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A Conformation-Dependent Neutralizing Monoclonal Antibody Specifically Targeting Receptor-Binding Domain in Middle East Respiratory Syndrome Coronavirus Spike Protein

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ABSTRACT

Prophylactic and therapeutic strategies are urgently needed to combat infections caused by the newly emerged Middle East respiratory syndrome coronavirus (MERS-CoV). Here, we have developed a neutralizing monoclonal antibody (MAb), designated Mersmab1, which potently blocks MERS-CoV entry into human cells. Biochemical assays reveal that Mersmab1 specifically binds to the receptor-binding domain (RBD) of the MERS-CoV spike protein and thereby competitively blocks the binding of the RBD to its cellular receptor, dipeptidyl peptidase 4 (DPP4). Furthermore, alanine scanning of the RBD has identified several residues at the DPP4-binding surface that serve as neutralizing epitopes for Mersmab1. These results suggest that if humanized, Mersmab1 could potentially function as a therapeutic antibody for treating and preventing MERS-CoV infections. Additionally, Mersmab1 may facilitate studies of the conformation and antigenicity of MERS-CoV RBD and thus will guide rational design of MERS-CoV subunit vaccines.

IMPORTANCE

MERS-CoV is spreading in the human population and causing severe respiratory diseases with over 40% fatality. No vaccine is currently available to prevent MERS-CoV infections. Here, we have produced a neutralizing monoclonal antibody with the capacity to effectively block MERS-CoV entry into permissive human cells. If humanized, this antibody may be used as a prophylactic and therapeutic agent against MERS-CoV infections. Specifically, when given to a person (e.g., a patient's family member or a health care worker) either before or after exposure to MERS-CoV, the humanized antibody may prevent or inhibit MERS-CoV infection, thereby stopping the spread of MERS-CoV in humans. This antibody can also serve as a useful tool to guide the design of effective MERS-CoV vaccines.

The newly emerged Middle East respiratory syndrome coronavirus (MERS-CoV) causes severe pneumonia and renal failure in infected patients and has led to 206 laboratory-confirmed MERS cases, including 86 deaths (a case fatality rate of ~42%) (1) (http://www.who.int/csr/don/2014_03_27_mers/en/). The symptoms caused by MERS-CoV infection are similar to those caused by the severe acute respiratory syndrome coronavirus (SARS-CoV), the latter of which led to over 8,000 infections and a fatality rate of ~10% during the 2002-2003 SARS epidemic (2, 3). While no new SARS-CoV case has been reported since 2005 (4), the number of reported cases for MERS-CoV infections is still rising. Despite the high fatality rate of MERS-CoV and its ongoing spread in the human population (5, 6), no vaccine or antiviral therapeutic is currently available to combat MERS-CoV infections. Therefore, the development of strategies to prevent and treat MERS-CoV infections is urgently needed. This study aims to develop such a strategy.

Both MERS-CoV and SARS-CoV belong to the *Betacoronavirus* genus of the coronavirus family (1, 7). Coronaviruses are enveloped and positive-stranded RNA viruses. The entry of coronavirus into host cells is mediated by a virus envelope-anchored spike protein (8–10). The spike protein contains a receptor-binding subunit, S1, and a membrane fusion subunit, S2. As a first step of viral entry, a defined receptor-binding domain (RBD) in the S1 subunit binds to a host receptor on the cell surface (4, 11, 12). The host receptors for MERS-CoV and SARS-CoV are dipeptidyl peptidase 4 (DPP4) and angiotensin-converting enzyme 2 (ACE2), respectively (13, 14). Structural studies show that the RBDs of MERS-CoV and SARS-CoV are comprised of a core structure and a receptor-binding motif (RBM) (12, 15–18). Whereas the core structures of these two RBDs are highly similar, their RBMs are significantly different, leading to different receptor-binding specificities. Following receptor binding, the S2 subunit of the spike protein undergoes a dramatic conformational change to fuse the viral and host membranes, allowing coronaviruses to penetrate cell membranes (10, 19). This knowledge has paved the way for

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possible human intervention to block the entry of coronaviruses into host cells.

Viral entry into host cells may be targeted in various ways (4). Vaccination remains one of the most effective approaches to control viral infections (20). In fact, both MERS-CoV and SARS-CoV RBDs can elicit strong neutralizing immune responses and, hence, potentially function as subunit vaccines (21–23). However, vaccines generally cannot provide immediate prophylactic protection or be used to treat ongoing viral infections. Instead, passive immunotherapeutics using neutralizing monoclonal antibodies (MAbs) have recently emerged as a powerful tool to provide prophylactic and therapeutic protections against viral infections (24, 25). For example, a potent therapeutic MAb, palivizumab, is currently used clinically to prevent and treat respiratory syncytial virus (RSV) infection in infants (26). In addition, several MAbs have been developed to combat SARS-CoV and influenza virus infections (24, 27). These therapeutic MAbs target the viral surface spike glycoproteins and block either the receptor-binding or the membrane fusion step (28–30). These studies suggest that therapeutic MAbs may be a promising approach to prevent and treat MERS-CoV infections.

In this study, we report the generation of a novel monoclonal antibody, Mermab1, which targets the MERS-CoV RBD and blocks MERS-CoV entry into host cells. We also characterize the neutralizing potency of, RBD-binding specificity of, and epitopes recognized by Mersmab1 (hereinafter referred to as "recognizing epitopes" of Mersmab1) and discuss its potential use in controlling MERS-CoV infections.

