INFECTIOUS DISEASE

Neutralizing the MERS Coronavirus Threat

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Human neutralizing monoclonal antibodies are candidates for prophylactic and therapeutic interventions against Middle East respiratory syndrome coronavirus (MERS-CoV) infection.

A CONTINUING THREAT

Middle East respiratory syndrome coronavirus (MERS-CoV) was recently discovered in humans with lower respiratory tract infection. Available data suggest that the clinical presentation of MERS-CoV infection ranges from asymptomatic to severe pneumonia, ultimately causing death (*1*). Chest radiograph findings are consistent with severe viral pneumonitis and acute respiratory distress syndrome (ARDS), although co-infections with other respiratory viruses as well as nosocomially acquired bacterial infections have been reported, and may complicate the clinical diagnosis. Most MERS patients seem to have underlying comorbidities, including type 2 diabetes, and the clinical presentation is usually more severe in immunocompromised individuals (*1*). In the absence of specific medication effectively targeting ARDS, general supportive care is currently the cornerstone of patient management.

The emerging severe acute respiratory syndrome (SARS) pandemic, caused by SARS-CoV, that started about a decade ago was successfully controlled through rapid identification of the agent, subsequent isolation of infected individuals, supportive care of isolated patients, and interruption of virus introductions from carnivore spill-over reservoirs. Recent studies indicate that a MERS-CoV similar to the virus found in humans is present in dromedary camels, which may be the zoonotic spill-over animal reservoir (*2*). Because seropositive dromedary camels are found in most countries of the Arabian Peninsula and even in parts of Africa, zoonotic transmission of MERS-CoV is probably difficult to control.

Therefore, a "One Health" approach—a combination of animal and human health measures—that effectively blocks the zoonotic spread and subsequent human-to-human spread of MERS-CoV should be pursued urgently. This may be achieved by the elimination of MERS-CoV–spreading camels; the introduction of camel vaccination programs with currently available vaccine candidates; the prevention of human-to-human transmission by early identification and isolation of MERS-CoV infected individuals; and by the development of virus-specific treatment options for MERS-CoV–infected humans. To fulfill the latter, in a recent issue of *Science Translational Medicine,* Jiang *et al.* describe the production and characterization of neutralizing human monoclonal antibodies that could be used in prophylactic and therapeutic interventions against MERS-CoV infection in humans (*3*). These antibodies offer the advantage of safe use in humans and the ability to be produced in large quantities by recombinant DNA technologies.

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INTERVENTION

In MERS patients, MERS-CoV can be detected in blood, urine, and stool, but at much lower viral loads than in the respiratory tract. The relatively high viral load in samples collected from the lower respiratory tract, as compared to those from the upper respiratory tract, suggests that the lungs are the primary site of infection. Although approximately 40% of the MERS patients identified have succumbed to severe lung damage leading to ARDS, the majority apparently mounted immune responses that eventually controlled the infection. Identification and characterization of these protective immune mechanisms in humans may therefore provide essential clues for intervention strategies that may be translated into clinical practice. Early in vitro observations already have indicated that, similar to SARS-CoV infection, interferons produced during the innate antiviral immune response inhibit MERS-CoV replication. Indeed, when combined with ribavirin, another antiviral compound, interferon-α inhibited MERS-CoV replication to some extent in a nonhuman primate model (*4*). However, the limited observational human data available suggest no clinical benefit with the same combination therapy.

Virus-neutralizing antibodies that block virus entry into the cell are considered powerful means to prevent infection. MERS-CoV uses its envelope spike glycoprotein to interact with a cellular receptor and entry into the target cell (Fig. 1). Determinants of host range and cellular tropism are located in the receptor-binding domain. Resolving the crystal structure of the MERS-CoV receptor binding domain complexed with the cellular receptor dipeptidyl peptidase 4 (DPP4, also called CD26) has revealed which amino acids are critically involved in binding of the spike protein to human DPP4. The virus's receptor-binding domain does not interact with the region that contains the hydrolase domain, which is responsible for its peptidase activity. Consequently, existing medications shown to inhibit this enzymatic activity, such as the type 2 diabetes drug linagliptin, do not inhibit MERS-CoV infection. Yet, antibodies capable of disrupting the spike protein–DPP4 interaction may block entry of MERS-CoV into the target cell. Indeed, immunization of rabbits with the MERS-CoV receptor binding domain induces such antibody production (*5*).

