

Current understanding of middle east respiratory syndrome coronavirus infection in human and animal models

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Abstract: Middle East respiratory syndrome (MERS) is a highly lethal respiratory disease caused by a novel betacoronavirus (MERS coronavirus, MERS-CoV). Since its first emergence in 2012, multiple transmission events of MERS-CoV (dromedary to human and human to human) have been reported, indicating that MERS-CoV has the potential to cause widespread outbreak. However, the epidemiology of MERS as well as immune responses against the virus in animal models and patients are still not well understood, hindering the vaccine and therapeutic developments. In this review, we summarize recent genetic and epidemic findings of MERS-CoV and the progress in animal model development, immune response studies in both animals and humans. At last, we discussed the breakthrough on vaccine and therapeutic development which are important against potential future MERS outbreak.

Keywords: Middle East respiratory syndrome coronavirus (MERS-CoV); epidemiology; animal model; immune response; antiviral therapy

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Introduction

Since the discovery of first human coronavirus (HCoV), HCoVs have been studied for over 50 years (1). Overall six HCoVs have been identified including HCoV-229E and HCoV-OC43 (first identified in the 1960s), SARS-CoV (in 2003), HCoV-NL63 (in 2004), HCoV-HKU1 (in 2005) and Middle East respiratory syndrome coronavirus (MERS-CoV) (in 2012) (1-5). Zoonotic coronaviruses such as SARS-CoV (6) and MERS-CoV (7), crossed barrier and lead to epidemic in humans. MERS-CoV as the most recent novel coronavirus emerged in human can cause severe, life-threatening disease and is a potential threat to global public health and economy. Since 2012, MERS cases, including those transmitted from dromedary camels were reported to

World Health Organization (WHO) almost every month. In May 2015, a returned traveler with MERS-CoV infection caused a significant outbreak in South Korea, which spread to 186 patients and over 16,000 people were quarantined (8). This outbreak raised the fear that a pandemic like SARS would reoccur. Here, we reviewed the epidemiology, animal model generations and most recent progress on vaccine and treatment developments against MERS-CoV.

Origin and evolution

MERS-CoV was first emerged in Saudi Arabia in 2012. As of January 2018, WHO has been notified of 2143 laboratory-confirmed cases including 750 deaths (mortality rate, 35%) from 27 countries (<http://www.who.int/emergencies/mers-cov/en/>)

