HUMAN ANTIBODIES TO MIDDLE EAST RESPIRATORY SYNDROME – CORONAVIRUS SPIKE PROTEIN

FIELD OF THE INVENTION

[001] The present invention is related to human antibodies and antigen-binding fragments of human antibodies that specifically bind to the spike protein of the Middle East Respiratory Syndrome – coronavirus (MERS-CoV), and therapeutic and diagnostic methods of using those antibodies.

STATEMENT OF RELATED ART

[002] Middle East Respiratory Syndrome – Coronavirus (MERS-CoV) is a newly-emergent betacoronavirus which causes severe acute respiratory disease. It was first isolated in Saudi Arabia in 2012 (Zaki et al 2012, NEJM 367: 1814-1820) and since then has spread to about 18 countries with most of the cases in Saudi Arabia and United Arab Emirates. As of May 15, 2014, the World Health Organization reported 571 cases of MERS, including 171 deaths. Two cases of MERS infection have recently been discovered in the US. Clinical features of MERS-CoV infection in humans range from an asymptomatic infection to very severe pneumonia, with potential development of acute respiratory distress syndrome, septic shock and multi-organ failure resulting in death.

[003] MERS-CoV shares similarity with bat coronaviruses HKU4 and HKU5. The virus uses its spike protein for interaction with a cellular receptor for entry into a target cell. Raj et al showed that the virus binds via the receptor binding domain of its spike protein to dipeptidyl peptidase 4 (DPP4) on human epithelial and endothelial cells (Raj et al 2013, Nature 495: 251-256). Lu et al 2013 have shown that MERS-CoV receptor binding domain consists of a core and a receptor binding subdomain that interacts with DPP4 (Lu et al 2013, Nature 500: 227-231).

[004] WO2014/045254 describes the isolation and characterization of MERS-CoV, spike protein and polyclonal antibodies against the receptor binding domain of the spike protein. Neutralizing monoclonal antibodies to the receptor binding domain of the spike protein have been disclosed by, *e.g.*, Du et al (2014, J. Virol.), Ying et al (2014, J. Virol.), Tang, et al., (2014, PNAS) and Jiang et al (2014, Sci. Transl. Med. Vol. 6, 234ra59).

[005] Thus far, there has been no vaccine or therapeutic agent to prevent or treat MERS infection. In view of the continuing threat to human health and high fatality rate (over 30%), there is an urgent need for preventive and therapeutic antiviral therapies for MERS control. Fully human antibodies that specifically bind to MERS-CoV spike protein with high affinity and inhibit virus infectivity could be important in the prevention and treatment of MERS infection.

BRIEF SUMMARY OF THE INVENTION

[006] The present invention provides antibodies and antigen-binding fragments thereof that bind MERS-CoV spike protein. The antibodies of the present invention are useful, *inter alia*, for

inhibiting or neutralizing the activity of MERS-CoV spike protein. In some embodiments, the antibodies are useful for blocking binding of the virus to its host cell receptor dipeptidyl peptidase 4 (DPP4) and for preventing the entry of MERS-Corona virus into host cells. In some embodiments, the antibodies function by inhibiting the cell-to-cell transmission of the virus. In certain embodiments, the antibodies are useful in preventing, treating or ameliorating at least one symptom of MERS-CoV infection in a subject. In certain embodiments, the antibodies may be administered prophylactically or therapeutically to a subject having or at risk of having MERS-CoV infection.

[007] The antibodies of the invention can be full-length (for example, an IgG1 or IgG4 antibody) or may comprise only an antigen-binding portion (for example, a Fab, $F(ab')_2$ or scFv fragment), and may be modified to affect functionality, e.g., to increase persistence in the host or to eliminate residual effector functions (Reddy et al., 2000, J. Immunol. 164:1925-1933). In certain embodiments, the antibodies may be bispecific.

[008] In a first aspect, the present invention provides isolated recombinant monoclonal antibodies or antigen-binding fragments thereof that bind specifically to the MERS-CoV spike protein. In some embodiments, the antibodies are fully human monoclonal antibodies. The antibodies and antigen-binding fragments thereof of the invention bind to an epitope within the receptor binding domain (RBD) of the spike protein of MERS-CoV. In some embodiments, the present invention provides antibodies and antigen-binding fragments thereof that bind to an amino acid selected from the amino acids 367 to 606 of GenBank Accession No. AFS88936.1 (SEQ ID NO: 457). In one embodiment, the antibodies of the present invention bind to the spike protein of MERS-CoV isolate EMC/2012. In certain embodiments, the antibodies bind to spike protein of different MERS-CoV isolates.

[009] Exemplary anti-MERS-CoV-S antibodies of the present invention are listed in Tables 2 and 3 herein. Table 2 sets forth the amino acid sequence identifiers of the heavy chain variable regions (HCVRs), light chain variable regions (LCVRs), heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3), and light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3) of exemplary anti-MERS-CoV-S antibodies. Table 3 sets forth the nucleic acid sequence identifiers of the HCVRs, LCVRs, HCDR1, HCDR2 HCDR3, LCDR1, LCDR2 and LCDR3 of the exemplary anti-MERS-CoV-S antibodies.

[010] The present invention provides antibodies, or antigen-binding fragments thereof, comprising an HCVR comprising an amino acid sequence selected from any of the HCVR amino acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[011] The present invention also provides antibodies, or antigen-binding fragments thereof, comprising an LCVR comprising an amino acid sequence selected from any of the LCVR amino acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[012] The present invention also provides antibodies, or antigen-binding fragments thereof,

comprising an HCVR and an LCVR amino acid sequence pair (HCVR/LCVR) comprising any of the HCVR amino acid sequences listed in Table 2 paired with any of the LCVR amino acid sequences listed in Table 2. According to certain embodiments, the present invention provides antibodies, or antigen-binding fragments thereof, comprising an HCVR/LCVR amino acid sequence pair contained within any of the exemplary anti-MERS-CoV-S antibodies listed in Table 2. In certain embodiments, the HCVR/LCVR amino acid sequence pair is selected from the group consisting of SEQ ID NOs: 2/10, 18/26, 34/42, 50/58, 66/74, 82/90, 98/106, 114/106, 122/106, 130/106, 138/106, 146/106, 154/162, 170/162, 178/162, 186/194, 202/210, 218/226, 234/242, 250/258, 266/274, 282/290, 298/306, 314/322, 330/338, 346/354, 362/370, 378/386, 394/402, 410/418, 426/434, and 442/450. In certain embodiments, the HCVR/LCVR amino acid sequence pair is selected from one of SEQ ID NOs: 2/10 (*e.g.*, H1H15177P), 18/26 (*e.g.*, H1H15188P), 66/74 (*e.g.*, H1H15211P), 114/106 (*e.g.*, H1H15231P2), 170/162 (*e.g.*, H1H15260P2) or 218/226 (*e.g.*, H1H15277N).

[013] The present invention also provides antibodies, or antigen-binding fragments thereof, comprising a heavy chain CDR1 (HCDR1) comprising an amino acid sequence selected from any of the HCDR1 amino acid sequences listed in Table 2 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[014] The present invention also provides antibodies, or antigen-binding fragments thereof, comprising a heavy chain CDR2 (HCDR2) comprising an amino acid sequence selected from any of the HCDR2 amino acid sequences listed in Table 2 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[015] The present invention also provides antibodies, or antigen-binding fragments thereof, comprising a heavy chain CDR3 (HCDR3) comprising an amino acid sequence selected from any of the HCDR3 amino acid sequences listed in Table 2 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[016] The present invention also provides antibodies, or antigen-binding fragments thereof, comprising a light chain CDR1 (LCDR1) comprising an amino acid sequence selected from any of the LCDR1 amino acid sequences listed in Table 2 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[017] The present invention also provides antibodies, or antigen-binding fragments thereof, comprising a light chain CDR2 (LCDR2) comprising an amino acid sequence selected from any of the LCDR2 amino acid sequences listed in Table 2 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[018] The present invention also provides antibodies, or antigen-binding fragments thereof, comprising a light chain CDR3 (LCDR3) comprising an amino acid sequence selected from any of the LCDR3 amino acid sequences listed in Table 2 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[019] The present invention also provides antibodies, or antigen-binding fragments thereof, comprising an HCDR3 and an LCDR3 amino acid sequence pair (HCDR3/LCDR3) comprising

any of the HCDR3 amino acid sequences listed in Table 2 paired with any of the LCDR3 amino acid sequences listed in Table 2. According to certain embodiments, the present invention provides antibodies, or antigen-binding fragments thereof, comprising an HCDR3/LCDR3 amino acid sequence pair contained within any of the exemplary anti-MERS-CoV-S antibodies listed in Table 2. In certain embodiments, the HCDR3/LCDR3 amino acid sequence pair is selected from the group consisting of SEQ ID NOs: 8/16 (*e.g.*, H1H15177P), 24/32 (*e.g.*, H1H15188P), 72/80 (*e.g.*, H1H15211P), 120/112 (*e.g.*, H1H15231P2), 176/168 (*e.g.*, H1H15260P2) and 224/232 (*e.g.*, H1H15277N).

[020] The present invention also provides antibodies, or antigen-binding fragments thereof, comprising a set of six CDRs (*i.e.*, HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3) contained within any of the exemplary anti-MERS-CoV-S antibodies listed in Table 2. In certain embodiments, the HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 amino acid sequence set is selected from the group consisting of SEQ ID NOs: 4-6-8-12-14-16 (*e.g.*, H1H15177P), 20-22-24-28-30-32 (*e.g.*, H1H15188P); 68-70-72-76-78-80 (*e.g.*, H1H15211P); 116-118-120-108-110-112 (*e.g.*, H1H15231P2); 172-174-176-164-166-168 (*e.g.*, H1H15260P2) and 220-222-224-228-230-232 (*e.g.*, H1H15277N).

[021] In a related embodiment, the present invention provides antibodies, or antigen-binding fragments thereof, comprising a set of six CDRs (*i.e.*, HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3) contained within an HCVR/LCVR amino acid sequence pair as defined by any of the exemplary anti-MERS-CoV-S antibodies listed in Table 2. For example, the present invention includes antibodies, or antigen-binding fragments thereof, comprising the HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 amino acid sequences set contained within an HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 2/10 (e.g., H1H15177P), 18/26 (*e.g.*, H1H15188P); 66/74 (*e.g.*, H1H15211P); 114/106 (*e.g.*, H1H15231P2); 170/162 (*e.g.*, H1H15260P2) and 218/226 (*e.g.*, H1H15277N). Methods and techniques for identifying CDRs within HCVR and LCVR amino acid sequences are well known in the art and can be used to identify CDRs within the specified HCVR and/or LCVR amino acid sequences disclosed herein. Exemplary conventions that can be used to identify the boundaries of CDRs include, *e.g.*, the Kabat definition, the Chothia definition, and the AbM definition. In general terms, the Kabat definition is based on sequence variability, the Chothia definition is based on the location of the structural loop regions, and the AbM definition is a compromise between the Kabat and Chothia approaches. *See*, *e.g.*, Kabat, "Sequences of Proteins of Immunological Interest," National Institutes of Health, Bethesda, Md. (1991); Al-Lazikani *et al.*, *J. Mol. Biol. 273*:927-948 (1997); and Martin *et al.*, *Proc. Natl. Acad. Sci. USA 86*:9268-9272 (1989). Public databases are also available for identifying CDR sequences within an antibody.

[022] The present invention includes anti-MERS-CoV-S antibodies having a modified glycosylation pattern. In some embodiments, modification to remove undesirable glycosylation sites may be useful, or an antibody lacking a fucose moiety present on the oligosaccharide chain, for example, to increase antibody dependent cellular cytotoxicity (ADCC) function (see

Shield et al. (2002) JBC 277:26733). In other applications, modification of galactosylation can be made in order to modify complement dependent cytotoxicity (CDC).

[023] The present invention also provides for antibodies and antigen-binding fragments thereof that compete for specific binding to MERS-CoV-S with an antibody or antigen-binding fragment thereof comprising the CDRs of a HCVR and the CDRs of a LCVR, wherein the HCVR and LCVR each has an amino acid sequence selected from the HCVR and LCVR sequences listed in Table 2.

[024] The present invention also provides antibodies and antigen-binding fragments thereof that cross-compete for binding to MERS-CoV-S with a reference antibody or antigen-binding fragment thereof comprising the CDRs of a HCVR and the CDRs of a LCVR, wherein the HCVR and LCVR each has an amino acid sequence selected from the HCVR and LCVR sequences listed in Table 2.

[025] In some embodiments, the antibody or antigen binding fragment thereof may bind specifically to MERS-CoV-S in an agonist manner, i.e., it may enhance or stimulate MERS-CoV-S binding and/or activity; in other embodiments, the antibody may bind specifically to MERS-CoV-S in an antagonist manner, i.e., it may block MERS-CoV-S from binding to its receptor (DPP4).

[026] The present invention also provides isolated antibodies and antigen-binding fragments thereof that block MERS-CoV spike protein binding to DPP4. In some embodiments, the antibody or antigen-binding fragment thereof that blocks MERS-CoV spike protein binding to DPP4 may bind to the same epitope on MERS-CoV spike protein as DPP4 or may bind to a different epitope on MERS-CoV spike protein as DPP4. In some embodiments, the present invention provides antibodies or antigen-binding fragments thereof that block the binding of MERS-CoV-S to human, camel or bat DPP4.

[027] In certain embodiments, the antibodies or antigen-binding fragments of the present invention are bispecific comprising a first binding specificity to a first epitope in the receptor binding domain of MERS-CoV spike protein and a second binding specificity to a second epitope in the receptor binding domain of MERS-CoV spike protein wherein the first and second epitopes are distinct and non-overlapping.

[028] In one embodiment, the invention provides an isolated antibody or antigen-binding fragment that has one or more of the following characteristics: (a) is a fully human monoclonal antibody; (b) is isolated from a hybridoma cell line selected from the group consisting of HBVX06H05, HBVX11H04, HBVX11D02, HBVZ10E10, HBVY09F08, HBVZ05G02, HBVZ09B06, HBVY01F08, HBVY10G02, HBVY04B06, HBVY07D10, HBVZ08A09, HBVZ05G04, HBVY06C07, HBVY03H06, HBVZ10G06, HBVZ04F10, HBVX11E09, HBVY06H09, HBVZ05B11, HBVY02E05 and HBVZ04C07; (c) interacts with one or more amino acid residues in the receptor binding domain of MERS-CoV spike protein selected from amino acid residues 367 to 606 of SEQ ID NO: 457; (d) binds to MERS-CoV spike protein with a dissociation constant (K_D) of less than 10^{-9}M, as measured in a surface plasmon resonance

assay; (e) blocks binding of MERS-CoV spike protein to dipeptidyl peptidase 4 (DPP4) by more than 90%, as measured in a blocking ELISA assay; (f) neutralizes MERS-CoV infectivity of human host cells by more than 90% and with an IC_{50} less than 4nM, as measured in a virus-like particle (VLP) neutralization assay; (g) neutralizes MERS-CoV infectivity wherein the MERS-CoV comprises an isolate of the virus selected from the group consisting of EMC/2012, Jordan-N3/2012, England-Qatar/2012, Al-Hasa_1_2013, Al-Hasa_2_2013, Al-Hasa_3_2013, Al-Hasa_4_2013, Al-Hasa_12, Al-Hasa_15, Al-Hasa_16, Al-Hasa_17, Al-Hasa_18, Al-Hasa_19, Al-Hasa_21, Al-hasa_25, Bisha_1, Buraidah_1, England 1, Hafr-Al-batin_1, Hafr-Al-Batin_2, Hafr-Al-Batin_6,Jeddah_1, KFU-HKU 1, KFU-HKU 13, Munich, Qatar3, Qatar4, Riyadh_1, Riyadh_2, Riyadh_3, Riyadh_3, Riyadh_4, Riyadh_5, Riyadh_9, Riyadh_14, Taif_1, UAE, and Wadi-Ad-Dawasir; and (h) is a bi-specific antibody comprising a first binding specificity to a first epitope in the receptor binding domain of MERS-CoV spike protein and a second binding specificity to a second epitope in the receptor binding domain of MERS-CoV spike protein wherein the first and second epitopes are distinct and non-overlapping.

[029] In a second aspect, the present invention provides nucleic acid molecules encoding anti-MERS-CoV-S antibodies or portions thereof. For example, the present invention provides nucleic acid molecules encoding any of the HCVR amino acid sequences listed in Table 2; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCVR nucleic acid sequences listed in Table 3, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[030] The present invention also provides nucleic acid molecules encoding any of the LCVR amino acid sequences listed in Table 2; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCVR nucleic acid sequences listed in Table 3, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[031] The present invention also provides nucleic acid molecules encoding any of the HCDR1 amino acid sequences listed in Table 2; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCDR1 nucleic acid sequences listed in Table 3, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[032] The present invention also provides nucleic acid molecules encoding any of the HCDR2 amino acid sequences listed in Table 2; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCDR2 nucleic acid sequences listed in Table 3, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[033] The present invention also provides nucleic acid molecules encoding any of the HCDR3 amino acid sequences listed in Table 2; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCDR3 nucleic acid sequences

listed in Table 3, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[034] The present invention also provides nucleic acid molecules encoding any of the LCDR1 amino acid sequences listed in Table 2; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCDR1 nucleic acid sequences listed in Table 3, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[035] The present invention also provides nucleic acid molecules encoding any of the LCDR2 amino acid sequences listed in Table 2; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCDR2 nucleic acid sequences listed in Table 3, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[036] The present invention also provides nucleic acid molecules encoding any of the LCDR3 amino acid sequences listed in Table 2; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCDR3 nucleic acid sequences listed in Table 3, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[037] The present invention also provides nucleic acid molecules encoding an HCVR, wherein the HCVR comprises a set of three CDRs (*i.e.*, HCDR1-HCDR2-HCDR3), wherein the HCDR1-HCDR2-HCDR3 amino acid sequence set is as defined by any of the exemplary anti-MERS-CoV-S antibodies listed in Table 2.

[038] The present invention also provides nucleic acid molecules encoding an LCVR, wherein the LCVR comprises a set of three CDRs (*i.e.*, LCDR1-LCDR2-LCDR3), wherein the LCDR1-LCDR2-LCDR3 amino acid sequence set is as defined by any of the exemplary anti-MERS-CoV-S antibodies listed in Table 2.

[039] The present invention also provides nucleic acid molecules encoding both an HCVR and an LCVR, wherein the HCVR comprises an amino acid sequence of any of the HCVR amino acid sequences listed in Table 2, and wherein the LCVR comprises an amino acid sequence of any of the LCVR amino acid sequences listed in Table 2. In certain embodiments, the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCVR nucleic acid sequences listed in Table 3, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto, and a polynucleotide sequence selected from any of the LCVR nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto. In certain embodiments according to this aspect of the invention, the nucleic acid molecule encodes an HCVR and LCVR, wherein the HCVR and LCVR are both derived from the same anti-MERS-CoV-S antibody listed in Table 2.

[040] The present invention provides nucleic acid molecules encoding any of the heavy chain amino acid sequences listed in Table 2. The present invention also provides nucleic acid

molecules encoding any of the light chain amino acid sequences listed in Table 2.

[041] In a related aspect, the present invention provides recombinant expression vectors capable of expressing a polypeptide comprising a heavy or light chain variable region of an anti-MERS-CoV-S antibody. For example, the present invention includes recombinant expression vectors comprising any of the nucleic acid molecules mentioned above, *i.e.*, nucleic acid molecules encoding any of the HCVR, LCVR, and/or CDR sequences as set forth in Table 3. Also included within the scope of the present invention are host cells into which such vectors have been introduced, as well as methods of producing the antibodies or portions thereof by culturing the host cells under conditions permitting production of the antibodies or antibody fragments, and recovering the antibodies and antibody fragments so produced.

[042] In a third aspect, the invention provides a pharmaceutical composition comprising a therapeutically effective amount of at least one recombinant monoclonal antibody or antigen-binding fragment thereof which specifically binds MERS-CoV spike protein and a pharmaceutically acceptable carrier. In a related aspect, the invention features a composition which is a combination of an anti-MERS-CoV-S antibody and a second therapeutic agent. In one embodiment, the second therapeutic agent is any agent that is advantageously combined with an anti-MERS-Co-V-S antibody. Exemplary agents that may be advantageously combined with an anti-MERS-CoV-S antibody include, without limitation, other agents that bind and/or inhibit MERS-CoV activity (including other antibodies or antigen-binding fragments thereof, etc.) and/or agents which do not directly bind MERS-CoV-S but nonetheless inhibit viral activity including infectivity of host cells. In certain embodiments, the invention provides for a pharmaceutical composition comprising: (a) a first anti-MERS-CoV-S antibody or antigen-binding fragment thereof; (b) a second anti-MERS-CoV-S antibody or antigen-binding fragment thereof, wherein the first antibody binds to a first epitope on MERS-CoV spike protein and the second antibody binds to a second epitope on MERS-CoV spike protein wherein the first and second epitopes are distinct and non-overlapping; and (c) a pharmaceutically acceptable carrier or diluent. In certain embodiments, the invention provides for a pharmaceutical composition comprising: (a) a first anti-MERS-CoV-S antibody or antigen-binding fragment thereof; (b) a second anti-MERS-CoV-S antibody or antigen-binding fragment thereof, wherein the first antibody does not cross-compete with the second antibody for binding to MERS-CoV spike protein; and (c) a pharmaceutically acceptable carrier or diluent. Additional combination therapies and co-formulations involving the anti-MERS-CoV-S antibodies of the present invention are disclosed elsewhere herein.

