becoming digitally connected.^{135,136} Each museum would also act as a biorepository for long-term preservation of biological materials, such as hides and skins, skeletons, cryogenically frozen tissues, and information, including sample collection location and occurrence, environment, and pathogen/symbiont relationships.¹³⁴

7.4 Factors driving zoonotic transmission

Several factors affect the ability of infectious agents, including coronaviruses, to undergo either transmission of pathogens from humans to animals or zoonotic transmission and, perhaps, lead to a life-threatening pandemic. These involve microbial, host-related, and environmental factors that permit microbes to adapt themselves to new host species.

7.4.1 Viral factors driving zoonotic transmission

Viral factors that influence interspecies transmission include the presence of an error-prone RdRp or ExoN, genetic recombination, viral quasispecies, selection pressure by the host immune system, viral variant replicative ability, and the number of generations of passage from one person to another. All of these encourage a variety of mutations in coronavirus strains.^{103,137} This may lead to an initial viral rapid growth process within cells, which results in a rapid increase in viral diversity that may produce viral variants with an expanded host range.^{103,138} Factors that aid in predicting successful zoonotic viral transmission with long-term persistence in humans include those that cause low host mortality, establish long-term chronic infections, and involve nonenveloped, **nonsegmented** viruses that are not transmitted by vectors.¹³¹ Coronaviruses display the last two traits, but not the others. Somewhat surprisingly, genome length, type, and recombination frequency have been reported to not be important predictive factors,¹³¹ although they have been so stated by many other studies, as described earlier in this chapter. The above factors, therefore, did not successfully predict the persistence of MERS-CoV and SARS-CoV-2 in humans.

7.4.2 Host-related factors driving zoonotic transmission

Some of the host species' biological properties increase or decrease the risk of infection as well as disease severity. These properties include the presence and extent of the pathogen's receptor or coreceptor expression, the efficacy of the immune response, and the host's body temperature and behaviors.¹⁰³

7.4.2.1 Host cell receptors and immune responses

If a potential host species do not have an adequate number of a microbe's receptors and coreceptors, it will not be naturally infected since the microbe will not be able to bind to or enter the hosts' cells. In coronaviruses, the viral component that binds to these receptors is the S protein, a major target of the immune response. A delicate balance exists in the extent and type of the host's immune response to a given microbe. If the immune response is too limited (immunosuppression), the host is more susceptible to infection, while if too great, dangerous inflammatory responses ensue, as described in Chapter 1. Moreover, the type of immune response is important. **Th1** immune responses are both antiviral and proinflammatory. By contrast, **T regulatory** responses downregulate the immune response in general and so decrease both beneficial antiviral activity as well as detrimental, excessive inflammation. Similar types of stimulatory and inhibitory macrophages also exist (**M1** and **M2 macrophages**, respectively) as do other such complementary immune **homeostatic** systems. The activity of stimulatory and regulatory immune operatives is constantly in flux to keep the protective aspects of the immune response in an optimal range while tamping down pathogenic, disproportionate responses, as described in Chapter 1.

7.4.2.2 Host body temperature and behaviors

One of the host's immune defense strategies is to raise body temperature since microbes are adapted to a certain temperature range. Some **cytokines**, especially **interleukin-1** and

tumor necrosis factor- α , raise the body's temperature above the high terminus of many microbes' optimal survival range, including SARS-CoV-2.¹³⁹ Cats and ferrets are highly susceptible to SARS-CoV-2 infection. Their body temperatures are 37.8°C (100°F) and 38.2°C to 38.8°C (100.8° to 101.8°F) for cats and ferrets, respectively. Pigs, with body temperatures of 39.3°C to 39.8°C (102.8° to 103.6°F), are not infected by SARS-CoV, despite having an ACE2 receptor with greater similarity to human ACE2 than those of cats and ferrets.¹⁰³ Ducks and chickens are also not infected by SARS-CoV. Their body temperatures are 41.2°C (106.2°F) and 41.6°C to 41.9°C (106.9° to 107.4°F), respectively.¹⁰³

Bats are a special case in that they have unusual, fluctuating changes in their daily body temperature due to the intense metabolic activity involved in flight at night and the **torpor** resting state during the day, in addition to winter hibernation in some bat species.¹⁴⁰ The decreases in body temperature and metabolism might reduce the bats' immune responses, delaying microbial clearance from their populations.¹⁴⁰

The spread of infectious disease agents also involves the host's behaviors, including population density.¹⁰³ Many species of bats live in large, dense colonies in caves or other enclosed spaces. Bats may roost in larger groups in abandoned mines rather than caves, thus human activity increases their population density and risk of intraspecies virus transmission.⁶⁸ Some bat species also partake in grooming activities. This close contact and intimate interaction may increase the risk of transmission of respiratory disease pathogens by inhalation or by contact with body fluids, including saliva.¹⁰³ The three bat species hosts of the SARS-CoV-2-like viruses BANAL-52, -103, and -236 inhabit caves. The effects of human behavior on infectious diseases are much more complex and will be described separately.

7.4.3 Environmental factors driving zoonotic transmission

Land changes lead to the loss of natural habitat. Deforestation allows poachers increased access to large areas that still contain animals for game and illegal trade.⁶⁸ Replacement of forested regions with agricultural or urban communities further increases human-to-animal contact and some of the animals, including rodent intermediate coronavirus hosts, adjust to human population centers and to agricultural fields, where humans may inhale viruses found in rodent feces and dried excreta during plowing, as in the case of American hemorrhagic fever viruses.^{141,142} Interestingly, reforestation sometimes also is at least partially responsible for the emergence of some infectious diseases as well. An example of this is Lyme disease, in which the increase in forested areas also increases the population of deer and deer ticks, the vector of Lyme disease.¹⁴³

Urbanization, the mass movement of people from rural to urban areas, can spread formerly localized infections.¹⁴³ Overcrowded and rapidly growing cities and surrounding slums reduce the proper implementation of public health measures, which might allow the establishment of newly introduced infections and their associated diseases, including HIV, cholera, and dengue fever.¹⁴³

Improvements in hunting and agricultural tools also increase the ability of hunters and farmers to harvest wildlife and transform both the fauna and flora of ecosystems.⁶⁸ Additionally, several human influenza pandemics of the 20th century appear to have been

associated with the agricultural practice of integrated pig-duck farming in China in which pigs serve as biological mixing vessels that allow the production of novel influenza strains that are very different from their predecessors.¹⁴⁴

Human dietary habits also affect ecosystems and spread potential pathogenic HCoVs. For example, some rare animal species are delicacies in China.¹⁴⁵ Pangolins are very difficult to raise on farms and so are taken from the wild.¹⁴⁶ As Chinese pangolins (*Manis pentadactyla Linnaeus*) are nearing extinction, the illegal trade for pangolins from other Southeast Asian countries has grown.⁶⁸

7.4.4 The “human factor” and modeling

Many of the predictions about the course of epidemics and pandemics are based on computer modeling. The models are only as good as the data upon which they are based. It is difficult to produce accurate predicative models for viral prevalence and disease severity due to several parameters, including human factors. People have complex lives and so are our responses to any given situation. As intelligent life forms, humans can and do alter other factors important to microbial diseases. We change our behaviors to prevent exposure to sources of viral transmission. While humans do not readily evolve our genetic and physical attributes, we can evolve our responses to emerging threats.

While predictive models can factor in the effects of changing behavioral responses, they cannot account for unexpected events. These include the production of readily available diagnostic tests, some of which can be delivered to and administered at homes, allowing rapid identification of asymptomatic as well as symptomatic infections. Other unpredictable factors include the discovery of new and more effective drugs, such as remdesivir, or the development of several safe, efficacious, and inexpensive vaccines. Additionally, a one-size-fits-all model cannot reflect all relevant factors, since conditions vary in different populations and over time. Predictive models are useful tools, but they often over- or underestimate the numbers of people who will become infected, the severity of the disease, and the number of deaths. Overestimation of disease severity may lead to panic and the enforcement of harsher than necessary protective policies, which may negatively affect individuals and cultures throughout the world. Underestimation of disease severity, however, leads to complacency and failure to institute isolation, distancing, masking, and vaccination measures, when appropriate, promptly.

7.4.5 The emergence and disease severity of severe acute respiratory system coronavirus-2 variants

During the early phase of the COVID-19 pandemic, SARS-CoV-2 variants containing the D614G mutation of the S protein were dominant in many areas, but by the late fall of 2020, other SARS-CoV-2 variants emerged that disseminated more rapidly, including the Alpha (B.1.1.7) variant from England, the Beta (B.1.351) variant from South Africa, and the Gamma (P.1) lineage from Brazil.¹⁴⁷ Mutation of other key nucleosides alters the ability of many viral proteins to block the immune response, particularly the production of the strongly antiviral **interferons**.¹⁴⁸

The Alpha variant of SARS-CoV-2 was first reported in England on December 14, 2020, but it was estimated to have emerged in September of that year.^{147,149} A 2021 model predicted a large surge in COVID-19 cases and deaths in 2021. The Alpha variant had a 43%–90% higher R_0 than prior SARS-CoV-2 variants. According to a report published in April 2021, the contagious Alpha variant soon represented greater than 98% of the SARS-CoV-2 infections in England and was responsible for a surge in COVID-19 cases and deaths. These led to a third national lockdown on January 5, 2021.¹⁴⁹ By mid-January 2021, thirty countries had reported infections with the Alpha variant, which was more readily transmissible than other SARS-CoV-2 variants known at that time.¹⁴⁷

The Delta variant (B.1.617.2) is almost twofold as contagious as prior SARS-CoV-2 variants and causes more severe disease. Both the Delta variant and the BA.1 Omicron subvariant can infect fully vaccinated people, who can subsequently transmit the virus to other, primarily unvaccinated, people.¹⁵⁰ The BA.1 Omicron subvariant is even more contagious than Delta. It contains 37 mutations in its S protein.^{151,152} In the three weeks between late November 2021 to early January 2022, this subvariant led to 90% of the new COVID-19 cases. Despite an increased vaccine breakthrough rate, the rate of hospitalization, the intensity of respiratory support, and the mortality rate were less than that associated with the Delta variant in Canada, the United States, and South Africa.^{151–153} Vaccination protects against severe disease in patients with this Omicron subvariant.¹⁵⁰ The decreased disease severity may be due in part to the younger age of the patients compared to those infected by the Delta variant.¹⁵²

As of the date of this writing (March 22, 2022), another Omicron subvariant, BA.2, has emerged that is more contagious, but leads to less severe disease, than the Delta variant. According to a statement by the World Health Organization issued near the end of February 2022,¹⁵⁴ the overall proportion of reported cases of the BA.2 Omicron subvariant is increasing relative to that of the BA.1 subvariant. The BA.2 subvariant is more contagious than BA.1, however, the difference in transmissibility is much smaller than that between the BA.1 Omicron subvariant and the Delta variant. The global circulation of BA.2 and all other SARS-CoV-2 variants was stated to be decreasing.

7.5 The continuing threat of emerging infectious diseases

Within a very short time period in 2003, researchers identified a highly pathogenic coronavirus, named SARS-CoV, isolated it, characterized it, and sequenced its genome.^{155–159} This was a tremendous feat, especially since coronaviruses up to this time typically were associated with the common cold. Due in large part to lessons learned from the studies of SARS/SARS-CoV and tremendous effort, in addition to advanced techniques, in 2020, researchers from around the world were able to identify the species of coronavirus responsible for the current COVID-19 pandemic, decipher its RNA genetic code, produce test kits, and begin the process of developing drugs to treat the infection and vaccines to prevent infection, within the period of three months (January to March). The mass production of huge numbers of masks, ventilators, and test kits for use by people who have symptoms of infection has also been extremely rapid.

7.5.1 Changes in infectious disease patterns over the last ten years

To gain an understanding of trends in emerging infectious disease agents, the author used as a proxy the number of articles whose titles included the names of various human microbes and other infectious agents in three time periods in different years for increments of four months each (December–March) in the CDC's *Emerging Infectious Diseases journal* (Beltz, unpublished data). The three-time periods were conducted at five-year intervals (2021–2022, 2016–2017, and 2011–2012). The results of this search are listed in [Tables 7.3 and 7.4](#) and include information about the types of microbe, the major disease(s) with which they are associated, and the major route(s) by which they are transmitted. The findings are summarized below. Please note that this information includes multiple reports of the same infectious disease agent so that the overall reporting of various categories of agents is being considered, not the number of new emerging infectious disease agents themselves.

7.5.1.1 Viruses

For the total of the three-time periods, the number of reports of viruses equaled that of bacteria. However, reports of viruses exceeded those of bacteria and other infectious disease agents during 2021–2022, and reports of bacteria exceed those of viruses during 2016–2017 and 2011–2012. The majority of viral reports were highest in the (+) single-stranded RNA Baltimore Class IV viruses, especially during the 2021–2022 time period. This number increased greatly with time since the number of reports was much lower during the 2011–2012 period. The number of reports of Class IV families was highest in Coronaviridae during 2021–2022, with all of these reports concerning SARS-CoV-2, except for one report of MERS-CoV. During the 2016–2017 interval, the highest number of reports was among members of Flaviviridae, the majority concerning the Zika virus. No overall trend was seen in the 2011–2012 interval in Class IV viruses.

The next highest number of reports among viruses was in the (–) single-stranded RNA Baltimore Class V viruses, especially during the 2011–2012 time period. The number of reports of Class V families was highest in the Bunyaviridae in 2021–2022 and Filoviridae in 2016–2017, the latter being primarily due to the 2014–2015 Ebola epidemic. During the 2011–2012 interval, the highest number of reports was among members of Orthomyxoviridae and Paramyxoviridae, the majority of which concerned the 2009–2010 H1N1 pandemic influenza virus. Very few reports were found in Class I (double-stranded DNA), Class III (double-stranded-RNA), or Class IV retroviruses.

Respiratory diseases were predominantly reported during 2021–2022, primarily due to the COVID-19 pandemic. The number of reports of nervous system diseases was highest during 2016–2017, primarily due to the Zika epidemic in Brazil. The number of reports of viral hemorrhagic fever was highest during 2016–2017 and 2011–2012, the former being primarily due to the Ebola outbreak in western Africa in 2014–2015.

The major route of reported disease transmission during 2021–2022 was by inhalation of contaminated respiratory droplets or their contact with mucus membranes, including those of the eyes. This was primarily due to the SARS-CoV-2-associated COVID-19 pandemic. The major route of disease transmission during both the 2016–2017 and 2011–2012

TABLE 7.3 Emerging Infectious Disease Agents—Viruses.

Class IV—(+) ss-RNA											
Time Period	Coronaviruses	Picornaviruses	Calciviruses	Flaviviruses	Hepeviruses	Togaviruses	Astroviruses	Rhabdoviruses	Total		
12/21–3/22	67	2	1	4	1	1	0	0	76		
12/16–3/17	3	3	4	25	8	1	1	0	45		
12/11–3/12	1	3	0	12	4	3	2	3	28		
Total	71	8	5	41	13	5	3	3	149		
Class V—(-) ss-RNA											
Time Period	Paramyxo-viruses	Nairoviruses	Phenui-viruses	Filoviruses	Bunya-viruses	Orthomyo-viruses	Hanta-viruses	Borna-viruses	Pneumono-viruses	Arenaviruses	Total
12/21–3/22	2	2	2	1	5	1	0	0	0	0	13
12/16–3/17	1	2	1	6	2	1	2	1	1	1	18
12/11–3/12	7	0	0	1	5	10	0	1	0	3	27
Total	10	4	3	8	12	12	2	2	1	4	58
Disease											
Time period	Respiratory	Digestive	Fever/HF	Nervous	Joint	Liver	Immune	Rash	Cancer	Heart	Multiple
12/21–3/22	70	2	7	9	1	3	2	0	0	0	1
12/16–3/17	6	5	14	24	1	9	0	2	1	0	0
12/11–3/12	18	2	19	12	3	4	2	2	1	1	2
Total	84	9	40	45	5	16	4	4	2	1	3
Transmission											
Time period	Respiratory	Fecal-oral	Tick/Insect	STD	Animal contact/excreta			Bodily fluids		Human contact	
12/21–3/22	68	4	11	1	4			4		0	
12/16–3/17	6	9	26	0	11			5		3	
12/11–3/12	16	4	21	0	16			3		2	
Total	90	17	58	1	31			12		5	

Also 2 Class III Reoviridae (ds-RNA) and 1 Class VI Retroviridae for 12/2021–3/2022.

Also 1 Class I Parvoviridae and 3 Class III Reoviridae for 12/2016–3/2017.

Also 1 Class I Herpesviridae.

The number of reports containing the names of emerging infectious viruses of humans from the *Emerging Infectious Diseases* journal are depicted by Baltimore Class and family and their major associated disease type(s) and major route(s) of transmission. These numbers indicate four-month time intervals (December–March) over three five-year periods (2011–2012, 2016–2017, and 2021–2022). The majority of viruses reported were from Class IV, followed by Class V. The majority of disease types were respiratory, fever/hemorrhagic fever, or nervous system illnesses. The majority of transmission occurred by the respiratory route or tick/insect bites. The virus families, prevalent diseases, and routes of transmission differed among the five-year time periods.

intervals was by the bite of infected mosquitoes, primarily due to the Zika and West Nile viruses, respectively, both members of the Class IV family Flaviviridae. SARS-CoV is a Class IV member of Coronaviridae. Ticks also transmit some viruses, including flaviviruses such as tick-borne encephalitis virus, louping-ill virus, and Powassan virus, which are common in some regions of Eurasia, the British Isles, and North America, respectively.^{160–162}

TABLE 7.4 Emerging Infectious Disease Agents—Bacteria, Prions, Protists, Fungi, Helminths, and Insects.

Bacteria										
Time period	(−) Cocci	(+) Cocci	(−) Bacilli	(+) Bacilli	Coccobacilli	Acid-fast	Spirochete	Other	Total	
12/2021–3/2022	3	4	9	5	2	8	1	2	29	
12/2016–3/2017	1	1	16	6	7	17	4	4	56	
12/2011–3/2012	2	5	16	6	8	15	3	9	64	
Total	6	10	41	17	17	40	8	15	149	

Disease										
Time period	Respiratory	Digestive	Fever	Nervous	Joint	Rash	Reproductive	Plague	Heart	Other
12/2021–3/2022	10	7	4	3	1	2	1	0	0	11
12/2016–3/2017	19	9	6	4	2	3	2	4	0	11
12/2011–3/2012	10	15	6	3	3	3	1	1	1	23
Total	39	24	16	10	6	8	4	5	1	45

Transmission										
Time period	Respiratory	Fecal-oral	Wounds	Tick/Fleas	Insects	STD	Animal bites	Animal contact	Body Fluids	Other
12/2021–3/2022	9	5	1	3	1	1	1	0	0	13
12/2016–3/2017	23	9	2	9	2	3	0	4	0	0
12/2011–3/2012	16	19	1	7	0	0	0	4	4	11
Total	48	33	4	19	3	4	1	8	4	24

Other Infectious Disease Agents						
Time period	Prions	Protists	Fungi	Helminths	Insects	Total
12/2021–3/2022	2	0	3	1	0	6
12/2016–3/2017	1	3	3	7	0	14
12/2011–3/2012	7	7	6	5	1	26
Total	10	10	12	13	1	46

The number of reports containing the names of emerging infectious disease agents of humans other than viruses from the *Emerging Infectious Diseases* journal are depicted by type and their major associated disease type(s) and major route(s) of transmission. These numbers indicate four-month time intervals (December–March) over three five-year periods (2011–2012, 2016–2017, and 2021–2022). The majority of the reported agents were Gram-negative bacilli and acid-fast bacteria. The majority of disease types were respiratory, digestive, and fever. The majority of transmission occurred by the respiratory or fecal-oral routes or tick bites. Very little differences were seen over the five-year time intervals reported.

7.5.1.2 Bacteria and other infectious disease agents

The author found that bacterial infections were reported more often in the *Emerging Infectious Diseases* journal in the December-March interval during 2011–2012 and 2016–2017 than during 2021–2022 (Beltz, unpublished data). The most commonly reported bacteria were Gram-negative bacilli and acid-fast bacteria (both *Mycobacteria*

tuberculosis and nontuberculosis *Mycobacteria*). The diseases that they cause are primarily respiratory (all three time periods) and digestive (2016–2017) and they were primarily transmitted via the respiratory and fecal-oral routes, respectively.

The other types of infectious disease agents were most frequently reported during 2011–2012. They are almost equally represented by prions, parasitic protozoa, helminths, and fungi, although a parasitic insect larva was also reported.

7.5.1.3 General trends and prediction of infectious diseases between 2011–2022

The author found that during the 2021–2022 and 2016–2017 time periods, the reports of Class IV viruses greatly exceeded that of Class V viruses, especially during 2021–2022 (Beltz, unpublished data). During 2021–2022, respiratory diseases predominated, while nervous system diseases predominated between 2016–2017, and respiratory/hemorrhagic fever was dominant during 2011–2012. The modes of transmission also varied between time periods, being predominately respiratory during 2021–2022, mosquito-borne during 2016–2017, and being a mixture of insect-borne, respiratory, and animal contact during 2011–2012. These trends reflect differences in the predominant disease epidemics and pandemics at those times: COVID-19 during 2021–2022, Zika and Ebola during 2016–2017, and H1N1 pandemic influenza during 2011–2012. Taken together, this overview suggests that it is difficult to model and predict the coming viral epidemics and pandemics, the types of diseases that they cause, and the route of transmission over time.

A separate group of researchers developed a model of the 2014–2016 Ebola disease epidemic in Western Africa that utilizes spatial contagion dynamics. It detects individual viruses that possess high rates of spatiotemporal propagation.¹⁶³ This model indicates that maximizing the knowledge about the virus and its host and the environment in which it resides, as well as where the pandemic begins, is critical to developing an effective model of viral disease spread. This model and the Temporary Epidemiology Field Assignee Program have also been applied to COVID-19.^{163,164} Models have been developed to predict factors involved in Zika-related diseases as well.^{165,166} These models mapped spatiotemporal features, disease burden, and week of initial detection. Nevertheless, the information in [Tables 7.3 and 7.4](#) cautions about the efficacy of such models in predicting intermediate to long-term epidemic emergence and spread of viruses and their associated diseases.

The bacteria and their diseases and routes of transmission were less variable than those of viruses. Most of the reported bacteria were Gram-positive bacilli and acid-fast bacteria (primarily nontuberculosis *Mycobacteria*) in all examined time periods, causing digestive and respiratory diseases, and transmitted by the fecal-oral and respiratory routes. Among the other infectious disease agents, no definite pattern was apparent during the examined disease periods. This suggests that the prediction of these bacteria and other disease agents is easier to accomplish than predictions of viruses.

7.5.1.4 General trends in infectious diseases between 1940 and 2004

A 2008 study examined the temporal origin of an emerging infectious disease (the original or cluster of cases of infectious disease as it initially emerges in humans) as an emerging infectious disease “event.”¹⁶⁷ For that study, the annual number of articles published in the *Journal of Infectious Diseases* was used. The study reported 335 emerging infectious

disease events between 1940 and 2004.¹⁶⁷ During that time interval, 54.3% of the events were due to bacterial or rickettsial infections. Of the remaining events, 25.4% were due to viruses or prions, 10.7% were protozoa, 6.3% were fungi and 3.3% were helminths.¹⁶⁷ Several prior analyses with overlapping authorships had indicated that 37%–44% of the emerging pathogenic agents were viruses or prions and 10%–30% were bacteria or rickettsia.^{168–170}

Regions with high densities of emerging infectious disease events in the 2008 study¹⁶⁷ were found in 30°–60° north and 30°–40° south latitudes, especially in medically advanced areas of the northeastern United States, western Europe, Japan, and southeastern Australia. Accordingly, many of the events were due to drug-resistant microbes for which each strain was considered separately. This may explain to some extent the difference between the 2008 study and a prior report in which the human pathogen species diversity was more equatorial.¹⁷¹

7.5.2 The next pandemics—thinking outside of the box

We must learn to prepare ourselves for the next pandemics, for it is almost a certainty that there will be other pandemics in our lifetimes as humans alter the environment in ways that bring us into contact with species of animals and their microbiomes that we would not otherwise encounter. We will doubtless be infected by some of the new infectious disease agents, some of which have the potential to sicken or kill large numbers of people or specific demographic groups. One question for these future encounters with outbreaks of these new microbial infections is whether we will have in place the strategies needed to prevent an outbreak from becoming an epidemic and if we can prevent an epidemic from becoming a pandemic.

