Title: SOLUBLE FRAGMENTS OF THE SARS-COV SPIKE GLYCOPROTEIN

Abstract: The invention provides isolated SARS Coronavirus polypeptides, anti-SARS antibodies, recombinant poxviruses and compositions that are useful for treating and inhibiting SARS Coronavirus infection.
**Soluble Fragments of the SARS-CoV Spike Glycoprotein**

This application claims benefit of the filing date of U.S. Provisional Application Ser. No. 60/558,995 filed April 5, 2004, which is hereby incorporated by reference in its entirety.

**Government Funding**

The invention described herein was developed with the support of the Department of Health and Human Services, National Institutes of Health. The United States Government has certain rights in the invention.

**Field of the Invention**

The invention relates to the treatment and prevent of severe acute respiratory syndrome (SARS) caused by the SARS-coronavirus (SARS-CoV).

**Background of the Invention**

Severe acute respiratory syndrome (SARS), an emerging infectious disease of humans, appeared in China in November 2002 and spread to thirty countries in early 2003. Before the epidemic ended, 8,098 probable cases of SARS and 774 associated deaths were reported to the World Health Organization. See website at cdc.gov/mmwr/mguide_sars.html. The etiologic agent of SARS was identified as a coronavirus (CoV) and the sequence of the SARS virus genome established that it was a new member of the family. See Rota et al. (2003) *Science* 300, 1394-1399; Marra et al. (2003) *Science* 300, 1399-1404. Closely related coronaviruses were recovered from civet cats and other animals in southern China, although the source of human SARS infection remained uncertain. Other members of the CoV family can cause fatal diseases of livestock, poultry and laboratory rodents. Holmes, K. V. (2003) *J. Clin. Invest.* 111, 1605-1609. The two previously identified human CoV, however, cause only mild upper respiratory infections. *Id.*

Although the 2002/2003 epidemic was eventually controlled by case isolation, the high morbidity and mortality, lack of specific treatment, and potential of re-emergence make it imperative to develop effective means to prevent or cure the disease should it reappear.
Summary of the Invention

The invention provides SARS Coronavirus polypeptides, antibodies directed against those polypeptides and recombinant viruses that can express SARS Coronavirus polypeptides. Administration of these SARS-related polypeptides, antibodies and recombinant viruses to animals is surprisingly effective for protecting those animals against SARS Coronavirus infection.

Therefore, one aspect of the invention is an isolated polypeptide consisting essentially of SEQ ID NO:4, 6 or 7. Another aspect of the invention is an isolated nucleic acid encoding a polypeptide consisting essentially of SEQ ID NO:4, 6 or 7. For example, such a nucleic acid can have SEQ ID NO:2 or 5.

Another aspect of the invention is an antibody that can bind to a SARS Coronavirus polypeptide consisting essentially of SEQ ID NO:4, 6 or 7.

Another aspect of the invention is a recombinant attenuated poxvirus comprising a genome with a nucleic acid insertion that encodes a SARS Coronavirus polypeptide consisting essentially of SEQ ID NO:1, 3, 4, 6 or 7. Nucleic acid insertions that can be used in the recombinant attenuated poxvirus can, for example, have SEQ ID NO:2 or 5. Many types of poxviruses are available for use. In one embodiment, the poxvirus is a modified MVA virus.

Another aspect of the invention is a recombinant attenuated baculovirus comprising a nucleic acid encoding a SARS Coronavirus polypeptide consisting essentially of SEQ ID NO:4, 6 or 7. For example, such a nucleic acid can have SEQ ID NO:2 or 5.

Another aspect of the invention is a DNA vaccine comprising a pharmaceutically acceptable carrier and a vector encoding a SARS Coronavirus polypeptide consisting essentially of SEQ ID NO:1, 3, 4, 6 or 7.

Another aspect of the invention is a composition comprising a carrier and an effective amount of SARS Coronavirus polypeptide consisting essentially of SEQ ID NO:4, 6, 7, or a combination thereof. The amount employed in the composition can be effective for generating antibody production in an animal.

Another aspect of the invention is a composition comprising a carrier and an effective amount of a recombinant attenuated poxvirus comprising a genome with a nucleic acid insertion that encodes a SARS Coronavirus polypeptide consisting essentially of SEQ ID NO:1, 3, 4, 6 or 7. The amount employed in
the composition can be effective for generating antibody production in an animal.

Another aspect of the invention is a composition comprising a carrier and an effective amount of antibody that can bind to a SARS Coronavirus polypeptide consisting essentially of SEQ ID NO:4, 6 or 7. The amount employed in this composition can be effective to inhibit SARS Coronavirus replication in the animal.

Another aspect of the invention is a method for generating an immune response in an animal against a SARS Coronavirus S polypeptide comprising: administering to the animal an immunologically effective amount of any of the polypeptide or poxvirus compositions of the invention.

Another aspect of the invention is a method for inhibiting SARS Coronavirus infection in an animal comprising: administering to the animal an immunologically effective amount of any of the polypeptide, poxvirus or antibody compositions of the invention.

Another aspect of the invention is a method for treating SARS Coronavirus infection in an animal comprising: administering to the animal an effective amount of the composition of the invention. For example, an effective amount is effective to inhibit SARS Coronavirus replication in the animal.

Another aspect of the invention is a diagnostic kit for detection of a SARS Coronavirus infection in a mammal comprising packaging material, an antibody that can bind to a SARS Coronavirus polypeptide consisting essentially of SEQ ID NO:4, 6 or 7, and instructions for detection of a SARS Coronavirus infection in a mammal.

Another aspect of the invention is a diagnostic kit for detection of a SARS Coronavirus infection in a mammal comprising packaging material, a SARS Coronavirus polypeptide consisting essentially of SEQ ID NO:4, 6 or 7, and instructions for detection of a SARS Coronavirus infection in a mammal.

Description of the Figures

FIG. 1A-B provides a diagram of a recombinant Spike polypeptide expression cassette within a MVA viral vector and illustrates expression from this construct. FIG. 1A provides a diagram of selected portion of MVA/S. The GFP and S open reading frames were inserted into a deletion site (del III) of the
MVA genome. The early/late mH5 and late P11 vaccinia virus promoters were used to regulate expression of S and GFP, respectively. MVA/S-HA has an identical structure except for the presence of a short segment of DNA encoding the influenza virus HA tag at the C-terminus of the S open reading frame. FIG. 1B provides a Western-blot analysis of SARS-CoV S protein expressed by cells infected with MVA or MVA/S-HA. Uninfected HeLa cells were used as a control. Eighteen hours after infection, the cells were harvested and the cleared cell lysates were analyzed by SDS-PAGE. The electrophoretically separated proteins were transferred to a nitrocellulose membrane and detected with anti-HA mAb (lanes 1, 2, and 3) or anti-SARS-CoV S polyclonal antibody (lanes 4, 5, and 6). The masses of marker proteins in kDa are shown on the left and the position of SARS-CoV S protein is indicated by an arrow on the right.

FIG. 2A-B illustrates that the SARS-CoV S protein is a glycoprotein. FIG. 1A shows that the molecular weight of the SARS-CoV S protein is sensitive to Endo H, which digests the N-linked high-mannose carbohydrate side chains of glycoproteins that are synthesized in the endoplasmic reticulum (ER), and PNGase F, which hydrolyzes all types of N-glycan chains. HeLa cells were uninfected (lanes 1, 5) or infected with MVA (lanes 2, 6) or MVA/S-HA (lanes 3, 4, 7, 8). After 18 h, the cells were lysed, cleared by centrifugation, and incubated with anti-HA affinity matrix (Roche). The bound proteins were treated with endo H or PNGase F as indicated by plus signs and analyzed by SDS-PAGE and western blotting with anti-HA mAb. The positions of two glycosylated forms of S and a non-glycosylated (ngS) form are shown by arrows. FIG. 1B illustrates the kinetics of endo H sensitivity. HeLa cells at 8 h after infection with MVA/S-HA were pulse-labeled with [35S]methionine and [35S]cysteine for 10 min and then washed and chased for 0, 20, 40, 60 and 80 min in medium supplemented with unlabeled cysteine and methionine. Cells were lysed immediately after the pulse or chase and the S was captured with anti-HA affinity matrix (Roche), subjected to endo H digestion, resolved by SDS-PAGE and visualized by autoradiography. The masses of marker proteins in kDa are shown on the left.

FIG. 3A-H illustrates the cellular localization of SARS-CoV S. Unfixed and unpermeabilized CEF (FIG. 3A-F) that had been infected with MVA (FIG. 3A-B), MVA/S (FIG. 3C-D) and MVA/S-HA (FIG. 3E-F) for 18 h were stained
with anti-SARS-CoV mouse serum (FIG. 3A-D) or anti-HA mAb (FIG. 3E-F) followed by Alexa 594-conjugated-anti-mouse IgG and viewed by confocal microscopy. CEF infected with MVA/S-HA (FIG. 3G-H) were fixed, permeabilized and stained with anti-HA mAb followed by Alexa 594-conjugated-anti-mouse IgG. Panels on the left and right show GFP and Alexa 594 fluorescence, respectively.

FIG. 4A-B illustrates the antibody responses after immunization with recombinant MVA/S by intranasal (IN) or intramuscular (IM) routes. FIG. 4A provides end-point ELISA titers of pooled serum (n=8), taken before (prebleed) or after immunizations, were determined using insect cell expressed S1 domain of the SARS-CoV S as the capture antigen. Sera from 2 mice were pooled after challenge and analyzed. Thin and thick arrows depict times of immunizations and challenge with SARS-CoV respectively. FIG. 4B shows the pre-challenge SARS-CoV neutralization titers of pooled serum were determined. The dilution of serum that completely prevented SARS-CoV cytopathic effect in 50% of the wells was calculated.

FIG. 5 illustrates that mice immunized with MVA/S, which expresses the SARS-CoV S polypeptide, were protected from subsequent challenge with live SARS-CoV. Groups of 8 BALB/c mice were mock vaccinated or vaccinated with MVA or MVA/S by the IN or IM routes at 0 time and 4 weeks and then challenged 4 weeks later with $10^4$ TCID$_{50}$ of SARS-CoV administered by the IN route. Two days later the titers of SARS-CoV in the lungs and nasal turbinates of 4 mice in each group were determined. Virus titers are expressed as $\log_{10}$ TCID$_{50}$/g of tissue. Statistical comparison of MVA/S titers to unvaccinated controls was performed using a Mann Whitney U non-parametric analysis; $^*p = 0.02$.

FIG. 6 provides amino acid and cDNA sequences (SEQ ID NO:4 and 5, respectively) for the SARS-CoV (Urbani strain) S$\Delta$TM+CT polypeptide containing spike protein amino acids 14-1195.

FIG. 7A-C illustrates the construction, expression and characterization of SARS-CoV nS glycoprotein, which include amino acids 14 to 762 (SEQ ID NO:6) of the SARS-CoV S polypeptide. FIG. 7A provides a schematic representation of pMelBacB-based baculovirus transfer vector. Abbreviations: $P_{ph}$ polyhedrin promoter; HBM, DNA encoding honeybee melittin signal
sequence; nS, DNA segment encoding amino acids (aa) 14-762 of the SARS-CoV S protein; His6, DNA encoding 6 histidine residues. FIG. 7B illustrates that the SARS CoV nS polypeptide is pure as analyzed by SDS polyacrylamide gel electrophoresis and Coomassie Blue staining (lane 1), silver staining (lane 2) and western blot analysis with anti-His mAb (lane 3) or anti-SARS CoV S polyclonal antibody (lane 4). FIG. 7C shows that the SARS-CoV nS polypeptide is glycosylated. Purified nS protein was (+) or was not (-) treated with peptide N-glycosidase F and was analyzed by SDS polyacrylamide gel electrophoresis and western blotting with anti-His mAb and anti-SARS-CoV S polyclonal antibody. Molecular masses of marker proteins in kDa are shown on the left.

FIG. 8A-H illustrates binding of antibodies from mice immunized with nS to full-length membrane-bound S. HeLa cells were uninfected (FIG. 8A-B), infected with non-recombinant MVA (FIG. 8C-D) or MVA expressing S (FIG. 8E-H) for 18 h. After fixation, the unpermeabilized cells were stained with pooled sera from mice immunized three times with nS and MPL + TDM (E-F) or nS and QS21 (FIG. 8A-D, G-H) followed by Alexa 594-conjugated-anti-mouse IgG and viewed by visible (FIG. 8A,C,E,G) or fluorescence (FIG. 8B,D,F,H) light microscopy.

FIG. 9A-B illustrates ELISA and neutralizing antibody responses to the nS (SEQ ID NO:6) polypeptide. Groups of 7 BALB/c mice were immunized subcutaneously with 10 μg of purified nS and QS21 or MPL + TDM adjuvant at 4-week intervals (arrows) and challenged intranasally with 10⁵ TCID₅₀ SARS-CoV on day 82 (arrow head). Control mice were immunized at the same times with purified soluble vaccinia virus L1R protein. FIG. 9A shows end-point ELISA titers of pooled sera collected on days indicated were measured using nS as the capture antigen. The absorbance obtained with serum from mice immunized with L1R was subtracted. FIG. 9B shows the dilution of serum that completely prevented cytopathic effects of SARS-CoV in 50% of wells containing Vero cells. Assays were performed on pooled serum collected on days 28 and 56 days and on individual mouse serum collected on day 78. Standard error bars are shown for the latter.