MATERIALS AND METHODS

Ethics statement. Female BALB/c mice aged 6 to 8 weeks were used for MAb production. The animal studies were carried out in strict accordance with the recommendations in the *Guide for the Care and Use of Laboratory Animals* of the U.S. National Institutes of Health (31) and of the State Key Laboratory of Pathogen and Biosecurity at the Beijing Institute of Microbiology and Epidemiology of China. The animal protocol was approved by the Committee on the Ethics of Animal Experiments of the Beijing Institute of Microbiology and Epidemiology (permit number PMB13.02).

Expression and purification of recombinant proteins. Recombinant MERS-CoV S1 or S2 protein fragments (strain EMC; GenBank accession no. AFS88936.1) were expressed and purified using a protocol as previously described (32). Briefly, the protein fragments were fused with either a C-terminal Fc tag of human IgG or a C-terminal His_6 tag and were transiently expressed in 293T cells. The protein fragments were harvested from the cell culture supernatants and purified using protein A Sepharose beads (GE Healthcare, NJ) (for Fc-tagged proteins) or Ni-nitrilotriacetic acid (Ni-NTA) Superflow (Qiagen, CA) (for His_6-tagged proteins).

Recombinant human DPP4 ectodomain (residues 39 to 766) was expressed and purified using a protocol as previously described for other coronavirus receptor proteins (16). Briefly, human DPP4 ectodomain with a C-terminal His_6 tag (DPP4-His_6) was expressed in insect cells using a Bac-to-Bac system (Life Technologies, CA). The protein was harvested from the cell culture supernatants and purified sequentially on a Ni-NTA column and a Superdex200 size exclusion column (GE Healthcare).

Generation of anti-MERS-CoV MAbs. Anti-MERS-CoV MAbs were generated using a protocol as previously described (24). Briefly, mice were immunized subcutaneously three times with MERS-CoV S1 subunit (residues 18 to 725) containing a C-terminal human IgG Fc tag (S1-Fc; 10 μg/mouse). Aluminum was used as an adjuvant (InvivoGen, CA). Mice were sacrificed 10 days after the last immunization, and their splenocytes were fused with mouse myeloma cells. Positive hybridomas were screened by enzyme-linked immunosorbent assay (ELISA) using a recombinant MERS-CoV S1 containing a C-terminal His_6 tag (S1-His) (33). Positive cells were expanded and subcloned to generate stable hybridoma cell lines. The MAbs were purified from ascites using protein A and G Sepharose 4 Fast Flow (GE Healthcare). To obtain the Fab region of MAbs, MAbs were digested using papain (Sigma, MO), and the resulting Fabs were purified as previously described (24).

Inhibition of MERS-CoV spike-mediated pseudovirus entry into target cells. Entry of MERS-CoV spike-mediated pseudoviruses into Huh-7 cells was inhibited by MAbs using a protocol as previously described (34). Briefly, 293T cells were cotransfected with a plasmid encoding Env-defective and luciferase-expressing HIV-1 genome (pNL4-3.luc.RE) and a plasmid expressing MERS-CoV spike protein. The produced pseudovirus particles were harvested 72 h posttransfection from the cell culture supernatant. The pseudovirus particles were subsequently incubated with the MAbs at 37°C for 1 h. The mixture of pseudovirus and MAbs was then added to DPP4-expressing Huh-7 cells, which had been preplated in 96-well tissue culture plates (10^4/well) 6 h before infection. After another 72 h, the cells were lysed with cell lysis buffer (Promega, WI). Lysates were transferred into 96-well luminometer plates, and the luciferase activity was determined using an Infinite M1000 luminometer (Tecan, CA).

MERS-CoV neutralization assay. The efficacy of MAbs in neutralizing MERS-CoV infection in DPP4-expressing Vero E6 or Calu-3 cells was determined using a microneutralization assay as previously described (22, 35). For the neutralizing assay using Vero E6 cells, each of the serially diluted anti-MERS-CoV MAbs was incubated with MERS-CoV (strain EMC) at a 0.1 multiplicity of infection (MOI) and a temperature of 37°C for 1 h. The mixture was then incubated with Vero E6 cells at 37°C for 72 h. The inhibitory capacity of each of the MAbs was assessed by determining the presence or absence of virus-induced cytopathic effect (CPE). The 50% neutralization dose (ND_{50}) was defined as the concentration of the MAb that completely inhibited virus-induced CPE in at least 50% of the wells (36). An anti-SARS-CoV RBD MAb, 33G4, was used as a control (27). For the neutralizing assay using Calu-3 cells, the mixture of Mersmab1 and virus was incubated with Calu-3 cells at 37°C for 24 h. The efficacy of Mersmab1 in attenuating MERS-CoV-induced CPE was observed with an inverted microscope (Olympus 1X51). Levels of CPE were scored as follows: CPE0 (no CPE), CPE1 (5 to 10%), CPE2 (10 to 25%), CPE3 (25 to 50%), or CPE4 (>50%).

AlphaScreen assay. An AlphaScreen assay was performed to detect the binding between Mersmab1 and MERS-CoV RBD. MERS-CoV RBD (residues 367 to 588) containing a C-terminal His_6 tag (15) was incubated with Mersmab1 at room temperature for 1 h. AlphaScreen nickel chelate donor beads and AlphaScreen protein A acceptor beads (5 μg/ml each) (PerkinElmer, Massachusetts) were then added to the mixture. After incubation at room temperature for 1 h, the AlphaScreen signal was detected using an EnSpire plate reader (PerkinElmer).

