Chapter 31

Coronaviruses

The family *Coronaviridae* encompasses a broad spectrum of animal and human viruses, all characterized by a distinctive morphology. Virions are enveloped and spherical (coronaviruses), or disc, kidney, or rod shaped (toroviruses). Each particle is surrounded by a fringe or "corona" representing the bulbous distal ends of embedded envelope glycoproteins. Prior to 2003 members of this family were believed to cause only mild respiratory illness in humans, other coronaviruses then known being largely of importance only to the livestock industry. But the emergence of severe acute respiratory virus (SARS-CoV) that year stimulated major research into these viruses, to the effect that many new coronaviruses have since been discovered, some with zoonotic potential of causing serious outbreaks of disease in humans. The more recent emergence of MERS-CoV is exemplary.

Coronaviruses are also noted for having the largest positive-sense RNA genome: coronavirus genes are mostly expressed by a complex procedure whereby nested mRNA transcripts are produced, the regulation of which governs the progression of the replication cycle.

PROPERTIES OF CORONAVIRUSES

Classification

The family *Coronaviridae* is one of three RNA virus families within the order *Nidovirales*, the other being the *Arteriviridae* and the *Roniviridae* containing pathogens of birds and insects, respectively. The family consists of two subfamilies, *Coronavirinae* and *Torovirinae*, the latter containing viruses causing mainly enteric infections of horses, cattle, pigs, cats, and goats. Although of economic importance, members of the *Torovirinae* subfamily are not as yet known to cause human infection, and thus are not dealt with further. All coronaviruses share a common morphology and possess a single-stranded RNA genome of up to 30 kb in length.

Members of the subfamily *Coronavirinae* are subdivided into four genera. The genus *Alphacoronavirus* contains the human virus HCoV-229E, one other human coronavirus (HCoV-NL63), and many animal viruses. The genus *Betacoronavirus* includes the prototype mouse hepatitis virus (MHV), the three human viruses HCoV-OC43, SARS-HCoV, and HCoV-HKU1, and the SARS-related coronavirus, Middle Eastern respiratory syndrome (MERS) coronavirus, together with a number of animal coronaviruses. The genus *Gammacoronavirus* contains viruses of cetaceans (whales) and birds, and the genus *Deltacoronavirus* contains viruses isolated from pigs and birds.

Since 2005, dozens of new coronaviruses have been isolated from bats, and there is evidence that human respiratory coronaviruses, SARS coronavirus, and MERS coronavirus, may each have originally emerged from ancestral bat viruses (Table 31.1, Fig. 31.1).

Structure and Genome

Coronaviruses virions contain three major structural proteins: the very large (200 K) glycoprotein S (for spike) that forms the bulky (15 to 20 nm) peplomers found in the viral envelope, an unusual transmembrane glycoprotein (M) and the internal phosphorylated nucleocapsid protein (N). In addition, there is a minor transmembrane protein E, and some

TABLE 31.1 Properties of Coronaviruses

- Virion is pleomorphic spherical 80 to 220 nm (coronaviruses); or disc, kidney, or rod shaped 120 to 140 nm (toroviruses)
- Envelope with large, widely spaced club-shaped peplomers
- Tubular nucleocapsid with helical symmetry
- Linear, plus sense ssRNA genome 27 to 33 kb, capped, polyadenylated, infectious; untranslated sequences at each end
- Three or four structural proteins: nucleoprotein (N), peplomer glycoprotein (S), transmembrane glycoprotein (M), sometimes hemagglutinin-esterase (HE)
- Genome encodes 3 to 10 further non-structural proteins, including the RNA-dependent RNA polymerase made up of subunits cleaved from two polyproteins translated from the 5′-end
- Replicates in cytoplasm; genome is transcribed to full-length negative sense RNA, from which is transcribed a 3′-coterminal nested set of mRNAs, only the unique 5′ sequences of which are translated
- Virions are assembled and bud into the endoplasmic reticulum and Golgi cisternae; release is by exocytosis
- Variant viruses arise readily, by mutation and recombination, and the use of different receptors influences the host range exhibited

Fenner and White's Medical Virology. DOI: http://dx.doi.org/10.1016/B978-0-12-375156-0.00031-X

