Human Coronavirus Respiratory Infections

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27.1 INTRODUCTION

 Coronaviruses are enveloped, positive-sense, single-stranded, ribonucleic acid (RNA) viruses in the family Coronaviridae, of the order Nidovirales.1-3 Viral particles are typically 120–160 nm in diameter and the genomic RNA is capped and polyadenylated with an average length of 27–31 kb.4 Virions are composed of a flexible core, formed by the viral RNA and multiple copies of the nucleocapsid (N) protein, surrounded by the viral membrane, which consists of the spike (S), envelope (E), and membrane (M) proteins.1 The S proteins are heavily glycosylated and this feature is necessary for establishment and maintenance of infection.1 The RNA genome contains at least six open reading frames (ORFs). The 5’ end of the genome encompasses ORF 1, which comprises the majority of the genome (approximately two-thirds). ORF1 is subdivided into ORF1a and ORF1b, which are translated to two polyproteins, pp1a and pp1ab (Figure 27.1). Translation of pp1ab follows pp1a after a −1 frameshift. These are then cleaved into up to 16 viral replicate proteins by the virus-encoded protease 3CLpro.5 The structural proteins, S, E, M, and N, are encoded by the remaining one-third of the genome (Figure 27.1). An additional hemagglutinin esterase (HE) protein is encoded by a subset of coronaviruses and plays an important role for infection in the target host species, but is not required for viral replication.3-6 S protein trimers are arranged radially within the envelope, forming peplomers that give the virus its crown-like (corona) morphology (Figure 27.2).7

 The N protein plays a critical role in viral encapsidation. It has also been shown to act as an interferon antagonist. The E proteins are involved in virus assembly and budding, and their absence leads to attenuation in vitro and in vivo due to partial or complete inhibition of viral release.8-10 In addition to the replication and structural proteins, there are accessory proteins that vary in number depending on viral strain. These are not required for viral replication in cell culture, but some evidence suggests they may be important for replication in the natural host.3,11

 Six coronaviruses are known to infect humans: human coronaviruses (HCoVs) 229E, OC43, NL63, HKU1, severe acute respiratory syndrome coronavirus (SARS-CoV), and Middle East respiratory syndrome coronavirus (MERS-CoV).2,12 HCoVs 229E and OC43 were first discovered in the
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FIGURE 27.1 (See color insert.) (a) Schematic diagram of representative genomes from each of the coronavirus groups. Approximately the first two-thirds of the 26–32 kb, positive-sense RNA genome encodes a large polyprotein (ORF1a/b; green) that is proteolytically cleaved to generate 15 or 16 nonstructural proteins (nsps; nsps for severe acute respiratory syndrome coronavirus [SARS-CoV] are illustrated). The 3′-end third of the genome encodes four structural proteins—spike (S), membrane (M), envelope (E), and nucleocapsid (N) (all shown in blue)—along with a set of accessory proteins that are unique to each virus species (shown in red). Some group 2 coronaviruses express an additional structural protein, hemagglutinin esterase (not shown). (b) Schematic diagram of the coronavirus virion. 2′OMT, ribose-2′-O-methyltransferase; ExoN, 3′5′ exonuclease; Hel, helicase; IBV, infection bronchitis virus; NendoU, uridylate-specific endoribonuclease; RDRP, RNA-dependent RNA polymerase; ssRBP, single-stranded RNA binding protein; ssRNA, single-stranded RNA; TGEV, transmissible gastroenteritis virus. (Reproduced with permission from Perlman S, Netland J. Nature Reviews Microbiology. 2009; 7(6): 439–50.)

FIGURE 27.2 Thin-section electron micrograph of the surface of an infected FRhK4 cell showing SARS coronavirus with spikes. Bar = 100 nm. (Reproduced with permission from Poon LL et al. The Lancet Infectious Diseases. 2004; 4(11): 663–71.)
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1960s, whereas SARS-CoV was identified in 2003 and NL63 and HKU1 were identified in 2004 and 2005, respectively. Four of these viruses (HCoV 229E, OC43, NL63, HKU1) are known to circulate continuously in the human population.

There are three serogroups of coronaviruses, and within these serogroups, viruses are further subdivided based on their host range and genomic sequence. Group 1 contains HCoVs 229E and NL63; group 2 contains HCoVs HKU1 and OC43 and SARS-CoV; and group 3 contains MERS-CoV.

