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Antiviral agents for the treatment, control and prevention of infections by coronaviruses

Abstract

The invention provides compositions and methods that are useful for preventing and treating a coronavirus infection in a subject. More specifically, the invention provides peptides and conjugates and pharmaceutical compositions containing those peptides and conjugates that block fusion of a coronavirus, such as the SARS virus, to a target cell. This blocking mechanism prevents or treats a coronavirus infection, such as a SARS infection, in a subject, such as a human subject.

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Parent Case Text

This application claims the benefit of U.S. Provisional Application No. 60/466,432, filed Apr. 30, 2003, and U.S. Provisional Application No. 60/465,782, filed Apr. 28, 2003. The entire contents of each of the above-identified applications are hereby incorporated by reference.

Claims

What is claimed is:

1. An antiviral molecule, wherein said molecule exhibits antiviral activity against a coronavirus, wherein said molecule comprises a peptide linked to human serum albumin, and wherein said peptide contains up to 40 amino acids and comprises the sequence: VVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIK (SEQ ID NO: 181).

2. A pharmaceutical composition comprising a molecule according to claim 1 and a pharmaceutically acceptable diluent, adjuvant and/or excipient.

3. A method of treating or a SARS infection in a subject, comprising administering to a patient suspected of suffering from said infection an effective amount of a composition according to claim 2.

4. The method according to claim 3, wherein said subject is a human.

5. An antiviral molecule, wherein said molecule exhibits antiviral activity against a coronavirus, wherein said molecule comprises a peptide linked to human serum albumin, and wherein said peptide consists of the sequence: VVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIK (SEQ ID NO: 181).

6. A pharmaceutical composition comprising a molecule according to claim 5 and a pharmaceutically acceptable diluent, adjuvant and/or excipient.

7. A method of treating a SARS infection in a subject, comprising administering to a patient suspected of suffering from said infection an effective amount of a composition according to claim 6.

8. The method according to claim 7, wherein said subject is a human.

Description

BACKGROUND

Severe Acute Respiratory Syndrome (SARS) is an emerging new infectious disease caused by a novel coronavirus that infects humans. See Ksiazek et al., New Engl. J. Med. (http://content.nejm.org/cgi/reprint/NEJMoa030781 v2.pdf, published Apr. 10, 2003). SARS is fatal in about 4 10% of cases reported so far. Initially described in mid February, 2003 in China's Guangdong province as atypical pneumonia, by mid-March, 2003 the World Health Organization (WHO) had received reports of more than 150 new suspected cases of unknown origin or cause. By mid April, 2003, over 4400 cases with 263 deaths of patients diagnosed with symptoms of SARS have been documented from 26 different countries, including Canada, China, Hong Kong, Indonesia, Philippines, Singapore, Thailand, Viet Nam and the United States. In light of the rapid spread of SARS to several countries in a short period of time, the World Health Organization issued a global alert and provided emergency guidance for travellers and airlines. In only a few months after the outbreak was first recognized, SARS became a worldwide threat to global health and global economies. There are presently no known therapies that are effective against SARS, and no vaccine is available. Accordingly, there is an urgent need for antiviral agents that can control or prevent SARS in infected individuals, and that can prevent SARS from spreading.

In general, SARS begins with a fever greater than 100.4.degree. F. [>38.0.degree. C.]. Other symptoms may include headache, an overall feeling of discomfort, and body aches. Some people also experience mild respiratory symptoms. After 2 to 7 days, SARS patients may develop a dry cough and have trouble breathing.

The primary way that SARS appears to spread is by close person-to-person contact. Most cases of SARS have involved people who cared for or lived with someone with SARS, or had direct contact with infectious material (for example, respiratory secretions) from a person who has SARS. Potential ways in which SARS can be spread include touching the skin of other people or objects that are contaminated with infectious droplets followed by touching of eye(s), nose, or mouth. This can happen when someone who is sick with SARS coughs or sneezes droplets onto themselves, other people, or nearby surfaces. It also is possible that SARS can be spread more broadly through the air or by other ways that are currently not known.

Scientists at the Centers for Disease Control and Prevention (CDC) and other laboratories around the world have detected a previously unrecognized coronavirus in patients with SARS. The evidence for a coronavirus was based on genetic fingerprint and electron microscopic ultrastructural studies and was widely reported in the popular press. Viologists at the CDC, WHO and numerous academic laboratories all reported that a coronavirus is the leading hypothesis for the cause of SARS.

The CDC recently reported sequencing the genome for SARS-CoV (Urbani strain), a strain of a novel human coronavirus believed to be responsible for SARS. The sequence data confirm that the SARS virus is a previously unrecognized coronavirus. The virus was cultured from cells taken from a throat culture taken from a SARS patients and grown in Vero cells (African green monkey kidney cells) in order to reproduce the ribonucleic acid (RNA) of the disease-causing coronavirus. The new sequence has 29,727 nucleotides, which places it well within the typical RNA boundaries for coronaviruses. Members of this viral family tend to have between 29,000 and 31,000 nucleotides. See Lai et al., Adv. Virus Res. 48:1, (1997). The genome organization of the SARS virus also is similar to that of other coronaviruses.

The genome sequence of SARS-CoV (Urbani) is available from GenBank at the Web site for the National Center for Biotechnology Information, National Library of Medicine http://www.ncbi.nim.nih.gov/. The accession number for the sequence of SARS-CoV (Urbani strain) is ay278741. The present inventors have used these sequence data to identify molecular targets that can be exploited to design safe and effective novel antiviral therapies that can be used to treat SARS and to stem the tide of the growing epidemic.

SUMMARY OF THE INVENTION

In accordance with a first aspect of the invention there is provided an antiviral peptide having between 7 and 50 amino acids, where the peptide exhibits antiviral activity against a coronavirus, and where the peptide contains a sequence comprising at least 7 contiguous amino acids from one of the following sequences:

DVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIK (SEQ ID NO: 1);

QIPFAMQMAYRFNGIGVTQNVLYENQKQIANQFNKAISQIQESLT (SEQ ID NO: 2);

ESLTTSTALGKLQDVVNQNAQALNTLVKQLSSNFGAISS (SEQ ID NO: 3);

GKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDKVEAE (SEQ ID NO: 4); and

RLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDF (SEQ ID NO: 5).

In accordance with a second aspect of the invention there is provided an antiviral peptide having between 7 and 50 amino acids, where the peptide exhibits antiviral activity against a coronavirus, and where the peptide contains a sequence comprising at least 7 contiguous amino acids from the sequence:

DVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIK (SEQ ID NO: 1);

where the amino acids at bold letter positions can be substituted with an amino acid selected from the group consisting of I, L, V, W, Y, F, N, Q, S, T, D, E, G, H, and M, and where amino acids in non-bold positions can be any amino acid except proline.