[043] In a fourth aspect, the invention provides therapeutic methods for treating a disease or disorder associated with MERS-CoV such as viral infection in a subject using an anti-MERS-CoV-S antibody or antigen-binding portion of an antibody of the invention, wherein the therapeutic methods comprise administering a therapeutically effective amount of a pharmaceutical composition comprising an antibody or antigen-binding fragment of an antibody of the invention to the subject in need thereof. The disorder treated is any disease or condition

which is improved, ameliorated, inhibited or prevented by inhibition of MERS-CoV activity. In certain embodiments, the invention provides methods to prevent, treat or ameliorate at least one symptom of MERS-CoV infection, the method comprising administering a therapeutically effective amount of an anti-MERS-CoV-S antibody or antigen-binding fragment thereof of the invention to a subject in need thereof. In some embodiments, the present invention provides methods to ameliorate or reduce the severity of at least one symptom or indication of MERS infection in a subject by administering an anti-MERS-CoV-S antibody of the invention, wherein the at least one symptom or indication is selected from the group consisting of inflammation in the lung, alveolar damage, fever, cough, shortness of breath, diarrhea, organ failure, pneumonia, septic shock and death. In certain embodiments, the invention provides methods to decrease viral load in a subject, the methods comprising administering to the subject an effective amount of an antibody or fragment thereof of the invention that binds MERS-CoV-S and blocks MERS-CoV-S binding to host cell receptor DPP4. In some embodiments, the antibody or antigen-binding fragment thereof may be administered prophylactically or therapeutically to a subject having or at risk of having MERS infection. The subjects at risk include, but are not limited to, an immunocompromised person, an elderly adult (more than 65 years of age), children younger than 2 years of age, travelers to countries in the Middle East (such as Saudi Arabia, United Arab Emirates, Qatar, etc.), healthcare workers, adults or children in close contact with a person(s) with confirmed or suspected MERS infection, and people with underlying medical conditions such as pulmonary infection, heart disease or diabetes. In certain embodiments, the antibody or antigen-binding fragment thereof the invention is administered in combination with a second therapeutic agent to the subject in need thereof. The second therapeutic agent may be selected from the group consisting of an anti-inflammatory drug (such as corticosteroids, and non-steroidal anti-inflammatory drugs), an anti-infective drug, a different antibody to MERS-CoV spike protein, an anti-viral drug, a vaccine for MERS-CoV, a dietary supplement such as anti-oxidants and any other drug or therapy known in the art. In certain embodiments, the second therapeutic agent may be an agent that helps to counteract or reduce any possible side effect(s) associated with an antibody or antigen-binding fragment thereof of the invention, if such side effect(s) should occur. The antibody or fragment thereof may be administered subcutaneously, intravenously, intradermally, intraperitoneally, orally, intramuscularly, or intracranially. In one embodiment, the antibody may be administered as a single intravenous infusion for maximum concentration of the antibody in the serum of the subject. The antibody or fragment thereof may be administered at a dose of about 0.1 mg/kg of body weight to about 100 mg/kg of body weight of the subject. In certain embodiments, an antibody of the present invention may be administered at one or more doses comprising between 50mg to 600mg.

[044] The present invention also includes use of an anti-MERS-CoV-S antibody or antigen-binding fragment thereof of the invention in the manufacture of a medicament for the treatment of a disease or disorder that would benefit from the blockade of MERS-CoV binding and/or

activity.

[045] Other embodiments will become apparent from a review of the ensuing detailed description.

BRIEF DESCRIPTION OF THE FIGURES

[046] Figure 1 is a table listing antibody hybridoma supernatants (listed in column 1 by their Sample ID) and their properties in binding, blocking and neutralization assays, as described elsewhere herein.

[047] Figure 2 is a matrix showing the results of an antibody cross-competition assay in which a first anti-MERS-CoV-S antibody (mAb-1) was applied to a MERS RBD-coated sensor tip, followed by treatment with a second anti-MERS-CoV-S antibody (mAb-2). Binding responses (numerical values -0.05 to 0.64) for each antibody combination tested are depicted. Light grey boxes with black font represent binding response for self-competition. Antibodies competing in both directions, independent of the order of antigen binding, are highlighted in black boxes with white font. No competition, suggesting distinct binding regions, is represented as white boxes with black font. Antibodies that show more than 0.18nm shift in binding do not cross-compete with one another.

[048] Figure 3 is a matrix showing the results of an antibody cross-competition assay in which a first anti-MERS-CoV-S antibody (mAb-1) was applied to a MERS RBD-coated sensor tip, followed by treatment with a second anti-MERS-CoV-S antibody (mAb-2). Binding responses (numerical values -0.01 to 0.55) for each antibody combination tested are depicted. Light grey boxes with black font represent binding response for self-competition. Antibodies competing in both directions, independent of the order of antigen binding, are highlighted in black boxes with white font. No competition, suggesting distinct binding regions, is represented as white boxes with black font. Antibodies that show more than 0.14nm shift in binding do not cross-compete with one another.

[049] Figure 4 shows quantitative PCR of MERS-CoV transcript (transcribed mRNA of the genome upstream of the envelope gene – UpE) in humanized DPP4 mice at days 2 and 4 post infection.

[050] Figure 5 shows quantitative PCR of MERS-CoV transcript (MERS-CoV genome – leader sequence) in humanized DPP4 mice at days 2 and 4 post infection.

[051] Figure 6 shows MERS-CoV viral titer quantitation of infected mouse lung at day 4 post infection. Mouse lung MERS-CoV levels were quantified and expressed as pfu/ml of homogenized mouse lung.

[052] Figure 7 shows quantitative PCR of MERS-CoV transcript (transcribed mRNA of the genome upstream of the envelope gene – UpE) from lungs of mice treated with 200µg, 20µg, or 2 µg of H1H15211P or H1H15277N antibodies, or with a hIgG isotype control one day before infection with MERS-CoV. All samples were compared to hIgG1 isotype control set at 100%.

[053] Figure 8 shows quantitative PCR of MERS-CoV transcript (MERS-CoV genome – leader

sequence) from lungs of mice treated with 200µg, 20µg, or 2 µg of H1H15211P or H1H15277N antibodies, or with a hIgG isotype control one day before infection with MERS-CoV. All samples were compared to hIgG1 isotype control set at 100%.

[054] Figure 9 shows analysis of viral titer in the lungs as quantitated by plaque assay and reported as pfu/ml from lungs of mice treated with 200µg, 20µg, or 2 µg of H1H15211P or H1H15277N antibodies, or with a hIgG isotype control one day before infection with MERS-CoV. All samples were compared to hIgG1 isotype control set at 100%.

[055] Figure 10 shows inflammatory scores from histological analysis of the lungs of mice treated with 200µg, 20µg, or 2 µg of H1H15211P or H1H15277N antibodies, or with a hIgG isotype control one day before infection with MERS-CoV.

[056] Figure 11 shows quantitative PCR of MERS-CoV transcript (transcribed mRNA of the genome upstream of the envelope gene – UpE) from lungs of mice treated with 200µg or 500µg of H1H15211P or a hIgG isotype control at one day post infection or with 200µg of H1H15211P at one day before infection with MERS-CoV. All samples were compared to hIgG1 isotype control set at 100%.

[057] Figure 12 shows quantitative PCR of MERS-CoV transcript (MERS-CoV genome – leader sequence) from lungs of mice treated with 200µg or 500µg of H1H15211P or a hIgG isotype control at one day post infection or with 200µg of H1H15211P at one day before infection with MERS-CoV. All samples were compared to hIgG1 isotype control set at 100%.

[058] Figure 13 shows analysis of viral titer in the lungs as quantitated by plaque assay and reported as pfu/ml from lungs of mice treated with 200µg or 500µg of H1H15211P or a hIgG isotype control at one day post infection or with 200µg of H1H15211P at one day before infection with MERS-CoV. All samples were compared to hIgG1 isotype control set at 100%.

[059] Figure 14 shows inflammatory scores from histological analysis of the lungs of mice treated with 200µg or 500µg of H1H15211P or with a hIgG isotype control one day post infection with MERS-CoV.

DETAILED DESCRIPTION

[060] Before the present methods are described, it is to be understood that this invention is not limited to particular methods, and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[061] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference in their entirety.

Definitions

[062] The term "MERS-CoV", also called as "MERS coronavirus", refers to the newly-emerged Middle East Respiratory Syndrome – Corona Virus which was first isolated in the Arabian peninsula in 2012 (Zaki et al 2012, NEJM 367: 1814-1820) and identified as the cause for the outbreak of severe acute respiratory disease. It was initially called human coronavirus-EMC (Erasmus Medical Centre; hCoV-EMC). It belongs to the betacoronavirus lineage 2c and causes severe respiratory disease, similar to the Severe Acute Respiratory Syndrome coronavirus (SARS-CoV) that emerged in China in 2002. The MERS coronavirus has been found to be closely related to coronaviruses found in bats and camels. It binds via the viral spike protein to human host cell receptor dipeptidyl peptidase 4 (DPP4). MERS-CoV spike protein has been found to bind to DPP4 of other species, especially bats and camels (Raj et al 2013, Nature 495: 251-254).

[063] The term "MERS-CoV-S", also called "S protein" refers to the spike protein of the MERS coronavirus. The MERS-CoV spike protein is a 1353 amino acid type I membrane glycoprotein which assembles into trimers that constitute the spikes or peplomers on the surface of the enveloped MERS coronavirus particle. The protein has two essential functions, host receptor binding and membrane fusion, which are attributed to the N-terminal (S1, amino acid residues 1 – 751) and C-terminal (S2, amino acid residues 752 – 1353) halves of the S protein. MERS-CoV-S binds to its cognate receptor, dipeptidyl peptidase 4 (DPP4) via about 230-amino acid long receptor binding domain (RBD) present in the S1 subunit. Mou et al (2013) have shown in J. Virology (vol 87, pages 9379-9383) that the MERS-CoV RBD is located within the residues 358 – 588 of the spike protein. The amino acid sequence of full-length MERS-CoV spike protein is exemplified by the amino acid sequence of spike protein of MERS-CoV isolate EMC/2012 provided in GenBank as accession number AFS88936.1 (SEQ ID NO: 457). The term "MERS-CoV-S" also includes protein variants of MERS-CoV spike protein isolated from different MERS-CoV isolates, *e.g.*, Jordan-N3/2012, England-Qatar/2012, Al-Hasa_1_2013, Al-Hasa_2_2013, Al-Hasa_3_2013, Al-Hasa_4_2013, Al-Hasa_12, Al-Hasa_15, Al-Hasa_16, Al-Hasa_17, Al-Hasa_18, Al-Hasa_19, Al-Hasa_21, Al-Hasa_25, Bisha_1, Buraidah_1, England 1, Hafr-Al-Batin_1, Hafr-Al-Batin_2, Hafr-Al-Batin_6,Jeddah_1, KFU-HKU 1, KFU-HKU 13, Munich, Qatar3, Qatar4, Riyadh_1, Riyadh_2, Riyadh_3, Riyadh_3, Riyadh_4, Riyadh_5, Riyadh_9, Riyadh_14, Taif_1, UAE, and Wadi-Ad-Dawasir. The term "MERS-CoV-S" includes recombinant MERS-CoV spike protein or a fragment thereof. The term also encompasses MERS-CoV spike protein or a fragment thereof coupled to, for example, histidine tag, mouse or human Fc, or a signal sequence such as ROR1. For example, the term includes sequences exemplified by the sequence shown in SEQ ID NO: 458, comprising a mouse Fc (mIgG2a) or human Fc (hIgG1) at the C-terminal, coupled to amino acid residues 367 – 606 of full-length MERS-CoV spike protein. The term also includes protein variants that comprise a histidine tag at the C-terminal, coupled to amino acid residues 367 – 606 of full-length MERS-CoV spike protein.

[064] The term "DPP4" refers to dipeptidyl peptidase 4, a receptor for MERS-CoV. DPP4 is a

766-amino acid type II transmembrane glycoprotein present in a dimeric form on the cell surface. It is an exopeptidase that cleaves dipeptides from hormones and chemokines after a proline amino acid residue, thereby regulating their bioactivity. In humans, DPP4 is primarily expressed on the epithelial cells in kidney, small intestine, liver and prostate, on ciliated and non-ciliated cells in the upper and lower respiratory tract, and on immune cells (i.e., CD4+, CD8+, dendritic cells and macrophages). Unless specified as being from a non-human species, the term “DPP4”, as used herein, means human DPP4.

[065] The term “MERS infection” or “MERS-CoV infection”, as used herein, also characterized as Middle East Respiratory Syndrome refers to the severe acute respiratory illness caused by MERS coronavirus and first reported in Saudi Arabia in 2012. The term includes respiratory tract infection, often in the lower respiratory tract. The symptoms include high fever, cough, shortness of breath pneumonia, gastro-intestinal symptoms such as diarrhea, organ failure (kidney failure and renal dysfunction), septic shock and death in severe cases.

[066] The term "antibody", as used herein, is intended to refer to immunoglobulin molecules comprised of four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds (*i.e.*, "full antibody molecules"), as well as multimers thereof (*e.g.* IgM) or antigen-binding fragments thereof. Each heavy chain is comprised of a heavy chain variable region (“HCVR” or “V_H”) and a heavy chain constant region (comprised of domains C_H1, C_H2 and C_H3). Each light chain is comprised of a light chain variable region (“LCVR or “V_L”) and a light chain constant region (C_L). The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. In certain embodiments of the invention, the FRs of the antibody (or antigen binding fragment thereof) may be identical to the human germline sequences, or may be naturally or artificially modified. An amino acid consensus sequence may be defined based on a side-by-side analysis of two or more CDRs.

[067] Substitution of one or more CDR residues or omission of one or more CDRs is also possible. Antibodies have been described in the scientific literature in which one or two CDRs can be dispensed with for binding. Padlan *et al.* (1995 FASEB J. 9:133-139) analyzed the contact regions between antibodies and their antigens, based on published crystal structures, and concluded that only about one fifth to one third of CDR residues actually contact the antigen. Padlan also found many antibodies in which one or two CDRs had no amino acids in contact with an antigen (see also, Vajdos *et al.* 2002 J Mol Biol 320:415-428).

[068] CDR residues not contacting antigen can be identified based on previous studies (for example residues H60-H65 in CDRH2 are often not required), from regions of Kabat CDRs lying outside Chothia CDRs, by molecular modeling and/or empirically. If a CDR or residue(s) thereof is omitted, it is usually substituted with an amino acid occupying the corresponding position in another human antibody sequence or a consensus of such sequences. Positions for

substitution within CDRs and amino acids to substitute can also be selected empirically. Empirical substitutions can be conservative or non-conservative substitutions.

[069] The fully human anti-MERS-CoV-S monoclonal antibodies disclosed herein may comprise one or more amino acid substitutions, insertions and/or deletions in the framework and/or CDR regions of the heavy and light chain variable domains as compared to the corresponding germline sequences. Such mutations can be readily ascertained by comparing the amino acid sequences disclosed herein to germline sequences available from, for example, public antibody sequence databases. The present invention includes antibodies, and antigen-binding fragments thereof, which are derived from any of the amino acid sequences disclosed herein, wherein one or more amino acids within one or more framework and/or CDR regions are mutated to the corresponding residue(s) of the germline sequence from which the antibody was derived, or to the corresponding residue(s) of another human germline sequence, or to a conservative amino acid substitution of the corresponding germline residue(s) (such sequence changes are referred to herein collectively as "germline mutations"). A person of ordinary skill in the art, starting with the heavy and light chain variable region sequences disclosed herein, can easily produce numerous antibodies and antigen-binding fragments which comprise one or more individual germline mutations or combinations thereof. In certain embodiments, all of the framework and/or CDR residues within the V_H and/or V_L domains are mutated back to the residues found in the original germline sequence from which the antibody was derived. In other embodiments, only certain residues are mutated back to the original germline sequence, *e.g.*, only the mutated residues found within the first 8 amino acids of FR1 or within the last 8 amino acids of FR4, or only the mutated residues found within CDR1, CDR2 or CDR3. In other embodiments, one or more of the framework and/or CDR residue(s) are mutated to the corresponding residue(s) of a different germline sequence (*i.e.*, a germline sequence that is different from the germline sequence from which the antibody was originally derived). Furthermore, the antibodies of the present invention may contain any combination of two or more germline mutations within the framework and/or CDR regions, *e.g.*, wherein certain individual residues are mutated to the corresponding residue of a particular germline sequence while certain other residues that differ from the original germline sequence are maintained or are mutated to the corresponding residue of a different germline sequence. Once obtained, antibodies and antigen-binding fragments that contain one or more germline mutations can be easily tested for one or more desired property such as, improved binding specificity, increased binding affinity, improved or enhanced antagonistic or agonistic biological properties (as the case may be), reduced immunogenicity, etc. Antibodies and antigen-binding fragments obtained in this general manner are encompassed within the present invention.

[070] The present invention also includes fully human anti-MERS-CoV-S monoclonal antibodies comprising variants of any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein having one or more conservative substitutions. For example, the present invention includes anti-MERS-CoV antibodies having HCVR, LCVR, and/or CDR amino acid sequences

with, *e.g.*, 10 or fewer, 8 or fewer, 6 or fewer, 4 or fewer, etc. conservative amino acid substitutions relative to any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein.

[071] The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human mAbs of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.,* mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs and in particular CDR3. However, the term "human antibody", as used herein, is not intended to include mAbs in which CDR sequences derived from the germline of another mammalian species (*e.g.*, mouse), have been grafted onto human FR sequences. The term includes antibodies recombinantly produced in a non-human mammal, or in cells of a non-human mammal. The term is not intended to include antibodies isolated from or generated in a human subject.

[072] The term “recombinant”, as used herein, refers to antibodies or antigen-binding fragments thereof of the invention created, expressed, isolated or obtained by technologies or methods known in the art as recombinant DNA technology which include, *e.g.*, DNA splicing and transgenic expression. The term refers to antibodies expressed in a non-human mammal (including transgenic non-human mammals, *e.g.*, transgenic mice), or a cell (*e.g.*, CHO cells) expression system or isolated from a recombinant combinatorial human antibody library.

[073] The term "specifically binds," or “binds specifically to”, or the like, means that an antibody or antigen-binding fragment thereof forms a complex with an antigen that is relatively stable under physiologic conditions. Specific binding can be characterized by an equilibrium dissociation constant of at least about $1x10^{-8}$ M or less (*e.g.*, a smaller K_D denotes a tighter binding). Methods for determining whether two molecules specifically bind are well known in the art and include, for example, equilibrium dialysis, surface plasmon resonance, and the like. As described herein, antibodies have been identified by surface plasmon resonance, *e.g.*, BIACORE™, which bind specifically to MERS-CoV-S. Moreover, multi-specific antibodies that bind to one domain in MERS-CoV-S and one or more additional antigens or a bi-specific that binds to two different regions of MERS-CoV-S are nonetheless considered antibodies that “specifically bind”, as used herein.

[074] The term “high affinity” antibody refers to those mAbs having a binding affinity to MERS-CoV-S, expressed as K_D, of at least 10^{-8} M; preferably 10^{-9} M; more preferably 10^{-10}M, even more preferably 10^{-11} M, even more preferably 10^{-12} M, as measured by surface plasmon resonance, e.g., BIACORE™ or solution-affinity ELISA.

[075] By the term “slow off rate”, “Koff” or “kd” is meant an antibody that dissociates from MERS-CoV, with a rate constant of 1×10^{-3} s^{-1} or less, preferably 1×10^{-4} s^{-1} or less, as determined by surface plasmon resonance, *e.g.*, BIACORE™.

[076] The terms "antigen-binding portion" of an antibody, "antigen-binding fragment" of an

antibody, and the like, as used herein, include any naturally occurring, enzymatically obtainable, synthetic, or genetically engineered polypeptide or glycoprotein that specifically binds an antigen to form a complex. The terms "antigen-binding fragment" of an antibody, or "antibody fragment", as used herein, refers to one or more fragments of an antibody that retain the ability to bind to MERS-CoV spike protein.

[077] In specific embodiments, antibody or antibody fragments of the invention may be conjugated to a moiety such a ligand or a therapeutic moiety ("immunoconjugate"), such as an anti-viral drug, a second anti-MERS-CoV-S antibody, or any other therapeutic moiety useful for treating an infection caused by MERS-CoV.

[078] An "isolated antibody", as used herein, is intended to refer to an antibody that is substantially free of other antibodies (Abs) having different antigenic specificities (*e.g.*, an isolated antibody that specifically binds MERS-CoV-S, or a fragment thereof, is substantially free of Abs that specifically bind antigens other than MERS-CoV-S.

[079] A "blocking antibody" or a "neutralizing antibody", as used herein (or an "antibody that neutralizes MERS-CoV-S activity" or "antagonist antibody"), is intended to refer to an antibody whose binding to MERS-CoV-S results in inhibition of at least one biological activity of MERS-CoV. For example, an antibody of the invention may prevent or block MERS-CoV binding to DPP4.

[080] The term "surface plasmon resonance", as used herein, refers to an optical phenomenon that allows for the analysis of real-time biomolecular interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIACORE™ system (Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, N.J.).

[081] The term "K_D ", as used herein, is intended to refer to the equilibrium dissociation constant of a particular antibody-antigen interaction.

[082] The term "epitope" refers to an antigenic determinant that interacts with a specific antigen binding site in the variable region of an antibody molecule known as a paratope. A single antigen may have more than one epitope. Thus, different antibodies may bind to different areas on an antigen and may have different biological effects. The term "epitope" also refers to a site on an antigen to which B and/or T cells respond. It also refers to a region of an antigen that is bound by an antibody. Epitopes may be defined as structural or functional. Functional epitopes are generally a subset of the structural epitopes and have those residues that directly contribute to the affinity of the interaction. Epitopes may also be conformational, that is, composed of non-linear amino acids. In certain embodiments, epitopes may include determinants that are chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl groups, or sulfonyl groups, and, in certain embodiments, may have specific three-dimensional structural characteristics, and/or specific charge characteristics.

[083] The term "cross-competes", as used herein, means an antibody or antigen-binding fragment thereof binds to an antigen and inhibits or blocks the binding of another antibody or antigen-binding fragment thereof. The term also includes competition between two antibodies in

both orientations, *i.e.*, a first antibody that binds and blocks binding of second antibody and vice-versa. In certain embodiments, the first antibody and second antibody may bind to the same epitope. Alternatively, the first and second antibodies may bind to different, but overlapping epitopes such that binding of one inhibits or blocks the binding of the second antibody, *e.g.*, *via* steric hindrance. Cross-competition between antibodies may be measured by methods known in the art, for example, by a real-time, label-free bio-layer interferometry assay. Cross-competition between two antibodies may be expressed as the binding of the second antibody that is less than the background signal due to self-self binding (wherein first and second antibodies is the same antibody). Cross-competition between 2 antibodies may be expressed, for example, as % binding of the second antibody that is less than the baseline self-self background binding (wherein first and second antibodies is the same antibody).