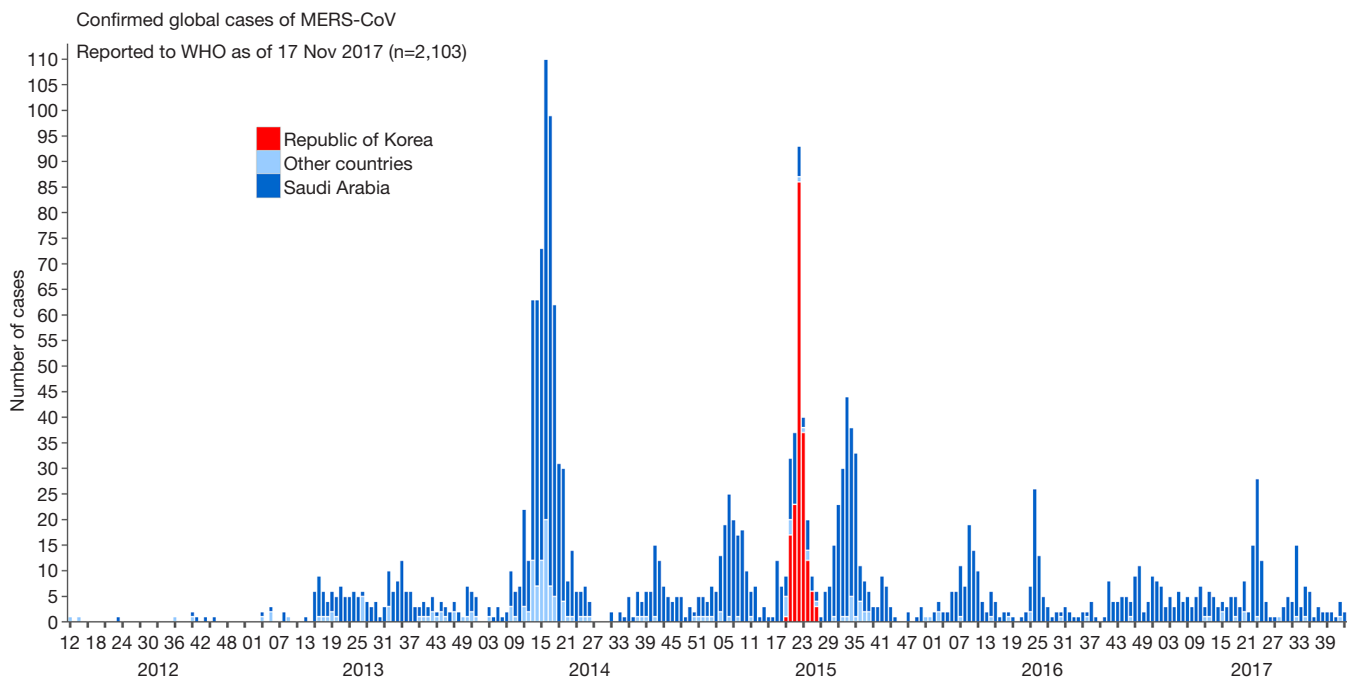


Figure 1 Confirmed global cases of MERS-CoV from World Health Organization. MERS-CoV, Middle East respiratory syndrome coronavirus.

(Figure 1). The evolutionary origins of MERS-CoV are still uncertain. Epidemiologic surveys showed that most of the dromedary camels in Middle East region are serological or MERS-CoV viral nucleic acid positive (9). Several MERS-CoV viruses have also been isolated from these camels indicating that they could be an intermediate host for MERS-CoV (10). In addition, MERS-CoV shares strong sequence similarities with bat CoVs, such as HKU4 and HKU5. HKU4 virus even uses the same DPP4 receptor for entry, and two critical mutations could license bat-to-human transmission of MERS-CoV (11). Whether MERS-CoV is originated from bats as SARS-CoV did remains unclear so far. Current evidence indicates that bats are likely to be the original source (12,13), and dromedary camels are considered to be a possible intermediate host for MERS-CoV (10) (Figure 2). MERS-CoV continuously crosses species and transmits from dromedary camels and/or bats to human population, which poses a significant threat to public health. Previous studies indicated that MERS-CoV infection are primarily due to repeated introductions of MERS-CoV from dromedary camels to human, while human to human transmission is limited (14,15). However, the 2015 MERS outbreak in South Korea with dozens of secondary- and tertiary-generation cases raised the concern

that MERS-CoV may have adapted to allow a more efficient spread in humans (16).

As for the evolution, CoVs are one of the most rapidly evolving viruses undergoing frequent genetic recombination and mutations. Compared to other RNA viruses, the estimated evolutionary rates in HCoV are moderate to high (17,18). For MERS-CoV the mutation rate in the complete genome was estimated to be 1.12×10^3 substitutions per site per year (19), while HCoV-OC43 and HCoV-229E represent an average mutation rate of about $3\text{--}6 \times 10^4$ substitutions per site per year (20,21). Deletion mutation also occasionally occurred in the genome of MERS-CoV, especially in the accessory proteins (22). Based on the analysis of MERS-CoV sequences which are available in genbank, diverse MERS-CoV strains are circulating in dromedaries and human to date, including Clade A and Clade B. Clade B can be further divided into five lineages (lineage 1–5 or group 1–5) (16,19). Evidence of genetic recombinant has also been found in MERS-CoV (16), as well as in other HCoVs, such as HCoV-OC43 (23), HCoV-NL63 (21), HCoV-HKU1 (24), SARS-CoV (25,26). Several groups also reported multiple recombinant MERS-CoV prevalent in dromedary camels and humans (26). The MERS-CoV outbreak in South Korea in 2015 also revealed