FIG. 10A-B illustrates that immunized mice are protected against SARS-CoV replication. Groups of 7 BALB/c mice were immunized and challenged with SARS-CoV as described in the legend to FIG. 9. Two days after the
challenge, the virus titers (mean log_{10} TCID_{50} per g tissue with standard error) were measured in the lower (FIG. 10A) and upper (FIG. 10B) respiratory tract.

**Detailed Description of the Invention**

As illustrated herein, a full-length Spike (S) polypeptide of SARS-CoV, expressed by an attenuated poxvirus, induces formation of neutralizing antibodies and protectively immunizes animals against a subsequent infection with SARS-CoV. Antiserum collected from animals immunized with the attenuated poxvirus reduced SARS viral replication in infected animals. As also described herein, a secreted, glycosylated S polypeptide including amino acids 14 to 762 of the SARS coronavirus (SARS-CoV) S protein provided complete protection of the upper and lower respiratory tract against SARS infection. Thus, the invention provides immunological compositions of SARS-CoV polypeptides, and of live attenuated viruses that can express such SARS-CoV polypeptides. In another embodiment, the invention provides anti-SARS-CoV S antibody compositions that are useful for passive immunization of animals that are infected, or may become infected, with SARS.

**Definitions**

"Attenuated recombinant virus" refers to a virus that has been rendered less virulent than wild type, typically by deletion of specific genes or by serial passage in a non-natural host cell line or at cold temperatures.

Nucleic acid-based vaccines" include both naked DNA and vectored DNA (within a viral capsid) where the nucleic acid encodes B-cell and T-cell epitopes and provides an immunoprotective response in the animal to which the vaccine has been administered.

"Poxviruses" are large, enveloped viruses with a genome of double-stranded DNA that is covalently closed at the ends. Poxviruses replicate entirely in the cytoplasm. They have been used as vaccines since the early 1980's (see, e.g., Panicali, D. et al. *Construction of live vaccines by using genetically engineered pox viruses: biological activity of recombinant vaccinia virus expressing influenza virus hemagglutinin*, Proc. Natl. Acad. Sci. USA 80:5364-5368, 1983).
"Viral load" is the amount of virus present in the blood of a patient. Viral load is also referred to as viral titer or viremia. Viral load can be measured using procedures available to one of skill in the art.

5 SARS Coronavirus Spike (S) Protein

SARS-coronavirus (SARS-CoV) has a nearly 30,000 nucleotides long RNA genome with eleven open reading frames that encode four major structural proteins consisting of nucleocapsid, spike (S), membrane and small envelope protein (Marra et al. (2003) Science 300, 1399-1404; Rota et al. (2003) Science 300, 1394-1399). The latter is a type-I transmembrane glycoprotein, which forms the characteristic corona of large protruding spikes on the virion surface and mediates binding to the host cell receptor and membrane fusion. In previously studied CoV, S was shown to be an important determinant of pathogenesis as well as the major target of protective immunity (8, 11). The S of SARS-CoV is quite divergent from those of other CoV, exhibiting only 20 to 27% overall amino acid identity (Rota et al. (2003) Science 300, 1394-1399). Recent studies have indicated that the SARS-CoV S polypeptide is expressed as a non-cleaved glycoprotein with an apparent mass of 180 to 200 kDa that interacts with a functional receptor identified as angiotensin-converting enzyme 2 (Li et al. (2003) Nature 426, 450-454; Xiao et al. (2003) Biochem. Biophys. Res. Commun. 312, 1159-1164.

As described herein, S polypeptides are useful antigens for generating an immune response against SARS-CoV. Several different strains of SARS-CoV have been isolated and sequenced. Nucleic acid and amino acid sequences for different S polypeptides, and the nucleic acids that encode them can be found in the art, for example, in the NCBI database. See website at ncbi.nlm.nih.gov. For example, one amino acid sequence for the S polypeptide from the Urbani strain of SARS-CoV can be found in the NCBI database as accession number AAP13441 (gi: 30027620). See website at ncbi.nlm.nih.gov. This Urbani S polypeptide sequence is provided below as follows (SEQ ID NO:1):

```
1 MFIFLLFLTL TSGSDLRCT TFDDVQAPNY TQHTSSMRGV
41 YYPDEIFRSD TLYLTDLFLF FFYSNVTFGP TINHTFGNPV
81 IPFKQDGIYFA ATEKSNVVRG WVFSTMNKK SQSVIIINS
121 TNVVIRACNF ELCDNPFPAV SKPMGTQHT TIFDNAPNCT
161 FEFYSDASFL DVEKSGNFK HLREFVFKNK DGFLYVYKY
201 QPIDVVRDLFP SGFNTLKPIF KLPLGINITN FRAILTAFSP
```
A nucleotide sequence for this SARS-CoV Urbani S polypeptide can be found in the nucleotide sequence having accession number AY278741 (gi: 30027617), which provides the complete nucleotide sequence for the Urbani genome. The S polypeptide sequence is encoded by nucleotides 21492 to 25259.

This S nucleic acid sequence is provided below for easy reference (SEQ ID NO:2).

21492 ATGTTTATT TTCTTATGAT TTCTTACTCT
21521 CACTAGTGGT AGTGACCTTG ACCGGTGACAC CACTTTTGAT
21561 GTGTTCAAAG CTCTAAACTA CATCTCAACT ACTCTCTTCA
21601 TGAGGGGCTG TTAATACCTC CATGAAATTT TAGTAGTACA
21641 CACTTCTTAT TTAATCCAGG ATTTATTTCT TCCATTTTAT
21681 TCTCAATTTGA CAGGGTTTTCA TACTATTATG CATAGTTTGG
21721 GCAACCCCTG TATACCTTTT AAGGATGTTA TTTATTTTGC
21761 TGCCACAGAG AAATCAAAGT TTGTCGTTGG TTGGTTTTTT
21801 GGGTTCTACCA TGAACACAAG GTCAAGTGCG GTGATATTGA
21841 TTAACAAATCC TACTAATGTT GTTACAGCAG CATGTAACCT
21881 TGAAATGTGGT GACAACCCCT TCTTGTCTGT TCTAAACCC
21921 ATGGTGACAC AGACACATAC TATGATATTC GATAATGCAT
21961 TTATATTGACAC TTTGAGTCAC ATATCTGATG CCTTTTCGCT
22001 TGATATGATCGAAAAATCTGAG ATATTTTAAAC ACTTACACGA
22041 GAGTTTGTGT TTTAAAATAT AGATGGGGTT CTCTATGTGT
<table>
<thead>
<tr>
<th>22081</th>
<th>ATAAGGGCTTA</th>
<th>TCAACCTATA</th>
<th>GATGTAGTTC</th>
<th>GTGATCTACC</th>
</tr>
</thead>
<tbody>
<tr>
<td>22121</td>
<td>TTCTGTTTTT</td>
<td>AACACTTGG</td>
<td>AACCCTATT</td>
<td>TARGTTGCC</td>
</tr>
<tr>
<td>22161</td>
<td>CTGGTGATTA</td>
<td>ACACTCAAA</td>
<td>TTTTAGAGCC</td>
<td>ATCTTACAG</td>
</tr>
<tr>
<td>22201</td>
<td>CCTTTTCAAC</td>
<td>TGCTCAAGAC</td>
<td>ATTTGGGGCA</td>
<td>CTGCAGCTG</td>
</tr>
<tr>
<td>5</td>
<td>22241</td>
<td>AGCCATATT</td>
<td>TGTTGCGATT</td>
<td>TAAGGCGCAAC</td>
</tr>
<tr>
<td>22281</td>
<td>CTCAAGATTTG</td>
<td>AGGAAAAATG</td>
<td>TACAACTCA</td>
<td>GATGCCTTGG</td>
</tr>
<tr>
<td>22321</td>
<td>ATTTGAATTCA</td>
<td>AAATCCACTT</td>
<td>GCTGAACCTA</td>
<td>AATGCTCTGT</td>
</tr>
<tr>
<td>22361</td>
<td>TATAGCCTTT</td>
<td>GAGATGACA</td>
<td>AAGGATTTTA</td>
<td>CCAAGACTCT</td>
</tr>
<tr>
<td>22401</td>
<td>AATTTTGCGG</td>
<td>TTTTTGCTTT</td>
<td>AAGAGATTGT</td>
<td>GTGAGATTTT</td>
</tr>
<tr>
<td>10</td>
<td>22441</td>
<td>CATTAATATT</td>
<td>AAACCTGTTG</td>
<td>CTTTTTGGAG</td>
</tr>
<tr>
<td>22481</td>
<td>TGCTACTAAA</td>
<td>TTCCCTCTGG</td>
<td>TCTTAGCATG</td>
<td>GAGAGAAGAA</td>
</tr>
<tr>
<td>22521</td>
<td>AAAATTTTCA</td>
<td>ATTTGGTTGC</td>
<td>TGATTCACTT</td>
<td>GTCTCCTCAG</td>
</tr>
<tr>
<td>22561</td>
<td>ACTCAAATTT</td>
<td>TTTTTCAACC</td>
<td>TTATAGTCTG</td>
<td>ATGCGGGTTC</td>
</tr>
<tr>
<td>22601</td>
<td>TGCCACTAAG</td>
<td>TTGGAGTATG</td>
<td>TTTGCTCTTC</td>
<td>CAAAGTGTAT</td>
</tr>
<tr>
<td>15</td>
<td>22641</td>
<td>GCAAGATTTT</td>
<td>TTCTATGCAA</td>
<td>GGGGAGATG</td>
</tr>
<tr>
<td>22681</td>
<td>TAGGCGCGAG</td>
<td>ACAAACCTGTG</td>
<td>GTCTATGCTG</td>
<td>ATTAAAATT</td>
</tr>
<tr>
<td>22721</td>
<td>TAAATTTGCGG</td>
<td>GTATGTTTCA</td>
<td>TGGGTGTTGT</td>
<td>CTCTCGCTTG</td>
</tr>
<tr>
<td>22761</td>
<td>AAATCGAGGA</td>
<td>AATGGATTGC</td>
<td>TACCTCACT</td>
<td>GCTTAAATA</td>
</tr>
<tr>
<td>22801</td>
<td>ATTTATAAATA</td>
<td>TAGTGATCTT</td>
<td>AGACATGGCA</td>
<td>AGCTTAGGCC</td>
</tr>
<tr>
<td>25</td>
<td>22841</td>
<td>CTTTGGAGAG</td>
<td>GACATATCTA</td>
<td>ATGGCCTTTT</td>
</tr>
<tr>
<td>22881</td>
<td>GGCGAAACCTT</td>
<td>GCACCCACC</td>
<td>TGCCCTTAAA</td>
<td>TTATTTGGGC</td>
</tr>
<tr>
<td>22921</td>
<td>CATTTAATAG</td>
<td>TTATGGTTTT</td>
<td>TACACACTA</td>
<td>CTGGCATTGG</td>
</tr>
<tr>
<td>22961</td>
<td>CTACCCAGTTT</td>
<td>TACAGATTGG</td>
<td>TAGTACTTTT</td>
<td>TTTGGAACCT</td>
</tr>
<tr>
<td>23001</td>
<td>TTAATATGAC</td>
<td>CGGCCAGGGT</td>
<td>TTGTGAGCCT</td>
<td>AATTTATCCA</td>
</tr>
<tr>
<td>23041</td>
<td>CTGACCTCTT</td>
<td>TAAAGACGAT</td>
<td>TGGTGCAAAT</td>
<td>TTAATTTAAA</td>
</tr>
<tr>
<td>23081</td>
<td>TGGACCTCATT</td>
<td>GGTACTGTTG</td>
<td>TGTAAAACCT</td>
<td>TTTCCTCAAAG</td>
</tr>
<tr>
<td>23121</td>
<td>AGATTTCAAC</td>
<td>CATTTCAACA</td>
<td>ATTTGGCGGT</td>
<td>GATGTTCCTG</td>
</tr>
<tr>
<td>23161</td>
<td>ATTTGCTAGA</td>
<td>TTGCCTGTCG</td>
<td>GATCTAATAA</td>
<td>CATCTGAAAT</td>
</tr>
<tr>
<td>23201</td>
<td>ATTAGACATT</td>
<td>TCAACCTTGGT</td>
<td>CTTTTGGGGA</td>
<td>TGTAAGTGA</td>
</tr>
<tr>
<td>30</td>
<td>23241</td>
<td>ATTTACACCTG</td>
<td>GAACCAATGC</td>
<td>TTCACTGCAA</td>
</tr>
<tr>
<td>23281</td>
<td>TATATCAAGA</td>
<td>TGTTAAGTGC</td>
<td>ACTGAGTTTT</td>
<td>CTACAGCAAT</td>
</tr>
<tr>
<td>23321</td>
<td>TCATGCAAGT</td>
<td>CAACCTACAC</td>
<td>CAGCTTGCGG</td>
<td>CATATATTCT</td>
</tr>
<tr>
<td>23361</td>
<td>ACTGGGAAACA</td>
<td>ATGTTATTCGA</td>
<td>GACTCAAGCA</td>
<td>GCTGTGCTTA</td>
</tr>
<tr>
<td>23401</td>
<td>TAGGAGCCTGA</td>
<td>GCATGTCGAC</td>
<td>ACTTTCATTG</td>
<td>AGTGGGACAT</td>
</tr>
<tr>
<td>35</td>
<td>23441</td>
<td>TCTTATTGGA</td>
<td>GCTGGCAATT</td>
<td>GTGCTAGTTA</td>
</tr>
<tr>
<td>23481</td>
<td>TCCTTATTAC</td>
<td>TAGTACTTAG</td>
<td>CAAAAATCCT</td>
<td>ATTTGTTGCT</td>
</tr>
<tr>
<td>23521</td>
<td>ATACTACTATG</td>
<td>TTTAGTGCTG</td>
<td>GATAGTTCAA</td>
<td>TTGGCTTACTC</td>
</tr>
<tr>
<td>23561</td>
<td>TATAACACAC</td>
<td>ATTTGCTATAC</td>
<td>CTACTAATCT</td>
<td>TCAATATTAG</td>
</tr>
<tr>
<td>23601</td>
<td>ATATTACAGA</td>
<td>AAGTAATGCC</td>
<td>TTGTCTTATG</td>
<td>GCTAAAACCT</td>
</tr>
<tr>
<td>40</td>
<td>23641</td>
<td>CGTGAGAATG</td>
<td>TAATATGCTG</td>
<td>ATCTGGCGAG</td>
</tr>
<tr>
<td>23681</td>
<td>ATGTGCTAAT</td>
<td>TTGCTTCTCC</td>
<td>AATTAGTTAG</td>
<td>CTTTTGCGCA</td>
</tr>
<tr>
<td>23721</td>
<td>CAACTAAATG</td>
<td>GTGCACTCTCT</td>
<td>AGTGATTTCT</td>
<td>GCTGAACAGG</td>
</tr>
<tr>
<td>23761</td>
<td>ATCGCAACAC</td>
<td>AGCTGAAATG</td>
<td>TTGGCTCAAG</td>
<td>TCCAAAATAT</td>
</tr>
<tr>
<td>23801</td>
<td>GTACAACACC</td>
<td>CCAAACCTTA</td>
<td>AATAATTTGG</td>
<td>TGGTTTTTAA</td>
</tr>
<tr>
<td>45</td>
<td>23841</td>
<td>TTTTCAAAAA</td>
<td>TATTACGTGA</td>
<td>CCCCCTAAG</td>
</tr>
<tr>
<td>23881</td>
<td>GGTCTTTTAT</td>
<td>TAGGAGACTG</td>
<td>TTCTTTAATA</td>
<td>AGTGTACACT</td>
</tr>
<tr>
<td>23921</td>
<td>GCCTGATGCT</td>
<td>GGCTTACAGA</td>
<td>AGCAATATTG</td>
<td>CGAATGCCCA</td>
</tr>
<tr>
<td>23961</td>
<td>GGCTGATATT</td>
<td>ATGTCTAGAG</td>
<td>TCTCATTTTG</td>
<td>GCACGAAACT</td>
</tr>
<tr>
<td>24001</td>
<td>TCAATGCAAGT</td>
<td>TACAGTTGG</td>
<td>CCAACTCTGC</td>
<td>TCACTGTAAG</td>
</tr>
<tr>
<td>50</td>
<td>24041</td>
<td>TAGATGCCTG</td>
<td>GCCTACACTG</td>
<td>CTGCTCTAGT</td>
</tr>
<tr>
<td>24081</td>
<td>GCCACCTGCTG</td>
<td>GATGGCAATT</td>
<td>TGTTGCTGGC</td>
<td>GCTGCTCTTC</td>
</tr>
</tbody>
</table>
Another example of a SARS-CoV S polypeptide, NS-1 strain, has accession number AAR91586 (gi: 40795747). See website at ncbi.nlm.nih.gov.