7.5.2.1 *Plans for rapid responses to outbreaks and innovative solutions*

It may be wise if all healthcare facilities have well-considered plans that allow the early identification of patients with highly contagious and virulent infectious diseases. Such plans may detail the procedures for immediate isolation of these patients. Infectious disease training of all healthcare personnel as well as custodial staff could be given before the appearance of an epidemic or pandemic, regardless of the nature of the disease or its route of transmission. A reserve of appropriate **personal protective equipment (PPE)** could be present to prevent transmission among healthcare personnel and, from them to other patients and visitors.¹⁷²

Large numbers of surgical masks for the public and N95/KN95 respirator masks for frontline healthcare providers, gloves, gowns, and disinfectants could be stockpiled and replaced regularly to ensure that they are used elsewhere if they near their expiration dates. These stockpiles could be placed in regional locations and plans made for their distribution to viral hotspots. During the 2009–2010 H1N1 influenza pandemic, there was a shortage of these respirators¹⁷³ that might have been devastating if that pandemic had produced a larger number of cases and without the help of industries, as described below. Plans could also be in place for the rapid production of additional equipment and supplies within the United States and other developed nations as well as plans to manufacture and

distribute essential material to developing regions, for both humanitarian reasons as well as to prevent the emergence of novel viral variants. It should be noted that respiratory viruses can survive for extended periods on N95 respirator material, posing a challenge to the reuse of these masks. Several technologies to disinfect these expensive masks are under consideration, including UV light, hydrogen peroxide vapor, and ethylene oxide.¹⁷⁴

In addition to PPE, adequate supplies of other materials are also needed. In dealing with the periodic upsurges of COVID-19, large numbers of ventilators were needed. Other medical supplies and equipment are needed for other types of diseases, such as diseases of the digestive tract and nervous and urinary systems. These are also affected by pathogenic HCoVs as well as other infectious disease agents. We cannot stockpile enough specialized equipment to cover every type of infectious disease, but we can make plans for innovative ways to retool our industries to rapidly produce the supplies and equipment appropriate to the threat. During the COVID-19 pandemic, this was evidenced in the United States by some distilleries producing alcohol for hand sanitizing,¹⁷⁵ the auto industry producing ventilators,¹⁷⁶ and the use of three-dimensional printing for production of PPE and medical equipment, including ventilators.¹⁷⁷

7.5.2.2 Dedicated infectious disease treatment centers

The United States designed over 90 specialized facilities in response to the large Ebola outbreak in West Africa between 2014 and 2016. According to the CDC, *Ebola treatment centers that provide comprehensive care to people diagnosed with EVD need specific competencies and resources.*¹⁷⁸ The guidelines for these centers included information concerning facility infrastructure, patient transportation, laboratory, staffing, training, PPE, waste management, worker safety, environmental services, clinical competency, operations coordination, and state/hospital selection as an Ebola Treatment Center.¹⁷⁸

This author suggests a more flexible approach to outbreaks and epidemics of infectious diseases. Rather than producing only specialized healthcare facilities, the suggested approach includes the production and maintenance of multifaceted regions of hospitals that can be quickly transformed to care for large numbers of critically ill or contagious patients with different kinds of infections. Facilities with such capacities, proposed to be named Infectious Disease Centers, could be similar to regional Trauma Centers. Infectious Disease Centers could have lists of the appropriate doctors and nurses needed for different disease categories—a list of virologists for viral infections, a different list for bacterial infections, a list of respiratory disease specialists, digestive disease specialists, and so on. The appropriate group of specialists could then be called upon during an outbreak. Between outbreaks, the Centers could be used to isolate patients with infectious diseases from the remainder of the hospital's patient population.

Additional specialized facilities could be included in plans for the Infectious Disease Centers. The additional healthcare spaces would not have to be permanent but could involve the conversion of passenger ships into hospital ships, as was performed during the early phase of the COVID-19 pandemic. An example of this type of innovative temporary facility occurred in Italy. One deck of a long-distance ferry ship was converted into a hospital ship for COVID-19 patients who continued to test positive for the virus following the acute phase but still required low to medium intensity care.¹⁷⁹ The ship contained “unsafe zones” and air treatment units that were previously present separated the air in

the unsafe zones from other areas of the ship. N99/KN99 masks, visors, and appropriate safety suits were used during contact with patients. Both healthcare staff and the ship's crew received periodic safety training. Special arrangements were made for the storage and removal of wastes and bed linens from the unsafe zone.¹⁷⁹ The presence of this hospital ship allowed the use of beds in conventional hospitals for patients in the acute phase of the disease. From March 23 to June 18, 2020, 191 patients were admitted onto the hospital ship and received multidisciplinary care. All patients had favorable outcomes.¹⁷⁹ This innovative work was performed with the cooperation and coordination of local and regional hospitals and healthcare authorities.

Military hospital ships were also used during the COVID-19 pandemic. Early during the pandemic, the United State Naval Ship COMFORT was deployed to New York City to increase the healthcare capacity for the region's overextended patient care system.¹⁸⁰ This military hospital ship contained 1000 beds and had open bay wards of approximately 30 beds each, operating rooms, and postanesthesia care units that were appropriate for a trauma hospital.¹⁸⁰ During this mission, intensive care unit bays were converted into use for COVID-19 patients and a dedicated negative pressure isolation room was designed with air passage return through high-efficiency particulate air filters. Despite being present in an area with a high rate of community transmission, the total rate of SARS-CoV-2 infection among the ship's personnel was 3.0% (13/432 responding personnel), all but one of whom were healthcare providers, with 61.5% being asymptomatic.¹⁸⁰ This contrasts with the 30% infection rate on a deployed United States destroyer and an aircraft carrier during the pandemic. The low prevalence rate demonstrates that this type of operation could be safely incorporated into the proposed Infectious Disease Center plan as well.

7.6 Infectious diseases and the developing world

As demonstrated by the 2014–2015 Ebola virus epidemic in West Africa, regions of the world, especially in developing countries, have inadequate health services and cannot quickly identify previously known or emerging infectious disease outbreaks. They cannot also treat the ill, isolate the exposed, and break the chain of disease transmission.¹²⁵ Community responses were often strongly negative and resulted in the deaths of some aid and medical personnel due to inappropriate medical interventions and foreign workers that were not knowledgeable about local cultural practices as well as not knowing the regional languages.^{125,181,182}

In addition to national, international, and global health surveillance, local surveillance is also needed.¹²⁵ Infrastructure, such as clinics and mobile hospitals, and import of supplies and equipment need to be rapidly brought into the affected area, such as provided by aid agencies, including the American Red Cross¹⁸³, the World Health Organization,¹⁸⁴ and the United Nations Children's Fund.¹⁸⁵ Equipment includes autoclaves and other sterilizers and beds, while supplies include gloves, gowns, other PPE, bedding, disinfectants, and, perhaps, three-dimensional printers. Volunteer healthcare providers are also needed. Some of the volunteer groups that provide healthcare personnel and surgeries include Médecins Sans Frontières (Doctors Without Borders)¹⁸⁶ and the World Medical Mission of Samaritan's Purse¹⁸⁷.

It has been said that during the Ebola outbreak, rapid point-of-care diagnostics, specific treatments, vaccines, adequate medical care facilities, caregivers, trained staff, and the necessary supplies and equipment were lacking and that international health agencies need to act with greater speed and alacrity to allow early resolution of the epidemic before its expansion.¹²⁵ While these inadequacies need to be addressed, this author notes that, in the case of Ebola hemorrhagic fever, there are no specific treatments or vaccines. These do exist, however, for at least some potential pandemic agents. Even in the case of infection with SARS-CoV and SARS-CoV-2, first reported in developed countries, diagnostics, treatments, and vaccines had not yet been developed and the causative agents had to be identified and their genomes sequenced before specific and sensitive diagnostics could be produced, let alone mass-produced and distributed to developed and developing countries. Rapid treatment and vaccine development also need to be tempered by safety and efficacy concerns that require clinical testing, even if expedited.

Logistic concerns are also involved, even in developed countries, since some medicines must be administered intravenously in a medical setting and most SARS-CoV-2 vaccines require a “cold chain” in which the vaccine needs to be kept and transported either frozen or refrigerated, depending upon the vaccine in question. The total time for transport and clinic workday should not exceed 8 hours.¹⁸⁸ This delivery and usage time is difficult in remote areas of developing nations, although portable vaccine refrigerators or freezers may be used during transport to off-site clinics or satellite facilities. If frozen vaccines require transportation, they should be placed in a portable vaccine freezer container and pack that reliably keeps the temperature at -50° to -15°C (-58°F to 5°F).¹⁸⁸

For SARS-CoV-2 vaccines approved for use in the United States, the Centers for Disease Control and Prevention has a detailed database for transportation, storage, and use.¹⁸⁸ Pfizer vaccines require storage at ultralow temperatures of -90°C to -60°C (-76°F to -130°F) in a specialized freezer, in a regular freezer for up to 14 days, or a refrigerator for 5 days.¹⁸⁸ Delivery is at an ultralow temperature range in a thermal shipping container containing dry ice. Moderna vaccines may be stored in a regular freezer or a refrigerator for up to 30 days and are delivered frozen. The Johnson and Johnson vaccine is transported and stored in refrigerated temperatures of 2°C – 8°C (36° – 46°F). Freezers, especially ultralow freezers, are difficult to obtain in remote areas of developing countries.

7.7 Author's note (March 2022)

We must not allow ourselves the luxury of ignoring serious infectious diseases in other, less developed areas of the world. People worldwide are too interconnected by rapid means of travel, social media, economic interdependences, and our common humanity to do so. During the COVID-19 pandemic, what had begun in a small part of China in December 2019 was introduced to most of the rest of the areas of the world in early 2020. During the Spanish influenza pandemic, what began as a respiratory disease in Kansas, United States, in 1917 spread throughout the world, aided by the First World War and movement of military personnel¹⁸⁹ and civilian populations fleeing combat zones. In the case of Ebola, a disease originating in Western Africa during 2014–2015 caused many deaths as well as panic around the world.¹⁶³ Much more recently, a major epidemic of

Ebola-related diseases with a mortality rate of approximately 30% circulated in the Democratic Republic of the Congo (formerly Zaire). News about this large outbreak was not being covered in most of the world and may only come to the attention of these regions if a case appears there. We cannot continue to ignore these “foreign” diseases since they may spread to other areas unexpectedly, as occurred in the case of the 1918 influenza and the AIDS pandemic.

It has been said that “natural disasters bring people together but outbreaks and epidemics of infectious diseases split them apart...”¹⁹⁰ Let us hope that we will, as human beings that are part of numerous societies; as leaders of cities, states, and nations; and as scientists and clinicians, learn from our mistakes and follow the science of ever emerging, mutating, and evolving viruses. We need to remember that the human factor is as important in the course of the disease as are the protective measures that we develop. Human behavior is often unpredictable, and crises bring out the worst as well as the best in people.

We need not let panic dictate our lives. To do so would greatly diminish them, individually and as members of society. If the world and our social interactions could survive the devastation and loss of 20–40 million lives following the 1918 influenza pandemic, we should be able to return to our lives after the current COVID-19 pandemic as well. Hopefully, we will also be wiser and carefully watch for serious disease outbreaks throughout the globe. If we work together, despite our differences and fears, perhaps we can come out of this pandemic, the next, and the epidemics and pandemics to follow, as a world full of better and more caring people. It truly is a small, interconnected world, and, working together, we can accomplish once unimagined achievements.

Any man’s death diminishes me, because I am involved in mankind, and therefore never send to know for whom the bell tolls; it tolls for thee. —*John Donne (1623)*

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I

Coronavirus disease overviews

Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV)

Disease(s)—Severe acute respiratory syndrome (SARS)

Host Species—Humans

Common Intermediate Hosts—Animals from wild-game animal markets (palm civets, raccoon dogs, ferret badgers)

Type of Agent—Betacoronavirus lineage B, subgenus *Sarbecovirus*

Host Cell Receptor—Angiotensin-converting enzyme 2 (ACE2) as well as c-type lectin receptor expressed by dendritic cells (DC-SIGN), L-SIGN, and vimentin

Mode of Transmission—Inhalation of respiratory secretions from infected animals or humans; contact with the mucus membranes of the eyes or contaminated surfaces; rarely fecal-oral

Site of Origin—China

Geographical Distribution—Highest incidence in parts of Asia and Canada

Year of emergence—2002

Middle East Respiratory Syndrome Coronavirus (MERS-CoV)

Disease(s)—Middle East respiratory syndrome (MERS)

Host Species—Humans

Common Intermediate Host—Dromedary camels

Type of Agent—Betacoronavirus lineage C, subgenus *Merbecovirus*

Host Cell Receptor—Dipeptidyl peptidase 4 (DPP4)

Mode of Transmission—Inhalation of respiratory secretions of camels or consumption of their raw milk or urine; inhalation of respiratory secretions from infected people or dromedary camels

Site of Origin—Saudi Arabia

Geographical Distribution—Highest incidence in Saudi Arabia; other parts of the Middle East

Year of emergence—2012

Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2)

Disease(s)—Coronavirus disease (COVID-19)

Host Species—Humans

Common Intermediate Host—Possibly pangolins

Type of Agent—Betacoronavirus lineage B, subgenus *Sarbecovirus*

Host Cell Receptor—Angiotensin-converting enzyme 2 (ACE2)

Mode of Transmission—Inhalation of respiratory secretions from infected people or animals

Site of Origin—China

Geographical Distribution—World-wide

Year of emergence—2019

HCoV-229E

Disease(s)—Typically mild upper respiratory tract illnesses, including the common cold; may act as an autoimmune trigger for multiple sclerosis

Host Species—Humans

Type of Agent—Alphacoronavirus, subgenus *Duvinacovirus*

Host Cell Receptor—Aminopeptidase N (APN)

Mode of Transmission—Inhalation of respiratory secretions from infected people

Geographical Distribution—World-wide

Year of emergence—1966

HCoV-OC43

Disease(s)—Typically mild upper respiratory tract illnesses, including the common cold; may cause severe neurological diseases, such as chronic demyelinating disease and acute encephalomyelitis

Host Species—Humans

Type of Agent—Betacoronavirus lineage A, subgenus *Embecovirus*

Virus Species—*Betacoronavirus-1*

Host Cell Receptor—N-acetyl-9-O-acetylneuraminic acid; sialic acid as attachment receptor and human leukocyte antigen class I (HLA-I) as an entry receptor

Mode of Transmission—Inhalation of respiratory secretions from infected people

Geographical Distribution—World-wide

Year of emergence—1967

HCoV-NL63

Disease(s)—Typically mild upper respiratory tract illnesses, including the common cold; may cause croup in children

Host Species—Humans

Type of Agent—Alphacoronavirus, subgenus *Setracovirus*

Host Cell Receptor—ACE2

Mode of Transmission—Inhalation of respiratory secretions from infected people

Geographical Distribution—World-wide

Year of emergence—2004

HCoV-HKU1

Disease(s)—Typically mild upper respiratory tract illnesses, including the common cold; may cause inflammation of the bronchial tubes and pneumonia

Host Species—Humans

Type of Agent—Betacoronavirus lineage A, subgenus *Embecovirus*

Host Species Receptor—N-acetyl-9-O-acetyl neuraminic acid receptor; HLA-I as an entry receptor

Mode of Transmission—Inhalation of respiratory secretions from infected people

Geographical Distribution—World-wide

Year of emergence—2005

Porcine Endemic Diarrhea Virus (PEDV)

Disease(s)—Severe, watery diarrhea, vomiting, and dehydration; rapidly fatal in piglets and causes weight loss in older pigs

Host Species—Pigs

Type of Agent—Alphacoronavirus, subgenus *Pedacovirus*

Host Species Receptor—Aminopeptidase N (APN); coreceptors include cholesterol, sialic acid, and occludin

Mode of Transmission—Fecal-oral route

Geographical Distribution—Asia, Europe, North America

Year of emergence—1971

Porcine Deltacoronavirus (PDCoV; also known as Porcine Coronavirus HKU15)

Disease(s)—Diarrhea, vomiting, potentially fatal dehydration, anorexia, weight loss, and malnutrition, especially in neonatal piglets

Host Species—Pigs, cattle, birds

Type of Agent—Deltacoronavirus, subgenus *Buldecovirus*

Host Species Receptor—Aminopeptidase N (APN)

Mode of Transmission—Fecal-oral; respiratory route

Geographical Distribution—Southeast Asia, North America

Year of emergence—2009

Transmissible Gastrointestinal Enteric Virus (TGEV)

Disease(s)—Vomiting, profuse diarrhea, and possibly fatal dehydration in pigs of all ages

Host Species—Pigs

Type of Agent—Alphacoronavirus, subgenus *Tegacovirus*

Virus Species—*Alphacoronavirus-1*

Host Species Receptor—Aminopeptidase N (APN), sialic acid

Mode of Transmission—Fecal-oral

Geographical Distribution—Eastern Asia; disappeared from North America and Europe

Year of emergence—1946

Porcine Respiratory Coronavirus (PRCV)

Disease(s)—Very mild, short-lived disease; coughing and respiratory distress

Host Species—Pigs

Type of Agent—Alphacoronavirus, subgenus *Tegacovirus*

Virus Species—*Alphacoronavirus-1*

Host Species Receptor—Aminopeptidase N (APN); sialic acid

Mode of Transmission—Inhalation of respiratory secretions from infected pigs; direct contact between pigs postweaning

Geographical Distribution—Europe, North America, Japan, and the Republic of Korea

Year of emergence—1984

Porcine Hemagglutinating Encephalomyelitis Virus (PHEV)

Disease(s)—Vomiting and wasting disease; encephalomyelitis

Host Species—Pigs

Type of Agent—Betacoronavirus lineage A, *Embecovirus* subgenus

Virus Species—*Betacoronavirus-1*

Host Species Receptor—Neural cell adhesion molecule (CD56)

Mode of Transmission—Inhalation of respiratory secretions from infected pigs; direct nose-to-nose contact

Geographical Distribution—World-wide

Year of emergence—1959

Swine Acute Diarrhea Syndrome Coronavirus (SADS-CoV)

Disease(s)—Acute diarrhea

Host Species—Pigs

Type of Agent—Alphacoronavirus, subgenus *Rhinacovirus*

Host Species Receptor—Unknown

Mode of Transmission—Fecal-oral

Geographical Distribution—China

Year of emergence—2017

Bovine Coronaviruses (Enteric and Respiratory Forms; BECV and BRCV, respectively)

Disease(s)—Life-threatening calf diarrhea (BECV); winter dysentery with hemorrhagic diarrhea in adult cattle (BECV); shipping fever of feedlot cattle with fever, coughing, and runny nose (BRCV)

Host Species—Cattle and other domestic and wild ruminants

Type of Agent—Betacoronavirus lineage A, subgenus *Embecovirus*

Virus Species—*Betacoronavirus-1*

Host Species Receptor—N-acetyl-9-O acetyl neuraminic acid and human leukocyte antigen class I (HLA-1)

Mode of Transmission—Fecal-oral; inhalation of respiratory secretions from infected cattle

Geographical Distribution—Worldwide

Year of emergence—1972 (BECV), 1982 (BRCV)

Dromedary Alphacoronavirus (Dromedary Camel CoV-229E)

Disease—Potential respiratory system disease

Host species—Dromedary camels

Type of Agent—Alphacoronavirus, subgenome *Duvinacovirus*

Host Species Receptor—Aminopeptidase N (APN)

Mode of Transmission—Inhalation of respiratory secretions from infected camels

Geographical Distribution—Middle East

Year of emergence—2014

DcCoV -HKU23

Disease(s)—Gastroenteritis; diarrhea in calves

Host Species—Dromedary camels

Type of Agent—Betacoronavirus lineage A, *Embecovirus* subgenus

Host Species Receptor—N-acetyl-9-O acetyl neuraminic acid

Mode of Transmission—Inhalation of respiratory secretions from infected camels

Geographical Distribution—Parts of eastern and northern Africa, the Middle East, and Pakistan

Year of emergence—2013

Alpaca 229E-Related Coronavirus

Disease(s)—Severe pulmonary congestion and edema; diffuse interstitial to bronchointerstitial pneumonia, high fever

Host Species—Alpacas

Type of Agent—Alphacoronavirus, subgenus *Duvinacovirus*

Host Cell Receptor—Aminopeptidase N (APN)

Mode of Transmission—Inhalation of respiratory secretions from infected animals

Geographical Distribution—Americas

Year of emergence—2007

Alpaca Enteric Coronavirus

Disease(s)—Diarrhea, severe weight loss

Host Species—Alpacas and llamas

Type of Agent—Betacoronavirus lineage A, subgenus *Embecovirus*

Host Species Receptor—N-acetyl-9-O acetyl neuraminic acid and human leukocyte antigen class

I (HLA-1)

Mode of Transmission—Fecal-oral

Geographical Distribution—Americas

Year of emergence—1998

Equine coronavirus (ECoV)

Disease(s)—Severe diffuse necrotizing enteritis, anorexia, watery diarrhea, dehydration, hyperammonemia encephalopathy, ataxia, abnormal proprioception, nystagmus, recumbency, head tilt, seizures

Host Species—Horses, donkeys

Type of Agent—Betacoronavirus lineage A; subgenome *Embecovirus*

Virus Species—*Betacoronavirus-1*

Host Species Receptor—N-acetyl-9-O acetyl neuraminic acid and human leukocyte antigen class I (HLA-1)

Mode of Transmission—Fecal-oral route
Geographical Distribution—Asia, Europe, North America
Year of emergence—1975

Feline Enteric Coronavirus (FECV) Biotype of Feline Coronavirus

Disease(s)—Asymptomatic or mild enteric disease (diarrhea)
Host Species—Cats
Type of Agent—Alphacoronavirus, *Tegacovirus* subgenus
Virus Species—*Alphacoronavirus-1*
Host Species Receptor—Aminopeptidase N (APN), sialic acid
Mode of Transmission—Fecal-oral route
Geographical Distribution—World-wide

Feline Infectious Peritonitis Virus (FIPV) Biotype of Feline Coronavirus

Disease(s)—A primarily neurological disease that is particularly severe in kittens; also inflamed testicles, glomeruli of the kidneys, and heart muscle; damage to the eyes
Host Species—Cats
Type of Agent—Alphacoronavirus, *Tegacovirus* subgenus
Host Species Receptor—Not applicable; arises from internal mutation of FECV
Mode of Transmission—Not applicable
Geographical Distribution—World-wide
Year of emergence—1963

Canine Coronavirus (CCoV)

Disease(s)—Mild digestive symptoms; severe gastroenteritis if coinfecting by parvoviruses
Host Species—Dogs
Type of Agent—Alphacoronavirus; *Tegacovirus* subgenus
Virus Species—*Alphacoronavirus-1*
Host Species Receptor—Aminopeptidase N (APN), sialic acid
Mode of Transmission—Fecal-oral
Geographical Distribution—World-wide
Year of emergence—2003

Canine respiratory coronavirus (CRCoV)

Disease(s)—Kennel cough, a mild respiratory illness
Host Species—Dogs
Type of Agent—Betacoronavirus lineage A, *Embecovirus*
Virus Species—*Betacoronavirus-1*
Host Species Receptor—Human leukocyte antigen class I (HLA-1)
Mode of Transmission—Respiratory route
Geographical Distribution—Europe, North America, Asia
Year of emergence—2003

Rabbit HKU14

Disease(s)—Unknown

Host Species—Rabbits

Type of Agent—Betacoronavirus lineage A

Host Species Receptor—Unknown

Mode of Transmission—Unknown

Geographical Distribution—Southern China

Year of emergence—2012

Rabbit Enteric Coronavirus

Disease—Enteritis

Host species—Rabbits

Type of Agent—Betacoronavirus lineage A, subgenus *Embecovirus*

Host Species Receptor—N-acetyl-9-O acetyl neuraminic acid

Mode of Transmission—Fecal-oral route

Geographical Distribution—North America

Year of emergence—1980

Mouse (Murine) Hepatitis Virus (MHV)

Disease(s)—Fulminant viral hepatitis, mild encephalitis, and subacute demyelination of white matter of nerves in the CNS; acute, self-limiting infection of white liver foci in adults; gaseous distention of intestines in sucklings; the high mortality rate

Host Species—Mice

Type of Agent—Betacoronavirus lineage A, subgenome *Embecovirus*

Host Species Receptor—Carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1 or CD66a)

Mode of Transmission—Fecal-oral; direct contact; inhalation of respiratory secretions from infected mice

Geographical Distribution—World-wide

Year of emergence—1949

China Rattus coronavirus HKU24 (ChRCoV HKU24)

Type of Agent—Betacoronavirus lineage A, subgenus *Embecovirus*

Geographical Distribution—China

Year of emergence—2015

Longquan Aa mouse coronavirus (LAMV)