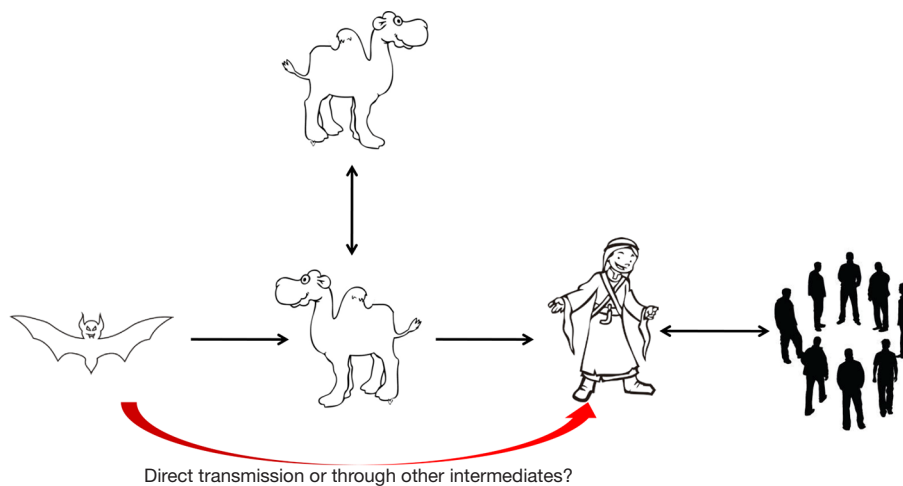


Figure 2 Transmission of MERS-CoV. MERS-CoV, Middle East respiratory syndrome coronavirus.

a probable recombinant event between two different parental Clade B viruses (16). Genetic recombination in MERS-CoV indicates that frequent co-infections with different lineages of MERS-CoV could occur in camels and humans (26). In summary, mutation and deletion in accessory proteins and genetic recombination play a major role in the evolution of MERS-CoV.

Virology and structure

Coronaviruses are the largest positive strand RNA viruses (26–32 kb) that are about 125 nm in diameter (27). Four coronavirus genera have been identified including alpha- (group 1), beta- (group 2), gamma- (group 3) and deltacoronavirus (group 4) genera (28). HCoV are among the alphacoronavirus (HCoV-NL63 and HCoV-229E) and betacoronavirus (HCoV-HKU1, HCoV-OC43, SARS-CoV and MERS-CoV) (*Figure 3*). Four HCoVs, including HCoV-NL63, HCoV-OC43, HCoV-229E and HCoV-HKU1, are circulating globally in human population and primarily contribute to 10–20% common cold (3,29), while MERS-CoV and SARS-CoV are the two major causes of severe pneumonia in humans (30,31), other four HCoVs sporadically causing severe pneumonia were also reported (32–35). In 2002–2004 pandemic, SARS-CoV infected over 8,000 patients and resulted in more than 800 deaths (36). MERS-CoV is the etiological agent responsible for the ongoing MERS pandemic in Middle East region. No specific vaccine and drug have so far been licensed for human use (37).

