Chapter 4
Emerging Animal Coronaviruses: First SARS and Now MERS

4.1 Introduction

The severe acute respiratory syndrome [SARS] first appeared in southern China [Guangdong Province] in November 2002, as an atypical community pneumonia [1]. Within a year, the World Health Organization [WHO] reported 8096 cases with 774 deaths [9.6% fatality] in >30 countries from five continents, and the outbreak was declared a pandemic infection [1]. In the elderly and subjects with significant comorbid illness, the mortality rate was up to 50%. Investigations revealed that SARS was due to a novel coronavirus [SARS-CoV] which was circulating among wild game animals in wet markets of southern China. The palm civet, a wild feline, was considered the amplification host that transmitted the virus to humans from occupational contact and handling by consumers during the preparation for consumption as a delicacy [2, 3]. SARS-CoV mainly spread from human to human by respiratory droplets or by contact of mucosae with contaminated fomites. Spread of SARS-CoV from mainland China to Hong Kong and other countries was from interpersonal transmission in healthcare facilities, homes, workplaces, and public transports [1].

The Middle East respiratory syndrome [MERS] coronavirus [MERS-CoV] was first isolated from a patient with fatal pneumonia in September 2012, in Jeddah, Saudi Arabia [4]. From September 2012 through July 2014, WHO reported at least 834 laboratory-confirmed cases of MERS with 288 deaths [34.5% fatality]; and known cases were directly or indirectly linked to countries in the Arabian Peninsula [5]. However, an outbreak of MERS was subsequently reported from South Korea in June 2015. The illness occurred in an older man who returned from traveling to Saudi Arabia, and within a month 17 secondary cases occurred in South Korea which was connected to the index case [6]. Within 2 months the local outbreak resulted in a total of 186 confirmed cases of MERS in South Korea, except for one case exported to China. There were 36 deaths attributed to MERS-CoV infection with a mortality rate of 19.4% [6].

4.2 Virology

Coronaviruses are the largest RNA viruses and they are enveloped with positive-strand genomes of 26–32 kb, and they are distributed globally in a wide range of animals and humans [7]. The coronaviruses are classified in four genera based on phylogenetic analysis: alpha-coronaviruses, beta-coronaviruses, gamma-coronaviruses, and delta-coronaviruses [7]. Some strains of coronaviruses, HCoV-OC43 [a beta-coronavirus] and HCoV-229E [an alpha-coronavirus], are causative agents of the common cold and rarely severe respiratory disease [8, 9]. Other newly discovered human coronaviruses, HCoV-NL634 and HCoV-HKU1, are occasionally associated with severe lower respiratory tract infection in infants and immunocompromised patients [10, 11]. SARS-CoV is a lineage B beta-coronavirus and MERS-CoV is a novel beta-coronavirus of lineage C, and both these viruses appear to have crossed the species barrier from bats to humans [12, 13]. Bats are reservoirs for many mammalian coronaviruses [13–15]. Various SARS-like coronaviruses have been found in bats from China, Asia, and Europe, but none were considered as direct progenitor of SARS-CoV. Recently, however, investigators from China reported whole genome sequences of two novel bat coronaviruses from Chinese horseshoe bats closely related to SARS-CoV [16]. The receptor-binding domain of the spike protein was very similar to that of SARS-CoV, and one of the isolates [bat SL-CoV-w1V1] uses the angiotensin-converting enzyme 2 [ACE2] from humans, civets, and Chinese horseshoe bats for cell entry [16]. SARS-CoV was previously shown to use human ACE2 molecule as its entry receptor, an outstanding feature of its cross-species transmissibility [17]. Thus, the bat SL-CoV-WIVI may be the ancestor virus that precedes the evolution of SARS-CoV in humans.

The respiratory epithelial cells of humans are the main targets of SARS-CoV, but the virus can also be found in immune cells in the circulation [lymphocytes and macrophages] and in various organs [18]. MERS-CoV, on the other hand, uses human and bat dipeptidyl peptidase-4 [DPP4] as receptor for cell entry [19]. Replication and RNA protein synthesis by MERS-CoV can occur in human airway epithelial cells, lung fibroblasts, microvascular endothelium, and alveolar type II pneumocytes [20]. Hence MERS-CoV has a broader tissue tropism than the SARS-CoV. The mechanism of the SARS-CoV and MERS-CoV interspecies transmission appears to be mediated by the S protein, by mediating receptor recognition and membrane fusion, a key factor in host specificity [21].