This sequence for this SARS-CoV S polypeptide is provided below (SEQ ID NO:3).

```plaintext
1 MFIFLLFELTL TSGSDLDRCT TFDDVQAPNY TQHTSSMRGV
41 YYPDEIFRSD TLYLTQDLFL PFYSNVTFGFH TINHTFGNPV
81 TPFFKDIYFA ATEKSNVVRG WVFSTMNKK SQSVIIINNS
121 TNVVRIRACNF ELCNPFPFAV SKFMGTQHTT MFDPNANCT
161 FEYISDAFSL DVSEKSGNFK HLRERFVFKN DGFLVYVKGY
201 QPIDVVRDLPL SGFNKLKPFF KLPLGGINTN FRAILTAFSP
241 AQDZWGTSAA AYFVGYLKPT TFMLKYGDEX TTDADVDCSQ
281 NPLAEELKCV KSFEIDQKGY QTSNFRVVPV GDVFIRWNIT
321 NLCFFGEVFN ATKFRPSVYW ERKKISNCVA DYSVLYNSTF
361 FSTFKCGYGS AKTLNDLCFS NYVADSVFVV GDDVRQATPG
401 QTGVIADYNK KLPPDFMGCW LAWNTRNIDA TSTGNYNKY
441 RYLHRHGLRPR FERDISNVFP SPDKGPCTPP ALNCYWPLND
481 YGFYTGTGIG YQPYRUVVLS FELLNAPATV CGPKLSTDLI
521 KNCVNFNFMN GLZGTGVLTP SSKRFQPFQQ FGRDVSDFTD
561 SVRDPKTFSEI LDISPSCSGG VSVTIPGNTA SSEVAVLYQD
```
In another embodiment, the invention provides antigenic fragments of SARS-CoV S polypeptides. In one embodiment, substantially full length SARS-CoV spike protein, with native signal sequences as well as transmembrane and cytoplasmic regions are deleted from the S polypeptide (ΔTM+CT). For the substantially full length S(ΔTM+CT) polypeptide, a cDNA encoding amino acids 14 to 1195 of the SARS-CoV (Urbani Strain) S protein was used (see GenBank accession no. AY278741, starting at nucleotide 21531) with a sequence for 6 histidine residues attached to its 3’end. The sequences of the S(ΔTM+CT) polypeptide (14-1195AA, SEQ ID NO:4) and cDNA (SEQ ID NO:5) are shown in Fig. 6 and are provided below. The S(ΔTM+CT) spike polypeptide sequence (14-1195AA, SEQ ID NO:4) is as follows.

```
14  SDLRDT CTFTDDQAPNY TQHTSSMRGV
41  YYPDEIFRSD TLTYLTQDLFL FYSNVTGFFH TINHTFGNPV
81  TFFKDIGYFA ATEKSNVVRG WFGSTMNNK SQSVIIINNS
121 TNVVIARCNF ELCDNFPFAV SKPMTQTHT MFNDAPNCT
161 FYFYSDAFSL DVEKSGNFK HLREFVFKNK DGFLYVYKGY
201 QPIDVVRDLDP SGFNTLKP1F KLPLGINITN FRAILTAFSP
241 AQD1WGTSA1 AYFVGYLKPT TFMLKYDENG TITDAVDCSQ
281 NPIAELKCSV KSFEDIKGIY QTSNFRVVPV GDVRFPNIT
321 NLCFPGEFVN ATKFPSVYAW EYKISNCVA DYSVLYNSTF
361 FSTFKDYGVS ATKMLNDCFS NVYADSFVVK GDVRIQAPG
401 QTGVIADYNN KLPDFOMGCV LAWNTRNIDA TSTGNYNYKY
441 RYLHKGKLRP FERDISNVFF SPDGKFCPP ALNCYWPND
481 YGFYTTTGGIG YQPYVYVLVS FELLNAPATV CGEKLSTDLL
521 KNQCVNPNFN GLTGTGVVLT PSKRFQPFFQ FGRDVSDFTD
```
561  SVRDPKTEI  LDSPCSFGG  VSVITPGTNA  SSEAVLYQD
561  560  VNCVDVSTAI  HADQITPFWR  IYSTGNNVFOQ  TQAGCLIGAE
561  560  HVTSYECDI  PIGAGICASY  HTVSSLRRST  QKSIVAYMTS
561  560  LGADDPXAYA  NTNIAAPTENF  SISITTEVMP  VSMATSVDCC
561  560  NMYICGDSTE  CANLLQYGQS  FCTQNLNRALS  GIAAEQRDRN
561  560  721  REVFAQVKQM  YKPTPLKTYG  GFNFSQILPD  PLKPTKRSFI
561  721  801  EDDLFPKVTI  ADAGFMMKQYG  ECLGDINARD  LICAQQKNGL
561  801  841  TTVLPLILDD  MIAAYTAALV  SGTTATAGWTF  GAGAALQIPF
561  841  881  AMQMYARFGNG  IGVTONVLYE  NQKQIANQFN  KAIQSIQBLSL
561  881  921  1571  TTSTSTALCKL  QDVQNQNAQA  LNTLVKQLLL  NFGAISVSLN
561  921  1001  DILRLDLKVE  AEVQIDRLIT  GRLQSLQTYV  TQOLIRAIREEI
561  1001  1041  RASANLAAKT  MSECVLGQSK  RVDFCGKGYH  LMSFQPAAHPH
561  1041  1081  GVVFLHVTYV  PSQERNFTTA  PAICHEGKAY  FPREGVFVFN
561  1081  1121  GTSWFITQRN  FFPSQIUTTD  NTFVSGNCDV  VIGIINMTYYV
561  1121  1161  DPLQPELDSF  KEKELDKYFKN  HTSPDVDLGD  ISGINASVVEN
561  1161  1201  IQKEIDRLNE  VAKNLNESLI  DLQELGKYEQ  YIKWPHHHH
561  1201  1201  H

The S(ΔTM±CT) cDNA (SEQ ID NO:5) sequence is as follows.