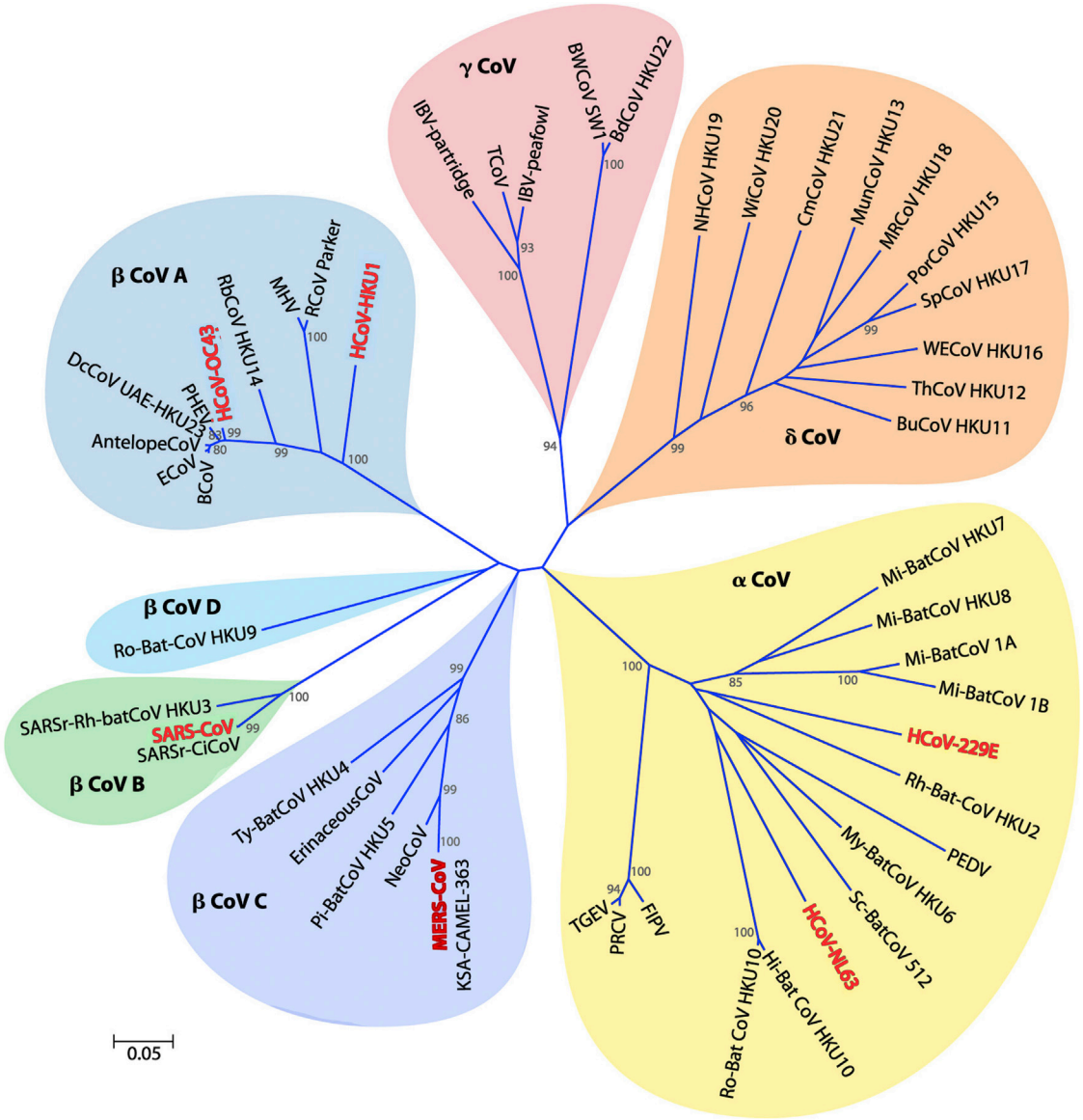

FIGURE 31.1 Phylogeny of coronaviruses. Phylogenetic tree of 50 coronaviruses constructed by the neighbor-joining method using MEGA 5.0 using partial nucleotide sequences of RNA-dependent RNA polymerase. The scale bar indicates the estimated number of substitutions per 20 nucleotides. Space does not permit providing full virus names, except for the human viruses, which are scattered among the viruses isolated from many other species (major pathogens shown in red): HCoV-229E, human coronavirus 229E; HCoV-HKU1, human coronavirus HKU1; HCoV-NL63, human coronavirus NL63; HCoV-OC43, human coronavirus OC43; KSA-CAMEL-363, KSA-CAMEL-363 isolate of Middle East respiratory syndrome coronavirus; MERS-CoV, Middle East respiratory syndrome coronavirus; MHV, murine hepatitis virus, the prototypic virus of the family; SARS-CoV, SARS coronavirus; SARSr-CiCoV, SARS-related palm civet coronavirus. A remarkable number of the viruses represented here are from bats, many different species of bats, and quite a few of these are rather closely related to SARS-CoV. *Modified from Chan, J.F., Lau, S.K., To, K.K., Cheng, V.C., Woo, P.C., Yuen, K.Y., 2015. Middle East respiratory syndrome coronavirus: another zoonotic betacoronavirus causing SARS-like disease. Clin. Microbiol. Rev. 28, 465–522, with permission.*

coronaviruses contain a further envelope protein with both hemagglutination and esterase functions (HE) (Fig. 31.2).

The 30 kb positive sense, single-stranded RNA genome is the largest RNA viral genome known. It is capped at the 5′-end and polyadenylated at the 3′-terminus, and is infectious. Due to its size the expression of individual genes occurs through a complex process whereby sets of nested mRNAs are produced, all sharing the same 5′-end sequence. Extensive rearrangements may occur as a result of heterologous RNA recombination. At the 5′-end of the genome is an untranslated (UTR) sequence of 65 to 98 nucleotides, termed the leader RNA, which is also present at the 5′-ends of all subgenomic mRNAs. At the 3′-end of the RNA genome is another untranslated sequence of 200 to 500 nucleotides, followed by a poly(A) tail. Both untranslated regions are important for regulating RNA replication and transcription.