4.3 Pathogenesis

It has been postulated that the primary mechanism of SARS is immune suppression resulting from damage to the immune cells of the spleen, lymph nodes, and lymphoid tissue with severe lymphopenia [18]. Furthermore, Gu et al. [18] estimated

that the extent of immune cell damage is a better predictor of outcome than the damage to the lungs. Other investigators judge the lung injury and subsequent acute respiratory distress syndrome [ARDS] and pulmonary fibrosis as the primary events leading to adverse outcome and death. Using multiple modes of investigation, modeling with gene sets, proteomic analysis, and histopathology correlation, these investigators conclude that the urokinase and the extracellular fibrinolytic pathways are the primary mechanisms involved in lung damage and overall SARS-CoV infection pathogenesis [22]. Events driven by these pathways result in imbalance between the host coagulation and fibrinolysin pathways, ultimately leading to diffuse alveolar and acute lung damage. It is likely, however, that both mechanisms are involved in the pathogenesis of SARS.

MERS-CoV infection has been associated with severe pneumonia and multiorgan dysfunction with higher mortality rate than SARS. A recent study compared the viral replication, cytokine/chemokine response, and antigen presentation in MERS-CoV-infected human monocyte-derived macrophages [MDMs] and SARS-CoV-infected MDMs [23]. Only MERS-CoV and not SARS-CoV could replicate in human macrophages, but both viruses could not stimulate the expression of antiviral cytokines, interferon-alpha [IFN-α], and IFN-β. Comparable levels of tumor necrosis factor [TNF]-α and interleukin [IL]-6 could be induced by both viruses. However, MERS-CoV induced significantly higher levels of IL-12, IFN-¥, and several chemokines than SARS-CoV [23]. In addition, the expression of major histocompatibility complex [HLA] class 1 and costimulatory molecules were greater in MERS-CoV-infected MDMs than SARS-CoV-infected cells. The establishment of productive infection in macrophages and dendritic cells results in impaired antigen-presenting pathway, and greater aberrant induction of cytokines/chemokines by MERS-CoV could explain the higher severity of infection and greater mortality than SARS-CoV infection [23]. Furthermore, it has recently been shown that MERS-CoV has the capacity to infect T lymphocytes and induce apoptosis of these cells by activation of the extrinsic and intrinsic apoptosis pathways [24]. MERS-CoV-derived proteins inhibit IFN-α/IFN-β expression [25], resulting in lower IFN levels in the respiratory tract [26], and lower expression of type 1 IFN in fatal cases [27]. Persistent expression of proinflammatory cytokines, neutrophil activation, and chemotactic response can result in damage to surrounding uninfected lung tissues [27]. Furthermore, MERS-CoV can impair activation of the adaptive immunity through multiple mechanisms: downregulation of antigen-presenting pathways to inhibit activation of T-cells [28], infection of CD4+ and CD8+ T-cells of peripheral blood lymphoid organs [tonsils and spleen], and extensive apoptosis of T-cells can result in impaired T- and B-cell function as the helper T-cells are also affected.

The spike [S] and nucleocapsid [N] proteins are the major immunogenic components of the coronaviruses and are produced in large quantities during infection. Antibodies against the S and N proteins have diagnostic and therapeutic potentials [28–30]. The S protein appears to be the main determinant of protective immunity and cross-species transmission in SARS-CoV and other emerging animal coronaviruses [31]. In mice antibodies against S protein protect against SARS-CoV challenge but antibodies against N protein produced limited protection [32].

4.4   Transmission

The initial cases of SARS in 2003 and the Guangdong outbreak of 2004 were related to game animals contact in wet markets, or handling for consumption [3]. However, secondary cases [the majority] were from exposure to infected droplets or contaminated fomites from other infected patients. Airborne transmission of SARS-CoV was considered rare or unlikely, but may have occurred in one community outbreak from generation of negative pressure by exhaust fans with dissemination of contaminated aerosols from sewage drains [33]. Nosocomial transmission of SARS-CoV, which was a major source of the outbreaks in Hong Kong and Toronto, was enhanced with the use of nebulizers, suction, intubation, bronchoscopy, or cardiopulmonary resuscitation, from generation of high amount of infectious droplets [1]. It was estimated during the height of the SARS pandemic that each single case resulted in an average of 2–4 secondary cases, but some patients were superspreaders of the virus and could infect larger number of people [1].

MERS-CoV infection outbreak epidemiology strongly suggested zoonotic transmission through an intermediate animal host. Genomic analysis of the virus indicated that the MERS-CoV arose from a bat coronavirus [34, 35]. This is supported by the presence of a small fragment of genomic sequences identical to the MERS-CoV Essen isolate [KC875821] in an Egyptian tomb bat [*Taphozous perforatus*] found in Saudi Arabia [36]. There is increasing evidence that camels are the intermediate hosts responsible for cross-species transmission to humans. Cross-reactive antibodies to MERS-CoV have been found in dromedary camels in Oman, Canary Islands, and Egypt [37, 38]. Further studies have also confirmed the presence of MERS-CoV RNA by real-time reverse transcriptase polymerase chain reactor [RT-PCR] assay and partial genome sequencing of viral RNA in 3 of 14 nasal samples collected from 14 camels in Qatar and two subjects from the same farm [39]. In addition, a fatal case of human MERS-CoV infection was transmitted through contact with an infected camel with rhinorrhea, and the full genome sequence of the isolates from the patient and the camel was identical [40]. It has been recently reported that a high proportion of dromedaries at a slaughterhouse shed nasal MERS-CoV, with a high-risk of human exposure and potential of driving the epidemic [41].

