

TITLE:

Molecular Pathogenesis of Middle East Respiratory Syndrome (MERS) Coronavirus

ABSTRACT:

PURPOSE OF REVIEW: Middle East respiratory syndrome coronavirus (MERS-CoV) emerged in 2012 and is listed in the World Health Organization's blueprint of priority diseases that need immediate research. Camels are reservoirs of this virus, and the virus spills over into humans through direct contact with camels. Human-to-human transmission and travel-associated cases have been identified as well. Limited studies have characterized the molecular pathogenesis of MERS-CoV. Most studies have used ectopic expression of viral proteins to characterize MERS-CoV and its ability to modulate antiviral responses in human cells. Studies with live virus are limited, largely due to the requirement of high containment laboratories. In this review, we have summarized current studies on MERS-CoV molecular pathogenesis and have mentioned some recent strategies that are being developed to control MERS-CoV infection. **RECENT FINDINGS:** Multiple antiviral molecules with the potential to inhibit MERS-CoV infection by disrupting virus-receptor interactions are being developed and tested. Although human vaccine candidates are still being developed, a candidate camel vaccine is being tested for efficacy. Combination of supportive treatment with interferon and antivirals is also being explored. **SUMMARY:** New antiviral molecules that inhibit MERS-CoV and host cell receptor interaction may become available in the future. Additional studies are required to identify and characterize the pathogenesis of MERS-CoV EMC/2012 and other circulating strains. An effective MERS-CoV vaccine, for humans and/or camels, along with an efficient combination antiviral therapy may help us prevent future MERS cases.

Introduction:

Coronaviruses have historically been associated with low impact human disease, such as the common cold [1, 2]. It was the sudden emergence of severe acute respiratory syndrome (SARS) coronavirus (CoV) in 2003 that made us aware of deadly versions of coronaviruses that can cause large outbreaks associated with high mortality and global panic. SARS-CoV caused an outbreak that was deemed a pandemic and an international threat by the World Health Organization (WHO). A total of 8098 people were infected and of these 774 died [3]. Extensive epidemiological studies and wildlife surveillance revealed that SARS-CoV had originated in bats and somehow spread to civet cats. The virus is speculated to have later "jumped" from civet cats to humans [4–7]. This outbreak established the importance of wildlife (i.e., bats) in the emergence of highly pathogenic coronaviruses.

Middle East respiratory syndrome coronavirus (MERS-CoV) emerged in September 2012 in the Kingdom of Saudi Arabia [8]. MERS-CoV causes a lower respiratory tract infection in humans with 35% mortality. Since its emergence, 2279 laboratory-confirmed cases have been identified in humans with 806 deaths. Twenty-seven countries have reported cases of MERS with many associated with travel. MERS-CoV has been listed on the WHO's blueprint of priority diseases needing immediate research.

Currently, only supportive treatment exists for MERS-CoV. MERS-CoV, like many other viruses, has evolved proteins that can effectively counteract human antiviral responses [9–11]. The sudden appearance of MERS, the high infectivity rate of the virus, and the need for a high containment level 3 lab for research have hindered progress in designing vaccines and therapeutics against this virus. Development of vaccine candidates is further complicated by the ability of coronaviruses to rapidly mutate and recombine [6]. Several quasispecies of the virus can be detected in camels [12], which are reservoirs of the virus, and it is extremely difficult to develop therapeutic strategies against a pathogen that can exist as several different variants.

The evolution of MERS-CoV is intriguing. It is speculated that MERS-CoV originally spilled over from bats to camels. Several MERS-like CoVs have been detected in bats [13–15, 16*, 17–19]. Current models propose that camels are reservoirs of the virus and are often the source of human infection [20–22]. Human-to-human transmission of MERS-CoV has also been documented [23]. In spite of these advances in our understanding of how the virus has evolved, there is a significant lack of knowledge about virus transmission dynamics and ecological and environmental factors that drive the emergence and evolution of MERS-CoV.