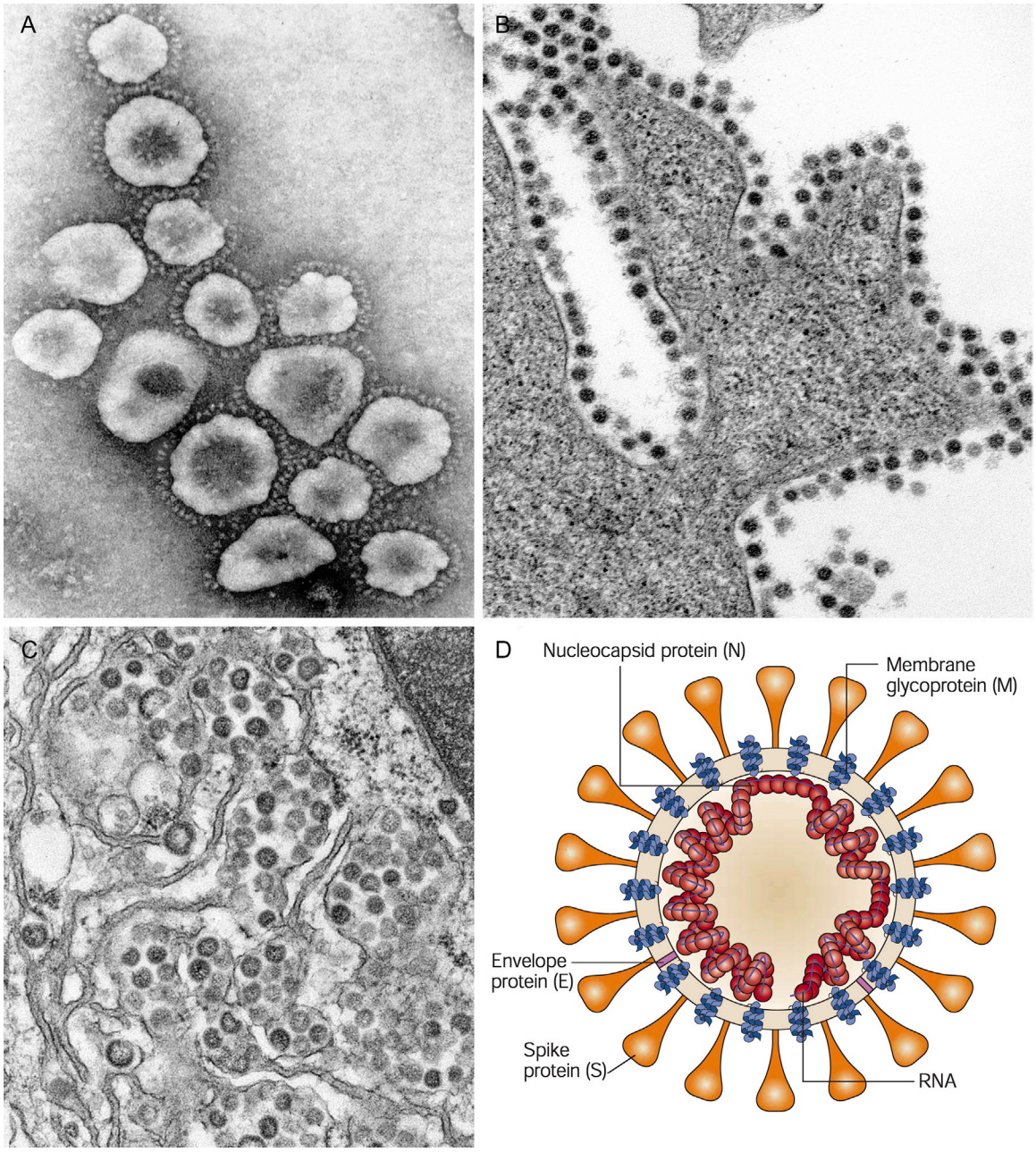

FIGURE 31.2 Coronavirus morphology and structure. (A) Negative contrast electron microscopy of SARS coronavirus (SARS-CoV), showing the large petal-shaped surface projections (spikes, peplomers). (B) Thin-section electron microscopy of SARS-CoV in cell culture, showing typical adherence of virions to the plasma membrane of a cell—virions adhere to infected and uninfected cells. (C) Thin-section electron microscopy of Middle Eastern respiratory syndrome virus (MERS-CoV) in cell culture, showing typical virion assembly in the lumen of the Golgi membrane system. (D) Model of coronavirus virion structure, showing the supercoiling of the viral nucleocapsid under the envelope. *(B) From Sandra Crameri, CSIRO, Geelong, Australia. (C) From Public Health Image Library, CDC. (D) Reproduced from Stadler, K., et al., 2003. SARS—beginning to understand a new virus. Nat. Rev. Microbiol. 1, 209–218. All with permission.*

The coronavirus genome contains 7 to 14 open reading frames (ORFs). Starting from the 5′-end, Gene 1, which comprises two-thirds of the genome, is about 20 to 22 kb in length. It consists of two overlapping ORFs (1a and 1b), collectively functioning as the viral RNA polymerase (Pol). The order of the other four genes of structural proteins are 5′-S (spike)–E (envelope)–M (membrane)–N (nucleocapsid)-3′. These genes are interspersed with several ORFs encoding non-structural proteins and the HE glycoprotein, when present. Each gene differs markedly among coronaviruses