The AlphaScreen assay was also used to detect whether Mersmab1 inhibited the binding between MERS-CoV RBD and recombinant DPP4. To this end, MERS-CoV RBD-Fc (residues 377 to 588 containing a C-terminal Fc tag) was mixed with recombinant DPP4 in the presence of Mersmab1-Fab. The remaining AlphaScreen assay was carried out as described above.

Flow cytometry. Flow cytometry was performed to detect whether Mersmab1 inhibited the binding between MERS-CoV RBD and cell surface DPP4 (22). Briefly, DPP4-expressing Huh-7 cells were incubated with MERS-CoV RBD-Fc (0.5 μg/ml) in the presence or absence of Mersmab1 at various concentrations at room temperature for 30 min. DyLight 488-labeled goat anti-human IgG antibody was then added. After incubation at room temperature for another 30 min, the flow cytometry signal was analyzed.

ELISA. Binding of Mersmab1 to different regions of the MERS-CoV spike or mutant MERS-CoV RBD proteins was detected by ELISA (24). Briefly, ELISA plates were coated overnight at 4°C with recombinant proteins (1 μg/ml) corresponding to various lengths of the MERS-CoV spike or mutant RBD proteins. After blocking at 37°C for 2 h, Mersmab1 or the

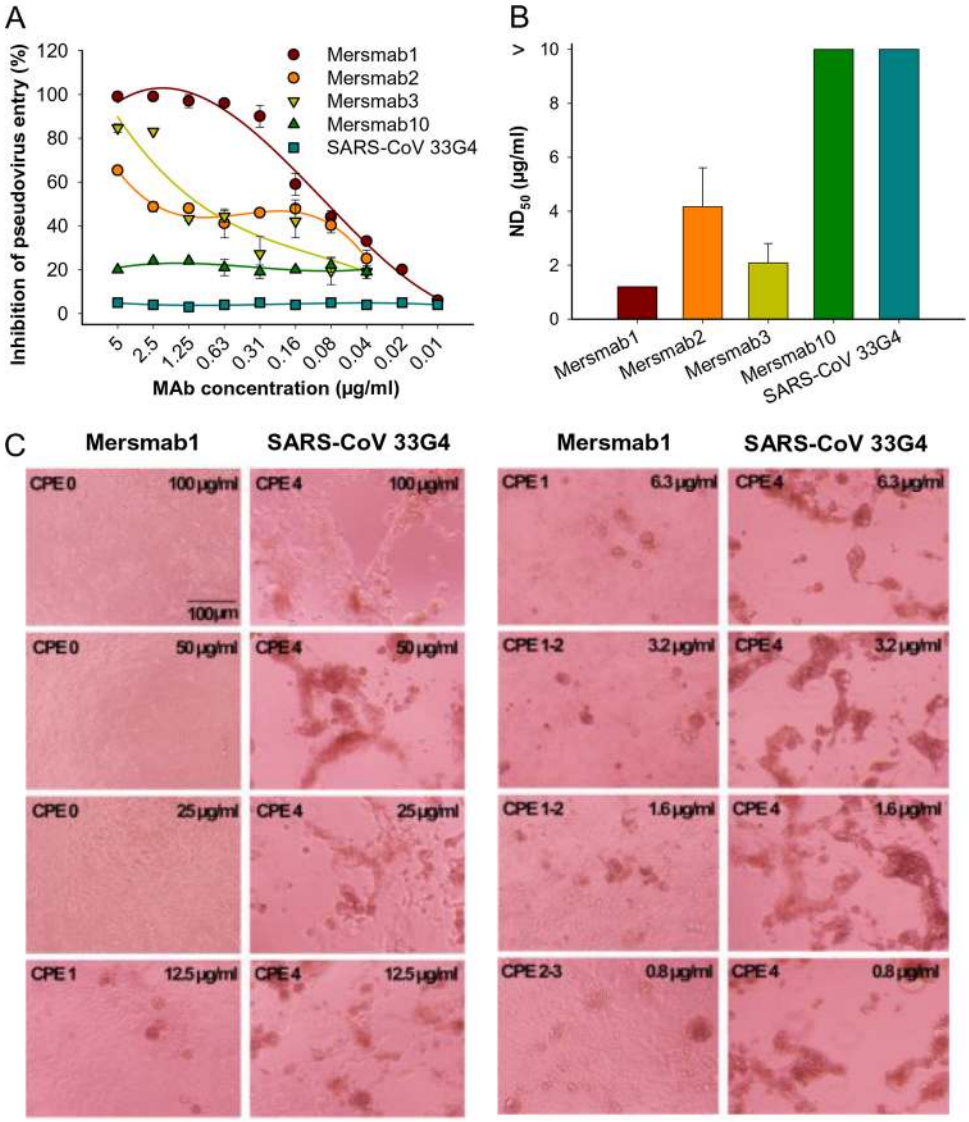

FIG 1 Mersmab1 inhibited MERS-CoV spike-mediated pseudovirus entry into DPP4-expressing Huh-7 cells and neutralized MERS-CoV infection in both Vero E6 and Calu-3 cells. An anti-SARS-CoV RBD MAb, 33G4, was included as a control. (A) Selected anti-MERS-CoV MAbs (Mersmab1, Mersmab2, Mersmab3, and Mersmab10) were tested for their inhibition of MERS-CoV spike-mediated pseudovirus entry into DPP4-expressing Huh-7 cells. The data are presented as mean percentages of inhibition ± standard deviations (SD) ($n = 4$ experiments). (B) Anti-MERS-CoV MAbs were tested for their neutralizing activity against infection by authentic MERS-CoV (EMC strain) in Vero E6 cells. The neutralization capability of MAbs was characterized using a 50% neutralization dose (ND_{50}), which was defined