[084] The term "substantial identity" or "substantially identical," when referring to a nucleic acid or fragment thereof, indicates that, when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 90%, and more preferably at least about 95%, 96%, 97%, 98% or 99% of the nucleotide bases, as measured by any well-known algorithm of sequence identity, such as FASTA, BLAST or GAP, as discussed below. A nucleic acid molecule having substantial identity to a reference nucleic acid molecule may, in certain instances, encode a polypeptide having the same or substantially similar amino acid sequence as the polypeptide encoded by the reference nucleic acid molecule.

[085] As applied to polypeptides, the term "substantial similarity" or “substantially similar” means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 90% sequence identity, even more preferably at least 95%, 98% or 99% sequence identity. Preferably, residue positions, which are not identical, differ by conservative amino acid substitutions. A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well known to those of skill in the art. See, *e.g.*, Pearson (1994) Methods Mol. Biol. 24: 307-331, which is herein incorporated by reference. Examples of groups of amino acids that have side chains with similar chemical properties include 1) aliphatic side chains: glycine, alanine, valine, leucine and isoleucine; 2) aliphatic-hydroxyl side chains: serine and threonine; 3) amide-containing side chains: asparagine and glutamine; 4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; 5) basic side chains: lysine, arginine, and histidine; 6) acidic side chains: aspartate and glutamate, and 7) sulfur-containing side chains: cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine,

phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine. Alternatively, a conservative replacement is any change having a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet *et al.* (1992) Science 256: 1443 45, herein incorporated by reference. A "moderately conservative" replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

[086] Sequence similarity for polypeptides is typically measured using sequence analysis software. Protein analysis software matches similar sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, GCG software contains programs such as GAP and BESTFIT which can be used with default parameters to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and a mutein thereof. See, *e.g.,* GCG Version 6.1. Polypeptide sequences also can be compared using FASTA with default or recommended parameters; a program in GCG Version 6.1. FASTA (*e.g.,* FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson (2000) *supra*). Another preferred algorithm when comparing a sequence of the invention to a database containing a large number of sequences from different organisms is the computer program BLAST, especially BLASTP or TBLASTN, using default parameters. See, *e.g.,* Altschul *et al.* (1990) J. Mol. Biol. 215: 403-410 and (1997) Nucleic Acids Res. 25:3389-3402, each of which is herein incorporated by reference.

[087] By the phrase “therapeutically effective amount” is meant an amount that produces the desired effect for which it is administered. The exact amount will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (see, for example, Lloyd (1999) The Art, Science and Technology of Pharmaceutical Compounding).

[088] As used herein, the term “subject” refers to an animal, preferably a mammal, more preferably a human, in need of amelioration, prevention and/or treatment of a disease or disorder such as viral infection. The term includes human subjects who have or are at risk of having MERS infection.

[089] As used herein, the terms “treat”, “treating”, or “treatment” refer to the reduction or amelioration of the severity of at least one symptom or indication of MERS infection due to the administration of a therapeutic agent such as an antibody of the present invention to a subject in need thereof. The terms include inhibition of progression of disease or of worsening of infection. The terms also include positive prognosis of disease, i.e., the subject may be free of infection or may have reduced or no viral titers upon administration of a therapeutic agent such as an antibody of the present invention. The therapeutic agent may be administered at a therapeutic dose to the subject.

[090] The terms “prevent”, “preventing” or “prevention” refer to inhibition of manifestation of MERS infection or any symptoms or indications of MERS infection upon administration of an antibody of the present invention. The term includes prevention of spread of infection in a

subject exposed to the virus or at risk of having MERS infection.

[091] As used herein, the term “anti-viral drug” refers to any anti-infective drug or therapy used to treat, prevent, or ameliorate a viral infection in a subject. The term “anti-viral drug” includes, but is not limited to ribavirin, oseltamivir, zanamivir, interferon-alpha2b, analgesics and corticosteroids. In the context of the present invention, the viral infections include infection caused by human coronaviruses, including but not limited to, MERS-CoV, HCoV_229E, HCoV_NL63, HCoV-OC43, HCoV_HKU1, and SARS-CoV.

General Description

[092] Passive immunotherapy for prophylaxis or treatment of infectious diseases has been used for more than a century, usually in the form of convalescent human sera that contains high titers of neutralizing antibodies (Good et al 1991; Cancer 68: 1415-1421). Today, multiple purified monoclonal antibodies are currently in preclinical and clinical development for use as anti-microbials (Marasco et al 2007; Nature Biotechnology 25: 1421-1434).

[093] The inventors have described herein fully human antibodies and antigen-binding fragments thereof that specifically bind to MERS-CoV-S and modulate the interaction of MERS-CoV-S with DPP4. The anti-MERS-CoV-S antibodies may bind to MERS-CoV-S with high affinity. In certain embodiments, the antibodies of the present invention are blocking antibodies wherein the antibodies may bind to MERS-CoV-S and block the interaction of MERS-CoV-S with DPP4. In some embodiments, the blocking antibodies of the invention may block the binding of MERS-CoV-S to DPP4 and/or inhibit or neutralize viral infectivity of host cells. In some embodiments, the blocking antibodies may be useful for treating a subject suffering from MERS infection. In certain embodiments, selected antibodies that do not cross-compete for binding to the spike protein are used in combination as a cocktail to reduce the ability of the virus to escape via mutation in response to the selective pressure from either component. The antibodies when administered to a subject in need thereof may reduce the infection by a virus such as MERS-CoV in the subject. They may be used to decrease viral loads in a subject. They may be used alone or as adjunct therapy with other therapeutic moieties or modalities known in the art for treating viral infection. It is also shown herein that these antibodies bind to epitopes on the S protein that have been conserved during the natural evolution of the virus during the past two years. Further, a novel transgenic mouse model was used to demonstrate that the identified antibodies can prophylactically protect mice from infection as well as ameliorate a previously established infection in a post-inoculation treatment paradigm. In Example 7, it is shown that administration of an anti-MERS-Cov-S antibody at 1 day before infection is able to reduce MERS-CoV replication to near the level of detection in the live virus assays and by 3 logs in the viral RNA assays. This antibody demonstrated dose-dependent protection, as lower doses of the antibody given 24 hours pre-infection were able to block MERS-CoV to a lesser degree. Additionally, histological analysis of lung tissue demonstrated that mice pre-treated with the antibody displayed reduced MERS-CoV induced peri-bronchiolar cuffing, alveolar wall

thickening, and overall inflammatory foci.

[094] The full-length amino acid sequence of full length MERS-CoV spike protein is shown in SEQ ID NO: 457. In certain embodiments, the antibodies of the invention are obtained from mice immunized with a primary immunogen, such as a full length MERS-CoV spike protein (SEQ ID NO: 457) or with a recombinant form of MERS-CoV-S or modified MERS-CoV-S fragments (*e.g.*, SEQ ID NO: 458), followed by immunization with a secondary immunogen, or with an immunogenically active fragment of MERS-CoV-S.

[095] The immunogen may be a biologically active and/or immunogenic fragment of MERS-CoV-S or DNA encoding the active fragment thereof. The fragment may be derived from the N-terminal or C-terminal domain of MERS-CoV-S. In certain embodiments of the invention, the immunogen is a fragment of MERS-CoV-S that ranges from amino acid residues 367 – 606 of SEQ ID NO: 457.

[096] The peptides may be modified to include addition or substitution of certain residues for tagging or for purposes of conjugation to carrier molecules, such as, KLH. For example, a cysteine may be added at either the N terminal or C terminal end of a peptide, or a linker sequence may be added to prepare the peptide for conjugation to, for example, KLH for immunization.

[097] Certain anti-MERS-CoV-S antibodies of the present invention are able to bind to and neutralize the activity of MERS-CoV-S, as determined by *in vitro* or *in vivo* assays. The ability of the antibodies of the invention to bind to and neutralize the activity of MERS-CoV-S may be measured using any standard method known to those skilled in the art, including binding assays, or activity assays, as described herein.

[098] Non-limiting, exemplary *in vitro* assays for measuring binding and blocking activity are illustrated in Examples 4 – 5, herein. In Example 4, the binding affinity and dissociation constants of anti-MERS-CoV-S antibodies for MERS-CoV-S were determined by surface plasmon resonance assay. In Example 5, neutralization assays were used to determine infectivity of MERS-CoV spike protein-containing virus-like particles.

[099] The antibodies specific for MERS-CoV-S may contain no additional labels or moieties, or they may contain an N-terminal or C-terminal label or moiety. In one embodiment, the label or moiety is biotin. In a binding assay, the location of a label (if any) may determine the orientation of the peptide relative to the surface upon which the peptide is bound. For example, if a surface is coated with avidin, a peptide containing an N-terminal biotin will be oriented such that the C-terminal portion of the peptide will be distal to the surface. In one embodiment, the label may be a radionuclide, a fluorescent dye or a MRI-detectable label. In certain embodiments, such labeled antibodies may be used in diagnostic assays including imaging assays.

Antigen-Binding Fragments of Antibodies

[0100] Unless specifically indicated otherwise, the term "antibody," as used herein, shall be understood to encompass antibody molecules comprising two immunoglobulin heavy chains and

two immunoglobulin light chains (*i.e.*, "full antibody molecules") as well as antigen-binding fragments thereof. The terms "antigen-binding portion" of an antibody, "antigen-binding fragment" of an antibody, and the like, as used herein, include any naturally occurring, enzymatically obtainable, synthetic, or genetically engineered polypeptide or glycoprotein that specifically binds an antigen to form a complex. The terms "antigen-binding fragment" of an antibody, or "antibody fragment", as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to MERS-CoV spike protein. An antibody fragment may include a Fab fragment, a $F(ab')_2$ fragment, a Fv fragment, a dAb fragment, a fragment containing a CDR, or an isolated CDR. In certain embodiments, the term "antigen-binding fragment" refers to a polypeptide fragment of a multi-specific antigen-binding molecule. Antigen-binding fragments of an antibody may be derived, *e.g.*, from full antibody molecules using any suitable standard techniques such as proteolytic digestion or recombinant genetic engineering techniques involving the manipulation and expression of DNA encoding antibody variable and (optionally) constant domains. Such DNA is known and/or is readily available from, *e.g.*, commercial sources, DNA libraries (including, *e.g.*, phage-antibody libraries), or can be synthesized. The DNA may be sequenced and manipulated chemically or by using molecular biology techniques, for example, to arrange one or more variable and/or constant domains into a suitable configuration, or to introduce codons, create cysteine residues, modify, add or delete amino acids, etc.

[0101] Non-limiting examples of antigen-binding fragments include: (i) Fab fragments; (ii) F(ab')2 fragments; (iii) Fd fragments; (iv) Fv fragments; (v) single-chain Fv (scFv) molecules; (vi) dAb fragments; and (vii) minimal recognition units consisting of the amino acid residues that mimic the hypervariable region of an antibody (*e.g.*, an isolated complementarity determining region (CDR) such as a CDR3 peptide), or a constrained FR3-CDR3-FR4 peptide. Other engineered molecules, such as domain-specific antibodies, single domain antibodies, domain-deleted antibodies, chimeric antibodies, CDR-grafted antibodies, diabodies, triabodies, tetrabodies, minibodies, nanobodies (*e.g.* monovalent nanobodies, bivalent nanobodies, etc.), small modular immunopharmaceuticals (SMIPs), and shark variable IgNAR domains, are also encompassed within the expression "antigen-binding fragment," as used herein.

[0102] An antigen-binding fragment of an antibody will typically comprise at least one variable domain. The variable domain may be of any size or amino acid composition and will generally comprise at least one CDR, which is adjacent to or in frame with one or more framework sequences. In antigen-binding fragments having a V_H domain associated with a V_L domain, the V_H and V_L domains may be situated relative to one another in any suitable arrangement. For example, the variable region may be dimeric and contain V_H - V_H, V_H - V_L or V_L - V_L dimers. Alternatively, the antigen-binding fragment of an antibody may contain a monomeric V_H or V_L domain.

[0103] In certain embodiments, an antigen-binding fragment of an antibody may contain at least one variable domain covalently linked to at least one constant domain. Non-limiting,

exemplary configurations of variable and constant domains that may be found within an antigen-binding fragment of an antibody of the present invention include: (i) V_H -C_H1; (ii) V_H -C_H2; (iii) V_H -C_H3; (iv) V_H -C_H1-C_H2; (v) V_H -C_H1-C_H2-C_H3; (vi) V_H -C_H2-C_H3; (vii) V_H -C_L; (viii) V_L -C_H1; (ix) V_L -C_H2; (x) V_L -C_H3; (xi) V_L -C_H1-C_H2; (xii) V_L -C_H1-C_H2-C_H3; (xiii) V_L -C_H2-C_H3; and (xiv) V_L -C_L. In any configuration of variable and constant domains, including any of the exemplary configurations listed above, the variable and constant domains may be either directly linked to one another or may be linked by a full or partial hinge or linker region. A hinge region may consist of at least 2 (*e.g.*, 5, 10, 15, 20, 40, 60 or more) amino acids, which result in a flexible or semi-flexible linkage between adjacent variable and/or constant domains in a single polypeptide molecule. Moreover, an antigen-binding fragment of an antibody of the present invention may comprise a homo-dimer or hetero-dimer (or other multimer) of any of the variable and constant domain configurations listed above in non-covalent association with one another and/or with one or more monomeric V_H or V_L domain (*e.g.*, by disulfide bond(s)).

[0104] As with full antibody molecules, antigen-binding fragments may be mono-specific or multi-specific (*e.g.*, bi-specific). A multi-specific antigen-binding fragment of an antibody will typically comprise at least two different variable domains, wherein each variable domain is capable of specifically binding to a separate antigen or to a different epitope on the same antigen. Any multi-specific antibody format, including the exemplary bi-specific antibody formats disclosed herein, may be adapted for use in the context of an antigen-binding fragment of an antibody of the present invention using routine techniques available in the art.

Preparation of Human Antibodies

[0105] Methods for generating human antibodies in transgenic mice are known in the art. Any such known methods can be used in the context of the present invention to make human antibodies that specifically bind to MERS-CoV spike protein.

[0106] An immunogen comprising any one of the following can be used to generate antibodies to MERS-CoV spike protein. In certain embodiments, the antibodies of the invention are obtained from mice immunized with a full length, native MERS-CoV spike protein (See, for example, GenBank accession number AFS88936.1) (SEQ ID NO: 457), or with DNA encoding the protein or fragment thereof. Alternatively, the spike protein or a fragment thereof may be produced using standard biochemical techniques and modified and used as immunogen. In one embodiment, the immunogen is the receptor binding domain (S1) of MERS-CoV spike protein. In certain embodiments of the invention, the immunogen is a fragment of MERS-CoV spike protein that ranges from about amino acid residues 367-606 of SEQ ID NO: 457.

[0107] In some embodiments, the immunogen may be a recombinant MERS-CoV spike protein receptor binding domain peptide expressed in *E. coli* or in any other eukaryotic or mammalian cells such as Chinese hamster ovary (CHO) cells.

[0108] Using VELOCIMMUNE® technology (see, for example, US 6,596,541, Regeneron Pharmaceuticals, VELOCIMMUNE®) or any other known method for generating monoclonal

antibodies, high affinity chimeric antibodies to MERS-CoV-S are initially isolated having a human variable region and a mouse constant region. The VELOCIMMUNE® technology involves generation of a transgenic mouse having a genome comprising human heavy and light chain variable regions operably linked to endogenous mouse constant region loci such that the mouse produces an antibody comprising a human variable region and a mouse constant region in response to antigenic stimulation. The DNA encoding the variable regions of the heavy and light chains of the antibody are isolated and operably linked to DNA encoding the human heavy and light chain constant regions. The DNA is then expressed in a cell capable of expressing the fully human antibody.

[0109] Generally, a VELOCIMMUNE® mouse is challenged with the antigen of interest, and lymphatic cells (such as B-cells) are recovered from the mice that express antibodies. The lymphatic cells may be fused with a myeloma cell line to prepare immortal hybridoma cell lines, and such hybridoma cell lines are screened and selected to identify hybridoma cell lines that produce antibodies specific to the antigen of interest. DNA encoding the variable regions of the heavy chain and light chain may be isolated and linked to desirable isotypic constant regions of the heavy chain and light chain. Such an antibody protein may be produced in a cell, such as a CHO cell. Alternatively, DNA encoding the antigen-specific chimeric antibodies or the variable domains of the light and heavy chains may be isolated directly from antigen-specific lymphocytes.

[0110] Initially, high affinity chimeric antibodies are isolated having a human variable region and a mouse constant region. As in the experimental section below, the antibodies are characterized and selected for desirable characteristics, including affinity, selectivity, epitope, etc. The mouse constant regions are replaced with a desired human constant region to generate the fully human antibody of the invention, for example wild-type or modified IgG1 or IgG4. While the constant region selected may vary according to specific use, high affinity antigen-binding and target specificity characteristics reside in the variable region.

Bioequivalents

[0111] The anti-MERS-CoV-S antibodies and antibody fragments of the present invention encompass proteins having amino acid sequences that vary from those of the described antibodies, but that retain the ability to bind MERS-CoV spike protein. Such variant antibodies and antibody fragments comprise one or more additions, deletions, or substitutions of amino acids when compared to parent sequence, but exhibit biological activity that is essentially equivalent to that of the described antibodies. Likewise, the antibody-encoding DNA sequences of the present invention encompass sequences that comprise one or more additions, deletions, or substitutions of nucleotides when compared to the disclosed sequence, but that encode an antibody or antibody fragment that is essentially bioequivalent to an antibody or antibody fragment of the invention.

[0112] Two antigen-binding proteins, or antibodies, are considered bioequivalent if, for

example, they are pharmaceutical equivalents or pharmaceutical alternatives whose rate and extent of absorption do not show a significant difference when administered at the same molar dose under similar experimental conditions, either single dose or multiple doses. Some antibodies will be considered equivalents or pharmaceutical alternatives if they are equivalent in the extent of their absorption but not in their rate of absorption and yet may be considered bioequivalent because such differences in the rate of absorption are intentional and are reflected in the labeling, are not essential to the attainment of effective body drug concentrations on, *e.g.*, chronic use, and are considered medically insignificant for the particular drug product studied.

[0113] In one embodiment, two antigen-binding proteins are bioequivalent if there are no clinically meaningful differences in their safety, purity, or potency.

[0114] In one embodiment, two antigen-binding proteins are bioequivalent if a patient can be switched one or more times between the reference product and the biological product without an expected increase in the risk of adverse effects, including a clinically significant change in immunogenicity, or diminished effectiveness, as compared to continued therapy without such switching.

[0115] In one embodiment, two antigen-binding proteins are bioequivalent if they both act by a common mechanism or mechanisms of action for the condition or conditions of use, to the extent that such mechanisms are known.

[0116] Bioequivalence may be demonstrated by *in vivo* and/or *in vitro* methods. Bioequivalence measures include, *e.g.*, (a) an *in vivo* test in humans or other mammals, in which the concentration of the antibody or its metabolites is measured in blood, plasma, serum, or other biological fluid as a function of time; (b) an *in vitro* test that has been correlated with and is reasonably predictive of human *in vivo* bioavailability data; (c) an *in vivo* test in humans or other mammals in which the appropriate acute pharmacological effect of the antibody (or its target) is measured as a function of time; and (d) in a well-controlled clinical trial that establishes safety, efficacy, or bioavailability or bioequivalence of an antibody.

[0117] Bioequivalent variants of the antibodies of the invention may be constructed by, for example, making various substitutions of residues or sequences or deleting terminal or internal residues or sequences not needed for biological activity. For example, cysteine residues not essential for biological activity can be deleted or replaced with other amino acids to prevent formation of unnecessary or incorrect intramolecular disulfide bridges upon renaturation. In other contexts, bioequivalent antibodies may include antibody variants comprising amino acid changes, which modify the glycosylation characteristics of the antibodies, *e.g.*, mutations that eliminate or remove glycosylation.

Anti-MERS-CoV-S Antibodies Comprising Fc Variants

[0118] According to certain embodiments of the present invention, anti-MERS-CoV-S antibodies are provided comprising an Fc domain comprising one or more mutations which enhance or diminish antibody binding to the FcRn receptor, *e.g.*, at acidic pH as compared to neutral pH.

For example, the present invention includes anti-MERS-CoV-S antibodies comprising a mutation in the C_H2 or a C_H3 region of the Fc domain, wherein the mutation(s) increases the affinity of the Fc domain to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0). Such mutations may result in an increase in serum half-life of the antibody when administered to an animal. Non-limiting examples of such Fc modifications include, *e.g.*, a modification at position 250 (e.g., E or Q); 250 and 428 (e.g., L or F); 252 (e.g., L/Y/F/W or T), 254 (e.g., S or T), and 256 (e.g., S/R/Q/E/D or T); or a modification at position 428 and/or 433 (e.g., H/L/R/S/P/Q or K) and/or 434 (*e.g.,* A, W, H, F or Y [N434A, N434W, N434H, N434F or N434Y]); or a modification at position 250 and/or 428; or a modification at position 307 or 308 (*e.g.,* 308F, V308F), and 434. In one embodiment, the modification comprises a 428L (*e.g.,* M428L) and 434S (*e.g.,* N434S) modification; a 428L, 259I (*e.g.,* V259I), and 308F (*e.g.,* V308F) modification; a 433K (*e.g.,* H433K) and a 434 (*e.g.,* 434Y) modification; a 252, 254, and 256 (*e.g.,* 252Y, 254T, and 256E) modification; a 250Q and 428L modification (*e.g.,* T250Q and M428L); and a 307 and/or 308 modification (*e.g.,* 308F or 308P). In yet another embodiment, the modification comprises a 265A (*e.g.*, D265A) and/or a 297A (*e.g.*, N297A) modification.