20  1  AGTACACTTGA  ACCGTCACAC  CACTTTTGTACTATTGAC  GATGTTCAAG
20  41  1  CTCCCTAATGA  CACTCAACAT  ACTTCATCTA  TGAGGGGGGT
20  81  1  TTACTCTATCT  GATGAAATTT  TTAGATCAGA  CACTCTTTAT
20  121  1  TTAACTCCAGG  ATTTATTCTT  TCCATTATTAT  TCTAATTGTA
20  161  1  CAGGGTTCTCA  TACTATTAAAT  CATACGTTTG  GCACCCTGGT
25  201  1  CATACCTTTT  AAGGATTGGA  TTATTTTGGC  TGCCACAGAG
25  241  1  AATACCAATGG  TTGTCGCTGG  TTGGGTGGTTT  GGTCTATCCA
25  281  1  TGACACACAA  GTCCAGCTGG  GTGAATTATTAT  TTACAAATTC
25  321  1  TAACTATTGTT  TTATACAGAG  CATGTAACCTT  TGAATTTGTTG
25  361  1  GACACACCTCT  TCTTTCTGCTT  TTTCAAAACC  ATGGGGTACAC
30  401  1  AGACACATAC  TATGATTATTAT  GATAATTCATT  TTAATTCGAC
30  441  1  TTTCTGAGATG  ATATCAGTATG  CTTTCTCTCCTG  TGAATTTTCA
30  481  1  GAAAGTGCTG  GTAAATTAAA  ACATCTACGG  GAGTTGTTGT
30  521  1  TTTAAATAGA  AGATGGGTCT  CTCTATGTTT  ATAGGGGCTA
30  561  1  TCACCCCTATA  GATGTTAGTCG  GTAGATCACC  TTCTGTTTTT
35  601  1  AACACTTTGGA  AACCTATTAT  TAAAGTGCTCT  CTTGGGATTAT
35  641  1  ACATACACAAA  TTATCGAGCC  ATCTTTTCAAG  CTTTTCTCAC
35  681  1  TGCTCAAGAT  ATTGGGGGCA  GTTCAGTCTG  AGCTTTTTTT
35  721  1  GTTGTAGTTT  TAAAGGCACAC  TACATTATTAT  CTCAAGTATG
35  761  1  AGAAGATCGG  TACAATCAGA  GATGCTGTCT  ATTGTCTACA
40  801  1  AAATCCCACTT  GCTGAACTCA  AAATCTGCTGT  TAAAGAGCTT
40  841  1  GATGTTGACA  AAGGAATTTA  CCGACACCTCT  AATTTCAAGG
40  881  1  TTGCCTTCTCG  AGGAGATGGT  GTGAGATTCT  CTAATTATAC
40  921  1  AAATCTTGTG  CTTTTTGAGG  AGTTTTTTAA  TGCTCATTAA
40  961  1  GCTCCCTCTTG  CTATAGCAGT  AGGAGAGAAAT  AAAATTCTCA
45  1001  1  ATTTGTGTTGC  TGATTACTCT  GTGCTCTACA  ACTCAACATT
45  1041  1  TTTTTCAACC  TTTAATGCTT  ATGGCCTTTCG  TGCCACTAAG
45  1081  1  TTGAATGTAC  TTTGCTTCTC  CAAATGCTAT  CGCAGTCTT
45  1121  1  TTGTACTCAAA  GGGACAGTG  GATAAGACAAA  TAGCCCGAAG
45  1161  1  ACAACTGCTT  GGTATGTCTGT  ATTATATTAT  TAAATGTGCA
50  1201  1  GATGATTCCA  TGGGTTGCTG  CCTTGCTTGG  AATACTAGGAA
50  1241  1  ACATTTGATG  TACTTCAAAT  GTGTAATTATA  ATTATAAATA
1281 TAGGTATCTT AGACATGGCA AGCCTAGGCC CTCTGAGAGA
1321 GACATATCTCA ATGGCGCTTT CTCGCCCTGAT GGCAAAACTT
1361 GCACCTCACC TGCTCTTGAAT TGTTGTTGAG CATTAAATG
1401 TTATGCTTTT TACACTACTA CTGGCATGAG CTTCAACACT
5 1441 TACAGAGTGG TAGTACTTCTC TTTTGAACTT TTAAATGCACT
1481 CGGCGCCGCTT TTTGGAGGACA AAATATCCA CGAGCCTTAT
1521 TAAGAACCAG TGGTCAATT TTAATTCTAA TGGACTCAGT
1561 GTGTGGTTGT TGGTATCTCC TCTCTGCAAG AGATTTCAC
1601 CATTCTCCAA AGTTGGCCGT TAGTCTTTGC TATTTACACT
10 1641 TCCCGTTCGA GATCCTAAA CATCTGGAAT ATTAGCATT
1681 TCACCTTGGT CTTTGGGGGC TGTAGTGTGA ATTAACACTG
1721 GAACAAATGC TTTACTCTGA GGTCTCTTGC TATATCAAG
1761 TGGTAACTGC ACTGATGTTT TACACGCAAT CATGCGAGAT
1801 CAACTACACAG CGGTGAGGCA CATATACTCT CATGCAAAAC
1841 ATGGATTCTCA GACCTAGGCA GCCTGCTCTA TAGGAGCTTG
1881 GCATGGCGAC ACTCTCTTATG AGTGCCAGAT TCTGTATTGA
1921 GCCTGCACTTT GTGCTAGTTA CCATTACATT CCTTTATTAC
1961 GTAGTACTAG CCAAATAATCT ATTTGTGGCT ATACTATGGC
20 2001 TTATGCTTGT CTAGTTCGAC CCTGTATCCT TAAATACCC
2041 ATGGCTATAC CTACTAACTT TCTAAATTGC ATTCATCAG
2081 AACATAGGCG TGGTTCTATG GCTAACAATC CGGTAGATTG
2121 TAATATCTGC ATCTCGCGAG ATCTCTACTG ATGCGTAAT
2161 TTGCTTCTCC TAAATGCTTG CTTTTGCAAA CAACTAATAC
2201 GTGCACCTCTC AGGATTGTGT GCTGAGAAAG ATCGCAACAC
2241 AGCTGAAATG TTTGCCTCAG TCAACAAATA GTCAAAACC
2281 CCAACCTTTGA AAATATTTGA TGGTTTAAAT TTTTACCAAA
2321 TAATACCTCG CCACTTAAAG CCAACTAAAG GCTTCTTTAT
2361 TGAGGACTTG CTTTTTATAA AGGGAGACCT CGCTATGCTT
2401 GCCTTCAGTA AGGCAATATAG CGAAGTCGATA AGTGGATTAA
2441 ATGGCTAGAGA TCTCATTGGT GGCAGAAGGT TCAATGGACT
2481 TACATGTTTG GCCACCTTGC TCATGCTGGA TATGATTGCT
2521 GCCTACACTCT GTGCTCTAGT TAGTGTTGACT GCCATGTGCT
2561 GAGGGGACATT TGTGCTCTGC GCTGTCTTCTA AAATACCTTT
2601 TGCTATGCCAA TGGCACTATA GTGCTCATTG AGTGGGATTG
30 2641 ACCCAAAATG TTTCTCTATG GACCAAAGAA CAAATCGCCA
2681 ACCAAATTTAA CAGGGCGATT AGTCAAATTC AAGAATCACT
2721 TACACACACA TCAACTCTAG TAGGCAAGCT GAAAGACGTT
2761 GTTACCAGAG ATGCTCAAAG ATTAACACA CTTGTAAAC
2801 AACTTAGCTC TAATTTGGGT GCAATTTCAA GTGGCGTTAA
35 2841 TGATATCCCT TGCGGACTTG ATAAAGTCGA GCCGGAGTTA
2881 CAAATGGACA GGTAAATTAC AGGCGACTT CAAAGCCCTC
2921 AACATATTTG AACAACAAAC CTAATCACGG CTGCTGAAAT
2961 CGGGCTCTT GCTAAATCTG CTGCTAATA AATGCTGAG
3001 TGCTGTCTTG GACAATCAA AAGATGGAC TTTGTTGGAA
40 3041 AGGCGTACCA CTTATAGGCC TTCCCAACAG CAGGCCCGCA
3081 TGGTGTTGGT TTTCTCATAT TCACGATGTG GCCATCCAG
3121 GAGGGGACAT TCACGCACGC GCCAGCAATT TGTCAAGAG
3161 GCAAGGCTTA CTCCCTCGTT GAAGGTTGTT TGTGTTTAAA
3201 TGGCACTTCT TGTTTATATA CACAGGAGAA CTCTTTTCTC
45 3241 CCACAAATAA TATACACAGA CAATACATTG GCTCCAGGAA
3281 ATTTGTGATGT CTTATTGGGC ATCAATAACA ACAGGTTTA
50
An S polypeptide encoding the N-terminal 14-762 amino acids is also
highly antigenic and is provided for use in an immunogenic composition or
vaccine. This S polypeptide fragment was selected on the basis of hydrophilicity
and secondary structure predictions using Kyte and Dolittle and Chou Fasman
algorithms (McVactor 7.2) and also because it encompasses the receptor binding
region as well as the region corresponding to S1 of other coronaviruses. The
sequence of this N-terminal 14-762 amino acid spike polypeptide is as follows
(SEQ ID NO:6).

```
14  SDLRCT TFDDVQAPNY TQHTSSMRGV
41  YYPDEIFRSD TLYLTQDLFL PFYSNVTFGH TINH7F8GPV
81  IPFKDGIYFA ATEKSNVVRG VVFXSTMKMN SQSVIIIIN
121 TNVVRACNF BLCDFPPFAV SKPMGTQHTH MIFMDNAFNT
161 FEYTSDFAFSL DVSEKSGNFK HLHVEFVKKN DGFLYVYKGY
201  QPIDVQRDLTP SGFNTLKPFP IFKLPGPAITIN TFAILTASAP
241 AQDINGTSSAA AYFVGKLYKPT TFMKLKDENG TITDAVDCSQ
281  NPLAELKCSV KSFEIDKGIY QTSNFRVVPVS GDVVRFPNIT
321  NLCPPFGEVFN ATKPSSVVAW ERKKISNCVA DYSVLHYNSTF
361  FSTFKCYGVST AKLNDFLCEF NVYADSFVVK GDDVRQIAPG
401  QTGGVIAVEN KLPSDFMGCIV LAWNTRNIDA TSTGNYNYKY
441  RYLHRHGKLLRP FERDSNNVF SPDGKPCSTTP ALNCWPLNND
481  QGFYYTTTGGQ QPYRYYVLLSP FELLNAPATV CPGKLDSDLI
521  KNQCVNPNNFN GLTGTGVILT PSSKRQFQFFQ FGRVDVSDFTDI
561  SVRDPKSETEI LDISPSCFSGG VSVITPNTA SBEAVLYQD
601  VNCTDQSTALP HADQLTQPAR IYSTGNNVFQ TQACCLIGAE
641  HVDSYECEDIG PIGAGICASY HTVSSLRSTS QKISIVATMS
681  LGADSSYAYS NNTTAIPNTNF SISITTEVMP VSMAKTSVDC
```

A C-terminal S polypeptide fragment is also provided for use in the
immunogenic compositions and vaccines of the invention. This C-terminal S
polypeptide fragment includes amino acids 763-1195. The sequence for this C-
terminal 763-1195 amino acid SARS-CoV S polypeptide is as follows (SEQ ID
NO:7).

```
763  VFAQVKQKM YKTPDKLYFG GFNFSQILPD PLKPTKRSFI
801  EDLLENKVTLL ADAGFMKQYG ECLGDDINARD LICAQKFNGL
```
Thus, the invention provides S polypeptides and antigenic fragments thereof that are useful for treating and preventing SARS infection. Moreover, peptide variants and derivatives of the S polypeptides and peptides are also useful in the practice of the invention. Such peptide variants and derivatives can have one or more amino acid substitutions, deletions, insertions or other modifications so long as the S polypeptide variant or derivative can induce an immune response against an S polypeptide or against SARS-CoV.

Amino acid residues of the S polypeptides can be genetically encoded L-amino acids, naturally occurring non-genetically encoded L-amino acids, synthetic L-amino acids or D-enantiomers of any of the above. The amino acid notations used herein for the twenty genetically encoded L-amino acids and common non-encoded amino acids are conventional and are as shown in Table 1.

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>One-Letter Symbol</th>
<th>Common Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>A</td>
<td>Ala</td>
</tr>
<tr>
<td>Arginine</td>
<td>R</td>
<td>Arg</td>
</tr>
<tr>
<td>Asparagine</td>
<td>N</td>
<td>Asn</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>D</td>
<td>Asp</td>
</tr>
<tr>
<td>Cysteine</td>
<td>C</td>
<td>Cys</td>
</tr>
<tr>
<td>Glutamine</td>
<td>Q</td>
<td>Gln</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>E</td>
<td>Glu</td>
</tr>
<tr>
<td>Glycine</td>
<td>G</td>
<td>Gly</td>
</tr>
<tr>
<td>Histidine</td>
<td>H</td>
<td>His</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>I</td>
<td>Ile</td>
</tr>
<tr>
<td>Leucine</td>
<td>L</td>
<td>Leu</td>
</tr>
<tr>
<td>Amino Acid</td>
<td>One-Letter Symbol</td>
<td>Common Abbreviation</td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>-------------------</td>
<td>--------------------</td>
</tr>
<tr>
<td>Lysine</td>
<td>K</td>
<td>Lys</td>
</tr>
<tr>
<td>Methionine</td>
<td>M</td>
<td>Met</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>F</td>
<td>Phe</td>
</tr>
<tr>
<td>Proline</td>
<td>P</td>
<td>Pro</td>
</tr>
<tr>
<td>Serine</td>
<td>S</td>
<td>Ser</td>
</tr>
<tr>
<td>Threonine</td>
<td>T</td>
<td>Thr</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>W</td>
<td>Trp</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>Y</td>
<td>Tyr</td>
</tr>
<tr>
<td>Valine</td>
<td>V</td>
<td>Val</td>
</tr>
<tr>
<td>β-Alanine</td>
<td></td>
<td>Bala</td>
</tr>
<tr>
<td>2,3-Diaminopropionic acid</td>
<td></td>
<td>Dpr</td>
</tr>
<tr>
<td>α-Aminoisobutyric acid</td>
<td></td>
<td>Aib</td>
</tr>
<tr>
<td>N-Methylglycine (sarcosine)</td>
<td></td>
<td>MeGly</td>
</tr>
<tr>
<td>Ornithine</td>
<td></td>
<td>Orn</td>
</tr>
<tr>
<td>Citrulline</td>
<td></td>
<td>Cit</td>
</tr>
<tr>
<td>t-Butylalanine</td>
<td></td>
<td>t-BuA</td>
</tr>
<tr>
<td>t-Butylglycine</td>
<td></td>
<td>t-BuG</td>
</tr>
<tr>
<td>N-methylisoleucine</td>
<td></td>
<td>Mlle</td>
</tr>
<tr>
<td>Phenylglycine</td>
<td></td>
<td>Phg</td>
</tr>
<tr>
<td>Cyclohexylalanine</td>
<td></td>
<td>Cha</td>
</tr>
<tr>
<td>Norleucine</td>
<td></td>
<td>Nle</td>
</tr>
<tr>
<td>Naphthylalanine</td>
<td></td>
<td>Nal</td>
</tr>
<tr>
<td>Pyridylanine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-Benzothienyl alanine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-Chlorophenylalanine</td>
<td></td>
<td>Phe(4-Cl)</td>
</tr>
<tr>
<td>2-Fluorophenylalanine</td>
<td></td>
<td>Phe(2-F)</td>
</tr>
<tr>
<td>3-Fluorophenylalanine</td>
<td></td>
<td>Phe(3-F)</td>
</tr>
<tr>
<td>4-Fluorophenylalanine</td>
<td></td>
<td>Phe(4-F)</td>
</tr>
<tr>
<td>Penicillamine</td>
<td></td>
<td>Pen</td>
</tr>
<tr>
<td>Amino Acid</td>
<td>One-Letter Symbol</td>
<td>Common Abbreviation</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>-------------------</td>
<td>--------------------</td>
</tr>
<tr>
<td>1,2,3,4-Tetrahydro-</td>
<td></td>
<td>Tic</td>
</tr>
<tr>
<td>isoquinoline-3-carboxylic acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-2-thienylalanine</td>
<td></td>
<td>Thi</td>
</tr>
<tr>
<td>Methionine sulfoxide</td>
<td></td>
<td>MSO</td>
</tr>
<tr>
<td>Homoarginine</td>
<td></td>
<td>hArg</td>
</tr>
<tr>
<td>N-acetyl lysine</td>
<td></td>
<td>AcLys</td>
</tr>
<tr>
<td>2,4-Diamino butyric acid</td>
<td></td>
<td>Dbu</td>
</tr>
<tr>
<td>ρ-Aminophenylalanine</td>
<td></td>
<td>Phe(pNH₂)</td>
</tr>
<tr>
<td>N-methylvaline</td>
<td></td>
<td>MeVal</td>
</tr>
<tr>
<td>Homocysteine</td>
<td></td>
<td>hCys</td>
</tr>
<tr>
<td>Homoserine</td>
<td></td>
<td>hSer</td>
</tr>
<tr>
<td>ε-Amino hexanoic acid</td>
<td></td>
<td>Aha</td>
</tr>
<tr>
<td>δ-Amino valeric acid</td>
<td></td>
<td>Ava</td>
</tr>
<tr>
<td>2,3-Diaminobutyric acid</td>
<td></td>
<td>Dab</td>
</tr>
</tbody>
</table>