Type of Agent—Betacoronavirus lineage A, subgenus *Embecovirus*

Virus Species—*Betacoronavirus-1*

Geographical Distribution—China

Year of emergence—2015

Lucheng Rn rat CoV (LRNV)

Type of Agent—Alphacoronavirus, subgenus *Luchacovirus*

Geographical Distribution—China

Year of emergence—2015

Longquan RI rat coronavirus (LRLV)

Type of Agent –Betacoronavirus lineage A, subgenus *Embecovirus*

Virus Species—*Betacoronavirus-1*

Geographical Distribution—Asia and Europe

Year of emergence—2015

Bat Coronaviruses (See [Table 5.1](#) for more complete information)

Bat Alphacoronaviruses

Rh-BatCoV HKU2 from *Rhinolophus sinicus*

My-BatCoV HKU6 from *Myotis ricketti*

Mi-BatCoV HKU8 from *Miniopterus pusillus*

Hi-BatCoV HKU10 from *Hipposideros pomona*

Bat CoVs— β -CoV, lineage B

Rh-BatCoV HKU3 from *Rhinolophus sinicus*

LYRa11 from *Rhinolophus affinis*

Rf1 from *Rhinolophus ferrumequinum*

Rs3367 from *Rhinolophus* species

RsSHC014 from *Rhinolophus* species

WIV1 from *Rhinolophus sinicus*

WIV16 from *Rhinolophus sinicus*

Bat CoVs— β -CoV lineage C

Ty-BatCoV HKU4 from *Tylonycteris pachypus*

Pi-BatCoV HKU5 from *Pipistrellus abramus*

Hy-BatCoV HKU25 from *Hypsugo pulveratus*

NeoCoV from *Neoromicia capensis* and *Neoromicia capensis*

PML/2011 from *Neoromicia zuluensis*

SC2013 from *Vespertilio superans*

Bat CoVs— β -CoV lineage D

Ro-BatCoV HKU9 from *Rousettus leschenaulti*

B

Glossary

- 2'–5' Oligoadenylate** RNase L RNA pathway – one of the interferon-inducible, RNA decay pathways that cleave viral RNA
- 2' O-methyltransferase** nonstructural protein 10 in coronaviruses; enzyme responsible for ribose 2'-O-methylation of mRNA
- 3-Chymotrypsin-like cysteine protease (CL^{Pro})** see “3CL^{Pro}”
- 3'-poly-A tail** long stretch of adenosines bound to the 3'-end of mature mRNA
- 5'-cap** protective methylated guanosine nucleotide bound to the 5'-end of precursor mRNA
- 25-hydroxycholesterol (25HC)** blocks entry of potential host cells by coronaviruses, including pathogenic human coronaviruses; decreases proinflammatory Th1 lymphocyte activity and plays a role in plasma membrane fluidity
- 3CL^{Pro} (3-chymotrypsin-like cysteine protease or main protease)** coronaviruses enzymes that cleaves viral polyproteins in 11 locations to produce individual, functional proteins
- β -carotene** antioxidant that protects against the toxic reactive oxygen species superoxide anion
- $\gamma\delta$ T cells** relatively uncommon class of T cells that use a receptor composed of γ and δ chains rather than the typical α and β chains
- γ -tocopherol** antioxidant that removes toxic reactive nitrogen species
- Abelson kinase (Abl kinase)** member of a class of enzymes that chemically modifies proteins by the addition of a phosphate ion; regulates several intracellular signaling pathways and contributes to cancer development
- Acetylcholine** neurotransmitter involved in muscle contraction, learning, and memory
- Acidosis** excessive amounts of acid in bodily fluids; causes rapid breathing and heart rate, confusion, tiredness, weakness, nausea, and potentially shock or death
- Action potential** changes in electrical charges on the opposite sides of the plasma membrane of muscle or nerve cell associated with the passage of an electrical impulse along the membrane
- Activating transcription factor 6 (ATF6)** transcription factor involved in the Unfolded Protein Response; signaling mediator of endoplasmic reticulum stress
- Active immunization** stimulates one to generate one's own immune response to a specific microbe, often by vaccination, for protection against infection by that specific microbe
- Acute adrenal insufficiency** acute interruption of the normally functioning adrenal or pituitary gland; leads to vomiting, nausea and diarrhea, low blood pressure causing dizziness or fainting upon standing, irritability, and depression
- Acute disseminated encephalomyelitis** brief, intense inflammation of the central nervous system and, occasionally, the optic nerves; which may result from viral infection
- Acute encephalomyelitis** temporary, widespread inflammation of the brain and spinal cord that damages myelin, the protective fatty layer that surrounds some nerves

- Acute flaccid paralysis** neurological condition characterized by rapid onset of muscular weakness, including respiratory and pharynx muscles, which may lead to respiratory failure; polio-like condition
- Acute ischemic stroke** stroke that results from a sudden drop in brain tissue blood flow and decreased levels of oxygen; symptoms include paralysis or numbness of the face, arms, or legs, usually on one side of the body, confusion, trouble speaking, headache with vomiting, decreased vision
- Acute kidney injury (AKI)** abrupt decrease in kidney function which leads to the retention of urea and other nitrogenous waste products, water, and electrolytes
- Acute necrotizing encephalopathy** brain damage following an acute disease with fever, often due to infection with viruses, including coronaviruses; symptoms may include seizures and disturbed consciousness, progressing to coma
- Acute-phase response** response to infection that increases levels of acute phase proteins in serum, especially C-reactive protein; acute phase proteins are produced in the liver and may either inhibit or mediate inflammation
- Acute renal failure** condition in which the kidneys abruptly lose their ability to filter waste products from the blood, allowing dangerous levels of waste to accumulate
- Acute respiratory distress syndrome (ARDS)** life-threatening, acute lung disease in which organs receive an insufficient amount of oxygen due to fluid buildup in the lungs
- Acute sensory neuropathy** extensive damage to the collection of neuron cell bodies that affects sensory recognition
- Acute telogen effluvium** temporary, large-scale hair loss during the resting stage of the hair cycle
- Acute tubular injury** damage to kidney tubules that may result in renal failure
- Acyl-CoA: cholesterol acyltransferase** enzyme that converts cholesterol to cholesteryl esters that clog arterial walls; may result in atherosclerosis
- Adaptive immune system** composed of B and CD4⁺ T helper and CD8⁺ T killer lymphocytes and their products, including antibodies and cytokines; produces memory cells that respond more quickly and strongly upon re-exposure to the same infectious agent or other protein
- Adenosine deaminase (ADA)** enzyme required to activate some T helper cells, increasing their production of proinflammatory molecules
- Adenovirus vector** nonpathogenic adenovirus that is engineered to contain a gene from another virus, including coronaviruses; used as vaccines
- Adiponectin** hormone produced by white adipose tissue that oxidizes fatty acids and inhibits glucose production
- Adjuvant** material added to a vaccine to produce stronger, longer-lived immune responses
- Adrenal gland** endocrine organ that produces a wide range of hormones, including dehydroepiandrosterone and cortisol
- Adrenal infarction (AAI)** loss of blood flow to the adrenal gland due to blood clots in the main adrenal vein or to microvascular thrombosis within the adrenal gland's tissue
- Adrenocorticotrophic hormone** hormone produced by the anterior region of the pituitary gland; regulates the release of cortisol from the adrenal cortex
- Affective disorders** mood disorders that include major depression and bipolar disorder
- Afferent and efferent arterioles** small blood vessels that bring blood to and from the nephrons of the kidneys
- Agglutinate** to clump together red blood cells or bacteria
- Agglutinin** material that causes clumping
- Alanine aminotransferase** enzyme found in the liver and kidneys; released into blood upon liver damage
- Alarmins** group of proteins that initiates several processes, including host defense, inflammation, cellular homeostasis, and wound healing
- Albuminuria** presence of albumin, the major blood protein, in the urine; due to disease conditions, dehydration, emotional stress, extreme cold, high fever, or strenuous exercise
- Aldosterone** hormone produced by the adrenal cortex that increases blood pressure
- Alkaloids** group of basic organic compounds containing at least one nitrogen atom
- Alleles** different variants of a given gene
- Alopecia** hair loss
- Alopecia areata** autoimmune attack upon the hair follicles in round patches
- Alopecic patches** patches of hair loss (bald spots)

- Alpaca respiratory syndrome (ARS)** respiratory disease of alpacas caused by infection with alpaca alphacoronavirus; disease severity ranges from a mild upper respiratory disease to a life-threatening lower respiratory tract disease
- Alpha1-acid glycoprotein (AGP)** binds pathogens and modulates immune cell activity
- Altered consciousness** altered state of mind; temporary change in a person's normal mental state
- Alternative pathway of complement activation** complement activation initiated by spontaneous production of C3b from C3
- Alveolar edema** fluid in the terminal air sacs of the lungs
- Alveolar macrophages** macrophages in the lung's alveoli; among the first lines of defense against microbial infections of the lower respiratory tract
- Alveolar pneumocytes** two types of cells found in the alveolar lining; both are infected by SARS-CoV, but at different times after infection
- Alveoli** small grape-like sacs at the end of the respiratory tract in which oxygen and carbon dioxide are exchanged between the blood and the lungs
- Aminopeptidase N (APN)** cellular protein that serves as a receptor for several coronaviruses of humans and animals
- Amphiregulin** one of the profibrogenic ligands of the epidermal growth factor receptor that promotes wound repair via differentiation of fibroblasts into myofibroblasts
- Amygdala** part of the limbic system; attaches emotions to memories and sensations
- Amylin** hormone secreted by β cells of the pancreatic islets of Langerhans; regulates blood sugar levels by slowing the stomach's emptying, promoting satiety, and decreasing food intake
- Amyopathic dermatomyositis** rash on the face, around the eyes, neck, forearms, and upper chest; red or violet bumps on the knuckles often accompanied by itch and light sensitivity, but not muscle abnormalities
- Anaphylactic shock** extreme, often a life-threatening allergic reaction to materials that are not in themselves harmful, such as peanuts, seafood, penicillin, and bee or snake bites
- Anaphylatoxins** chemotactic C3a, C4a, and C5a components of the complement cascade; induce inflammatory responses, smooth muscle contraction, dilation of blood vessels, and histamine release
- Androgen receptor (AR)** nuclear receptor that binds androgens and acts as a transcription factor, promoting expression of genes associated with androgen functions; found in cells of the reproductive, nervous, immune, muscular, skeletal, endocrine, and cardiovascular systems
- Androgenetic alopecia** in men, hair loss that begins above both temples, followed by receding hairline and thinning near the top of the head, often results in partial or complete baldness ("male pattern baldness"); in women, hair thins all over the head without a receding hairline ("female pattern baldness")
- Androgens** hormones responsible for male characteristics as well as bone density, muscle strength, and body fat; including testosterone and dihydrotestosterone
- Anemia** low number of red blood cells in the blood
- Anergic** state of nonresponsiveness to stimuli
- Angioedema** swelling under the skin of the face, throat, or genital areas caused by allergic reactions
- Angiogenesis** production of new blood vessels
- Angiotensin-converting enzyme (ACE)** key enzyme that raises blood pressure via the renin-angiotensin-aldosterone pathway; its activity is opposed by angiotensin-converting enzyme-2 (ACE2)
- Angiotensin-converting enzyme-2 (ACE2)** cellular receptor for several coronaviruses, including SARS-CoV and SARS-CoV-2; counterbalances ACE activity, decreasing blood pressure to keep it in the optimal range for bodily functions
- Angiotensinogen** precursor to angiotensin, a molecule involved in the renin-angiotensin-aldosterone system that increases blood pressure
- Animal husbandry** care of domestic animals
- Anorexia** eating disorder that leads to abnormally low body weight
- Anosmia** loss of the sense of smell
- Antagonists (antagonistic)** molecules that block a process
- Anterior pituitary (adenohypophysis)** "the master gland," endocrine gland that secretes the following hormones: adrenocorticotrophic hormone, thyroid-stimulating hormone, follicle-stimulating hormone, luteinizing hormone, growth hormone, prolactin, and beta-endorphin

- Anterograde axonal transport** transportation of material from the axon back towards the neuron's cell body through its cytoplasm
- Antibody** molecule produced by B lymphocytes that attaches to small areas of material that are recognized as "foreign"; classes are IgG, IgM, IgA, IgD, and IgE
- Antibody-dependent enhancement (ADE)** pathogenic condition in which early, suboptimal antibody levels against a microbe are not able to clear the microbe but rather increase the entry of microbe-antibody complexes into macrophages, where the microbes multiply
- Anti-CCP antibodies** autoantibodies that replace the amino acid arginine with citrulline; high levels are present in rheumatoid arthritis
- Anticoagulant** blood-thinner
- Anti-GD1b antibodies** autoimmune condition in which antibodies attack the ganglioside GD1b; characterized by extreme tiredness, paralysis of the eye muscles, altered gait, and bulbar palsy (progressive loss of speech, weakness in the limbs, difficulty swallowing)
- Antigen** portion of a protein that is recognized by the immune system
- Antigen presentation** displaying a processed antigen on a major histocompatibility complex type II molecule; activates CD4⁺ T helper cells
- Antigen-presenting cell** cell that expresses major histocompatibility complex type II molecules necessary to activate CD4⁺ T helper cells; includes dendritic cells, B lymphocytes, and monocytes/macrophages
- Anti-nuclear autoantibodies** antibodies against double- or single-stranded DNA and histones
- Antiphospholipid syndrome** autoimmune disease caused by antibodies attacking the phospholipids of the inner lining of blood vessels, leading to the production of blood clots in arteries or veins
- Apgar score** assesses the physical condition of newborns based upon heart rate, respiration, muscle tone, skin color, and response to stimuli
- Apoptosis (apoptotic death)** form of cell death in which the cell turns on a self-destruct pathway that may be beneficial if the cell is damaged, no longer useful, or acting as a microbial host; the process is detrimental when a normal, functional cell dies, especially in nondividing cells, such as neurons and skeletal muscle cells
- Archaea** category of single-celled organisms that lack membrane-enclosed organelles; similar to, but distinct from, bacteria; often present in extreme environments, with high levels of sulfur or salt, temperature, or pressure
- Arginase-1 (Arg-1)** enzyme that catalyzes the breakdown of arginine to urea and ornithine; inhibits replication of T cells
- Argonaute 2** a member of a group of proteins responsible for cleavage of specific RNAs during RNA interference
- Arrhythmia** abnormal rhythm of heartbeats, giving one the feeling of a fluttering or racing heart
- Arteriosclerosis obliterans** arteries are narrowed from arteriosclerosis (thickening and hardening of the walls of the arteries; "hardening of the arteries")
- Ascarid** type of parasitic intestinal worm
- Ascites** excessive fluid build-up in the abdominal cavity
- Aspartate aminotransferase** increased levels of this enzyme in the blood indicate liver damage
- Astrocytes** most abundant type of glial cells in the central nervous system; form the blood-brain barrier which regulates the flow of material from the circulatory system into the brain and is also active in the repair and scarring of the brain and spinal cord after infection or traumatic events
- Astrocytosis** abnormally high number of astrocytes in an area of the central nervous system
- Asymptomatic** infection that does not cause apparent illness
- Ataxia** Loss of muscle control or coordination, including walking and picking up objects
- Atherosclerosis** fatty plaques deposit on the inner walls of arteries
- Atrial fibrillation** rapid, weak contractions of the upper chambers of the heart; that may result in formation of blood clots or lead to strokes
- Atrophy** wasting of cells, typically due to their degeneration
- Attenuated viruses** form of the virus that has been rendered nonpathogenic but is still "alive"; used in many vaccines since they are more effective in activating CD8⁺ T killer cells than are "killed" viruses
- Atypical hemolytic uremic syndrome** life-threatening thrombotic microangiopathy that primarily affects the kidneys; usually caused by dysregulation of the alternative pathway of complement activation
- Autophagocytosis** self-consuming; used into disposal of damaged organelles within a cell, often through the action of lysosomes

- Autophagy** process of consumption of one's own tissues
- Avascular necrosis (osteonecrosis)** degenerative bone disorder caused by the lack of an adequate blood supply, leading to the bone's eventual collapse; often associated with long-term use of high-doses of corticosteroids, excessive alcohol use, and smoking
- Avian** pertaining to birds; avian coronaviruses generally are either gammacoronaviruses or deltacoronaviruses; not known to infect humans, but may infect pigs (porcine deltacoronavirus)
- Axon** type of process proceeding from the cell body of neurons, carrying electrical impulses away from the cell body; form first part of neural synapses
- Axonal transport** process that transports materials or microbes through a neuron's cytoplasm down the axons from the neuron cell body
- B lineage SARS-CoV-2** lineage of SARS-CoV-2 that includes Delta and Omicron variants
- B lymphocytes (B cells)** adaptive immune system cells that produce antibodies and stimulate CD4⁺ T helper lymphocytes
- Bacteremia** presence of bacteria in the blood
- Bacteriophages** viruses that infect bacteria; source of novel bacterial RNA
- Bacteroidetes** major group of bacteria that are anaerobic, nonspore-forming, Gram-negative bacilli
- Bactrian camels** two-humped camels from colder regions of Asia; do not serve as reservoirs of MERS-CoV
- Baltimore Class IV viruses** genome is composed of (+) single-stranded RNA; including coronaviruses
- Baltimore Class V viruses** genome is composed of (−) single-stranded RNA
- Baltimore Classification System of viruses** common system of classifying viruses into 7 categories that are based on whether the virus's genome consists of double- or single-stranded DNA or RNA
- Bam** proapoptotic protein that helps to trigger self-destruction of abnormal or infected cells
- Basal ganglia** clusters of neurons (substantia nigra, caudate nucleus, putamen, and globus pallidus) involved in involuntary movements, such as tremors, athetosis, and chorea
- Basal lamina** thin layer of the basement membrane found between epithelial cells and the connective tissue under them; consists of proteins, especially collagen
- Basement membrane** extracellular matrices that underlie the basal portion of epithelial and endothelial cells; cells attach to it, allowing them to remain in their correct location
- Basic reproduction number (R_0)** number of secondary cases arising from an index case in a fully susceptible population; R_0 above 1 indicates epidemic potential
- Basophils** rare blood leukocytes whose compounds produce allergic reactions by releasing histamines and other powerful molecules
- Bax** compound involved in apoptosis; increases permeabilization of the mitochondrial outer membrane
- Bcl-2 (B-cell lymphoma 2)** antiapoptotic protein that inhibits the self-destruction of abnormal or infected cells
- Betacoronavirus-1** species of coronavirus that includes bovine coronavirus of cattle, equine coronavirus of horses, canine respiratory coronavirus of dogs, porcine hemagglutinating encephalomyelitis virus of pigs, and HCoV-OC43 of humans
- Bicarbonate** buffer that helps regulate pH, particularly of the digestive and respiratory systems
- Bickerstaff's encephalitis** triad of ophthalmoplegia (paralysis of muscles around the eye), ataxia (incoordination of skeletal muscle activity), and decreased consciousness
- Bid** proapoptotic molecule that aids in the self-destruction of the cell
- Bile duct** tube that carries bile from the liver to the gall bladder
- Bilirubin** neurotoxic compound produced by the break-down of hemoglobin, especially when large numbers of red blood cells lyse; normally transformed in the liver into a part of bile that digests fats. High levels of this yellowish toxin accumulate in those with liver diseases, including cirrhosis and hepatitis, giving the whites of the eyes a yellowing tinge
- Bim** proapoptotic compound
- Binding affinity** strength of binding interaction between a molecule and its binding partner
- Biosafety Level 3** containment for work with biological agents that cause potentially fatal diseases; requires negative pressure and an autoclave in the compartment
- Biosafety Level 4** self-contained compartment for work with biological agents that are extremely contagious and cause potentially fatal diseases for which no vaccine or therapy is available; requires work in specialized suits with air supply external to the work area

- Bipolar I disorder** psychiatric disease in which a person's emotions swing between depression and mania
- Bix** proapoptotic protein that helps trigger self-destruction of abnormal or infected cells
- Blood:brain barrier (BBB)** typically an impermeable barrier formed by a type of neuroglial cell that surrounds the blood vessels of the brain and controls which molecules or cells may pass from the blood into the brain tissue
- Blood-ocular barrier** composed of the blood-retinal barrier and blood-aqueous barrier; prevents entry of toxic or unwanted substances into the eye and maintains its homeostasis
- Blood-retinal barrier** cells joined tightly together to regulate materials, including ions, proteins, and water, from entering or leaving the retina
- Bone marrow (red)** site of production of all types of blood cells and platelets
- Bone marrow plasma cells (BMPCs)** long-lived antibody-producing B lymphocytes present in red bone marrow
- Bovine** pertaining to cattle
- Bovine enteric coronavirus (BECV)** coronavirus of cattle that causes calf diarrhea in young animals and winter dysentery with hemorrhagic diarrhea in adult cattle
- Bovine respiratory coronavirus (BRCV)** coronavirus of cattle that causes shipping fever, a respiratory illness
- Bovine respiratory disease complex (BRD)** complex, multifactorial respiratory illness in cattle that results from interactions among environmental factors, host factors, and bacteria and viruses, including bovine coronavirus; symptoms include rapid shallow breathing, coughing, watery and then puss-like bloody discharge from the nasal cavity, and eye discharge
- Bowman's capsule** cup-like membranous structure around glomeruli of kidney nephrons
- Brainstem** lowermost region of the brain that connects it with the spinal cord; contains vital centers that are required for life
- Brainstem nuclei** central network in the brainstem where nerve cells and nerves originate
- Bronchial tubes** large diameter tubes that branch from the trachea into the increasingly smaller bronchioles
- Bronchioalveolar lavage** process during which saline is injected through a tube inserted into a bronchiole; the fluids and cells from the lower lungs are removed to examine them for diseases, including viral infections
- Bronchioalveolitis** concurrent inflammation of bronchi, bronchioles, and alveoli
- Bronchiolar epithelium** cells that line the bronchioles, including goblet cells that produce mucus to trap microbes and particulate matter, ciliated cells that sweep mucus up the branches of the respiratory tree and away from the lungs, and basal cells that reproduce to replace lost cells
- Bronchioles** branches of the bronchi; series of tubules that branch multiple times, becoming smaller in diameter each time until terminating in the alveoli
- Brush border** region of the lumen of the small intestine where nutrient absorption occurs and carbohydrates are digested
- Brush border of the kidneys** microvilli on the plasma membrane of the luminal surface of epithelial cells of kidney tubules; resorb material from the glomerular fluid into the surrounding capillaries
- C3** pivotal component of all 3 pathways of complement activation; cleaved into C3a and C3b
- C3a** proinflammatory breakdown product of the C3 component of complement; multifunctional activities include recruitment of leukocytes
- C4a** proinflammatory breakdown product of the C4 component of complement produced during the classical pathway of activation; multifunctional activities include recruitment of leukocytes
- C5a** proinflammatory breakdown product of the C5 component of complement; multifunctional activities include recruitment of leukocytes
- Calcifediol** form of Vitamin D used to increase levels of blood calcium and phosphate
- Calcitonin** thyroid hormone that decreases blood calcium levels and increases bone density
- Calcitriol** hormone produced by chemical modification of Vitamin D in the liver and kidneys; increases blood calcium levels
- Calf diarrhea** severe, malabsorptive diarrhea in calves due to infection with bovine coronavirus; has a high mortality rate
- Calicivirus** group of positive-sense, single-stranded RNA viruses; the best-known example is norovirus ("cruise ship virus")
- Camelid** pertaining to camels and related species, including llamas and alpacas
- cANCA (antineutrophil cytoplasmic antibodies)** autoantibodies that target material in the cytoplasm of neutrophils

Canine pertaining to dogs

Canine infectious respiratory disease (CIRD or kennel cough) disease of dogs due to infection with one of many viruses, including canine respiratory coronaviruses; characterized by a dry, hacking cough and high morbidity but low mortality

Capillaries smallest type of blood vessel which consist of a single layer of flattened cells; allow the exchange of oxygen, nutrients, and ions from the circulatory system into the tissues and remove carbon dioxide and waste products in the blood from the tissues via the lungs and kidneys, respectively

Carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5) molecule that aids entry of MERS-CoV into cells; normally involved in the adhesion of epithelial cells

Cardiac muscles muscles in the wall of the heart that push blood through the heart chambers and out the pulmonary trunk or aorta to the lungs or remainder of the body, respectively

Cardiac output (stroke volume) amount of blood ejected from a ventricle during a single heartbeat multiplied by the heart rate; normally 4–6 liters/minute

Cardiac troponin I protein that regulates contraction of cardiac muscles; elevated levels indicate myocardial damage