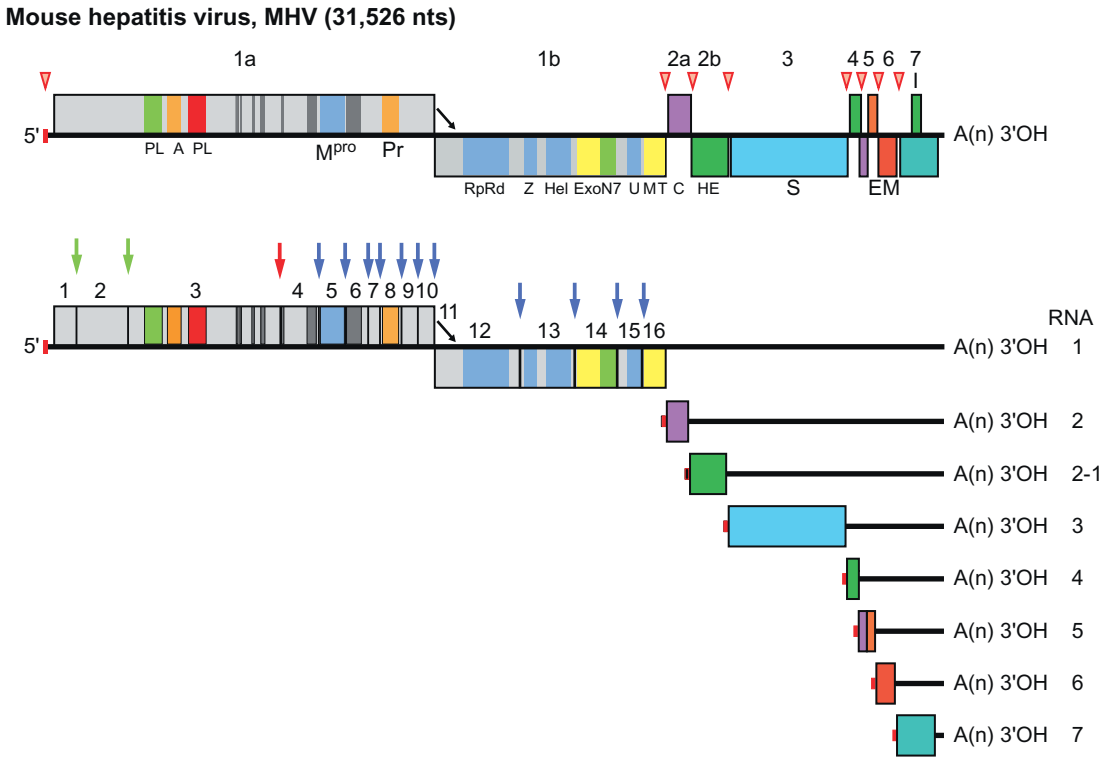

FIGURE 31.3 Coronavirus genome organization and expression. (Upper panel) Genome organization of mouse hepatitis virus. ORFs are represented by boxes, with a number above and the protein acronym below. The diagonal arrow between ORF 1a and 1b represents the ribosomal frameshift site. Red arrowheads indicate the locations of transcriptional-regulating sequences (TRSs). (Lower panel) Map of the viral mRNAs, which are overlapping and coterminal at the 3′ end. The different sub-genomic mRNAs are numbered by convention from large to small, beginning with the genome as RNA1. The two huge replicase polyproteins (1a and 1ab) are cleaved by virus-coded proteases as shown by colored ORFs and colored arrows; green, papain-like protease 1(PL1^{pro}); red, papain-like protease 2 (PL2^{pro}); blue, 3C-like main protease (M^{pro}). *Reproduced from King, A.M.Q., et al., 2012. Virus taxonomy. In: Ninth Report of the International Committee on Taxonomy of Viruses, Academic Press, London, p. 808, with permission.*

in number, nucleotide sequence, gene order, and method of expression, although these are conserved within the same serogroup. The SARS-CoV genome encodes several smaller ORFs located in the 3′ region of the genome not present in other coronaviruses. These ORFs are predicted to express eight novel proteins termed accessory proteins. Antibodies reactive against all of the SARS-CoV proteins have been detected in sera isolated from SARS patients, indicating that these proteins are expressed by the virus *in vivo* (Fig. 31.3).

ORFs 1a and 1b are first translated into two polyproteins, both identical at the N terminus but one of which has a C-terminal extension due to frame-shifting. These are precursors of proteins in the transcription–replication complex. All coronaviruses encode a chymotrypsin-like protease, termed M^{pro} (main protease) or 3CL^{pro} since it shares some similarities with the 3C proteases of picornaviruses. This protease is responsible for processing the remainder of the polyprotein, producing as many as 16 non-structural proteins. SARS-CoV contains the largest number of these non-structural proteins. For example, nsp3 is a multifunctional protein with protease and ADP-ribose 1″phosphatase activity. Two proteins (nsp 7 and nsp 8) form a cylinder-like structure that may be important in coronavirus RNA synthesis, and a single-strand RNA-binding protein (nsp 9). ORF1b encodes the viral RNA-dependent RNA polymerase and a multifunctional helicase protein. In addition to helicase activity, this protein has NTPase and dNTPase activities as well as 5′ triphosphatase activity.

These non-structural protein gene products are not essential for virus replication, but deletion of one or more often causes viral attenuation. At least one, the product of ORF3a, is now recognized to be a structural protein. The ORF3a product is an O-glycosylated, triple-membrane-spanning protein capable of binding to N, M, and S proteins, suggesting a role in viral biogenesis.

Viral Replication

The N-terminal half of the S protein contains the receptor binding domain, and the C-terminal half is membrane anchored and has fusogenic activity. The specificity of binding is important for the host spectrum of individual coronaviruses. Soon after the virus was isolated, the

receptor for SARS-CoV was identified as angiotensin-converting enzyme 2 (ACE2), a cellular protein expressed on the surface of cells of the lungs, heart, kidney, and small intestine as well as other tissues. Other proteins, such as CD209L (L-SIGN), DC-SIGN, and LSECtin, can support the entry of SARS-CoV into cells, but cannot by themselves confer susceptibility to a cell lacking ACE2. Receptors of other coronaviruses have also been identified. For instance, the receptor for murine hepatitis virus (MHV) is a murine biliary glycoprotein belonging to the carcinoembryonic antigen (CEA) family in the Ig superfamily (CEACAM1). Individual strains of MHV may exhibit different preferences for viral entry. For example, only the MHV-3 strain infects T and B cells, with subsequent lymphopenia. Although most strains of coronavirus exhibit strict species specificity, like other RNA viruses, coronaviruses can readily mutate under selective pressure during passage *in vitro* or *in vivo* in response to environmental conditions, and an S protein with extended host range is thus selected. These variants can efficiently use human CEA as receptors for infection of human cells. Such a mechanism may account for the development of cross-species infection, exemplified in the outbreak of the SARS epidemic.

After binding to the cellular receptor, the viral S protein acts as a class I fusion protein, undergoing conformational changes that lead to fusion between the viral envelope with either the plasma or endosomal membranes. SARS-CoV requires an acidic pH for entry, but not for S glycoprotein-mediated cell-to-cell fusion. Subsequently, the viral nucleocapsid is released into the cytoplasm and the RNA is uncoated prior to transcription.

During the first stage of virus replication, the positive strand viral RNA serves as mRNA for translation of the two large open reading frames (ORF 1a and 1b), each encoding units of the RNA-dependent RNA polymerase. After cleavage, these proteins assemble to form the active RNA polymerase which transcribes full-length complementary (negative-sense) RNA. This, in turn, is transcribed not only into full-length genomic RNA, but also a nested set of 3′-coterminal overlapping subgenomic mRNAs described above. Each species of viral mRNA differs in length but shares a common 3′-end and a 70 nucleotide 5′-leader sequence. Only the unique sequence not shared with the next smallest mRNA in the nested set is translated to yield viral protein. Due to the large size of the genome, genetic recombination occurs at high frequency between the genomes of different but related coronaviruses. This mechanism may be important for generation of the genetic diversity of coronaviruses in nature.