TABLE-US-00001 SVVNIQK (SEQ ID NO:6) VVNIQKE (SEQ ID NO:7) VNIQKEI (SEQ ID NO:8) NIQKEID (SEQ ID NO:9) IQKEIDR (SEQ ID NO:10) QKEIDRL (SEQ ID NO:11) KEIDRLN (SEQ ID NO:12) EIDRLNE (SEQ ID NO:13) IDRLNEV (SEQ ID NO:14) DRLNEVA (SEQ ID NO:15) RLNEVAK (SEQ ID NO:16) LNEVAKN (SEQ ID NO:17) NEVAKNL (SEQ ID NO:18) EVAKNLN (SEQ ID NO:19) VAKNLNE (SEQ ID NO:20) AKNLNES (SEQ ID NO:21) KNLNESL (SEQ ID NO:22) NLNESLI (SEQ ID NO:23) LNESLID (SEQ ID NO:24) NESLIDL (SEQ ID NO:25) ESLIDLQ (SEQ ID NO:26) SLIDLQE (SEQ ID NO:27) LIDLQEL (SEQ ID NO:28) IDLQELG (SEQ ID NO:29) DLQELGK (SEQ ID NO:30) LQELGKY (SEQ ID NO:31) QELGKYE (SEQ ID NO:32) ELGKYEQ (SEQ ID NO:33) LGKYEQY (SEQ ID NO:34) GKYEQYI (SEQ ID NO:35) KYEQYIK (SEQ ID NO:36) QIPFAMQ (SEQ ID NO:37) IPFAMQM (SEQ ID NO:38) PFAMQMA (SEQ ID NO:39) FAMQMAY (SEQ ID NO:40) AMQMAYR (SEQ ID NO:41) MQMAYRF (SEQ ID NO:42) QMAYRFN (SEQ ID NO:43) MAYRFNG (SEQ ID NO:44) AYRFNGI (SEQ ID NO:45) YRFNGIG (SEQ ID NO:46) RFNGIGV (SEQ ID NO:47) FNGIGVT (SEQ ID NO:48) NGIGVTQ (SEQ ID NO:49) IGVTQNV (SEQ ID NO:50) GVTQNVL (SEQ ID NO:51) VTQNVLY (SEQ ID NO:52) TQNVLYE (SEQ ID NO:53) QNVLYEN (SEQ ID NO:54) NVLYENQ (SEQ ID NO:55) VLYENQK (SEQ ID NO:56) LYENQKQ (SEQ ID NO:57) YENQKQI (SEQ ID NO:58) ENQKQIA (SEQ ID NO:59) NQKQIAN (SEQ ID NO:60) QKQIANQ (SEQ ID NO:61) KQIANQF (SEQ ID NO:62) QIANQFN (SEQ ID NO:63) IANQFNK (SEQ ID NO:64) ANQFNKA (SEQ ID NO:65) NQFNKAI (SEQ ID NO:66) QFNKAIS (SEQ ID NO:67) FNKAISQ (SEQ ID NO:68) NKAISQI (SEQ ID NO:69) KAISQIQ (SEQ ID NO:70) AISQIQE (SEQ ID NO:71) ISQIQES (SEQ ID NO:72) SQIQESL (SEQ ID NO:73) QIQESLT (SEQ ID NO:74) ESLTTTS (SEQ ID NO:75) SLTTTST (SEQ ID NO:76) LTTTSTA (SEQ ID NO:77) TTTSTAL (SEQ ID NO:78) TTSTALG (SEQ ID NO:79) TSTALGK (SEQ ID NO:80) STALGKL (SEQ ID NO:81) TALGKLQ (SEQ ID NO:82) ALGKLQD (SEQ ID NO:83) LGKLQDV (SEQ ID NO:84) GKLQDVV (SEQ ID NO:85) KLQDVVN (SEQ ID NO:86) LQDVVNQ (SEQ ID NO:87) QDVVNQN (SEQ ID NO:88) DVVNQNA (SEQ ID NO:89) VVNQNAQ (SEQ ID NO:90) VNQNAQA (SEQ ID NO:91) NQNAQAL (SEQ ID NO:92) QNAQALN (SEQ ID NO:93) NAQALNT (SEQ ID NO:94) AQALNTL (SEQ ID NO:95) QALNTLV (SEQ ID NO:96) ALNTLVK (SEQ ID NO:97) LNTLVKQ (SEQ ID NO:98) NTLVKQL (SEQ ID NO:99) TLVKQLS (SEQ ID NO:100) LVKQLSS (SEQ ID NO:101) VKQLSSN (SEQ ID NO:102) KQLSSNF (SEQ ID NO:103) QLSSNFG (SEQ ID NO:104) LSSNFGA (SEQ ID NO:105) SSNFGAI (SEQ ID NO:106) SNFGAIS (SEQ ID NO:107) NFGAISS (SEQ ID NO:108) LQDVVNQ (SEQ ID NO:109) QDVVNQN (SEQ ID NO:110) DVVNQNA (SEQ ID NO:111) VVNQNAQ (SEQ ID NO:112) VNQNAQA (SEQ ID NO:113) NQNAQAL (SEQ ID NO:114) QNAQALN (SEQ ID NO:115) NAQALNT (SEQ ID NO:116) AQALNTL (SEQ ID NO:117) QALNTLV (SEQ ID NO:118) ALNTLVK (SEQ ID NO:119) LNTLVKQ (SEQ ID NO:120) NTLVKQL (SEQ ID NO:121) TLVKQLS (SEQ ID NO:122) LVKQLSS (SEQ ID NO:123) VKQLSSN (SEQ ID NO:124) KQLSSNF (SEQ ID NO:125) QLSSNFG (SEQ ID NO:126) LSSNFGA (SEQ ID NO:127) SSNFGAI (SEQ ID NO:128) SNFGAIS (SEQ ID NO:129) NFGAISS (SEQ ID NO:130)

FGAISSV (SEQ ID NO:131) GAISSVL (SEQ ID NO:132) AISSVLN (SEQ ID NO:133) ISSVLND (SEQ ID NO:134) SSVLNDI (SEQ ID NO:135) SVLNDIL (SEQ ID NO:136) VLNDILS (SEQ ID NO:137) LNDILSR (SEQ ID NO:138) NDILSRL (SEQ ID NO:139) DILSRLD (SEQ ID NO:140) ILSRLDK (SEQ ID NO:141) LSRLDK (SEQ ID NO:142) SRLDKV (SEQ ID NO:143) RLDKVE (SEQ ID NO:144) LDKVEA, (SEQ ID NO:145) RLITGRL (SEQ ID NO:146) LITGRLQ (SEQ ID NO:147) ITGRLQS (SEQ ID NO:148) TGRLQSL (SEQ ID NO:149) GRLQSLQ (SEQ ID NO:150) RLQSLQT (SEQ ID NO:151) LQSLQTY (SEQ ID NO:152) QSLQTYV (SEQ ID NO:153) SLQTYVT (SEQ ID NO:154) LQTYVTQ (SEQ ID NO:155) QTYVTQQ (SEQ ID NO:156) TYVTQQL (SEQ ID NO:157) YVTQQLI (SEQ ID NO:158) VTQQLIR (SEQ ID NO:159) TQQLIRA (SEQ ID NO:160) QQLIRAA (SEQ ID NO:161)