[0119] For example, the present invention includes anti-MERS-CoV-S antibodies comprising an Fc domain comprising one or more pairs or groups of mutations selected from the group consisting of: 250Q and 248L (*e.g.*, T250Q and M248L); 252Y, 254T and 256E (*e.g.*, M252Y, S254T and T256E); 428L and 434S (*e.g.*, M428L and N434S); 257I and 311I (*e.g.*, P257I and Q311I); 257I and 434H (*e.g.*, P257I and N434H); 376V and 434H (*e.g.*, D376V and N434H); 307A, 380A and 434A (*e.g.*, T307A, E380A and N434A); and 433K and 434F (*e.g.*, H433K and N434F). All possible combinations of the foregoing Fc domain mutations and other mutations within the antibody variable domains disclosed herein, are contemplated within the scope of the present invention.

[0120] The present invention also includes anti-MERS-CoV-S antibodies comprising a chimeric heavy chain constant (C_H) region, wherein the chimeric C_H region comprises segments derived from the C_H regions of more than one immunoglobulin isotype. For example, the antibodies of the invention may comprise a chimeric C_H region comprising part or all of a C_H2 domain derived from a human IgG1, human IgG2 or human IgG4 molecule, combined with part or all of a C_H3 domain derived from a human IgG1, human IgG2 or human IgG4 molecule. According to certain embodiments, the antibodies of the invention comprise a chimeric C_H region having a chimeric hinge region. For example, a chimeric hinge may comprise an "upper hinge" amino acid sequence (amino acid residues from positions 216 to 227 according to EU numbering) derived from a human IgG1, a human IgG2 or a human IgG4 hinge region, combined with a "lower hinge" sequence (amino acid residues from positions 228 to 236 according to EU numbering) derived from a human IgG1, a human IgG2 or a human IgG4 hinge region. According to certain embodiments, the chimeric hinge region comprises amino acid residues derived from a human IgG1 or a human IgG4 upper hinge and amino acid residues derived from a human IgG2 lower hinge. An antibody comprising a chimeric C_H region as described herein

may, in certain embodiments, exhibit modified Fc effector functions without adversely affecting the therapeutic or pharmacokinetic properties of the antibody. (*See*, *e.g.*, U.S. Provisional Appl. No. 61/759,578, filed February 1, 2013, the disclosure of which is hereby incorporated by reference in its entirety).

Biological Characteristics of the Antibodies

[0121] In general, the antibodies of the present invention function by binding to MERS-CoV spike protein. In certain embodiments, the antibodies of the present invention bind with high affinity to one or more amino acids in the receptor binding domain (RBD) of the spike protein of MERS-CoV. For example, the present invention includes antibodies and antigen-binding fragments of antibodies that bind dimeric MERS-CoV spike protein RBD (*e.g.*, at 25°C or at 37°C) with a K_D of less than 20nM as measured by surface plasmon resonance, e.g., using the assay format as defined in Example 4 herein. In certain embodiments, the antibodies or antigen-binding fragments thereof bind dimeric MERS-CoV-S with a K_D of less than about 20nM, less than about 10nM, less than about 5nM, less than about 2nM, less than about 1nM, less than about 500pM, less than 250pM, or less than 100pM, as measured by surface plasmon resonance, e.g., using the assay format as defined in Example 4 herein, or a substantially similar assay.

[0122] The present invention also includes antibodies and antigen-binding fragments thereof that bind MERS-CoV spike protein with a dissociative half-life (t½) of greater than about 2.1 minutes as measured by surface plasmon resonance at 25ºC, *e.g.*, using an assay format as defined in Example 4 herein, or a substantially similar assay. In certain embodiments, the antibodies or antigen-binding fragments of the present invention bind MERS-CoV spike protein with a t½ of greater than about 5 minutes, greater than about 10 minutes, greater than about 30 minutes, greater than about 50 minutes, greater than about 100 minutes, greater than about 150 minutes, greater than about 200 minutes, or greater than about 250 minutes, as measured by surface plasmon resonance at 25ºC, *e.g.*, using an assay format as defined in Example 4 herein (*e.g.*, mAb-capture or antigen-capture format), or a substantially similar assay.

[0123] The present invention also includes antibodies and antigen-binding fragments thereof that bind MERS-CoV spike protein with a dissociative half-life (t½) of greater than about 1.5 minutes as measured by surface plasmon resonance at 37ºC, *e.g.*, using an assay format as defined in Example 4 herein, or a substantially similar assay. In certain embodiments, the antibodies or antigen-binding fragments of the present invention bind MERS-CoV spike protein with a t½ of greater than about 2 minutes, greater than about 5 minutes, greater than about 10 minutes, greater than about 25 minutes, greater than about 50 minutes, greater than about 100 minutes, greater than about 150 minutes, or greater than about 200 minutes, as measured by surface plasmon resonance at 37ºC, *e.g.*, using an assay format as defined in Example 4 herein (*e.g.*, mAb-capture or antigen-capture format), or a substantially similar assay.

[0124] The present invention also includes antibodies or antigen-binding fragments thereof

that block more than 90% MERS-CoV-S binding to DPP4 as determined using a ELISA-based immunoassay assay, e.g., as shown in Example 2, or a substantially similar assay.

[0125] The present invention also includes antibodies or antigen-binding fragments thereof that neutralize or inhibit the infectivity of MERS-CoV for its host cells. In certain embodiments, the antibodies neutralize the infectivity of MERS-CoV-like pseudoparticles (MERSpp). In some embodiments, the antibodies inhibited more than 90% binding of MERS-CoV on human host cells in an optimized virus-like pseudo-particle (VLP) neutralization assay, e.g., as shown in Example 5, or a substantially similar assay. The antibodies neutralized the MERSpp infectivity with IC50 ranging from 58.9pM to 2.93nM.

[0126] In some embodiments, the antibodies of the present invention bind to the receptor binding domain of MERS-CoV spike protein or to a fragment of the domain. In some embodiments, the antibodies of the present invention may bind to more than one domain (cross-reactive antibodies). In certain embodiments, the antibodies of the present invention may bind to an epitope located in the receptor binding domain comprising amino acid residues 367 – 606 of MERS-CoV-S. In one embodiment, the antibodies may bind to an epitope comprising one or more amino acids selected from the group consisting of amino acid residues 367 – 606 of the sequence set forth in SEQ ID NO: 457.

[0127] In certain embodiments, the antibodies of the present invention may function by blocking or inhibiting the DPP4-binding activity associated with MERS-CoV spike protein by binding to any other region or fragment of the full length protein, the amino acid sequence of which is set forth in SEQ ID NO: 457.

[0128] In certain embodiments, the antibodies of the present invention may be bi-specific antibodies. The bi-specific antibodies of the invention may bind one epitope in one domain and may also bind a second epitope in the same or a different domain of MERS-CoV spike protein. In certain embodiments, the bi-specific antibodies of the invention may bind two different epitopes in the same domain.

[0129] In one embodiment, the invention provides an isolated recombinant antibody or antigen-binding fragment thereof that binds specifically to MERS-CoV spike protein, wherein the antibody or fragment thereof exhibits one or more of the following characteristics: (a) is a fully human monoclonal antibody; (b) interacts with one or more amino acid residues in the receptor binding domain of the spike protein selected from amino acid residues 367 to 606 of the sequence shown in SEQ ID NO: 457; (c) binds to MERS-CoV spike protein with a dissociation constant (K_D) of less than 18.5nM, as measured in a surface plasmon resonance assay; (d) blocks binding of MERS-CoV spike protein to dipeptidyl peptidase 4 by more than 90%, as measured in a blocking ELISA assay; (e) neutralizes MERS-CoV infectivity of human host cells by more than 90% and with an IC_{50} less than 4nM, as measured in a VLP neutralization assay; (f) neutralizes MERS-CoV infectivity wherein the MERS-CoV comprises an isolate of the virus selected from the group consisting of EMC/2012, Jordan-N3/2012, England-Qatar/2012, Al-Hasa_1_2013, Al-Hasa_2_2013, Al-Hasa_3_2013, Al-Hasa_4_2013, Al-Hasa_12, Al-Hasa_15,

Al-Hasa_16, Al-Hasa_17, Al-Hasa_18, Al-Hasa_19, Al-Hasa_21, Al-hasa_25, Bisha_1, Buraidah_1, England 1, Hafr-Al-batin_1, Hafr-Al-Batin_2, Hafr-Al-Batin_6,Jeddah_1, KFU-HKU 1, KFU-HKU 13, Munich, Qatar3, Qatar4, Riyadh_1, Riyadh_2, Riyadh_3, Riyadh_3, Riyadh_4, Riyadh_5, Riyadh_9, Riyadh_14, Taif_1, UAE, and Wadi-Ad-Dawasir; (g) blocks MERS-CoV replication *in vivo* in a subject infected with MERS-CoV; and (h) is a bi-specific antibody comprising a first binding specificity to a first epitope in the receptor binding domain of MERS-CoV spike protein and a second binding specificity to a second epitope in the receptor binding domain of MERS-CoV spike protein wherein the first and second epitopes are distinct and non-overlapping.

[0130] The antibodies of the present invention may possess one or more of the aforementioned biological characteristics, or any combinations thereof. Other biological characteristics of the antibodies of the present invention will be evident to a person of ordinary skill in the art from a review of the present disclosure including the working Examples herein.

Epitope Mapping and Related Technologies

[0131] The present invention includes anti-MERS-CoV-S antibodies which interact with one or more amino acids found within one or more domains of the MERS-CoV spike protein molecule including, the N-terminal S1 domain (amino acid residues 1-751) and C-terminal S2 domain (amino acid residues 752-1353). The epitope to which the antibodies bind may consist of a single contiguous sequence of 3 or more (*e.g.*, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more) amino acids located within any of the aforementioned domains of the MERS-CoV spike protein molecule (*e.g.* a linear epitope in a domain). Alternatively, the epitope may consist of a plurality of non-contiguous amino acids (or amino acid sequences) located within either or both of the aforementioned domains of the spike protein molecule (*e.g.* a conformational epitope).

[0132] Various techniques known to persons of ordinary skill in the art can be used to determine whether an antibody "interacts with one or more amino acids" within a polypeptide or protein. Exemplary techniques include, for example, routine cross-blocking assays, such as that described in Antibodies, Harlow and Lane (Cold Spring Harbor Press, Cold Spring Harbor, NY). Other methods include alanine scanning mutational analysis, peptide blot analysis (Reineke (2004) Methods Mol. Biol. 248: 443-63), peptide cleavage analysis crystallographic studies and NMR analysis. In addition, methods such as epitope excision, epitope extraction and chemical modification of antigens can be employed (Tomer (2000) Prot. Sci. 9: 487-496). Another method that can be used to identify the amino acids within a polypeptide with which an antibody interacts is hydrogen/deuterium exchange detected by mass spectrometry. In general terms, the hydrogen/deuterium exchange method involves deuterium-labeling the protein of interest, followed by binding the antibody to the deuterium-labeled protein. Next, the protein/antibody complex is transferred to water and exchangeable protons within amino acids that are protected by the antibody complex undergo deuterium-to-hydrogen back-exchange at a slower rate than

exchangeable protons within amino acids that are not part of the interface. As a result, amino acids that form part of the protein/antibody interface may retain deuterium and therefore exhibit relatively higher mass compared to amino acids not included in the interface. After dissociation of the antibody, the target protein is subjected to protease cleavage and mass spectrometry analysis, thereby revealing the deuterium-labeled residues which correspond to the specific amino acids with which the antibody interacts. *See, e.g.*, Ehring (1999) Analytical Biochemistry 267: 252-259; Engen and Smith (2001) Anal. Chem. 73: 256A-265A.

[0133] The term "epitope" refers to a site on an antigen to which B and/or T cells respond. B-cell epitopes can be formed both from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents, whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation.

[0134] Modification-Assisted Profiling (MAP), also known as Antigen Structure-based Antibody Profiling (ASAP) is a method that categorizes large numbers of monoclonal antibodies (mAbs) directed against the same antigen according to the similarities of the binding profile of each antibody to chemically or enzymatically modified antigen surfaces (see US 2004/0101920, herein specifically incorporated by reference in its entirety). Each category may reflect a unique epitope either distinctly different from or partially overlapping with epitope represented by another category. This technology allows rapid filtering of genetically identical antibodies, such that characterization can be focused on genetically distinct antibodies. When applied to hybridoma screening, MAP may facilitate identification of rare hybridoma clones that produce mAbs having the desired characteristics. MAP may be used to sort the antibodies of the invention into groups of antibodies binding different epitopes.

[0135] In certain embodiments, the anti-MERS-CoV-S antibodies or antigen-binding fragments thereof bind an epitope within any one or more of the regions exemplified in MERS-CoV spike protein, either in natural form, as exemplified in SEQ ID NO: 457, or recombinantly produced, as exemplified in SEQ ID NO: 458, or to a fragment thereof. In some embodiments, the antibodies of the invention bind to an extracellular region comprising one or more amino acids selected from the group consisting of amino acid residues 367 – 606 of MERS-CoV spike protein.

[0136] In certain embodiments, the antibodies of the invention, interact with at least one amino acid sequence selected from the group consisting of amino acid residues ranging from about position 358 to about position 450; or amino acid residues ranging from about position 451 to about position 606 of SEQ ID NO: 457.

[0137] The present invention includes anti-MERS-CoV-S antibodies that bind to the same epitope, or a portion of the epitope, as any of the specific exemplary antibodies obtained from the cell lines listed in Figure 1. Likewise, the present invention also includes anti-MERS-CoV-S antibodies that compete for binding to MERS-CoV spike protein or a fragment thereof with any of the specific exemplary antibodies obtained from the hybridomas listed in Figure 1. For

example, the present invention includes anti-MERS-CoV-S antibodies that cross-compete for binding to MERS-CoV spike protein with one or more antibodies obtained from the hybridomas listed in Figure 1.

[0138] One can easily determine whether an antibody binds to the same epitope as, or competes for binding with, a reference anti-MERS-CoV-S antibody by using routine methods known in the art. For example, to determine if a test antibody binds to the same epitope as a reference anti-MERS-CoV-S antibody of the invention, the reference antibody is allowed to bind to a MERS-CoV spike protein or peptide under saturating conditions. Next, the ability of a test antibody to bind to the MERS-CoV spike protein molecule is assessed. If the test antibody is able to bind to MERS-CoV-S following saturation binding with the reference anti-MERS-CoV-S antibody, it can be concluded that the test antibody binds to a different epitope than the reference anti-MERS-CoV-S antibody. On the other hand, if the test antibody is not able to bind to the MERS-CoV spike protein following saturation binding with the reference anti-MERS-CoV-S antibody, then the test antibody may bind to the same epitope as the epitope bound by the reference anti-MERS-CoV-S antibody of the invention.

[0139] To determine if an antibody competes for binding with a reference anti-MERS-CoV-S antibody, the above-described binding methodology is performed in two orientations: In a first orientation, the reference antibody is allowed to bind to a MERS-CoV spike protein under saturating conditions followed by assessment of binding of the test antibody to the MERS-CoV-S molecule. In a second orientation, the test antibody is allowed to bind to a MERS-CoV-S molecule under saturating conditions followed by assessment of binding of the reference antibody to the MERS-CoV-S molecule. If, in both orientations, only the first (saturating) antibody is capable of binding to the MERS-CoV-S molecule, then it is concluded that the test antibody and the reference antibody compete for binding to MERS-CoV-S. As will be appreciated by a person of ordinary skill in the art, an antibody that competes for binding with a reference antibody may not necessarily bind to the identical epitope as the reference antibody, but may sterically block binding of the reference antibody by binding an overlapping or adjacent epitope.

[0140] Two antibodies bind to the same or overlapping epitope if each competitively inhibits (blocks) binding of the other to the antigen. That is, a 1-, 5-, 10-, 20- or 100-fold excess of one antibody inhibits binding of the other by at least 50% but preferably 75%, 90% or even 99% as measured in a competitive binding assay (see, *e.g.*, Junghans *et al.*, Cancer Res. 1990 50:1495-1502). Alternatively, two antibodies have the same epitope if essentially all amino acid mutations in the antigen that reduce or eliminate binding of one antibody reduce or eliminate binding of the other. Two antibodies have overlapping epitopes if some amino acid mutations that reduce or eliminate binding of one antibody reduce or eliminate binding of the other.

[0141] Additional routine experimentation (*e.g.*, peptide mutation and binding analyses) can then be carried out to confirm whether the observed lack of binding of the test antibody is in fact due to binding to the same epitope as the reference antibody or if steric blocking (or another

phenomenon) is responsible for the lack of observed binding. Experiments of this sort can be performed using ELISA, RIA, surface plasmon resonance, flow cytometry or any other quantitative or qualitative antibody-binding assay available in the art.

Immunoconjugates

[0142] The invention encompasses a human anti-MERS-CoV-S monoclonal antibody conjugated to a therapeutic moiety ("immunoconjugate"), such as a toxoid or an anti-viral drug to treat MERS infection. As used herein, the term "immunoconjugate" refers to an antibody which is chemically or biologically linked to a radioactive agent, a cytokine, an interferon, a target or reporter moiety, an enzyme, a peptide or protein or a therapeutic agent. The antibody may be linked to the radioactive agent, cytokine, interferon, target or reporter moiety, enzyme, peptide or therapeutic agent at any location along the molecule so long as it is able to bind its target. Examples of immunoconjugates include antibody drug conjugates and antibody-toxin fusion proteins. In one embodiment, the agent may be a second different antibody to MERS-CoV spike protein. In certain embodiments, the antibody may be conjugated to an agent specific for a virally infected cell. The type of therapeutic moiety that may be conjugated to the anti-MERS-CoV-S antibody and will take into account the condition to be treated and the desired therapeutic effect to be achieved. Examples of suitable agents for forming immunoconjugates are known in the art; see for example, WO 05/103081.

Multi-specific Antibodies

[0143] The antibodies of the present invention may be mono-specific, bi-specific, or multi-specific. Multi-specific antibodies may be specific for different epitopes of one target polypeptide or may contain antigen-binding domains specific for more than one target polypeptide. See, *e.g.*, Tutt et al., 1991, J. Immunol. 147:60-69; Kufer et al., 2004, Trends Biotechnol. 22:238-244.

[0144] Any of the multi-specific antigen-binding molecules of the invention, or variants thereof, may be constructed using standard molecular biological techniques (e.g., recombinant DNA and protein expression technology), as will be known to a person of ordinary skill in the art.

[0145] In some embodiments, MERS-CoV-S-specific antibodies are generated in a bi-specific format (a "bi-specific") in which variable regions binding to distinct domains of MERS-CoV spike protein are linked together to confer dual-domain specificity within a single binding molecule. Appropriately designed bi-specifics may enhance overall MERS-CoV-spike-protein inhibitory efficacy through increasing both specificity and binding avidity. Variable regions with specificity for individual domains, (*e.g.*, segments of the N-terminal domain), or that can bind to different regions within one domain, are paired on a structural scaffold that allows each region to bind simultaneously to the separate epitopes, or to different regions within one domain. In one example for a bi-specific, heavy chain variable regions (V_H) from a binder with specificity for one domain are recombined with light chain variable regions (V_L) from a series of binders with specificity for a second domain to identify non-cognate V_L partners that can be paired with an

original V_H without disrupting the original specificity for that V_H. In this way, a single V_L segment (*e.g.,* V_L1) can be combined with two different V_H domains (*e.g.,* V_H1 and V_H2) to generate a bi-specific comprised of two binding "arms" (V_H1- V_L1 and V_H2- V_L1). Use of a single V_L segment reduces the complexity of the system and thereby simplifies and increases efficiency in cloning, expression, and purification processes used to generate the bi-specific (See, for example, USSN13/022759 and US2010/0331527).

[0146] Alternatively, antibodies that bind more than one domains and a second target, such as, but not limited to, for example, a second different anti-MERS-CoV-S antibody, may be prepared in a bi-specific format using techniques described herein, or other techniques known to those skilled in the art. Antibody variable regions binding to distinct regions may be linked together with variable regions that bind to relevant sites on, for example, the extracellular domain of MERS-CoV-S, to confer dual-antigen specificity within a single binding molecule. Appropriately designed bi-specifics of this nature serve a dual function. Variable regions with specificity for the extracellular domain are combined with a variable region with specificity for outside the extracellular domain and are paired on a structural scaffold that allows each variable region to bind to the separate antigens.

[0147] An exemplary bi-specific antibody format that can be used in the context of the present invention involves the use of a first immunoglobulin (Ig) C_H3 domain and a second Ig C_H3 domain, wherein the first and second Ig C_H3 domains differ from one another by at least one amino acid, and wherein at least one amino acid difference reduces binding of the bi-specific antibody to Protein A as compared to a bi-specific antibody lacking the amino acid difference. In one embodiment, the first Ig C_H3 domain binds Protein A and the second Ig C_H3 domain contains a mutation that reduces or abolishes Protein A binding such as an H95R modification (by IMGT exon numbering; H435R by EU numbering). The second C_H3 may further comprise a Y96F modification (by IMGT; Y436F by EU). Further modifications that may be found within the second C_H3 include: D16E, L18M, N44S, K52N, V57M, and V82I (by IMGT; D356E, L358M, N384S, K392N, V397M, and V422I by EU) in the case of IgG1 antibodies; N44S, K52N, and V82I (IMGT; N384S, K392N, and V422I by EU) in the case of IgG2 antibodies; and Q15R, N44S, K52N, V57M, R69K, E79Q, and V82I (by IMGT; Q355R, N384S, K392N, V397M, R409K, E419Q, and V422I by EU) in the case of IgG4 antibodies. Variations on the bi-specific antibody format described above are contemplated within the scope of the present invention.