During maturation and assembly, the S protein is cotranslationally inserted into the rough endoplasmic reticulum (RER) and glycosylated with N-linked glycans. Glycosylation is essential for the proper folding and transport of the S protein. The S protein forms trimers before it is exported out of the endoplasmic reticulum (ER), and then interacts with M and E proteins to migrate to the site of virus assembly. In infected cells, the M protein is localized mostly in the virus-budding compartment, while at a later stage of viral replication, N protein is transported to the site of virus assembly. SARS-CoV expresses another structural protein, 3a, that is not only associated with intracellular and plasma membranes, but is also secreted and induces apoptosis. S protein is crucial for virus entry, but not necessarily required for assembly, as spikeless virus-like particles can form in the absence of S protein.

Pathogenesis and Immunity

Most coronaviruses first replicate in epithelial cells of the respiratory or enteric tracts. Because coronaviruses are enveloped, virions are less stable in the environment and in clinical specimens compared to most non-enveloped viruses. Although transmission is usually associated with close contact, SARS-CoV is surprisingly stable on environmental surfaces. It is not clear how these enveloped viruses retain infectivity in the presence of bile and proteolytic enzymes present in the enteric tract. Perhaps the virions may be more resistant to proteolytic degradation because coronavirus envelope glycoproteins are extensively glycosylated.

The pathogenesis and immune responses of coronaviruses have been most studied in animal coronavirus infections. For example, mouse hepatitis virus (MHV), the prototype of betacoronaviruses, includes a spectrum of strains with different tropism, causing enteric, hepatic, respiratory, or CNS infections. Since neurological disease caused by MHV simulates multiple sclerosis (MS) in humans, the pathogenesis and immune responses have been studied in detail. Highly neurovirulent, moderately neurovirulent, and attenuated strains cause different clinical manifestations involving different patterns of infection of neurons, oligodendrocytes, microglia, and astrocytes. Demyelination can occur, which is mostly immune-mediated; irradiated or congenitally immunodeficient mice do not develop demyelination after infection, but when these mice, which lack T and B cells, are reconstituted with virus-specific T cells, demyelination rapidly develops. Demyelination is accompanied by infiltration of macrophages and activated microglia into the white matter of the spinal cord. Both CD4 and CD8 T cells are also required for virus clearance from the central nervous system (CNS), with CD8 T cells as the most important in this process.

On the other hand, the efficacy of the innate immune response also determines the extent of initial virus replication and the levels of virus seen. Coronaviruses have developed strategies to counter the innate immune responses. For example, N protein and the SARS-CoV accessory proteins ORF6 and ORF3b prevent IFN induction, and the N protein prevents nuclear translocation of proteins containing

classical nuclear import signals, including STAT1, a crucial component of IFNα, IFNβ, and IFNγ signaling pathways.

HUMAN CORONAVIRUS INFECTIONS

Respiratory Coronavirus Infections

Prior to 2003 the interest in human coronaviruses was primarily driven by having a role in relatively mild upper respiratory tract infections. Viral strains HCoV-229E and HCoV-OC43 were isolated from patients with upper respiratory tract infections in the 1960s. Then, shortly after the discovery of SARS-CoV in 2003, two new human coronaviruses were isolated, HCoV-NL63 in the Netherlands and HKU1 in Hong Kong. The host cell receptor for HCoV-229E aminopeptidase N, while that for HCoV-NL63 is ACE2. Interest in the possible role of human coronaviruses in multiple sclerosis has initiated a search for human coronaviruses in human brain tissues. One study found HCoV-OC43 sequences in 36% of brains from MS patients and 14% in those of controls, but the significance of this remains unclear.

Clinical Features

The typical coronavirus "common cold" is mild and the virus remains localized to the epithelium of the upper respiratory tract and elicits a poor immune response, hence the high rate of reinfection. There is no cross-immunity between human coronavirus-229E and human coronavirus-OC43, and it is likely that new strains are continually arising by mutation selection.

Human volunteer studies have shown that these viruses cause an illness of some seven days, with typical symptoms of a sore throat, rhinorrhea, fever, cough, and headache, indistinguishable from the common colds caused by rhinoviruses. Asymptomatic infections are frequent as measured by the detection of virus in the upper respiratory tract.

Occasionally human coronaviruses HCoV-229E and HCoV-OC43 cause lower respiratory tract infections and otitis media. There is no evidence of either of these viruses causing enteric disease in humans, despite the finding of coronavirus-like particles in the stools of such patients.

Laboratory Diagnosis

Laboratory diagnosis of the "common" human respiratory coronaviruses is not often called for, but is sometimes included in the panel of respiratory pathogens incorporated in multiplex RT-PCR assay systems. These viruses are difficult to grow in cultured cells, hence are rarely recovered from clinical systems. The exception is HCoV-NL63 which, like SARS-CoV, grows readily in a number of cell lines; this may be linked to the finding that HCoV-NL63 and SARS-CoV are unique in using ACE2 as a receptor. Human coronavirus-OC43 and related strains were originally isolated in organ cultures of human embryonic trachea or nasal epithelium. Organ culture is too intricate a technique for a diagnostic laboratory, but some strains can be isolated directly in diploid fibroblastic lines from human embryonic lung or intestine. Foci of "granular" cells become evident after a week and may progress to vacuolation before disintegrating; syncytia may form in some cell types. Hemadsorption and hemagglutination are demonstrable with OC43 only.

Epidemiology

As with many respiratory infections, spread is by direct contact between infected individuals or via fomites. HCoV-229E and HCoV-OC43 viruses are most often detected between November and May in the northern hemisphere, although the incidence of infection varies from year to year. It is thought these two viruses together account for 5 to 30% of all common colds. Little is known with regard to the epidemiology of HC-V-NL63 and HCoV-HKU1.