QLIRAAE (SEQ ID NO:162) LIRAAEI (SEQ ID NO:163) IRAAEIR (SEQ ID NO:164) RAAEIRA (SEQ ID NO:165) AAEIRAS (SEQ ID NO:166) AEIRASA (SEQ ID NO:167) EIRASAN (SEQ ID NO:168) IRASANL (SEQ ID NO:169) RASANLA (SEQ ID NO:170) ASANLAA (SEQ ID NO:171) SANLAAT (SEQ ID NO:172) ANLAATK (SEQ ID NO:173) NLAATKM (SEQ ID NO:174) LAATKMS (SEQ ID NO:175) AATKMSE (SEQ ID NO:176) ATKMSEC (SEQ ID NO:177) TKMSECV (SEQ ID NO:178) KMSECVL and (SEQ ID NO:179) MSECVLG. (SEQ ID NO:180)

The peptide may contain at least 10, 15, 20, 25, 30, 35, or 40 contiguous amino acids from one of the sequences:

VVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIK (SEQ ID NO: 181);

QIPFAMQMAYRFNGIGVTQNVLYENQKQIANQFNKAISQIQESLT (SEQ ID NO: 2);

ESLTTSTALGKLQDVVNQNAQALNTLVKQLSSNFGAISS (SEQ ID NO: 3);

GKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDKVEAE (SEQ ID NO: 4); and

RLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDF (SEQ ID NO: 5).

Any of the peptides above may be linked to a carrier protein, such as human serum albumin, for example.

In accordance with a third aspect of the invention there is provided an antiviral composition comprising a peptide X having between 7 and 50 amino acids, where the peptide exhibits antiviral activity against a coronavirus, and where the composition has the structure:

B--X--Z,

where B is an amino acid sequence containing up to about 43 amino acids, or B is an amino group, an acetyl group, a 9-fluorenylmethoxy-carbonyl group, a hydrophobic group, or a macromolecule carrier group, or B is a carrier protein, in which case B may contain more than 8 amino acids, and may also comprises a linker peptide sequence that connects the antiviral sequence to the carrier protein; Z is an amino acid sequence containing up to about 43 amino acids, or Z comprises a carboxyl group, an amido group, a hydrophobic group, or a macromolecular carrier group, or Z is a carrier protein, such as HSA, in which case Z may contain more than 8 amino acids, and may also comprise a linker peptide sequence that connects the antiviral sequence to the carrier protein; where when considered together B and Z must contain at least 8 amino acids between the B and Z groups; and where X is a peptide sequence comprising at least 7 contiguous amino acids from one of the following sequences:

VVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIK (SEQ ID NO: 181);

QIPFAMQMAYRFNGIGVTQNVLYENQKQIANQFNKAISQIQESLT (SEQ ID NO: 2);

ESLTTSTALGKLQDVVNQNAQALNTLVKQLSSNFGAISS (SEQ ID NO: 3);

GKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDKVEAE (SEQ ID NO: 4); and

RLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDF (SEQ ID NO: 5).

X may contain, for example, at least 10, 15, 20, 25, 30, 35, or 40 contiguous amino acids from one of the following sequences:

VVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIK (SEQ ID NO: 181);

QIPFAMQMAYRFNGIGVTQNVLYENQKQIANQFNKAISQIQESLT (SEQ ID NO: 2);

ESLTTTSTALGKLQDVVNQNAQALNTLVKQLSSNFGAISS (SEQ ID NO: 3);

GKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDKVEAE (SEQ ID NO: 4); and

RLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDF (SEQ ID NO: 5).

In accordance with a fourth aspect of the invention there is provided an antiviral peptide having between 7 and 50 amino acids, where the peptide exhibits antiviral activity against a coronavirus, and where the peptide comprises a sequence that exhibits identity in any two of the seven positions of a contiguous heptapeptide, where the contiguous heptapeptide comprises 7 contiguous amino acids from one of the following sequences:

VVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIK (SEQ ID NO: 181);

QIPFAMQMAYRFNGIGVTQNVLYENQKQIANQFNKAISQIQESLT (SEQ ID NO: 2);

ESLTTTSTALGKLQDVVNQNAQALNTLVKQLSSNFGAISS (SEQ ID NO: 3);

GKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDKVEAE (SEQ ID NO: 4); and

RLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDF (SEQ ID NO: 5).

The sequence identity may be located, for example, in the ith and i+4th positions in the contiguous heptapeptides.

In accordance with another aspect of the invention there is provided a pharmaceutical composition comprising a peptide or composition as described above and a pharmaceutically acceptable diluent, adjuvant and/or excipient.

In accordance with yet another aspect of the invention there is provided a method of treating or preventing a coronavirus infection in a subject, comprising administering to a patient suspected of suffering from the infection an effective amount of a peptide or composition as described above. The subject may be a human, a cow, pig, or chicken.

Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows a model of SARS-CoV fusing with the host cell and of an inhibitor blocking fusion between SARS-CoV and the host cell membrane. SARS-CoV contains a surface spike protein consisting of S1 and S2 domains. SARS-CoV binds to the host cell through interaction between S1 domain and the host cell receptor, ACE2. The HR2 helices of the S2 protein fold over and interact with the HR1 helices to form "hairpin-like" structures, which draw both the viral and host cell membranes together for fusion. An inhibitor is shown in

the lower portion binding to the HR1 trimer and blocking association of HR1 and HR2, thereby inhibiting SARSCoV fusion with the host cell. Model from www.nybloodcenter.org/pdf/Anti-SARS%20Peptide %20Model.pdf by Dr. Shibo Jiang.

FIG. 2 shows some representative examples of results of secondary structure prediction and homology analyses on peptides (SEQ ID NOS 197 and 2, respectively in order of appearance) from SARS coronavirus, isolate Tor2, E2 glycoprotein precursor. Amino acids in the most highly predicted helical regions are listed in bold. E, H and L designations are from the secondary structure prediction algorithm and refer to extended or coil, helix and loop regions, respectively. Numbers refer to the probable accuracy of the prediction, from lowest (0) to highest (9).

DESCRIPTION OF THE INVENTION

The invention provides compositions and methods that are useful for preventing and treating a coronavirus infection in a subject. More specifically, the invention provides peptides and conjugates and pharmaceutical compositions containing those peptides and conjugates that block fusion of a coronavirus, such as the SARS virus, to a target cell. This blocking mechanism prevents or treats a coronavirus infection, such as a SARS infection, in a subject, such as a human subject.

SARS-CoV is a Novel Coronavirus

The sequence of the genome of the SARS-CoV was downloaded from the CDC website and translated into ten putative open reading frames (ORFs). The amino acid sequences corresponding to putative proteins encoded by the ten ORFs were analyzed for homology to existing proteins in the proteome using BLAST, a protein database searching program. See Altschul et al, Nucleic Acids Res. 25: 3389 (1997). A number of open reading frames were found to encode proteins with significant sequence homology to proteins from known coronaviruses. For example, ORF1 corresponds to a coronavirus polymerase protein (polymerase 1a, 1b), and ORF3 corresponds to a coronavirus spike protein (S). The homology and organization of the genome provide additional convincing evidence that the SARS virus is a coronavirus.

Coronaviruses previously have been grouped into three categories based on cross-reactivity of antibodies backed up by genetic data. The two previously identified human coronaviruses fall into two different groups. One of these groups includes a number of enteric coronoviruses that cause gastroenteritis. The other includes coronaviruses that cause respiratory or neurological diseases in diverse species. The third group includes coronaviruses isolated from avian species.