S polypeptides that are within the scope of the invention can have one or more amino acids substituted with an amino acid of similar chemical and/or physical properties, so long as these variant or derivative S polypeptides retain the ability to induce an immune response in an animal against SARS-CoV.

Amino acids that are substitutable for each other generally reside within similar classes or subclasses. As known to one of skill in the art, amino acids can be placed into three main classes: hydrophilic amino acids, hydrophobic amino acids and cysteine-like amino acids, depending primarily on the characteristics of the amino acid side chain. These main classes may be further divided into subclasses. Hydrophilic amino acids include amino acids having acidic, basic or polar side chains and hydrophobic amino acids include amino acids having aromatic or apolar side chains. Apolar amino acids may be further subdivided to include, among others, aliphatic amino acids. The definitions of the classes of amino acids as used herein are as follows:

“Hydrophobic Amino Acid” refers to an amino acid having a side chain that is uncharged at physiological pH and that is repelled by aqueous solution.
Examples of genetically encoded hydrophobic amino acids include Ile, Leu and Val. Examples of non-genetically encoded hydrophobic amino acids include t-BuA.

“Aromatic Amino Acid” refers to a hydrophobic amino acid having a side chain containing at least one ring having a conjugated π-electron system (aromatic group). The aromatic group may be further substituted with substituent groups such as alkyl, alkenyl, alkynyl, hydroxyl, sulfonyl, nitro and amino groups, as well as others. Examples of genetically encoded aromatic amino acids include phenylalanine, tyrosine and tryptophan. Commonly encountered non-genetically encoded aromatic amino acids include phenylglycine, 2-naphthylalanine, β-2-thienylalanine, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, 4-chlorophenylalanine, 2-fluorophenylalanine, 3-fluorophenylalanine and 4-fluorophenylalanine.

“Apolar Amino Acid” refers to a hydrophobic amino acid having a side chain that is generally uncharged at physiological pH and that is not polar. Examples of genetically encoded apolar amino acids include glycine, proline and methionine. Examples of non-encoded apolar amino acids include Cha.

“Aliphatic Amino Acid” refers to an apolar amino acid having a saturated or unsaturated straight chain, branched or cyclic hydrocarbon side chain. Examples of genetically encoded aliphatic amino acids include Ala, Leu, Val and Ile. Examples of non-encoded aliphatic amino acids include Nle.

“Hydrophilic Amino Acid” refers to an amino acid having a side chain that is attracted by aqueous solution. Examples of genetically encoded hydrophilic amino acids include Ser and Lys. Examples of non-encoded hydrophilic amino acids include Cit and hCys.

“Acidic Amino Acid” refers to a hydrophilic amino acid having a side chain pK value of less than 7. Acidic amino acids typically have negatively charged side chains at physiological pH due to loss of a hydrogen ion. Examples of genetically encoded acidic amino acids include aspartic acid (aspartate) and glutamic acid (glutamate).

“Basic Amino Acid” refers to a hydrophilic amino acid having a side chain pK value of greater than 7. Basic amino acids typically have positively charged side chains at physiological pH due to association with hydronium ion. Examples of genetically encoded basic amino acids include arginine, lysine and
histidine. Examples of non-genetically encoded basic amino acids include the
non-cyclic amino acids ornithine, 2,3-diaminopropionic acid, 2,4-diaminobutyric
acid and homoarginine.

"Polar Amino Acid" refers to a hydrophilic amino acid having a side
chain that is uncharged at physiological pH, but which has a bond in which the
pair of electrons shared in common by two atoms is held more closely by one of
the atoms. Examples of genetically encoded polar amino acids include
asparagine and glutamine. Examples of non-genetically encoded polar amino
acids include citrulline, N-acetyl lysine and methionine sulfoxide.

"Cysteine-Like Amino Acid" refers to an amino acid having a side chain
capable of forming a covalent linkage with a side chain of another amino acid
residue, such as a disulfide linkage. Typically, cysteine-like amino acids
generally have a side chain containing at least one thiol (SH) group. Examples
of genetically encoded cysteine-like amino acids include cysteine. Examples of
non-genetically encoded cysteine-like amino acids include homocysteine and
penicillamine.

As will be appreciated by those having skill in the art, the above
classifications are not absolute. Several amino acids exhibit more than one
characteristic property, and can therefore be included in more than one category.
For example, tyrosine has both an aromatic ring and a polar hydroxyl group.
Thus, tyrosine has dual properties and can be included in both the aromatic and
polar categories. Similarly, in addition to being able to form disulfide linkages,
cysteine also has apolar character. Thus, while not strictly classified as a
hydrophobic or apolar amino acid, in many instances cysteine can be used to
confer hydrophobicity to a peptide.

Certain commonly encountered amino acids that are not genetically
encoded and that can be present, or substituted for an amino acid, in the peptides
and peptide analogues include, but are not limited to, β-alanine (β-Ala) and other
omega-amino acids such as 3-aminopropionic acid (Dap), 2,3-diaminopropionic
acid (Dpr), 4-aminoisobutyric acid and so forth; α-aminoisobutyric acid (Aib); ε-
aminohexanoic acid (Aha); δ-aminovaleric acid (Av); methylglycine (MeGly);
ornithine (Orn); citrulline (Cit); t-butyllalanine (t-BuA); t-butylglycine (t-BuG);
N-methylisoleucine (MeIle); phenylglycine (Phg); cyclohexylalanine (Cha);
norleucine (Nle); 2-naphthylalanine (2-Nal); 4-chlorophenylalanine (Phe(4-Cl));
2-fluorophenylalanine (Phe(2-F)); 3-fluorophenylalanine (Phe(3-F)); 4-
fluorophenylalanine (Phe(4-F)); penicillamine (Pen); 1,2,3,4-
tetrahydroisoquinoline-3-carboxylic acid (Tic); β-2-thienylalanine (Thi);
methionine sulfoxide (MSO); homoarginine (hArg); N-acetyl lysine (AcLys);
2,3-diaminobutyric acid (Dab); 2,3-diaminobutyric acid (Dbu); p-
aminophenylalanine (Phe(pNH2)); N-methyl valine (MeVal); homocysteine
(hCys) and homoserine (hSer). These amino acids also fall into the categories
defined above.

The classifications of the above-described genetically encoded and non-
encoded amino acids are summarized in Table 2. It is to be understood that
Table 2 is for illustrative purposes only and does not purport to be an exhaustive
list of amino acid residues that may include the peptides and peptide analogues
described herein. Other amino acid residues that are useful for making the
peptides and peptide analogues described herein can be found, e.g., in Fasman,
1989, CRC Practical Handbook of Biochemistry and Molecular Biology, CRC
Press, Inc., and the references cited therein. Amino acids not specifically
mentioned herein can be conveniently classified into the above-described
categories on the basis of known behavior and/or their characteristic chemical
and/or physical properties as compared with amino acids specifically identified.

<table>
<thead>
<tr>
<th>Classification</th>
<th>Genetically Encoded</th>
<th>Genetically Non-Encoded</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrophobic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aromatic</td>
<td>F, Y, W</td>
<td>Phg, Nal, Thi, Tic, Phe(4-Cl), Phe(2-F), Phe(3-F), Phe(4-F), Pyridyl Ala, Benzo thi enyl Ala</td>
</tr>
<tr>
<td>Apolar</td>
<td>M, G, P</td>
<td></td>
</tr>
<tr>
<td>Aliphatic</td>
<td>A, V, L, I</td>
<td>t-BuA, t-BuG, Melle, Nle, MeVal, Cha, bAla, MeGly, Aib</td>
</tr>
<tr>
<td>Hydrophilic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acidic</td>
<td>D, E</td>
<td></td>
</tr>
<tr>
<td>Classification</td>
<td>Genetically Encoded</td>
<td>Genetically Non-Encoded</td>
</tr>
<tr>
<td>------------------</td>
<td>---------------------</td>
<td>-------------------------------------------------</td>
</tr>
<tr>
<td>Basic</td>
<td>H, K, R</td>
<td>Dpr, Orn, hArg, Phe(p-NH₂), DBU, A₂BU</td>
</tr>
<tr>
<td>Polar</td>
<td>Q, N, S, T, Y</td>
<td>Cit, AcLys, MSO, hSer</td>
</tr>
<tr>
<td>Cysteine-Like</td>
<td>C</td>
<td>Pen, hCys, β-methyl Cys</td>
</tr>
</tbody>
</table>

Polypeptides can have any amino acid substituted by any similarly classified amino acid to create a variant or derivative peptide, so long as the peptide variant or derivative retains the ability to induce an immune response in an animal. Preferably, the immune response is against SARS-CoV.

The ability of S polypeptides, derivatives and variants thereof to generate an immune response in an animal can be assessed by procedures available to one of skill in the art. For example, the S polypeptide, derivative or variant thereof can be administered to the animal and, after a time period sufficient for production of antibodies, serum can be collected from the animal to ascertain whether the animal has produced circulating antibodies that are reactive with the S polypeptide, derivative or variant thereof.

One of skill in the art may also choose to test the S polypeptides, derivatives and variants thereof to ascertain whether they are useful for inhibiting SARS viral replication in an animal. A rodent animal model has been developed in which SARS-CoV replicates but does not cause disease (Subbarao et al. (2004) J. Virol. 78, 3572-3577). An S polypeptide, derivative or variant thereof can be administered to such a rodent animal, the animal can then be exposed to SARS-CoV and the respiratory tract or lungs of the animal can be monitored for SARS-CoV viral load. If administration of the S polypeptide, derivative or variant thereof reduces the viral load relative to animals exposed to the SARS-CoV but not immunized with the S polypeptide, derivative or variant thereof, then the S polypeptide, derivative or variant is an effective immunogen that can be used to treat or protect an animal against SARS-CoV infections.

**Attenuated Recombinant Viruses Encoding SARS-CoV Antigens**

Attenuated recombinant viruses that express SARS-CoV specific epitopes are of use in immunological compositions of this invention. Attenuated viruses are modified from their wild type virulent form to a non-infective or
weakened form when administered to humans. Among the recombinant viruses that can be used are adenoviruses, adeno-associated viruses, retroviruses and poxviruses. For example, a recombinant, attenuated virus for use in an immunogenic composition or vaccine is a virus wherein the genome of the virus is defective with respect to a gene that is essential for the efficient production of an infectious virus. The mutant virus acts as a vector for production of an immunogenic SARS-CoV S epitope or antigenic SARS-CoV S polypeptide by virtue of insertion of S polypeptide DNA into the genome of the virus. Expression of the SARS-CoV S epitopes or antigens provokes or stimulates an immune response against S polypeptides and against SARS-CoV.