Cardio- (cardiac) pertaining to the heart

Cardiomyocytes muscle cells of the heart

Cardiomyopathy injury to the cardiac muscles

Cardiotonic steroids group of several specific ligands of the Na^+ , K^+ -ATPase enzyme involved in inflammation, host defense, and survival mechanisms, as well as many chronic illnesses, neurodegenerative diseases, and mood disorders

Caspase 3 essential component of an apoptotic pathway that activates caspases 6, 7, and 9

Caspase 8 dependent extrinsic pathway of apoptosis – apoptotic pathway initiated by death receptors

Caspase apoptotic pathway one of two pathways that induce programmed cell death; including the extrinsic and intrinsic pathways

Caspases group of proteases that function in a cascade that induces apoptotic cell death

Catalase iron-containing enzyme that converts the toxic reactive oxygen species hydrogen peroxide into water and oxygen

Catarrhine primate primates whose nostrils are close together and directed downward; include humans, apes, and Old World monkeys

Catatonia neuropsychiatric disorder affecting behavior and motor function; results in unresponsiveness to stimuli

Cathelicidin antimicrobial peptide primarily stored in lysosomes of phagocytic cells (macrophages and neutrophils)

Cathepsin member of a class of protease enzymes; present in most animal cells but is particularly plentiful in the liver, spleen, kidneys, and intestines

Cathepsin B lysosomal papain-like exopeptidase

Cathepsin L lysosomal endopeptidase

Caudate nucleus one of the basal ganglia that control movement, memory, and learning

Caveolin-dependent endocytosis process of plasma membrane invaginating material into vesicles and moving this material into the cell using caveolae, small flask-shaped pits, via caveolin, a cholesterol-binding protein

CCL1 chemokine that recruits monocytes but not neutrophils

CCL2 (macrophage chemotactic protein-2; MCP-1) chemokine that recruits monocytes

CCL3 (macrophage inflammatory protein-1; MIP-1 α) chemokine that recruits T killer cells and B cells

CCL4 (macrophage inflammatory protein-1; MIP-1 β) chemokine that recruits monocytes, T lymphocytes, dendritic cells, NK cells, and platelets, as well as activating granulocytes (neutrophils, eosinophils, and basophils)

CCL5 (RANTES) chemokine that recruits effector and memory T cells

CCL6 chemokine that is only identified in rodents; involved in the pathogenesis of IL-13-induced inflammation and tissue remodeling

CCL8 chemokine recruits a wide range of leukocytes, including monocytes, T cells, NK cells, mast cells, eosinophils, and basophils

CCL9 (macrophage inflammatory protein-1 gamma or MIP-1 γ) present on osteoclasts of the bone; regulates bone resorption

- CCL11 (eosinophil chemotactic protein or eotaxin-1)** chemokine that recruits eosinophils: implicated in allergic responses
- CCL20 (C-C motif chemokine ligand 20)** chemokine that strongly recruits lymphocytes and weakly recruits neutrophils
- CCL22 (C-C motif chemokine ligand 22)** chemokine that recruits monocytes, dendritic cells, natural killer cells, and chronically activated T lymphocytes
- CCR5 (C-C motif chemokine receptor 5)** chemokine receptor that recruits T cells, macrophages, and immature dendritic cells
- CD4⁺ T helper cells** T cells that secrete cytokines and regulate the activity of other leukocytes
- CD8⁺ T killer cells** T cells that kill abnormal cells, including cells infected by viruses
- CD11 (cluster of differentiation 11)** one of the alpha chains of various integrins that mediate leukocyte adhesion; often binds CD18
- CD18 (cluster of differentiation 18)** one of the beta chains of integrins that mediate cellular adhesion and cell surface signaling; often binds CD11
- CD40 (cluster of differentiation 40)** costimulatory protein for T lymphocytes; present on antigen-presenting cells
- CD83 (cluster of differentiation 83)** cell surface protein found primarily on leukocytes; plays an important role in cell adhesion, signal transduction, and calcium signaling
- CD86 (cluster of differentiation 86 or B7-2)** cell surface protein constitutively expressed on antigen-presenting cells; working together with CD80, CD86 is a costimulatory protein required for T cell activation and survival
- CEACAM** see "Carcinoembryonic antigen-related cell adhesion molecule"
- Cecum** pouch-like region at the beginning of the large intestine
- Celiac disease (gluten-intolerance)** autoimmune disease triggered by the ingestion of food containing gluten; damages villi of small intestinal cells, leading to a major decrease in nutrient absorption
- Cell cycle** 4-staged cycle through which cells pass during cell division; during the G₁ stage, cells begin to produce enough materials and organelles needed for two daughter cells; during the S phase, DNA replication occurs; during the G₂ stage, cells finish acquiring materials needed for daughter cells; and during the M stage (mitosis), cells separate their DNA and cytoplasm and form two daughter cells
- Cell-mediated immune response** immunity that involves T lymphocytes, natural killer cells, monocytes/macrophages, and other leukocytes but not B lymphocytes or antibodies
- Cellular tetraspanin scaffolding protease (CD9)** enzyme that aids in the distribution of proteins into highly organized regions involved in cellular adhesion, signaling, and trafficking
- Cellulitis** skin is swollen, red, and painful due to serious bacterial infection
- Central memory cells** T cells are found predominantly in lymph nodes and tonsils that express CD45RO, C-C chemokine receptor type 7, and L-selectin; secrete IL-2, 4, IFN- γ , TNF, and CD40L
- Central nervous system (CNS)** brain and spinal cord, but not nerves elsewhere in the body
- Cerebellum** area of the lower, back of the brain that coordinates muscle movements, including balance and posture
- Cerebral cortex** outer, gray portion of the brain's cerebrum which controls the higher functions of the brain, including sensory information
- Cerebral gyri** ridges on the outer portion of the cerebrum
- Cerebral infarction** death of part of the cerebrum due to an obstruction of its blood supply
- Cerebrospinal fluid (CSF)** fluid that bathes and provides nutrients to the brain and spinal cord
- Cerebrovascular disease** medical condition that affects blood vessels of the brain and cerebral circulation; includes various forms of strokes
- Cerebrum** large area of the brain responsible for "higher brain" functions, including interpretation of sensory data, thought, memory, speech, emotions, self-awareness
- Ceruloplasmin (CP)** major copper-carrying protein in the blood; involved in iron metabolism
- Chemiluminescence immunoassay assay** fluorometric assay that detects antibodies or other proteins using a reaction between an enzyme bound to a specific antibody and an appropriate substrate
- Chemokine (C-C motif) ligand 2** recruits and activates monocytes during inflammation
- Chemokines** class of immune mediators that attract specific cell types, especially leukocytes, into an area; normally protective activity since it eliminates microbes and is necessary for wound repair, but, when in excess or chronic, it is inflammatory

- Chemotactic** drawing cells in an area in response to chemokines; during inflammation, often involves monocytes, neutrophils, and lymphocytes
- Chilblain lupus** skin manifestation of systemic lupus erythematosus; due to exposure to cold
- Chimeric** contains components from different species
- Cholesterol** fatty molecule involved in cells' plasma membrane flexibility and serves as a precursor for steroid hormones, including sex hormones and the immunosuppressive hormone cortisol; excessive blood levels contribute to plaque formation that narrows arteries, preventing adequate blood flow to areas, including the brain and heart
- Cholinergic agonists** agents that stimulate cholinergic receptors activated by acetylcholine
- Choroiditis** inflammation of the retina and choroid layers of the eye
- Chromatin condensation** chromosomes shorten and become compact by multiple rounds of folding; transforming DNA from chromatin to chromosomes to prepare for mitosis
- Chronic demyelinating disease** autoimmune central nervous system disease targeting the fatty myelin sheath surrounding some axons or the cells producing and maintaining the lipid coat; results in inflammation and injures the sheath and the axons that it surrounds
- Chronic granulomatous hepatitis** inherited immunodeficiency disorder that increases the risk of fungi and bacteria infection and granuloma formation
- Chronic obstructive pulmonary disease (COPD)** severe lung disease that includes asthma, chronic bronchitis, and emphysema
- Cilia** short, hair-like projections found in cells lining much of the respiratory tract; sweep mucus and material caught within it, including microbes and particulate material, away from the lower parts of the lungs, decreasing the risk of lung infection and cancer
- Cirrhosis** degradation of an organ, including scarring; is often associated with diseased livers
- Clade** categorization of organisms based upon evolutionary ancestors
- Clara cells** cuboidal, nonciliated cells in terminal bronchioles of the lungs; play a major role in barrier maintenance, secretion, and metabolism
- Classical pathway of complement activation** initiated by IgM or IgG binding antigen on the cellular or microbial membrane
- Class IV viruses** see "Baltimore Class IV viruses"
- Clathrin-coated pits** areas of the plasma membrane enriched in clathrin; site from which clathrin-dependent endocytosis occurs
- Clathrin-dependent endocytosis (receptor-mediated endocytosis)** process involving clathrin by which materials, including microbes, enter cells by inward budding of the plasma membrane; forms intracellular vesicles containing absorbed substances
- Coagulation** formation of blood clots
- Coagulation pathways** pathways that result in the formation of blood clots that stop bleeding; if clots break loose, they may travel to the heart or brain, causing heart attacks or strokes
- Cofactor** chemical, excluding proteins, that aids enzymes in chemical reactions
- Cold chain** maintaining materials to be tested under cold conditions from the point of sampling to the testing site
- Collaborative Cross** large panel of new inbred mouse strains derived from an 8-way cross using several mouse founder strains, including 3 outbred strains
- Collagen** fibrous protein that composes about 1/3 of the proteins in humans; strands serve as supporting structures and anchor cells to each other and collagen fibers provide strength and elasticity to the skin and help to form a network of cells upon which new cells grow, aiding in replacement of damaged or skin cells, while in the cells' cytoskeleton, they help to provide structure and shape
- Collapsing glomerulopathy** kidney injury with the segmental or global collapse of glomerular capillaries; most common in people of African descent; rapidly progressive and may lead to abrupt-onset kidney failure
- Colloidal gold immunochromatographic assay** simple, inexpensive, rapid immunochromatographic test using monoclonal antibodies recognizing different sections of test materials; similar to the enzyme-linked immunosorbent assay, but uses chromatography that separates material based on differences in movement on paper strips
- Colon (large intestine)** part of the digestive system that follows the small intestine; most of the fluid from food or drinks is taken into the body from the colon

- Colonic ridges** ridges present in the interior of the colon
- Colostrum** 1st fluid secreted from the breasts after birth; rich in antibodies, primarily IgA, which help to protect the infant until it develops its adaptive immune response
- Columnar epithelial cells** elongated cells that cover or line organs
- Commensal microbes** microbes inhabitants that are harmless or beneficial to their hosts; including common forms of *E. coli* that help produce vitamins and prevent the growth of pathogenic microbes
- Comorbidity** medical disorders that include chronic obstructive pulmonary disease (COPD), diabetes, obesity, infections, autoimmune diseases, renal dialysis, and cancer treatments
- Complement cascade** immune system pathway which produces large pores in infected cells and recruits leukocytes to the site of infection to kill or ingest microbes; activated by classical, alternative, or lectin pathways
- Computed tomography (CT or CAT scan)** produces cross-sectional images of bones, blood vessels, and soft tissues by computerized processing of a series of X-ray images from different angles around the body
- Congenital** condition present at birth
- Congenital heart disease** heart disease present at birth; involves walls, valves, or blood vessels
- Congestion of the lungs and liver** blood vessels of the lungs and liver distend, causing alveoli to fill with blood; slows blood flow through the liver
- Congestive heart failure** heart beats weakly, resulting in fluid buildup in the lungs
- Conjugation** “bacterial sex”; bacteria of different mating types exchange DNA via extensions between them (sex pili)
- Conjunctiva** membrane lining the inside of the eyelids and the front of the eye
- Conserved genes or conserved regions** genes or gene sections that do not often vary from genes found in different members of the same or different host species
- Consolidation** when in the lungs, fluid accumulates, causing them to become stiff and unable to exchange oxygen and carbon dioxide; symptoms include chest pain, cough, and fever
- Contact tracing** in public health, identification of all people who may have had contact with an infected person and people whom they have contacted; contacts are tested for the microbe, monitored, isolated, or treated to reduce the spread of an infectious agent through populations; used to halt the spread of SARS in 2012–2013
- Convalescent plasma or serum** antibody-rich fluid portion of nonclotted or clotted blood, respectively, from people who recovered from an infectious disease; administration to infected people, may decrease the extent of disease in the recipient but does not induce recipients’ own immune response
- Copper gluconate** copper salt of D-gluconic acid; antiinflammatory, antiviral molecule
- Copper/zinc superoxide dismutase** see “Superoxide dismutase”
- Correlate, correlation** linkage of 2 things over time; during positive correlation, as 1 thing increases, so does the other, while during negative correlation, as 1 thing increases, the other decreases; may or may not indicate that 1 thing causes the other
- Cortisol** immunosuppressive hormone from the adrenal glands that decreases inflammation
- Coronaviruses** group of viruses using (+) single-stranded RNA for genetic material; surrounded by a crown (corona) of projecting spike proteins; examples include SARS-CoV, SARS-CoV-2, MERS, and other related viruses of humans or animals
- Corpus luteum** endocrine organ produced from ovarian follicles in women following ovulation; produces high levels of progesterone and low levels of estradiol
- Corticosteroids** immunosuppressive hormones, such as prednisone and cortisol, which decrease inflammation by inhibiting the activity of several types of leukocytes, cytokines, or chemokines; lower immunity to infections and cancer
- C-reactive protein** acute-phase protein produced by the liver whose blood levels increase over 25% during inflammation; blood levels serve as indicators of acute inflammatory conditions or severity of chronic diseases
- COVID toes** skin of the toes or fingers that begins with bright red which gradually turns purple
- Cowper’s glands** part of the male reproduction system that produces a preejaculate fluid that is secreted during sexual arousal; neutralizes the acidity of the urethra
- Cranial nerve palsies** partial or complete loss of function of at least 1 cranial nerve
- Cranial nerves** 12 pairs of nerves that arise from the brain rather than from the spinal cord
- Creatinine** breakdown product of important nitrogenous metabolite creatine that is excreted in the urine; high levels may indicate kidney disorders

- Creatinine kinase** enzyme that transfers high-energy phosphate from ATP to creatine, forming phosphocreatine
- Cria** baby camelid
- Cribiform plate** flattened upper portion of ethmoid bone; contains small holes through which extensions of olfactory nerve pass on to the cerebrum
- Critical illness polyneuropathy** rapid onset of widespread weakness in critically ill patients, especially in muscles of the extremities and diaphragm
- Crohn's disease** chronic, progressive, inflammatory autoimmune response that damages the intestines; symptoms include diarrhea, cramping, abdominal pain, weight loss, and fever
- Cross-reactive** reaction of an antibody with a molecule other than the one which gave rise to it
- Croup** infection and inflammation of the throat, vocal cords, trachea, and lungs in children; causes breathing difficulties, but is usually not severe
- Cuboidal epithelial cells** cube-shaped cells that line or cover organs or tissues
- Cutaneous** refers to the skin
- CXCL1** chemokine that recruits neutrophils
- CXCL3** chemokine that recruits monocytes
- CXCL9 (monokine induced by gamma interferon; MIG)** chemokine involved in leukocyte proliferation, Th1 cell differentiation, and recruitment of stimulated T and NK cells
- CXCL10 (interferon gamma-induced protein 10)** chemokine that recruits monocytes/macrophages, T cells, NK cells, and dendritic cells
- Crypt hyperplasia** changes in the shape of the finger-like villi of the small intestine in which the regions between the "fingers" (crypts) close off at the tops, flattening the mucosa, and deepening the area between crypts which become filled with dividing cells
- Cyclooxygenase 2 (COX-2)** enzyme involved in the formation of prostaglandins, thromboxane, and leukotrienes that cause inflammation and perception of pain; drugs that inhibit its activity are used to treat inflammatory conditions or lower blood pressure
- Cystic fibrosis** disease affects the ability to move thickened mucus up the respiratory tract away from the lungs, leading to bacterial infections of the lower respiratory tract, and the inability to release pancreatic enzymes into the small intestines; due to decreased activity of the chloride ion channel
- Cytochrome c** small protein loosely associated with the mitochondria's inner membrane; involved in the electron transport chain that produces ATP as well as triggering apoptosis
- Cytochrome c-oxidase** enzyme encoded by mitochondrial DNA; component of the mitochondrial electron transport chain that produces ATP during aerobic respiration
- Cytokine release syndrome** see "cytokine storm"
- Cytokine storm** overactive, uncontrolled immune response in which excessive levels of proinflammatory cytokines trigger large numbers of leukocytes to gather in an infected area; results in inflammation, tissue damage, and organ failure
- Cytokines** immune messenger molecules are involved in intercellular communication and influence other immune cells' activities
- Cytolysis** fatal rupture of a cell
- Cytomegaly** abnormal enlargement of cells, usually due to viral infections
- Cytopathic effect** formation of large groups of conjoined, dying cells
- Cytoplasm** intracellular material lying between the plasma membrane and the nucleus; composed of a fluid portion (the cytosol) and organelles
- Cytoskeletal proteins** 3 types of elongated protein strands in a cell's cytoplasm that provide cellular structure and movement, as well as the movement of material in the cell's interior; classified as microfilaments, intermediate filaments, and microtubules
- Cytoskeleton** network of the 3 cytoskeletal proteins
- D-dimer** protein product of fibrin degradation during fibrinolysis and destruction of blood clots
- De novo synthesis** the synthesis of a complex molecule from simpler ones; often used to refer to the novel synthesis of nucleotides
- Death domain** adapter involved in tumor necrosis factor- α -induced apoptosis
- Deep vein thrombosis** clots in veins deep below the skin
- Dehydroepiandrosterone** adrenal gland hormone that is a precursor to steroid hormones

- DeISGylation** removal of interferon-stimulated gene 15
- Demyelination** nerve extensions (some axons) lose their fatty myelin covering. Demyelination slows signal conditions in the nervous system and decreases the protection of the underlying axons
- Dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN)** cell surface pathogen immune recognition molecule on macrophages and dendritic cells that binds the high-mannose type of N-glycans found on viruses, bacteria, and fungi; may act as a receptor for several viruses, including some coronaviruses
- Dendritic cells (DCs)** innate immune system cells that release molecules that powerfully stimulate T cell activity; their infection by coronaviruses is important to viral dissemination by the circulatory and lymphatic systems
- Dengue fever ("breakbone fever")** extremely painful, but self-resolving and nonfatal, a disease caused by infection with dengue viruses; symptoms include high fever, rash, and severe muscle and joint pain
- Dengue hemorrhagic fever** a severe, often fatal, manifestation of infection by dengue viruses; symptoms include severe bleeding and a sudden, large drop in blood pressure
- Dengue shock syndrome** severe, often fatal, manifestation of infection by dengue viruses that typically occurs in children under the age of 10 years; symptoms include abdominal pain, excessive bleeding, and a large drop in blood pressure that may lead to shock
- Deoxyribonucleic acid (DNA)** long string composed of the nucleotides thymine, cytidine, guanine, and adenine; contains genetic information for all forms of life and some viruses
- Dermal** refers to the layer of the skin immediately beneath the epidermis
- Dermatological lesions** types of skin lesions, including macular, vesicular, papular, and pustular
- Desiccation (desiccated)** process of dehydration; rapidly kills most microbes, particularly those on external surfaces, shortening the time during which they can infect a new host
- Deubiquitinase** enzyme that removes ubiquitin from molecules
- Deubiquitination** process of removing ubiquitin from molecules; may save them from proteolysis in the proteasomes
- Developed countries** countries with a high standard of living and an economy with a high level of industrial and technological structure
- Developing countries ("Third World" countries)** primarily agricultural countries having low standards of living or industrial production
- Diabetic ketoacidosis** potentially life-threatening condition in which blood ketone levels are increased due to the use of fats, rather than glucose, in ATP production; causes excessive thirst, frequent urination, fatigue, vomiting, and a fruity smell of the breath
- Diastolic** time interval during which the heart is resting between beats; the lower number of blood pressure measurements
- Dicer** RNase that divides double-stranded RNA and premicroRNA into short double-stranded RNA fragments of small interfering RNA and microRNA, respectively; inhibits the production of specific proteins by blocking translation or cleaving mRNA
- Diffuse alveolar damage (DAD)** condition characterized by pulmonary edema, inflammation, severe hypoxia, and, in some cases, pulmonary fibrosis
- Diffuse necrotizing enteritis** widespread bacterial invasion of the wall of the intestine; results in local infection and inflammation that may eventually destroy the intestinal wall
- Dilated cardiomyopathy** impaired the heart's ability to pump blood that is due to enlargement and weakening of the left ventricle
- Dipeptidyl peptidase 4 (DDP4)** cell-surface enzyme found on most cell types that serve as the receptor for Middle East respiratory virus; functions include regulation of the immune response, signal transduction, and apoptosis
- Disseminated intravascular coagulation** serious bleeding condition due to a large reduction in molecules used in blood clotting, resulting from their previous depletion
- Distal renal (convoluted) tubules** small tubes in the kidneys that aid in urine formation
- Double-stranded RNA** form of viral RNA produced during the reproduction of some viruses
- Down's syndrome** disease resulting from three copies of chromosome 21; results in mental retardation and early-onset dementia
- Dromedary (Arabian) camels** one-humped camels that are primarily found in northern Africa, the Middle East, and Central Asia; reservoir hosts for MERS-CoV that transmit the virus to humans by the respiratory route or by drinking unpasteurized camel milk or urine

- Drosha** ribonuclease involved in microRNA processing in correlation with Dicer
- Dynamin** GTPase involved in several types of endocytosis as well as phagocytosis
- Dyspnea** difficulty or labored breathing
- E2-conjugating enzyme (ubiquitin-conjugating enzyme)** enzyme that plays a role in the addition of ubiquitin to proteins that are targeted for degradation in the proteasome
- E3 ubiquitin ligase** enzyme that transfers ubiquitin from E2-conjugating enzymes to a specific protein for degradation in the proteasome
- E-cadherin** type of cell adhesion molecule; used in the formation of adherens junctions that allow adhesion between cells
- Edema** swelling, particularly in the hands, arms, feet, ankles, or legs, caused by excess fluid trapped in the tissues; may also accumulate in the lungs, resulting in pulmonary edema or congestive heart failure
- Effector memory cells** T cell variety expresses CD45RO, but not C-C chemokine receptor type 7 or L-selectin; functions include secretion of IL-4, IL-5, IFN- γ , and perforin; located primarily in the lungs, liver, and intestines
- Effusions** escape fluids from an area, such as the abdominal cavity
- Eigen paradox** in evolutionary theory, the error threshold limits self-replicating molecules to a size that is much shorter than that necessary to encode their genetic information
- eIF4A (eukaryotic initiation factor 4A)** 3 closely-related proteins, EIF4A1, EIF4A2, and EIF4A3, required for mRNA binding to the 40S subunit of ribosomes during translation
- Elastase** pancreatic enzyme that digests elastin, a component of the extracellular matrix that stretches and recoils, allowing cells to resume their shape after elongating or contracting
- Electrolyte disorder** abnormally high or low levels of electrolytes in the blood
- Emergency myelopoiesis** rapid, inflammation-induced production and release of myeloid cells by the bone marrow in response to infection
- Encephalitis** inflammation of the brain
- Encephalomyelitis** inflammation of the brain and spinal cord, typically due to acute viral infection; often has flu-like symptoms, but may include confused thinking, seizures, problems with movement or sight and hearing, or death
- Encephalopathy** disease of the brain's structure or activity
- Endemic** conditions that are regularly found in populations of a given area
- Endocrine system** glands and organs that release hormones; including the pituitary, hypothalamus, pancreas, and thyroid glands
- Endoglin** component of the membrane TGF- β receptor complex that promotes wound repair; the soluble form is produced by metalloprotease 14 and increases inflammation of the damaged area, recruiting cell types, such as endothelial cells, that remove damaged molecules, followed by cell proliferation to repair the wound
- Endonucleases** enzymes that cut DNA or RNA in the center, rather than from the ends
- Endoplasmic reticulum (ER)** cytoplasmic, membranous tubular system; the rough ER acts in concert with attached ribosomes to produce and modify proteins during their maturation
- Endoplasmic Reticulum Associated Degradation (ERAD)** quality-control mechanism that adds ubiquitin to misfolded proteins in the endoplasmic reticulum prior to their degradation
- Endoplasmic reticulum chaperone proteins** proteins in the lumen of the endoplasmic reticulum that ensure correct protein folding and degradation of misfolded proteins
- Endoribonuclease** enzyme that cuts RNA in the center, rather than from the ends
- Endosome** vesicle formed by invagination and pinching off the cell membrane, bringing extracellular material, including microbes, into the cell's interior where they may be released into the cytoplasm or taken to the lysosomes for degradation
- Endothelial cells** flattened cells that line blood vessels and allow the exchange of materials between the blood and the tissues
- Endothelial dysfunction** malfunction of endothelial cells lining blood vessels during arterial diseases, microvascular lung thrombosis, and arteriole and venous thromboembolisms
- Endothelial nitric oxide synthase** endothelial enzyme that produces nitric oxide which, in this case, leads to vasodilation (increased diameter of blood vessels)
- Endotoxemia** presence of lipopolysaccharide from Gram-negative bacteria in the blood; results in an excessive and potentially fatal immune response

