Middle East Respiratory Syndrome Virus Pathogenesis

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Abstract

Coronaviruses (CoVs) are enveloped RNA viruses that infect birds, mammals, and humans. Infections caused by human coronaviruses (hCoVs) are mostly associated with the respiratory, enteric, and nervous systems. The hCoVs only occasionally induce lower respiratory tract disease, including bronchitis, bronchiolitis, and pneumonia. In 2002 to 2003, a global outbreak of severe acute respiratory syndrome (SARS) was the seminal detection of a novel CoV (SARS-CoV). A decade later (June 2012), another novel CoV was implicated as the cause of Middle East respiratory syndrome (MERS) in Saudi Arabia. Although bats might serve as a reservoir of MERS-CoV, it is unlikely that they are the direct source for most human cases. Severe lines of evidence suggest that dromedary camels have been the major cause of transmission to humans. The emergence of MERS-CoV has triggered serious concerns about the potential for a widespread outbreak. All MERS cases were linked directly or indirectly to the Middle East region including Saudi Arabia, Jordan, Qatar, Oman, Kuwait, and UAE. MERS cases have also been reported in the later phases in the United Kingdom, France, Germany, Italy, Spain, and Tunisia. Most of these MERS cases were linked with the Middle East. The high mortality rates in family-based and hospital-based outbreaks were reported among patients with comorbidities such as diabetes and renal failure. MERS-CoV causes an acute, highly lethal pneumonia and renal dysfunction. The major complications reported in fatal cases are hyperkalemia with associated ventricular tachycardia, disseminated intravascular coagulation, pericarditis, and multi-organ failure. The case-fatality rate seems to be higher for MERS-CoV (around 30%) than for SARS-CoV (9.6%). The combination regimen of type 1 interferon + lopinavir/ritonavir is considered as the first-line therapy for MERS. Antiviral treatment is generally recommended for 10 to 14 days in patients with MERS-CoV infection. Convalescent plasma therapy has shown some efficacy among patients refractory to antiviral drugs if administered within 2 weeks of the onset of the disease.

Keywords
► coronavirus
► SARS-CoV
► MERS-CoV

Coronaviruses (CoVs) are enveloped, positive sense RNA viruses, infecting birds and mammals. CoVs belong to the family of Coronaviridae and the subfamily Coronavirinae. Infections caused by human coronaviruses (hCoVs) are mostly associated with the respiratory, enteric, and nervous systems. These are divided into four genera: α, β, γ, and δ. Among the hCoVs, the HKU1, NL63, 229E, and OC43 are endemic in the human population and responsible for mild, self-limiting upper respiratory tract infections.[1,2] In addition, the hCoVs also induce lower respiratory tract disease, including bronchitis, bronchiolitis, and pneumonia.[3–6] Severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) are genetically subgrouped into lineages B and C, respectively.

The first case of MERS was reported in Jeddah, Saudi Arabia, in June 2012. The outbreak of MERS-CoV infections started a