Similar to the epidemiology of SARS, most cases of MERS occurred from human-to-human transmission from respiratory droplets, contamination of mucosae with infected fomites, and direct contact in various settings. There is no direct evidence of airborne transmission of infectious aerosols. Household contact clusters were associated with 26 index patients infected with MERS-CoV in 2013 in Saudi Arabia. Investigations for secondary transmission to 280 household contacts, using serology and RT-PCR from throat swabs, identified secondary transmission in 6 of 26 clusters [23%], but only 12 secondary infected persons for a transmission rate of only 5% [42]. However, most cases of MERS in the Arabian Peninsula resulted from transmission were associated with direct or indirect contact with healthcare facilities, from patients with unrecognized infection to other patients, visitors, and healthcare personnel [39]. In the largest single outbreak in Jeddah,

Saudi Arabia, in 2014, the majority of patients with MERS-CoV infection had contact with healthcare facility, other patients, or both [44]. There were 255 laboratory-confirmed MERS-CoV infection of which 64 subjects [25.1%] were asymptomatic and 93 of the ill patients died, with an overall mortality of 36.5%. However, many of "asymptomatic" subjects could recall symptoms consistent with a mild respiratory infection or illness. Over 90% of the symptomatic patients [excluding healthcare personnel] had contact with healthcare facility, persons with confirmed MERS, or someone with severe respiratory illness in the preceding 14 days before onset of their illness [44].

The largest outbreak of MERS outside of the Middle East occurred in South Korea in 2015, with 186 confirmed cases and 36 [19%] deaths. Healthcare facilities were the major sources of the outbreak, with four hospital clusters accounting for 82% of all the cases. Investigation in isolation wards found extensive viable MERS-CoV contamination of the air and surrounding materials in MERS units, raising concern of the adequacy of current infection control procedures [45].

The interhuman transmissibility of MERS-CoV has been estimated by using Bayesian analysis to calculate the basic reproduction number [Ro]. The estimated Ro for MERS-CoV was 0.60–0.69 [95% confidence interval (CI) 0.42–0.92], whereas prepandemic SARS-CoV Ro was 0.80, CI 0.54–1.13 [46]. When Ro is above 1.0, a pandemic potential exists; hence MERS-CoV was not considered of pandemic potential. The cycles of transmission of SARS-CoV and MERS-CoV are demonstrated in Fig. 4.1a, b.