Several novel approaches are being currently developed to combat MERS-CoV, including small molecule inhibitors, monoclonal antibodies and vaccines. In this mini-review, we have discussed

the pathogenesis of MERS-CoV and the role of various viral proteins in subverting antiviral responses in humans. We also discuss current approaches that are being developed and tested to control MERS-CoV replication and associated disease. Finally, we bring forward additional strategies to identify therapeutic targets and molecules to improve the outcome of MERS-CoV infections in humans.

Coronavirus: Structure and Replication:

A mature CoV particle is spherical in shape and has a diameter of around 100 nm [24]. Virus particles have a protein coat, called the capsid, which protects the virus' genetic material. CoVs have an additional envelop of lipids surrounding the protein capsid. This layer is derived from portions of host cell membranes (phospholipids and proteins) but also include some viral glycoproteins. CoV virions contain four essential viral glycoproteins—spike (S), membrane (M), envelop (E), and nucleocapsid (N), all of which are encoded within the 3' end of the viral genome [25]. S protein is club-shaped and located on the surface of the virion, giving CoVs a distinct appearance of a solar corona, hence the name—Coronaviruses. S protein is critical for the initial attachment of the virion to the host cell receptor, dipeptidyl peptidase 4 (DPP4) [26]. Recent studies have demonstrated that the spike protein can adapt to interact with DPP4 from different species of bats to enable cross-species entry and replication of MERS-CoV [27••]. The structure of CoVs is maintained by M protein while E protein facilitates the assembly and release of the virus. CoVs have helically symmetrical nucleocapsids and the only structural protein associated with the nucleocapsid is the N protein, which helps in the packaging of the virus genome (reviewed here [25, 28, 29]).

Viruses use host cell machinery to replicate (Fig. 1). CoVs have large, non-segmented positive-sense (+) RNA genomes of ~ 30 kb and use their own RNA replicase enzymes to create copies of their genomes in the cytoplasm. CoVs complete their life cycle in 4 steps: (1) entry, (2) expression of the replicase protein, (3) replication and transcription of their genome, and (4) release of assembled progeny virions (reviewed here [25] and Fig. 1). CoVs interact through S protein with its cognate receptor on the surface of susceptible cells. The specificity of this interaction determines the host range and tissue tropism of CoVs. Many CoVs bind peptidases as their cognate receptor for entry, but the choice of receptor has been reported to vary across different CoVs, for example, SARS-CoV uses an angiotensin-converting enzyme (ACE2) [30] and MERS-CoV binds to DPP4 [26]. Subsequently, S protein undergoes acid-dependent proteolytic cleavage, resulting in the fusion of viral and cellular membranes and the release of the viral genome into the cytoplasm (reviewed here [29]).

Coronavirus genomes include a 5' cap structure and a 3' poly (A) tail, allowing them to act as messenger RNA (mRNA; positive-sense RNA) templates for translation of replicase polyproteins. The replicase gene encodes two large open reading frames (ORFs), rep1a and rep1ab, which express two co-terminal polyproteins, pp1a and pp1ab. These polyproteins are cleaved into individual non-structural proteins (Nsps). Nsp3 encodes a papain-like protease (PLpro) that cleaves Nsp1/2, Nsp2/3 and Nsp3/4 boundaries. Serine-type protease, also known as main protease (Mpro), which is encoded by Nsp5, cleaves the polyproteins into remaining Nsps. Nsps facilitate viral RNA synthesis, for example, Nsp12 encodes RNA-dependent RNA polymerase (RdRp). The formation of a replicase-transcriptase complex (RTC) containing Nsps initiates viral RNA synthesis. The RTC complex localizes to modified intracellular membranes which are derived from the rough endoplasmic reticulum (ER) and initiates the production of negative-sense RNAs through both replication and transcription. Full-length copies of CoV negative-sense RNA genomes serve as a template to synthesize full-length positive-sense RNA genomes that eventually get packaged into new virions. CoVs utilize discontinuous transcription to generate a subset of sub-genomic negative-sense RNAs, including those encoding structural proteins [31]. Varying lengths of the 3' end of the genome is combined with the 5' leader sequence to make sub-genomic negative-sense RNAs. These sub-genomic negative-sense RNAs are then transcribed into sub-genomic positive-sense mRNAs for translation.