MERS-CoV genome contains a 5' terminal cap structure

along with poly (A) tails at the 3' end, the replicase gene encoding the non-structural protein makes up approximately two-third of the genome at the 5' end of genome, which contains 16 non-structural proteins (nsp1–16). Four structural protein, including spike (S), envelope (E), membrane (M) and nucleocapsid (N) protein, and five accessory proteins (ORF3, ORF4a, ORF4b, ORF5 and ORF8) make up about 10kb at the 3' end of genome (*Figure 4A*). In summary, MERS-CoV genome is typically arranged in the order of 5'terminal-ORF1a-ORF1b-S-E-M-N-3'terminal, accessory proteins are interspersed along the structural genes. The viral membrane contains S, E and M protein, and spike protein plays vital roles in viral entry. MERS-CoV attaches human host-cell receptor dipeptidyl peptidase 4 (hDPP4, CD26) (38) via receptor binding domain (RBD) of spike protein (39). M and E proteins play important role in viral assembly, N protein is required for RNA synthesis (*Figure 4B*) (28).

Animal models

Robust animal models for MERS infection are urgently needed to elucidate MERS pathogenesis and develop antiviral drugs and vaccines. However, small laboratory animals that generally used for emerging virus studies, such as mice (40,41), ferrets (42), guinea pig (43) and hamster (44) are not susceptible to MERS-CoV infection since their homologous DPP4 molecules do not fit as the receptors for MERS-CoV entry. No effective viral replications were detected in these animals after challenged with high dose of MERS-CoV (45). Upon intratracheally (IT) and intranasally