The sequence of the S protein from SARS-CoV shares about 30 35% identity throughout its 1260 or so amino acid length with S proteins from all three groups of coronaviruses, including coronaviruses from humans, cows, pigs, mice and chickens. Based on the sequence homology analysis, the SARS coronavirus represents the first, and so far only, member of a new fourth coronavirus group.

Spike Proteins are Required for Viral Entry

Numerous studies have shown that entry of enveloped viruses into host cells requires membrane fusion between virus and host cell. For most animal viruses, this fusion function is mediated by a single envelope glycoprotein on virions. The S protein has been shown to be the fusion protein that mediates cellular entry for coronavirus. See Spaan et al., J. Gen. Virol. 69:2939 (1988).

The S protein forms the peplomer projections that protrude from the virion surface as seen in electron micrographs. Peplomers are thought to be composed of three oligomerized S protein molecules. See Delmas et al., J. Virol. 64:5367 (1990).

The S protein is cleaved by host proteases during virus assembly into two similarly-sized subunits: S1 and S2. The C-terminal S2 subunit, which associates non-covalently with the N-terminal S1, anchors the S protein to the membrane through a transmembrane domain, while the S1 subunit contains the receptor binding activity of the S protein.

Helical Heptad Repeats in the S2 Subunit are Required for Fusion

Reviews of the roles of the coronavirus spike proteins in viral entry and pathogenesis can be found in Gallagher et al., Virology 279:371 (2001) and Luo et al, J. Virol. 73:8152 (1999). Several studies suggest that the S2 subunit is required for viral fusion. Functional mutagenesis studies indicate that critical residues for fusion are located within two regions in S2 that have been identified as heptad repeat regions. Helical heptad repeats are found in fusion proteins from other enveloped viruses, including paramyxoviruses, such as influenza virus, retroviruses, such as HIV, and filoviruses, such as Ebola virus. The existence of two heptad repeats, HR1 and HR2, separated by a non-helical spacer in the S2 subunit of coronaviruses is suggestive of the formation of a coiled-coil or "trimer of hairpins" fusogenic complex similar to the fusogenic structures thought to be formed by the helical heptad repeats in the fusion glycoproteins of, for example, HIV, influenza virus and Ebola virus.

In the case of HIV, a peptide sequence that mimics either HR1 or HR2 can prevent HIV fusion and block viral replication. Enfurvitide, a 38-residue peptide based on the sequence of HR2 of the HIV glycoprotein, is used clinically to treat HIV/AIDS. The present inventors reasoned that peptides that can bind to coiled-coil intermediates of coronaviruses should block the formation of a productive fusogenic complex and prevent virus entry (FIG. 1).

SARS-CoV Contains High Homology Regions with HR1 and HR2 of Coronaviruses that are Predicted to be Helical.

The amino acid sequence homology between the S protein of SARS-CoV and other coronaviruses was evaluated throughout its length. The highest homology resides in regions that overlap with the HR1 and HR2 heptad repeats in known coronoaviruses. Analysis of the S protein using secondary structure prediction methods (see for example program PROF as implemented on the CUBIST protein prediction server @http://cubic.bioc.columbia.edu) revealed that the HR1 and HR2 regions are strongly predicted to be helical (FIG. 2). The HR1 and HR2 regions were divided into five contiguous amino acids segments that are most strongly predicted to be helical. In the same fashion as observed with HIV gp41, heptad-containing sequences derived from the HR1 segments bind to HR2 helices. Similarly, heptad-containing sequences derived from the HR2 segments can bind to the HR1 helices. Peptides that bind to either HR1 or HR2 prevent virus entry, possibly by disrupting formation of the fusogenic complex. Such peptides, and compositions containing these peptides, are useful for treating infections caused by coronaviruses, and as prophylactics against coronavirus infection. These peptides are particularly useful for preventing and treating SARS infection.

The N peptides are predicted to form a trimeric coiled-coil with 3-fold symmetry similar to HR1 of HIV-gp41. The C peptides are predicted to form helices that bind in the grooves formed by adjacent N-helices in the coiled coil, similar to HR2 in HIV gp41. These peptide sequences are predicted to form continuous alpha helices constructed of series of contiguous, or nearly contiguous, helical heptad repeats in which the ith and i+4th residues are important or critical for oligomer formation.

The peptides contained in the S protein have the following sequences (most strongly predicted helical sequences are shown in bold)

C-terminal, HR2 peptide 1 (SEQ ID NO: 182)

PDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIK

N-Terminal, HR1 peptide 2 (SEQ ID NO: 2)
QIPFAMQMAYRFNGIGVTQNVLYENQKQIANQFNKAISQIQESLT

N-Terminal, HR1 peptide 3 (SEQ ID NO: 3) ESLTTTSTALGKLQDVVNQNAQALNTLVKQLSSNFGAISS

N-Terminal, HR1 peptide 4 (SEQ ID NO: 4)
GKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDKVEAE

N-Terminal, HR1 peptide 5 (SEQ ID NO: 183)
QALNTLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLITGRLQS

N-Terminal, HR1 peptide 6 (SEQ ID NO: 5)
RLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDF

These sequences were used to design peptide inhibitors having between about 15 and about 50 amino acids, advantageously having 15 to 50 amino acids and containing at least 7 contiguous amino acids from one of the sequences shown above. Thus, for example, the peptides may contain one of the following 7 amino acid sequences:

for the C-terminal HR2 peptide 1 the peptide may contain one of the following sequences