Enteric refers to the small or large intestine

Enteric nervous system controls involuntary activities of the intestines, including the movement of material through the intestine, fluid transport, blood flow, and hormone release

Enteritis inflammation of the small or large intestine; symptoms include loss of appetite, diarrhea, nausea, and vomiting

Enterocytes cells in the intestines whose villi absorb ingested material, including ions, water, sugars, amino acids, fatty materials, and vitamin B₁₂

Enteroviruses viruses that infect the gastrointestinal tract; often cause diarrhea and vomiting

Entomology study of insects

Envelope outer covering of some viruses; composed primarily of host cell lipid bilayer together with viral surface proteins

Enzyme-linked immunosorbent assay (ELISA) colorimetric or fluorometric assay that detects antibodies or other proteins using a reaction between an enzyme bound to a specific antibody and an appropriate substrate

Enzymes proteins that increase the rate of chemical reactions by lowering their energy of activation

Eosinophilia condition of possessing an excessive number of eosinophils

Eosinophils relatively uncommon type of leukocyte that is involved in allergic responses, typically present in higher numbers during allergies or parasitic worm infections

Ependymal cells glial cells that surround ventricles in the central nervous system; control the composition of cerebrospinal fluid

Epididymitis inflammation of the epididymis, the part of the male reproductive system that stores sperm as they mature

Epidemic widespread outbreak of an infectious disease in a community over a short time period

Epidermal growth factor (EGF) protein that induces cell growth, wound healing, and tumor formation

Epidermal growth factor receptor (EGFR) cell surface receptor for epidermal growth factor; binding between them sets in motion a pathway that stimulates cell division

Epidermis upper layer of the skin; composed of five layers

Epinephrine “fight-or-flight” hormone produced by the adrenal medulla; released during stress

Epithelial cells cells that line and cover organs or cavities; flattened, cube-shaped, or elongated

Epithelial necrosis death of epithelial cells

Epithelium tissue lining or covering structures

Epitopes small regions of a protein (9–11 amino acids) that are recognized by lymphocytes

Epizootic catarrhal gastroenteritis (“green slime disease”) disease characterized by the production of profuse amounts of green mucoid diarrhea, severe dehydration, or starvation

Equine pertaining to horses

ER chaperones see “Endoplasmic reticulum chaperone proteins”

Erythema reddening of the skin

Erythema multiforme-like lesions skin lesions that have the appearance of targets

Erythrocytes red blood cells

Erythroderma potentially life-threatening inflammation of most of the body’s skin; may result from an adverse reaction to a medicine

Erythropoietin cytokine produced by the kidneys that signal bone marrow to produce more erythrocytes

Estradiol most important sex hormone during a female’s reproductive years; is required for reproduction; produced by the ovaries, adrenal gland, and placenta during pregnancy

Estrogen female hormone found primarily in women that aids in the formation, maturation, and function of female structures; also decreases blood calcium and body fat levels, increases muscle growth and activity, strengthens bones, and increases nervous system activity

Eukaryote organism whose cells contain membrane-enclosed organelles and nuclei; includes single-celled protists, mosses, fungi, plants, and animals

Eukaryotic translation initiation factor 2 signaling pathway 1 of the 2 pathways that regulate translation initiation; activated in response to stress or infection by certain viruses

Euthanize to humanely put an animal to death

Ex vivo (“outside of life”) experimental studies performed on living tissue in an artificial environment outside of the organism

- Exanthematous pustulosis** sudden fluid-filled skin eruptions appearing about five days after beginning a medication
- Exfoliative erythroderma** form of erythroderma in which the skin's upper layers are shed
- Exocrine** unlike hormones, a type of cellular secretion that is released through ducts; includes tears, saliva, digestive enzymes, milk, and sweat
- Exocytosis** process by which material within vesicles is released from a cell
- Exonuclease N (ExoN)** proofreading enzyme that corrects mistakes made during replication
- Exoribonucleases** enzymes that remove nucleic acid bases from the ends of DNA or RNA
- Extracellular matrix (ECM)** network of large extracellular proteins that include collagen, enzymes, and glycoproteins found outside of and between cells; many ECMs play a role in cell adhesion and cell-to-cell communication
- Extracellular-signal-regulated kinases (ERKs)** enzymes that are part of the mitogen-activated protein kinase family of signaling molecules; the ERK1/2 signaling pathway is involved in cells' proliferation, differentiation, migration, survival, metabolism, and transcription
- Extracorporeal membrane oxygenation (heart-lung bypass)** procedure is used to oxygenate blood with an external pump that passes blood through an artificial lung and back into the body
- Extravasation** leakage of fluid into the surrounding area, particularly plasma
- Exudate** fluid and materials that had seeped out of blood vessels or tissues
- Factor II/IIa** component of the extrinsic pathway of coagulation that works in collaboration with tissue factor of neutrophils during wound repair
- Factor Va** component of the intrinsic coagulation pathway; cofactor for factor Xa
- Factor Xa** enzyme component of the intrinsic coagulation pathway; part of the prothrombinase complex that produced thrombin
- "False negative" test** test in which infected people test negative
- "False positive" test** test in which uninfected people test positive
- Farrowing** sows giving birth to piglets
- Fas/FasL** transmembrane proteins of the tumor necrosis factor family; binding of FasL to Fas induces an apoptotic program
- Fatty degeneration** small fat droplets accumulate in the cytoplasm of cells
- Fc region** part of the antibody which differs between antibody classes; does not bind to the antigen
- Febrile seizures** convulsions in children between the ages of six months and five years having a temperature greater than 38°C (100.4° F)
- Feline** pertaining to cats
- Feline coronaviruses (FCoVs)** 2 biotypes of similar coronaviruses of cats
- Feline enteric coronavirus (FECV)** coronavirus of the intestine of cats that causes atypical or minor digestive system symptoms
- Feline infectious peritonitis (FIP)** extreme-to-fatal inflammatory reaction in tissues around the abdomen, kidney, and brain
- Feline infectious peritonitis virus (FIPV)** highly pathogenic coronavirus of cats that arises from postinfection mutations of feline enteric coronavirus
- Femur head** "ball" of the ball and socket joint of the hip
- Fenton reaction** conversion of hydrogen peroxide to the highly toxic hydroxyl ion while increasing the oxidation state of iron
- Ferritin** iron-binding protein that prevents microbes from acquiring this vital ion
- Fibrin** fibrous protein involved in coagulation; formed by the protease thrombin, which causes its fibrinogen precursor to polymerize into insoluble strands that work together with platelets to form a clot
- Fibrin thrombi (clots)** final products of the coagulation process; consist of plugs containing aggregates of platelets and red blood cells together with a mesh of cross-linked fibrin
- Fibrinogen** protein is involved in coagulation; the soluble precursor of the insoluble fibrin protein
- Fibrinogen-like protein 2 (FLP2)** member of the fibrinogen superfamily that acts as a pro-coagulative membrane protein or as a soluble immunosuppressive protein
- Fibrinolysis** cleavage of cross-linked fibrin by plasmin
- Fibrinopurulent bronchopneumonia** bronchopneumonia that exudes fibrin and pus
- Fibrinous exudates** exudate consisting largely of fibrinogen and fibrin

- Fibroblast growth factor 2 (FGF2)** molecule that stimulates growth and development of new blood vessels; involved in wound repair
- Fibroblasts** relatively nonspecific spindle-shaped cells present in most tissue types; normally participate in wound control, but may also cause pathogenic fibrosis (scarring)
- Fibrocytes** primitive cells produced by monocyte precursors with inflammatory and tissue remodeling characteristics of macrophages and fibroblasts, respectively
- Fibronectin** compound present during development and responses to cellular injury; increases survival of human stem cells
- Filopodia** protrusions from the plasma membrane that allow cells to sense their external environment, information which is important for cell migration and wound healing
- Filterable agents** viruses; pass through almost all filters
- Firmicutes** group of Gram-negative bacteria present in the human gut; many produce butyrate, a compound that maintains colon health
- First-generation** group of patients infected by the first wave of an infectious disease
- Flaviviruses** (+)-sense, single-stranded RNA viruses; including West Nile and Zika viruses
- Flavonoids** antioxidant plant compounds with various phenolic structures; found in fruits, seeds, wine, tea, etc.
- Fluorescence immunochromatographic assay** antibodies against the tested material are labeled with fluorescent particles and detected and quantified by the fluorescent intensity of a paper test strip
- Foamy macrophages** macrophages containing large amounts of cholesterol and other fatty materials that are found in arterial plaques; less inflammatory than other macrophages
- Follicle stimulating hormone** stimulates the development of ovarian follicles in women and gonads in men; produces estrogen and progesterone in women and sperm production in men
- Follicular epithelium of the thyroid gland** cells that produce and secrete the thyroid hormones thyroxine (T4) and triiodothyronine (T3) which regulate metabolism
- Follicular hyperplasia** increased the number and size of follicles in lymph nodes, tonsils, spleen, and other lymphoid tissues; follicles consist primarily of B cells surrounded by T cells
- Fomites** inanimate objects that, when contaminated with microbial agents, may transfer the microbe to a new host
- Forkhead box P3 (FOXP3)** master regulator of T regulatory cells; decreases T cell functions
- Founder's effect** genetic traits and mutations present in a population generated by a small number of individuals tend to amplify the prevalence of traits from their original founders
- Fractalkine** chemokine that recruits lymphocytes and monocytes
- Frontal lobe of the cerebrum** a brain region that is vital to higher cognitive functions, including thought, memory, attention, and motivation
- Frugivorous** fruit-eating
- Fulminant myocarditis** acute form of myocarditis with a rapidly progressive disease course
- Fulminant viral hepatitis (FH)** disease that begins like hepatitis but rapidly develops into life-threatening liver failure; symptoms include unconsciousness or coma, blood-clotting defects, and edema of the abdominal cavity, arms, and legs
- Furin** cellular protease that cleaves some coronaviruses' spike proteins at the S1/S2 junction
- Gain-of-function** microbe becomes more virulent or utilizes a greater range of host cells or host species; accomplished by natural or artificial means
- Gamma-aminobutyric acid (GABA)** major inhibitory neurotransmitter
- Gastric inhibitory polypeptide** hormone produced by enteroendocrine K cells in the intestines; stimulates insulin secretion and inhibits hydrochloric acid secretion by the stomach
- Genetic recombination** exchange of genomic RNA between or within host species
- Genome** complete set of genes for an organism; contained in DNA for all organisms, except some types of viruses, including coronaviruses, which have an RNA genome
- Geographic information systems (GIS)** computerized method of capturing, storing, and displaying information related to positions on the Earth's surface
- Geospatial study** study of objects and events with regards to location on or near Earth's surface
- Germ cells** sperm and eggs
- Germinal center** inner area of lymphoid follicles of the spleen, lymph nodes, and tonsils that contains activated B cells that secrete antibodies; increases in size during infections

- Ghrelin** (“hunger hormone”) hormone produced by the stomach that increases appetite
- Glia (neuroglia)** cells that support neurons; also communicate via gliotransmitters
- Glial fibrillary acidic protein (GFAP)** member of the intermediate filament family; a marker of the activation of Müller cells and astrocytes in the retina
- Gliosis** dense fibrous network of glia in the nervous system that may cause scarring
- Glomerular capillaries** tangle of capillaries surrounded by the glomerular capsule of the kidneys’ nephrons; fluid is pushed out of them and into the nephron’s tubular system during urine production
- Glomerular filtration rate** rate at which material is forced out of the glomerular capillaries into the glomerular capsule and then into the renal tubule system
- Glomerular fluid** fluid pushed from the glomerular capillaries into the glomerular capsule and then into the proximal convoluted tubules during urine production
- Glomeruli** see “glomerular capillaries”
- Glomerulonephritis** severe inflammation of the glomeruli of the kidney’s nephrons
- Globus pallidus** part of the basal ganglia that regulate voluntary movement
- Glucagon** hormone produced by α cells of the pancreatic islets; increases blood glucose levels
- Glucagon-like peptide-1** hormone that increases the release of insulin, suppresses the release of glucagon, lowers blood glucose levels, and reduces food intake; produced by intestinal enteroendocrine L-cells and some brain-stem neurons after eating
- Glucose-6-phosphate dehydrogenase deficiency (G6PDd)** genetic disorder in which red blood cells are destroyed prematurely; symptoms include dark urine, rapid heart rate, shortness of breath, heart murmur, enlarged spleen and liver, and jaundice (yellow skin and eyes)
- Glutamate** major excitatory neurotransmitter
- Glutathione** antioxidant; critical to immune system function
- Glutathione peroxidase-3** selenocysteine-containing enzyme that converts hydrogen peroxide into water
- Glycemic control** regulation of blood sugar levels
- Glycosylation** process of adding sugar groups onto a molecule
- Goblet cells** cells that secrete mucus
- Golgi apparatus** organelle composed of stacked membranous structures; site of the completion of protein maturation and package into vesicles for delivery to their appropriate destination
- Gonadotropic hormone** hormone that stimulates the secretion of follicle-stimulating hormone and luteinizing hormone in women and male sex hormones, such as testosterone, in men
- Gram-negative bacteria** bacteria that appear pink after Gram staining; often more pathogenic than Gram-positive bacteria due to lipopolysaccharide in their membranes that may induce excessive, pathogenic immune responses
- Granulocyte** leukocytes containing granules; neutrophils, eosinophils, basophils, and mast cells
- Granulocyte colony-stimulating factor (G-CSF)** cytokine that stimulates granulocyte production
- Granulocyte-monocyte colony-stimulating factor (GM-CSF)** similar to G-CSF but also stimulates monocyte production
- Granuloma** tumor-like aggregate of macrophages
- Granulosa cells** ovarian cells are closely associated with developing eggs
- Granzyme** compounds produced by T killer cells and NK cells that results in apoptosis
- Graves’ disease** autoimmune disease in which thyroid hormones are produced in excessive amounts
- Gray matter** neuron cell bodies, dendrites, and axons that are not surrounded by myelin
- Ground-glass opacity** hazy gray areas seen in CAT scans or X-rays of the lungs; indicates the increased density of the area
- Growth and differentiation factor 15 (GDF15; macrophage inhibitory cytokine-1)** member of the transforming growth factor β superfamily that promotes lipid cleavage; produced by skeletal muscles during exercise
- GTPase** group of enzymes that cleave a phosphate group from guanosine triphosphate (GTP) during intracellular signal transmission; generally when other members of the signaling pathways are bound to GTP, they are active and are inactivated by the actions of GTPases
- Guano** bat feces
- Guillain-Barré syndrome** acute or chronic autoimmune disorder whose symptoms include weakness or tingling in the legs and hands; pins-and-needles sensations in fingers, toes, ankles, or wrists; inability to walk or climb stairs, loss of some sensations in the limbs; difficulty in controlling bowel and bladder functions
- Gustation** sense of taste

- Halo sign** ground glass opacity surrounding pulmonary nodules or masses in the lungs
- Haptoglobin** compound present in serum which removes free hemoglobin from the blood
- Hashimoto's thyroiditis** autoimmune condition targeting the thyroid gland, decreasing its ability to produce normal hormone levels; resulting in slow metabolism and obesity
- Heart block** condition in which the heart beats too slowly due to malfunctions in its electrical system
- Heat-shock protein 40** inhibitor of newly produced polypeptides translocating into the endoplasmic reticulum
- Helicase** enzyme that separates the two strands of double-stranded RNA
- Hemadsorption** adsorption of red blood cells to the plasma membranes of infected cells
- Hemagglutinating** causing red blood cell clumping
- Hemagglutinating encephalomyelitis (vomiting and wasting disease)** disease of piglets that induces vomiting, wasting, and neurological pathology
- Hemagglutination inhibiting antibodies (HI antibodies)** antibodies that prevent hemagglutination
- Hemagglutinin-esterase enzyme** viral envelope glycoprotein involved in reversible attachment to sialic acids by acting both as a lectin (sugar-binding molecule) and as receptor-destroying enzyme; present in some betacoronaviruses
- Hematocrit** percentage of red blood cells in the blood
- Hematopoiesis (hematopoietic)** process of producing all blood cells and platelets, typically in red bone marrow
- Hematuria** abnormal presence of blood in the urine
- Hemophagocytosis** phagocytic cells ingest red blood cells
- Hemorrhagic fever** often a fatal disease that involves massive hemorrhaging, high fever, petechiae, and disseminated intravascular coagulation; primarily caused by viruses
- Hemosiderin granules** abnormal yellowish-brown granules consisting of iron hydroxides, proteins, and polysaccharides present in cells of people having excessive levels of iron
- Heparan sulfate** multifunctional molecule found on cell surfaces; one of its roles is to maintain the functioning of the β cells of the islets of Langerhans in the pancreas
- Heparin-binding EGF-like growth factor** member of the epidermal growth factor family that stimulates growth and differentiation of cells
- Hepatitis** inflammation of the liver
- Hepatic** pertaining to the liver
- Hepatocytes** liver cells
- Hepatotropic** attracted to the liver
- Hepcidin** liver molecule that transports iron; "master regulator" of iron metabolism
- Herd immunity** indirect protection of people who are not immune to a given disease; results from a large portion of a population being immune to that specific disease either by having contracted and survived the disease or through vaccination
- Herpetology** study of amphibians and reptiles
- Heterodimer** structure composed of 2 different units
- Heterologous challenge** following vaccination, an animal is administered a different strain of microbe to test vaccine efficacy against the multiple strains of microbe present in nature
- Heterologous recombination** in coronaviruses, exchanging parts of the genomic RNA with a member of another virus species
- High-density lipoprotein ("good cholesterol")** lipid-containing compound that has high levels of proteins; involved in cholesterol transport
- High-efficiency particulate air (HEPA) filters** filter that removes 99.95% to 99.995% of particles ≥ 0.1 microns in size
- Hippocampus** part of the limbic system that plays a critical role in learning and memory; 1 of the 2 areas of the brain that contains functioning neural stem cells throughout life
- Hipposiderid bats** roundleaf bats
- Histamine** molecule associated with Type I Hypersensitivity (rapidly-induced allergies); may cause runny nose, sneezing, itching, constriction of lung bronchioles, fatal food allergies
- Homeostasis** process of bringing bodily functions back to a state of optimal functioning
- Homologous** sameness
- Homologous challenge** following vaccination, an animal is administered the same strain of microbe to test vaccine efficacy; since multiple strains of microbe are present in nature, this type of assay needs to be repeated using other, heterologous microbe strains

- Homologous recombination** in coronaviruses, exchanging parts of the genomic RNA with another virus of the same species
- Homozygous** in genetics, having two of the same copy of an allele of a gene
- Hormone** compound produced by an endocrine organ; produces reactions that are relatively slow to develop and long-lasting
- Human growth hormone (somatotropin)** hormone that regulates growth and development in children; in children and adults, it regulates energy release from food, synthesis of lipids, proteins, and glucose, as well as production of red blood cells and increased muscle mass
- Hyaline membrane** glassy membrane composed of proteins and dead cells that lines the alveoli, severely restricting the exchange of oxygen and carbon dioxide between the blood and lungs
- Hybrid microbe** microbe containing genes from more than 1 microbe species due to gene “swapping,” between different viral species; hybrid viruses may be highly pathogenic since they differ greatly from their parental viruses and are not readily recognized by the immune system of either host species
- Hydrogen peroxide** H_2O_2 ; reactive oxygen species formed by the dismutation of superoxide
- Hydroxyl radical** $\bullet\text{OH}$; one of the most toxic reactive oxygen species
- Hyperammonemia-associated encephalopathy (hyperammonemia encephalopathy)** brain injury and death due to excessive levels of ammonia in the blood
- Hyperandrogenism** condition in which increased levels of androgens are present in women
- Hypercarbic respiratory failure** condition in which tissues levels of CO_2 are excessive
- Hypercytokinemia** high levels of inflammatory mediators produced by stimulated T cells and macrophages leads to potentially fatal acute respiratory distress syndrome or systemic inflammatory responses
- Hyperesthesia** excessive sensitivity to sensory information
- Hypergammaglobulinemia** high level of antibodies or other gamma globulins in the blood
- Hyperglycemia** high blood sugar levels
- Hyperkalemia** high blood potassium levels
- Hyperlipidemia** high blood lipid levels
- Hyperpigmentation** excessive skin coloration
- Hyperplasia** enlargement of an organ or tissue due to increased cellular reproduction
- Hypertension** high blood pressure
- Hypoferremia** low blood iron levels
- Hypogeusia** reduced the sense of taste
- Hypoglycemia** low blood sugar levels
- Hypoperfusion** lowered blood flow through a region of the body
- Hyperproteinemia** low blood protein levels
- Hypotension** low blood pressure
- Hypertensive nephrosclerosis** hardening and thickening of kidney tissues due to chronic high blood pressure; results in narrowing of the blood vessels that restricts tissues from receiving adequate amounts of oxygen
- Hyperthyroidism** high levels of thyroid hormones; results in increased metabolism, bone density, nervous system, and muscle activity and decreased blood calcium levels
- Hypothalamic–pituitary–adrenal (HPA) axis** complex, dynamic interactions of the central nervous (hypothalamus) and endocrine (pituitary and adrenal glands) systems during stress
- Hypothalamic–pituitary–thyroid (HPT) axis** complex interactions between the hypothalamus, pituitary, and thyroid; regulates development, energy metabolism, and growth
- Hypothalamus** area of the brain is responsible for homeostatic regulation of many of the body’s critical functions; also part of the limbic system
- Hypothermia** low body temperature
- Hypothyroidism** low levels of thyroid hormones; may result in decreased metabolism, bone density, nervous system and muscle activity, and increased blood calcium levels
- Hypotonic lysis** rupture of a structure due to lower internal than external solute levels
- Hypovolemia** low blood volume; may result in rapid heartbeat, weak pulse, confusion, loss of consciousness, or shock
- Hypoxemic respiratory failure** condition in which tissues receive too little oxygen
- Hypoxia** low blood oxygen levels

- Idiopathic perniosis** inflammation of small skin blood vessels due to an abnormal response to the cold; origin is unknown
- IFN-stimulated genes** see “interferon-stimulated genes”
- IgA antibody** prevalent class of antibodies in mucus membranes and secretions, including mucus, saliva, tears, and milk; protects against microbes entering entry via these membranes and also protects breastfeeding infants
- IgG antibody** prevalent class of antibodies in the blood and helps prevent infection by blood-borne microbes: passes through the placenta to protect fetuses
- IgM antibody** pentameric antibody that is the first to be produced during an immune response; first produced by babies at approximately 6 months of age
- IL-6 (interleukin-6)** major proinflammation cytokine that increases the production of IL-17; produced by T helper cells and some tissue macrophages and epithelial cells
- IL-7 (interleukin-7)** cytokine that is necessary for early T cell development and homeostasis
- IL-8 (interleukin-8)** proinflammatory chemokine; recruits neutrophils, increases phagocytosis
- IL-10 (interleukin-10)** antiinflammatory T regulatory cell cytokine
- IL-12 (interleukin-12)** cytokine that aids in the differentiation of Th1 cells
- IL-15 (interleukin-15)** promotes natural killer cell differentiation, neutrophil phagocytic activity, and immune responses to intracellular microbes
- IL-17 (interleukin-17)** proinflammatory cytokine; increases the production of CXCL1 and CXCL2
- IL-23 (interleukin-23)** chemokine that recruits neutrophils and aids in Th17 cell development
- Ileum** final region of the small intestine; absorbs nutrients into blood vessels
- Immune complex** large, mesh-like structure that contains antibodies and antigens whose size increases the chance of being phagocytized; large complexes may be deposited in joints or kidney tubules, producing painful to fatal inflammation in these regions
- Immune thrombocytopenic purpura** low platelet count due to their consumption by excessive bleeding; symptoms include easy bruising, bleeding from the gums and nose, and bloody urine and feces
- Immunocompetent** producing a normal immune response
- Immunogenic** substance that induces an immune response
- Immunoglobulin superfamily** immune system molecules with structures similar to antibodies
- Immunoglobulin-like transcript 3 (ILT3)** inhibitory receptor molecule expressed by dendritic cells, monocytes, and endothelial cells; suppresses the activity of myeloid cells, primarily macrophages and neutrophils
- Immunological memory** lymphocyte responses that are produced more rapidly, stronger, and long-lasting after the 2nd and subsequent exposures to microbes or other foreign material
- Immunosenescence** decreased immune responsiveness, usually due to aging
- In vitro (“in glass”)** performed in the laboratory
- In vivo (“in life”)** performed within living organisms
- Inactive virus** a permanently noninfectious form to a virus: a “dead” virus
- Inbred** repeated breeding of related members of a species; eventually produces a distinct group of plants and animals that are genetically identical
- Incontinence** inability to control urination or fecal release
- Index case** initial case of a disease in a given location
- Indoleamine-pyrrole 2,3-dioxygenase (IDO-1)** enzyme that contains iron which binds oxygen; antioxidant involved in antimicrobial/antitumor defense and immunoregulation
- Inducible nitric oxide synthetase (iNOS)** enzyme found in monocytes/macrophages that produce toxic nitric oxide upon stimulation
- Infarction** cell death to insufficient oxygenation
- Inflammatory monocytes/macrophages (IMM)** activated monocytes and macrophages that produce excessive levels of proinflammatory cytokines that contribute to tissue injury
- Inflammatory transitional CD14⁺CD16⁺ monocytes** monocytes that express variable levels of inflammatory cytokines, possess the intermediate phagocytic capacity and are highly potent in antigen presentation
- Inhibitor of nuclear factor kappa-B kinase subunit epsilon (inhibitor of NF- κ B kinase epsilon; IKK ϵ)** enzyme that activates NF- κ B by the addition of a phosphate ion
- Innate immune system** rapidly active, nonspecific cells and their secretions; do not result in immune memory; response is weaker than that of the adaptive immune system