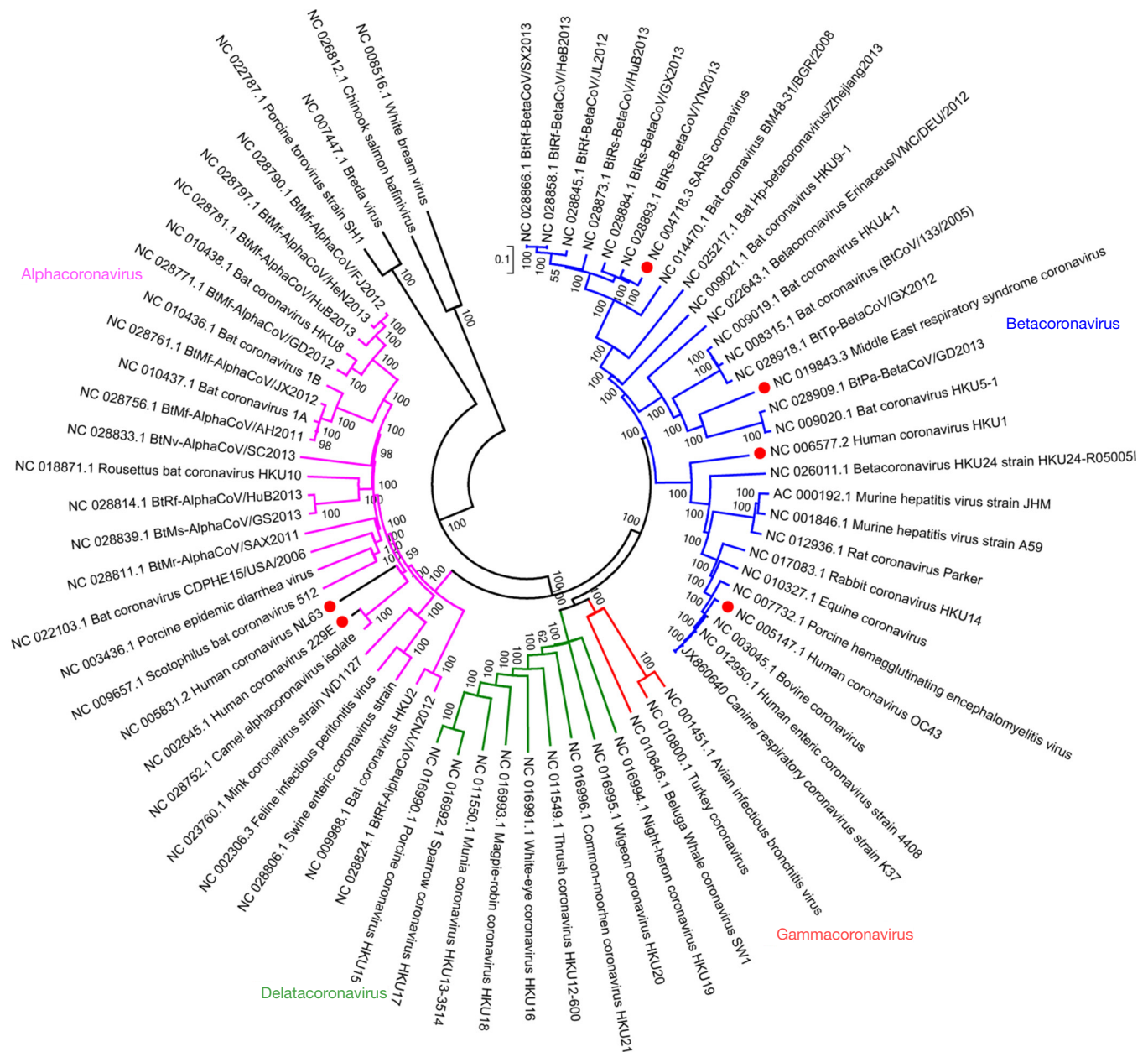


Figure 3 Phylogenetic analysis of coronaviruses based on complete genomes.

(IN) inoculation with MERS-CoV, New Zealand white rabbits remained free of clinical sing of disease (46), whereas viral RNA was detected in the respiratory tract, and moderate necrosis was observed in nasal turbinates. Dromedary camels, as a reservoir of MERS-CoV, developed mild upper respiratory infections after MERS-CoV infection (47). Alpacas, a close relative within the Camelidae family, secreted live virus after oronasal infection and remained asymptomatic without showing any upper or

lower respiratory tract diseases (48,49). In addition, due to their cost and relatively larger size, these animal models are not suitable for high-throughput research for MERS.

Nonhuman primates

Nonhuman primates are useful models for pathogenesis studies and vaccine evaluations for a lot of human infectious diseases. MERS-CoV caused transient lower respiratory

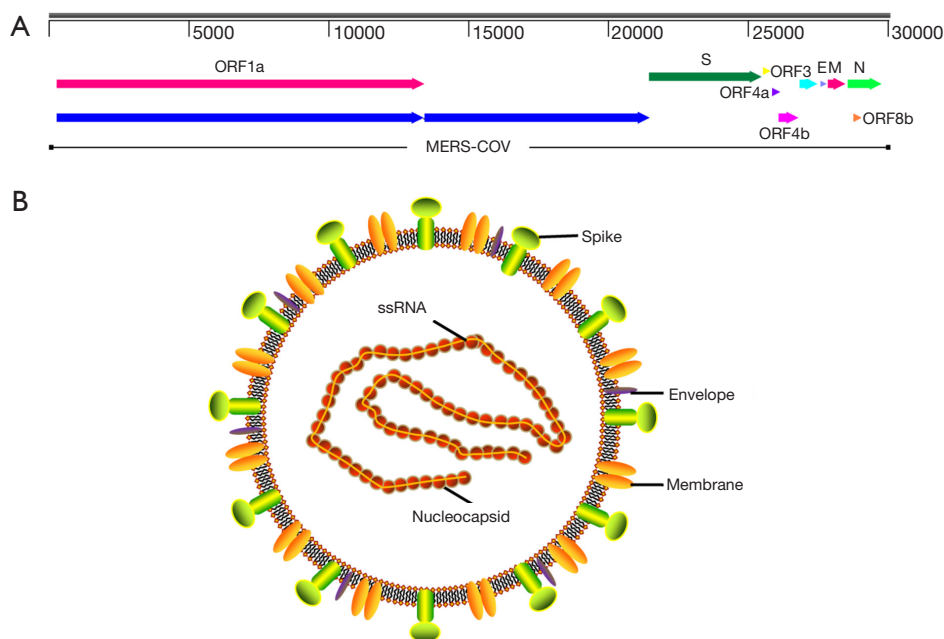


Figure 4 The MERS-CoV genome structure and virion. MERS-CoV, Middle East respiratory syndrome coronavirus.