decade after the outbreak of the SARS-CoV epidemic in 2002–2003.[7,8] The inefficient spread between infected patients suggested the strong possibility of zoonotic transmission, but the exact source of infection is still not known in human population. The *Tylonycteris* and *Pipistrellus* bats have been recently reported as the reservoir of MERS-CoV.[9] Although bats might serve as a reservoir of MERS-CoV, it is unlikely that they are the direct source for most of the human cases because human contacts with bats are not very common. The phylogenetic studies revealed the presence of CoV RNA sequences in the bat fecal samples in Europe, Africa, and Asia, including the Middle East. Some of the RNA sequences have been reported to be closely related to MERS-CoV sequences.[10–14] The MERS-CoV spilled over in human population either from bats or through other animals as intermediate hosts.[10] However, other evidences indicated the origin of MERS-CoV from dromedary camels. High levels of neutralizing antibodies, viral RNA, and infectious viruses have been reported in dromedary camels, suggesting a potential for camels to be the source for human transmission.[15,16] The presence of MERS-CoV neutralizing antibodies in dromedary camels suggested either a past infection with MERS-CoV or a closely related virus in camels.[17,18] Unpasteurized camel milk has also been suggested as a potential source because the virus has been detected in the camel milk.[8,19,20] The World Health Organization (WHO), Saudi Arabia, and Qatar have issued recommendation for not consuming unpasteurized camel milk and to be cautious in cases of close association with dromedary camels.[20] The full-genome sequencing data have shown the sequence similarity in the MERS-CoV isolated from a man and his camel in Saudi Arabia. Such sequence similarity indicated camels as a strong possible link in the cases of camel-to-human transmission.[21] The phylogenetic studies provided the evidence of interspecies jumps of CoVs.[22,23] The emergence of MERS-CoV has triggered serious concerns about the potential for a widespread outbreak. All MERS cases were linked directly or indirectly to the Middle East region including Saudi Arabia, Jordan, Qatar, Oman, Kuwait, and UAE.[11,24] The MERS cases have also been reported in the later phases in the United Kingdom, France, Germany, Italy, Spain, and Tunisia.[25] Most of these MERS cases were linked with the Middle East.[25] The high mortality rates in family-based and hospital-based outbreaks were reported among patients with comorbidities such as diabetes and renal failure.[24,26,27]

Molecular Virology of MERS-CoV

The MERS-CoV genome consists of 30,119 nucleotides and 11 open reading frames (ORFs).[2,28] The single positive-stranded RNA genome has 5′-UTR (278 nucleotides) and 3′-UTR (300 nucleotides) (►**Fig. 1**). The genes located at the 5′-UTR play an important role in viral replication, whereas the genes at the 3′-UTR encode accessory and structural proteins.[13] The 5′-UTR has two overlapping ORFs (ORF1a and ORF1b), which gets translated to two polyproteins (polyprotein 1a [pp1a] and polyprotein 1ab [pp1ab]) (►**Fig. 1**).[28] These polyproteins are cleaved into 16 functional nonstructural proteins (nsps) through proteolytic activity of two viral proteases called papain-like protease (PLpro) and 3C-like protease (3CLPro)

after their self-cleavage from pp1ab (►**Fig. 1**).[2,25,29,30] PLpro and 3CLpro are located on nsp3 and ns5, respectively.[31] Other nsps encoded by ORFs are nsp12 (for RNA-dependent RNA polymerase activity), nsp14 (for exoribonuclease activity), nsp15 (for exoribonuclease activity), and nsp16 (for methyl transferase activity) (►**Fig. 1**).[2,32,33] The nsp14 plays an important role in proofreading activity to monitor the rate of mutation.[33] The other genes downstream to ORF1ab encode for structural proteins such as Spike (S), envelope (E), membrane (M), and nucleocapsid (N) and accessory proteins.[25] The accessory proteins might help the virus in immune evasion by interfering with the innate immune response. MERS-CoV has five accessory proteins and SARS-CoV has eight different accessory proteins.[32] These differences might lead to the differences in the effects on induction and signaling of type 1 interferons (IFNs),[32] which could explain the greater sensitivity of IFN to MERS-CoV than SARS-CoV.[34,35] The viral RNA is encapsidated in the N protein and transported to the endoplasmic reticulum-Golgi intermediate compartment (ERGIC), the site of assembly. Viral RNA encapsidated in the N protein and then buds into vesicles lined with the S, M, and E proteins.[32] The dipeptidyl-peptidase 4 (DPP4/CD26) was identified as the host-cell receptor for the entry of MERS-CoV.[13,36] Both MERS-CoV and SARS-CoV bind to large ectopeptidases (DPP4 and ACE2, respectively) to invade the cells.[36,37] The aminopeptidase N (APN) has been reported as entry receptors for several α-CoVs.[38] All hCoV receptors reported so far are exopeptidases, although their proteolytic activity is not necessary for the virus to bind to the receptors, or for entering in the host cells.[5,39–41] MERS-CoV can bind to the DPP4 of the several species, but the binding site of DPP4 is different in different species, explaining the variation in the susceptibility of MERS-CoV infections in various animals.[42] The S glycoprotein located on the surface of the MERS-CoV virion interacts with the receptor binding domain of DPP4 to enter in the host cells.[2,36,43] The S protein consists of a globular S1 domain at the N-terminal region, an S2 domain with two heptad repeats (HR1 and HR2), and a transmembrane domain.[3] Lower rate of mutation has been reported so far in the MERS-CoV during transmission in human populations in the recent outbreaks.