The viral structural proteins, such as S, E, and M, are translated from sub-genomic positive-sense mRNAs. The structural proteins are inserted into the endoplasmic reticulum-Golgi intermediate compartment (ERGIC). The viral genomes, encapsidated by N protein, bud into ERGIC containing the structural proteins for assembly. The newly assembled mature virions are then transported to the cell surface in vesicles and are released from the infected cell by exocytosis. The genome organization, replication and associated replicase-transcriptase proteins of murine hepatitis virus (MHV) have been extensively studied [32]. All identified coronaviruses use a similar replication strategy (reviewed here [25, 29]).

Pathogenesis of MERS-CoV:

Pathological changes in a MERS-CoV infected patient have been studied with the help of a single research-oriented autopsy. The pathology in this fatal case primarily included exudated diffuse alveolar damage with hyaline membranes, pulmonary edema, type 2 pneumocyte hyperplasia, interstitial pneumonia that was predominantly lymphocytic, and multinucleate syncytial cells [33, 34].

Several studies have characterized MERS-CoV in cell lines and animal models. Like other viruses, MERS-CoV has evolved strategies to evade innate antiviral responses. The ability of the virus to suppress antiviral responses [11] and promote an exaggerated inflammatory response [35•] contributes to its pathogenesis. As described above, MERS-CoV is made up of several structural, non-structural, and accessory proteins. MERS-CoV accessory proteins are dispensable for virus replication in tissue culture [36] but play an important role in modulating the host's antiviral defensive responses. Human and most mammalian cells have evolved evolutionarily conserved molecules (pattern recognition receptors or PRRs) that can detect conserved structures in viruses called pathogen-associated molecular patterns. When a coronavirus infects and enters a cell, it releases its RNA genome, which rapidly initiates the process of translation and RNA replication and transcription. This process produces long strands of single and double-stranded RNA (dsRNA). The cells detect exogenous single and dsRNA through PRRs such as Toll-like receptors (TLRs), a retinoic acid-inducible gene I (RIG-I), and melanoma differentiation-associated protein 5 (MDA5). PRRs initiate a series of signaling events in these cells to stimulate the expression of antiviral proteins such as interferons (IFNs), which in turn express interferon-stimulated genes (ISGs) in the infected cells (autocrine effect) and neighboring uninfected cells (paracrine effect) (reviewed here [37–39]). Thus, the battle between the host (infected individual) and the pathogen (MERS-CoV) begins at a molecular level.

MERS-CoV has evolved several accessory proteins that can inhibit innate antiviral responses by modulating the signaling pathway at several stages (Fig. 2). MERS-CoV protein 4a (ORF 4a) inhibits PACT-induced activation of RIG-I and MDA5 by directly binding with dsRNA [9] (Fig. 2). PACT is a cellular dsRNA binding protein that activates RIG-I to initiate downstream antiviral responses [40]. Thus, ORF 4a can inhibit antiviral responses at the very first step of viral nucleic acid sensing. Interferon regulatory factor 3 (IRF3) is a central mediator of antiviral signaling in human cells [41] and thus, the activation of IRF3 is also the target of several viral proteins, including proteins from MERS-CoV. In human cells, once TLRs, RIG-I, and MDA-5 have sensed viral nucleic acid, the cells activate kinases such as TANK-binding kinase 1 (TBK1) and inhibitor of nuclear factor kappa-B kinase subunit epsilon (IKKε), which phosphorylate IRF3. Phosphorylated IRF3 forms dimers in the cytoplasm and localizes to the nucleus of the cells to initiate the expression of IFNs. MERS-CoV ORFs 4a, 4b, 5, and protein M inhibit kinase-mediated phosphorylation of IRF3 (Fig. 2). Ectopic expression of these proteins inhibits nuclear localization of IRF3 in human cells and downstream antiviral signaling in response to virus infection and synthetic dsRNA (polyI:C) stimulation [10, 11]. Although the cellular interacting partners of all MERS-CoV viral proteins have not been identified, M protein has been shown to inactivate TBK1-mediated phosphorylation of IRF3 by disrupting TRAF3-TBK1 association [42•]. MERS-CoV ORF 4b also inhibits nuclear factor kappa-light-chain-enhancer of activated B cell (NFκB)-dependent antiviral responses by abrogating nuclear import of signal transducer and activator of transcription 1 (STAT1) [43•] (Fig. 2).