Neutralizing antibodies may be found in the plasma of patients who have survived MERS-CoV infection, although such convalescent plasma samples may be limited in availability. On the other hand, MERS-CoV cross-reactive neutralizing antibodies have not been detected in serum obtained from human blood donors. Therefore, more sophisticated technologies are needed to identify strongly neutralizing monoclonal antibodies that can be produced in large quantities. To this end, Jiang and colleagues isolated and characterized two potent receptor-binding domain–specific human neutralizing monoclonal antibodies (*3*). These two antibodies, named MERS-4 and MERS-27, were derived from single-chain variable region fragments of a non-immune human antibody library. Purified soluble MERS-CoV receptor binding domain was used as an antigen bait to select yeast cells that expressed diverse antibody fragments on their surface. [Alternatively, human antibodies may be derived from B cells isolated from MERS patients, as was also shown for SARS-CoV–specific antibodies (*6*)]. The selected antibodies inhibited infection in vitro with both pseudotyped (lentiviral

particles bearing MERS-CoV spike proteins on their surface) and live MERS-CoV, with IC50 values at nanomolar concentrations. Moreover, combining MERS-4 and MERS-27 antibodies demonstrated a synergistic neutralizing effect against pseudotyped MERS-CoV in vitro.

Further mechanistic analysis indicated that MERS-4 and MERS-27 blocked the spike protein receptor-binding domain interaction with the DPP4 receptor on the surface of Huh7 (hepatocellular carcinoma) cells (Fig. 1). Mutagenesis analysis demonstrated that MERS-4 and MERS-27 recognized distinct regions in the receptor-binding domain. These results suggest that these antibodies are promising candidates for preventing MERS-CoV infection (*3*). In contrast to antibodies derived from animal species, these human antibodies are nonimmunogenic when administered in people. A recent study in *Proceedings of the National Academy of Sciences* also reported several neutralizing human antibodies that can prevent binding of the MERS-CoV spike protein to DPP4 on human cells (*7*). This study further supports the role for human antibodies in thwarting viral infection, and the utility of nonimmune antibody-phage libraries for discovering such promising antibody candidates for MERS-CoV and other emerging infectious diseases. Furthermore, having the DNA sequences that code for these human antibodies will allow for production in large quantities using recombinant DNA technologies.