SEVERE ACUTE RESPIRATORY VIRUS (SARS-CoV)

SARS-CoV infection of humans is a serious lower respiratory tract illness that emerged with dramatic suddenness in China in 2002. Up to 20% of infections needed intensive care; the overall fatality rate initially was around 10% but approached 50% in elderly patients and those with underlying illness. The epidemic was halted in 2003 by a highly effective national and global health response, and the virus is no longer circulating in humans, although it is endemic in horseshoe bats.

Clinical Features and Pathogenesis

The disease began initially with an influenza-like prodrome starting two to seven days after exposure. This was followed after a further three or more days by the lower respiratory tract phase, comprising dry cough, dyspnea, and increasing respiratory distress sometimes requiring mechanical ventilation. Most patients showed lymphopenia (70 to 95%), with a substantial drop in both CD4 and CD8 T cells. Asymptomatic or mild illness was uncommon, as illustrated by studies of exposed health-care workers in which less than 1% of those without a SARS-like illness had serological evidence of infection. However, in the year following the epidemic of 2003, only a few SARS patients were found and these had mild disease. SARS-CoV infected both upper

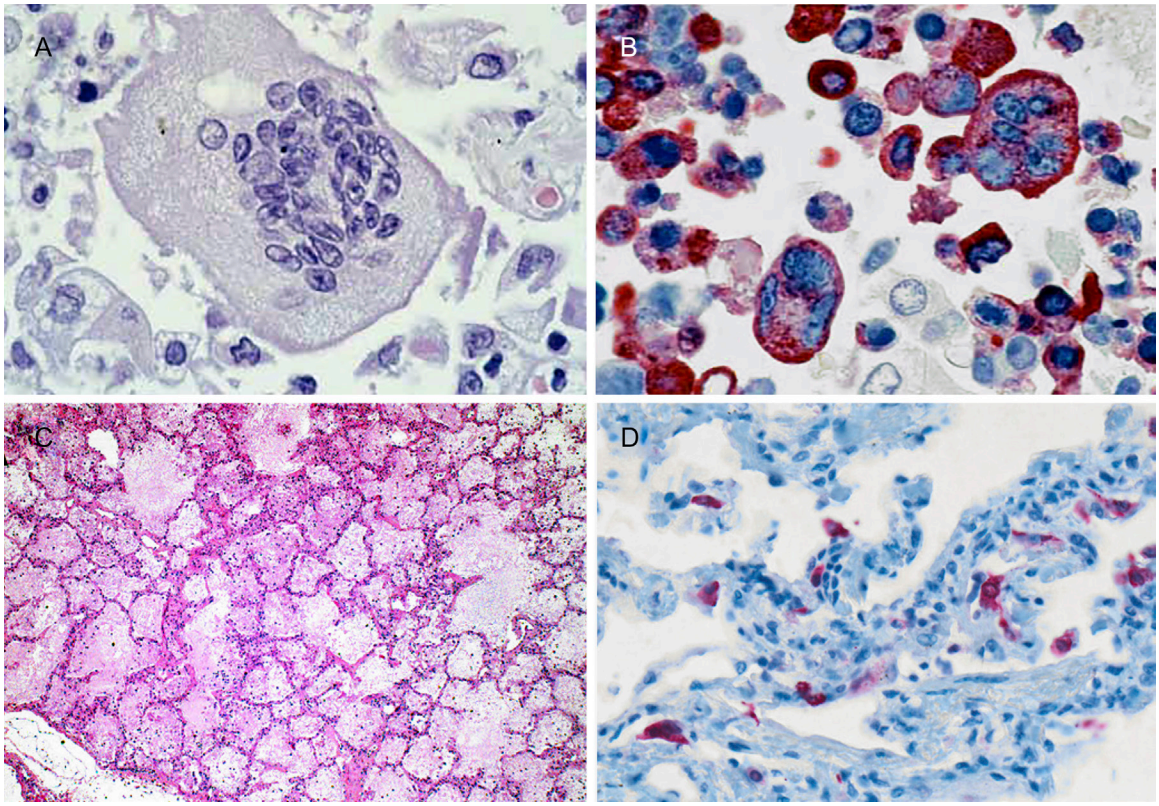

FIGURE 31.4 Histopathology and immunohistochemistry of SARS and MERS acute respiratory distress syndrome (ARDS). (A) SARS: lesions have consisted of diffuse alveolar damage at various levels of progression and severity—changes have included interstitial mononuclear inflammatory infiltration, hyaline-membrane formation, desquamation of pneumocytes and necrotic inflammatory debris into small airways, focal intra-alveolar hemorrhage, and as shown multinucleated syncytial cells. H&E. (B) SARS: immunohistochemical staining of SARS-CoV-infected cells free in interstitial space in small airway. Viral antigens in the cytoplasm of cells, including syncytial cells, with most intense immunostaining near margins of cells. Immunoalkaline phosphatase system, napthol–fast red substrate, hematoxylin counterstain. (C) MERS: pulmonary edema with mononuclear inflammatory cells in alveoli. H&E. (D) MERS: immunohistochemical staining of MERS-CoV-infected pneumocytes within alveoli at a site where normal lung architecture is still intact. Commercial avidin–alkaline phosphatase complex system with naphthol–fast red substrate. *From Thomas Ksiazek, University of Texas Medical Branch, Galveston and Sherif Zaki, U.S. Centers for Disease Control and Prevention, and their many colleagues in these studies: Ksiazek, T.G., et al., 2003. A novel coronavirus associated with severe acute respiratory syndrome. N. Engl. J. Med. 348, 1953–1966; and Ng, D.L., et al. 2016. Clinicopathologic, immunohistochemical, and ultrastructural findings of a fatal case of middle east respiratory syndrome coronavirus infection in the United Arab Emirates, April 2014. Am. J. Pathol. 186, 652–658.*

airway and alveolar epithelial cells, resulting in lung injury. Virus or viral products were also detected in other organs, such as the kidney, liver, and small intestine, as well as in stools. Although the lung is recognized as the organ most severely affected by SARS-CoV, the exact mechanism of lung injury is controversial. Levels of infectious virus diminished as the clinical disease worsened, suggesting that lung injury was due to an immunopathological mechanism. Similar to mice with MHV-mediated demyelination, large numbers of macrophages were detected in infected lung. However, this conclusion derived from samples of live patients—from nasopharyngeal aspirates, not from the lungs or other organs (Fig. 31.4). In common with other coronaviruses, SARS-CoV infected macrophages and dendritic cells, but the replication cycle is not completed in these cells. Several proinflammatory cytokines and chemokines, such as IP-10, MCP-1, MIP-1, RANTES and MCP-2, TNF-α, and IL-6 are expressed by infected dendritic cells; many of these molecules were also expressed at elevated levels in the serum of infected patients.