as the concentration of the MAb that reduced CPE by 50%. The data are presented as mean ND_{50}s ± SD ($n = 3$). (C) Mersmab1 neutralized MERS-CoV infection in Calu-3 cells. A standard microneutralization assay was used to assess the potency of Mersmab1 in neutralizing MERS-CoV infection. Levels of CPE were scored as follows: CPE0 (no CPE), CPE1 (5 to 10%), CPE2 (10 to 25%), CPE3 (25 to 50%), and CPE4 (>50%).

SARS-CoV 33G4 MAb control was added to the plates. After incubation at 37°C for 1 h, one of the following reagents was added to the plates: horseradish peroxidase (HRP)-conjugated anti-mouse IgG (1:3,000, GE Healthcare), IgG1 (1:2,000), IgG2a (1:5,000), IgG2b (1:2,000), or IgG3 (1:2,000) (Invitrogen, CA). After incubation for another hour at 37°C, the substrate 3,3′,5,5′-tetramethylbenzidine (TMB) (Life Technologies) was added. The reaction continued for 10 min and was subsequently stopped by adding 25 μl of 1N H_2SO_4. The ELISA signal was measured using an ELISA plate reader (Tecan).

To detect the binding between Mersmab1 and denatured MERS-CoV spike protein fragments, ELISA plates were coated with recombinant spike protein fragments (1 μg/ml) at 4°C overnight and then treated with dithiothreitol (DTT) (10 mM; Sigma) at 37°C for 1 h, followed by the addition of iodoacetamide (50 mM; Sigma) at 37°C for 1 h to stop the reaction (24). After three washes, regular ELISA was performed as described above.

RESULTS

Generation of Mersmab1 that potently neutralizes MERS-CoV cell entry. To generate MAbs capable of neutralizing MERS-CoV infection, mice were immunized with recombinant MERS-CoV S1 fused to a C-terminal Fc tag (S1-Fc). Subsequently, stable hybridoma cell lines were generated for screening of positive clones using ELISA. These selected clones reacted not only to recombinant MERS-CoV S1-Fc protein but also to recombinant MERS-CoV S1 containing a C-terminal His_6 tag (S1-His). The latter step aimed to eliminate the selection of the clones targeting