Both hACE2 and DPP4 have been reported to shed from cell surface.[38,44] The loss of hACE2 results in more severe pulmonary disease.[45] The DPP4 has been reported as a neutrophil chemorepellent; therefore, the variations in DPP4 shedding during MERS-CoV infection could influence the composition of the immune infiltrate, and ultimately to the outcome of the infection. DPP4 has also been reported to be expressed on immune cells, including T cell lymphocytes, and required for optimal function of these cells.[38] The events occurring immediately after MERS-CoV binding with the DPP4 receptor appear to be similar to other CoVs. Cleavage of the surface glycoprotein is one of the necessary steps, which exposes the fusion peptide and facilitates the fusion of virus-cell membrane, which results into the release of the nucleocapsid in the cell cytoplasm. Several host proteases, including cathepsin B and members of the transmembrane protease, serine 2 (TMPRSS) family, have been implicated in this process.[38,46]
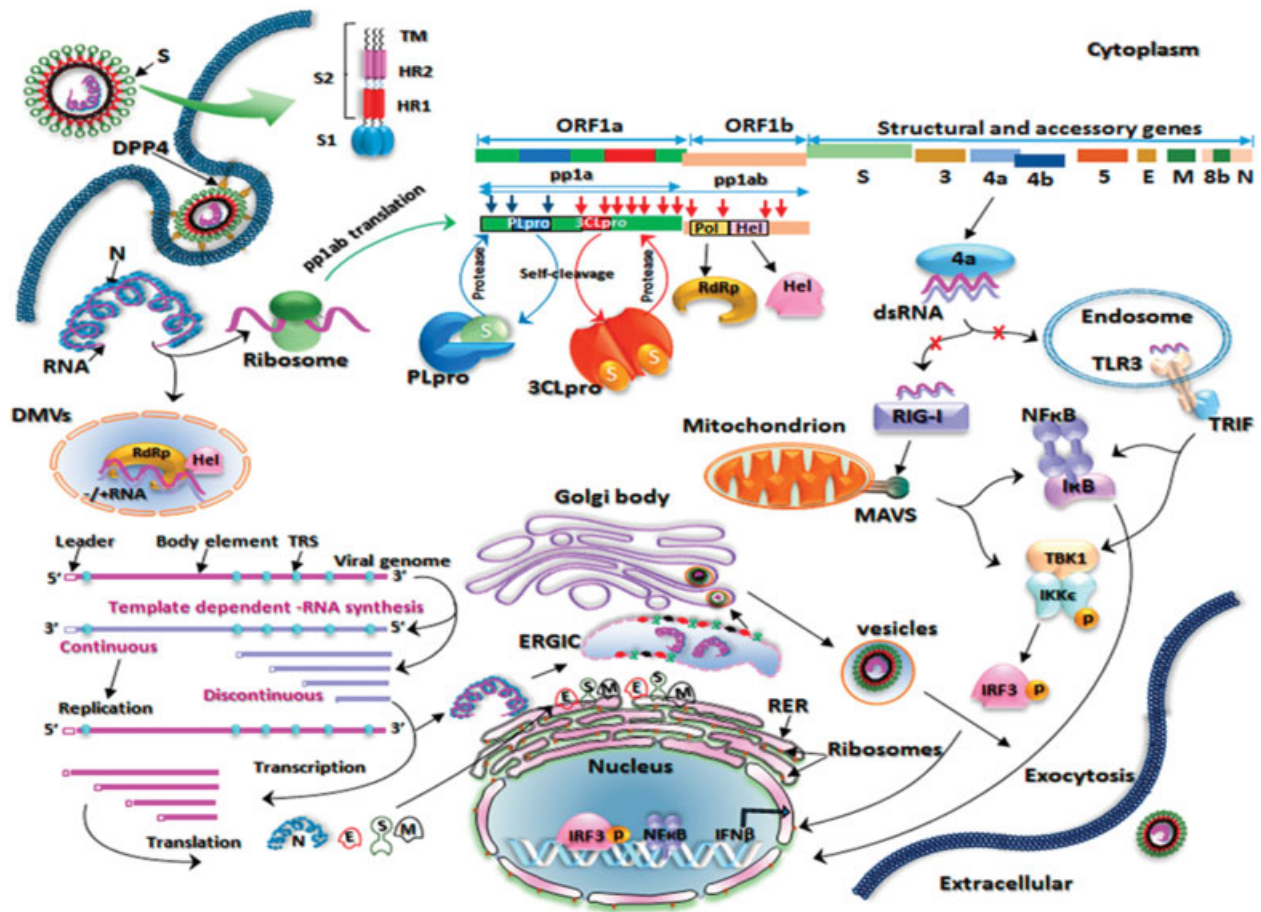
Fig. 1 Schematic of the replication cycle of Middle East respiratory syndrome coronavirus (MERS-CoV). MERS-CoV binds to dipeptidyl peptidase 4 (DPP4) on the host cell through its receptor-binding domain (RBD) in the S1 subunit of the spike (S) glycoprotein, which leads to virus–cell fusion and the release of genomic RNA into the cytoplasm. Initially open reading frame 1a (ORF-1a) and ORF-1b are translated into polyproteins, polyprotein 1a (pp1a) and pp1ab, respectively, which are cleaved by the virus-encoded proteases papain-like protease (PLpro) and 3C-like protease (3CLpro) into 16 mature nonstructural proteins (nsps). The proteins involved