A variety of attenuated viruses can be used. Examples of viral expression vectors include adenoviruses as described in M. Eloit et al., *Construction of a Defective Adenovirus Vector Expressing the Pseudorabies Virus Glycoprotein gp50 and its Use as a Live Vaccine*, J. Gen. Virol. 71(10):2425-2431 (Oct., 1990), adeno-associated viruses (see, e.g., Samulski et al., J. Virol. 61: 3096-3101 (1987); Samulski et al., J. Virol. 63:3822-3828 (1989)), papillomavirus, Epstein Barr virus (EBV) and Rhinoviruses (see, e.g., U.S. Patent No. 5,714,374). Human parainfluenza viruses are also reported to be useful, especially JS CP45 HPIV-3 strain. The viral vector may be derived from herpes simplex virus (HSV) in which, for example, the gene encoding glycoprotein H (gH) has been inactivated or deleted. Other suitable viral vectors include retroviruses (see, e.g., Miller, Human Gene Ther. 1:5-14 (1990); Ausubel et al., Current Protocols in Molecular Biology).

Poxviruses can be used in the compositions of this invention. There are a variety of attenuated poxviruses that are available for use as an immunological composition against SARS-CoV. These include attenuated vaccinia virus, cowpox virus and canarypox virus.

Techniques for inserting SARS-CoV S polypeptides into the recombinant virus are available. For example, one technique for inserting foreign genes into live infectious poxvirus involves a recombination between pox DNA sequences flanking a foreign genetic element in a donor plasmid and homologous sequences present in the rescuing poxvirus as described in Piccini et al., Methods in Enzymology 153, 545-563 (1987). In some embodiments, the recombinant poxviruses are constructed in two steps using procedures like those for creating
synthetic recombinants of poxviruses such as the vaccinia virus and avipox virus as described in U.S. Patent No. 4,769,330, U.S. Patent No. 4,722,848, U.S. Patent No. 4,603,112, U.S. Patent No. 5,110,587 and U.S. Patent No. 5,174,993, the disclosures of which are incorporated herein by reference.

Thus, for example, a nucleic acid segment encoding an antigenic S polypeptide sequence, such as an identified or known T-cell epitope, is selected to be inserted into the virus. The nucleic acid segment to be inserted is generally operably ligated to a promoter. The promoter-SARS-CoV segment is positioned in the plasmid construct so that the promoter-gene linkage is flanked on both ends by DNA homologous to a DNA sequence flanking a region of pox DNA encoding a nonessential function.

The resulting plasmid construct is then amplified by growth in a host cell, for example, within *E. coli* cells. Thus, the isolated vector or plasmid containing the SARS-CoV sequence to be inserted into the poxviral genome is transfected into animal cells (e.g. chick embryo fibroblasts), along with the poxvirus. Recombination between homologous pox DNA in the plasmid and the viral genome gives a poxvirus with a SARS-CoV insertion. In general, one of skill in the art selects a nonessential region of the poxvirus genome to insert the foreign (SARS-CoV) DNA sequences.

Attenuated recombinant pox viruses are often used as viral vectors in the compositions of the invention. A review of this technology is found in U.S. Patent No. 5,863,542, which is incorporated by reference herein. Representative examples of recombinant pox viruses include MVA, ALVAC, TROVAC, NYVAC, and vCP205 (ALVAC-MN120TMG). These viruses have been deposited under the terms of the Budapest Treaty with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Md., 20852, USA. The NYVAC virus has been deposited under ATCC accession number VR-2559 on Mar. 6, 1997. The vCP205 (ALVAC-MN120TMG) virus has been deposited under ATCC accession number VR-2557 on Mar. 6, 1997. The MVA virus has been deposited under ATCC accession number VR-1508 or VR-1566. The TROVAC virus has been deposited under ATCC accession number VR-2553 on Feb. 6, 1997, and the ALVAC virus has been deposited under ATCC accession number VR-2547 on Nov. 14, 1996.
NYVAC is a genetically engineered vaccinia virus strain generated by the specific deletion of eighteen open reading frames encoding gene products associated with virulence and host range. NYVAC is highly attenuated by a number of criteria including: (a) decreased virulence after intracerebral inoculation in newborn mice, (b) inoquity in genetically (nu+/nu+) or chemically (cyclophosphamide) immunocompromised mice; (c) failure to cause disseminated infection in immunocompromised mice, (d) lack of significant induration and ulceration on rabbit skin; (e) rapid clearance from the site of inoculation; and (f) greatly reduced replication competency on a number of tissue culture cell lines including those of human origin.

TROVAC refers to an attenuated fowlpox that is a plaque-cloned isolate derived from the FP-1 vaccine strain of fowlpoxvirus, which is licensed for vaccination of one-day old chicks.

ALVAC is an attenuated canarypox virus-based vector that was a plaque-cloned derivative of the canarypox vaccine, Kanapox (Taraglia et al., AIDS Res. Hum. Retroviruses 8:1445-47 (1992)). ALVAC has some general properties which are similar to the Kanapox. ALVAC-based recombinant viruses expressing extrinsic immunogens have also been demonstrated to be efficacious as vaccine vectors. This avipox vector is restricted to avian species for productive replication. In human cell cultures, canarypox virus replication is aborted early in the viral replication cycle prior to viral DNA synthesis. Nevertheless, when engineered to express immunogens, authentic expression and processing are observed in vitro in mammalian cells and inoculation into numerous mammalian species induces antibody and cellular immune responses to the extrinsic immunogen and provides protection against challenge with the cognate pathogen.

NYVAC, ALVAC ad TROVAC have also been recognized as unique among poxviruses in the National Institutes of Health (U.S. Public Health Service), Recombinant DNA Advisory Committee, which issues guidelines for the physical containment of genetic material such as viruses and vectors. This Committee granted a reduction in physical containment level for NYVAC, ALVAC and TROVAC from BSL2 to BSL1.

Another attenuated poxvirus for use in the invention is the Modified Vaccinia virus Ankara (MVA), which acquired defects in its replication ability
in humans, as well as most mammalian cells, following over 500 serial passages in chicken fibroblasts (see, e.g., Mayr et al., Infection 3: 6-14 (1975); Carrol, M and Moss, B., Virology 238: 198-211 (1997)). MVA retains its original immunogenicity and its variola-protective effect and longer has any virulence and contagiousness for animals and humans. As for the NYVAC and ALVAC viruses, expression of recombinant polypeptides by MVA occurs during an abortive infection of human cells, thus providing a safe, yet effective, delivery system for antigenic S polypeptides.

Vaccinia virus vectors, including the highly attenuated modified vaccinia virus Ankara (MVA) strain, have been used to express and characterize glycoproteins of numerous pathogens and some of those are being evaluated as candidate prophylactic and therapeutic vaccines (Moss, B. (1996) Proc. Natl. Acad. Sci. USA 93, 11341-11348). MVA accumulated multiple deletions and other mutations during more than 500 passages in chicken embryo fibroblasts (CEF) resulting in a severe host range restriction in most mammalian cells. Because the restriction occurs at a late stage of virus assembly, MVA expresses viral and recombinant proteins in non-permissive as well as in permissive cells. MVA is highly attenuated due to its replication defect in mammalian cells and no adverse effects were reported even when high doses of MVA were given to immune deficient non-human primates or severe combined immunodeficiency disease mice.

Hence, nucleic acids encoding antigenic SARS-CoV S polypeptides can be inserted into viral genomes such as those of the poxviruses described herein, to generate a recombinant virus that can express the SARS-CoV S polypeptide after administration to an animal (e.g. a human).

The recombinant virus is introduced into an animal (e.g. a human) by standard methods for administering immunogenic compositions or for vaccination with live vaccines. A composition containing live recombinant virus can be administered at, for example, about $10^4$ - $10^8$ organisms/dose, or $10^6$ to $10^{10}$ pfu per dose. For example, NYVAC, ALVAC or MVA recombinant poxviruses can be administered by an intramuscular route using a dosage of about $10^7$ to $10^9$ pfu per inoculation, for a patient of about 100 to 200 pounds. Compositions containing such recombinant viruses can be delivered in a physiologically compatible solution such and phosphate buffered saline in a
volume of about 0.05 to about 1.5 ml. Such dosages can be administered once or several times in a continuous or intermittent fashion, using a regimen that is readily determined by one of ordinary skill in the field.

5 Nucleic Acid-Based Immunological Composition

Alternatively, an immunological or vaccine composition of the invention may contain DNA encoding one or more of the SARS S polypeptides described herein, such that the polypeptide is generated in situ. The DNA may thus be "naked," as described, for example, in Ulmer et al., Science 259:1745-1749 (1993), and reviewed by Cohen, Science 259:1691-1692 (1993). In such compositions, the DNA may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid expression systems, bacteria and viral expression systems. Appropriate nucleic acid expression systems contain the necessary DNA sequences for expression in the patient (such as a suitable promoter and terminating signal). Bacterial delivery systems involve the administration of a bacterium (such as Bacillus-Calmette-Guerrin) that expresses an immunogenic portion of the polypeptide on its cell surface.

Any of the conventional vectors used for expression in eukaryotic cells may be used directly introducing DNA into tissue. Expression vectors containing regulatory elements from eukaryotic viruses are often used in eukaryotic expression vectors, for example, SV40 vectors, pMSG, PAV009/A+, pMAMneo-5, baculovirus pDSVE, and other vectors that permit expression of proteins under the direction of promoters such as the SV40 early promoter, SV40 later promoter, metallothionein promoter, human cytomegalovirus promoter, murine mammary tumor virus promoter, Rous sarcoma virus promoter, polyhedron promoter, or other promoters effective for expression in eukaryotic cells.

Therapeutic quantities of plasmid DNA can be produced, for example, by expansion in E. coli, followed by purification. Aliquots from the working cell bank are used to inoculate growth medium, and the cells are grown to saturation in shaker flasks or bioreactors using procedures available in the art. Plasmid DNA can be purified using available bioseparation techniques such as solid
phase anion-exchange resins. If required, supercoiled DNA can be isolated from the open circular and linear forms using gel electrophoresis or other methods.

Preferred plasmid DNA can be prepared for administration using a variety of formulations. The simplest is reconstitution of lyophilized DNA in sterile phosphate-buffer saline (PBS). This formulation, known as “naked DNA,” is particularly suitable for intramuscular (IM) or intradermal (ID) administrations. To maximize the immunotherapeutic effects of plasmid DNA vaccines, alternate methods for formulating purified plasmid DNA may be desirable. A variety of methods has been described, and such methods are available to one of skill in the art. Cationic lipids can be used in the formulation, for example, as described by WO 93/24640; Mannino & Gould-Fogerite, BioTechniques 6(7): 682 (1988); U.S. Patent No. 5,279,833; WO 91/06309; and Felgner et al., Proc. Nat'l Acad. Sci. USA 84: 7413 (1987). In addition, glycolipids, fusogenic liposomes, peptides and compounds referred to collectively as protective, interactive, non-condensing compounds (PINC) can also be complexed to purified plasmid DNA to influence variables such as stability, intramuscular dispersion or trafficking to specific organs or cell types. The uptake of naked DNA may be increased by coating the DNA onto biodegradable beads, which are efficiently transported into the cells.

**Antibody Preparations**

In another embodiment, the invention provides a preparation of antibodies that can bind to a SARS-CoV, or a SARS CoV S polypeptide, derivative or variant thereof. For example, the antibody can be directed against an SARS-CoV S polypeptide comprising any one of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, or a combination thereof. In some embodiments, the antibody preparations are useful for treating and preventing SARS-CoV infection in an animal.

The invention provides an antibody that binds to a polypeptide or peptide fragment of the invention, or a variant thereof. In some embodiments, the antibody is an antigen-binding antibody fragment. In other embodiments, the antibody is a polyclonal antibody. In further embodiments, the antibody is a single-chain antibody. In other embodiments, the antibody is a monoclonal antibody. In some preferred embodiments, the antibody is a humanized
antibody. The antibody may be coupled to a detectable tag. For example, the
detectable tag can be a radiolabel. In some embodiments, the detectable tag is an
affinity tag. In other embodiments, the detectable tag is an enzyme. In further
embodiments, the detectable tag is a fluorescent protein. In some embodiments,
the detectable tag is a fluorescent molecule. The antibody may also be coupled
to a toxin.

All antibody molecules belong to a family of plasma proteins called
immunoglobulins, whose basic building block, the immunoglobulin fold or
domain, is used in various forms in many molecules of the immune system and
other biological recognition systems. A typical immunoglobulin has four
polypeptide chains, containing an antigen binding region known as a variable
region and a non-varying region known as the constant region.