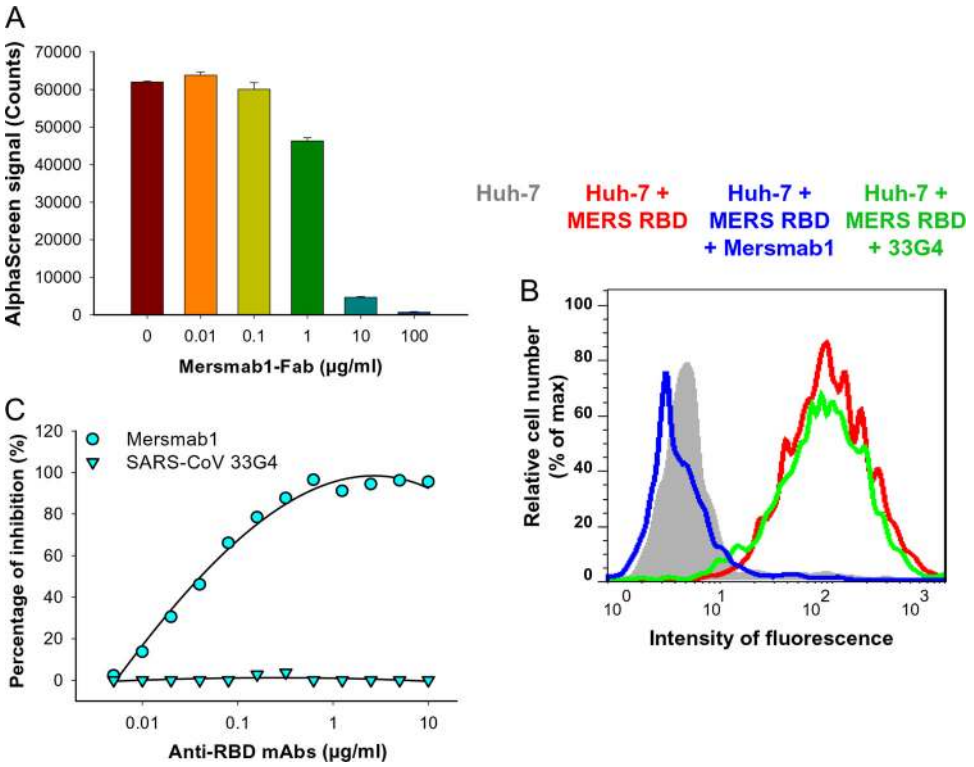

FIG 2 Mersmab1 blocked the binding between MERS-CoV RBD and its receptor, DPP4. (A) AlphaScreen assay was performed to detect whether Mersmab1 could block the binding between recombinant MERS-CoV RBD and recombinant DPP4. MERS-CoV RBD-Fc (0.01 μg/ml) was mixed with DPP4-His (0.27 μg/ml) in the presence of Mersmab1-Fab. (B) Flow cytometry assay was carried out to detect whether Mersmab1 could block the binding between MERS-CoV RBD and host cells expressing DPP4 on their surface. Gray shading, Huh-7 cell control; red line, binding of MERS-CoV RBD-Fc (MERS RBD, 0.5 μg/ml) to Huh-7 cells; blue line, Mersmab1 inhibits MERS RBD (0.5 μg/ml) binding of Huh-7 cells; green line, anti-SARS-CoV MAb 33G4 (0.5 μg/ml) control. (C) Flow cytometry assay shows that Mersmab1 inhibits the binding between MERS-CoV RBD and DPP4 in a dose-dependent fashion. The data in panels A and C are presented as means ± SD ($n = 4$) and those in panel C as percentages of inhibition.

the Fc fusion tag, ensuring that the MAbs generated specifically targeted the MERS-CoV S1 region.

The selected anti-MERS-CoV MAbs were tested for their inhibition of pseudovirus entry mediated by MERS-CoV spike protein and for their neutralizing activity against live MERS-CoV infection in DPP4-expressing Vero E6 cells. Among the four selected MAbs (designated Mersmab1, Mersmab2, Mersmab3, and Mermab10), Mersmab1 demonstrated the most potent anti-MERS-CoV activities (Fig. 1A and B). Mersmab1 not only was highly effective in blocking the entry of MERS-CoV spike-mediated pseudoviruses into DPP4-expressing Huh-7 cells (Fig. 1A) but also potently neutralized live MERS-CoV infection of permissive Vero E6 cells by inhibiting the formation of MERS-CoV-induced CPE (Fig. 1B). In addition, Mersmab1 efficiently attenuated the formation of MERS-CoV-induced CPE in permissive Calu-3 cells, confirming its potent anti-MERS-CoV neutralizing activity (Fig. 1C). As a control, an anti-SARS-CoV RBD MAb, 33G4, did not exhibit any anti-MERS-CoV activities (Fig. 1A to C) (27). Accordingly, Mersmab1 was chosen for further characterization and evaluation.

Mechanism of Mersmab1 in neutralizing MERS-CoV cell entry. To investigate the mechanism of Mersmab1 in neutralizing MERS-CoV cell entry, we performed RBD/receptor-binding assays in the presence of Mersmab1. First, an AlphaScreen assay was carried out by mixing recombinant MERS-CoV RBD-Fc and recombinant DPP4-His in the presence of Mersmab1-Fab, which is the Fab portion of Mersmab1. The AlphaScreen signal decreased with increased concentrations of Mersmab1-Fab, indicating that Mersmab1 inhibited the binding of MERS-CoV RBD to DPP4 in a dose-dependent manner (Fig. 2A). Second, a flow cytometry assay was performed by incubating MERS-CoV RBD-Fc with DPP4-expressing Huh-7 cells in the presence of Mersmab1. The results show that the binding between MERS-CoV RBD and DPP4-expressing Huh-7 cells was blocked by Mersmab1 (Fig. 2B) and that the blockade was also in a dose-dependent manner (Fig. 2C). In contrast, the anti-SARS-CoV MAb 33G4 was unable to inhibit the binding between MERS-CoV RBD and DPP4-expressing cells (Fig. 2B and C). These results suggest that Mersmab1 neutralizes MERS-CoV entry into host cells by blocking the binding of MERS-CoV RBD to its host receptor, DPP4.

To further identify the binding partner for Mersmab1, we measured direct binding interactions between MERS-CoV RBD and Mersmab1 using both AlphaScreen assay and ELISA. The AlphaScreen assay showed that Mersmab1 specifically bound to MERS-CoV RBD but not to SARS-CoV RBD (Fig. 3A). ELISA demonstrated that Mersmab1 specifically bound to MERS-CoV S1-Fc and RBD-Fc but not to the MERS-CoV S1 N-terminal domain Fc (S-NTD-Fc), S2-Fc, or human Fc (hFc) (Fig. 3B). As a control, anti-SARS-CoV MAb 33G4 bound only to SARS-CoV RBD and not to any of the MERS-CoV spike protein fragments (Fig. 3A and B). ELISA also showed that the antibody subtype of Mersmab1 was mainly IgG1 (Fig. 3C). ELISA further revealed that