4.5 Clinical Features

The clinical features of SARS and MERS are very similar and usually mimic influenza infection or nonspecific viral illness initially. The incubation period of SARS was estimated to be 2–14 days with most cases occurring within 10 days after exposure, and transmission from symptomatic patients usually occurred after 5 days of illness [1]. This was related to the rising viral load in nasopharyngeal secretions, which peaked around day 10 of illness. In SARS the initial symptoms were fever, chills, myalgia, malaise, and nonproductive cough, and sore throat and rhinorrhea were less frequent [1]. Clinical deterioration in those with severe illness usually occurred after several days of infection, often heralded by development of diarrhea, evidence of pneumonia, and then respiratory distress. The most common extrapulmonary manifestations of SARS were diarrhea, hepatic dysfunction, cardiac impairment, myositis, and seizures [1]. Milder disease occurred in children in both SARS and MERS, and increased morbidity and mortality were seen in the elderly, those with significant comorbid illnesses [diabetes, heart disease, etc.], and pregnancy. In severe cases with ARDS requiring mechanical ventilation, renal failure was a complication. Survivors of severe SARS could develop residual pulmonary fibrosis, muscle weakness, and depression even 6 months after the acute illness [1].
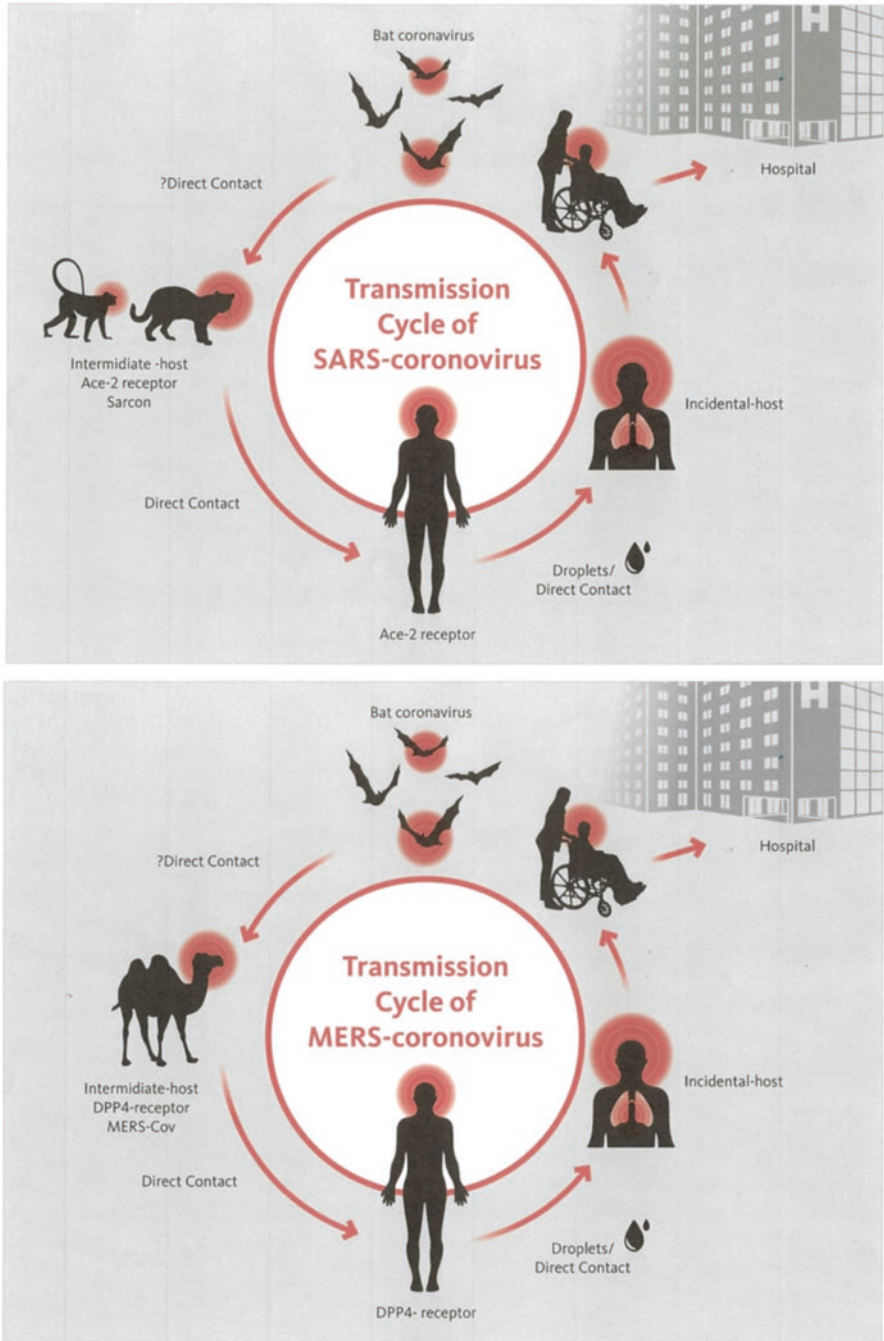
Fig. 4.1 (**a**) Transmission cycle of SARS coronavirus. (**b**) Transmission cycle of MERS-coronavirus

Infection with MERS-CoV results in disease clinically indistinguishable from SARS but a greater risk for severe pneumonia. The incubation period is also very similar, ranging from 1.9 to 14.7 days, with a median time of 5 days [43]. Both MERS-CoV and SARS-CoV were more likely to be transmitted from symptomatic patients, as the viral concentration in pharyngeal secretion for those with asymptomatic or mild infection was very low. Current data indicate that most people with MERS-CoV infection develop clinical illness. In symptomatic patients the most common presentations are fever [62–89%], cough [50–89%], shortness of breath [42–56%], chest pain, fatigue [35%], nausea and vomiting [23%], rhinorrhea and sore throat [19% each], diarrhea [15%], and muscle pain or headaches [12% each] [44, 47, 48]. Laboratory findings in MERS may include leucopenia or lymphopenia, thrombocytopenia, elevated liver enzymes, and elevated serum creatinine [47–49]. A combination of more than one system involvement was present in 88%. Pneumonia developed in about two-thirds of the patients with MERS-CoV infection [41], which could lead to respiratory failure, ARDS, acute renal failure, and death.