Native antibodies and immunoglobulins are usually heterotetrameric
glycoproteins of about 150,000 Daltons, composed of two identical light (L)
chains and two identical heavy (H) chains. Each light chain is linked to a heavy
chain by one covalent disulfide bond, while the number of disulfide linkages
varies between the heavy chains of different immunoglobulin isotypes. Each
heavy and light chain also has regularly spaced intrachain disulfide bridges.
Each heavy chain has at one end a variable domain (VH) followed by a number
of constant domains. Each light chain has a variable domain at one end (VL) and
a constant domain at its other end. The constant domain of the light chain is
aligned with the first constant domain of the heavy chain, and the light chain
variable domain is aligned with the variable domain of the heavy chain.
Particular amino acid residues are believed to form an interface between the light
and heavy chain variable domains (Clothia et al., J. Mol. Biol. 186, 651-66,

Depending on the amino acid sequences of the constant domain of their
heavy chains, immunoglobulins can be assigned to different classes. There are at
least five (5) major classes of immunoglobulins: IgA, IgD, IgE, IgG and IgM,
and several of these may be further divided into subclasses (isotypes), e.g. IgG-
1, IgG-2, IgG-3 and IgG-4; IgA-1 and IgA-2. The heavy chains constant
domains that correspond to the different classes of immunoglobulins are called
alpha (α), delta (δ), epsilon (ε), gamma (γ) and mu (μ), respectively. The light
chains of antibodies can be assigned to one of two clearly distinct types, called
kappa (κ) and lambda (λ), based on the amino sequences of their constant domain. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

The term "variable" in the context of variable domain of antibodies, refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies. The variable domains are for binding and determine the specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed through the variable domains of antibodies. It is concentrated in three segments called complementarity determining regions (CDRs) also known as hypervariable regions both in the light chain and the heavy chain variable domains.

The more highly conserved portions of variable domains are called the framework (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a β-sheet configuration, connected by three CDRs, which form loops connecting, and in some cases forming part of, the β-sheet structure. The CDRs in each chain are held together in close proximity by the FR regions and, with the CDRs from the other chain, contribute to the formation of the antigen binding site of antibodies. The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector function, such as participation of the antibody in antibody-dependent cellular toxicity.

An antibody that is contemplated for use in the present invention thus can be in any of a variety of forms, including a whole immunoglobulin, an antibody fragment such as Fv, Fab, and similar fragments, a single chain antibody that includes the variable domain complementarity determining regions (CDR), and the like forms, all of which fall under the broad term "antibody," as used herein. The present invention contemplates the use of any specificity of an antibody, polyclonal or monoclonal, and is not limited to antibodies that recognize and immunoreact with a specific epitope. In some embodiments, however, the antibodies of the invention may react with selected epitopes within various domains of the SARS-CoV S protein.

The term "antibody fragment" refers to a portion of a full-length antibody, generally the antigen binding or variable region. Examples of antibody fragments include Fab, Fab', F(ab')₂ and Fv fragments. Papain digestion of
antibodies produces two identical antigen binding fragments, called the Fab fragment, each with a single antigen binding site, and a residual "Fc" fragment, so-called for its ability to crystallize readily. Pepsin treatment yields an F(ab')$_2$ fragment that has two antigen binding fragments, which are capable of cross-linking antigen, and a residual other fragment (which is termed pFc'). Additional fragments can include diabodies, linear antibodies, single-chain antibody molecules, and multispecific antibodies formed from antibody fragments. As used herein, "functional fragment" with respect to antibodies, refers to Fv, F(ab) and F(ab')$_2$ fragments.

Antibody fragments retain some ability to selectively bind with its antigen or receptor and are defined as follows:

1. Fab is the fragment that contains a monovalent antigen-binding fragment of an antibody molecule. A Fab fragment can be produced by digestion of whole antibody with the enzyme papain to yield an intact light chain and a portion of one heavy chain.

2. Fab' is the fragment of an antibody molecule can be obtained by treating whole antibody with pepsin, followed by reduction, to yield an intact light chain and a portion of the heavy chain. Two Fab' fragments are obtained per antibody molecule. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxyl terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region.

3. (Fab')$_2$ is the fragment of an antibody that can be obtained by treating whole antibody with the enzyme pepsin without subsequent reduction. F(ab')$_2$ is a dimer of two Fab' fragments held together by two disulfide bonds.

4. Fv is the minimum antibody fragment that contains a complete antigen recognition and binding site. This region consists of a dimer of one heavy and one light chain variable domain in a tight, non-covalent association ($V_H-V_L$ dimer). It is in this configuration that the three CDRs of each variable domain interact to define an antigen binding site on the surface of the $V_H-V_L$ dimer. Collectively, the six CDRs confer antigen binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.
Single chain antibody ("SCA"), defined as a genetically engineered molecule containing the variable region of the light chain and the variable region of the heavy chain, linked by a suitable polypeptide linker as a genetically fused single chain molecule. Such single chain antibodies are also referred to as "single-chain Fv" or "sFv" antibody fragments. Generally, the Fv polypeptide further comprises a polypeptide linker between the VH and VL domains that enables the sFv to form the desired structure for antigen binding. For a review of sFv see Pluckthun in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenberg and Moore eds. Springer-Verlag, N.Y., pp. 269-315 (1994).

The term "diabodies" refers to a small antibody fragments with two antigen-binding sites, which fragments comprise a heavy chain variable domain (VH) connected to a light chain variable domain (VL) in the same polypeptide chain (VH-VL). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161, and Hollinger et al., Proc. Natl. Acad Sci. USA 90: 6444-6448 (1993).

The preparation of polyclonal antibodies is well-known to those skilled in the art. See, for example, Green, et al., Production of Polyclonal Antisera, in: Immunochemical Protocols (Manson, ed.), pages 1-5 (Humana Press); Coligan, et al., Production of Polyclonal Antisera in Rabbits, Rats Mice and Hamsters, in: Current Protocols in Immunology, section 2.4.1 (1992), which are hereby incorporated by reference.

The preparation of monoclonal antibodies likewise is conventional. See, for example, Kohler & Milstein, Nature, 256:495 (1975); Coligan, et al., sections 2.5.1-2.6.7; and Harlow, et al., in: Antibodies: A Laboratory Manual, page 726 (Cold Spring Harbor Pub. (1988)), which are hereby incorporated by reference. Methods of in vitro and in vivo manipulation of monoclonal antibodies are also available to those skilled in the art. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler and Milstein, Nature 256, 495 (1975), or they may be made by recombinant methods, for example, as described in U.S. Patent No. 4,816,567. The monoclonal antibodies for use with the present invention

Monoclonal antibodies can be isolated and purified from hybridoma cultures by a variety of well-established techniques. Such isolation techniques include affinity chromatography with Protein-A Sepharose, size-exclusion chromatography, and ion-exchange chromatography. See, e.g., Coligan, et al., sections 2.7.1-2.7.12 and sections 2.9.1-2.9.3; Barnes, et al., Purification of Immunoglobulin G (IgG), in: Methods in Molecular Biology, Vol. 10, pages 79-104 (Humana Press (1992)).

Another method for generating antibodies involves a Selected Lymphocyte Antibody Method (SLAM). The SLAM technology permits the generation, isolation and manipulation of monoclonal antibodies without the process of hybridoma generation. The methodology principally involves the growth of antibody forming cells, the physical selection of specifically selected antibody forming cells, the isolation of the genes encoding the antibody and the subsequent cloning and expression of those genes.

More specifically, an animal is immunized with a source of specific antigen. The animal can be a rabbit, mouse, rat, or any other convenient animal. This immunization may consist of purified protein, in either native or recombinant form, peptides, DNA encoding the protein of interest or cells expressing the protein of interest. After a suitable period, during which antibodies can be detected in the serum of the animal (usually weeks to months), blood, spleen or other tissues are harvested from the animal. Lymphocytes are isolated from the blood and cultured under specific conditions to generate antibody-forming cells, with antibody being secreted into the culture medium. These cells are detected by any of several means (complement mediated lysis of antigen-bearing cells, fluorescence detection or other) and then isolated using micromanipulation technology. The individual antibody forming cells are then processed for eventual single cell PCR to obtain the expressed Heavy and Light chain genes that encode the specific antibody. Once obtained and sequenced, these genes are cloned into an appropriate expression vector and recombinant, monoclonal antibody produced in a heterologous cell system. These antibodies are then purified via standard methodologies such as the use of protein A affinity


The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional polyclonal antibody preparations that typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In additional to their specificity, the monoclonal antibodies are advantageous in that they are synthesized by the hybridoma culture, uncontaminated by other immunoglobulins. The modifier "monoclonal"

indicates the antibody is obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method.

The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Pat. No. 4,816,567); Morrison et al. Proc. Natl. Acad Sci. 81, 6851-6855 (1984).

Methods of making antibody fragments are also known in the art (see for example, Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, New York, (1988), incorporated herein by reference).
Antibody fragments of the present invention can be prepared by proteolytic hydrolysis of the antibody or by expression in E. coli of DNA encoding the fragment. Antibody fragments can be obtained by pepsin or papain digestion of whole antibodies conventional methods. For example, antibody fragments can be produced by enzymatic cleavage of antibodies with pepsin to provide a 5S fragment denoted F(ab')2. This fragment can be further cleaved using a thiol reducing agent, and optionally a blocking group for the sulfhydryl groups resulting from cleavage of disulfide linkages, to produce 3.5S Fab monovalent fragments. Alternatively, an enzymatic cleavage using pepsin produces two monovalent Fab’ fragments and an Fc fragment directly. These methods are described, for example, in U.S. Patents No. 4,036,945 and No. 4,331,647, and references contained therein. These patents are hereby incorporated in their entireties by reference.

Other methods of cleaving antibodies, such as separation of heavy chains to form monovalent light-heavy chain fragments, further cleavage of fragments, or other enzymatic, chemical, or genetic techniques may also be used, so long as the fragments bind to the antigen that is recognized by the intact antibody. For example, Fv fragments comprise an association of VH and VL chains. This association may be noncovalent or the variable chains can be linked by an intermolecular disulfide bond or cross-linked by chemicals such as glutaraldehyde. Preferably, the Fv fragments comprise VH and VL chains connected by a peptide linker. These single-chain antigen binding proteins (sFv) are prepared by constructing a structural gene comprising DNA sequences encoding the VH and VL domains connected by an oligonucleotide. The structural gene is inserted into an expression vector, which is subsequently introduced into a host cell such as E. coli. The recombinant host cells synthesize a single polypeptide chain with a linker peptide bridging the two V domains. Methods for producing sFvs are described, for example, by Whitlow, et al., Methods: a Companion to Methods in Enzymology, Vol. 2, page 97 (1991); Bird, et al., Science 242:423-426 (1988); Ladner, et al, US Patent No. 4,946,778; and Pack, et al., Bio/Technology 11:1271-77 (1993).

Another form of an antibody fragment is a peptide coding for a single complementarity-determining region (CDR). CDR peptides ("minimal recognition units") can be obtained by constructing genes encoding the CDR of
an antibody of interest. Such genes are prepared, for example, by using the polymerase chain reaction to synthesize the variable region from RNA of antibody-producing cells. See, for example, Larrick, et al., Methods: a Companion to Methods in Enzymology, Vol. 2, page 106 (1991).

The invention further contemplates human and humanized forms of non-human (e.g. murine) antibodies. Such humanized antibodies can be chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')2 or other antigen-binding subsequences of antibodies) that contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a nonhuman species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity.

In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are found neither in the recipient antibody nor in the imported CDR or framework sequences. These modifications are made to further refine and optimize antibody performance. In general, humanized antibodies can comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the Fv regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see: Jones et al., Nature 321, 522-525 (1986); Reichmann et al., Nature 332, 323-329 (1988); Presta, Curr. Op. Struct. Biol. 2, 593-596 (1992); Holmes, et al., J. Immunol., 158:2192-2201 (1997) and Vaswani, et al., Annals Allergy, Asthma & Immunol., 81:105-115 (1998); U.S. Patent Nos. 4,816,567 and 6,331,415; PCT/GB84/00094; PCT/US86/02269; PCT/US89/00077; PCT/US88/02514; and WO91/09967, each of which is incorporated herein by reference in its entirety.

The invention also provides methods of mutating antibodies to optimize their affinity, selectivity, binding strength or other desirable property. A mutant antibody refers to an amino acid sequence variant of an antibody. In general,
one or more of the amino acid residues in the mutant antibody is different from what is present in the reference antibody. Such mutant antibodies necessarily have less than 100% sequence identity or similarity with the reference amino acid sequence. In general, mutant antibodies have at least 75% amino acid sequence identity or similarity with the amino acid sequence of either the heavy or light chain variable domain of the reference antibody. Preferably, mutant antibodies have at least 80%, more preferably at least 85%, even more preferably at least 90%, and most preferably at least 95% amino acid sequence identity or similarity with the amino acid sequence of either the heavy or light chain variable domain of the reference antibody.

The antibodies of the invention are isolated antibodies. An isolated antibody is one that has been identified and separated and/or recovered from the environment in which it was produced. In general, the isolated antibodies of the invention are substantially free of at least some contaminant components of the environment in which they were produced. Contaminant components of its production environment are materials that would interfere with diagnostic or therapeutic uses for the antibody, and may include cells, enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. The term "isolated antibody" also includes antibodies within recombinant cells because at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

If desired, the antibodies of the invention can be purified by any available procedure. For example, the antibodies can be affinity purified by binding an antibody preparation to a solid support to which the antigen used to raise the antibodies is bound. After washing off contaminants, the antibody can be eluted by known procedures. Those of skill in the art will know of various techniques common in the immunology arts for purification and/or concentration of polyclonal antibodies, as well as monoclonal antibodies (see for example, Coligan, et al., Unit 9, Current Protocols in Immunology, Wiley Interscience, 1991, incorporated by reference).