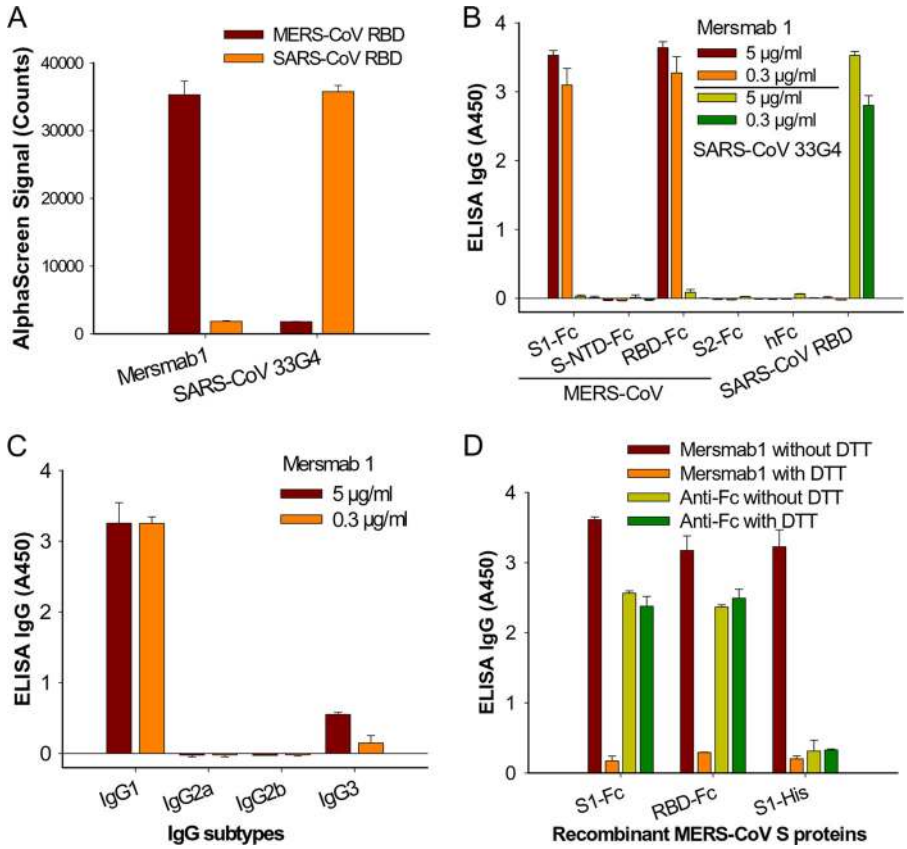

FIG 3 Mersmab1 recognizes MERS-CoV spike protein RBD in a conformation-dependent manner. (A) AlphaScreen assay was performed to detect the binding between Mersmab1 and MERS-CoV RBD. MERS-CoV RBD-His (2.8 μg/ml) was mixed with Mersmab1 (0.5 μg/ml). SARS-CoV 33G4 MAb and SARS-CoV RBD protein were used as controls. (B) ELISA was carried out to detect the binding between Mersmab1 (containing mouse IgG Fc) and MERS-CoV spike protein fragments (containing human IgG Fc). Recombinant human Fc (hFc), SARS-CoV RBD, and anti-SARS-CoV MAb 33G4 were used as controls. (C) ELISA was performed to identify Mersmab1 IgG subtypes using antibodies that target mouse IgG1, IgG2a, IgG2b, and IgG3, respectively. (D) ELISA was used to detect the binding between Mersmab1 and MERS-CoV S1 protein fragments in the presence or absence of DTT. An anti-Fc MAb (Sigma) was used as the control. All data are presented as means ± SD ($n = 4$). A450, absorbance at 450 nm.

Mersmab1 lost most of its binding affinity for MERS-CoV S1 or RBD in the presence of DTT, an agent that disrupts protein disulfide bonds and causes disulfide bond-stabilized proteins to lose their native conformation. In contrast, an anti-Fc MAb bound the Fc region of MERS-CoV S1-Fc or RBD-Fc, but did not bind MERS-CoV S1-His without Fc, in the presence or absence of DTT (Fig. 3D). These results are consistent with the fact that MERS-CoV RBD contains four pairs of disulfide bonds, whereas the Fc region contains none; hence, DTT distorts the native conformation of MERS-CoV RBD but not that of the Fc region. Taken together, these results suggest that Mersmab1 binds MERS-CoV RBD through recognizing conformational epitopes on the RBD.

Mapping neutralizing epitopes in MERS-CoV RBD that are recognized by Mersmab1. Based on the previously determined crystal structure of MERS-CoV RBD in complex with DPP4 (11, 12), we mutated a number of RBD key residues to alanines and then measured how each of these mutations affected the binding of Mersmab1. All of these RBD residues directly contact DPP4; they are L506, D510, R511, E513, D539, W553, and V555. Two other residues, E536 and E565, which do not directly contact DPP4, were also mutated to alanines as controls. Each of the mutant RBD-Fc proteins containing one of the aforementioned mutations was expressed and purified without a significant change in its expression levels or solubility (Fig. 4A). This observation indicates that none of these mutations affected the native structure of the RBD, which is consistent with the fact that all of the selected residues are located on the protein surface and not involved in protein folding. ELISA showed that a number of these RBD mutations, including L506A, D510A, R511A, E513A, and W553A, led to a significant loss of binding affinity for Mersmab1. In particular, mutation R511A completely abolished the binding of RBD-Fc to Mersmab1. In comparison, mutation D539A did not affect the binding between the RBD and Mersmab1 at all, nor did the two control mutations, E536A and E565A (Fig. 4B). A pseudovirus entry assay demonstrated that mutation R511A in the MERS-CoV spike protein slightly reduced pseudovirus entry into target cells, but it significantly reduced the inhibitory effect of Mersmab1 on pseudovirus entry (Fig. 4C and D). These results suggest that the R511A mutation decreases the binding affinities of the RBD for DPP4 slightly and for Mersmab1 significantly. Taken together, these results have identified the neutralizing epitopes in MERS-CoV RBD that are recognized by Mersmab1.