4.6   Diagnosis

During the SARS outbreak, the diagnosis was based on the potential exposure of the individual to the SARS-CoV at the time and presentation with acute febrile flu-like illness. The diagnosis was confirmed by real-time RT-PCR from a nasopharyngeal aspirate, with a sensitivity of 80% in the first 3 days of illness but very high specificity [50]. Antibody testing was done by various methods, and the virus could be recovered from respiratory secretions, fecal, and occasionally urine specimens by viral culture [1]. Neutralizing antibodies from acute and convalescent sera 3–4 weeks later could confirm the diagnosis, and indirect immunofluorescent antibody test was more commonly used [1]. Enzyme immunoassay [EIA] using recombinant nucleocapsid was a rapid screening test with high sensitivity after 5 days of illness, but could cross-react with other human coronaviruses and needed Western blot test for confirmation [51].

MERS diagnosis was based on clinical presentation with flu-like illness in the appropriate epidemiological setting or recent travel to the Arabian Peninsula or countries with local outbreaks. Real-time RT-PCR from a throat swab has been the primary means of confirming the diagnosis, and serological tests were used primarily for epidemiological investigations [42, 43]. Serological tests include recombinant ELISA with the use of the S1 domain of the MERS-CoV spike protein, recombinant immunofluorescence assay with the full spike protein, and plaque-reduction neutralizing assay. RT-PCR is the preferred diagnostic test for acute cases and the virus can also be cultured in Vero cells [40]. Testing for MERS-CoV should be done not only from nasopharyngeal secretions but also from lower respiratory secretions and serum, as detectable virus had been found on occasion from these sites with negative test from the upper respiratory secretions [52]. This is related to the higher viral load of MERS-CoV in lower respiratory tract than the

upper respiratory tract. The WHO recommends RT-PCR targets of upE, ORF1a, or ORFb, but recent evaluation indicates targeting ORF1b is less sensitive and should not be used for diagnosis [53].

4.7 Pathology and Immunology

There is extensive data on the lung pathology and immunology in patients with SARS, but very little so far on patients with MERS. However, it is expected that the lung pathology of severe MERS cases will be similar to that of severe SARS cases. In patients who died within 10 days of onset of SARS, diffuse alveolar damage with edema was the prominent findings [1]. These findings were accompanied by other changes that can be found in ARDS, hyaline membranes, interstitial infiltrates with inflammatory cells, bronchiolar epithelial cells injury with denudation and loss of cilia, fibrin deposition, and exposure of basement membrane. After 10 days of illness, the pathological changes consist of a mixture of acute changes and reactive process: interstitial and airspace fibroblast proliferation, type II pneumocytes hyperplasia, squamous metaplasia of the bronchial epithelium, alveolar infiltration with macrophages, desquamated pneumocytes, and multinucleated cells [1]. Some cases reveal hemophagocytosis in the alveolar exudate and thrombosis of venules. Rarely [one report], histology of the lungs had revealed vasculitis of the walls of the small veins with edema, fibrinoid necrosis, and infiltration with lymphocytes, monocytes, and plasma cells [54].

Pathological changes outside the lungs in SARS consist predominantly of necrosis and atrophy of lymph nodes and the white pulp of the spleen. Even though the virus can be detected in the enterocytes of the intestines, there was no cellular damage or inflammation. Studies on patients with severe SARS soon after hospitalization had shown decreased natural killer cells, CD4+ and CD8+ T lymphocytes, and B lymphocytes [55, 56]. During the first 2 weeks of SARS, there is intense inflammatory response with elevated proinflammatory cytokines and high viral load [1]. Specific serum antibodies, detected by indirect immunofluorescence or neutralization, appeared around day 10, peak and plateau at about the second month, and persisted for more than 12 months [1].

There is limited data on the pathology and the immune response to MERS-CoV infection in humans. In one study of two patients with MERS, one died and the other recovered; there was evidence that IFN-α generation was critical to initiate a robust immune response [57]. IFN-α usually promotes antigen presentation to drive the antiviral Th1 immune response, mediated by IL-12 and IFN-¥ to clear the virus. MERS-CoV could also upregulate IL-17 expression in humans. In the patient who died, there were low IFN-α and regulatory factors that are involved in the recognition of the virus, whereas these molecules were elevated in the survivor. In addition there were elevated chemokine ligand levels, CXCL10, and IL-10, associated with low IFN-¥ expression in the non-survivor [57]. It is unclear from this report whether the difference in immune response described in the two patients

with different outcome was related to differences in innate immunity or due to viral factors with overwhelming infection suppressing the immunity in the non-survivor. In vitro studies suggest that MERS-CoV induces greater dysfunction of the immune response than SARS-CoV, with downregulation of genes involved in the antigen presentation pathway [28]. To date there has not been any detailed pathological findings of severe MERS cases, probably because of religious customs in the Middle East.