Infection with SARS-CoV triggers a series of humoral and cellular immune responses in patients. Specific IgG and IgM antibodies against SARS-CoV were detected approximately two weeks postinfection, reaching a peak 60 days post-infection and remained at high levels for at least 180 days post-infection. High titers of neutralizing antibodies and SARS-CoV-specific cytotoxic T lymphocytes were detected in patients who had recovered from SARS, with high levels correlating well with a favorable outcome. This suggests that both humoral and cellular immune responses are crucial for the clearance of infection by SARS-CoV.

An important unresolved issue is how SARS-CoV caused such severe disease in humans. SARS-CoV infects several species of animals, including mice, ferrets, hamsters, cats, and cynomolgus macaques, but these animals develop either mild or subclinical disease. This may be due to the fact that these animals have already experienced subclinical infection with a coronavirus from the same group as SARS-CoV. Consistent with this, the clinical course of SARS-CoV in patients in Guangzhou, China, was mild in the year following the outbreak of severe cases of SARS.

Laboratory Diagnosis

Frequently a combination of serological and RT-PCR assays was used to detect and confirm infection. With very sensitive PCR assays (e.g., a nested or real-time PCR assay) and RNA extraction procedures that increased the amount of specimen used in the assay, the positivity rate in respiratory specimens increased from less than 40% to more than 80% during the second or third day of illness. Results by EIA showed the presence of SARS-CoV antibodies against the N protein in 50% to more than 80% of sera collected during the first week of illness, and in more than 50% of respiratory and stool specimens collected during the second and third weeks of illness.

SARS antibodies were detected as early as six days after onset of illness, but nearly always by 14 days after the onset of the illness, (in rare instances antibodies did not appear until four weeks after the onset of illness). Because sera from persons not infected with SARS during the 2002 to 2003 outbreak rarely tested positive for SARS-CoV antibodies, a single specimen positive for SARS-CoV antibodies was usually considered diagnostic for infection, and a negative test on a serum specimen collected late in the illness (28 days or later after onset of illness) could be used to rule out SARS-CoV infection. Because diagnosis of a re-emergence of SARS-CoV would have substantial public health, social, and economic impact, a future case putatively diagnosed as SARS would have to be confirmed by a national reference laboratory before international response actions could be initiated–false positives do occur.

During the SARS-CoV outbreak, rapid diagnosis was best made by RT-PCR using defined primers, usually derived from the viral N sequence. For early diagnosis, specimens from throat or nasal pharyngeal swabs can be used for RT-PCR, and serum samples were also used to detect viral RNA during the first week of illness. Both stool and respiratory specimens were assayed for viral RNA during the second week of illness. In contrast, serological assays usually provide the best way to confirm or rule out infections *ex post facto*. EIAs for viral N and S antigens were also developed to screen for suspected patients in rural areas.

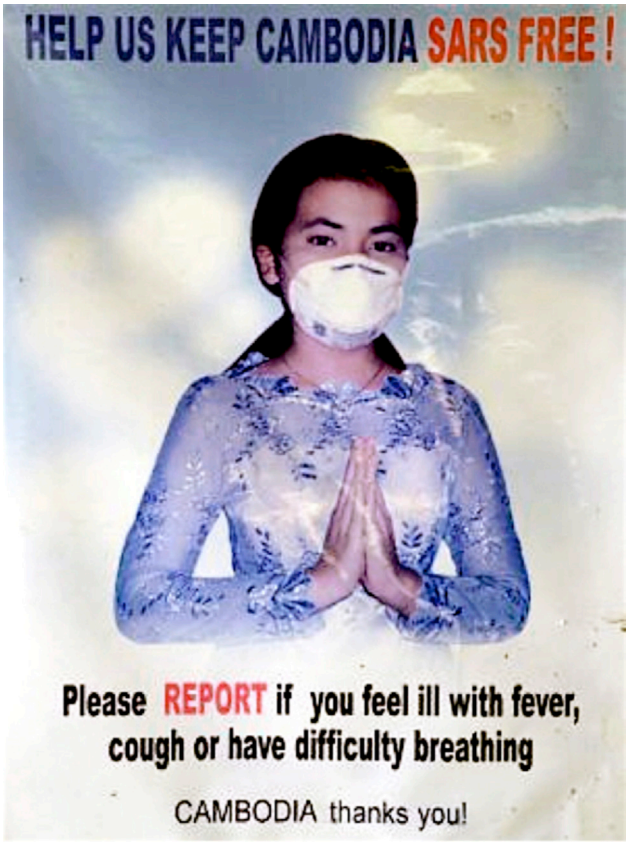

FIGURE 31.5 The sudden emergence of human cases of SARS, with its high mortality and rapid intercontinental dissemination, caused significant disruption to many international activities, and led to heightened awareness and respect for the possibility of new epidemics in the 21st century. *From a wall poster, Department of Immigration, Cambodia.*

Epidemiology

The high mortality and rapid intercontinental spread of clinical cases of SARS led to intensive epidemiological and virological investigations. It caused significant disruption to affected cities and many activities that involved travel. Anxiety and warnings impacted on many parts of the world (Fig. 31.5). Molecular analysis and field epidemiological studies for identifying the source of SARS-CoV revealed that horseshoe bats were most likely the natural reservoir of this virus. Sequences of several distinct SARS-like coronaviruses have been amplified from horseshoe bats from Hong Kong and several provinces in China, and 30% to 85% of this species of bat had antibodies to a SARS-like coronavirus. Initially, SARS-CoV was thought to originate in civet cats, but these were later found to act as intermediary hosts providing a source of transmission to humans. Multiple serological studies demonstrated that SARS-CoV had not circulated to any significant extent in humans prior to the outbreak in 2002 and 2003. Some persons working in wild animal markets in China had serological evidence

of a SARS-CoV-like infection acquired before the 2003 outbreak, but reported no SARS-like respiratory illness.