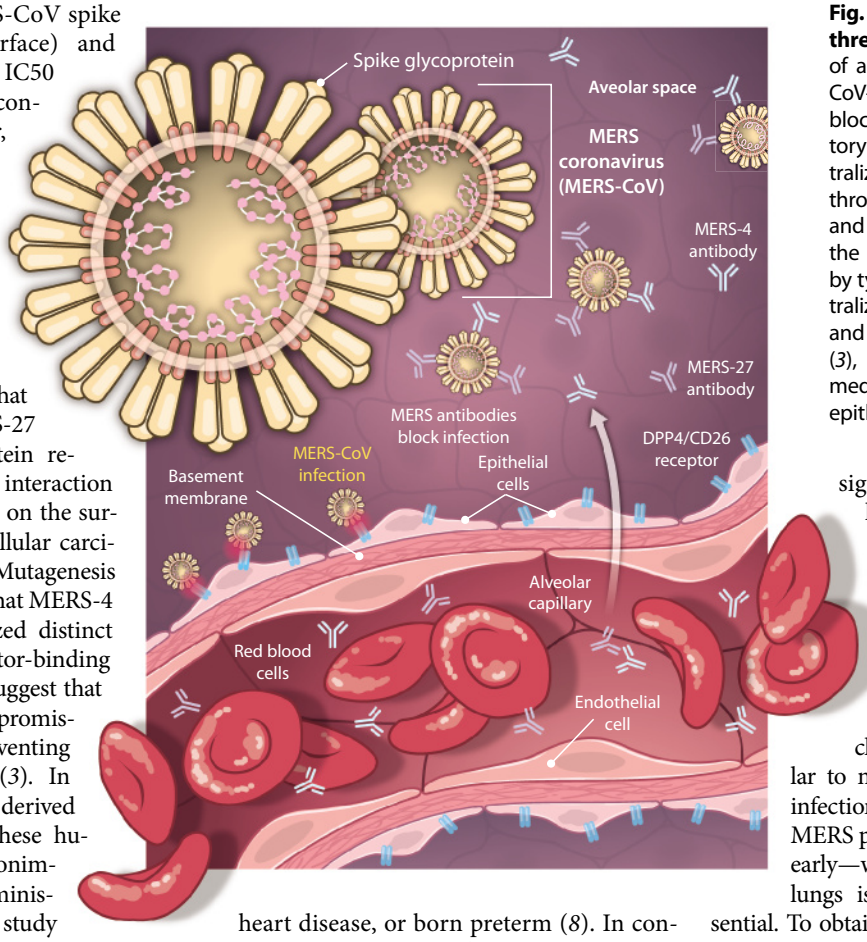

Fig. 1. Neutralizing the viral threat. Shown is the mechanism of action by which human MERS-CoV–neutralizing antibodies may block the virus in the lower respiratory tract. Circulating human neutralizing antibodies need to pass through the endothelial cell layer and the basal membrane to reach the alveolar space, which is lined by type I and II pneumocytes. Neutralizing antibodies, such MERS-7 and -24 discovered by Jiang *et al.* (*3*), can then block DPP4/CD26-mediated entry of MERS-CoV into epithelial cells.

TRANSLATING A MERS THERAPEUTIC ANTIBODY

Thus far, the best-studied human antiviral monoclonal antibody that made it to the clinic is palivizumab, which neutralizes respiratory syncytial virus (RSV). There is strong evidence that palivizumab prophylaxis is effective in reducing the frequency of hospitalizations due to RSV infection in children at high risk for infection, such as those with chronic lung disease, congenital heart disease, or born preterm (*8*). In contrast to prophylactic efficacy, therapeutic efficacy of the palivizumab antibody has not been demonstrated.

It will be a major challenge to test human MERS-CoV–neutralizing antibodies for a possible therapeutic benefit. First, to obtain further proof for the efficacy of MERS-CoV neutralizing antibodies MERS-4 and -27, testing in animal models is needed. However, development of animal models for MERS continues to be a challenge. MERS-CoV does not easily infect other animal species, such as mice, ferrets, and hamsters. Detailed analysis of the MERS-CoV spike protein binding region in DPP4 in different animal species revealed two divergent loops in the DPP4 propeller region. To circumvent this problem, the human *DPP4* gene has been delivered by an adenovirus to sensitize mice for MERS-CoV infection (*9*). Although this partially solved the problem, the MERS-CoV–infected mice showed little inflammatory changes in the lungs, as was also observed in MERS-CoV–infected nonhuman primates (*5*). Importantly, in *DPP4*-transfected mice administration of MERS-CoV neutralizing antibodies did significantly inhibit virus replication (*9*).

Second, it will be difficult to test the therapeutic benefit of these human antibodies in MERS patients. Given the acute nature of this respiratory infection and a clinical disease spectrum similar to many other respiratory virus infections, rapid diagnosis to identify MERS patients and to start treatment early—while viral replication in the lungs is still ongoing—will be essential. To obtain definitive proof of efficacy, well-controlled clinical trials will need to be performed. A thorough review of therapeutic options used for SARS clearly demonstrated the lack of evidence for therapeutic potential of nearly all of the intervention strategies explored during the outbreak (*10*). Some studies of convalescent plasma given in addition to steroids and ribavirin have been inconclusive, although observational data suggested efficacy (*10*).These promising results, combined with the identification and characterization of potent human MERS-CoV–neutralizing monoclonal antibodies (*3*, *7*), are important steps on the path to achieve the ultimate goal of prophylactically—and eventually even therapeutically—blocking MERS-CoV replication in humans.

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