[0148] Other exemplary bispecific formats that can be used in the context of the present invention include, without limitation, *e.g.*, scFv-based or diabody bispecific formats, IgG-scFv fusions, dual variable domain (DVD)-Ig, Quadroma, knobs-into-holes, common light chain (*e.g.*, common light chain with knobs-into-holes, etc.), CrossMab, CrossFab, (SEED)body, leucine zipper, Duobody, IgG1/IgG2, dual acting Fab (DAF)-IgG, and Mab^2 bispecific formats (*see, e.g.*, Klein *et al.* 2012, mAbs 4:6, 1-11, and references cited therein, for a review of the foregoing formats). Bispecific antibodies can also be constructed using peptide/nucleic acid conjugation, *e.g.*, wherein unnatural amino acids with orthogonal chemical reactivity are used to generate

site-specific antibody-oligonucleotide conjugates which then self-assemble into multimeric complexes with defined composition, valency and geometry. (*See*, *e.g.*, Kazane *et al.*, *J. Am. Chem. Soc.* [*Epub*: *Dec. 4, 2012*]).

Therapeutic Administration and Formulations

[0149] The invention provides therapeutic compositions comprising the anti-MERS-CoV-S antibodies or antigen-binding fragments thereof of the present invention. Therapeutic compositions in accordance with the invention will be administered with suitable carriers, excipients, and other agents that are incorporated into formulations to provide improved transfer, delivery, tolerance, and the like. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as LIPOFECTIN™), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. See also Powell *et al.* "Compendium of excipients for parenteral formulations" PDA (1998) J Pharm Sci Technol 52:238-311.

[0150] The dose of antibody may vary depending upon the age and the size of a subject to be administered, target disease, conditions, route of administration, and the like. When an antibody of the present invention is used for treating a disease or disorder in an adult patient, or for preventing such a disease, it is advantageous to administer the antibody of the present invention normally at a single dose of about 0.1 to about 60 mg/kg body weight, more preferably about 5 to about 60, about 10 to about 50, or about 20 to about 50 mg/kg body weight. Depending on the severity of the condition, the frequency and the duration of the treatment can be adjusted. In certain embodiments, the antibody or antigen-binding fragment thereof of the invention can be administered as an initial dose of at least about 0.1 mg to about 800 mg, about 1 to about 500 mg, about 5 to about 300 mg, or about 10 to about 200 mg, to about 100 mg, or to about 50 mg. In certain embodiments, the initial dose may be followed by administration of a second or a plurality of subsequent doses of the antibody or antigen-binding fragment thereof in an amount that can be approximately the same or less than that of the initial dose, wherein the subsequent doses are separated by at least 1 day to 3 days; at least one week, at least 2 weeks; at least 3 weeks; at least 4 weeks; at least 5 weeks; at least 6 weeks; at least 7 weeks; at least 8 weeks; at least 9 weeks; at least 10 weeks; at least 12 weeks; or at least 14 weeks.

[0151] Various delivery systems are known and can be used to administer the pharmaceutical composition of the invention, *e.g.,* encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the mutant viruses, receptor mediated endocytosis (see, *e.g.*, Wu et al. (1987) J. Biol. Chem. 262:4429-4432). Methods of introduction include, but are not limited to, intradermal, transdermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural and oral routes. The composition may be administered by

any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.,* oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. The pharmaceutical composition can be also delivered in a vesicle, in particular a liposome (see, for example, Langer (1990) Science 249:1527-1533).

[0152] The use of nanoparticles to deliver the antibodies of the present invention is also contemplated herein. Antibody-conjugated nanoparticles may be used both for therapeutic and diagnostic applications. Antibody-conjugated nanoparticles and methods of preparation and use are described in detail by Arruebo, M., et al. 2009 ("Antibody-conjugated nanoparticles for biomedical applications" in J. Nanomat. Volume 2009, Article ID 439389, 24 pages, doi: 10.1155/2009/439389), incorporated herein by reference. Nanoparticles may be developed and conjugated to antibodies contained in pharmaceutical compositions to target virally infected cells. Nanoparticles for drug delivery have also been described in, for example, US 8257740, or US 8246995, each incorporated herein in its entirety.

[0153] In certain situations, the pharmaceutical composition can be delivered in a controlled release system. In one embodiment, a pump may be used. In another embodiment, polymeric materials can be used. In yet another embodiment, a controlled release system can be placed in proximity of the composition's target, thus requiring only a fraction of the systemic dose.

[0154] The injectable preparations may include dosage forms for intravenous, subcutaneous, intracutaneous, intracranial, intraperitoneal and intramuscular injections, drip infusions, etc. These injectable preparations may be prepared by methods publicly known. For example, the injectable preparations may be prepared, *e.g.*, by dissolving, suspending or emulsifying the antibody or its salt described above in a sterile aqueous medium or an oily medium conventionally used for injections. As the aqueous medium for injections, there are, for example, physiological saline, an isotonic solution containing glucose and other auxiliary agents, etc., which may be used in combination with an appropriate solubilizing agent such as an alcohol (*e.g.*, ethanol), a polyalcohol (e.g., propylene glycol, polyethylene glycol), a nonionic surfactant [e.g., polysorbate 80, HCO-50 (polyoxyethylene (50 mol) adduct of hydrogenated castor oil)], etc. As the oily medium, there are employed, *e.g.*, sesame oil, soybean oil, etc., which may be used in combination with a solubilizing agent such as benzyl benzoate, benzyl alcohol, etc. The injection thus prepared is preferably filled in an appropriate ampoule.

[0155] A pharmaceutical composition of the present invention can be delivered subcutaneously or intravenously with a standard needle and syringe. In addition, with respect to subcutaneous delivery, a pen delivery device readily has applications in delivering a pharmaceutical composition of the present invention. Such a pen delivery device can be reusable or disposable. A reusable pen delivery device generally utilizes a replaceable cartridge that contains a pharmaceutical composition. Once all of the pharmaceutical composition within the cartridge has been administered and the cartridge is empty, the empty cartridge can readily be discarded and replaced with a new cartridge that contains the pharmaceutical composition. The pen delivery

device can then be reused. In a disposable pen delivery device, there is no replaceable cartridge. Rather, the disposable pen delivery device comes prefilled with the pharmaceutical composition held in a reservoir within the device. Once the reservoir is emptied of the pharmaceutical composition, the entire device is discarded.

[0156] Numerous reusable pen and autoinjector delivery devices have applications in the subcutaneous delivery of a pharmaceutical composition of the present invention. Examples include, but certainly are not limited to AUTOPEN™ (Owen Mumford, Inc., Woodstock, UK), DISETRONIC™ pen (Disetronic Medical Systems, Burghdorf, Switzerland), HUMALOG MIX 75/25™ pen, HUMALOG™ pen, HUMALIN 70/30™ pen (Eli Lilly and Co., Indianapolis, IN), NOVOPEN™ I, II and III (Novo Nordisk, Copenhagen, Denmark), NOVOPEN JUNIOR™ (Novo Nordisk, Copenhagen, Denmark), BD™ pen (Becton Dickinson, Franklin Lakes, NJ), OPTIPEN™, OPTIPEN PRO™, OPTIPEN STARLET™, and OPTICLIK™ (Sanofi-Aventis, Frankfurt, Germany), to name only a few. Examples of disposable pen delivery devices having applications in subcutaneous delivery of a pharmaceutical composition of the present invention include, but certainly are not limited to the SOLOSTAR™ pen (Sanofi-Aventis), the FLEXPEN™ (Novo Nordisk), and the KWIKPEN™ (Eli Lilly), the SURECLICK ™ Autoinjector (Amgen, Thousand Oaks, CA), the PENLET ™ (Haselmeier, Stuttgart, Germany), the EPIPEN (Dey, L.P.) and the HUMIRA ™ Pen (Abbott Labs, Abbott Park, IL), to name only a few.

[0157] Advantageously, the pharmaceutical compositions for oral or parenteral use described above are prepared into dosage forms in a unit dose suited to fit a dose of the active ingredients. Such dosage forms in a unit dose include, for example, tablets, pills, capsules, injections (ampoules), suppositories, etc. The amount of the antibody contained is generally about 5 to about 500 mg per dosage form in a unit dose; especially in the form of injection, it is preferred that the antibody is contained in about 5 to about 100 mg and in about 10 to about 250 mg for the other dosage forms.

Therapeutic Uses of the Antibodies

[0158] The antibodies of the present invention are useful for the treatment, and/or prevention of a disease or disorder or condition associated with MERS-coronavirus such as MERS infection and/or for ameliorating at least one symptom associated with such disease, disorder or condition. In one embodiment, an antibody or antigen-binding fragment thereof the invention may be administered at a therapeutic dose to a patient with MERS infection.

[0159] In certain embodiments, the antibodies of the invention are useful to treat subjects suffering from the severe and acute respiratory infection caused by MERS-coronavirus. In some embodiments, the antibodies of the invention are useful in decreasing viral titer or reducing viral load in the host. In one embodiment, the antibodies of the present invention are useful in preventing or reducing inflammation in the lung of a subject with MERS. In one embodiment, the antibodies of the present invention are useful in preventing or reducing interstitial, peribronchiolar or perivascular inflammation, alveolar damage and pleural changes in a subject

with MERS.

[0160] One or more antibodies of the present invention may be administered to relieve or prevent or decrease the severity of one or more of the symptoms or conditions of the disease or disorder. The antibodies may be used to ameliorate or reduce the severity of at least one symptom of MERS infection including, but not limited to consisting of fever, cough, shortness of breath, pneumonia, diarrhea, organ failure (e.g., kidney failure and renal dysfunction), septic shock and death.

[0161] It is also contemplated herein to use one or more antibodies of the present invention prophylactically to subjects at risk for developing MERS infection such as immunocompromised individuals, elderly adults (more than 65 years of age), children younger than 2 years of age, travelers to countries in the Middle East (e.g., Saudi Arabia, United Arab Emirates, and Qatar), healthcare workers, persons with occupational or recreational contact with camels or bats, family members in close proximity to a MERS patient, adults or children with contact with persons with confirmed or suspected MERS infection, and patients with a medical history (e.g., increased risk of pulmonary infection, heart disease or diabetes).

[0162] In a further embodiment of the invention the present antibodies are used for the preparation of a pharmaceutical composition or medicament for treating patients suffering from MERS infection. In another embodiment of the invention, the present antibodies are used as adjunct therapy with any other agent or any other therapy known to those skilled in the art useful for treating or ameliorating MERS infection.

Combination Therapies

[0163] Combination therapies may include an anti-MERS-CoV-S antibody of the invention and any additional therapeutic agent that may be advantageously combined with an antibody of the invention, or with a biologically active fragment of an antibody of the invention. The antibodies of the present invention may be combined synergistically with one or more drugs or therapy used to treat MERS. In some embodiments, the antibodies of the invention may be combined with a second therapeutic agent to reduce the viral load in a patient with MERS infection, or to ameliorate one or more symptoms of the infection.

[0164] The antibodies of the present invention may be used in combination with an anti-inflammatory drug (*e.g.*, corticosteroids, and non-steroidal anti-inflammatory drugs), an anti-infective drug, a different antibody to MERS-CoV spike protein, an anti-viral drug, interferon-alpha-2b plus intramuscular ribavirin, convalescent plasma, an inhibitor of the main viral protease, and entry/fusion inhibitors targeting the MERS-CoV spike protein, a vaccine for MERS-CoV, antibiotics, a dietary supplement such as anti-oxidants or any other palliative therapy to treat MERS infection.

[0165] In certain embodiments, the second therapeutic agent is another antibody to MERS-CoV spike protein. It is contemplated herein to use a combination (“cocktail”) of antibodies with broad neutralization or inhibitory activity against MERS-CoV. In some embodiments, non-competing

antibodies may be combined and administered to a subject in need thereof, to reduce the ability of MERS virus to escape due to rapid mutation as a result of selection pressure. In some embodiments, the antibodies comprising the combination bind to distinct non-overlapping epitopes on the spike protein. The antibodies comprising the combination may block the MERS-CoV binding to DPP4 or may prevent/inhibit membrane fusion. The antibodies comprising the combination may inhibit MERS-CoV activity of one or more MERS-CoV isolates including, but not limited to, EMC/2012, Jordan-N3/2012, England-Qatar/2012, Al-Hasa_1_2013, Al-Hasa_2_2013, Al-Hasa_3_2013, Al-Hasa_4_2013, Al-Hasa_12, Al-Hasa_15, Al-Hasa_16, Al-Hasa_17, Al-Hasa_18, Al-Hasa_19, Al-Hasa_21, Al-hasa_25, Bisha_1, Buraidah_1, England 1, Hafr-Al-batin_1, Hafr-Al-Batin_2, Hafr-Al-Batin_6, Jeddah_1, KFU-HKU 1, KFU-HKU 13, Munich, Qatar3, Qatar4, Riyadh_1, Riyadh_2, Riyadh_3, Riyadh_3, Riyadh_4, Riyadh_5, Riyadh_9, Riyadh_14, Taif_1, UAE, and Wadi-Ad-Dawasir.

[0166] It is also contemplated herein to use a combination of anti-MERS-CoV-S antibodies of the present invention, wherein the combination comprises one or more antibodies that do not cross-compete; In some embodiments, the combination includes a first antibody with broad neutralization activity with a second antibody with activity against a narrow spectrum of isolates and that does not cross-compete with the first antibody.

[0167] As used herein, the term “in combination with” means that additional therapeutically active component(s) may be administered prior to, concurrent with, or after the administration of the anti-MERS-CoV-S antibody of the present invention. The term “in combination with” also includes sequential or concomitant administration of an anti-MERS-CoV-S antibody and a second therapeutic agent.

[0168] The additional therapeutically active component(s) may be administered to a subject prior to administration of an anti-MERS-CoV-S antibody of the present invention. For example, a first component may be deemed to be administered "prior to" a second component if the first component is administered 1 week before, 72 hours before, 60 hours before, 48 hours before, 36 hours before, 24 hours before, 12 hours before, 6 hours before, 5 hours before, 4 hours before, 3 hours before, 2 hours before, 1 hour before, 30 minutes before, 15 minutes before, 10 minutes before, 5 minutes before, or less than 1 minute before administration of the second component. In other embodiments, the additional therapeutically active component(s) may be administered to a subject after administration of an anti-MERS-CoV-S antibody of the present invention. For example, a first component may be deemed to be administered "after" a second component if the first component is administered 1 minute after, 5 minutes after, 10 minutes after, 15 minutes after, 30 minutes after, 1 hour after, 2 hours after, 3 hours after, 4 hours after, 5 hours after, 6 hours after, 12 hours after, 24 hours after, 36 hours after, 48 hours after, 60 hours after, 72 hours after administration of the second component. In yet other embodiments, the additional therapeutically active component(s) may be administered to a subject concurrent with administration of an anti-MERS-CoV-S antibody of the present invention. "Concurrent" administration, for purposes of the present invention, includes, *e.g.*, administration of an anti-

MERS-CoV-S antibody and an additional therapeutically active component to a subject in a single dosage form, or in separate dosage forms administered to the subject within about 30 minutes or less of each other. If administered in separate dosage forms, each dosage form may be administered via the same route (*e.g.*, both the anti-MERS-CoV-S antibody and the additional therapeutically active component may be administered intravenously, etc.); alternatively, each dosage form may be administered via a different route (*e.g.*, the anti-MERS-CoV-S antibody may be administered intravenously, and the additional therapeutically active component may be administered orally). In any event, administering the components in a single dosage from, in separate dosage forms by the same route, or in separate dosage forms by different routes are all considered "concurrent administration," for purposes of the present disclosure. For purposes of the present disclosure, administration of an anti-MERS-CoV-S antibody "prior to", "concurrent with," or "after" (as those terms are defined herein above) administration of an additional therapeutically active component is considered administration of an anti-MERS-CoV-S antibody "in combination with" an additional therapeutically active component.

[0169] The present invention includes pharmaceutical compositions in which an anti-MERS-CoV-S antibody of the present invention is co-formulated with one or more of the additional therapeutically active component(s) as described elsewhere herein.

Administration Regimens

[0170] According to certain embodiments, a single dose of an anti-MERS-CoV-S antibody of the invention (or a pharmaceutical composition comprising a combination of an anti-MERS-CoV-S antibody and any of the additional therapeutically active agents mentioned herein) may be administered to a subject in need thereof. According to certain embodiments of the present invention, multiple doses of an anti-MERS-CoV-S antibody (or a pharmaceutical composition comprising a combination of an anti-MERS-CoV-S antibody and any of the additional therapeutically active agents mentioned herein) may be administered to a subject over a defined time course. The methods according to this aspect of the invention comprise sequentially administering to a subject multiple doses of an anti-MERS-CoV-S antibody of the invention. As used herein, "sequentially administering" means that each dose of anti-MERS-CoV-S antibody is administered to the subject at a different point in time, *e.g.*, on different days separated by a predetermined interval (*e.g.*, hours, days, weeks or months). The present invention includes methods which comprise sequentially administering to the patient a single initial dose of an anti-MERS-CoV-S antibody, followed by one or more secondary doses of the anti-MERS-CoV-S antibody, and optionally followed by one or more tertiary doses of the anti-MERS-CoV-S antibody.

[0171] The terms "initial dose," "secondary doses," and "tertiary doses," refer to the temporal sequence of administration of the anti-MERS-CoV-S antibody of the invention. Thus, the "initial dose" is the dose which is administered at the beginning of the treatment regimen (also referred

to as the "baseline dose"); the "secondary doses" are the doses which are administered after the initial dose; and the "tertiary doses" are the doses which are administered after the secondary doses. The initial, secondary, and tertiary doses may all contain the same amount of anti-MERS-CoV-S antibody, but generally may differ from one another in terms of frequency of administration. In certain embodiments, however, the amount of anti-MERS-CoV-S antibody contained in the initial, secondary and/or tertiary doses varies from one another (*e.g.*, adjusted up or down as appropriate) during the course of treatment. In certain embodiments, two or more (*e.g.*, 2, 3, 4, or 5) doses are administered at the beginning of the treatment regimen as "loading doses" followed by subsequent doses that are administered on a less frequent basis (*e.g.*, "maintenance doses").

[0172] In certain exemplary embodiments of the present invention, each secondary and/or tertiary dose is administered 1 to 48 hours (*e.g.*, 1, 1½, 2, 2½, 3, 3½, 4, 4½, 5, 5½, 6, 6½, 7, 7½, 8, 8½, 9, 9½, 10, 10½, 11, 11½, 12, 12½, 13, 13½, 14, 14½, 15, 15½, 16, 16½, 17, 17½, 18, 18½, 19, 19½, 20, 20½, 21, 21½, 22, 22½, 23, 23½, 24, 24½, 25, 25½, 26, 26½, or more) after the immediately preceding dose. The phrase "the immediately preceding dose," as used herein, means, in a sequence of multiple administrations, the dose of anti-MERS-CoV-S antibody which is administered to a patient prior to the administration of the very next dose in the sequence with no intervening doses.

[0173] The methods according to this aspect of the invention may comprise administering to a patient any number of secondary and/or tertiary doses of an anti-MERS-CoV-S antibody. For example, in certain embodiments, only a single secondary dose is administered to the patient. In other embodiments, two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, or more) secondary doses are administered to the patient. Likewise, in certain embodiments, only a single tertiary dose is administered to the patient. In other embodiments, two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, or more) tertiary doses are administered to the patient.

[0174] In certain embodiments of the invention, the frequency at which the secondary and/or tertiary doses are administered to a patient can vary over the course of the treatment regimen. The frequency of administration may also be adjusted during the course of treatment by a physician depending on the needs of the individual patient following clinical examination.

Diagnostic Uses of the Antibodies

[0175] The anti-MERS-CoV-S antibodies of the present invention may be used to detect and/or measure MERS-CoV in a sample, *e.g.*, for diagnostic purposes. Some embodiments contemplate the use of one or more antibodies of the present invention in assays to detect a disease or disorder such as viral infection. Exemplary diagnostic assays for MERS-CoV may comprise, *e.g.*, contacting a sample, obtained from a patient, with an anti-MERS-CoV-S antibody of the invention, wherein the anti-MERS-CoV-S antibody is labeled with a detectable label or reporter molecule or used as a capture ligand to selectively isolate MERS-CoV from patient samples. Alternatively, an unlabeled anti-MERS-CoV-S antibody can be used in

diagnostic applications in combination with a secondary antibody which is itself detectably labeled. The detectable label or reporter molecule can be a radioisotope, such as ^{3}H, ^{14}C, ^{32}P, ^{35}S, or ^{125}I; a fluorescent or chemiluminescent moiety such as fluorescein isothiocyanate, or rhodamine; or an enzyme such as alkaline phosphatase, β-galactosidase, horseradish peroxidase, or luciferase. Specific exemplary assays that can be used to detect or measure MERS-CoV in a sample include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence-activated cell sorting (FACS).

[0176] Samples that can be used in MERS-CoV diagnostic assays according to the present invention include any tissue or fluid sample obtainable from a patient, which contains detectable quantities of either MERS-CoV spike protein, or fragments thereof, under normal or pathological conditions. Generally, levels of MERS-CoV spike protein in a particular sample obtained from a healthy patient (*e.g.*, a patient not afflicted with a disease associated with MERS-CoV) will be measured to initially establish a baseline, or standard, level of MERS-CoV. This baseline level of MERS-CoV can then be compared against the levels of MERS-CoV measured in samples obtained from individuals suspected of having a MERS-CoV-associated condition, or symptoms associated with such condition.

[0177] The antibodies specific for MERS-CoV spike protein may contain no additional labels or moieties, or they may contain an N-terminal or C-terminal label or moiety. In one embodiment, the label or moiety is biotin. In a binding assay, the location of a label (if any) may determine the orientation of the peptide relative to the surface upon which the peptide is bound. For example, if a surface is coated with avidin, a peptide containing an N-terminal biotin will be oriented such that the C-terminal portion of the peptide will be distal to the surface.

EXAMPLES

[0178] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the methods and compositions of the invention, and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees Centigrade, room temperature is about 25°C, and pressure is at or near atmospheric.

Example 1: Generation of Human Antibodies to MERS-CoV spike protein

[0179] Human antibodies to MERS-CoV spike protein were generated in a VELOCIMMUNE® mouse comprising DNA encoding human Immunoglobulin heavy and kappa light chain variable regions. The mice were immunized with a DNA construct encoding the full-length MERS-CoV spike protein followed by a booster dose comprising a purified fragment of the spike protein that ranges from amino acids 367 – 606 of GenBank Accession AFS88936.1 (SEQ ID NO: 457).