tract infection in rhesus macaques. Clinical signs were observed by 1-day post infection (dpi) and resolved as early as 4dpi. No fatal cases observed in rhesus macaque model, the infection is not lethal to the rhesus macaque. This model can be used for studying the pathogenesis of mild MERS-CoV infection in humans (50). The use of common marmosets is still controversial. While a study showed that the infection could developed a progressive severe pneumonia (51), other groups found that common marmosets only developed mild to moderate nonlethal respiratory diseases through intratracheal infection with MERS-CoV (52).

Mice

Wild-type rodents are not susceptible to MERS-CoV infection (41). However, researchers have developed several models that rendered mice susceptible to MERS-CoV infection (53-55). In 2014, the first mouse model for MERS infection was generated (53). In this study, mice were transduced intranasally with recombinant adenovirus 5 encoding hDPP4 molecule. This model supports MERS-CoV replication in the lungs, and mice developed signs of interstitial pneumonia, including inflammatory cells infiltration, alveolar thickening and mild edema (53). This model enables research to use mice for MERS study

although there are some limitation, such as uncontrolled level of the hDPP4 expression and their tissue distribution. In 2015, the hDPP4 transgenic mice have been developed (54). These mice could be efficiently infected by MERS-CoV. However, global hDPP4 expression led to multiple organ damage (54), resulting in the death of the animals probably due to the lethal brain infection, as observed in ACE2 transgenic mice infected with SARS-CoV (56). Most recently, several MERS mouse models have been generated by replacing mouse DPP4 gene with homologous human DPP4 gene (55,57). Li and colleagues developed human DPP4 knockin (KI) mice, where mouse DPP4 gene fragments had been replaced by homologous human DPP4 fragments responsible for receptor binding. Further they serially passaged WT MERS-CoV in the respiratory tract of these mice for 30 times, resulting a mouse adapted MERS-CoV strain (MERSma). MERSma contained 13-22 mutations that caused significant weight loss and mortality in human DPP4-KI mice (55), this model is so far the best mouse model for MERS.

Immunopathogenesis

The lack of human autopsy data as well as good animal models hindered our understanding on the immunity and pathogenesis of MERS-CoV infection. Based on our

knowledge from the studies of SARS, several factors could be involved in MERS pathogenesis, including viral and host factors, interferon induction, dysregulation of cytokines and adaptive immune responses (58,59).

Virus and host interactions

SARS and MERS are both severe pneumonias caused by novel CoVs and they shared some similarities in clinical and laboratory features (58). For instances, the elderly and immunocompromised individuals are more susceptible to both SARS-CoV and MERS-CoV infections (60). However, the average comorbidity rate are much higher in MERS patients than that of SARS patients (76% vs. 10–30%) (61,62). The mortality rate of MERS so far is about 35% which is much higher than that of SARS (~10%) (7). Although some of the mild MERS patient were not readily identified in Middle East region (63), it might also partially due to the difference immunopathogenesis of these two viruses. Unlike SARS-CoV, MERS-CoV could efficiently infect human dendritic cells (64) and macrophages (65) *in vitro* which would help the virus to dysregulate the immune system. MERS-CoV also has the ability to infect T cells through their highly expression of CD26, leading to T cell apoptosis (66), which might potentially disrupt anti-viral T cell responses. As published previously, the clearance of MERS-CoV and SARS-CoV both required virus-specific T cell responses (67,68).

Some host factors might also be involved in MERS-CoV infection. As the increasing expression level of prostaglandin D2 (PGD2) in aged lungs impaired respiratory DC migration from lung to the draining lymph nodes, which in turn diminished the anti-viral CD8 T cell responses and resulted in increased mortality following SARS-CoV infection (69). Whether any host factors are involved in MERS-CoV infection and increase mortality rate in patients with comorbidity remain unknown.