4.8   Management

Clinical management of SARS and MERS were largely supportive care, depending on the severity of the illness. In healthcare settings prompt diagnosis, single room accommodation, and droplet and contact precautions were necessary to prevent nosocomial transmission. Special precautions to prevent airborne transmission were recognized to be important during the SARS outbreak for certain settings in the hospital, during tracheal suctioning, use of nebulizer, bronchoscopy, etc. [58]. Eye protection and airborne precautions should also be applied when caring for proven or suspected MERS-CoV-infected patients when performing aerosol-generating procedures [59, 60]. Antibiotics for treatment of possible community-acquired bacterial pneumonia were usually implemented until the diagnosis of coronavirus infection was confirmed. Severe cases of SARS or MERS usually require intensive care and management of fluid and electrolyte disturbances, mechanical ventilation for respiratory failure, and hemodialysis for renal failure in some cases.

No specific antiviral agents or immune modulators, such as corticosteroids, were of any significant value during the SARS outbreak. Although there was evidence of in vitro activity of IFN-α and IFN-β against SARS-CoV, the results of studies were inconsistent, and in vitro activity of the antiviral agent, ribavirin, used in combination with IFN-α was actually low [1]. However, there was a report of synergistic activity with the combination of IFN and ribavirin against SARS-CoV [61]. Pegylated IFN-α-2a was shown to be effective in reducing viral load and lung pathology in early treatment of SARS-CoV infection in a nonhuman primate model [62]. Treatment of severe cases of SARS with convalescent plasma with high neutralizing antibodies had been used with questionable value [63].

Similar to the experience during the SARS outbreak, no specific therapy had been shown to be of any definite value in severe MERS cases. In a retrospective cohort study of severely MERS-CoV-infected patients, 20 subjects were treated with ribavirin combined with IFN-α-2a compared with 24 patients treated only with supportive care. There was improved survival at 14 days but not at 28 days [64]. In another more recent report, 32 cases of MERS were treated with the combination of ribavirin and IFN-α-2a or IFN-β, with no promising results as the overall mortality rate was 69% [65]. Factors that were associated with increased mortality included age >50 years [odds ratio (OR) = 26.1], diabetes mellitus [OR = 15.74], renal failure

requiring dialysis [100% mortality], and a positive plasma PCR for MERS-CoV, 90% mortality compared to those with negative plasma virus with a mortality of 44% [65].

4.8.1   Animal Experiments

Nonhuman primate models have shown varying response to SARS-CoV challenge. Cynomolgus macaques [*Macaca fascicularis*] demonstrated clinical and pathological features similar to humans infected with SARS-CoV [66]. However, other studies have not reported any overt disease in SARS-CoV-infected cynomolgus, rhesus, and African green monkeys [67, 68]. In the African green monkeys, pathology demonstrated a mild interstitial pneumonitis which resolved by 4 days [68]. A diverse range of animals had been shown to be susceptible to SARS-CoV infection including palm civets, pigs, raccoons, dogs, ferrets, and golden Syrian hamsters; but while viral replication could occur in domestic cats and BALB/c mice, they remain asymptomatic [1].

Several animal species have been experimentally infected with MERS-CoV, rhesus macaques, cynomolgus macaques, marmosets, ferrets, mice, Syrian hamsters, rabbits, and dromedary camels [69]. The outcome and development of lower respiratory tract disease were quite variable in these models. Infection of rhesus macaques resulted in transient clinical signs such as increased body temperature, increased respiratory rate, and cough [70]. Localized pulmonary infiltration and interstitial markings were visible on radiographic imaging. Histopathology after 3 days post-inoculation revealed mild-moderate interstitial pneumonia, with little inflammation in the septa but thickening with edema and fibrin; intra-alveolar infiltration with macrophages, neutrophils, multinucleated giant cells, fibrin, and sloughed epithelial cells; and perivascular inflammatory infiltrate in the interstitium [70]. At day 3 the MERS-CoV could be detected in the lungs by RT-PCR but not in extrapulmonary organs, oropharyngeal and rectal swabs. At day 6 post-infection, there was type II pneumocyte hyperplasia with alveolar edema, fibrin deposition, and hyaline membrane [71]. The viral RNA and antigen could be detected in type I and II pneumocytes and alveolar macrophages. Increased levels of proinflammatory cytokines and chemokines, such as IL-6, CXCL1, and matrix metalloproteinase, were found in serum [71].

Marmosets infected with MERS-CoV developed more severe disease, with clinical signs of respiratory distress, progressive interstitial pulmonary infiltrate visible on imaging but with resolution by day 13 [72]. In other animal species including camels, the virus caused mild or no clinical disease. In dromedary camels nasal discharge with nasal excretion of the virus can be present for 2–14 days [69, 73]. Commonly used laboratory animals such as mice, Syrian hamsters, and ferrets are not susceptible to MERS-CoV infection because of differences in the receptor dipeptidyl peptidase 4 [74]. However, transgenic mice with expression of human DPP4 had been developed that demonstrated severe and lethal respiratory disease

with MERS-CoV infection [74]. Thus transgenic mice with humanized DPP4 receptor are a suitable animal model to study new therapeutics and vaccines for MERS-CoV infection.