We mapped the recognizing epitopes of Mersmab1 on the determined structural model of MERS-CoV RBD (12, 15). All of the residues critical for MAb binding are located on the left ridge of the MERS-CoV receptor-binding motif (RBM) (Fig. 5A), which

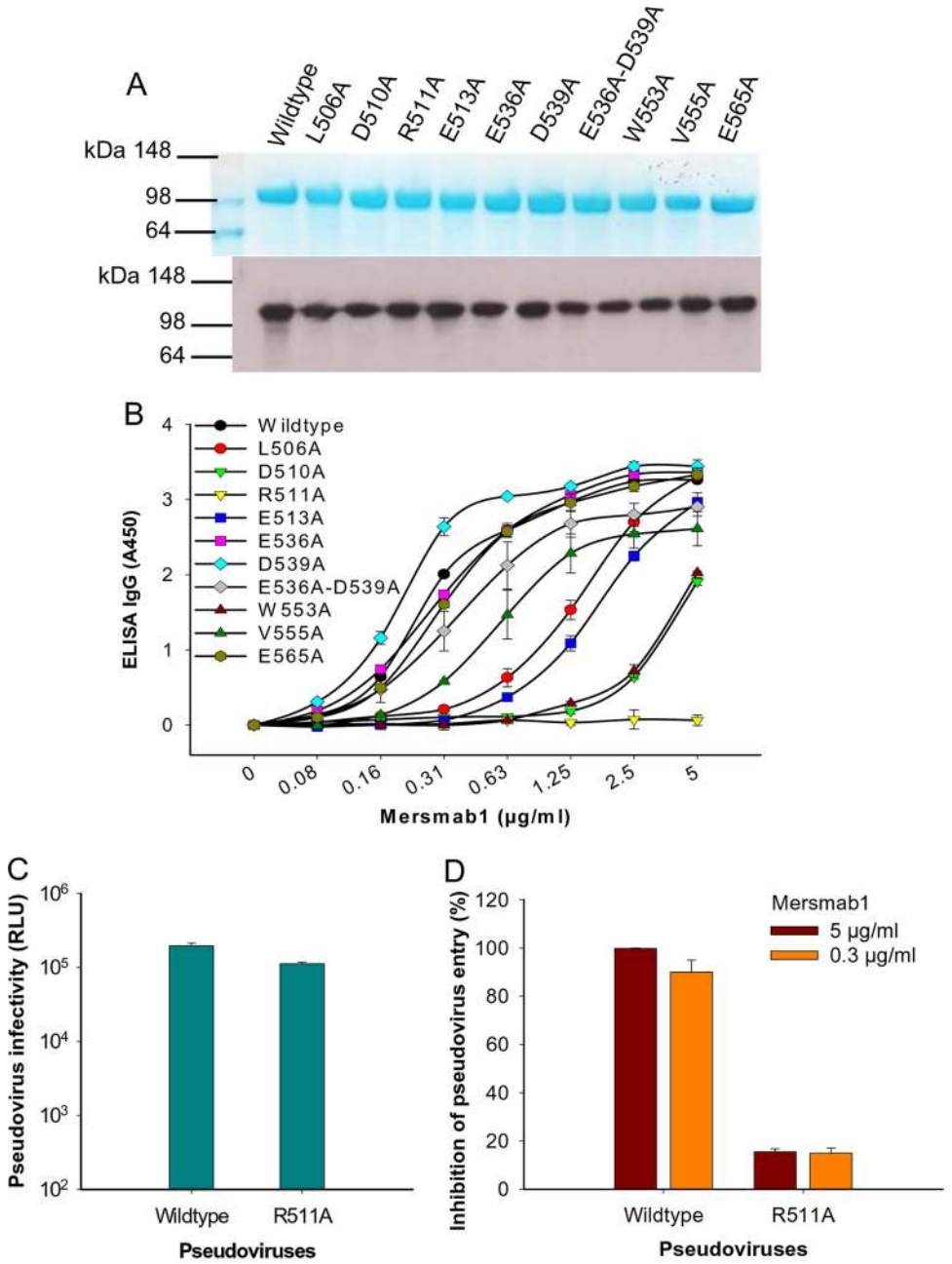

FIG 4 Mapping of recognizing epitopes of Mersmab1 in MERS-CoV RBD. (A) Expression levels of mutant MERS-CoV RBDs in the 293T cell culture supernatant were detected by SDS-PAGE (stained by Coomassie blue) (top) and Western blotting (recognized by anti-MERS-CoV-S1 polyclonal antibodies) (bottom). Protein molecular mass markers (kDa) are indicated on the left. (B) ELISA was performed to detect the binding of Mersmab1 to mutant MERS-CoV RBD proteins. (C) The R511A mutation in the MERS-CoV spike protein slightly reduced MERS-CoV spike-mediated pseudovirus entry into DPP4-expressing Huh-7 cells. (D) R511A mutation in the MERS-CoV spike protein significantly reduced the inhibitory effect of Mersmab1 on MERS-CoV spike-mediated pseudovirus entry. All data are presented as means ± SD ($n = 2$). The MERS-CoV RBD protein wild type was used as the control for panels A and B, while the pseudovirus wild type was included as the control for panels C and D.

overlaps the DPP4-binding region in MERS-CoV RBD (Fig. 5B). Therefore, the neutralization mechanism of Mersmab1 is based on the competitive blocking of MERS-CoV RBD binding to DPP4. Interestingly, these recognizing epitopes appear to differ from the recognizing epitopes of anti-SARS-CoV RBD MAbs. Currently, three crystal structures are available for SARS-CoV RBD in complex with neutralizing MAbs (37–39). Among the three anti-SARS-CoV MAbs, two bind to the right ridge of the SARS-CoV RBM, whereas one covers the center of the SARS-CoV RBM. Recognizing epitopes of all three anti-SARS-CoV RBD MAbs overlap the ACE2-binding region in SARS-CoV RBD. The different recognizing epitopes of anti-MERS-CoV and anti-SARS-CoV MAbs provide a structural basis for studying the antigenicity of different coronavirus RBDs. The identified recognizing epitopes for