in replication and transcription are gathered into replication-transcription complexes (RTCs) that associate with double-membrane vesicles (DMVs) derived from the endoplasmic reticulum (ER). The genomic RNA contains adenylate uridylate (AU)-rich sequences called transcription regulation sequences (TRSs). If the TRSs are recognized by RTCs, then RNA of subgenomic length for transcription will be generated, otherwise a full-length template RNA of genomic length for replication will be synthesized. The newly produced genomic RNAs are encapsidated in the nucleocapsid (N) proteins in the cytoplasm and then transported to the ER–Golgi intermediate compartment (ERGIC) for further assembly. The S, membrane (M), and envelope (E) proteins are inserted into the membrane of the rough ER (RER), from where they are transported to the ERGIC to interact with the RNA-encapsidated N proteins and assemble into viral particles. The budded vesicles containing mature viral particles are then transported to the cell surface for release after maturation in the Golgi bodies. Double-stranded RNAs (dsRNAs) are partially generated during viral replication. The 4a competes with Toll-like receptor 3 (TLR3) and retinoic acid-inducible gene I product (RIG-I)-like helicases (RIG-I and melanoma differentiation-associated protein 5 [MDA5]) to bind to dsRNAs and evades the host immune response. (Adapted from Durai P, Batool M, Shah M, Choi S., Middle East respiratory syndrome coronavirus: transmission, virology and therapeutic targeting to aid in outbreak control. Exp Mol Med 2015;47:e181; doi:10.1038/emm.2015.76 under Creative Commons CC-BY license (Creative Commons Attribution 4.0 International License.)[2]