TABLE-US-00002 PDVDLGD (SEQ ID NO:184) DVDLGDI (SEQ ID NO:185) VDLGDIS (SEQ ID NO:186) DLGDISG (SEQ ID NO:187) LGDISGIN (SEQ ID NO:188) GDISGIN (SEQ ID NO:189) DISGINA (SEQ ID NO:190) ISGINAS (SEQ ID NO:191) SGINASV (SEQ ID NO:192) GINASVV (SEQ ID NO:193) INASVVN (SEQ ID NO:194) NASVVNI (SEQ ID NO:195) ASVVNIQ (SEQ ID NO:196) SVVNIQK (SEQ ID NO:6) VVNIQKE (SEQ ID NO:7) VNIQKEI (SEQ ID NO:8) NIQKEID (SEQ ID NO:9) IQKEIDR (SEQ ID NO:10) QKEIDRL (SEQ ID NO:11) KEIDRLN (SEQ ID NO:12) EIDRLNE (SEQ ID NO:13) IDRLNEV (SEQ ID NO:14) DRLNEVA (SEQ ID NO:15) RLNEVAK (SEQ ID NO:16) LNEVAKN (SEQ ID NO:17) NEVAKNL (SEQ ID NO:18) EVAKNLN (SEQ ID NO:19) VAKNLNE (SEQ ID NO:20) AKNLNES (SEQ ID NO:21) KNLNESL (SEQ ID NO:22) NLNESLI (SEQ ID NO:23) LNESLID (SEQ lID NO:24) NESLIDL (SEQ ID NO:25) ESLIDLQ (SEQ ID NO:26) SLIDLQE (SEQ ID NO:27) LIDLQEL (SEQ ID NO:28) IDLQELG (SEQ ID NO:29) DLQELGK (SEQ ID NO:30) LQELGKY (SEQ ID NO:31) QELGKYE (SEQ ID NO:32) ELGKYEQ (SEQ ID NO:33) LGKYEQY (SEQ ID NO:34) GKYEQYI (SEQ ID NO:35) KYEQYIK (SEQ ID NO:36) for the N-Terminal, HR1 peptide 2 the peptide may contain one of the following sequences QIPFAMQ (SEQ ID NO:37) IPFAMQM (SEQ ID NO:38) PFAMQMA (SEQ ID NO:39) FAMQMAY (SEQ ID NO:40) AMQMAYR (SEQ ID NO:41) MQMAYRF (SEQ ID NO:42) QMAYRFN (SEQ ID NO:43) MAYRFNG (SEQ ID NO:44) AYRFNGI (SEQ ID NO:45) YRFNGIG (SEQ ID NO:46) RFNGIGV (SEQ ID NO:47) FNGIGVT (SEQ ID NO:48) NGIGVTQ (SEQ ID NO:49) IGVTQNV (SEQ ID NO:50) GVTQNVL (SEQ ID NO:51) VTQNVLY (SEQ ID NO:52) TQNVLYE (SEQ ID NO:53) QNYLYEN (SEQ ID NO:54) NVLYENQ (SEQ ID NO:55) VLYENQK (SEQ ID NO:56) LYENQKQ (SEQ ID NO:57) YENQKQI (SEQ ID NO:58) ENQKQIA (SEQ ID NO:59) NQKQIAN (SEQ ID NO:60) QKQIANQ (SEQ ID NO:61) KQIANQF (SEQ ID NO:62) QIANQFN (SEQ ID NO:63) IANQFNK (SEQ ID NO:64) ANQFNKA (SEQ ID NO:65) NQFNKAI (SEQ ID NO:66) QFNKAIS (SEQ ID NO:67) FNKAISQ (SEQ ID NO:68) NKAISQI (SEQ ID NO:69) KAISQIQ (SEQ ID NO:70) AISQIQE (SEQ ID NO:71) ISQIQES (SEQ ID NO:72) SQIQESL (SEQ ID NO:73) QIQESLT (SEQ ID NO:74) for the N-Terminal, HR1 peptide 3, the peptide may contain one of the following sequences ESLTTTS (SEQ ID NO:75) SLTTTST (SEQ ID NO:76) LTTTSTA (SEQ ID NO:77) TTTSTAL (SEQ ID NO:78) TTSTALG (SEQ ID NO:79) TSTALGK (SEQ ID NO:80) STALGKL (SEQ ID NO:81) TALGKLQ (SEQ ID NO:82) ALGKLQD (SEQ ID NO:83) LGKLQDV (SEQ ID NO:84) GKLQDVV (SEQ ID NO:85)

KLQDVVN (SEQ ID NO:86) LQDVVNQ (SEQ ID NO:87) QDVVNQN (SEQ ID NO:88) DVVNQNA (SEQ ID NO:89) VVNQNAQ (SEQ ID NO:90) VNQNAQA (SEQ ID NO:91) NQNAQAL (SEQ ID NO:92) QNAQALN (SEQ ID NO:93) NAQALNT (SEQ ID NO:94) AQALNTL (SEQ ID NO:95) QALNTLV (SEQ ID NO:96) ALNTLVK (SEQ ID NO:97) LNTLVKQ (SEQ ID NO:98) NTLVKQL (SEQ ID NO:99) TLVKQLS (SEQ LB NO:100) LVKQLSS (SEQ ID NO:101) VKQLSSN (SEQ ID NO:102) KQLSSNF (SEQ ID NO:103) QLSSNFG (SEQ ID NO:104) LSSNFGA (SEQ ID NO:105) SSNFGAI (SEQ ID NO:106) SNFGAIS (SEQ ID NO:107) NFGAISS (SEQ ID NO:108) for the N-Terminal, HR1 peptide 4, the peptide may contain one of the following sequences: LQDVVNQ (SEQ ID NO:109) QDVVNQN (SEQ ID NO:110) DVVNQNA (SEQ ID NO:111) VVNQNAQ (SEQ ID NO:112) VNQNAQA (SEQ ID NO:113) NQNAQAL (SEQ ID NO:114)

QNAQALN (SEQ ID NO:115) NAQALNT (SEQ ID NO:116) AQALNTL (SEQ ID NO:117) QALNTLV (SEQ ID NO:118) ALNTLVK (SEQ ID NO:119) LNTLVKQ (SEQ ID NO:120) NTLVKQL (SEQ ID NO:121) TLVKQLS (SEQ ID NO:122) LVKQLSS (SEQ ID NO:123) VKQLSSN (SEQ ID NO:124) KQLSSNF (SEQ ID NO:125) QLSSNFG (SEQ ID NO:126) LSSNFGA (SEQ ID NO:127) SSNFGAI (SEQ ID NO:128) SNFGAIS (SEQ ID NO:129) NEGAISS (SEQ ID NO:130) FGAISSV (SEQ ID NO:131) GAISSVL (SEQ ID NO:132) AISSVLN (SEQ ID NO:133) ISSVLND (SEQ ID NO:134) SSVLNDI (SEQ ID NO:135) SVLNDIL (SEQ ID NO:136) VLNDILS (SEQ ID NO:137) LNDILSR (SEQ ID NO:138) NDILSRL (SEQ ID NO:139) DILSRLD (SEQ ID NO:140) ILSRLDK (SEQ ID NO:141) LSRLDK (SEQ ID NO:142) SRLDKV (SEQ ID NO:143) RLDKVE (SEQ ID NO:144) LDKVEA, (SEQ ID NO:145) and for the N-Terminal, HR1 peptide 5 the peptide may contain one of the following sequences: RLITGRL (SEQ ID NO:146) LITGRLQ (SEQ ID NO:147) ITGRLQS (SEQ ID NO:148) TGRLQSL (SEQ ID NO:149) GRLQSLQ (SEQ ID NO:150) RLQSLQT (SEQ ID NO:151) LQSLQTY (SEQ ID NO:152) QSLQTYV (SEQ ID NO:153) SLQTYVT (SEQ ID NO:154) LQTYVTQ (SEQ ID NO:155) QTYVTQQ (SEQ ID NO:156) TYVTQQL (SEQ ID NO:157) YVTQQLI (SEQ ID NO:158) VTQQLIR (SEQ ID NO:159) TQQLIRA (SEQ ID NO:160) QQLIRAA (SEQ ID NO:161) QLIRAAE (SEQ ID NO:162) LIRAAEI (SEQ ID NO:163) IRAAEIR (SEQ ID NO:164) RAAEIRA (SEQ ID NO:165) AAEIRAS (SEQ ID NO:166) AFIRASA (SEQ ID NO:167) EIRASAN (SEQ ID NO:168) IRASANL (SEQ ID NO:169) RASANLA (SEQ ID NO:170) ASANLAA (SEQ ID NO:171) SANLAAT (SEQ ID NO:172) ANLAATK (SEQ ID NO:173) NLAATKM (SEQ ID NO:174) LAATKMS (SEQ ID NO:175) AATKMSE (SEQ ID NO:176) ATKMSEC (SEQ ID NO:177) TKMSECV (SEQ ID NO:178) KMSECVL (SEQ ID NO:179) MSECVLG (SEQ ID NO:180)