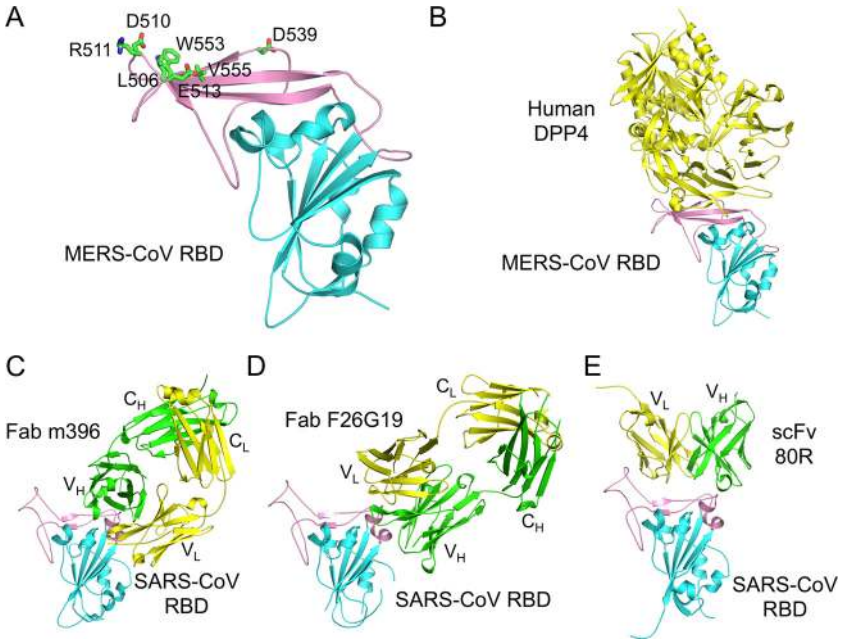

FIG 5 Structural analysis of the recognizing epitopes of anti-MERS-CoV RBD and anti-SARS-CoV RBD MAbs. (A) Crystal structure of MERS-CoV RBD. The core structure is in cyan, and the RBM is in pink. Critical residues at the RBD-DPP4 binding interface are in green (Protein Data Bank [PDB] accession no. 4KQZ). (B) Crystal structure of MERS-CoV RBD (cyan) complexed with its receptor, human DPP4 (yellow) (PDB accession no. 4KR0). (C) Crystal structure of SARS-CoV RBD complexed with anti-SARS-CoV MAb m396 Fab (PDB accession no. 2DD8). The light chain and heavy chain of the MAb are in yellow and green, respectively. (D) Crystal structure of SARS-CoV RBD complexed with anti-SARS-CoV MAb F26G19 Fab (PDB accession no. 3BGF). (E) Crystal structure of SARS-CoV RBD complexed with anti-SARS-CoV MAb 80R scFv (PDB accession no. 2GHW). C_H, C_L, V_H, and V_L, constant heavy, constant light, variable heavy, and variable light chain domains, respectively. scFV, single-chain variable fragment.

Mersmab1 can also guide structure-based design of effective anti-MERS-CoV subunit vaccines. Future structure determination of MERS-CoV RBD in complex with Mersmab1 will reveal more detailed knowledge about the neutralizing epitopes in MERS-CoV RBD.

DISCUSSION

The newly emerged MERS-CoV poses a continuing threat to human health. The high fatality rate (over 40%) associated with MERS-CoV infections is particularly worrying. Prevention and treatment strategies to control MERS-CoV infection are urgently needed. Although vaccines remain one of the most important approaches against viral infections, they generally take a long time to develop. In addition, vaccines cannot provide immediate prophylactic protection or treat ongoing infections. Instead, the successful clinical application of a therapeutic MAb in preventing and treating RSV infections in infants (26, 40) strongly suggests that therapeutic MAbs can be a promising approach to control MERS-CoV infections in humans.

In this study, we identified and characterized a novel monoclonal antibody, Mersmab1, which neutralizes MERS-CoV infection. Mersmab1 inhibits MERS-CoV entry into host cells through binding to the MERS-CoV spike protein RBD and thereby competitively blocks the binding of MERS-CoV RBD to its host receptor, DPP4. The neutralizing epitopes recognized by Mersmab1 are located on one edge of the DPP4-binding surface in MERS-CoV RBD. The overlap between these neutralizing epitopes and the DPP4-binding region explains the mechanism by which Mersmab1 potently neutralizes MERS-CoV infection. These results suggest that Mersmab1 can be humanized and serve as a potent passive immunotherapeutic agent for preventing and treating MERS-CoV infections. Like the therapeutic MAb against RSV infection (41, 42), humanized Mersmab1 could be given to patients who are at risk of possible exposure to MERS-CoV and to those who are already infected. This approach will be particularly helpful for high-risk populations, such as immunocompromised people, patients' family members, and health care workers. Therefore, the anti-MERS-CoV neutralizing MAb identified in this study, Mersmab1, provides a promising approach to combating and controlling the ongoing spread of MERS-CoV in human populations. In addition, Mersmab1 could be used a tool for studying the conformation and antigenicity of the MERS-CoV spike protein and for guiding the design of anti-MERS-CoV vaccines.

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