Immunopathogenesis of MERS-CoV

MERS-CoV causes an acute, highly lethal pneumonia and renal dysfunction. Renal dysfunctions take place either due to hypoxic damage or direct infection of the kidney. The entry receptor (DPP4) for MERS-CoV is expressed at high levels in the kidney.[47] Neutrophil and macrophage infiltration and alveolar edema have been reported in infected lung tissues in the MERS animal models.[32] The cell-line susceptibility study indicated that the MERS-CoV may infect several human cell lines, including lower respiratory, kidney, intestinal, and liver cells, as well as histiocytes.[48] The range of MERS-CoV tissue tropism in vitro was found to be broader than any other HCoV.

MERS-CoV has been reported to elicit attenuated innate immune responses with delayed proinflammatory cytokine induction in in vitro and in vivo,[49–51] which might lead to immune dysfunctions. Similar findings have been reported among SARS patients.[32,52] The ineffective B cell and T cell responses with prolonged cytokine expression have also been observed among the MERS patients during advanced stages of the disease. Clinical symptoms include fever, cough, sore throat, myalgia, chest pain, and gastrointestinal symptoms, such as diarrhea, vomiting, and abdominal pain.[5,53,54] Respiratory symptoms indicate the lower respiratory tract complications (dyspnea, cough, and fever); however, the upper respiratory tract complications are rare. The virus is detected in the upper

respiratory tract only during early course of the infections, and lower respiratory tract during late stages of infections.[21,55–57]

In some cases, the virus is detected in urine and blood of the patients, which indicates the possibility of systemic infection. The multiple specimens should be collected from different sites at different time points to increase the possibility of detecting MERS-CoV. Priority should be given to the respiratory specimens (lower tract if obtainable and in all cases of severe disease; upper tract if the disease is mild and lower tract specimens cannot be obtained). Potential risk factors include obesity, diabetes mellitus, end-stage renal disease, cardiac disease, hypertension, lung disease including asthma and cystic fibrosis, and immunosuppressive conditions.[24,26,53,54] The major complications reported in fatal cases are hyperkalemia with associated ventricular tachycardia, disseminated intravascular coagulation, pericarditis, and multiorgan failure.[5] Coinfections have also been reported frequently in the cases of MERS.[5,27,54] Hematological manifestations include higher leukocyte counts and lymphopenia, while a few cases showed lymphocytosis, thrombocytopenia, and coagulopathy.[5,27] Elevated levels of creatinine, lactate dehydrogenase, alanine aminotransferase, and aspartate aminotransferase are indicative of renal and liver dysfunctions.[27,58,59]

The median incubation period for MERS-CoV (5.2 days) is slightly longer than the incubation period of SARS-CoV (4 days). MERS and SARS are respiratory viruses, but gastrointestinal manifestations have been reported among patients suffering from SARS-CoV and MERS-CoV infections. The case-fatality rate seems to be higher for MERS-CoV (around 30%) than for SARS-CoV (9.6%).[60] The lesions and opacities in chest radiographs of the MERS patients have been reported to closely resemble the pneumonia patients infected with H1N1pdm09.[5,59] The lesions in MERS patients are different from the lesions in SARS patients showing fibrocellular intra-alveolar organization with a bronchiolitis obliterans organizing pneumonia (BOOP)-like pattern.[60,61] MERS-CoV has been reported to induce greater dysregulation of the host response to infection than SARS-CoV. MERS-CoV has been reported to downregulate the genes involved in the antigen presentation, as an immune evasion strategy.[62] The decreased expression of IFN-α, retinoic inducible acid gene (RIG)-1, melanoma differentiation associate (MDA5), and IFN regulatory factors IRF-3 and IRF-7 have been reported in bronchoalveolar lavage (BAL) and serum of patients in advanced stages of MERS. However, the higher levels of CXC-motif chemokines ligand (CXCL) 10 and interleukin (IL)-10 have been reported among patients severely suffering from MERS, which might have resulted in the lower IFN-γ expression and higher levels of IL-17A and IL-23.[63]