Interferon antagonism

It is generally accepted that innate immune response is essential for the control of coronavirus infection, and it also determines the extent of initial virus replication and immune response activation (70). MERS-CoV replication is highly sensitive to type I interferon (IFN-I) treatment in cell culture suggesting that IFN-I treatment could be a possible therapeutic approach in clinical practice (71). The combination of IFN- α 2b or IFN- β 1a with ribavirin was

effective in reducing MERS-CoV replication *in vitro* and improves outcome in MERS-CoV-infected rhesus macaques (72,73). It is well known that CoVs have developed several strategies to evade the innate immune response. In SARS-CoV infection, SARS-CoV accessory proteins ORF3b and ORF6 decreased IFN-expression (74), and ORF6 inhibited nuclear translocation of STAT1 which is the key molecule governing the expression of interferon-stimulated genes (ISG) with antiviral activity (75-77). Similarly, MERS-CoV structural and accessory proteins, including M, ORF4a, ORF4b, and ORF5 had all been proved that could antagonize IFN-I signaling and inhibit ISG productions (78,79).

Antibody and T cell responses

Convalescent serum from MERS and SARS patients could accelerate virus clearance (80,81). Neutralizing antibodies generated *in vitro* or by vaccination could efficiently prevent the secondary infection with the same strain of CoVs in animal models (82). However, antibody response in patients previously infected with SARS-CoV and MERS-CoV tend to be short lived (68,83). On the other hand, T cell response often target highly conserved internal proteins and are long lived. SARS-CoV-specific memory T cells but not B cells could be detected 6 years after infection in SARS survivors (84). A recent study showed that CD8 T cell response could be detected in patients with undetectable neutralizing antibody in convalescent MERS patients (63). Immunodominant epitopes recognized by T cells in MERS-CoV infected mice were found in structural protein S, M, N (53,85). These MERS-CoV-specific CD8 T cells efficiently lysed the target cells in cytotoxicity assays (53). It also had been shown previously, that adoptive transfer of SARS-CoV-specific CD4 or CD8 T cells reduced virus titers in the infected mouse lungs (85).

Vaccine and antiviral drug developments

Vaccine

There were still no vaccines and effective antiviral therapeutics against MERS-CoV infection (86). The spike protein of MERS-CoV, which is responsible for MERS-CoV entry is considered as a key target for vaccine development against MERS-CoV infection (87). Multiple vaccine candidates targeting S protein were developed, including DNA vaccines (88,89), subunit vaccines (90,91) and recombinant vector vaccines (92,93). DNA vaccine