- Inosine monophosphate dehydrogenase** enzyme that converts inosine monophosphate to xanthosine monophosphate during the *de novo* synthesis of guanine nucleotides
- Inositol-requiring enzyme 1 (IRE-1)** endoplasmic reticulum stress endonuclease that is part of the unfolded protein response
- Insectivorous** species that eat insects
- Insulin** hormone from β cells of the pancreatic islets of Langerhans; lowers blood sugar levels
- Integumentary system** composed of the skin, hair, and associated glands and nerve structures
- Intercellular** within a cell
- Interferon (IFN)** group of cytokines with antiviral activity; 3 general types- I, II, and III
- Interferon- α (IFN- α)** type I interferon with an antiviral and anticancer activity that is produced by virus-infected and cancer cells; decreases cell proliferation while increasing macrophage and T-lymphocyte activity
- Interferon- β (IFN- β)** type I interferon with an antiviral and anticancer activity that is produced by fibroblasts; decreases cell proliferation while increasing macrophage and T-lymphocyte activity
- Interferon- β promoter stimulator (IPS-1)** molecule with a critical role in producing type I interferon and proinflammatory cytokines
- Interferon- γ (IFN- γ)** inflammatory type II interferon that has antiviral and anticancer activity that is produced by T lymphocytes and natural killer cells; stimulates natural killer cells and neutrophils and is the primary activator of macrophages
- Interferon- γ -inducible protein 10 (IP-10)** chemokine that recruits T cells
- Interferon- λ (IFN- λ)** group of type III interferons with antiviral activity in a limited number of cell types; produced by peripheral blood mononuclear cells stimulated by IFN- α ; their activities are similar to IFN- α but bind to different receptors
- Interferon-inducible 2,5-oligoadenylate synthetase (OAS)** antiviral enzyme that recognizes viral single- and double-stranded RNA; leads to RNA destabilization via the RNase L enzyme
- Interferon-inducible transmembrane protein (IFITM)** protein whose expression is induced by interferon; impairs some enveloped viruses from target cell entry
- Interferon regulatory factor 3** transcriptional regulator of type I interferon genes; upon phosphorylation, it enters the nucleus, binds its target genes, and stimulates transcription
- Interferon regulatory factors (IRFs)** transcription factors that induce IL-28 production and regulate the production of type I interferon, Toll-like receptor signaling, and differentiation of CD4⁺ T helper cells
- Interferon response factor 3 (IRF-3)** part of a regulatory complex that enters the nucleus and activates the transcription of interferon- α and - β
- Interferon response factor 8 (IRF8)** transcription factor needed for normal B cell differentiation
- Interferons** cytokines which directly or indirectly inhibit viral replication; produced by cells of the immune system or connective tissue and regulate the functions of other immune cells
- Interferon-stimulated gene 15 (ISG15)** ubiquitin-like molecule important for antimicrobial defense; expression is stimulated by type I interferons
- Interferon-stimulated genes (ISGs)** molecules produced in response to type I interferon; have major roles in increasing the innate immune systems' defense against microbial infection
- Interleukins (ILs)** cytokines regulate many activities of both the innate and adaptive immune systems. See [Table 1.6](#) for a listing of interleukins mentioned in this book
- Intermediate host** animal species infected by microbes from the reservoir host prior to its subsequent transmission to another animal species, including humans
- Interspecies genetic recombination** process of exchanging genes between different species
- Interstitial edema** abnormal accumulation of fluid in tissues
- Interstitial fluid** fluid in the spaces between cells; leaks out of capillaries and brings oxygen and nutrients to cells and removes waste products
- Interstitial macrophages** macrophages in the interstitial spaces; those in the lungs cooperate with interstitial lymphocytes to produce a stronger and specific immune response
- Interstitial spaces** fluid-filled areas surrounding cells within a tissue
- Intestinal crypts** invaginations of the epithelium around the villi; secreted by epithelial cells
- Intracellular signaling** signals are transmitted within the cell by signaling pathways; often terminate by transcription factors entering the nucleus and binding specific regions of DNA, resulting in transcription of the corresponding gene

- Intracerebral hemorrhage** bleeding in the cerebrum of the brain
- Intracerebral** inoculated into the cerebrum
- Intradermal** inoculated into the skin
- Intranasal** inoculated via the nasal cavity
- Intraperitoneal** inoculated into the abdominal cavity
- Intraspecies transmission** transmission between members of a species
- Intratracheal** infected via the trachea
- Intravascular thrombosis** arteries or veins containing blood clots that impede blood flow
- Ion channel** pore in a membrane that opens to let ions to pass into or out of a membrane-enclosed structure or closes to retain them on the inside or outside
- Ionophore** chemical that binds up a free ion, removing it from circulation
- IP-10** chemokine that recruits monocytes and CD4⁺ T helper and CD8⁺ T killer cells
- Iron lung** device that brings air into and out of the lungs; were used primarily for people suffering from severe polio and who were unable to breathe independently
- Ischemia** reduced blood flow to a region of the body
- Ischemic stroke** stroke induced by reduced blood flow to the brain
- Islets of Langerhans** endocrine portion of the pancreas that regulates blood sugar levels; contains α , β , and γ cells that produce glucagon, insulin, and somatostatin, respectively
- Isovolumic relaxation time** time between the closing of the aortic heart valve and opening of the mitral (bicuspid) valve to allow filling of the left ventricle
- Ito cells** mesenchymal cells that produce collagen and other extracellular matrix proteins
- JAK/STAT (Janus kinase/signal transducer and transcription activator)** intracellular signaling pathway that involves a series of phosphorylation events that eventually lead to transcription of proteins involved in cell proliferation, differentiation, migration, apoptosis, and survival
- Janus kinase-1 (JAK-1)** kinase (an enzyme that adds the phosphate ion to other molecules) that is part of the JAK/STAT signaling pathway
- Jejunum** center area of the small intestine; absorbs nutrients into the blood
- Kawasaki disease (KD)** inflammation of blood vessels throughout the body, especially the coronary arteries of the heart; most common in children
- Kawasaki disease-like in children (MIS-C)** inflammatory multisystem syndrome affects the cardiac, gastrointestinal, renal, hematologic, dermatologic, and neurologic systems
- Kepi** subunit of protein phosphatase 1 that inhibits its activity
- Keratinocytes** type of epidermal cells of the skin that produces keratin to form a barrier against water loss, heat, ultraviolet radiation, and microbial entry into the body; microbial invasion of the upper part of the epidermis stimulates them to produce pro-inflammatory chemokines which recruit monocytes, natural killer cells, T cells, and dendritic cells
- Kidney replacement therapy** processes that include dialysis, hemofiltration, or kidney transplantation to replace the normal blood-filtering functions of the kidneys
- Kruppel-like factor 6 (Klf6)** tumor suppressor gene; decreases the production of toxic nitric oxide that may cause cell death
- Kupffer cells** resident liver macrophages
- Lactating** producing milk
- Lactoferrin** iron-binding protein in the milk that deprives microbes of iron
- Lamina propria** part of the basement membrane; a thin, vascular layer of connective tissue under the epithelium of mucous membranes
- Large vessel vasculitis** autoimmune condition characterized by inflammation of medium and large-sized arteries supplying the legs and arms or the aorta; may result in loss of vision, stroke, or difficulty with thought and memory
- Lectin** sugar-binding molecule
- Lectin pathway of complement activation** initiation of the complement system by mannose-binding lectin binding to terminal mannose of glycoproteins on the target surface
- Left shift** in the immune system, the population of leukocytes is “shifted” towards its more immature precursors
- Left ventricle** largest, most powerful of the heart chambers; pumps blood from the heart to the rest of the body with the exception of the lungs

- Leptin** hormone produced by white adipose tissue; signals satiety
- Leptomeninges** 2 innermost coverings of the brain and spinal cord (arachnoid and pia mater)
- Lethal dose₅₀ (LD₅₀)** amount of toxin that kills 50% of the test population of animals
- Leukocytes** white blood cells; primary cells of the immune response
- Leukotrienes** inflammatory products of arachidonic acid that are associated with strong allergic responses, such as asthma, constriction of lung bronchial tubes, and anaphylactic shock
- Leydig cells** cells within testes that secrete testosterone
- Ligand** compounds that trigger chemical reactions upon binding to its specific receptor
- Limbic system** brain regions that trigger strong emotions, such as rage, lust, and pleasure
- Lipid nanoparticles** very small particles of lipid surrounding material, including coronavirus RNA and transports material through phospholipid membranes; the basis of the Pfizer and Moderna anti-SARS-CoV-2 vaccines
- Lipid raft-mediated endocytosis** process of invaginating portions of the plasma membrane that are named lipid rafts because they contain large amounts of cholesterol and sphingolipids
- Lipocalin-2** antibacterial and antiinflammatory hormone that increases levels of matrix metalloproteinase 9 in neutrophils and destroys the extracellular matrix
- Lipopolysaccharide** lipid (fatty material) that is bound to a sugar molecule
- Liposomes** “fat bodies” that fuse with fatty membranes, such as the plasma membrane, and release materials within them into the cell
- Lipoteichoic acid** major part of the cell wall of Gram-positive bacteria
- Livedo racemose** red or violet, broken, branched, areas of the skin
- Liver sinusoidal endothelial cells (LSECs)** form the wall of liver sinusoids to produce a porous barrier between material in the blood and the liver cells
- Liver/lymph node-specific intercellular adhesion molecule-3-grabbing integrin (L-SIGN)** helps to attach several enveloped viruses to their target cells prior to cell entry; also captures viruses and spreads them to other target cells
- Long COVID syndrome** abnormal conditions lasting at least 12 weeks after onset of acute COVID-19 symptoms
- Long QT-interval** abnormally lengthened portion of the heartbeat that is detected by electrocardiograms; during stress, the affected person may die of sudden cardiac failure
- Lower respiratory tract** area of the respiratory system that is found within the chest, includes the lungs
- Lumen** inner cavity of a tube or tubular organ
- Lung consolidation** lung tissue is filled with liquid and solid material that displaces the air
- Lung-kidney axis** interaction between respiratory and urinary systems in which damage to the kidneys triggers an inflammatory response in the lungs, typically due to excessive IL-6
- Luteinizing hormone** in women, a hormone from the anterior pituitary gland that stimulates ovulation (release of an egg)
- Lymphatic system** one of the two transportation systems of the body; consists of lymph vessels, lymph, lymph nodes, and lymphocytes
- Lymphocytes** leukocytes that direct the actions of other immune cells, kill virus-infected cells, or produce antibodies; the 3 major categories are T helper cells, T killer cells, and B cells
- Lymphopenia** low levels of blood lymphocytes
- Lysosomes** acidic, membrane-bound cellular organelles that contain powerful digestive enzymes and reactive oxygen species; fuse with endosomes bringing material, including viruses, into the cell, then digesting this material
- M1 macrophages (inflammatory monocyte/macrophages)** inflammatory, stimulatory, antiviral macrophage subset
- M2 macrophages (alternatively activated macrophages)** antiinflammatory, inhibitory macrophages that remove cell debris after completion of wound repair
- M-calpain** calcium-dependent protease located in the central nervous system
- Macrophage-inflammatory protein (MIP)-1 α** see “CCL3”
- Macrophages** mature, tissue forms of monocytes that ingest and digest microbes and debris; produce inflammatory cytokines and chemokines
- Macular degeneration** destruction of the part of the eye upon which the image is focused (macula); degeneration leads to the inability to see images near the center of vision

Macules small discolored regions of the skin

Maculopapular rash flattened red areas of the skin

Main protease (M^{Pro}; nsp5) see “3CL^{Pro}”

Major histocompatibility complex class 1 molecules (MHC 1) cell surface molecules required for CD8⁺ T killer cells to kill infected or cancer cells; present on almost all cell types

Major histocompatibility complex class 2 molecules (MHC 2) cell surface molecules required for CD4⁺ T helper cell activation; present on B cells, monocytes/macrophages, and dendritic cells

Malabsorptive diarrhea diarrhea with fatty stools accompanied by malnutrition, weight loss, abdominal pain, and anemia

Malaise general feeling of unwellness

Mammalogy study of mammals

Mannose binding lectin molecule that activates complement via the lectin pathway by binding mannose

Mannose binding lectin (MBL)-associated serine protease (MASP)-2 enzyme that cleaves C4 and C2 to assemble a C3 convertase via the lectin pathway of complement activation

Mast cells innate immune system cells found in mucus membrane; involved in the causation of many allergies (Type I Hypersensitivity) by releasing histamines and leukotrienes

Matrix metalloproteinase-9 (MMP9) regulates tissue remodeling by degrading extracellular matrix proteins and stimulating cytokines and chemokines involved in wound repair

Matrix metalloproteinases family of enzymes responsible for the degradation of proteins of the extracellular matrix during wound repair

Mechanical ventilation artificial ventilation in which mechanical ventilators assist or replace spontaneous breathing in people with dysfunctional lungs; used to maintain blood oxygen and carbon dioxide levels

Medial temporal lobe part of the cerebrum involved in conscious memory of facts and events; consists of the hippocampus and perirhinal, entorhinal, and parahippocampal cortices

Medulla oblongata 1st portion of the brainstem; performs vital functions required to survive

Melanin dark pigment in the epidermis of the skin and hair that inhibits ultraviolet light from reaching live skin cells and underlying tissue; decreases the risk of sunburn and skin cancer

Melanocytes cells in the epidermis's stratum basal that produce the brownish pigment melanin

Melanoma differentiation-associated protein 5 (MDA5) pattern recognition receptor that recognizes double-stranded RNA in the host cell's cytoplasm

Melatonin sleep-inducing hormone produced by the pineal gland in the dark; possesses antiinflammatory activity

Memory T cells rapidly produced, highly active T cells formed after initial exposure to antigen

Membrane attack complex component of the complement cascade that produces large pores in cells, leading to their death; consists of complement components C5b-polyC9

Membrane protein (M protein) major structural protein in the envelope of coronaviruses; one of the major targets of the immune response to coronaviruses

Meningeal congestion excessive accumulation of body fluid in the covering of the brain

Meninges three-layered covering of the brain and spinal cords

Meningitis potentially fatal inflammation of the meninges

Meningoencephalitis severe central nervous system disease in which the brain and its coverings (meninges) are inflamed

Meningoencephalomyelitis severe central nervous system disease in which the brain and spinal and their coverings are inflamed

Mesenteric adipose tissue fatty material in the connective tissue of the abdomen

Mesenteric lymph nodes concentration of lymph nodes in the mesentery, a contiguous layer of connective tissue that holds the organs in place; contain large numbers of lymphocytes that protect against infection via the digestive system

Metabolic acidosis excessive acid levels in the body fluids

Metabolic syndrome metabolic disorder that is characterized by at least 3 of the following: obesity, diabetes, hypertension, and low levels of high-density lipoprotein cholesterol

Metalloenzyme enzyme that contains a metal; examples include iron in heme proteins and copper in the antioxidant enzyme superoxide dismutase

- Methyltransferases** enzymes that add methyl groups to other molecules
- Microbiome** assortment of all microbes that normally live in or on an individual's body, including bacteria, viruses, protists, and fungi
- Microglia** brain macrophages; part of the innate immune system
- Micronutrients** chemicals that are required in small amounts for normal growth and development; include trace elements and vitamins
- Microthrombosis (microthrombi)** microscopic clumps of fibrin, platelets, and red blood cells
- Mitochondrial antiviral signaling (MAVS) protein** protein involved in triggering interferon- β signaling pathways by viruses
- Mitogen-activated protein kinase p38 (p38)** mitogen-activated protein kinase that reacts to stressors, including UV irradiation and heat and osmotic shock; p38 is involved in cellular differentiation, apoptosis, and autophagy
- Mitogen-activated protein kinases (MAP kinases)** group of three enzymes involved in intracellular signaling by adding phosphate groups onto other proteins
- Molecular mimicry** similarity between the structure of the microbe and host components leads to immunological cross-reactivity; immune responses to the microbe also attack the host
- Monoclonal antibodies (mAbs)** set of identical antibodies that are the progeny of a single B cell
- Monocytes** immature, blood forms of tissue macrophages
- Monocyte chemoattractant protein 1 (MCP1)** chemokines that recruit monocytes, memory T cells, and dendritic cells
- Monocytic myeloid-derived suppressor cells** monocytic T cell suppressor cells whose numbers increase during inflammation
- Monokine induced by IFN- γ (MIG)** see "CXCL9"
- Mononuclear leukocytes** white blood cells whose nucleus contains a single lobe; typically monocytes/macrophages, lymphocytes, and natural killer cells
- Morbidity** extent of suffering from a disease
- Morphogenesis** changes in a structure's shape
- Mortality rate** rate of death in a population
- Mucocutaneous dyspigmentation** gray-blue area of the skin or mucus membranes
- Mucocutaneous lesions** lesions of the epithelium of the skin and other mucosal sites
- Mucositis** inflammation of mucus membranes
- Mucosal regions** areas of the body not covered by skin, including the lining of the digestive, respiratory, and urogenital systems; produce mucus that traps and helps remove unwanted material from the body
- Mucosal tissue** composed of mucus-producing cells in mucus membranes of the respiratory, digestive, and urogenital systems
- Mucous membrane** epithelial tissue whose goblet cells secrete mucus; lines many body cavities and tubular organs, including the digestive, respiratory, and urogenital passages
- Müller cells** type of neuroglial cell that supports local cells in the retina of the eye
- Multinucleated giant cells** group of fused, dying cells produced during some viral infections
- Multiple sclerosis** autoimmune, demyelinating disease of the nervous system; symptoms include double-vision or blindness, tingling or weakness, paralysis, dizziness, muscle spasms, tremors, slurred speech
- Multiplexed grafting-coupled fluorescent plasmonics** diagnostic test that detects antibodies to specific proteins, including microbial components, in dried blood and serum
- Multisystem inflammatory syndrome in children (MIS-C)** see "Kawasaki disease-like in children"
- MxA (myxovirus resistance protein 1)** interferon-stimulated gene that triggers a rapid inflammatory response in infected epithelial cells of the respiratory tract during infections
- Myalgia** muscle pain
- Myasthenia gravis** life-threatening autoimmune disorder in which antibodies block the binding of the neurotransmitter acetylcholine to its receptor on muscles; characterized by progressive loss of voluntary muscle contractions, including those of the diaphragm
- Myelin** fatty material that surrounds the axons of some nerves; protects axons and aids in their repair as well as increases the speed of nervous system electrical impulses
- Myeloid dendritic cells (mDCs)** antigen-presenting cells that capture antigens in peripheral body regions, then migrate to lymphoid organs to initiate T helper cell responses

- Myeloid-derived suppressor cells (MDSCs)** heterogeneous group of myeloid lineage immune cells (primarily macrophages and neutrophils) that suppress T cell activity
- Myeloid differentiation response 88 (MYD88)** external adapter protein that communicates signals from the outside of cells to intracellular signaling pathways
- Myeloperoxidase** enzyme in neutrophil granules; produces hypochlorous acid to kill microbes
- Myocardial** pertaining to the heart muscle
- Myocardial fibrosis** excess deposition of fibrous material in the heart muscle
- Myocardial infarction** death of heart cells
- Myocarditis** inflammation of the heart muscle
- Myocytes** heart muscle cells
- Myocyte hypertrophy** thickening of heart's ventricle walls; reduces the ability to pump blood
- Myofibroblasts** specialized form of fibroblasts in the lungs that interacts with the extracellular matrix and helps to regulate its organization and contracture during wound healing
- Myoglobin** oxygen-bearing molecules in muscles similar to hemoglobin in the blood
- Myxovirus resistance protein 1 (Mx1)** antiviral protein that protects against many RNA viruses; produced in response to type I interferon signals
- N-acetyl-9-O-acetylneuraminic acid** receptor for bovine coronavirus
- NADPH oxidase (NOX2)** membrane-bound enzyme that transfers electrons to O₂, producing superoxide or hydrogen peroxide using NADPH as the electron donor; kills microbes within phagocytic cells
- Naïve B cells** B lymphocytes prior to exposure to their antigen
- Naïve T cells** T lymphocytes prior to exposure to their antigen
- Nanoparticle-based lateral-flow assay** paper strip-based, on-site diagnostic test for material, including viral proteins, that is inexpensive, rapid, and easy to use without special equipment, training, or personnel
- Nasal turbinates** small protrusions in the nasal cavity that causes recirculation of inhaled air, detaining it briefly in the cavity, allowing time for the air to warm slightly
- "Natural immunity"** strong, specific immune state resulting from infection with a microbe
- Natural killer cells (NK cells)** immune system cells that are 1 of the 2 best defenses against viruses; form pores in infected cells or induces apoptosis
- Necrosis** form of premature cell death resulting from disease, injury, or loss of blood supply; cells rupture and release toxic compounds onto surrounding cells
- Necrotic death** see "Necrosis"
- Necrotizing lobar pneumonia** massive necrosis of the tissue from one or more lobes of the lungs
- Negative-sense genomic RNA** single-stranded RNA that is complementary to mRNA (does not code for protein); used as the genetic material of some viruses
- Neonatal** pertaining to newborn children or animals
- Nephron** functional unit of the kidneys; consists of glomerulus, proximal and distal convoluted tubules, and the loop of Henle
- Neural cell adhesion molecule (NCAM; CD56)** glycoprotein expressed on the surface of neurons, neuroglia, skeletal muscle, natural killer cells, and activated CD8⁺ T killer cells; involved in cell-to-cell adhesion, learning, and memory
- Neural synapses** very small gap between the axon of one neuron and the dendrite of the next; neurotransmitters cross this gap to allow signal transduction between neurons
- Neuroglial cells (glia)** nonneuronal cells of the nervous system; form blood:brain barrier, remove debris in the central nervous system, control contents of cerebrospinal fluid, speed nerve transmission, repair nerves, communicate via gliotransmitters
- Neuromyelitis optica** autoimmune diseases of the central nervous system, particularly the optic nerve and spinal cord; symptoms include blindness, limb paralysis, loss of sensation, and spasms
- Neuropeptides** short chains of amino acids that regulate long-lasting synaptic transmission; may serve as neurotransmitters
- Neuropilin-1** plasma membrane coreceptor for SARS-CoV-2; involved in entry into target cells
- Neurotropic** attracted to the nervous system
- Neutropenia** low levels of blood neutrophils