Codon optimized cDNA sequence encoding for MERS-CoV S protein (EMC/2012 – GenBank JX869059) was synthesized by GeneArt and cloned into standard expression vectors. Plasmids encoding MERS-CoV S protein variants in the Receptor Binding Domain (RBD) were generated by mutagenesis using QuikChange II (Agilent Technologies) according to the manufacturer's instructions.

[0180] The antibody immune response was monitored by a MERS-CoV-S-specific immunoassay. When a desired immune response was achieved splenocytes were harvested and fused with mouse myeloma cells to preserve their viability and form hybridoma cell lines. The hybridoma cell lines were screened and selected to identify cell lines that produce MERS-CoV-S-specific antibodies. Of a total of 696 hybridomas that express antigen-specific antibodies, 182 were found to block the interaction between DPP4 and spike protein and 123 blocked entry of MERSpp into target cells. Exemplary cell lines generated in this manner were designated as HBVX06H05, HBVX11H04, HBVX11D02, HBVZ10E10, HBVY09F08, HBVZ05G02, HBVZ09B06, HBVY01F08, HBVY10G02, HBVY04B06, HBVY07D10, HBVZ08A09, HBVZ05G04, HBVY06C07, HBVY03H06, HBVZ10G06, HBVZ04F10, HBVX11E09, HBVY06H09, HBVZ05B11, HBVY02E05 and HBVZ04C07. The cell lines were used to obtain several anti-MERS-CoV-S chimeric antibodies (*i.e.*, antibodies possessing human variable domains and mouse constant domains); exemplary antibodies generated in this manner were designated as H1M15277N, H2aM15279N, H1M15280N, H2aM15278N, H2aM15271N, H1M15267N, H2bM15292N, H2bM15291N, H1M15290N, H1M15293N, H1M15289N, H1M15269N, H2aM15287N, H1M15288N, H2aM15268N, H2aM15270N, H2aM15281N, and H2aM15272N.

[0181] Table 1 shows selected chimeric antibodies obtained from the corresponding hybridoma supernatants.

Table 1

Ab PID #	Clone Name
H1M15267N	HBVX11H04
H1M15269N	HBVY07D10
H1M15277N	HBVX06H05
H1M15280N	HBVZ09B06
H1M15288N	HBVY02E05
H1M15289N	HBVY03H06
H1M15290N	HBVZ04F10
H1M15293N	HBVZ10G06
H2aM15268N	HBVY04B06
H2aM15270N	HBVY09F08
H2aM15271N	HBVY10G02
H2aM15272N	HBVZ05G02
H2aM15278N	HBVX11D02
H2aM15279N	HBVY01F08
H2aM15281N	HBVZ10E10

H2aM15287N	HBVX11E09
H2bM15291N	HBVZ05G04
H2bM15292N	HBVZ08A09

[0182] Anti-MERS-CoV-S antibodies were also isolated directly from antigen-positive mouse B cells without fusion to myeloma cells, as described in U.S. Patent 7582298, herein specifically incorporated by reference in its entirety. Using this method, several fully human anti-MERS-CoV-S antibodies (*i.e.*, antibodies possessing human variable domains and human constant domains) were obtained; exemplary antibodies generated in this manner were designated as H4sH15188P, H1H15188P, H1H15211P, H1H15177P, H4sH15211P, H1H15260P2, H1H15259P2, H1H15203P, H4sH15260P2, H4sH15231P2, H1H15237P2, H1H15208P, H1H15228P2, H1H15233P2, H1H15264P2, H1H15231P2, H1H15253P2, H1H15215P, and H1H15249P2.

[0183] The biological properties of the exemplary antibodies generated in accordance with the methods of this Example are described in detail in the Examples set forth below.

Example 2: Characterization of hybridoma supernatants

[0184] An ELISA binding assay was performed to identify antibody supernatants (obtained from the hybridomas described above) that bound to the MERS-CoV spike protein. A protein composed of the receptor binding domain of MERS-CoV-S (aa E367-Y606) expressed with the Fc portion of the human IgG1 molecule at the C-terminus (MERS RBD-hFc; SEQ ID NO: 458) was coated at 2 μg/ml on a 96-well plate in PBS buffer overnight at 4°C. Nonspecific binding sites were subsequently blocked using a 0.5% (w/v) solution of BSA in PBS. Hybridoma supernatants were diluted 1:50 in the blocking buffer and allowed to bind to the MERS RBD coated plates for 1 hour at room temperature. After washing, bound antibodies were detected using an HRP conjugated a-hFc polyclonal antibody (Jackson Immunochemical). Samples were developed with a 3,3',5,5' - tetramethylbenzidine solution (TMB; BD Biosciences) to produce a colorimetric reaction and then neutralized with 1M sulfuric acid before measuring absorbance at 450nm on a Victor plate reader (Perkin Elmer). The results are shown in Figure 1. Intensity of signal at 450nm was used to select antibodies for further characterization.

[0185] Binding association and dissociation rate constants (k_a and k_d, respectively), equilibrium dissociation constants and dissociation half-lives (K_D and $t_{½}$, respectively) for antigen binding to hybridoma supernatants were determined using a real-time surface plasmon resonance biosensor assay on a Biacore 4000 or Biacore T200 instrument (details described in Example 4). As shown in Figure 1, 21 of 22 hybridoma antibodies bound to MERS-CoV spike protein with K_D values ranging from 245pM to 21.5nM.

[0186] The ability of anti-MERS coronavirus spike protein antibodies to block the receptor binding domain of MERS (MERS-RBD) binding to a cognate binding partner, human dipeptidyl peptidase 4 (hDPP4), was evaluated with an ELISA-based immunoassay. Briefly, human

dipeptidyl peptidase 4 expressed with a 6xHistidine tag at the c-terminus (hDPP4-6His; R&D cat#1180-SE) was coated at 2μg/mL on a 96-well plate in PBS buffer overnight at 4°C. Nonspecific binding sites were subsequently blocked using a 0.5% (w/v) solution of BSA in PBS. This plate was used to measure MERS-RBD (aa E367-Y606) expressed with the Fc portion of the human IgG1 molecule at the c-terminus (MERS RBD-hFc; SEQ ID NO: 458) in a MERS RBD-hFc solution pre-equilibrated with a dilution of anti-MERS antibody supernatants. A constant concentration of 300pM of MERS RBD-hFc was pre-mixed with a 10% volume of anti-MERS antibody supernatants, followed by an 1 hour incubation at room temperature (RT) to allow antibody-antigen binding to reach equilibrium. The equilibrated sample solutions were then transferred to hDPP4-6his-coated plates. After 1 hour of binding at RT, the plates were washed and bound MERS RBD was detected using HRP conjugated a-hFc polyclonal antibody (Jackson Immunochemical). Samples were developed with a TMB solution (BD Biosciences, #51-2606KC and #51-2607KC) to produce a colorimetric reaction and then neutralized with 1M sulfuric acid before measuring absorbance at 450nm on a Victor X5 plate reader. The absorbance measured for the constant concentration of MERS RBD-hFc alone is defined as 0% blocking and the absorbance measured for no added MERS RBD-hFc is defined as 100% blocking. Percent blockade was calculated as the ratio of the reduction in signal observed in the presence of antibody relative to the difference between the signal with MERS RBD-hFc alone and background (signal from HRP conjugated a-hFc antibody alone) subtracted from 100% blocking as defined previously.

[0187] As shown in Figure 1, 17 of 22 anti-MERS-CoV-S antibodies derived from separate independent hybridomas blocked more than 90% binding of MERS-CoV spike protein to DPP4.

[0188] The anti-MERS-CoV-S antibodies derived from separate independent hybridomas were tested for neutralization of MERS-infectivity (assay details in Example 5). As shown in Figure 1, 12 of 22 cell anti-MERS-CoV-S hybridoma antibodies inhibited or neutralized more than 90% of MERS-infectivity and with IC_{50} ranging from 68.4pM to 3.58nM.

Example 3: Heavy and Light Chain Variable Region Amino Acid and Nucleotide Sequences

[0189] Table 2 sets forth the amino acid sequence identifiers of the heavy and light chain variable regions and CDRs of selected anti-MERS-CoV-S antibodies of the invention.

Table 2: Amino Acid Sequence Identifiers

	SEQ ID NOs:							
Antibody Designation	**HCVR**	**HCDR1**	**HCDR2**	**HCDR3**	**LCVR**	**LCDR1**	**LCDR2**	**LCDR3**
H1H15177P	2	4	6	8	10	12	14	16
H1H15188P	18	20	22	24	26	28	30	32
H1H15203P	34	36	38	40	42	44	46	48

H1H15208P	50	52	54	56	58	60	62	64
H1H15211P	66	68	70	72	74	76	78	80
H1H15215P	82	84	86	88	90	92	94	96
H1H15228P2	98	100	102	104	106	108	110	112
H1H15231P2	114	116	118	120	106	108	110	112
H1H15233P2	122	124	126	128	106	108	110	112
H1H15237P2	130	132	134	136	106	108	110	112
H1H15249P2	138	140	142	144	106	108	110	112
H1H15253P2	146	148	150	152	106	108	110	112
H1H15259P2	154	156	158	160	162	164	166	168
H1H15260P2	170	172	174	176	162	164	166	168
H1H15264P2	178	180	182	184	162	164	166	168
H1M15267N2	186	188	190	192	194	196	198	200
H1M15269N	202	204	206	208	210	212	214	216
H1M15277N	218	220	222	224	226	228	230	232
H1M15280N	234	236	238	240	242	244	246	248
H1M15289N	250	252	254	256	258	260	262	264
H1M15290N	266	268	270	272	274	276	278	280
H1M15293N	282	284	286	288	290	292	294	296
H2M15268N	298	300	302	304	306	308	310	312
H2M15270N	314	316	318	320	322	324	326	328
H2M15271N	330	332	334	336	338	340	342	344
H2M15272N	346	348	350	352	354	356	358	360
H2M15278N	362	364	366	368	370	372	374	376
H2M15279N	378	380	382	384	386	388	390	392
H2M15281N	394	396	398	400	402	404	406	408
H2M15287N	410	412	414	416	418	420	422	424
H2M15291N	426	428	430	432	434	436	438	440
H2M15292N	442	444	446	448	450	452	454	456

[0190] The corresponding nucleic acid sequence identifiers are set forth in Table 3.

Table 3: Nucleic Acid Sequence Identifiers

	SEQ ID NOs:							
Antibody Designation	**HCVR**	**HCDR1**	**HCDR2**	**HCDR3**	**LCVR**	**LCDR1**	**LCDR2**	**LCDR3**
H1H15177P	1	3	5	7	9	11	13	15
H1H15188P	17	19	21	23	25	27	29	31

H1H15203P	33	35	37	39	41	43	45	47
H1H15208P	49	51	53	55	57	59	61	63
H1H15211P	65	67	69	71	73	75	77	79
H1H15215P	81	83	85	87	89	91	93	95
H1H15228P2	97	99	101	103	105	107	109	111
H1H15231P2	113	115	117	119	105	107	109	111
H1H15233P2	121	123	125	127	105	107	109	111
H1H15237P2	129	131	133	135	105	107	109	111
H1H15249P2	137	139	141	143	105	107	109	111
H1H15253P2	145	147	149	151	105	107	109	111
H1H15259P2	153	155	157	159	161	163	165	167
H1H15260P2	169	171	173	175	161	163	165	167
H1H15264P2	177	179	181	183	161	163	165	167
H1M15267N2	185	187	189	191	193	195	197	199
H1M15269N	201	203	205	207	209	211	213	215
H1M15277N	217	219	221	223	225	227	229	231
H1M15280N	233	235	237	239	241	243	245	247
H1M15289N	249	251	253	255	257	259	261	263
H1M15290N	265	267	269	271	273	275	277	279
H1M15293N	281	283	285	287	289	291	293	295
H2M15268N	297	299	301	303	305	307	309	311
H2M15270N	313	315	317	319	321	323	325	327
H2M15271N	329	331	333	335	337	339	341	343
H2M15272N	345	347	349	351	353	355	357	359
H2M15278N	361	363	365	367	369	371	373	375
H2M15279N	377	379	381	383	385	387	389	391
H2M15281N	393	395	397	399	401	403	405	407
H2M15287N	409	411	413	415	417	419	421	423
H2M15291N	425	427	429	431	433	435	437	439
H2M15292N	441	443	445	447	449	451	453	455

[0191] Antibodies are typically referred to herein according to the following nomenclature: Fc prefix (*e.g.* "H4xH," "H1M," "H2M," etc.), followed by a numerical identifier (*e.g.* "15177," "15228," "15268," etc., as shown in Table 2), followed by a "P," "P2," "N," or "B" suffix. Thus, according to this nomenclature, an antibody may be referred to herein as, *e.g.*, "H1H15177P," "H1H15228P2," "H2M15268N," etc. The H4sH, H1M, and H2M prefixes on the antibody designations used herein indicate the particular Fc region isotype of the antibody. For example, an "H4sH" antibody has a human IgG4 Fc with 2 or more amino acid changes as disclosed in

US20100331527, an "H1M" antibody has a mouse IgG1 Fc, and an "H2M" antibody has a mouse IgG2 Fc (a or b isotype) (all variable regions are fully human as denoted by the first 'H' in the antibody designation). As will be appreciated by a person of ordinary skill in the art, an antibody having a particular Fc isotype can be converted to an antibody with a different Fc isotype (*e.g.*, an antibody with a mouse IgG1 Fc can be converted to an antibody with a human IgG4, etc.), but in any event, the variable domains (including the CDRs) – which are indicated by the numerical identifiers shown in Table 2 – will remain the same, and the binding properties to antigen are expected to be identical or substantially similar regardless of the nature of the Fc domain.

Example 4: Antibody binding to MERS-CoV-S as determined by Surface Plasmon Resonance

[0192] Binding association and dissociation rate constants (k_a and k_d, respectively), equilibrium dissociation constants and dissociation half-lives (K_D and $t_{½}$, respectively) for antigen binding to purified anti-MERS-CoV-S antibodies or hybridoma supernatants were determined using a real-time surface plasmon resonance biosensor assay on a Biacore 4000 or Biacore T200 instrument. The Biacore sensor surface was derivatized with either a polyclonal rabbit anti-mouse antibody (GE, # BR-1008-38) or with a monoclonal mouse anti-human Fc antibody (GE, # BR-1008-39) to capture approximately 100-900 RUs of anti-MERS-CoV-S monoclonal antibodies, expressed with either a mouse Fc or a human Fc, respectively. The MERS-CoV reagents tested for binding to the anti-MERS-CoV-S antibodies included recombinant MERS-CoV-S receptor binding domain expressed with a C-terminal human IgG1 Fc (MERS RBD-hFc; SEQ ID NO: 458). Different concentrations of MERS-CoV reagent ranging from 3.7nM to 200nM were injected over the anti-MERS-CoV-S monoclonal antibody captured surface at a flow rate of 30µL/min on Biacore 4000 or at 50µL/min on Biacore T200. The binding of the MERS-CoV reagent to captured monoclonal antibodies was monitored for 3 to 5 minutes while their dissociation from the antibodies was monitored for 7 to 10 minutes in HBST running buffer (0.01 M HEPES pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.05% v/v Surfactant P20). Experiments were performed at 25°C. Kinetic association (k_a) and dissociation (k_d) rate constants were determined by processing and fitting the data to a 1:1 binding model using Scrubber 2.0c curve fitting software. Binding dissociation equilibrium constants (K_D) and dissociative half-lives ($t_{1/2}$) were then calculated from the kinetic rate constants as: K_D (M) = k_d / k_a and $t_{½}$ (min) = [ln2/(60*k_d)].

[0193] In an initial experiment, binding dissociation equilibrium constants and dissociative half-lives were determined for 22 hybridoma supernatants (described in Example 2).

[0194] In subsequent experiments, the purified antibodies were tested for binding to MERS-CoV spike protein using the surface plasmon resonance assay described above.

Table 4

	Binding Kinetics @ 25°C

mAb Captured	mAb Capture Level (RU)	100nM Ag Bound (RU)	ka (1/Ms)	kd (1/s)	KD (M)	t½ (min)
H2aM15281N	223 ± 0.8	127	2.10E+06	8.18E-04	3.90E-10	14
H1M15289N	224 ± 1.5	88	2.10E+05	4.41E-05	2.10E-10	262
H1M15267N	173 ± 0.7	109	8.43E+05	1.53E-04	1.81E-10	75
H2aM15272N	229 ± 1.1	120	1.65E+06	7.33E-04	4.43E-10	16
H2aM15279N	195 ± 0.7	127	8.70E+05	8.91E-05	1.02E-10	130
H2aM15278N	230 ± 1	118	1.72E+06	8.73E-04	5.06E-10	13
H2aM15268N	174 ± 0.6	121	2.68E+06	9.50E-04	3.55E-10	12
H2aM15270N	182 ± 0.7	107	7.79E+05	6.05E-04	7.76E-10	19
H1M15277N	211 ± 1	150	2.63E+06	9.10E-04	3.46E-10	13
H2bM15292N	185 ± 1.2	95	4.57E+05	1.55E-04	3.40E-10	75
H2aM15271N	227 ± 1.4	112	9.08E+05	1.24E-03	1.37E-09	9
H1M15269N	268 ± 2.8	83	1.76E+05	1.08E-04	6.16E-10	107
H1M15290N	210 ± 1.9	108	4.82E+05	2.11E-04	4.38E-10	55
H1M15293N	187 ± 0.9	91	6.04E+05	1.98E-04	3.28E-10	58
H2bM15291N	179 ± 1.8	83	2.52E+05	1.41E-04	5.59E-10	82
H1M15288N	149 ± 1	55	1.73E+05	7.16E-04	4.15E-09	16
H2aM15287N	186 ± 1.9	50	6.33E+04	4.42E-04	6.98E-09	26
H1M15280N	168 ± 0.8	56	2.83E+05	5.25E-03	1.85E-08	2.2
H1H15233P2	250 ± 13.2	141	1.99E+05	4.56E-05	2.29E-10	253
H1H15231P2	228 ± 3.8	154	6.39E+05	7.44E-05	1.16E-10	155
H1H15188P	189 ± 1.4	158	1.48E+06	1.22E-04	8.25E-11	95
H1H15211P	226 ± 1.1	180	1.13E+06	8.72E-05	7.75E-11	133
H1H15249P2	195 ± 1.2	99	3.49E+05	7.91E-05	2.27E-10	146
H1H15260P2	242 ± 2	164	1.16E+06	1.60E-04	1.38E-10	72
H1H15203P	128 ± 1.4	100	4.43E+05	1.21E-04	2.73E-10	95
H1H15237P2	171 ± 2.2	131	4.39E+05	1.20E-04	2.74E-10	96
H1H15264P2	110 ± 2.9	66	2.23E+05	1.86E-04	8.33E-10	62
H1H15228P2	144 ± 4.3	113	4.43E+05	1.85E-04	4.18E-10	63
H1H15208P	114 ± 4.3	98	4.39E+05	2.43E-04	5.53E-10	48
H1H15177P	135 ± 1.3	105	4.93E+05	1.92E-04	3.90E-10	60
H1H15253P2	127 ± 1.7	94	3.56E+05	1.24E-04	3.48E-10	93
H1H15215P	171 ± 1	86	1.44E+05	9.67E-05	6.71E-10	119
H1H15259P2	234 ± 2.1	142	2.26E+05	3.77E-04	1.67E-09	31

Table 5

	Binding Kinetics @ 37°C					
mAb Captured	mAb Capture Level (RU)	100nM Ag (RU)	ka (1/Ms)	kd (1/s)	KD (M)	t½ (min)
H2aM15281N	276 ± 1.6	140	2.55E+06	5.43E-04	2.13E-10	21
H1M15289N	271 ± 2.2	119	4.59E+05	1.26E-04	2.75E-10	92
H1M15267N	202 ± 1.1	132	2.37E+06	7.45E-04	3.14E-10	16
H2aM15272N	284 ± 1.9	135	2.00E+06	6.34E-04	3.16E-10	18
H2aM15279N	229 ± 1	150	1.39E+06	4.45E-04	3.20E-10	26
H2aM15278N	266 ± 1.1	127	2.17E+06	1.13E-03	5.19E-10	10
H2aM15268N	208 ± 1.1	126	3.75E+06	2.02E-03	5.37E-10	6
H2aM15270N	219 ± 0.8	128	1.75E+06	1.14E-03	6.51E-10	10
H1M15277N	246 ± 1.1	156	3.24E+06	2.18E-03	6.71E-10	5
H2bM15292N	242 ± 0.6	117	5.44E+05	5.24E-04	9.63E-10	22
H2aM15271N	276 ± 1.5	117	1.45E+06	1.45E-03	1.00E-09	8
H1M15269N	319 ± 1.7	107	2.54E+05	3.39E-04	1.34E-09	34
H1M15290N	243 ± 1.8	117	6.66E+05	9.84E-04	1.48E-09	12
H1M15293N	213 ± 1.7	112	5.12E+05	8.53E-04	1.66E-09	14
H2bM15291N	229 ± 1.7	97	3.42E+05	6.40E-04	1.87E-09	18
H1M15288N	176 ± 1	69	2.73E+05	2.08E-03	7.61E-09	6

H2aM15287N	228 ± 1.4	61	1.74E+05	1.89E-03	1.09E-08	6
H1M15280N	196 ± 1	59	4.97E+05	6.57E-03	1.32E-08	1.8
H1H15233P2	317 ± 6.9	184	3.74E+05	4.74E-05	1.27E-10	244
H1H15231P2	306 ± 4.3	228	6.59E+05	9.19E-05	1.39E-10	126
H1H15188P	267 ± 1.6	217	1.59E+06	2.65E-04	1.67E-10	44
H1H15211P	305 ± 2.7	232	8.27E+05	1.60E-04	1.94E-10	72
H1H15249P2	260 ± 2.3	157	4.42E+05	1.27E-04	2.88E-10	91
H1H15260P2	322 ± 2.5	220	1.05E+06	5.47E-04	5.19E-10	21
H1H15203P	179 ± 1.2	106	5.01E+05	3.23E-04	6.45E-10	36
H1H15237P2	218 ± 1.4	181	5.14E+05	4.09E-04	7.97E-10	28
H1H15264P2	149 ± 2.1	106	5.07E+05	4.75E-04	9.36E-10	24
H1H15228P2	188 ± 2	157	4.76E+05	4.64E-04	9.75E-10	25
H1H15208P	151 ± 3.6	145	5.25E+05	5.31E-04	1.01E-09	22
H1H15177P	181 ± 1.5	149	5.68E+05	7.01E-04	1.23E-09	16
H1H15253P2	172 ± 0.7	139	4.36E+05	6.29E-04	1.44E-09	18
H1H15215P	224 ± 2.1	96	1.38E+05	2.33E-04	1.68E-09	50
H1H15259P2	282 ± 3.8	173	4.78E+05	8.88E-04	1.86E-09	13

[0195] As shown in Tables 4 and 5, the antibodies bound to MERS-CoV spike protein with K_D values ranging from 77.5pM to 18.5nM at 25°C and from 127pM to 13.2nM at 37°C. The antibodies showed dissociative half-life (t½) values ranging from 2.2 to 262 minutes at 25°C and from 1.8 to 244 minutes at 37°C.

