Editorial

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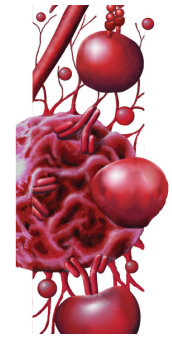
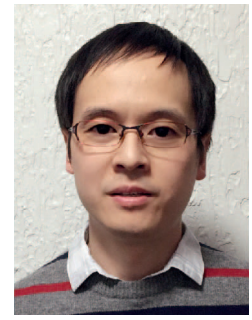
Immunotherapy



Human neutralizing antibodies against MERS coronavirus: implications for future immunotherapy

"The high mortality rate (~37%) and ongoing epidemic have raised concern of a more widespread regional outbreak or even global spread."

Keywords: animal model • biodefense • emerging pathogen • human monoclonal antibodies • humoral immunity • IGHV1–69 • MERS-CoV • passive immunotherapy • spike protein • zoonosis



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Middle East respiratory syndrome coronavirus (MERS-CoV) was first reported in September 2012 [1]. Dromedary camels are a putative source for human MERS-CoV since dromedaries have shown high rates of seropositivity (>90%) in serum samples collected over 30 years ago and virus was recently isolated from camels [2]. The high prevalence of MERS-CoV infection in camels and the difficulty of eliminating this virus reservoir suggest that the recurrence of MERS is very likely to occur. Indeed, during early 2015 an increasing number of MERS cases in the Kingdom of Saudi Arabia have been seen. As of 11 March 2015, 1060 confirmed cases, including at least 394 related deaths have officially been reported to WHO [3]. The high mortality rate (~37%) and ongoing epidemic have raised concern of a more widespread regional outbreak or even global spread.

Since this virus was reported, tremendous efforts have been made in the search for effective anti-MERS-CoV agents. Combinations of treatment with IFN-α2b and ribavirin were reported to improve clinical outcomes in MERS-CoV-infected rhesus macaques [4]. A synthesized peptide (HR2P) was found to inhibit MERS-CoV replication and its S protein mediated cell–cell fusion [5]. Some compounds with anti-MERS-CoV activity in cell culture were found by screening small libraries of US FDA approved drugs [6,7]. Neutralizing antibodies (nAbs) could be used in an outbreak setting for the prophylaxis and early treatment of emerging viral pathogens. During SARS outbreak, convalescent plasma from recovered SARS patients have been shown to help clearing SARS-CoV efficiently [8,9]. Convalescent sera were recommended for MERS treatment by the International Severe Acute Respiratory and Emerging Infection Consortium [10]. Monoclonal antibodies (mAbs) have demonstrated host protection against viral infections [11]. In this review, we summarize the recent progress in potential therapeutic mAbs and animal models for MERS.

Current nAbs against MERS-CoV

In April 2014, three independent studies reported the identification of human nAbs against MERS-CoV [12–14]. In our study, seven nAbs were identified from a well-characterized phage-displayed scFv library [12]. The panning target was full-length Spike incorporated on the surface of paramagnetic proteoliposomes and mammalian cells. This kind of bait was used to mimic the native structure of Spike protein

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on viral particles. With this panning strategy, seven nAbs were identified and all bound to the receptor-binding domain (RBD) in S1 of MERS-CoV with nanomolar-picomolar binding affinities. A competition assay revealed that these seven mAbs could be categorized into three groups and bind to at least three distinct epitopes. One epitope is located centrally in RBD and another two are flanking. All seven mAbs can neutralize pseudotyped and live MERS-CoV infection in cell culture through a mechanism of blocking the interaction between MERS-CoV S protein and its hDPP4 receptor. Virus evolution under nAb pressure was further investigated by identifying escape mutants. Interestingly, all the escape mutations were mapped onto the receptor-binding motif (RBM) that interfaces with the hDPP4, except for one mutation in S2 domain. These escape mutations reflected virus evolution under mAb mediated immune pressure as well as the binding footprints of each mAbs. These escape mutations not only affect RBD-Ab interaction, but also affect RBD–hDPP4 interaction. In addition, although MERS-CoV can escape these mAbs' neutralization, these mutations lead to a significant cost in viral fitness.

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With a yeast displayed scFv library and purified RBD as panning target, Jiang *et al.* identified two antibodies (MERS-4 and MERS-27) that can neutralize MERS-CoV infection as well as inhibit syncytia formation [13]. These two mAbs bind to RBD with nanomolar binding affinities. Combination of these two mAbs demonstrated a synergistic effect in neutralization. By alanine-scanning, the epitope of MERS-4 was mapped onto the interface of RBD-DPP4 while epitope of MERS-27 was not clear yet.

With a phage-displayed Fab library and purified RBD as selecting antigen, Ying *et al.* identified three nAbs [14]. The IgG form of three mAbs bind to RBD with sub-nanomolar affinities. Binding competition and alanine-scanning indicated that the epitopes bounded by these antibodies overlap with each other. These three mAbs also compete with the receptor DPP4 for binding to the S protein. Antibody modeling and docking results suggest a possible dominant role of the heavy chain in the mAb paratopes.