Although animals were the original source of SARS, its global spread occurred by human-to-human transmission. Transmission appeared to occur through close contact or infectious droplets and probably aerosols in some instances. There was also substantial patient-to-patient variation in efficiency of transmission, which in part was associated with degree of severity of illness and possibly associated with the virulence of viral strains. Since the SARS outbreak was controlled in June 2003, only 17 cases of SARS have been confirmed and none of these occurred after June 2004.

The underlying basis for the mysterious outbreak and sudden disappearance of SARS has not yet been fully explained.

Treatment and Prevention

Prevention has been based on careful identification and isolation of cases and contacts until ten days after symptoms had cleared, combined with investigation of particular environmental circumstances responsible for clusters of cases. This approach successfully stopped the outbreak within 4 months of the start of its global spread.

For treatment of SARS, several drugs were tried clinically with no clear benefit, and since then a number of drugs including protease inhibitors used in HIV have been investigated for efficacy *in vitro*. The viral S protein has been suggested as a candidate for developing a preventative vaccine against SARS or other coronaviruses. However, the genetic variability of these viruses, and poor immunity after natural infection, indicate the challenges involved.

MIDDLE EAST RESPIRATORY VIRUS (MERS-CoV)

The increasing numbers of coronaviruses isolated from animals and birds illustrate that it is likely that more members of this family will emerge in the years to come, posing further threats to public health in affected areas. The first case of MERS-CoV occurred in Jeddah, Saudi Arabia in June 2012 with a patient presenting with acute pneumonia and renal failure. As of May 2016, 1733 laboratory-confirmed cases of MERS had been reported to WHO, including at least 628 deaths (case fatality rate 36%). Evidence of the presence of MERS coronavirus has been reported from 27 countries, but still mostly from Saudi Arabia and Korea.

Clinical Features and Pathogenesis

The clinical presentation ranges from asymptomatic infection, to a mild flu-like illness or severe pneumonia accompanied by acute respiratory distress syndrome (ARDS), septic shock, and multi-organ failure preceding death. The course of infection is more severe among immunocompromised individuals and those with underlying medical conditions, especially chronic renal failure, heart disease, and diabetes.

The disease starts with fever and cough, sore throat, chills, arthralgia, and myalgia, soon followed by dyspnea. The infection rapidly progresses to severe pneumonia (Fig. 31.4). Around one-third of cases also show gastrointestinal symptoms such as diarrhea and vomiting.

The course of the illness is relatively short, with the disease progressing rapidly through a number of stages by 7 to 11 days after presentation. The lower respiratory tract is involved early in acute infection. Chest radiographs are consistent with viral pneumonitis, with bilateral infiltrates, segmented or lobular opacities and pleural effusions.

In common with other human coronaviruses, MERS-CoV evades the host innate immune response and causes a rapid dysregulation of cellular transcription pathways. The result is extensive apoptosis of bystander cells, to a far greater extent than is the case in SARS.

The cellular receptor for MERS-CoV has been identified as CD26, a dipeptyl peptidase, in contrast to the angiotensin-converting enzyme 2 (ACE2) used by SARS-CoV. This receptor is involved in the regulation of cytokine responses as well as glucose metabolism. Antibodies to the S binding domain of MERS-CoV efficiently neutralize infectivity.

Laboratory Diagnosis

MERS-CoV RNA can be detected in blood, urine, and stool as well as in respiratory aspirates by RT-PCR. In investigating and controlling a new outbreak such as this, it is essential that a consistent policy for testing and interpreting test results is applied to the diagnosis, management, quarantine, and reporting of cases. WHO provide such recommendations on their website www.who.int.csr.

Epidemiology

Dromedary camels have been implicated as the primary source of infection for humans, with a high percentage possessing viral antibodies. However, the exact route of transmission from camels is not clear. A recent report of MERS-CoV sequences in bats trapped in Saudi Arabia adds to the puzzle. Although the majority of hospitalized cases are thought to be secondary cases resulting from human-to-human transmission in health-care settings, MERS-CoV is not considered to be highly transmissible with an R_0 at <1—there is no evidence for ongoing spread within the community (see Chapter 13: Epidemiology of Viral Infections for further details of virus transmissibility).

Phylogenetic analysis of related sequences suggests MERS-CoV has diverged from a common ancestor as recently as 2007 to 2010. The discovery of another

coronavirus in camels closely related to bovine coronavirus suggests that camels could be acting as intermediate hosts. Closely related viruses have been recovered from bats, and two amino acid changes in the spike protein of one of these viruses allows it to be activated by human proteases and to infect human cells. This suggests a mechanism for this virus to jump species from bats to humans.

Treatment and Prevention

To date, treatment has been focused on supportive therapy in the absence of any specific intervention measures. The use of antimicrobials to minimize the risk of opportunistic infection has been employed. Attempts to reverse the progression of respiratory distress and fibrosis through the use of corticosteroids have been unsuccessful.

Prevention of infection involves avoiding exposure to camels including consuming raw camel milk and inadequately cooked meat, particularly for those with diabetes, chronic lung disease, renal impairment, the immunocompromised, or the elderly. Confirmed cases should be isolated to avoid nosocomial spread.

FURTHER READING

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