Although receptors for many coronaviruses are still unknown, several receptors have been identified. These include aminopeptidase N for multiple group 1 coronaviruses, 9-O-acetylated sialic acid for multiple group 2 coronaviruses, including HCoV-OC43, and angiotensin converting enzyme 2 (ACE2) for HCoV-NL63 and SARS-CoV. Although both HCoV-NL63 and SARS-CoV bind to ACE2, these viruses bind to different portions of the receptor and with different affinity. This difference contributes, in part, to the different clinical outcomes: mild disease for HCoV-NL63 infection and severe respiratory disease for SARS-CoV.

HCoV-NL63 was first isolated in the Netherlands from a 7-month-old child suffering from bronchiolitis, conjunctivitis, and fever. The virus was isolated using a virus-discovery-cDNA-amplified restriction fragment-length polymorphism technique (VIDISCA). VIDISCA is a novel and rapid technique for amplification of unknown genomes based on the cDNA AFLP technique and it does not require prior knowledge of the genomic sequence. Based on the genomic sequence, this virus is classified as a group 1 coronavirus and is most closely related to HCoV-229E. However, unlike HCoV-229E, which is fastidious and exhibits a narrow host range in vitro, HCoV-NL63 replicates efficiently in monkey kidney cells. The HCoV-NL63 viral genome contains several notable features, including a unique N-terminal fragment within the S protein.

### 27.2 EPIDEMIOLOGY

Infections of the respiratory tract rank as one of the top three causes of death in children under the age of 5 years worldwide, and while many of these infections are of unknown cause, HCoVs have been suggested as the etiological agent for up to 10% of all respiratory diseases.

The first cases of severe acute respiratory syndrome (SARS) were reported in Foshan, in the Guangdong province of China in November 2002. Within months, the disease spread to 29 countries worldwide, ultimately affecting greater than 8000 patients and causing close to 800 fatalities. An important feature that contributed to the 2002–2003 SARS epidemic was the ability of SARS-CoV to cross species from animals to humans. The virus was isolated from Himalayan palm civets, raccoon dogs, and Chinese ferret badgers found in a live-animal market in Guangdon, China where transmission to animal handlers occurred. However, subsequent screening suggested that these animals were not the primary reservoir for SARS-CoV. Rather, Chinese horseshoe bats have been suggested as an animal reservoir, and the virus may have spread from bats, to the mammals listed above, to humans (Figure 27.3). One patient who acquired SARS in Guangdong traveled to Hong Kong, and served as the index case for greater than 50% of the total cases of SARS, highlighting the efficacy of modern global travel for spreading infectious disease. SARS-CoV was spread primarily via airborne droplets and fomites.

HCoV-NL63 and HCoV-HKU1 are associated with acute respiratory disease in young children (less than 1 year of age) and immunocompromised adults. Based on RT-PCR screening, HCoV-NL63 virus was detected in 7% of patients suffering from respiratory disease at one hospital during January 2003, whereas no samples collected during the spring or summer of 2003 were positive.

In June, 2012, a novel coronavirus was isolated from the sputum of a man in Jeddah, Saudi Arabia who presented with acute pneumonia and respiratory failure. This isolate was provisionally termed human coronavirus Erasmus Medical Center (HCoV-EMC), and later renamed Middle East respiratory syndrome coronavirus (MERS-CoV). Since the index case in Saudi Arabia, 94
laboratory-confirmed and an additional 16 probable cases of MERS-CoV have been reported to the World Health Organization (WHO) as of August 2013. Of the 47 laboratory-confirmed MERS-CoV cases, 60% were fatal. The majority of these patients had preexisting medical disorders, including diabetes mellitus, hypertension, and chronic cardiac or renal disease, or immunosuppression. MERS-CoV has spread throughout the Arabian peninsula and Europe, and laboratory-confirmed cases have been reported in the following countries as of August 2013: Saudi Arabia, Jordan, Qatar, United Kingdom, Germany, France, Italy, Tunisia, and the United Arab Emirates. Infection with MERS-CoV in the index case from the United Kingdom was linked to travel to Pakistan and Saudi Arabia.

The specific mode of viral transmission for MERS-CoV is currently unknown, but most likely includes droplet and contact transmission. Limited person-to-person transmission has been documented, and the timing of symptom progression in the index and secondary cases suggested an incubation period ranging from 1 to 9 days.