In addition to accessory proteins, Nsps that are generated from the polyproteins pp1a and pp1ab also play a role in modulating host processes. MERS-CoV Nsp1 selectively targets mRNAs that are generated in the nucleus and transported to the cytoplasm (cellular mRNA) for translation inhibition and mRNA degradation. Nsp1 spares mRNAs that are introduced directly in the cytoplasm or virus-like mRNAs that originate in the cytoplasm [44]. Thus Nsp1 inhibits cellular processes while promoting viral replication.

There are limited studies with MERS-CoV, largely due to containment requirements. The modulation of adaptive immune responses by MERS-CoV has not been extensively studied, and cytokine responses in infected dendritic cells, macrophages, and other white blood cells have not been fully explored. In one study, antibody-mediated responses or seroconversion was observed in individuals that developed an asymptomatic infection with MERS-CoV. Patients that seroconverted at later time points developed pneumonia with or without respiratory failure.

Although the rate of seroconversion increased with disease severity, 75% of the deceased MERS-CoV infected patients who developed pneumonia did not seroconvert [45]. Another study by Choi et al. demonstrated the absence of neutralizing antibodies one year after successful treatment of

MERS-CoV infected patients who had developed mild pneumonia [46]. Their observation is consistent with the previous study where disease severity correlated with seroconversion. The inability to produce long-term neutralizing antibodies would put these individuals at risk for re-infection with MERS-CoV in the future. This possibility needs to be monitored and studied. These observations warrant further investigations on the ability of MERS-CoV to modulate humoral responses in patients.

The role of T cells in MERS-CoV infections has been recently reported. Zhao et al. analyzed antibody and T cell responses in peripheral blood mononuclear cells (PBMCs) and sera from twenty-one MERS survivors. The authors identified that patients with higher antibody and CD4⁺ T cell responses had longer intensive care unit stays and demonstrated prolonged virus shedding. In the same study, MERS survivors with undetectable MERS-CoV-specific antibody responses mounted CD8⁺ T cell responses [47]. This observation adds more detail to previous studies where neutralizing antibody levels were not detected in MERS survivors and disease severity correlated with seroconversion [45, 46]. These observations raise an important question: can MERS-CoV infection be augmented by the presence of antibodies? MERS-CoV can infect and propagate in macrophages. MERS-CoV Nsp15 is an endoribonuclease, and it is speculated to suppress the activation of dsRNA sensors in infected cells, such as macrophages. Although the mechanism of action for Nsp15 is not known, Nsp15 mutant MERS-CoV displays significant dispersal of dsRNA associated with replication complexes relative to wild-type virus [48]. Since macrophages display Fc receptors that bind to the Fc portion of antibodies [49], it is of interest to identify if antibodies bound to MERS-CoV can enhance virus uptake by macrophages and subsequent virus replication. In addition, the replication potential of MERS-CoV in other Fc receptor-bearing immune cells needs to be explored as well.

Current Approaches to Tackle MERS-CoV:

There are currently no approved treatment or vaccine for MERS-CoV, but different approaches are being tested and developed to inhibit virus replication with promising preliminary data. These approaches have been extensively reviewed in recent reviews [50–52]. We mention broad strategies that are currently being discovered to orient readers to the possibilities of future treatment and preventative options.