- Neutrophil elastase** highly destructive enzyme released by neutrophils during inflammation; degrades microbes and may damage host tissue, causing chronic lung disease
- Neutrophil extracellular traps (NETs)** networks of extracellular fibers containing DNA and histones plus the neutrophil-derived enzymes myeloperoxidase and elastase; entrap and kill extracellular microbes, but may cause autoimmune and inflammatory disorders
- Neutrophilia** elevated blood neutrophil levels
- Neutrophils** leukocytes that ingest and destroy foreign material and release toxic compounds that damage neighboring cells and extracellular microbes
- Neutralizing antibodies** antibodies that physically block viruses from binding to their receptors and entering cells
- NF- κ B (nuclear factor kappa-light-chain-enhancer of activated B cells)** protein complex that regulates transcription of DNA, cytokine production, and cell survival; respond to threatening stimuli, including infection, stress, cytokines, free radicals, and UV light
- Nicotine** highly addictive stimulant at low doses, but blocks activities of autonomic nerves and skeletal muscle cells at higher levels; improves short and long-term memory formation; binds nicotinic acetylcholine receptors in the brain
- Nicotinic acetylcholine receptor (nAChR) $\alpha 7$** one of the receptors for acetylcholine that has very high calcium permeability; a component of the cholinergic antiinflammatory pathway involved in cognition and memory formation
- Nitric oxide** reactive nitrogen molecule that may be pathogenic if it kills or mutates normal, healthy cells, but is beneficial when it kills infected or tumor cells; small amounts dilate blood vessels and increase blood flow to various body regions and lower blood pressure
- NKG2A receptor** most important inhibitor of natural killer cell activation
- NKG2D** cell membrane molecule that activates natural killer cells
- NKT cells** rare type of T cells that bear both T cell and natural killer cell surface molecules; rapidly release large amounts of cytokines
- NLRP3 inflammasome** innate immune system structure that activates caspase-1 during apoptosis and releases pro-inflammatory interleukin-1 β /interleukin-8 in reaction to infection or cell damage
- N-methyl-D-aspartate (NMDA) receptor** receptor for the major excitatory neurotransmitter glutamate; binding increases levels of free intracellular calcium, leading to neuron death if in excess
- NMDA-receptor encephalitis** autoimmune condition of the central nervous system in which antibodies cause brain inflammation; resulting in psychotic thoughts and hallucinations
- Nodular dermatitis** coin-shaped spots on the skin
- Nonclassical (CD14^{neg}CD16⁺) monocytes** antiinflammatory monocytes that maintain vascular homeostasis; recognize and eliminate microbes
- Nonsegmented** in viruses, possessing only one DNA or RNA strand
- Nonstructural proteins (nsp's)** up to 16 coronavirus proteins that are not part of the viral structure; functions include unwinding and replication of viral RNA, cleaving viral polyproteins, and inhibiting the immune system, particularly interferons
- Norepinephrine** "fight-or-flight" stimulatory hormone produced by the adrenal medulla; similar to epinephrine but released at low levels continually
- Nosocomial infection** infection associated with health care systems
- N-terminal binding domain (NBD)** amino terminus of the N protein that binds to viral RNA in the nucleocapsid
- Nuclear factor kappa-light-chain-enhancer of activated B cells** see "NF- κ B"
- Nucleocapsid protein (N) protein** coronavirus structural protein that encases viral RNA
- Nucleosides** uracil, cytosine, guanosine, and adenosine; compose RNA
- Nucleotides** thymine, cytosine, guanine, and adenine; compose DNA
- Nystagmus** rapid, involuntary eye movement
- Obligate intracellular parasite** organism that must live at least part of its life within cells
- Occludin** component of tight junctions between cells
- Ocular** pertaining to the eye
- Olfaction** detection of smell
- Olfactory bulb** enlarged beginning of the olfactory nerve

- Olfactory nerve** 1st cranial nerve; transports smell-associated signals between the brain and the nasal region; found in close proximity to the brain
- Oligoadenylylate synthetase/RNase L pathway** part of an antiviral innate immune response in which 2'-5'-oligoadenylylate synthetase enzymes detect viral double-stranded RNA in the cell's cytosol and activate ribonuclease L to cleave the viral RNA
- Oligodendrocytes** glial cells of the central nervous system that produce the myelin sheath which covers and protects some nerves
- Oligomeric** polymer consisting of several repeating units
- Oocyte** immature egg cell found within an ovarian follicle
- Open reading frames (ORFs)** portion of an RNA molecule that contains no stop codons
- Opisthotonos** backward arching of the head, neck, and spine
- Optic chiasm** X-shaped area where some of the fibers of the optic nerves from the right and left sides of the eyes cross to the opposite side
- Optic nerve** cranial nerve #2; transmits visual signals from optic receptors in the retina to the cerebrum of the brain
- Optic neuritis** inflammation of the optic nerve; may cause temporary vision loss
- Orchitis** inflammation and damage to the testicles; may lead to their loss, resulting in infertility
- Ornithology** study of birds
- Orthologs** closely-related forms of molecules found in a different host; may or may not have the same functions
- Osteoclasts** bone macrophages that tear down bone and release calcium into the blood to maintain calcium homeostasis
- Osteonecrotic death** necrotic death of bone cells due to reduced blood flow to the region
- Outbred** opposite of inbred; members of a species that are not genetically identical
- Ovarian follicle** aggregates of spherical cells containing an immature egg; produces estrogen and progesterone to aid in egg maturation prior to release during a menstrual cycle
- Ovarian stroma** ovary tissue; consists of connective tissue containing blood vessels, spindle-shaped ovarian cells, and multiple follicles containing immature eggs
- Oxidative burst** rapid release of the reactive oxygen species superoxide and hydrogen peroxide from neutrophils and macrophages to kill internalized microbes
- P lineage SARS-CoV-2** lineage of SARS-CoV-2 that includes Gamma variant
- Palm civet cats** wild felines that transmitted SARS-CoV to humans
- Palmitoylation** attaching fatty acids to proteins
- Palpitations** rapid, strong, or irregular heartbeat
- pANCA (perinuclear antineutrophil cytoplasmic antibodies)** antibodies that target the material around the nucleus of neutrophils
- Pancoronavirus drug** drug that is active against many or all coronaviruses
- Pancreas** digestive and endocrine system organ that releases digestive enzymes into the small intestine and produces and releases hormones involved in blood sugar homeostasis
- Pancreatic polypeptide** hormone produced in the pancreatic islets of Langerhans; increases insulin sensitivity in the liver and decreases the production of glucose, stimulating the secretion of hydrochloric acid and pepsin by the stomach
- Pandemic** very widespread infectious disease epidemic with a high morbidity or mortality rate
- Panencephalitis** encephalitis that affects gray and white matter simultaneously
- Pangolins** spiny anteaters; proposed amplifying host for SARS-CoV-2
- Pantropic** able to live within multiple tissue types
- Papain-like protease (PL^{Pro})** one of the proteases that cleave coronavirus polyproteins; a domain of nsp3
- Papulosquamous lesions** skin lesions that appear as red, raised, flaky or scaly bumps
- Parafollicular cells of the thyroid** cells that secrete calcitonin, a hormone that decreases blood calcium levels
- Paraphyletic** species belonging to a group that contains some, but not all, descendants of a common ancestor
- Parathyroid gland** endocrine organ that is composed of four areas that are posterior to the thyroid gland; releases parathyroid hormone that stimulates bone degradation to release calcium into the blood and maintain calcium homeostasis
- Parenchyma** tissue proper of an organ; does not include other tissue types of the area, such as vascular and connective tissue

- Parenchymal consolidation** build-up of fluid in the tissue proper; may refer to lung tissue
- Parturition** birthing process
- Passive immunization** provides temporary immunity against a specific microbe by the transfer of immune components, such as serum containing antibodies, from a person who has recovered from an infection into a recipient to prevent or decrease disease severity; the recipient is not protected from reinfection by the same microbe
- Pathogen pattern recognition receptors** immune cell receptors that recognize molecular components unique to a particular type of microbes, such as double-stranded RNA or the lack of terminal sialic acids on glycoproteins
- Patient zero** first patient in a chain of human-to-human transmission
- Pattern-recognition receptor** see "Pathogen pattern recognition receptors"
- Peptide** short strand of amino acids
- Peptide YY** hormone produced by intestinal L cells; reduces appetite, promotes satiety
- Perforin** molecule released by CD8⁺ T killer cells and natural killer cells; forms large pores in cells, leading to a great influx of water into the cell and cell rupture
- Pericardial effusion** excessive fluid in the sac-like structure around the heart
- Pericarditis** life-threatening inflammation of the connective tissue around the heart
- Peridomestic** abiding near residences
- Periphelebitis** inflammation of the outer coat of a vein or of tissues surrounding the vein
- Peritoneal** pertaining to the abdominal cavity
- Peritoneal cavity** regions of the abdominal cavity not occupied by organs
- Peritoneal macrophages** macrophages residing in the abdominal cavity
- Peritonitis** inflammation of the membranous wall surrounding the peritoneal cavity
- Perivascular cuffing** accumulation of leukocytes in a dense mass around a blood vessel
- Perivascular granulomatous inflammation** inflammatory reaction around blood vessels with small, tight, ball-like structures bordered by lymphocytes
- Perivascular inflammatory cell infiltrate** influx of leukocytes into the area around blood vessels
- Pernio-like lesions** redness and swelling of the toes and fingers; may involve type I interferon
- Peroxisome** organelle that regulates levels of hydrogen peroxide and metabolism of fatty compounds; also produces type III IFN (IFN- λ)
- Peroxynitrite** reactive oxygen species formed by the interaction of hydrogen peroxide and nitrite
- Personal protective equipment (PPE)** materials used to protect the wearer against infection; include gowns, masks, gloves, and face shields
- Peyer's patches** clusters of lymphoid tissue under the lining of the small intestine; help to protect the digestive tract from infection
- Phagocyte** cell type that engulfs and destroys large material, including microbes and red blood cells, in the blood or tissues
- Phagocytic** having the ability to engulf and destroy large material in blood or tissues
- Pharynx** throat cavity
- Phlebitis** inflammation of the walls of a vein
- Phosphatase** enzyme that removes phosphate ions from molecules, including proteins and lipids
- Phosphodiesterase** group of enzymes that break phosphodiester bonds typically in cyclical nucleosides, such as cyclic guanosine monophosphate (cGMP)
- Phosphorylation** addition of a phosphate ion to a molecule
- Phylogenetic analysis** in-depth study of mechanisms of species evolution by studying genetic changes
- Phylogeneticists** those who engage in phylogenetic analysis
- PI3K/Akt** component of an intracellular signaling pathway that regulates the cell cycle and proliferation
- Picornaviruses** small, positive-sense, single-stranded RNA viruses; including poliovirus, rhinoviruses, and hepatitis A virus
- Pigmented casts** microscopic clusters of metabolic breakdown products; may contain bilirubin during liver disease, hemoglobin during anemia, myoglobin during rhabdomyolysis
- Pituitary gland** endocrine organ that secretes multiple hormones that regulate the release of hormones from other endocrine organs
- PKR-like ER kinase (PERK)** rough endoplasmic reticulum-associated stress sensor protein that is induced by type I IFN; decreases translation by phosphorylating eukaryotic initiation factor 2 α (eIF2 α)

Plasma fluid portion of nonclotted blood

Plasma cells B lymphocytes that are actively secreting antibodies

Plasma membrane phospholipid bilayer studded with glycoproteins; surrounds cells of all organisms, except viruses, which are not cellular

Plasmacytoid dendritic cells rare type of dendritic cell that produces very large amounts of IFN

Plasmid small, nongenomic, circular DNA that enters and exits bacteria; replicates independently of bacterial chromosomal DNA replication

Plasmin enzyme that destroys blood clots by degrading fibrin

Plasminogen precursor to plasmin

Plasminogen activator inhibitor-1 (PAI-1) serine protease that blocks the activity of tissue plasminogen activator and urokinase, thus inhibiting fibrinolysis and preserving blood clots

Platelet-derived growth factor growth factor released when platelets rupture; aids in wound repair

Platelets formed elements in the blood that, upon rupture, release blood-clotting factors and growth factors

Pleural effusion excessive amounts of fluid in the pleural cavity surrounding the lungs

Pleuritis inflammation of the pleural cavity surrounding the lungs

Pneumocytes see "Alveolar pneumocytes"

Pneumoenteric virus virus that grows in the respiratory tract and intestines

Pneumonitis inflammation of the lungs

Podocytes epithelial cells of the renal glomerulus which have footlike radiating processes

Point mutations alterations of a single nucleotide or nucleoside

Point-of-care performed on-site rather than at a special outside laboratory

Polycystic ovary syndrome multiple cysts grow on ovary surfaces; results in irregular and heavy menstrual periods, excess body and facial hair, difficulty becoming pregnant

Polymerase chain reaction (PCR) technique that rapidly produces large quantities of a given DNA segment using multiple rounds of separating and annealing two strands of DNA

Polymorphic having multiple structural forms

Polymorphonuclear myeloid-derived suppressor cells neutrophilic cells that act as T lymphocyte suppressors

Polyphenols group of plant-based compounds that are composed of multiple phenol units; many of these are antioxidants

Polyprotein initial form of protein produced by some viruses; consists of many, joined proteins that must be enzymatically cleaved into their individual proteins before they become active

Porcine pertaining to pigs

Porcine epidemic diarrhea virus coronavirus of swine that causes profuse diarrhea, vomiting, and dehydration; responsible for weight loss in adult pigs and is rapidly fatal in piglets

Positive-sense in genomic viral RNA, this strand codes for a protein

Postpartum time following childbirth

Posttranscriptional alterations of RNA made after transcription; includes the addition of a 5' cap and a 3' polyadenosine tail and the removal of introns (areas that do not code for protein)

Post-traumatic distress syndrome (PTSD) mental condition characterized by intrusive thoughts about the incident, recurrent distress, anxiety, flashbacks, and avoidance of similar situations; occurs following very shocking or difficult experiences

Postural tachycardia syndrome (POTS) condition in which heart rate increases upon changing posture (lying to sitting up or standing)

Poult young domestic fowl, especially turkeys, but also chickens, pheasants, and other fowl

Pressure gradient difference in pressure between two compartments; BioSafety 2–4 laboratories have a negative pressure gradient so that air flows into, rather than out of, the area

Primary cell cultures ex vivo cultures of primary cells

Primary cells cells taken directly from a person without growth for long time periods in the laboratory; only undergo a limited number of reproductions before senescence or death

Primary infection in the case of infectious disease, the first person to become infected

Procoagulant activity (PCA) processes that thicken the blood; may lead to clot formation

Prodrug drug precursor that is acted upon by an enzyme to produce the active form of the drug

- Progesterone** steroid hormone produced by the corpus luteum; following ovulation, it thickens the endometrium to accept a fertilized egg during pregnancy
- Programmed cell death 1 (PD1)** molecule expressed by activated T cells that inhibits their proliferation, differentiation, cytokine secretion, and cytolytic function
- Programmed death-ligand (PD-L1)** molecule that binds to programmed cell death 1; plays a major role in suppressing adaptive immunity
- Prokaryotes** major class of living organisms that lack membrane-bound intracellular organelles, including the nucleus; class includes bacteria
- Prolactin** hormone that stimulates mammary gland development and milk secretion after birth
- Proliferation** growth in number
- Proline** amino acid that disrupts helices; partially responsible for the protein's folding pattern
- Prolonged prothrombin time** time that it takes blood to clot; increases during severe acute respiratory distress syndrome in COVID-19 patients with pneumonia
- Proofreading** correcting mistakes made during the replication of DNA or RNA
- Prophylactical** given prior to infection to protect at-risk populations
- Proprioception** awareness of the relative positions of body parts
- Prostaglandin D synthase** enzyme that produces prostaglandin D in mast cells; recruits Th2 cells, eosinophils, and basophils and aids in the development of type I allergic reactions
- Prostaglandin E₂ (PGE₂)** inflammatory lipid produced from arachidonic acid; contributes to pain awareness
- Prostaglandins** group of lipid hormones derived from arachidonic acid; increase pain perception and serve as pro-inflammatory mediators
- Proteases** enzymes that cleave specific proteins
- Protein activator of protein kinase R (PACT)** interferon-stimulated, double-stranded RNA-binding protein involved in microRNA biogenesis
- Protein kinase R (PKR)** enzyme that is activated by double-stranded RNA produced during viral replication; blocks protein synthesis by phosphorylating the α subunit of the translation initiation factor eIF2
- Protein kinases** enzymes that add phosphate ions onto proteins; often result in their activation
- Protein phosphatase 1 (PP1)** enzyme that removes phosphate ions from proteins; inhibits TNF- α signaling
- Proteinuria** presence of proteins in the urine; is typically indicative of kidney damage
- Proteolytic storm** release of high levels of serine protease activators due to degradation of neutrophils; results from an imbalance of the coagulation, complement, fibrinolytic, and kallikrein pathways
- Proteosomes** cellular organelles that degrade proteins tagged with ubiquitin
- Prothrombin** inactive precursor of thrombin
- Proximal renal (convoluted) tubules** 1st part of the renal tubular system used in urine formation
- Pruritus** severe itchy skin
- Pseudomembrane** membrane-like layer of cells and fluid that leaks out of blood vessels or an organ on the surface of the skin or mucous membrane
- Pseudoviruses** recombinant viral particles containing a portion of the envelope protein of another virus
- Psoriasis** chronic skin condition characterized by scaly, often itchy patches
- Psychosis** severe mental disorder in which thoughts and emotions are impaired to the extent that contact with external reality is lost; may involve hallucinations or delusions
- Pulmonary** pertaining to the lungs
- Pulmonary edema** fluid buildup in the lungs
- Pulmonary embolisms** clots in the lungs
- Pulmonary fibrosis** scarring of the lungs that may impair breathing
- Pulse oximetry** test that measures levels of blood oxygen; levels should be greater than 92%
- Purpuric lesions** reddish-brown skin lesions caused by leaky capillaries
- Putamen** part of the basal ganglia; regulates movement and controls various types of learning
- Pyogranulomatous inflammation** infiltration of neutrophils into a chronically inflamed area containing large numbers of macrophages and lymphocytes
- Pyogranulomatous leptomeningitis** neutrophil-associated inflammation of the pia and arachnoid mater surrounding the brain

- Pyogranulomatous masses** neutrophils invade masses consisting of mononuclear cells, especially macrophages
- Pyogranulomatous meningitis** masses of macrophages and neutrophils permeate meninges and central nervous system tissue
- Pyramidal neurons** neurons that play a role in vision-guided motor function and cognition; located in the cerebral cortex, hippocampus, and amygdala
- QT interval** time interval during which the ventricles contract and then relax; detected by an electrocardiogram
- Qualitative test** test that determines the presence of a material
- Quantitative test** test that determines the amount of a material
- Quasispecies** population consists of extremely large numbers of variants of a species, usually due to a very high mutation rate
- Raccoon dogs** member of the Canidae family that resembles a raccoon but is genetically related to domestic dogs, foxes, and wolves
- Radiculopathy** injury to a nerve root, associated with pain, numbness, or weakness in the region supplied by that nerve
- RANTES (regulated on activation, normal T cell expressed and secreted)** see “CCL5”
- Rapid antigen diagnostic test** antibody-based test that detects material, such as microbial proteins, within minutes; may be performed at home with no specialized training, equipment, or personnel
- Ras-ERK-AP-1 signaling pathway** signaling cascade that transfers a message from a cell surface to the nucleus to increase the transcription of genes that aid in cell division, differentiation into more specialized cells, and survival
- Ras/Mitogen-activated protein kinase (Ras/MAPK)** Ras, a small GTPase, activates MAPK signaling pathways
- Reactive oxygen species (ROS)** highly reactive toxic by-products of oxygen formed during cellular respiration that chemically modify DNA, RNA, proteins, and lipids; including superoxide, hydrogen peroxide, hydroxyl radicals, and singlet oxygen
- Reactive thrombosis** compensatory increased platelet production following thrombocytopenia
- Real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR)** form of PCR that identifies the amount of a specific RNA in a sample at that time
- Receptor** membrane molecule that binds to a specific ligand to initiate chemical signals
- Receptor activator of nuclear factor kappa- β ligand (RANKL)** member of the tumor necrosis factor cytokine family expressed by CD4⁺ T helper cells; functions include dendritic cell maturation and survival and down-regulates apoptosis
- Receptor-binding domain (RBD)** in coronaviruses, the area of the S1 domain of the spike protein that binds to its cellular receptor
- Recombination in coronaviruses** sections of RNA from one coronavirus species or strain that are exchanged with that section of RNA from a different coronavirus species or strain
- Recumbency** leaning or reclining
- Red pulp** portion of the spleen that filters and stores red blood cells and macrophages
- Reduction** reaction in which hydrogen are added onto a molecule
- Remyelination** adds the fatty material myelin back onto demyelinated neuronal axons
- Renal** pertaining to the kidneys
- Renal cortical infarct** region of dead cells within the renal cortex (outer region of the kidneys)
- Renal hypoperfusion** less blood reaches the kidneys
- Renal replacement therapy** use of kidney dialysis or kidney transplantation
- Renal tubular cells** cells located in the tubular system of the kidney’s nephrons; responsible for reabsorbing appropriate materials into the circulatory system from the glomerular filtrate
- Renin-angiotensin-aldosterone system** pathway that increases the production of aldosterone, a hormone that raises blood pressure; ACE and ACE2 have opposing roles in activating or blocking the pathway, help to maintain homeostatic blood pressure levels
- Replicase gene complex** complex of viral and cellular proteins that direct production of genomic and subgenomic mRNAs
- Replicate** process of producing genomic DNA or RNA identical to the original copy
- Replication-transcription complexes** in coronaviruses, a cluster of primarily nonstructural proteins responsible for viral replication and transcription

- Repolarization** electrical changes in cardiac and skeletal muscles that proceed their relaxation phases
- Repurposed drug** drug produced for one purpose and used for another; an example is the use of the antimalarial drug chloroquine to treat SARS-CoV and SARS-CoV-2
- Renal tubular cells** cells that compose the tubules of the kidneys; return the proper amounts of materials from the glomerular filtrate to renal capillaries during the formation of urine
- Renin-angiotensin-aldosterone response** homeostatic pathway that increases blood pressure
- Reservoir host** species infected by a microbe that serves as a source of infection for other organisms; typically is not seriously damaged by the microbe
- Residual volume** amount of air remaining in the lungs after a forceful exhalation
- Retiform purpura** angulated or branched hemorrhagic skin lesions caused by leakage of red blood cells into the skin
- Retinoic acid-inducible gene 1 (RIG-I)** intracellular molecule that recognizes viral nucleic acids and activates a signaling pathway that leads to the production of type I interferon
- Retinoic acid-inducible gene-like receptors (RLRs)** cytoplasmic viral sensor that recognizes viral RNA and initiates a signaling pathway that induces expression of type I interferon
- Reverse transcription-polymerase chain reaction** technique that rapidly produces large quantities of a given DNA segment from RNA
- Rhombencephalitis** inflammation of the brainstem
- Rhabdomyolysis** destruction of striated muscle cells that releases myoglobin, a protein involved in oxygen storage and release; excessive levels of myoglobin may damage the kidneys
- Rhinorrhea** runny nose
- Ribonuclease L (RNase L)** interferon-induced ribonuclease that destroys all cellular RNA, whether of cellular or viral origin
- Ribonucleic acid (RNA)** strand composed of nucleosides uracil, cytidine, guanosine, and adenosine; the template for protein production and genetic information for RNA viruses
- Ribose** sugar component of ribonucleic acid (RNA)
- Ribosomes** organelles that serve as the site of protein production
- RIG-1** cellular enzyme that recognizes viral double-stranded RNA, triggering its destruction
- RIG-I-like receptor** receptor that binds to RIG-1
- Rigors** cyclic feelings of extreme cold followed by extreme heat with copious sweating
- Ring vaccination** vaccination of all susceptible individuals in an area around an infectious disease to prevent its widespread dissemination
- RNA cap** modified guanosine nucleoside attached to the 5' end of mRNA via a 5' to 5' triphosphate linkage; contains methylation at the 7 positions of the RNA
- RNA-dependent RNA-polymerase (RdRp)** enzyme that produces new genetic RNA using RNA as a template
- Rough endoplasmic reticulum** large, complex, double-membraned organelle that is coated with ribosomes; receives and folds proteins produced by the ribosomes
- Sarcoidosis** potentially life-threatening autoimmune condition in which inflammatory cells cause clumps in the lungs, skin, or lymph nodes
- SARS-associated coronavirus (SARS-CoV)** Coronaviridae family member that causes SARS
- Schizophrenia** mental illness whose symptoms include delusion, hallucinations, disordered thought, and behavior; lack of emotional expression; agitation; phobias, lack of pleasure or interest in activities
- Sclera** whites of the eyes; the outer layer of the eye
- Scleroderma** hardened area of the skin
- Sebaceous glands** "oil glands" of the skin
- Second-generation** group of patients infected by person-to-person transmission of an infectious disease agent
- Secondary follicles** circular lymphocyte-containing regions in lymphoid tissues in which lymphocytes proliferate in response to infection; the center portion contains large numbers of activated B lymphocytes that produce microbe-specific antibodies
- Secondary infection** infection of an infected person with a different microbial species; for example, people infected by influenza virus may develop a secondary bacterial lung infection; infection with some microbial types lowers immunity to other microbes
- Secondary transmission** person-to-person transmission of an infectious disease