27.3 CLINICAL FEATURES

The first two human coronaviruses, HCoV-OC43 and HCoV-229E, were identified in the 1960s. These viruses cause mild upper respiratory tract infections, similar to the common cold, and are occasionally associated with more severe lower respiratory tract disease in newborn, elderly, or immunocompromised individuals.

The newer CoVs, NL63 and HKU1, are associated with mild infection of the upper respiratory tract, although severe lower respiratory tract disease is uncommonly reported. Affected patients are primarily young children or adults with underlying disease or immunosuppression.

SARS emerged in the Guandong province of China in November 2002. Patients with SARS-CoV initially developed fever, a nonproductive cough, and myalgia. This progressed to dyspnea and hypoxia approximately 7–14 days following onset of clinical signs, often with concurrent diarrhea. A subset of patients developed rapidly progressive respiratory failure, leading to acute respiratory distress syndrome (ARDS), requiring mechanical ventilation. A peculiar feature of SARS was apparent worsening of clinical signs as the infection cleared. This was later determined to be due to tissue damage secondary to an overexuberant host immune response to the virus. In addition, patients were not typically contagious until the development of lower respiratory tract signs.

The initial case of MERS-CoV was identified in a 60-year-old man from Bisha, Saudi Arabia who presented to a hospital with a week-long history of fever, cough, expectoration, and shortness of breath.
Diagnostic imaging was consistent with pneumonia, and the patient had no previous history of smoking or cardiopulmonary disease. Despite antimicrobial therapy, the patient’s respiratory signs worsened and he underwent intubation for mechanical ventilation. Blood urea nitrogen and creatinine progressively increased, beginning 3 days after admission, indicating renal failure. In addition, a progressive leukocytosis, characterized by neutrophilia, lymphopenia, and thrombocytopenia, began 8 days following admission. The patient died 11 days after hospital admission of respiratory and renal failure and no autopsy was performed. In the majority of patients infected with MERS-CoV, respiratory disease runs a similar rapidly progressive and often fatal clinical course. Typical clinical signs include fever, chills or rigor, cough, hemoptysis, shortness of breath, dyspnea, and myalgia. Concurrent gastrointestinal signs are typically present, including diarrhea, vomiting, and abdominal pain. Elevated lactate dehydrogenase and aspartate aminotransferase, thrombocytopenia, and lymphopenia are consistent abnormal clinical laboratory findings.

Recent evidence suggests that a spectrum of disease severity may be observed. In a family cluster of MERS-CoV cases, three young men, who became infected from an elderly male relative, displayed a range of clinical signs. One patient presented with fever, anorexia, and a productive cough with blood-tinged sputum, and later died. The other two family members had mild fever, malaise, cough, and sore throat, which resolved. The elderly male relative was hospitalized with fever, urinary retention, flank pain, diarrhea, renal colic, and urinary tract infection. He later died of cardiogenic and septic shock, although, interestingly, none of the 124 attending staff members at the hospital became ill, and an additional 24 family members living in the same household were presumably exposed and did not contract the disease. Additional case reports from Tunisia, the United Kingdom, Saudi Arabia, France, Germany, and Italy support the possibility of nonfatal respiratory illness due to MERS-CoV infection. Thus, the spectrum of respiratory disease may range from mild to severe in cases of MERS-CoV. The presenting clinical symptoms and presentation of MERS-CoV bear some similarities to those reported during the SARS-CoV outbreak, including the mean incubation period, propensity for infecting adults, and common clinical signs. However, there are some notable differences: the incidence of MERS-CoV is approximately three times higher in males than females, compared to an equal sex distribution for SARS-CoV; comorbidities are more frequent (>90%) in patients with MERS-CoV compared to SARS-CoV (10–30%), and MERS-CoV tends to be more rapidly fatal, necessitating greater ventilatory support compared to SARS-CoV.

27.4 PATHOGENESIS AND IMMUNITY

An overactive host immune response has been associated with many of the diseases associated with coronavirus infections. A similar phenotype was observed in human patients infected with SARS-CoV, namely, that clinical disease worsened 1–2 weeks following initial infection. This was due to bystander destruction of the respiratory system following the host immune response to viral infection. An inadequate T cell response, resulting in delayed viral clearance, contributes in part to this phenomenon. However, the relative success or failure of the initial innate immune response determines the extent of initial virus replication. The host interferon response is critical in limiting viral replication, and coronaviruses have developed multiple strategies to subvert interferon induction. SARS-CoV replicates in double-membrane vesicles, and it has been suggested that these serve to shield viral double-stranded RNA (a potent interferon stimulator) from infected cells, thus preventing signaling through RIG-I, MDA-5, and TLR3. Multiple viral proteins have been shown to directly inhibit induction of interferons, including nspl, nsp3, N protein, and the accessory proteins ORF6 and ORF3b. Specifically, the N protein of SARS-CoV inhibits NF-κB. In addition to acting on interferons, SARS-CoV induces multiple proinflammatory chemokines and cytokines, including IL-1, IL-6, IL-12, IL-8 CCL2, and CXCL10.