Moreover, because only certain of the amino acids of the peptide make contact in the grooves formed by adjacent N-helices in the coiled coil, amino acids at non-groove binding positions can be replaced with essentially any other amino acid to make "mutated" peptide inhibitors. In addition, amino acids at positions that make groove contact also may be replaced with certain preferred amino acids. Thus, in the peptide

DVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIK; (SEQ ID NO: 1)

the amino acids at bold letter positions can be substituted with an amino acid selected from the group consisting of I, L, V, W, Y, F, N, Q, S, T, D, E, G, H, and M. Of these amino acids, I, L, V, W, and Y are most preferred, and F, N, Q, S, and T are next most preferred, although D, E, G, H, and M also may be used. The amino acids in non-bold positions can be any amino acid except proline, which is predicted to break the helical structure and therefore prevent groove binding. As with the above peptide, a peptide containing any seven contiguous residues of such a mutated peptide can be used.

These peptide sequences are contained within a longer peptide sequence containing at least 15 and up to 50 amino acids. The additional amino acids may be at the N and/or C termini of the sequences shown.

The peptide inhibitors may also contain at least 10, 15, 20, 25, 30, 35, or 40 contiguous amino acids from one

of the sequences shown above, while remaining within the length limitations described above. The peptides may also be linked to carriers, such as carrier proteins, described in more detail below, in which case the entire molecule may contain more than 50 amino acids, but the portion of the molecule responsible for cell binding will still contain up to 50 amino acids.

The peptide of the invention may have the following general structure:

B--X--Z,

where B is an amino acid sequence containing up to about 43 amino acids, or B is an amino group, an acetyl group, a 9-fluorenylmethoxy-carbonyl group, a hydrophobic group, or a macromolecule carrier group. B also may comprise a carrier protein, such as HSA, in which case B may contain more than 8 amino acids, and may also comprises a linker peptide sequence that connects the antiviral sequence to the carrier protein.

Z is an amino acid sequence containing up to about 43 amino acids, or Z comprises a carboxyl group, an amido group, a hydrophobic group, or a macromolecular carrier group. Z also may comprise a carrier protein, such as HSA, in which case Z may contain more than 8 amino acids, and may also comprise a linker peptide sequence that connects the antiviral sequence to the carrier protein. The peptide and the carrier may also be linked as a chemical conjugate, via a linker such as a maleimide linker of the type that is commercially available from, for example, Pierce (Rockford, Ill.).

When considered together B and Z must contain at least 8 amino acids between the B and Z groups. Typically, only one of B and Z is a macromolecule or carrier protein

X is any 7, 10, 15, 20, 25, 30, 35, or 40 contiguous amino acids from the C or N peptides identified above.

The peptides of the invention also may comprise peptide sequences that exhibit 70% or more sequence identity with at least 7 10, 15, 20, 25, 30, 35, or 40 contiguous amino acids from one of the sequences shown above, while remaining within the length limitations described above.

The peptides of the invention also may comprise peptide sequences that exhibit identity in any two of the seven positions of the contiguous heptapeptide peptides described above, while remaining within the length limitations described above. This sequence identity advantageously may be located in the ith and i+4th positions in the contiguous heptapeptide peptides described above.

Ex vivo conjugation of the peptides of the invention moiety to a macromolecule such as HSA produces a highly soluble conjugate that can be purified and administered in tightly controlled dosage. The cloaked conjugate is biologically active as the conjugate, i.e. it does not act as a prodrug that releases the peptide moiety from the conjugate and cleavage of the conjugate is not required for biological activity. Moreover, once administered to a subject the conjugate has a surprisingly long in vivo half-life, has excellent tissue distribution and produces sustained activity corresponding to the activity of the biologically active moiety of the conjugate.

Advantageously, the peptide and the carrier protein and the macromolecule are linked in an approximately 1:1 ratio, to avoid "haptenization" of the biologically active moiety and generation of an immune response to the conjugate. Moreover, the peptide is advantageously appended to a single site in the macromolecule. For example, selective linkage to the unusually reactive cysteine 34 (C34) of HSA may be used. Methods for selective linkage to C34 using, for example, a maleimide containing linker, are known in the art.

In the event that more than one molecule of peptide is linked to the macromolecule, this is advantageously achieved via a "multivalent" linker that is attached to a single point of the macromolecule. For example, a

linker can be appended to C34 of HSA that permits attachment of a plurality of peptides to the linker. Multivalent linkers are known in the art and can contain, for example, a thiophilic group for reaction with C34 of HSA, and multiple nucleophilic (such as NH or OH) or electrophilic (such as activated ester) groups that permit attachment of a plurality of peptides to the linker.

Preparation of Peptides of the Invention

The peptides of the invention may be synthesized or prepared by techniques well known in the art. Peptide synthesizers are commercially available from, for example, Applied Biosystems or Milligen/Biosearch. See also, for example, Creighton, 1983, Proteins: Structures and Molecular Principles, W. H. Freeman and Co., N.Y., which is incorporated herein by reference in its entirety. Short peptides, for example, can be synthesized on a solid support or in solution. Longer peptides, or fusions of longer peptides with carrier proteins such as human serum albumin, may be made using recombinant DNA techniques. Nucleotide sequences encoding the desired peptides or fusion proteins containing the peptides may be synthesized, and/or cloned, and expressed according to techniques well known to those of ordinary skill in the art. See, for example, Sambrook, et al., 1989, Molecular Cloning, A Laboratory Manual, Vols. 1 3, Cold Spring Harbor Press, N.Y.

The peptides also may be synthesized such that one or more of the bonds linking the amino acid residues of the peptides are non-peptide bonds. Alternative non-peptide bonds may be formed by reactions well known to those in the art, and may include, but are not limited to imino, ester, hydrazide, semicarbazide, and azo bonds. In yet another embodiment of the invention, peptides comprising the sequences described above may be synthesized with additional chemical groups present at their amino and/or carboxy termini, such that, for example, the stability, bioavailability, and/or inhibitory activity of the peptides is enhanced. For example, hydrophobic groups such as carbobenzoxyl, dansyl, or t-butyloxycarbonyl groups, may be added to a peptide's amino terminus. Likewise, an acetyl group or a 9-fluorenylmethoxy-carbonyl group may be placed at a peptide's amino terminus. Additionally, a hydrophobic group, t-butyloxycarbonyl, or an amido group may be added to a peptide's carboxy terminus. Further, non-naturally occurring amino acids can be used to improve a peptide's stability, bioavailability, or binding/inhibitory characteristics. For example, methionine can be replaced with norleucine. Other non-naturally occurring amino acid residues are well known.