- Secretory vesicle** type of vesicle produced by the Golgi apparatus; delivers proteins to the plasma membrane for secretion from the cell
- Selective packaging** preferential incorporation of genomic RNA into nascent virions, while other types of cellular or viral RNA are excluded
- Selenium** antioxidative trace element essential for immune system functions; enzyme cofactor
- Selenocysteine** amino acid cysteine in which selenium replaces sulfur
- Selenoenzymes** enzymes that require selenium to function
- Selenoprotein P (SELENOP)** transporter of serum selenium; extracellular antioxidant
- Seminiferous tubules** region of the testes in which sperm are produced and mature
- Sensitivity** ability of a diagnostic test to correctly identify patients who have a disease from those who do not; low sensitivity produces many false-negative results
- Septicemia** presence of toxins in the blood
- Serial interval** time between successive cases of an infectious disease
- Serine proteases** enzymes containing the amino acid serine at their active sites
- Seronegative** lacking detectable levels of antibodies against a specific microbe
- Seropositive** presence of detectable levels of antibodies against a specific microbe
- Serosal inflammation** inflammation due to large amounts of clear serous fluid in the absence of white blood cells
- Serosal surfaces** outermost coat of an organ in the abdominal or thoracic body cavities
- Serotonin (5-HT)** mood-regulating neurotransmitter and immune system pro-inflammatory molecule; activates T cells that orchestrate adaptive immune system responses
- Serous membranes** coverings of organs and walls of body cavities that secrete a lubricating fluid to reduce friction and allows materials within the cavities to slide past each other
- Sertoli cells** “nurse” cells of the seminiferous tubule; aid in sperm production
- Serum** fluid from clotted blood
- Serum amyloid A** acute-phase protein that triggers leukocyte recruitment to inflammatory sites
- Serum sickness** allergic reaction due to repeated exposure to serum from an animal, usually a horse
- Severe acute respiratory syndrome (SARS)** severe lower respiratory tract infection that often caused an unusually severe type of viral pneumonia; emerged in humans in China in 2002
- Shipping fever (bovine respiratory disease)** respiratory system of disease of calves often due to infection by bovine respiratory coronavirus; symptoms include diarrhea, rapid or labored breathing, coughing, staggering, increased secretion of nasal discharge, sudden death
- Sialic acid** sugar moiety present on the terminus of human, but not microbial, glycoproteins
- Signal transducer and activator of transcription 2 (STAT2)** member of cellular signaling pathways; upon activation by type I interferons and phosphorylation, it translocates to the nucleus and serves as a transcription activator
- Simple cuboidal epithelium** single layer of cube-shaped cells lining or covering body regions
- Simple squamous epithelium** single layer of flattened cells lining or covering body regions; their flattened surface permits materials, such as nutrients, oxygen, and carbon dioxide, to pass through this layer in the digestive tract or lung capillaries
- S-INDEL** another name for members of the G1b strains of porcine epidemic diarrhea virus
- Skeletal muscles** voluntary muscles involved in moving body parts
- Skin fragility syndrome (plakophilin 1 deficiency)** decreased expression of plakophilin 1, a protein involved in desmosome formation; desmosomes are a type of junction that anchors cytoskeleton components of adjacent cells to each other; defective desmosomes cause trauma-induced skin blistering and defective hair follicles
- Smad3 and Smad4** compounds that form a pro-apoptotic complex
- Smad7** downstream mediator of transforming growth factor- β cytokine; its activity is vital to the development and function of the heart and the protection of the kidneys
- Small noncoding RNAs** viral RNAs that do not produce proteins but regulate the production of proteins from mRNA
- Small viral RNAs (svRNAs)** small RNAs that do not code for protein production; during SARS, some svRNAs decrease lung pathology and increase levels of proinflammatory cytokines and chemokines
- Smooth muscles** involuntary muscles whose activity moves materials through passageways

- Social distance (social distancing)** maintaining distance between people; during the COVID-19 pandemic, the standard difference is 6 feet
- Species restriction** microbe that enters and reproduces in only certain specific host species
- Specificity** ability of a diagnostic test to correctly identify people with a disease from those who do not have it; low specificity yields many false-positive results
- Spermatids** cells resulting from the second division of meiosis; differentiate into spermatozoa
- Spermatogonia** immature sperm cell
- Spike protein** coronavirus structural protein that binds to its cellular receptor during viral entry and fusion with its target cells
- Spillover** see “Zoonotic transmission”
- Splenomegaly** grossly enlarged spleen due to extensive production of B lymphocytes during microbial infection
- Spongiosis** edema between the keratinocytes of the epidermis of the skin
- Sputum** combination of saliva and mucus that is coughed up during infection or other disease condition
- Squamous epithelial cells** flattened cells that cover or line organs or tracts
- Squamous metaplasia** conversion of a mature tissue type to another; for example, the bronchiolar epithelium is replaced by stratified squamous epithelium
- Src intracellular signaling pathway** Src is an intracellular tyrosine kinase that, together with cell surface growth factor receptors, stimulates cell proliferation
- STAT1 (signal transducer and transcription activator)** transcription factor activated by type I interferons binding their receptors; STAT1 phosphorylation induces the production of interferon-stimulated genes whose protein products produce an antiviral state
- Steatosis** hardening of a body component, including arteries
- Stimulator of interferon genes (STING)** part of a pathway that controls innate signaling triggered by microbes with DNA genomes; when chronically active, STING is partially responsible for some inflammatory diseases
- Stratified squamous epithelium** more than 1 layer of flattened cells lining or covering a body component, such as the epidermis of the skin
- Subacute thyroiditis** form of thyroiditis that may cause either hyper- or hypothyroidism; often results from viral infection
- Subcutaneous** region immediately beneath the skin’s dermis; contains large amounts of fat cells
- Subgenomic RNA** viral RNA that is translated into 1–3 proteins; encoded by the 5’ open reading frames in mRNA
- Submucosal** middle layer of the basement membrane, located under the mucosal layer; composed of dense irregular connective tissue
- Substantia nigra** region of the midbrain is involved in reward and movement; produces the neurotransmitter dopamine
- Superantigen** compound that produces an extreme, potentially fatal, immune response
- Superoxide** reactive oxygen species molecule produced during aerobic respiration
- Superoxide dismutase** key enzyme in the elimination of superoxide by producing hydrogen peroxide; some forms require copper and zinc for activity
- “Superspreaders”** individuals cause an unusually large number of secondary infections, spreading the disease to large numbers of people
- Suppurative pancreatitis** release of pus-like fluid by an inflamed pancreas
- Surfactant** decreases the surface tension of fluid in the lungs; keeps alveoli from collapsing during exhalation
- Synapse** in the nervous system, a region in which 2 neurons communicate; in the muscular system, motor neuron triggers muscle contraction
- Synaptic plasticity** ability of synapses to strengthen or weaken over time, depending on their amount of activity; performs a major role in learning and memory
- Synaptic transmission** passage of information between neurons or between neurons and muscle cells
- Syncytial cells (syncytium)** multinucleated cells formed by the fusion of nuclear cells with only 1 nucleus
- Syringomyelia** cavities in the uppermost region of the spinal cord that cause muscle wasting in the hands and loss of sensation
- Systemic** presence of an object or microbes throughout most of the body, often transported via the blood or lymphatic systems

- Systemic lupus erythematosus ("lupus")** autoimmune condition characterized by a combination of symptoms, including the presence of anti-DNA or anti-RNA antibodies, a butterfly-like rash on the face, fatigue, hair loss, painful joints
- Systolic** refers to the stage of the cardiac cycle in which the heart is actively pumping blood
- T cell immunoglobulin and mucin domain-containing-3 (Tim-3)** immunoregulatory molecule primarily found on differentiated Th1 cells; inhibits IFN- γ production
- T cell-independent antibody** unlike the typical T cell-stimulated production of antibodies, this form of antibody production does not require T helper cell assistance
- T helper cells** kind of lymphocytes that control the activity of the other immune cells
- T helper 1 cells (Th1)** type of inflammatory CD4⁺ T helper cell; primarily antiviral and opposes T helper 2 cell activity
- T helper 2 cells (Th2)** type of antiinflammatory CD4⁺ T helper cell that is primarily antibacterial and opposes T helper 1 cell activity
- T killer cells** one of the best defenses against viral infection; induce apoptosis and form large holes in infected cells, killing them
- T lymphocytes (T cells)** major groups of cells of the adaptive immune response; divided into CD4⁺ T helper cells and CD8⁺ T killer cells
- T memory cells** T cells that are formed during an immune response that is readily activated by subsequent exposures to the same antigen; act rapidly and strongly, long-lived
- T regulatory cells (Tregs)** subset of CD4⁺ T helper lymphocytes that regulate other T cell actions to maintain homeostatic immune responses to prevent autoimmune conditions without compromising antimicrobial activity
- Tachycardia** increased heart rate
- TANK-binding kinase 1 (TBK1)** protein kinase that coordinates the activation of IRF3 and NF- κ B after the innate immune response has been stimulated by TLR3
- Telogen** resting phase of the hair cycle
- Temporal** refers to time
- Template switching** during replication of RNA, RNA polymerase switches to a different RNA; a nonprocessive form of replication used by coronaviruses
- Temporospatial-linked** associated with time and location
- Tachycardia** rapid heart rate
- Tachypnea** rapid breathing rate
- TAP1** chaperone protein is needed for the cell surface expression of MHC class I molecules
- Teratogenic** compound that leads to craniofacial and limb defects in a fetus
- Terpenoids** unsaturated molecules composed of linked isoprene units; that disrupt membranes, including those of coronaviruses
- Terminal glycosylation** sialic acid is the sugar molecule at the end of glycoproteins; human, but not viral, glycoproteins have this type of glycosylation
- Testosterone** major androgen; is produced primarily by men and is responsible for the development of male sexual characteristics (see androgens); small amounts are found in women
- Th1 cells** see "T helper 1 cells"
- Th2 cells** see "T helper 2 cells"
- Th17 cells** subset of CD4⁺ T helper cells that protect against unusual microbes, such as intracellular bacteria and some fungi
- Thalamus** region of the brain that directs sensory input to the correct region of the cerebrum
- Thiol proteins** chemicals similar to alcohols and phenols but contain sulfur rather than oxygen; antioxidants that remove hydrogen peroxide
- Thrombin** protease converts soluble fibrinogen into insoluble strands of fibrin during the formation of blood clots (thrombi)
- Thrombocytopenia** low levels of platelets in the blood; impedes the formation of blood clots and causes excessive bleeding or hemorrhaging in extreme cases
- Thrombocytosis** elevated numbers of platelets; may lead to the production of blood clots
- Thromboembolism** blood clot becomes dislodged and obstructs a blood vessel

Thrombosis formation of blood clots

Thrombotic microangiopathies microscopic blood clots in capillaries and small arteries; characterized by hemolytic anemia and thrombocytopenia

Thymectomize to remove the thymus, the primary organ in which T lymphocytes mature

Thymic involution age-related process in which fatty material replaces normal thymus tissue

Thymus small gland in the upper chest located above the heart and covers part of the esophagus; immature T cells travel from the bone marrow to the thymus, where they become mature and functional

Thyroid gland endocrine gland releases hormones that boost metabolism and decrease blood calcium levels

Thyroid stimulating hormone (TSH) hormone from the pituitary gland that stimulates the thyroid gland to release proteins that regulate metabolism

Thyroiditis autoimmune disorder in which the thyroid gland is inflamed; characterized by weight loss, anxiety or irritability, and trouble sleeping

Thyroxine (T4) hormone produced by the thyroid gland that regulates metabolism

Ticam2 molecule is required for the TLR4 pathway to activate human macrophages

Tissue factor (TF) receptor on neutrophils that works together with clotting factor IIa during the extrinsic pathway of coagulation; aids in wound repair

Tissue inhibitor of matrix metalloproteinase 1 (TIMP1) inhibitor of the enzyme matrix metalloproteinase 1; used as a marker of fibrosis

Tissue plasminogen activator protease that cleaves inactive plasminogen into active plasmin

Titubation staggering gait or swaying or shaking of the torso or head are often associated with disease of the cerebellum

TMPRSS2 (transmembrane protease, serine 2) enzyme that cleaves and activates glycoproteins on the envelope of some viruses, including the human coronaviruses, SARS-CoV, SARS-CoV-2, MERS-CoV, and HCoV-229E

TNF receptor-associated factor 3 involved in NF- κ B kinase and MAP kinase activation; regulates B-cell survival and signaling pathways which lead to cytokine production

Toll-like receptors molecules that detect the presence of various categories of pathogens, including coronaviruses, and signals cellular defense mechanisms

Tongue papillae small, nipple-like structures on the upper surface of the tongue that contain taste buds

Total lung capacity sum of the amount of air that can be forcefully inhaled and exhaled plus the residual air remaining trapped within the lungs

Trace elements elements, including iron, zinc, and copper, that are required in very small amounts to sustain life; may function as coenzymes that are required for an enzymatic activity to occur

Trachea tube that transports air from the throat to the lungs

Transcription process of producing a specific RNA using a genomic DNA or RNA template

Transcription factor molecule that enters the nucleus and turns “on” the transcription of the mRNA, which then produces a specific protein

Transdifferentiation rare event in which cells other than stem cells are transformed into a different cell type

Transduction process by which bacteria receive new DNA during infection by bacteriophages

Transferrin iron-binding protein in the blood that deprives microbes of iron

Transformation process by which bacteria take up exogenous DNA from their environment

Transforming growth factor- β (TGF- β) cytokine produced by T regulatory cells and M2 regulatory macrophages that dampens immune responses; maintains T cell homeostasis

Transgenic organism possessing genes from a different species

Translation process of producing a specific protein from messenger RNA (mRNA)

Transmembrane serine protease 2 (TMPRSS2) cell surface enzyme that is primarily expressed by endothelial cells in the respiratory and digestive tracts; cleaves the spike protein of some coronaviruses, including SARS-CoV and SARS-CoV-2, into S1 and S2 domains

Transmissible gastroenteritis coronavirus coronavirus of swine that leads to profuse diarrhea, vomiting, and life-threatening dehydration in pigs of all ages

Triacylglycerol major dietary lipid that consists of glycerol linked to three fatty acids; may adhere to the walls of blood vessels, occluding blood flow

TRLIF (toll/interleukin-1 receptor-domain-containing adapter-inducing interferon- β) adapter protein for TLR4; only adapter molecule for TLR3-mediated pathogen detection pathway

- Trigeminal nerve** fifth cranial nerve; responsible for sensation and motor activity in the face
- Triglycerides** energy storage molecules; are composed of 3 fatty acids linked to a glycerol backbone
- Triiodothyronine (T3)** hormone produced by the thyroid gland that regulates metabolism
- Tripartite motif-containing 25 (TRIM25)** enzyme that links ubiquitin to molecules to be degraded in the proteasome
- Triterpene** compounds consist of three terpene units with the molecular formula of $C_{30}H_{48}$
- Tropism** chemically-induced movement toward a specific target
- Troponin** molecule that regulates muscle contraction
- Truncated** shortened molecules that often are dysfunctional
- Trypsin** serine protease present in the digestive system; produced by the pancreas and released into the small intestine
- Tumor necrosis factor- α (TNF- α)** proinflammatory cytokine that decreases blood pressure and increases body temperature; excessive levels may cause high fever, wasting, and life-threatening shock
- Type 1 pneumocytes** one of the cell types lining the alveoli; involved in gas exchange between the blood and the air in the alveoli
- Type 2 pneumocytes** one of the cell types lining the alveoli; secrete fluids that allow the alveoli to remain open during exhalation by decreasing the alveolar surface tension
- Type I interferon (IFN)** antiviral and proinflammatory cytokines; IFN- α , - β , and - ω
- Type II interferon (IFN)** antiviral and proinflammatory cytokine; IFN- γ
- Type III interferon (IFN)** antiviral cytokine; various forms of IFN- λ
- Type III hypersensitivity reaction** allergic reaction due to the formation of large immune complexes that become lodged in an inappropriate location where they stimulate an inflammatory reaction, such as arthritis when lodged in joints or glomerulonephritis when lodged in kidney tubules
- Type IV collagen** major component of the basement membrane under epithelial cells
- Type IV hypersensitivity reaction** slowly developing allergic reactions that involve T lymphocytes, macrophages, neutrophils, and their secretions; triggers inflammatory reactions to poison ivy or inexpensive jewelry
- Ubiquitin** member of a group of compounds that tags defective or unneeded proteins for destruction in the proteasome
- Ubiquitin-proteasome system** system in which proteasomes degrade proteins that are tagged by the correct combination of ubiquitin molecules
- Ubiquitination** process of adding ubiquitin to molecules
- Ultrasound** use of sound having an ultrasonic frequency; used in medical imaging
- Umbilical cord stem cells** stem cells present in the umbilical cord; during cell division, they can form another such stem cell and a more differentiated (specialized) cell
- Unfolded protein response** survival mechanism that allows cells to withstand the stress of excessive amounts of misfolded proteins in the endoplasmic reticulum by decreasing the synthesis of most proteins but an increased synthesis of those that can correct the defect
- Upper respiratory tract** portion of the respiratory tract that lies above the larynx (voice box); consists of the nasal cavity and throat
- Urea nitrogen** breakdown product of proteins that is removed by the kidneys; increased levels in blood indicates kidney damage
- Uric acid** breakdown product of purines; a major component of urine
- Uridine** nucleoside containing uracil linked to ribose
- Urokinase (urokinase-type plasminogen activator)** enzyme that cleaves proteins and breaks down blood clots in the lungs
- Urticaria** hives
- Uveitis** Inflammation of the middle layer of the eye
- Vaccination** inoculation with inactivated or attenuated (weakened and nonpathogenic) microbes or microbe components to induce a protective secondary adaptive immune system response upon exposure to the natural, pathogenic form of the microbe
- Vaccinia** virus is used in the smallpox vaccine; due to potentially severe side effects, it is no longer given to the general public
- Vagus nerve** 10th cranial nerve; carries sensation information for the larynx, esophagus, trachea, lungs, heart, and digestive tract to the brain

- Varicella** virus that causes chickenpox
- Variola major** predominant and severe species of smallpox virus
- Variola minor** less common and milder species of smallpox virus
- Variolation** controlled inoculation with live variola virus to protect against subsequent natural infection
- Vascular endothelial growth factor (VEGF)** molecule that stimulates the growth of blood vessels
- Vascular leakage** loss of large amounts of fluid (plasma) from the circulatory system
- Vasculature** blood vessels in a region of the body; including arteries, capillaries, and veins
- Vasculitis** inflammation of blood vessels
- Vasoactive peptides** small chains of amino acids that lower blood pressure by regulating heart contraction, increasing the diameter of blood vessels, and relaxing the smooth muscle in the trachea to allow increased air-flow into and out of the lower respiratory system
- Vasoconstrictors** compounds that decrease the diameter of arteries to decrease blood coming into an area; increase blood pressure
- Vasodilatation (vasodilators)** increase the diameter of arteries increase to bring more blood to an area; decrease blood pressure
- Venous thromboembolism** blood clots that block blood flow through the veins
- Ventricles** four fluid-filled spaces in the brain through which the cerebrospinal flows
- Ventricular dilation** pathologic condition in which the left ventricle of the heart increases in size and cannot pump blood effectively
- Ventricular fibrillation** weak, irregular “fluttering” of the heart muscle; that may be fatal
- Ventricular tachyarrhythmia** condition characterized by rapid contractions of the ventricles of the heart
- Vesicular rash** fluid-filled skin lesions
- V-set immunoglobulin-domain-containing 4 (VSIG4)** complement receptor found in resting macrophages that downregulates some inflammatory conditions; negatively regulates T lymphocyte responses
- Villi** finger-like projections of the cells of the small intestine; increase the surface area of the outer part of the intestines to provide more area in which to absorb nutrients
- Villous enterocytes** intestinal cells found in the villi
- Vimentin** cytoskeletal protein; intracellular, intermediate filament
- Viperin (virus inhibitory protein, endoplasmic reticulum-associated, interferon-inducible: CIG5)** interferon-stimulated gene that inhibits viral release from its host cell by altering the plasma membrane’s lipid rafts
- Viral myocarditis** inflammation of the heart muscles due to viruses, including coronaviruses
- Viral neutralization tests** diagnostic tests that detect the presence of antibodies in a sample that can inhibit virus replication; previously the “gold standard” against which all other diagnostic tests were measured, but must be performed in Biosafety Level 3 facilities
- Viral vectors** mild virus containing portions of a more pathogenic virus; used in some vaccines
- Viremia** presence of viruses in the blood
- Viroporin** small viral protein that produces pores or ion channels in the cell’s plasma membrane, allowing the exit of newly-formed viruses
- Virulence factor** factor that increases the ability of an organism to cause disease
- Visceral peritoneum** lining that covers and holds the abdominal organs in place
- Vital capacity** maximum amount of air entering and leaving the lungs after forceful inhalation and exhalation
- Vitamin C** antioxidant that protects the body against reactive oxygen species
- Vitamin D** precursor to calcitriol; needed for calcium uptake from the digestive tract and is also vital to proper immune system functioning
- Wastewater-based epidemiology (WBE)** analysis of materials in raw wastewater that obtains qualitative and quantitative data on the activity or microbial infection of inhabitants in a wastewater catchment
- Wasting** extreme, potentially fatal, loss of weight
- “Wet markets”** outdoor markets in which wild animals are sold, including palm civets and raccoon dogs that serve as intermediate hosts for SARS-CoV and pangolins, putative intermediate hosts for SARS-CoV-2
- White adipose tissue** primary form of fat cells; stores lipids
- White matter** axons that are surrounded by a myelin sheath
- White pulp** area of the spleen that contains lymphocytes
- Wild-type** normal, nonmutated forms of species

Winter dysentery severe diarrhetic disease of adult cattle caused by a bovine enteric coronavirus

Zinc finger motif small structure of some proteins that contain at least one zinc ion; used to stabilize protein folding

ZO-1 member of a group of molecules that form tight junctions between cells

Zoonotic transmission (spillover) event in which a microbe that normally exists in found in animals is transmitted to humans; may result in mild-to-severe disease in both animals and humans

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PATHOGENIC CORONAVIRUSES OF HUMANS AND ANIMALS

SARS, MERS, COVID-19, and Animal Coronaviruses
with Zoonotic Potential

Lisa A. Beltz, Ph.D.

Pathogenic Coronaviruses of Humans and Animals provides relevant information about human coronaviruses, including SARS-CoV, SARS-CoV-2, MERS-CoV, and other more common human coronaviruses that may mutate in a way that increases their virulence. The addition of animal coronaviruses allows awareness of the potential of zoonotic transmission of coronaviruses not only from wild animals such as bats and rodents, but also from domestic agricultural and companion animals that we have close contact with.

The book opens with an introductory chapter with coverage of viruses, the immune system, as well as coronaviruses, their classifications, prevention, and protection. The book goes on to cover the history, disease, causative virus, immune response, diagnosis, treatment, prevention, and surveillance of SARS-CoV, MERS-CoV, and SARS-CoV-2. The remaining chapters discuss coronaviruses with the possible zoonotic transmission of domestic, semi-domestic animals, and companion animals. This book concludes with future perspectives of coronavirus mutations, protective measures against their zoonotic transmission, and a discussion about pandemics and infectious diseases around the globe.

Key features

- Covers SARS-CoV, MERS-CoV, and SARS-CoV-2 as well as coronaviruses with possible zoonotic transmission of domestic, semi-domestic animals, and companion animals
- Complements previous studies by bringing together information comparing human and animal coronaviruses
- Includes appendices of a glossary and coronavirus disease overviews of humans and animals

About the author

Dr. Lisa A. Beltz received her Bachelor's degree in Biology at Malone University in Canton, Ohio. She began her career in infectious disease research in the Department of Microbiology and Public Health at Michigan State University. She then spent 7 years as a postdoctoral fellow at the Johns Hopkins University Hospital System and at the University of Pittsburgh. Her research during this period focused on how simian and human immunodeficiency viruses, respectively, interact with simian and human bone marrow and blood. Dr. Beltz then accepted a faculty position at the University of Northern Iowa and continued teaching while authoring articles and books and giving conference presentations about infectious diseases of humans and bats. Dr. Beltz has previously written two books: *Emerging Infectious Diseases: A Guide to Diseases, Causative Agents, and Surveillance* and *Bats and Human Health: Ebola, SARS, Rabies, and Beyond* as well as recent Elsevier title, *Zika and Other Neglected and Emerging Flaviviruses*.



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