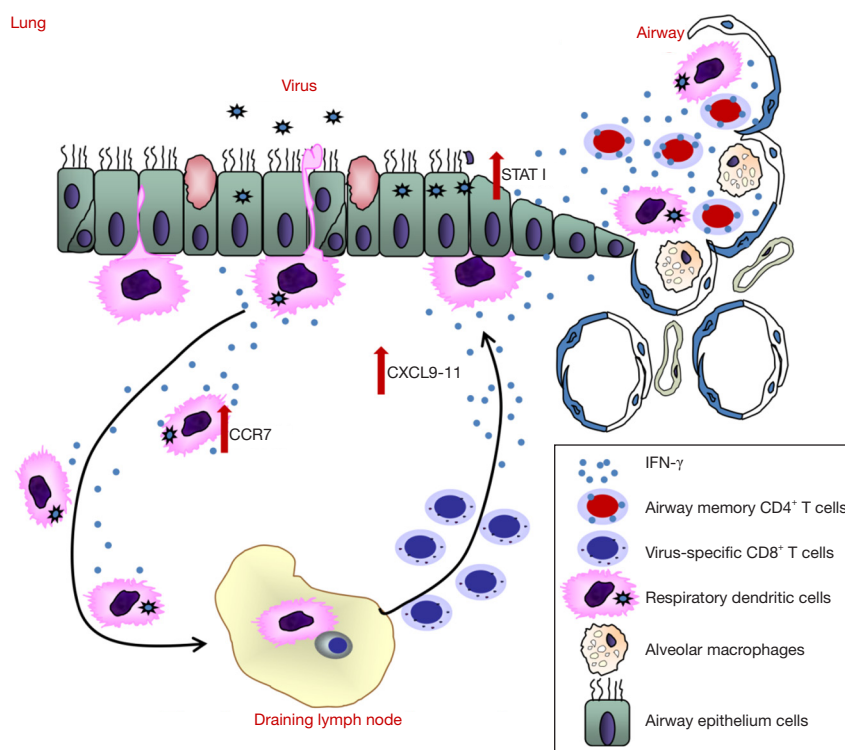


Figure 5 Airway memory CD4⁺ T cells mediate protective immunity against emerging respiratory coronaviruses.

expressing MERS-CoV S1 gene induced antigen-specific humoral and cellular immune responses in mice (88). In addition, RBD fragment induced the highest-titer IgG antibodies in mice compared with other region of S protein (90). Recombinant vectors (modified vaccinia Ankara and adenovirus vector) expressing MERS-CoV S glycoprotein showed immunogenic in mice (94). Attenuated live vaccine also has been shown to be protective; however the worries of its inadequate attenuation hindered its application (95). The protective role of virus specific CD4T cells is less studied, especially in respiratory CoV infections (63). Most recently, Zhao and colleagues showed that airway memory CD4 T cells were generated after intranasal vaccination with CoV N protein and mediated protection in following CoV challenge (68). These cells could upregulated anti-viral innate response at very early stage of infection and promoted CD8 T cell response by accelerating rDC migration and CD8 T cell mobilization (68). More importantly, they also found that these CD4 T cells targeting a conserved epitope within N protein cross reacted with several other CoVs, indicating that induction of airway memory CD4 T cells should be considered as a component of any universal human coronavirus vaccine (68) (Figure 5).

The combination of memory CD4 T cells with those able to elicit strong neutralizing antibodies and memory CD8 T cells could provide better protection against MERS-CoV as well as other respiratory CoV infections.

mAb and antiviral drugs

Neutralizing monoclonal antibodies bind to MERS-CoV spike protein and prevent virus-entry and following membrane fusion, therefore inhibit viral replication and reduce clinical diseases in animal models and humans (86). Neutralizing human monoclonal antibody (mAb) can be used for prophylactic and post-exposure treatments. Several potent mAbs have been developed from MERS infected patient (96), by humanizing mouse mAb against MERS RBD (97) or by screening human antibody phage library (82). All of these mAbs targeted RBD region of spike protein (98). To avoid viral escape mutants, combination of at least two monoclonal antibodies targeting different regions of spike protein had been prove to be more effective (86).

In addition, several antiviral drugs had been developed, including peptide fusion inhibitor targeting heptad-repeat region (HR) of S2 (99), small molecular entry inhibitor

targeting S (100), IFN- β , IFN- γ and ribavirin (101). Recently, GS-5734, small-molecule monophosphoramidate, prodrug of an adenosine analog which targeted RdRp, had been found to inhibit MERS-CoV and SARS-CoV replication in multiple *in vitro* systems (102).

Summary

With the MERS-CoV circulating in the dromedary camels in the Middle East region, efforts regarding epidemic surveillance, phylogenetic analysis, vaccine and antiviral drug developments were still needed to response to the global public health threat posed by this virus. Unlike SARS-CoV, the virus and host interaction as well as pathogenesis and immune responses after MERS-CoV infection in animals and humans are less investigated. Understanding these basic informations will not only enhance emerging CoV research but also will aid our public health preparedness against MERS-CoV.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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