The development of antiviral T cells and neutralizing antibody (NA) are critical for virus clearance. The primary determinant of NA production for SARS-CoV is the S glycoprotein. Within
the S glycoprotein, the receptor-binding domain of the S1 region contains multiple epitopes that induce NA.45

Patients that succumbed during early SARS had diffuse alveolar damage that included hyaline membrane formation, edema, vascular thrombosis, fibrinous exudate, pneumocyte loss and sloughing, and mixed inflammation consisting of lymphocytes, macrophages, and neutrophils. Those that died following chronic infection exhibited lung lesions that included type II pneumocyte hyperplasia, squamous metaplasia, syncytial cell formation, and bronchiolitis obliterans (Figure 27.4).7

27.5 DIAGNOSIS

Following the initial outbreak of respiratory disease in the SARS epidemic, SARS-CoV was identified as the causative agent within weeks, and initial protocols for diagnosis were rapidly disseminated. Koch’s postulates were later fulfilled by infecting cynomolgus macaques (Macaca fascicularis) with SARS-CoV.46,47 This progress in identifying the etiology and development of laboratory diagnostic tests was achieved through a WHO-facilitated virtual multinational laboratory network, which selflessly shared data in real time. The virus was first successfully cultured in Vero-E6 and FRhK-4 cells.22 SARS-CoV was detected via RT-PCR and culture in samples from the respiratory tract, gastrointestinal tract, feces, urine, and cerebrospinal fluid. Although viral RNA has been identified in high levels in feces from SARS patients, some reports indicate that fecal samples may not be suitable for detecting early infections due to low initial levels of viral RNA in the gastrointestinal tract.48-50 The WHO criteria for confirmation of SARS-CoV infection require: (1) detection of viral RNA by PCR, (2) increase in viral antibody titers in body fluids, or (3) isolation of SARS-CoV from clinical isolates.
Three primary laboratory diagnostic methods for SARS-CoV have been developed: (1) viral RNA detection using reverse transcription polymerase chain reaction (RT-PCR), (2) antibody detection using immunofluorescence assay (IFA), and (3) enzyme-linked immunosorbent assay (ELISA) targeting the N protein. ELISA is frequently used for routine screening for SARS-CoV, as it is a rapid and inexpensive test. However, serodiagnosis using indirect IFA is reported to be the gold standard for confirmatory diagnosis of SARS-CoV. RT-PCR assays have been developed for rapid detection that target the polymerase 1b (pol 1b) region of the 5' replicase gene and the nucleocapsid gene. One study investigating the detection rate of SARC-CoV in various clinical samples using RT-PCR determined that optimal detection occurred 2 weeks after the onset of clinical signs for respiratory samples (tracheal/nasopharyngeal aspirates and nasal swabs) and between 2 and 3 weeks for fecal or rectal swabs. One-step RT-PCR assays are available that detect multiple coronaviruses, including SARC-CoV, HCoV-OC43, and HCoV-229E. Indirect IFA has been validated as a highly specific test for the early detection of anti-N antibodies during early SARS-CoV infection. One study comparing IFA to Western blot and ELISA reported 89% sensitivity and 100% specificity for IFA. Detection of viral antigen in various body fluids, including serum, is accomplished using ELISA assays that recognize the highly conserved N protein. One specific ELISA capture was found to detect SARS-CoV with a sensitivity of 84.6% and a specificity of 98.5% when samples were obtained between 6 and 10 days of infection. Virus isolation is more cumbersome than the previously described diagnostic techniques for SARS-CoV, as it is less sensitive and requires a BSL-3 facility.

Similar to SARS-CoV, RT-PCR assays have been developed for the detection of MERS-CoV. One assay targeting the region upstream of the E gene (upE assay) was developed as a screening tool for rapid detection of MERS-CoV. Highly sensitive confirmatory RT-PCR assays targeting the ORF 1a and 1b genes have also been described.