The peptides of the invention also may contain amino acid substitutions, which may be of a conserved or non-conserved nature. Conserved amino acid substitutions consist of replacing one or more amino acids in a peptide sequence with amino acids of similar charge, size, and/or hydrophobicity characteristics, such as, for example, a glutamic acid (E) to aspartic acid (D) amino acid substitution. When only conserved substitutions are made, the resulting peptide retains the functionality of the unsubstituted peptide. Non-conserved substitutions consist of replacing one or more amino acids of a peptide sequence with amino acids possessing dissimilar charge, size, and/or hydrophobicity characteristics, such as, for example, a glutamic acid (E) to valine (V) substitution. The peptides of the present invention may advantageously contain amino acid substitutions of a conserved nature.

The stability of the peptides of the invention may be increased by either in vivo or ex vivo linkage to a carrier protein, such as a blood component. Suitable blood components for use in the present invention are known in the art. Human serum albumin ("HSA") is a predominant component of human blood and is particularly suited for use in the present invention. In particular, HSA has an exposed surface cysteine residue that provides a reactive thiol moiety for covalent linkage of the peptides compounds to the protein. Activated linkers that are particularly suited for linkage to thiols include unsaturated cyclic imides such as maleimides, .alpha.-halo esters, such as .alpha.-iodo- and .alpha.-bromo acetates, and vinyl pyridine derivative. Such linkers can be added to the peptides during synthesis and can be added at any point in the sequence although the N and/or C terminus advantageously is used. Suitable activated linkers are commercially available from, for example, Pierce Chemical (Rockford, Ill.). Methods for preparing suitable activated compounds for linking to HSA are known in art. See for example, U.S. Pat. No. 5,612,034, which is incorporated herein in

its entirety.

Moreover, the gene for HSA has been cloned, which permits the ready preparation of fusion proteins of the peptides and HSA. Methods of making fusion proteins are known in the art. See, for example, WO01/79271 and WO01/79258, the contents of which are hereby incorporated by reference in their entirety. The preparation of fusion proteins is useful for preparing persistent derivatives of the present anti-viral peptides.

Another blood component that is suitable for linkage to the anti-viral compounds is an immunoglobulin ("Ig") molecule. Igs are persistent and are present in relatively high concentration in the blood. For in vitro coupling, Igs have the advantage of being readily stable and readily isolated, and methods of making Ig conjugates are well known in the art. Moreover, Ig genes may readily be cloned and recombinant Ig and Ig fusion proteins prepared. Methods for obtaining fully human Igs are well known in the art. See for example, U.S. Pat. Nos. 5,969,108 and 6,300,064, the contents of which are hereby incorporated by reference in their entirety. In addition, phage display methods for selecting Igs having a particularly desired binding activity, for example, for binding to HSA, are well known in the art. See U.S. Pat. Nos. 5,885,793, 5,969,108 and 6,300,064. In the context of the present invention, an Ig refers to any suitable immunoglobulin or immunogolobulin derivative known in the art, and includes, for example, whole IgG, IgM, Fab fragments, F(ab')2 fragments, and single chain Fv fragments.

Other blood components suitable for use in the present invention include transferrin, ferritin, steroid binding proteins, thyroxin binding protein, and .alpha.-2-macroglobulin.

In the peptides, the activated linkers also may be coupled to reactive side chain residues, such as lysine side chains. For example, a linker containing an active ester moiety and a maleimide moiety can be selectively reacted at the active ester (such as an N-hydroxysuccinimidyl ester) via lysine side chains or at the N-terminus of the peptide.

Both natural and recombinant HSA and human Igs are commercially available and are suitable for use in the present invention.

The peptides also may have a non-peptide macromolecular carrier group covalently attached to their amino and/or carboxy termini. Such macromolecular carrier groups may include, for example, lipid-fatty acid conjugates, polyethylene glycol, or carbohydrates.

Use of the Peptides

The peptides of the invention exhibit potent antiviral activity against coronaviruses, such as for example, the SARS virus. As such, the peptides may be used as inhibitors of human and non-human coronoviruses, especially SARS, transmission to uninfected cells. Various peptides from the C-terminal HR2 domains of S2 proteins of SARS virus and murine hepatitis virus (MHV) have been shown to exhibit antiviral activity against these viruses in cell culture assays(see for example Liu et al., Lancet 363: 938 (2004); and Bosch et al., J. Virol. 77:8801 (2003). The human SARS viruses whose transmission may be inhibited by the peptides of the invention include, but are not limited to all strains of the SARS virus. The non-human retroviruses whose transmission may be inhibited by the peptides of the invention include, but are not limited to coronaviruses that infect domestic animals and livestock, for example, coronaviruses from cows, pigs, mice- and chickens. However, as will be appreciated by one skilled in the art, the peptides used for preventing coronaviruses will be most effective when derived using the specific sequence of the infecting virus strain.

With respect to SARS in humans, the peptides of the invention may be used as a therapeutic in the treatment of SARS infections. The peptides of the invention may be administered using techniques well known to those in the art. Preferably, agents are formulated and administered systemically. Techniques for formulation and

administration may be found in "Remington's Pharmaceutical Sciences" 18th ed., 1990 Mack Publishing Co., Easton, Pa. Suitable routes may include oral, rectal, transmucosal, or intestinal administration; parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections, just to name a few. Most preferably, administration is intravenous. For injection, the agents of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiological saline buffer. For such transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art. Other diluents, adjuvants, and excipients are known in the art.

In addition, the peptides may be used as a prophylactic measure in previously uninfected individuals after acute exposure to a SARS virus. Examples of such prophylactic use of the peptides may include, but are not limited to, settings where the likelihood of SARS transmission exists, such as, for example, in hospitals and transport termini such as airports and train stations. The peptides of the invention in such cases may serve the role of a prophylactic vaccine, wherein the host raises antibodies against the peptides of the invention, which then serve to neutralize SARS viruses by, for example, inhibiting further SARS infection. Administration of the peptides of the invention as a prophylactic vaccine, therefore, would comprise administering to a host a concentration of peptides effective in raising an immune response which is sufficient to neutralize SARS or a related coronavirus, by, for example, inhibiting SARS ability to infect cells. The exact concentration will depend upon the specific peptide to be administered, but may be determined by using standard techniques for assaying the development of an immune response which are well known to those of ordinary skill in the art. The peptides to be used as vaccines are usually administered intramuscularly.

The peptides may be formulated with a suitable adjuvant in order to enhance the immunological response. Such adjuvants may include, but are not limited to mineral gels such as aluminum hydroxide; surface active substances such as lysolecithin, pluronic polyols, polyanions; other peptides; oil emulsions; and potentially useful human adjuvants such as BCG and Corynebacterium parvum. Many methods may be used to introduce the vaccine formulations described here. These methods include but are not limited to oral, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, and intranasal routes.

Alternatively, an effective concentration of polyclonal or monoclonal antibodies raised against the peptides of the invention may be administered to a host so that no uninfected cells become infected by the SARS virus or other coronavirus. The exact concentration of such antibodies will vary according to each specific antibody preparation, but may be determined using standard techniques well known to those of ordinary skill in the art. Administration of the antibodies may be accomplished using a variety of techniques, including, but not limited to those described in this section.

Effective dosages of the peptides of the invention to be administered may be determined through procedures well known to those in the art which address such parameters as biological half-life, bioavailability, and toxicity.