Although different antibody libraries and panning strategies were used in the above studies, it is surprising that all identified neutralizing mAbs bind to receptor binding motif of MERS-CoV spike protein, despite the full-length spike was used as panning antigen. This demonstrates that the RBM is dominant in the selection systems, suggesting the RBM as a critical target for the development of MERS-CoV vaccines and therapeutics. Coronavirus S1 is responsible for virus attachment to cellular receptor, while S2 is responsible for viral and cellular membrane fusion. S2 is more conserved than S1 protein, suggesting that the S2 epitope may provide broader nAbs. The combination of Abs targeting divergent epitopes, that we termed 'divergent combination immunotherapy', would be more potent to prevent viral infection and neutralization escape [15]. Another interesting finding is that 8/12 of these mAbs used IGHV1–69 germline gene. IGHV1–69 germline gene has been reported preferentially used by many antiviral mAbs [11]. Finally, all these mAbs bind to RBD with nanomolar to picomolar affinities, but they showed different neutralization potencies according to each study. This is most likely because virus, cells and experiment conditions were different in each lab, or these potent mAbs do have different neutralization potencies *in vitro* despite they bind to closed epitopes with similar affinities. Most importantly, studies need to be conducted to verify if these mAbs can prevent MERS-CoV infection *in vivo*.

Animal models for MERS-CoV infection

Animal disease model is critical for studying the viral pathogenesis and evaluating effective countermeasures. However, unlike SARS-CoV, which readily infects many animals, the MERS-CoV does not seem to cause disease in small lab animals, such as mice, hamsters or ferrets. Rhesus macaque was the first animal model tested for MERS [4,16]. Upon a combined route of MERS-CoV inoculation via intratracheal, ocular, oral and intranasal administration, rhesus macaques developed a transient lower respiratory tract infection [16]. Viral RNA could be detected in the nasal mucosa, trachea and mediastinal lymph nodes, conjunctiva, tonsils, oronasopharynx and in the left and right bronchus on 3 days postinfection (dpi). However, viral loads were lower or undetectable in these tissues by 6 dpi. Compared with uninfected animals, the lung pathology, regulation of host gene expression and production of cytokines in infected animals showed significant differences on 3 dpi but not 6 dpi. These results suggest that MERS-CoV causes only transient infection in rhesus macaque resulting in mild to moderate clinical disease. The rhesus macaque model does not exactly recapitulate the more severe disease observed in humans [16].

Zhao *et al.* developed a mouse model for MERS by transducing human DPP4 into mouse lungs with

adenoviral vectors to sensitize mice for infection [17]. After MERS-CoV infection, virus could be detected in lungs but cleared by days 6–8 in young mice and days 10–14 in old mice; young mice failed to gain weight while aged mice lost weight. Several strains of immunocompromised mice were persistently infected, but did not lost weight. This suggested that this mouse model may recapitulate the respiratory disease observed in mild or moderate human cases but not the fatal cases or the occasionally occurring kidney disease. This model can be difficult as an evaluative model for therapeutics since clinical signs are mild; pathology and viral replication are limited.

Falzarano *et al.* tested the common marmoset as a MERS-CoV model. After MERS-CoV inoculation combined via intranasal, intratracheal, oral and ocular routes, most of the marmosets developed respiratory diseases that ranged from moderate to severe as indicated by progressive severe pneumonia with extensive lung pathology. Two out of nine animals had to be euthanized due to the severity of disease. Viral loads in the lungs were up to 1000-times higher than those in the rhesus macaque lungs and did not decrease between 3 and 6 dpi. Viral RNA was also detected in the blood of infected marmosets, suggesting a more systemic dissemination. This is the first animal model for MERS-CoV that showed severe to lethal disease. Common marmosets may be the best model for evaluating the efficacy of vaccines and treatment strategies to date. However the availability is limited.

Conclusion

The high fatality rate and epidemic of MERS emphasizes the need for effective vaccine or antivirals. Recently identified human neutralizing mAbs have shown potent neutralizing activities *in vitro*. Assessment of these mAbs in relevant animal models is yet to be conducted before clinical trials. It is worth to note that all human neutralizing mAbs mentioned above were identified from phage/yeast-displayed antibody libraries. This is particularly important when early access to patient specimens is problematic, in part because of government, regulatory and biocontainment restrictions. All these mAbs, despite being identified from different library with different methods, target the RBD of MERS-CoV S protein and have a similar mechanism of neutralization, blocking the interaction of MERS-CoV spike with its receptor DPP4. These mAbs have nanomolar to picomolar binding affinities to MERS-CoV RBD. They appear to recognize adjacent but nonoverlapping epitopes, suggesting that these mAb cocktails, while being directed to a similar region on S1 may still be sufficient for divergent combination immunotherapy. In addition, therapeutic approaches should not be limited to the combination of different antiMERS-CoV mAbs, but rather include the combination of mAbs with MERS-CoV inhibitors. We expect that these efforts will result in some potent therapeutic approaches to treat MERS patients and prevent MERS-CoV infection in high-risk populations, such as healthcare workers and patient families.

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