4.9 Experimental Antivirals and Vaccines

Several agents were found to have in vitro antiviral activity against SARS-CoV including glycyrrhizin, baicalin, reserpine, niclosamide, chloroquine, and nelfinavir [1], but were never tested in a suitable animal model or developed further probably because of cessation of the SARS outbreak and no further cases. Investigation in animal model also demonstrated that it was feasible to develop a beneficial vaccine for SARS. A protective antibody response could be generated by targeting the viral spike [S] antigen. Mucosal immunization of the African green monkey with a recombinant attenuated parainfluenza-SARS-CoV spike protein chimeric virus resulted in significant neutralizing antibodies to protect against virus replication in the upper and lower respiratory tract after SARS-CoV challenge [67]. Several other methods to deliver the S protein or nucleoprotein were investigated: adenoviral vector in rhesus macaques, inactivated whole virus vaccine in mice, S protein fragments in mice and rabbits, DNA vaccination with nucleoprotein in mice, and plasmid DNA vaccine carrying S protein encoded by human codons in a mouse model [1]. None of these studies used animal models with clinical pneumonia or showed protection against clinical disease. Further development of these vaccines was not pursued, but the studies provided some evidence of proof of concept.

Intense investigations have been implemented in various research centers to identify new therapeutic agents to combat MERS-CoV since the recognition of the outbreak in the Middle East. However, development of new drugs and vaccines take many years to become readily available, usually 10–12 years. A pragmatic approach to meet current needs or demand for the near future is to assess drugs or compounds already developed. In one such study, the in vitro activity of IFN products, ribavirin, and mycophenolic acid against MERS-CoV were assessed [75]. Of all the IFNs tested, IFN-β showed the greatest activity, 41-fold more potent than IFN-α-2b. Ribavirin did not inhibit the virus at concentrations achieved by doses used in humans. Mycophenolic acid showed marked inhibition of MERS-CoV [75]. However, mycophenolate mofetil has immunosuppressive properties and is used in organ transplant patients and could lead to superinfection and adverse outcome. In another study by the same group of investigators, 290 developed pharmaceutical compounds were screened for in vitro activity against the MERS-CoV [76]. A total of 27 agents showed antiviral activity against both SARS-CoV and MERS-CoV, from 13 different classes of pharmaceuticals, including inhibitors of dopamine receptors used as antipsychotics and inhibitors of estrogen receptors used for cancer treatment [76].

Probably the most clinically relevant study on repurposed drugs for therapeutics in severe MERS-CoV infection was just recently published. Three commercially

available drugs with potent in vitro activity against MERS-CoV were assessed in the common marmosets with severe disease resembling MERS in humans. The lopinavir/ritonavir [a protease inhibitor combination used for treating human immunodeficiency virus (HIV)] and IFN-β-1b-treated animals demonstrated significantly better outcome than untreated animals, with improved clinical, radiological, and pathological findings, and lower mean viral load in lungs and other tissues [77]. Animals treated with mycophenolate mofetil, in contrast, developed severe and fatal disease with higher mean viral loads than the untreated animals. Hence, clinical trials or pilot assessment in patients with severe MERS warrant trial of lopinavir/ritonavir and IFN-β-1b in combination or alone.

A novel approach for treatment and prevention of severe MERS is the use of neutralizing monoclonal antibodies [MAbs]. One such MAb, designated MERSMab1, potently blocks MERS-CoV entry into human cells [78]. MERSMab1 specifically binds to the receptor-binding domain of the MERS-CoV S protein, to block the binding to the cellular receptor DPP4. Thus, development of a humanized monoclonal antibody could be used therapeutically and prophylactically in healthcare workers and family members exposed to a patients with MERS-CoV infection [78]. Further development in this area included the isolation of a potent MERS-CoV neutralizing antibody from memory B lymphocytes of an infected subject [79]. The antibody, labeled LCA60, interfered with the binding to the cellular receptor CD26 [DPP4] and also could be used for treatment or prophylaxis. This is particularly relevant as, during the most recent MERS outbreak in South Korea, secondary and tertiary cases were largely from transmission to non-healthcare workers [80]. For more urgent need in future severe cases of MERS, it is reasonable to administer convalescent sera from previously infected and recovered subjects with MERS. It has been shown in experimental animals that MERS-immune sera from infected camel augment MERS-CoV clearance and reduced the pathological changes in the infected lungs [81].