The most commonly studied strategy is the inhibition of virus entry in human cells (Fig. 3). As previously mentioned, MERS-CoV binds to the human receptor DPP4 via its receptor-binding domain (RBD) in the spike protein. Monoclonal antibodies against the RBD and the receptor-binding motif within the RBD are being developed to inhibit this interaction (reviewed here [51]). Small molecules capable of disrupting the interaction between MERS-CoV spike protein and DPP4 are also being developed and tested (reviewed here [51]). MERS-CoV entry is accomplished through the fusion of the virus and cellular membranes that is mediated by the S2 region of the virus spike protein [53]. Inhibitory peptides targeting the S2 region of the spike protein to prevent fusion, and thus, entry of the virus are also being developed [52].

Current studies have focused on disrupting the interaction between MERS-CoV spike protein and the cellular DPP4 receptor. The recent discovery of an attachment factor, membrane-associated 78-kDa glucose-regulated protein (GRP78), that augments MERS-CoV entry [54] further complicates this approach. There is a need to identify and target additional interacting partners that may facilitate MERS-CoV binding and entry. An alternate, but interesting approach led Xia et al. to identify peptides that can inhibit MERS-CoV fusion by studying the bat HKU4 coronavirus. The authors identified inhibitory peptides in the HR2 domain of the HKU4 spike protein. These peptides were able to significantly inhibit MERS-CoV-mediated cell-cell fusion and viral infection in human cells [52]. We have also shown that MERS-CoV (EMC/2012 strain) cannot inhibit antiviral responses in insectivorous bat cells, unlike human cells where wild-type virus can efficiently inhibit these responses [55]. Future studies exploring the antiviral adaptations in bats and camelid reservoir species may enable us to identify better therapeutic targets in spillover mammals, such as humans.

While attempts are ongoing to develop vaccines and therapeutics for humans, considerable progress is being made to develop a vaccine for camels. Since MERS-CoV transmission has been primarily documented from camels to humans, it is of interest to develop a vaccine to inhibit virus replication in camels that would limit the cycle of transmission. A recent study provides promising data for an adjuvanted MERS-CoV spike protein subunit vaccine that confers complete protection from MERS-CoV disease in alpaca and results in reduced and delayed viral shedding in the upper airways of dromedary camels [56]. Other relevant advances in developing MERS-CoV vaccines have been reviewed here [50].

Conclusion:

MERS-CoV is an emerging pathogen that necessitates immediate research to develop therapeutics and vaccine candidates. Unlike SARS-CoV, MERS-CoV has not emerged to spread and cause a global pandemic, yet. We risk the emergence of an efficient human-to-human transmissible strain unless we develop intervention strategies for the current circulating strains of MERS-CoV.

The lack of resources and the need for high containment labs have hindered our attempts to study this virus. There is a need to isolate other strains of MERS-CoV to determine variations in the circulating strains and associated pathogenesis. The correlates of protection in surviving patients are not well defined, but early intervention seems to offer some protection. However, it is easy to confuse early symptoms of MERS with that of the common cold. We need to establish a public communication strategy and prioritize patients that have had known contacts with camels. In an observation study, combination treatment with interferon-beta and ribavirin was identified to be effective in early-stage patients but not in patients who were diagnosed late [57]. An ongoing clinical trial is studying the efficacy of interferon and antiviral combination treatments in MERS-CoV infected patients [58]. These studies will shed more light on possible ways to treat MERS patients. In the meantime, ongoing research is identifying several novel strategies to inhibit MERS-CoV entry and replication in cells. Thus, in the future, it might be possible to use these experimental treatment options, in combination or alone, to prevent the onset of severe disease in patients that have likely been exposed to MERS-CoV. However, the timing of the treatment will be crucial and it will be essential to identify patients during the early stages of infection. This further emphasizes the need for effective differential diagnostics and appropriate training of healthcare professionals. Vaccines for coronaviruses have been challenging to develop due to the ability of coronaviruses to recombine [59] and the possibility of exacerbating disease symptoms post an immune challenge [60, 61]. However, subunit vaccines using the spike protein have generated promising results in Camelids and may one day be suitable for human-use, although not in the near future.