Example 5: Antibody-mediated neutralization of MERS infectivity

Materials and Methods

Generation of pseudoparticles

[0196] MERS pseudoparticles (MERSpp; also called as virus like particle – VLP) were generated by co-transfecting 293T cells with a mix of plasmid constructs expressing MERS spike protein, HIV gag-pol, and an HIV proviral vector encoding for firefly luciferase. Supernatants containing MERSpp were harvested at 72 hours post transfection, clarified using centrifugation, concentrated, aliquoted and frozen at -80°C. Control pseudoparticles were generated by substituting the plasmid expressing MERS-CoV Spike protein with a plasmid encoding for Vesicular Stomatitis Virus Glycoprotein (VSVg).

MERSpp-based assay

[0197] Dilutions of antibodies were incubated with MERSpp for 1h at room temperature. Huh7 cells in complete DMEM media were detached using 1% EDTA, washed and incubated with the antibody/MERSpp mixtures for 72h. Infection efficiency was quantitated by luciferase detection with the BrightGlo® luciferase assay (Promega, San Luis Obispo, CA, USA) and read in a Victor® X3 plate reader (Perkin Elmer, Waltham, MA, USA) for light production.

Virus-based assay

[0198] $1x10^4$ Vero E6 cells per well were seeded into 96-well plates 1 day prior to the experiment. 500 $TCID_{50}$ of MERS-CoV EMC/2012 strain was mixed with the stated amount of antibody in 100ul normal Vero E6 growth media and incubated at room temperature for 30 minutes and the mixture was then added to the Vero E6 cells. At 2 days post-infection, cell

death was assessed using the CellTiter-Glo® Luminescent Cell Viability Assay (Promega) according to the manufacturers' instructions. Luminescence was read on Spectramax M plate reader (Molecular Devices). Data was expressed as percent of mock-infected controls.

Results

Neutralization of MERS-CoV by anti-MERS-CoV-S antibodies

[0199] In an initial experiment, the anti-MERS-CoV-S antibodies derived from separate independent hybridomas were tested for neutralization of MERS-infectivity. As shown in Figure 1, 12 of 22 cell anti-MERS-CoV-S hybridoma antibodies inhibited or neutralized more than 90% of MERS-infectivity and with IC_{50} ranging from 68.4pM to 3.58nM.

[0200] In subsequent experiments, the purified antibodies were tested for neutralization of MERSpp and live MERS virus (Isolate: EMC/2012). The results are shown in Tables 6 and 7.

Table 6

Antibody	IC50 against MERSpp (M)	IC50 against live virus (EMC2012) (M)
H1M15277N	6.46E-11	2.87E-10
H2aM15279N	9.39E-11	8.25E-10
H1M15280N	1.11E-10	7.63E-10
H2aM15278N	1.42E-10	6.44E-10
H2aM15271N	1.27E-10	1.03E-09
H1M15267N	9.98E-11	8.91E-10
H2bM15292N	1.13E-10	2.26E-09
H2bM15291N	1.86E-10	2.22E-09
H1M15290N	2.10E-10	2.55E-09
H1M15293N	2.58E-10	2.58E-09
H1M15289N	2.81E-10	3.15E-09
H1M15269N	6.88E-10	6.80E-09
H2aM15287N	2.93E-09	8.92E-09
H1M15288N	~0.1209	2.66E-08
H2aM15268N	5.89E-11	2.72E-10
H2aM15270N	1.02E-10	7.11E-10
H2aM15281N	7.85E-11	2.92E-10
H2aM15272N	7.23E-11	5.69E-10

Table 7

Antibody	IC50 against MERSpp (M)	IC50 against live virus (EMC2012) (M)
H1H15211P	4.74E-11	3.69E-10
H1H15188P	6.20E-11	5.05E-10
H1H15177P	4.57E-11	6.29E-10
H1H15203P	7.65E-11	8.26E-10
H1H15228P2	9.75E-11	8.98E-10
H1H15231P2	1.02E-10	9.20E-10
H1H15237P2	6.60E-11	1.01E-09
H1H15233P2	8.73E-11	1.37E-09
H1H15259P2	7.64E-11	1.63E-09
H1H15264P2	1.34E-10	2.39E-09
H1H15208P	8.27E-11	2.41E-09
H1H15253P2	1.51E-10	2.42E-09
H1H15215P	1.92E-10	2.67E-09
H1H15260P2	6.43E-11	6.70E-09
H1H15249P2	2.27E-10	1.20E-08
H4sH15188P	2.91E-11	NA

H4sH15211P	6.08E-11	NA
H4sH15260P2	6.24E-11	NA
H4sH15231P2	7.33E-11	NA

[0201] The data above suggest that the anti-MERS-CoV-S antibodies of the present invention potently block the entry of MERS-CoV into susceptible cells and neutralize infectivity.

Neutralization of clinical isolates by anti-MERS-CoV-S antibodies

[0202] RNA viruses encode low-fidelity genome replication machinery, which allows them to rapidly adapt to their host environment (Malpica et al 2002; Genetics 162: 1505-1511). However, coronaviruses, such as MERS-CoV, encode for a non-structural protein with a 3'-to-5' exo-ribonuclease activity, that provides a proof-reading function during their replication and greatly increases the coding capacity of the genome without leading to mutational catastrophe (Cotton et al 2013; Lancet doi:10.1016/S0140-6736(13)61887-5). Despite that, sequencing of multiple clinical isolates sampled during the first two years of the MERS-CoV epidemic has revealed that the MERS-CoV S protein of the virus is evolving (Cotton et al 2014; MBio 5 doi:10.1128/mBio.01062-13). For this study, 38 NCBI deposited sequences of MERS-CoV clinical isolates were aligned and compared to the sequence of the first isolated strain, EMC/2012. Based on the design of the screening assay, the antibodies of the present invention were expected to bind within the RBD of the MERS-CoV S protein, so the comparison of the sequences specifically focused on amino acids 367-606. Seven different amino acids were identified that differ between the sequenced clinical isolates and the prototype EMC/2012 sequence, A431P, S457G, S460F, A482V, L506F, D509G and V534A (Table 8).

Table 8

Amino Acid change	Isolate
A431P	Riyadh_9
S457G	KFU-HKU 1; KFU-HKU 13
S469F	Qatar3; Qatar4
A482V	Riyadh_9
L506F	England 1; England-Qatar/2012
D509G	Bisha_1; Riyadh_1
V534A	Riyadh_2

[0203] To test the ability of the antibodies to bind to conserved regions within the RBD and neutralize all MERS-CoV clinical isolates sequenced as of July 2014, site-directed mutagenesis was used to create plasmid constructs encoding for all S protein variants. These were used to generate MERSpp pseudotyped with the modified S proteins and performed neutralization assays. The purified antibodies (described in Example 1) were tested for neutralization of MERS-infectivity using MERSpp generated with the above-mentioned mutations. Tables 9 and 10 show the percent neutralization of MERSpp variants.

Table 9

RBD Variant	wt	A431P	S457G	S460F	A482V	L506F	D509G
Antibody µg/mL	0.02	0.02	0.02	0.02	0.02	0.02	0.02
H1M15267N	71.65%	68.15%	70.81%	76.82%	66.78%	57.11%	84.91%
H1M15269N	7.19%	11.87%	27.48%	19.57%	7.74%	21.09%	7.08%
H1M15280N	56.69%	59.42%	74.42%	73.14%	65.89%	65.90%	38.21%
H1M15277N	75.83%	89.05%	90.07%	89.35%	75.14%	83.27%	62.26%
H2aM15268N	79.86%	89.51%	93.08%	89.11%	83.50%	88.63%	87.74%
H2aM15270N	63.45%	81.24%	81.64%	80.51%	67.96%	79.63%	45.28%
H2aM15271N	53.38%	63.32%	67.50%	65.52%	52.90%	4.79%	38.21%
H2aM15272N	62.88%	72.97%	78.34%	80.51%	58.90%	16.58%	7.08%
H2aM15281N	78.99%	85.38%	86.76%	83.70%	73.96%	17.87%	19.81%
H2aM15279N	48.92%	68.84%	69.91%	65.27%	55.36%	71.27%	53.77%
H2aM15278N	50.50%	65.39%	70.81%	73.87%	48.97%	56.25%	-29.72%
H1M15288N	1.29%	11.64%	20.86%	26.45%	23.68%	8.22%	-2.83%
H1M15289N	43.74%	44.26%	45.54%	51.27%	36.86%	42.32%	28.30%
H1M15290N	36.55%	39.20%	43.73%	48.08%	22.89%	-0.14%	-39.62%
H1M15293N	27.91%	29.10%	34.40%	40.21%	20.33%	1.57%	-15.57%
H2aM15287N	11.65%	5.90%	12.14%	15.15%	19.06%	15.30%	-1.42%
H2bM15291N	45.90%	38.74%	50.05%	49.80%	28.30%	-3.57%	18.40%
H2bM15292N	42.01%	41.04%	49.45%	51.02%	40.21%	14.87%	-16.98%
Isotype control (mIgG1)	-0.14%	10.03%	-3.81%	1.39%	-5.25%	2.64%	-24.06%
Isotype control (mIgG2a)	5.32%	-1.00%	7.32%	8.76%	10.30%	5.65%	-26.89%
Isotype control (mIgG2b)	0.43%	-5.13%	26.88%	5.08%	13.35%	6.08%	-4.25%
H1H15211P	87.77%	90.20%	93.98%	93.78%	88.82%	91.85%	80.66%
H4sH15211P	91.65%	95.02%	98.80%	91.81%	95.41%	96.35%	67.92%
Isotype control (hIgG1)	11.80%	10.26%	-11.63%	3.60%	15.61%	6.08%	-8.49%
Isotype control (hIgG4)	-4.60%	-0.54%	-0.50%	-0.33%	13.84%	-5.50%	8.49%

Table 10

RBD variant	wt	A431P	S457G	S460F	A482V	L506F	D509G
Ab ID µg/mL	0.02	0.02	0.02	0.02	0.02	0.02	0.02
H4sH15188P	81.6%	89.7%	95.7%	92.6%	74.2%	93.6%	101.4%
H4sH15211P	77.1%	91.6%	88.1%	86.0%	73.8%	88.2%	57.2%
H4sH15231P2	70.6%	72.3%	74.5%	75.7%	70.5%	80.6%	60.0%
H4sH15260P2	73.3%	82.8%	77.7%	74.8%	79.0%	73.3%	73.8%
H1H15177P	82.8%	83.2%	84.4%	84.5%	75.9%	90.9%	35.2%
H1H15188P	81.4%	88.9%	86.8%	85.5%	86.4%	92.4%	90.3%
H1H15203P	61.0%	80.5%	74.5%	74.0%	74.0%	61.3%	35.2%
H1H15208P	53.9%	79.3%	73.8%	77.7%	65.2%	76.5%	82.1%
H1H15211P	77.8%	87.3%	93.5%	94.6%	80.2%	82.6%	79.3%
H1H15215P	20.2%	43.0%	42.4%	44.0%	41.7%	15.8%	-47.6%
H1H15228P2	47.8%	64.1%	68.4%	57.4%	63.1%	74.8%	62.8%
H1H15231P2	48.1%	60.6%	71.4%	55.7%	60.0%	55.2%	46.2%
H1H15233P2	55.4%	66.8%	69.7%	59.9%	65.0%	50.8%	65.5%
H1H15237P2	52.6%	74.4%	82.7%	73.5%	70.5%	73.8%	79.3%

H1H15249P2	15.6%	34.6%	19.8%	34.2%	37.2%	30.7%	29.7%
H1H15253P2	52.9%	66.4%	56.0%	57.7%	58.2%	56.9%	51.7%
H1H15259P2	61.2%	79.3%	77.1%	63.8%	68.1%	73.0%	79.3%
H1H15260P2	68.8%	85.6%	74.5%	86.7%	80.4%	82.1%	54.5%
H1H15264P2	23.1%	55.5%	52.3%	52.3%	40.1%	47.4%	-20.0%
Isotype control (hIgG1)	-38.0%	-0.3%	1.6%	-36.9%	0.8%	-16.9%	15.9%
Isotype control (hIgG4)	-19.9%	1.1%	-4.5%	-8.1%	6.7%	-7.4%	15.9%
H1M15277N	35.2%	86.7%	83.1%	65.0%	75.1%	73.0%	71.0%
Isotype control (mIgG1)	-8.8%	2.6%	-3.6%	3.7%	0.2%	-9.6%	26.9%

[0204] These data suggest that the antibodies of the present invention bind to regions of the MERS-CoV spike protein that are conserved during natural evolution of the virus.

Anti-MERS-CoV-S antibodies of the inventions are more effective neutralizers that previously isolated anti-MERS-CoV antibodies

[0205] The potency of selected antibodies of the invention was compared with previously isolated monoclonal antibodies. Three groups have used *in vitro* antibody isolation methods, *i.e.* phage display (Tang et al 2014, PNAS doi:10.1073/pnas.1402074111; and Ying et al 2014, J. Virol. doi:10.1128/JVI.00912-14) and yeast display (Jiang et al 2014, Sci. Transl. Med. doi:10.1126/scitranslmed.3008140), to select for antibodies that bind to MERS-CoV S protein and block virus entry. For this study, a panel of three antibodies with published variable domain sequences which were reported to neutralize MERS-CoV and bind to diverse epitopes was selected. Sequences for antibodies 3B12 (Tang et al 2014, PNAS doi:10.1073/pnas.1402074111), MERS-4 and MERS-27 (Jiang et al 2014, Sci. Transl. Med. doi:10.1126/scitranslmed.3008140) were cloned onto human IgG1 constant domains, then expressed and purified similarly to selected antibodies of the present invention. The neutralization efficacy of all antibodies was tested using pseudoparticles generated with the prototypical EMC/2012 sequence and all clinical isolates described above.

[0206] The neutralization IC50 of selected antibodies against MERSpp variants is shown in Table 11. As seen in Table 11, H1H15211P was able to neutralize all isolates with IC_{50} values ranging from 1.3E-11M to 6.5E-11M. Similar values were also observed for H1H15277N, with the exception of the V534A variant, which exhibited a partial resistance to the antibody. It is worth noting however, that this amino acid change was only observed in a single MERS-CoV isolate (Riyadh_2), which was isolated in 2012 and is part of a dead branch of the MERS-CoV evolutionary tree.

[0207] H1H15211P and H1H277N neutralize MERSpp with IC_{50} values at least one log lower than the most potent comparator antibody, MERS-4. In addition, both 3B12 and MERS-27 appear to bind to less conserved sites on the MERS-CoV S protein, as pseudoparticles with sequences of multiple clinical isolates cannot be neutralized with these antibodies.

Table 11

	H1H15177P	H1H15188P	H1H15211P	H1H15277N	H1H15267N2	3B12	MERS-4	MERS-27
wt	5.374E-11	4.819E-11	6.525E-11	6.978E-11	7.621E-11	1.69E-09	3.90E-10	8.36E-09
A431P	1.924E-11	1.323E-11	2.15E-11	3.162E-11	4.877E-11	6.35E-10	2.02E-10	4.50E-09
S457G	3.022E-11	1.99E-11	2.578E-11	3.532E-11	4.973E-11	7.43E-10	2.81E-10	5.06E-09
S460F	2.435E-11	1.49E-11	2.031E-11	3.19E-11	6.805E-11	6.69E-10	3.14E-10	6.36E-09
A482V	2.044E-11	1.955E-11	2.735E-11	8.094E-11	6.533E-11	9.58E-10	6.83E-10	1.26E-08
L506F	1.624E-11	1.017E-11	1.341E-11	3.967E-11	3.537E-11	~0.001534	5.64E-10	6.64E-09
D509G	1.792E-10	1.682E-11	4.036E-11	4.322E-11	4.846E-11	1.17E-07	1.37E-09	4.57E-09
V534A	3.663E-11	2.034E-11	3.195E-11	7.064E-09	4.317E-10	5.63E-08	2.19E-10	1.31E-07

[0208] These data suggest that antibodies of the present invention are able to neutralize a broader range of MERS-CoV isolates with improved potency compared to several antibodies isolated solely based on *in vitro* biochemical properties.

Example 6: Octet cross-competition between anti-MERS-CoV-S antibodies

[0209] Binding competition between MERS-CoV S antibodies was determined using a real time, label-free bio-layer interferometry assay on an Octet® RED96 biosensor (Pall ForteBio Corp). The entire experiment was performed at 25°C in HBS-P Octet buffer (10mM HEPES, 150mM NaCl, and 0.05% v/v Surfactant Tween-20, pH 7.4, 1mg/mL BSA) with the plate shaking at the speed of 1000rpm. To assess whether antibodies are able to compete with one another for binding to their respective epitopes on a recombinantly expressed MERS spike protein receptor binding domain fused to a human Fc tag (MERS RBD-hFc; SEQ ID: 458), a pre-mix assay format was adopted and 50nM of MERS-CoV-RBD-mFc was pre-incubated with 500nM of different anti-MERS monoclonal antibody (subsequently referred to as mAb-2) for at least 2 hours prior to performing the binding competition assay. A non-specific monoclonal antibody was incubated with MERS-RBS.mFc as an Isotype control. Octet biosensors coated with an anti-hFc polyclonal antibody (Pall ForteBio Corp., Cat# 18-5060) were first submerged into wells containing 50µg/mL solution for 4 minutes to capture around 2nm of anti-MERS antibody (subsequently referred to as mAb-1). Following the capture step, unoccupied anti-hFc polyclonal antibodies were then saturated by submerging Octet biosensors into wells containing 100µg/mL of non-specific fully human monoclonal antibody. Finally, the biosensors were immersed for 4 minutes into wells containing the pre-mixed samples of 50nM of MERS-RBD-mFc and 500nM of mAb-2. The biosensors were washed in HBS-P Octet buffer in between every step of the experiment. The real-time binding response was monitored during the course of the experiment and the binding response at the end of every step was measured. The response of mAb-1 binding to the pre-complex of MERS-RBD-mFc and mAb-2 was compared and competitive/non-competitive behavior of different anti-MERS monoclonal antibodies was determined.

[0210] Under the experimental conditions used in this Example, cross competition (*i.e.*, competition between antibodies in both orientations) was observed, for example, for: (a) H2aM15281N, H1M15290N, H1M15293N, and H2bM15291N; (b) H2aM15272N, H2bM15292N, and H2aM15271N; and (c) H2aM15268N, H1M15267N, H2M15279N, H1M15289N, and H1M15277N (Figure 2).

[0211] In another experiment, binding competition between selected antibodies isolated directly from antigen-positive mouse B cells (described in Example 1) was determined. Under the experimental conditions used in this Example, cross competition (*i.e.*, competition between antibodies in both orientations) was observed, for example, for: (a) H1H15215P, H1H15177P, H1H15203P, and H1H15211P; (b) H1H15188P, H1H15208P, H1H15228P2, H1H15233P2, H1H15237P2, H1H15253P2, and H1H15259P2; and (c) H1H15231P2, H1H15249P2, H1H15260P2, and H1H15264P2 (Figure 3).

[0212] In another experiment, the binding competition between two antibodies H1H15211P and H1H15277N was monitored.

Table 12

		Binding of pre-complex of mAb2 and MERS-RBD-mFc to captured mAb1			
mAb1	mAb1 capture level (nm)	H1H15277N	H1H15211P	Isotype control mAb	No mAb
H1H15277N	2.19	0.22	0.55	1.03	0.96
H1H15211P	2.08	0.68	0.22	0.73	0.69

[0213] Table 12 shows that the two antibodies do not inhibit each other and that binding of one antibody to MERS-CoV RBD still allows binding of the second antibody. These data suggest that the two antibodies bind to discrete, non-overlapping epitopes. Mutation of one epitope as a result of selective pressure by one antibody should not affect binding of the other one.

Example 7: In vivo efficacy of anti-MERS-CoV-S antibodies

Materials and Methods

Generation of human DPP4 knockin mice

[0214] Since the MERS spike protein does not interact with mouse DPP4, a humanized model of MERS-CoV infection was generated using VelociGene® technology (Valenzuela et al 2003, Nat. Biotechnol. 21: 652-659). Briefly, a large targeting vector (LTVEC) was constructed to contain 82 kb human DPP4 genomic DNA from exons 2 to 26 including 3' UTR to replace 79 kb mouse Dpp4 counterparts sequence. Human BAC RP11-68L22 and mouse BAC RP23-362N15 containing the Dpp4 gene were identified using Blast and sequence confirmed using Illumina bench top sequencer MiSeq. The LTVEC was electroporated in F1 hybrid

(129S6SvEvTac/C57BL6NTac) ES cells. G418-resistant colonies were picked 10 days after electroporation and screened for correct targeting by the loss-of-allele assay. The VelociMouse® method was used (Poueymirou, et. al. 2007; Nature Biotechnology 25: 91-99), in which targeted ES cells were injected into un-compacted 8-cell stage Swiss Webster embryos, to produce fully ES cell-derived F0 generation mice carrying the human DPP4 gene allele. All animal procedures were carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the NIH. The protocol was approved by the Regeneron Pharmaceuticals Institutional Animal Care and Use Committee (IACUC). The method is described in detail in US Patent Application No: 62/051,626, filed on September 17, 2014, incorporated herein in its entirety. Transgenic mice were also generated by random insertion of the hDPP4 gene in the mouse genome.