### 27.6 TREATMENT

Although respiratory coronavirus infections were brought to the forefront of the medical and scientific scenes with the 2003 SARS outbreak, clinically approved drug treatments and vaccinations are still absent a decade later. The most promising methods of treatment and prevention identified thus far include antiviral therapy, passive immunotherapy with human monoclonal antibodies, and active immunization.

Some advancement has been made with regard to identifying potential antiviral candidates, most of which target the S protein, nsp 13 helicase, or the 3C-like protease (3CLpro). Synthetic peptides that competitively bind S protein can inhibit viral entry by preventing the conformational changes necessary for S protein during viral fusion. Lectins, and other substances that bind carbohydrates, can diminish infection by binding viral glycoproteins like S protein and creating steric hindrance during attachment, fusion, and entry. The efficacy of lectins as a therapeutic route depends upon the amount of glycosylation present on the host cell, which can vary depending upon the cell type infected.

Peptidomimetic inhibitors, consisting of a peptide with specificity for the ligand of interest and a chemically reactive warhead, also afford potential therapeutics. One such inhibitor, possessing a peptide specific for the 3CLpro catalytic site and a nitrile warhead, demonstrated inhibition of 3CLpro. Broad-spectrum protection can theoretically be achieved with such peptidomimetic inhibitors that target 3CL proteases, since these proteins are highly conserved among coronaviruses, caliciviruses, and picornaviruses. As a natural alternative, Salvia miltiorrhiza root, a common remedy for cardiac maladies in Asia, contains tanshinones as an active component, which exert specific inhibition against SARS-CoV proteases 3CLpro and PLpro. Several natural and synthetic chemicals can also inhibit nsp13, either by interfering with the ATPase activity or with the DNA unwinding ability.

During the 2003 SARS outbreak, several broad-spectrum antiviral drugs were tested in infected individuals but none were shown to have been effective, and several may have even had deleterious
Currently, no antiviral drugs have advanced to clinical testing in humans. More research is necessary in vitro and in vivo in animal models to ensure safety and efficacy.

Monoclonal and polyclonal antibodies are capable of interfering with coronavirus attachment, and the simultaneous use of multiple monoclonal antibodies has been tested successfully in vivo in animal models. In addition to offering better protection, using multiple antibodies can also prevent the potential problem of developing antibody-resistant viral strains. Several neutralizing human monoclonal antibodies have been developed; most bind within or near the receptor binding domain of the S protein and offer good therapeutic potential.

While some SARS-CoV NAs provide protection in vitro, others induce infection in immune cells by antibody-dependent enhancement (ADE), and the extent of ADE directly correlates with the neutralization titer. In one study, several protective neutralizing SARS-CoV antibodies were tested as candidate vaccines in a mouse model but were found to elicit a Th2 response, indicating an immune hypersensitivity that would be inadequate for direct progression to clinical trials. Using a mouse model to study the difference in vaccine efficacy between young and old individuals, Bolles et al. found that young mice receive better protection against both homologous and heterologous challenge than did older mice. Thus, ADE and immunosensitivity are current hurdles for the development of efficacious vaccines, and more study is needed to elucidate how age and other similar factors may affect vaccine efficacy.

Vaccine candidates have been identified and tested in vitro and in animal models but have not reached clinical testing stages. Inactivated coronavirus vaccines and subunit coronavirus vaccines have been developed, and some testing has been undertaken in vivo. PIKA, derived from Poly (I:C), has been shown to be a helpful adjuvant for SARS-CoV inactivated vaccines in order to achieve adequate mucosal immunity. More in vitro and in vivo study is necessary with these and other vaccine candidates to ensure safety and efficacy prior to proceeding to clinical trials.

Many chemical compounds have been identified as potential adjuvants or scaffolding for vaccines or as antiviral substances themselves, including both natural plant-based compounds and synthetic compounds. Some have been designed to provide specific protection against coronaviruses while others provide more broad-range protection against viruses with differing taxonomy. As with the current vaccine candidates, these chemical compounds require further study before they can be utilized in clinical trials.

Respiratory coronaviruses afford several options for therapeutic targets, including the S protein at viral attachment and entry; the nsp13 helicase, which functions during replication; and the 3CLpro needed for viral proteolytic cleavage. Research in recent years has identified a variety of possible antiviral treatments and preventative vaccine candidates. Future endeavors toward establishing a human respiratory coronavirus treatment should focus on establishing safety and efficacy in vitro and in vivo so that clinical trials can proceed.

REFERENCES