Although it is feasible to develop an effective vaccine for the MERS-CoV, as supported by recent experiments with subunit or full-length MERS-CoV protein/antigen [82, 83], it appears that this is unlikely to occur. There are too few cases of MERS and the virus has so far not mutated to become more easily transmissible; thus development of a vaccine would not be a commercially viable enterprise. Table 4.1 summarizes the comparative features of SARS and MERS.

4.9.1 Future Direction

Although the future is unpredictable, it seems more likely that the MERS-CoV, unlike SARS-CoV, may continue to cause sporadic human infections or local outbreaks, as the virus appears to be entrenched or endemic in dromedary camels of the Middle East. It also has the potential to spread and maintain reservoirs in other animal in the region that carry similar DPP4 receptors such as horses, goats, sheep, and cows [84, 85].

Table 4.1 Comparative features of SARS and MERS

Features	SARS	MERS
Etiology	Zoonotic coronavirus [SARS-CoV]	Zoonotic coronavirus [MERS-CoV]
Source	Bats	Bats
Transmitting host	Palm civet feline	Dromedary camels
Country of origin	Southern China	Saudi Arabia
Human to human	Droplets/direct contact	Droplets/direct contact
Transmissibility	High/pandemic potential	Medium/non-pandemic
Incubation	2–14 days/median 5 days	2–14 days/median 5–7 days
Clinical aspects	Flu-like illness/severe pneumonia	Flu-like illness/severe pneumonia
Diagnosis	Real-time RT-PCR/viral culture	Real-time RT-PCR/viral culture
Management	Supportive care/ventilation	Supportive care/ventilation
Prevention	Contact/droplet isolation	Contact/droplet isolation
Mortality rate	Overall 10%	19% to 41%
Future recurrence	Unlikely	Probably likely

It is important, however, to prepare not just for future MERS outbreak but for other zoonotic coronaviruses that may "jump" the species barrier to produce another novel unexpected zoonosis epidemic. In order to prevent future coronavirus zoonosis emerging, we need more basic research to fully understand the mechanisms of cross-species virus transmission. Current investigations indicate that the surface S protein of the coronavirus and the host proteases that cleave the protein before membrane fusion are key factors for interspecies transmission [86]. It is believed that two mutations may have allowed the bat coronavirus HJKU4 to enter human cells, enabling the S protein to be activated by human proteases [87]. Could there be environmental or extrinsic factors that facilitate key mutations to enable cross-species transmission from bats to camels to humans, and are these modifiable? These are areas for future research.

Rather than developing specific agents to treat MERS-CoV or vaccines for prevention, which may be after the fact, it would be more prudent to develop new treatment and prevention that could be effective against all zoonotic coronaviruses that may emerge in the future. All coronaviruses require proteolytic activity of nsp 5 protease [3CL-pro] during replication, and this has been identified as a common target for development of a general anti-coronavirus agent [88]. Development of a universal antiviral agent for animal coronaviruses may be feasible, but could be difficult, as screening of a peptidomimetic library identified 43 compounds with good to excellent inhibitory potency against a bat coronavirus [HKU4-CoV] [89]. Another target for multiple coronaviruses is the coronavirus helicase [nsp 13], which is also important in viral replication. A replication inhibitor of the viral helicases of SARS-CoV, mouse hepatitis virus, and MERS-CoV, SSYA10-001, may be a suitable candidate as a broad spectrum coronavirus inhibitor [90].

Designing a universal vaccine for current and future zoonotic coronaviruses may be a very difficult undertaking. Neutralizing antibodies against the spike glycopro-

tein were shown to be strain specific with very little cross-reactivity within or across subgroups [91]. In addition, the nucleocapsid proteins do not share cross-reactive epitopes across subgroups of coronaviruses. It has been proposed that vaccine designed for emerging animal coronaviruses should include chimeric spike proteins containing neutralizing epitopes from multiple strains across subgroups [91]. Vaccine manufacturing companies would likely not be enticed on such seemingly nonprofitable enterprise, but scientist should still pursue such a venture, even for proof of concept with animal model experiments, as the need for a universal coronavirus vaccine may arise sometime in the future.

4.9.2   Conclusion

MERS-CoV is the most lethal of the six known human coronaviruses and produces a higher mortality than SARS-CoV. Moreover, it is likely to continue to afflict humans for the foreseeable future unlike SARS-CoV which has not reappeared since its first appearance over a decade ago. MERS-CoV uses various methods to evade the host innate antiviral immunity, which may explain its high pathogenic capability. Recent advances of our understanding of the immunopathogenesis of MERS may lead to more effective therapy. Although the overall mortality of clinical recognizable cases of MERS globally is about 41%, treatment in South Korea reduced the death rate to 19% [45].

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