Animal Experiments

[0215] Six- to eight-week-old humanized DPP4 mice were injected intraperitoneally (i.p.) with indicated amounts of antibody or sterile saline as a control at 1 day pre-infection or 1 day post-infection, depending on the experiment. Prior to intranasal inoculation, mice were anaesthetized by intraperitoneal injection using a mix of xylazine (0.38mg/mouse; Lloyd laboratories) and ketamine (1.3mg/mouse; Henry Schein animal health), diluted in PBS to make a total volume of 50µl per mouse. Once anaesthetized, mice were intranasally inoculated with either PBS or $2x10^5$ pfu of MERS-CoV (Jordan) diluted into PBS for a total inoculum of 50µl. During the experiment, mice were weighed prior to infection and every day of the experiment to assess MERS-CoV induced weight loss. Mice were euthanized at day 2 or day 4 post-infection using a lethal dose of isofluorane (Butler Animal Health Supply). Lungs were harvested for further analysis of MERS-CoV replication and pathology.

MERS-CoV RNA analysis

[0216] RNA was extracted from mouse lung by homogenization in 1ml of Trizol® (Life Technologies Inc) using a Magnalyzer (Roche) according to manufacturer's instructions. Levels of MERS-CoV RNA were assessed using the Taqman® Fast virus one-step master mix (Applied Biosystems) according to the manufacturers' instructions using a duplex of primers obtained from Life Technologies targeting a region of the genome upstream of the envelope gene (UpE); the leader sequence of the nucleocapsid messenger RNA (leader primer) and compared to mouse 18S rRNA (endogenous control). qPCR reactions in Microamp® fast optical reaction plates (Applied Biosystems) were read on a 7500 fast DX real-time PCR instrument (Applied Biosystems) and data was analyzed using the delta Ct method, with an uninfected control set to 1. Percent MERS-CoV RNA detected was expressed relative to levels of RNA detected in infected mice treated with isotype-matched control antibodies.

Plaque assay for MERS-CoV titers

[0217] Mouse lung was homogenized in 1ml of PBS with glass beads for 60 seconds at 6000rpm using a Magnalyzer (Roche). Homogenate was then centrifuged for 10 minutes at

10,000rpm and supernatant analyzed by plaque assay on Vero E6 cells to quantitate levels of virus remaining after treatment. Plaque assays were performed as previously described, except that plates were left for 3 days for plaques to appear.

Histological Analysis

[0218] Histological slides were prepared from 5-µm sections of fixed, paraffin-embedded tissues, and stained with hematoxylin and eosin. Fields were examined by light microscopy and analyzed. The degrees of interstitial, peribronchiolar and perivascular inflammation were scored from 0 to 5. For each experimental group, the slides were blinded and scored from 0 to 5 and tabulated to compare strains, time points and treatments. Other histologic features, such as the presence of bronchiolar epithelial and alveolar damage, pleural changes and the extent of peribronchovascular inflammation, were also noted for each group in the text. The overall inflammatory scores for each mouse were averaged for each group, and presented as average scores of all mice in each group and time-point.

Results

Humanized mice for DPP4 are susceptible to MERS-CoV infection

[0219] *In vivo* testing of anti-viral molecules requires a small animal model that is susceptible to MERS-CoV infection. Mice are not susceptible to MERS-CoV infection. Sequence comparison of the sequence of mouse and human DPP4 revealed that the amino acids that have previously been identified as contact sites between MERS-CoV S protein and its receptor differ between the two species. In addition, expression of human DPP4 in mouse cells allows for MERSpp entry and MERS-CoV propagation, indicating that entry of the virus is the limiting step in infection of mouse cells and the lack of interaction between mouse DPP4 and the MERS-CoV glycoprotein defines the species tropism *in vitro*.

[0220] The inventors hypothesized that mice expressing human DPP4 in place of mouse DPP4 would be susceptible to MERS-CoV and allow for *in vivo* testing of anti-MERS-CoV therapeutics. VelociGene® technology was employed to replace the 79kb of the mouse Dpp4 gene with 82kb of its human ortholog. The resulting mice express fully human DPP4 under the control of the mouse regulatory elements, to preserve the proper expression regulation and protein tissue distribution.

[0221] To test if humanized mice and transgenic mice could support MERS-CoV infection, in one initial experiment, transgenic mice were treated intraperitoneally with 200 µg of anti-MERS-CoV-S antibodies or isotype control on day -1 and infected intranasally with MERS-CoV (~10^6 pfu of EMC2012) on day 0. Four days post-infection, the lungs were harvested and RNA levels of the virus were measured by RT-PCR to check the effect of the antibodies on viral load. Table 13 shows the average levels of viral genomic RNA and the replicating RNA expressed as percentage of isotype controls. Treatment with H1H15211P resulted in about 500-fold reduction of viral RNA in the infected mice.

Table 13

Antibody	Genomic RNA	Replicating RNA (leader)
H1H15177P	0.356839562	0.273565089
H1H15211P	0.254493202	0.206006238
H4sH15211P	1.989548316	1.112094283
IgG1 isotype control	104.0889287	101.2578723
IgG4 isotype control	100	100

[0222] In another experiment, 6 to 8 week old humanized DPP4 (huDPP4) mice were intranasally inoculated with MERS-CoV. Although no mortality or clinical signs of disease was observed up to day 4, at days 2 and 4 post-inoculation mice were euthanized and their lungs were dissected. To obtain virus RNA levels, lungs were homogenized in Trizol®, and analyzed by Realtime PCR using primers specific to MERS-CoV (Figures 4 and 5). To obtain virus titers, lungs were homogenized in PBS, clarified by centrifugation and titered on Vero E6 cells (Figure 6). Robust MERS-CoV replication in the lungs was evident at 2 and 4 days post infection. RNA quantification, using a primer set specific for MERS-CoV leader, which were designed to only amplify replicating MERS-CoV, demonstrated high levels of MERS-CoV replicating RNA in lungs collected at day 2, and these levels were maintained through day 4 post infection (Figure 5). Plaque assay of lung homogenate on Vero E6 cells quantified MERS-CoV (Jordan) levels of ~$7.27x10^4$ pfu/g lung at day 2 and ~$3.75x10^5$ pfu/g lung at 4 days post infection (Figure 6), demonstrating active replication of MERS-CoV in the lungs of the huDPP4 mice. These data suggest that humanization of the receptor DPP4 using VelociGene® technology has created a robust model of MERS-CoV in mice that can be used to assess MERS-CoV treatment *in vivo*.

[0223] Lungs from huDPP4 mice intranasally inoculated with either MERS-CoV (Jordan strain) or PBS (mock infected) were analyzed for pathological changes. At day 2 post infection, peri-bronchiolar inflammation was evident with alterations in bronchiolar cell structure found throughout the lungs. Minimal peri-vascular inflammation or effects on alveolar structures were noted at this time point. At 4 days post infection, significant interstitial infiltration was observed with peri-vascular cuffing and extensive alveolar thickening. Bronchiolar alterations were still present as well. Importantly, this pathology is consistent with the radiographic findings of development of interstitial pneumonia and significant lung disease seen in humans with MERS-CoV, suggesting that this humanized DPP4 *in vivo* model of MERS-CoV infection recapitulates pathological sequelae that are seen in MERS-CoV infection of humans.

[0224] In further experiments (see below), the infected mice were administered with one or more doses of purified antibodies (as described in Example 1) or a combination of antibodies prophylactically and/or therapeutically to test their efficacy against MERS infection.

H1H15211P and H1H15277N protect humanized DPP4 mice from MERS-CoV infection

[0225] Having established that humanized DPP4 mice were susceptible to MERS-CoV, this model was used to evaluate the activity of two monoclonal antibodies *in vivo*. Mice were i.p. injected with 200ug, 20ug or 2ug of either H1H15211P, or of H1H15277N or with 200ug of hIgG1 isotype control antibody 24 hours before intranasal infection with $1x10^5$ pfu of MERS-CoV

(Jordan strain). As seen in Figures 7 and 8, both antibodies were able to significantly decrease MERS-CoV specific RNA levels in the lungs by over 2 logs at the 200ug per mouse dose, compared to the isotype control antibody. H1H15211P was more effective at reducing MERS-CoV RNA levels at the 20ug dose compared to H1H15277N at the same dose. The 2ug dosing of either antibody was ineffective at reducing viral RNA levels compared to isotype control treated mice. When MERS-CoV titer was analyzed in the lungs (Figure 9) it was found that both the 200ug and 20ug dose of H1H15211P reduces virus levels to near the level of detection in the assay ($2x10^3$ pfu/ml). H1H15277N is equally efficient at the 200ug dose as H1H15211P, while the 20ug and 2ug display a dose dependent inhibition of viral inhibition. These data suggest that H1H15211P and H1H15277N can effectively block MERS-CoV infection *in vivo*.

[0226] Histological analysis was also performed on lungs from mice treated 24 hours pre-infection with H1H15277N, H1H15211P or hIgG1 isotype control antibody at 4 days post infection. Lungs from mice pre-treated with hIgG isotype control mice displayed significant lung pathology with increased interstitial inflammation, perivascular cuffing, and thickening of alveolar septa. Mice treated with 200ug of either H1H15277N or H1H15211P had reduced inflammation with minimal foci of inflammatory cells in the interstitium and minor bronchiolar cuffing. In mice pre-treated with 20ug of H1H15277N and H1H15211P, moderate levels of perivascular cuffing and interstitial inflammation were found as compared to the higher antibody group. In contrast, the 2ug antibody pre-treated group had similar pathology to the hIgG1 isotype control displaying significant interstitial inflammation and predominant peri-vascular inflammation. Blinded histological scoring (Figure 10) reflects the reduced inflammation scores for treated mice. These findings demonstrate that H1H15277N and REGN 3051 confer a dose-dependent reduction in lung pathology following MERS-CoV infection corroborating viral RNA levels and virus titers determined for these mice.

[0227] Taken together, these data indicate that H1H15211P and H1H15277N can block MERS-CoV infection and disease *in vivo* when injected 1 day before infection. To our knowledge, H1H15211P and H1H15277N are the first fully-human antibodies that have been shown to display efficacy in an *in vivo* model of MERS-CoV infection.

<u>H1H15211P and H1H15277N can treat humanized DPP4 mice infected with MERS-CoV</u>

[0228] The ability to inhibit MERS-CoV replication and lung pathology after infection is a desired trait in a potential therapeutic. To assess whether H1H15211P is able have an effect therapeutically, huDPP4 mice were infected with MERS-CoV, and then 24 hours later injected i.p. with either 500ug of hIgG isotype control or H1H15211P at 500ug or 200ug. At 4 days post infection mice were euthanized and mouse lungs were analyzed for viral RNA, virus titer and lung pathology. Both the 500ug and 200ug doses of H1H15211P were able to reduce viral RNA levels by about 10 fold in the lungs of mice compared to control antibody treated mice (Figure 11 and 12). Lung titers of the same mice demonstrated significant reduction in viral levels in the lungs with a greater than 2 log reduction at day 4 post infection (Figure 13). These data

demonstrate H1H15211P can significantly inhibit viral replication even when administered 24 hours following viral inoculation.

[0229] Histological analysis was performed on mice treated 24 hours post infection with hIgG control antibody, 500ug or 200ug H1H15211P. Mice treated with control antibody displayed similar pathology to the control above with significant interstitial inflammation, peri-vascular cuffing and thickening of alveolar septa. Mice treated with either 200ug or 500ug of H1H15211P had minimal interstitial inflammation and with reduced and only focal peri-vascular inflammation throughout the lungs. Blinded histological scoring demonstrates reduced inflammation scores for treated mice (Figure 14). These data demonstrates that therapeutic doses of H1H15211P reduce MERS-CoV induced lung pathology even when given 24 hour post-infection.

[0230] The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

What is claimed is:

1. A human monoclonal antibody or antigen-binding fragment thereof that specifically binds to Middle East Respiratory Syndrome corona virus (MERS-CoV) spike protein, wherein the antibody or antigen-binding fragment comprises three heavy chain complementarity determining regions (CDRs) (HCDR1, HCDR2 and HCDR3) contained within any one of the heavy chain variable region (HCVR) sequences selected from the group consisting of SEQ ID NOs: 2, 18, 34, 50, 66, 82, 98, 114, 122, 130, 138, 146, 154, 170, 178, 186, 202, 218, 234, 250, 266, 282, 298, 314, 330, 346, 362, 378, 394, 410, 426, and 442; and three light chain CDRs (LCDR1, LCDR2 and LCDR3) contained within any one of the light chain variable region (LCVR) sequences selected from the group consisting of SEQ ID NOs: 10, 26, 42, 58, 74, 90, 106, 162, 194, 210, 226, 242, 258, 274, 290, 306, 322, 338, 354, 370, 386, 402, 418, 434, and 450, wherein the antibody or antigen-binding fragment comprises a HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 2/10, 18/26, 34/42, 50/58, 66/74, 82/90, 98/106, 114/106, 122/106, 130/106, 138/106, 146/106, 154/162, 170/162, 178/162, 186/194, 202/210, 218/226, 234/242, 250/258, 266/274, 282/290, 298/306, 314/322, 330/338, 346/354, 362/370, 378/386, 394/402, 410/418, 426/434, and 442/450.

2. The antibody or antigen-binding fragment thereof of claim 1, wherein the antibody has one or more of the following characteristics:

(a) interacts with one or more amino acid residues in the receptor binding domain of the MERS-CoV spike protein selected from amino acid residues 367 to 606 of SEQ ID NO: 457;

(b) binds to MERS-CoV spike protein with a dissociation constant (K_D) of less than 18.5 nM, as measured in a surface plasmon resonance assay;

(c) blocks binding of MERS-CoV spike protein to dipeptidyl peptidase 4 (DPP4) by more than 90%, as measured in a blocking ELISA assay;

(d) neutralizes MERS-CoV infectivity of human host cells by more than 90% and with IC_{50} less than 4 nM, as measured in a virus-like particle (VLP) neutralization assay;

(e) neutralizes MERS-CoV infectivity wherein the MERS-CoV comprises an isolate of the virus selected from the group consisting of EMC/2012, Jordan-N3/2012, England-Qatar/2012, Al-Hasa_1_2013, Al-Hasa_2_2013, Al-Hasa_3_2013, Al-Hasa_4_2013, Al-Hasa_12, Al-Hasa_15, Al-Hasa_16, Al-Hasa_17, Al-Hasa_18, Al-Hasa_19, Al-Hasa_21, Al-hasa_25, Bisha_1, Buraidah_1, England 1, Hafr-Al-batin_1, Hafr-Al-Batin_2, Hafr-Al-Batin_6, Jeddah_1,

KFU-HKU 1, KFU-HKU 13, Munich, Qatar3, Qatar4, Riyadh_1, Riyadh_2, Riyadh_3, Riyadh_3, Riyadh_4, Riyadh_5, Riyadh_9, Riyadh_14, Taif_1, UAE, and Wadi-Ad-Dawasir; and

(f) prevents entry of MERS-CoV into a host cell.

3. The antibody or antigen-binding fragment thereof of claim 1, comprising a HCVR having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 18, 34, 50, 66, 82, 98, 114, 122, 130, 138, 146, 154, 170, 178, 186, 202, 218, 234, 250, 266, 282, 298, 314, 330, 346, 362, 378, 394, 410, 426, and 442.

4. The antibody or antigen-binding fragment thereof of claim 1, comprising a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 26, 42, 58, 74, 90, 106, 162, 194, 210, 226, 242, 258, 274, 290, 306, 322, 338, 354, 370, 386, 402, 418, 434, and 450.

5. The antibody or antigen-binding fragment thereof of claim 1, comprising:

(a) a HCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 20, 36, 52, 68, 84, 100, 116, 124, 132, 140, 148, 156, 172, 180, 188, 204, 220, 236, 252, 268, 284, 300, 316, 332, 348, 364, 380, 396, 412, 428, and 444;

(b) a HCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 6, 22, 38, 54, 70, 86, 102, 118, 126, 134, 142, 150, 158, 174, 182, 190, 206, 222, 238, 254, 270, 286, 302, 318, 334, 350, 366, 382, 398, 414, 430, and 446;

(c) a HCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 24, 40, 56, 72, 88, 104, 120, 128, 136, 144, 152, 160, 176, 184, 192, 208, 224, 240, 256, 272, 288, 304, 320, 336, 352, 368, 384, 400, 416, 432, and 448;

(d) a LCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 12, 28, 44, 60, 76, 92, 108, 164, 196, 212, 228, 244, 260, 276, 292, 308, 324, 340, 356, 372, 388, 404, 420, 436, and 452;

(e) a LCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 14, 30, 46, 62, 78, 94, 110, 166, 198, 214, 230, 246, 262, 278, 294, 310, 326, 342, 358, 374, 390, 406, 422, 438, and 454; and

(f) a LCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 32, 48, 64, 80, 96, 112, 168, 200, 216, 232, 248, 264, 280, 296, 312, 328, 344, 360, 376, 392, 408, 424, 440, and 456.

6. An isolated human antibody or antigen-binding fragment thereof that competes for binding to MERS-CoV-S with the antibody or antigen-binding fragment of claim 1.

7. The antibody or antigen-binding fragment thereof of claim 6, wherein the antibody or antigen-binding fragment thereof interacts with the spike protein of a MERS-CoV isolate selected from the group consisting of EMC/2012, Jordan-N3/2012, England-Qatar/2012, Al-Hasa_1_2013, Al-Hasa_2_2013, Al-Hasa_3_2013, Al-Hasa_4_2013, Al-Hasa_12, Al-Hasa_15, Al-Hasa_16, Al-Hasa_17, Al-Hasa_18, Al-Hasa_19, Al-Hasa_21, Al-hasa_25, Bisha_1, Buraidah_1, England 1, Hafr-Al-batin_1, Hafr-Al-Batin_2, Hafr-Al-Batin_6, Jeddah_1, KFU-HKU 1, KFU-HKU 13, Munich, Qatar3, Qatar4, Riyadh_1, Riyadh_2, Riyadh_3, Riyadh_3, Riyadh_4, Riyadh_5, Riyadh_9, Riyadh_14, Taif_1, UAE, and Wadi-Ad-Dawasir.

8. The antibody or antigen-binding fragment thereof of claim 9, wherein the antibody or antigen-binding fragment thereof blocks the binding of MERS-CoV to DPP4 on human cells.

9. The antibody or antigen-binding fragment thereof of claim 7, wherein the antibody or antigen-binding fragment thereof is isolated from a hybridoma cell line selected from the group consisting of HBVX06H05, HBVX11H04, HBVX11D02, HBVZ10E10, HBVY09F08, HBVZ05G02, HBVZ09B06, HBVY01F08, HBVY10G02, HBVY04B06, HBVY07D10, HBVZ08A09, HBVZ05G04, HBVY06C07, HBVY03H06, HBVZ10G06, HBVZ04F10, HBVX11E09, HBVY06H09, HBVZ05B11, HBVY02E05 and HBVZ04C07.

10. An isolated recombinant human monoclonal antibody or antigen-binding fragment thereof that binds to the same epitope as the antibody or antigen-binding fragment of claim 1.

11. A pharmaceutical composition comprising the antibody or antigen-binding fragment thereof of claim 1 and a pharmaceutically acceptable carrier or diluent.

12. A pharmaceutical composition comprising: (a) a first antibody or antigen-binding fragment thereof that binds to MERS-CoV spike protein at a first epitope; (b) a second antibody or antigen-binding fragment thereof that binds to MERS-CoV spike protein at a second epitope, and (c) a pharmaceutically acceptable carrier or diluent, wherein the first antibody or antigen-

binding fragment thereof is a human antibody or antigen-binding fragment comprising a HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 2/10, 18/26, 34/42, 50/58, 66/74, 82/90, 98/106, 114/106, 122/106, 130/106, 138/106, 146/106, 154/162, 170/162, 178/162, 186/194, 202/210, 218/226, 234/242, 250/258, 266/274, 282/290, 298/306, 314/322, 330/338, 346/354, 362/370, 378/386, 394/402, 410/418, 426/434, and 442/450.

13. The pharmaceutical composition of claim 12, wherein at least the first antibody or antigen-binding fragment thereof or the second antibody or antigen-binding fragment thereof blocks MERS-CoV-S binding to DPP4.

14. The pharmaceutical composition of claim 13, wherein the first and second epitopes are present in the receptor binding domain of MERS-CoV spike protein and are distinct and non-overlapping.

15. The pharmaceutical composition of claim 14, wherein the first antibody or antigen-binding fragment thereof comprises a HCVR, wherein the HCVR comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 18, 66, 114, 170, and 218; and a LCVR, wherein the LCVR comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 26, 74, 106, 162, and 226.

16. The antibody or antigen-binding fragment thereof of claim 5 comprising a HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 66/74 and 218/226.

17. The antibody or antigen-binding fragment thereof of claim 16 comprising a HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 66/74.

18. The antibody or antigen-binding fragment thereof of claim 16 comprising a HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 218/226.

19. The antibody or antigen-binding fragment thereof of claim 5 comprising HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 domains of SEQ ID NOs: 68-70-72-76-78-80.

20. The antibody or antigen-binding fragment thereof of claim 5 comprising HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 domains of SEQ ID NOs: 220-222-224-228-230-232.

21. A pharmaceutical composition comprising one or more antibodies or antigen-binding fragments thereof of claim 5 and a pharmaceutically acceptable carrier or diluent.

22. The pharmaceutical composition of claim 15, wherein the first antibody or antigen-binding fragment thereof comprises a HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 66/74 and 218/226.

23. The pharmaceutical composition of claim 22, wherein the first antibody or antigen-binding fragment thereof comprises a HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 66/74.

24. The pharmaceutical composition of claim 22, wherein the first antibody or antigen-binding fragment thereof comprises a HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 218/226.

25. The pharmaceutical composition of claim 23, wherein the second antibody or antigen-binding fragment thereof comprises a HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 218/226.