Diagnostics and Therapeutics

MERS-CoV seems more sensitive to prophylactic and therapeutics in vitro than SARS-CoV.[36] Immunofluorescence assays have been used to detect MERS-CoV antibodies.[64] IgG and IgM antibodies in serum samples could be determined using an anti-MERS-CoV indirect immunofluorescence assay.[16,65] In addition, the enzyme-linked immunosorbent assays (ELISA), protein microarray technology, and micro-neutralization (MN) assays

have also been used to detect MERS-CoV antibodies.[65,66] The real-time PCR (RT-PCR) assays targeting RNA upstream of the E gene (upE) and ORF-1b and ORF-1a have been also used for detection.[32] According to the WHO, the screening using RT-PCR targeting upE gene should be conducted on samples from suspected MERS patients. All positive samples should undergo confirmatory testing by targeting ORF-1a, ORF-1b, or N genes.[16,67] These RT-PCR assays have been reported to be very specific without any cross-reactivity with other respiratory viruses including hCoVs.[32] Respiratory samples should be collected at least every 2 to 4 days to confirm virus clearance in the cases where two consecutive results are negative.[32,68] Western blotting is also useful in the diagnosis of MERS-CoV.[68,69] The samples from lower respiratory tract such as BAL and tracheal aspirates are better suited for the testing due to the higher viral loads in lower respiratory tract samples.[70]

Antiviral treatment under the supervision of medical experts is recommended for MERS patients with or without comorbidities, with clear symptoms of pneumonia.[71] The efficacy of lopinavir/ritonavir was found to be similar to IFN-β1b in experimental trials using marmosets.[72] IFN-β1b or lopinavir/ritonavir can be considered for treating patients, in whom the use of ribavirin is not possible due to renal dysfunction or other adverse effects.[71] The combination regimen of type 1 IFN + lopinavir/ritonavir may be considered as the first-line drug for MERS patients.[71] One must monitor creatinine clearance according to renal function during the use of ribavirin.[71] Antiviral treatment is generally recommended for 10 to 14 days in patients with MERS-CoV infection.[71] The levels of hemoglobin, bilirubin, haptoglobin, and reticulocyte must be monitored carefully during the use of ribavirin. The use of lopinavir/ritonavir has been recommended instead of ribavirin in cases of hemolytic anemia.[71] Since ribavirin is teratogenic, patients are advised not to plan for pregnancy for 6 months after treatment.[73] Convalescent plasma therapy has been shown some efficacy among patients refractory to antiviral drugs if administered within 2 weeks of the onset of the disease.[71,74] Convalescent plasma therapy could help some patients with severe disease.[10,32,75] MERS-CoV-specific peptide fusion inhibitors have been reported as a novel approach to treat MERS patients.[71]

Conclusion

There is a need to understand the framework of establishment of the MERS-CoV infection in the society to develop control measures for the larger outbreaks. The involvement of pattern recognition receptors and downstream signaling at the cellular level is required to understand the molecular pathogenesis of MERS-CoV. The role of accessory proteins of MERS-CoV should be dissected out to understand their diverse roles in the immune evasion strategies adopted by MERS-CoV to inhibit IFN-β expression. There is need to develop suitable animal models to study the MERS-CoV pathogenesis for testing antiviral drugs and vaccines. The threat of potential pandemics is always there because pathogenic CoVs continue to spill over from animals to the human population. The specific diagnostics and control measures are required to avoid the spread among healthcare workers and nosocomial infections among other patients. The

knowledge about the dynamics and molecular characteristics of MERS-CoV and other closely related CoVs in circulation is very important to understand their emergence and to strengthen disease surveillance and disease preparedness.

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