The antiviral activity of the peptides of the invention may show a pronounced type and subtype specificity, i.e., specific peptides may be effective in inhibiting the activity of only specific coronaviruses. This feature of the invention presents many advantages. One such advantage, for example, lies in the field of diagnostics, wherein one can use the antiviral specificity of the peptide of the invention to ascertain the identity of a viral isolate. With respect to coronaviruses, one may easily determine whether a viral isolate consists of a coronavirus that causes SARS or a virus that causes milder cold-like symptoms. For example, uninfected cells may be co-infected with a coronavirus isolate which has been identified as containing a SARS virus. A peptide of the invention may be added which is known to be active against the SARS virus, after which the retroviral activity of cell supernatants may be assayed, using known methods. Those isolates whose viral activity is completely or nearly completely inhibited contain the SARS virus. Those isolates whose viral

activity is unchanged or only reduced by a small amount, may be considered to not contain the SARS virus. Such an isolate may then be treated with one or more of the other peptides of the invention, and subsequently be tested for its viral activity in order to determine the identify of the viral isolate.

SEQUENCE LISTINGS

1

197149PRTArtificial SequenceDescription of Artificial Sequence Synthetic peptide 1Asp Val Asp Leu Gly Asp Ile Ser Gly Ile Asn Ala Ser Val Val Asn1 5 10 15Ile Gln Lys Glu Ile Asp Arg Leu Asn Glu Val Ala Lys Asn Leu Asn 20 25 30Glu Ser Leu Ile Asp Leu Gln Glu Leu Gly Lys Tyr Glu Gln Tyr Ile 35 40 45Lys245PRTArtificial SequenceDescription of Artificial Sequence Synthetic peptide 2Gln Ile Pro Phe Ala Met Gln Met Ala Tyr Arg Phe Asn Gly Ile Gly1 5 10 15Val Thr Gln Asn Val Leu Tyr Glu Asn Gln Lys Gln Ile Ala Asn Gln 20 25 30Phe Asn Lys Ala Ile Ser Gln Ile Gln Glu Ser Leu Thr 35 40 45340PRTArtificial SequenceDescription of Artificial Sequence Synthetic peptide 3Glu Ser Leu Thr Thr Thr Ser Thr Ala Leu Gly Lys Leu Gln Asp Val1 5 10 15Val Asn Gln Asn Ala Gln Ala Leu Asn Thr Leu Val Lys Gln Leu Ser 20 25 30Ser Asn Phe Gly Ala Ile Ser Ser 35 40445PRTArtificial SequenceDescription of Artificial Sequence Synthetic peptide 4Gly Lys Leu Gln Asp Val Val Asn Gln Asn Ala Gln Ala Leu Asn Thr1 5 10 15Leu Val Lys Gln Leu Ser Ser Asn Phe Gly Ala Ile Ser Ser Val Leu 20 25 30Asn Asp Ile Leu Ser Arg Leu Asp Lys Val Glu Ala Glu 35 40 45548PRTArtificial SequenceDescription of Artificial Sequence Synthetic peptide 5Arg Leu Ile Thr Gly Arg Leu Gln Ser Leu Gln Thr Tyr Val Thr Gln1 5 10 15Gln Leu Ile Arg Ala Ala Glu Ile Arg Ala Ser Ala Asn Leu Ala Ala 20 25 30Thr Lys Met Ser Glu Cys Val Leu Gly Gln Ser Lys Arg Val Asp Phe 35 40 4567PRTArtificial SequenceDescription of Artificial Sequence Synthetic peptide 6Ser Val Val Asn Ile Gln Lys1 577PRTArtificial SequenceDescription of Artificial Sequence Synthetic peptide 7Val Val Asn Ile Gln Lys Glu1 587PRTArtificial SequenceDescription of Artificial Sequence Synthetic peptide 8Val Asn Ile Gln Lys Glu Ile1 597PRTArtificial SequenceDescription of Artificial Sequence Synthetic peptide 9Asn Ile Gln Lys Glu Ile Asp1 5107PRTArtificial SequenceDescription of Artificial Sequence Synthetic peptide 10Ile Gln Lys Glu Ile Asp Arg1 5117PRTArtificial SequenceDescription of Artificial Sequence Synthetic peptide 11Gln Lys Glu Ile Asp Arg Leu1 5127PRTArtificial SequenceDescription of Artificial Sequence Synthetic peptide 12Lys Glu Ile Asp Arg Leu Asn1 5137PRTArtificial SequenceDescription of Artificial Sequence Synthetic peptide 13Glu Ile Asp Arg Leu Asn Glu1 5147PRTArtificial SequenceDescription of Artificial Sequence Synthetic peptide 14Ile Asp Arg Leu Asn Glu Val1 5157PRTArtificial SequenceDescription of Artificial Sequence Synthetic peptide 15Asp Arg Leu Asn Glu Val Ala1 5167PRTArtificial SequenceDescription of Artificial Sequence Synthetic peptide 16Arg Leu Asn Glu Val Ala Lys1 5177PRTArtificial SequenceDescription of Artificial Sequence Synthetic peptide 17Leu Asn Glu Val Ala Lys Asn1 5187PRTArtificial SequenceDescription of Artificial Sequence Synthetic peptide 18Asn Glu Val Ala Lys Asn Leu1 5197PRTArtificial SequenceDescription of Artificial Sequence Synthetic peptide 19Glu Val Ala Lys Asn Leu Asn1 5207PRTArtificial SequenceDescription of Artificial Sequence Synthetic peptide 20Val Ala Lys Asn Leu Asn Glu1 5217PRTArtificial SequenceDescription of Artificial Sequence Synthetic peptide 21Ala Lys Asn Leu Asn Glu Ser1 5227PRTArtificial SequenceDescription of Artificial Sequence Synthetic peptide 22Lys Asn Leu Asn Glu Ser Leu1 5237PRTArtificial SequenceDescription of Artificial Sequence Synthetic peptide 23Asn Leu Asn Glu Ser Leu Ile1 5247PRTArtificial SequenceDescription of Artificial Sequence Synthetic peptide 24Leu Asn Glu Ser Leu Ile Asp1 5257PRTArtificial SequenceDescription of Artificial Sequence Synthetic peptide 25Asn Glu Ser Leu Ile Asp Leu1 5267PRTArtificial SequenceDescription of Artificial Sequence Synthetic peptide 26Glu Ser Leu Ile Asp Leu Gln1 5277PRTArtificial SequenceDescription of Artificial Sequence Synthetic peptide 27Ser Leu Ile Asp Leu Gln Glu1 5287PRTArtificial SequenceDescription of Artificial Sequence Synthetic peptide 28Leu Ile Asp Leu Gln Glu Leu1 5297PRTArtificial SequenceDescription of Artificial Sequence Synthetic peptide 29Ile Asp Leu Gln Glu Leu Gly1 5307PRTArtificial SequenceDescription of Artificial Sequence Synthetic peptide 30Asp Leu Gln Glu Leu Gly Lys1 5317PRTArtificial SequenceDescription of Artificial Sequence Synthetic peptide 31Leu Gln Glu Leu Gly Lys Tyr1 5327PRTArtificial SequenceDescription of Artificial

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