In some embodiments, the antibody will be purified as measurable by at least three different methods: 1) to greater than 95% by weight of antibody as determined by the Lowry method, and most preferably more than 99% by weight; 2) to a degree sufficient to obtain at least 15 residues of N-terminal or
internal amino acid sequence by use of a spinning cup sequenator; or 3) to
homogeneity by SDS-PAGE under reducing or non-reducing conditions using
Coomassie blue or, preferably, silver stain.

5 Methods to immunize, treat, and diagnose an animal against SARS

The invention provides a method to immunize an animal against severe
acute respiratory syndrome. In one embodiment, the method involves
administering to an animal a therapeutically effective amount of a SARS-CoV S
polypeptide having, for example, SEQ ID NO: 1, 3, 4, 6, 7 or a fragment of SEQ
ID NO: 1, or a conservative variant thereof. In another embodiment, the method
involves administering to an animal a therapeutically effective amount of an
antibody that binds to a SARS-CoV S polypeptide, for example, a polypeptide
having SEQ ID NO: 1, 3, 4, 6, 7 or a fragment thereof, or a conservative variant
thereof. In another embodiment, the method involves administering to an animal
an effective amount of a live recombinant virus that encodes and can express a
SARS-CoV S polypeptide, for example, one having SEQ ID NO: 1, 3, 4, 6, 7 or a
fragment thereof, or a variant thereof. The animal may be a mammal, such as a
human. Methods to administer vaccines and immune compositions have been
described herein and are available in the art.

An animal may also be treated for infection by SARS-CoV through
passive immunization according to the invention. For example, antibodies that
bind to an amino acid sequence such as SEQ ID NO: 1, 3, 4, 6, 7 or a fragment
of SEQ ID NO: 1, or a conservative variant thereof may be administered to an
animal, such as a human, that is infected with SARS-CoV. Such administration
may be suitable in a variety of situations, for example, where a patient is
immunocompromised and is unable to mount an effective immune response
against SARS-CoV, or to a vaccine or immune composition.

The invention provides a method to diagnose severe acute respiratory
syndrome in an animal that involves contacting a biological sample obtained
from the animal, such as tissue samples, blood, mucus, or saliva, with an
antibody that binds to an amino acid sequence as set forth in SEQ ID NO: 1, 3,
4, 6, 7 or a fragment of SEQ ID NO: 1, and determining if the antibody binds to
the biological sample. Diagnostic assays that utilize antibodies to detect the
presence of an antigen in a biological sample are available in the art. Briefly, an
antibody of the invention may be immobilized on a surface. A biological sample can then be contacted with the immobilized antibody such that an antigen contained in the sample is bound by the antibody to form an antibody-antigen complex. The sample may then be optionally washed to remove unbound materials. A second antibody of the invention that is coupled to a detectable tag, such as an enzyme, fluorophore or radiolabel, can then be contacted with the antibody-antigen complex such that the enzyme, fluorophore or radiolabel is immobilized on the surface. The detectable tag can then be detected to determine if an antigen was present in the biological sample. In another example, a biological sample can be immobilized on a surface. An antibody of the invention that is coupled to a detectable tag is then contacted with the immobilized biological sample and any unbound material is washed away. The presence of the detectable tag is then detected to determine whether the biological sample contained an antigen. Examples of such assays are available in the art and include, enzyme-linked immunosorbant assays, sandwich assays, radioimmuno assays, and the like.

Nucleic acid based methods may also be used to diagnose severe acute respiratory syndrome. In one example, polymerase chain reaction (PCR) may be used to diagnose SARS-CoV infection. Briefly, a biological sample, such as a tissue sample, blood, mucus, or saliva, is obtained from an animal. The nucleic acids within the sample are then extracted using common methods, such as organic extraction. The extracted nucleic acids are then mixed with forward and reverse primers that anneal to nucleic acids that encode SARS proteins, polymerase, nucleotides, and typically a buffer that includes components that allow the polymerase to extend the forward and reverse primers using the SARS nucleic acid as a template. The presence of amplified DNA between the forward and reverse primers is then detected to determine if the sample contained SARS originated nucleic acid. Nucleic acid hybridization techniques, such as Northern and Southern blotting, may also be used to detect the presence of SARS nucleic acids in a biological sample.

Compositions

A SARS-CoV S polypeptide, S polypeptide derivative, S polypeptide variant, recombinant virus encoding a S polypeptide or an anti-S polypeptide
antibody can be formulated as a pharmaceutical composition. A pharmaceutical composition of the invention includes a SARS-CoV S polypeptide, S polypeptide derivative, S polypeptide variant, recombinant virus encoding a S polypeptide or an anti-S polypeptide antibody in combination with a pharmaceutically acceptable carrier. The compositions of the invention can be immune (or immunogenic) compositions or vaccines.

Thus the compositions can contain any S polypeptide or fragment thereof, for example, an S polypeptide having any one of SEQ ID NO:1, 3, 4, 6, 7 or a combination thereof. The invention also provides pharmaceutical compositions containing an antibody that binds to an S polypeptide, for example, any of SEQ ID NO: 1, 3, 4, 6, 7 or a combination thereof, and a pharmaceutically acceptable carrier. In some embodiments, the antibody binds to a peptide having SEQ ID NO:4 or 6. Antibodies that bind to the polypeptide including amino acids 14 to 762 of the SARS coronavirus (SARS-CoV) spike protein (SEQ ID NO:6) are highly effective, and can inhibit viral replication in vivo. In other embodiments, the compositions can include a live recombinant virus that can express a SARS-CoV S polypeptide. Thus, as described herein a substantially full-length Spike (S) polypeptide of SARS-CoV having SEQ ID NO:1, which was encoded within and expressed by a recombinant MVA, induces formation of neutralizing antibodies. An immunogenic composition of this recombinant MVA-SARS-CoV S poxvirus protectively immunized mice against a subsequent infection with SARS-CoV. Hence, the invention provides compositions of live recombinant viruses that encode and express SARS-CoV antigens.

In some embodiments, the compositions may contain an adjuvant. Examples of adjuvants that can be used in the compositions of the invention include, for example, a combination of monophosphoryl lipid A (e.g. 3-de-O-acylated monophosphoryl lipid A (3D-MPL)), and a saponin derivative such as combination of QS21 and 3D-MPL as described in WO 94/00153. In other embodiments, monophosphoryl lipid A can be combined with an aluminum salt to form an adjuvant for use in the compositions of the invention. MPL adjuvants are available from Corixa Corporation (Seattle, Wash.; see U.S. Pat. Nos. 4,436,727; 4,877,611; 4,866,034 and 4,912,094). CpG-containing oligonucleotides (in which the CpG dinucleotide is unmethylated) can also be
used in the compositions of the invention. Such oligonucleotides are available and are described, for example, in WO 96/02555 and WO 99/33488.

Immunostimulatory DNA sequences are also described, for example, by Sato et al., Science 273:352, 1996.

In some embodiments, the adjuvant that can be used is a saponin, preferably QS21 (Aquilla Biopharmaceuticals Inc., Framingham, Mass.), which may be used alone or in combination with other adjuvants. For example, a combination of a monophosphoryl lipid A and saponin derivative can be employed, as described above. In other embodiments, a less reactogenic composition is used where the QS21 is quenched with cholesterol, as described in WO 96/33739. As described herein, the excellent results were obtained with a combination of QS21 and an S polypeptide, which provided the highest antibody response as well as complete protection of the upper and lower respiratory tract.

Other formulations of the invention comprise an oil-in-water emulsion and tocopherol. A particularly potent adjuvant formulation involving QS21, 3D-MPL and tocopherol in an oil-in-water emulsion is described in WO 95/17210. Additional adjuvants that may be employed include Montanide ISA 720 (Seppic, France), SAF (Chiron, Calif., United States), ISCOMS (CSL), MF-59 (Chiron), the SBAS series of adjuvants (e.g., SBAS-2 or SBAS-4, available from SmithKline Beecham, Rixensart, Belgium), Detox (Ribi ImmunoChem Research Inc., Hamilton, Mont.), RC-529 (Ribi ImmunoChem Research Inc., Hamilton, Mont.) and Aminoalkyl glucosaminide 4-phosphates (AGPs).

An immune composition or vaccine may be administered by any conventional route used in the field of vaccines. For example, an immune composition or vaccine can be administered orally or by intravenous infusion, or injected subcutaneously, intramuscularly, intraperitoneally, intrarectally, intravaginally, intranasally, intragastrically, intratracheally, or intrapulmonarily. The choice of the administration route depends on a number of parameters such as the nature of the active principle; the identity of the polypeptide, peptide fragment, immunopeptide, recombinant virus, DNA vaccine; or the adjuvant that is combined with the aforementioned molecules.

Administration of an immune composition may take place in a single dose or in a dose repeated once or several times over a certain period. The
appropriate dosage varies according to various parameters. Such parameters include the individual treated (adult or child), the immune composition or antigen itself, the mode and frequency of administration, the presence or absence of adjuvant and, if present, the type of adjuvant and the desired effect (e.g. protection or treatment), as will be determined by persons skilled in the art.

The pharmaceutical compositions of the invention may be prepared in many forms that include tablets, hard or soft gelatin capsules, aqueous solutions, suspensions, and liposomes and other slow-release formulations, such as shaped polymeric gels. Oral liquid pharmaceutical compositions may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid pharmaceutical compositions may contain conventional additives such as suspending agents, emulsifying agents, non-aqueous vehicles (which may include edible oils), or preservatives.

An oral dosage form may be formulated such that the SARS-CoV S polypeptide, S polypeptide derivative, S polypeptide variant, live recombinant virus or anti-S polypeptide antibody is released into the intestine after passing through the stomach. Such formulations are described in U.S. Patent No. 6,306,434 and in the references contained therein.

Oral liquid pharmaceutical compositions may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid pharmaceutical compositions may contain conventional additives such as suspending agents, emulsifying agents, non-aqueous vehicles (which may include edible oils), or preservatives.

A SARS-CoV S polypeptide, S polypeptide derivative, S polypeptide variant, or anti-S polypeptide antibody can be formulated for parenteral administration (e.g., by injection, for example, bolus injection or continuous infusion) and may be presented in unit dosage form in ampoules, pre-filled syringes, small volume infusion containers or multi-dose containers with an added preservative. The pharmaceutical compositions may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the SARS-CoV S polypeptide, S polypeptide derivative, S
polypeptide variant, or anti-S polypeptide antibody may be in powder form, obtained by lyophilization from solution, for constitution with a suitable vehicle, e.g., sterile saline, before use.

An antibody can be formulated for parenteral administration (e.g., by injection, for example, bolus injection or continuous infusion) and may be presented in unit dosage form in ampoules, prefilled syringes, small volume infusion containers or multi-dose containers with an added preservative. The pharmaceutical compositions may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles. The antibody compositions may also contain formulary agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical compositions suitable for rectal administration can be prepared as unit dose suppositories. Suitable carriers include saline solution and other materials commonly used in the art.

For administration by inhalation, a SARS-CoV S polypeptide, S polypeptide derivative, S polypeptide variant, or anti-S polypeptide antibody can be conveniently delivered from an insufflator, nebulizer or a pressurized pack or other convenient means of delivering an aerosol spray. Pressurized packs may comprise a suitable propellant such as dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount.

Alternatively, for administration by inhalation or insufflation, a SARS-CoV S polypeptide, S polypeptide derivative, S polypeptide variant, or anti-S polypeptide antibody may take the form of a dry powder composition, for example, a powder mix of a modulator and a suitable powder base such as lactose or starch. The powder composition may be presented in unit dosage form in, for example, capsules or cartridges or, e.g., gelatin or blister packs from which the powder may be administered with the aid of an inhalator or insufflator. For intra-nasal administration, a SARS-CoV S polypeptide, S polypeptide derivative, S polypeptide variant, or anti-S polypeptide antibody may be administered via a liquid spray, such as via a plastic bottle atomizer.

Pharmaceutical compositions of the invention may also contain other ingredients such as flavorings, colorings, anti-microbial agents, anti-
inflammatory agents or preservatives. It will be appreciated that the amount of a SARS-CoV S polypeptide, S polypeptide derivative, S polypeptide variant, live recombinant virus or anti-S polypeptide antibody required for use in treatment will vary not only with the particular carrier selected but also with the route of administration, the severity of the infection being treated and the age and condition of the patient. Ultimately the attendant health care provider may determine proper dosage.

Kits

The invention provides a kit which contains packaging material and a SARS-CoV S polypeptide, for example, an S polypeptide having any one of SEQ ID NO: 1, 3, 4, 6, 7 or a fragment of SEQ ID NO: 1, or a conservative variant thereof. The kit may also contain a syringe to allow for injection of the polypeptide contained within the kit into an animal, such as a human. In another embodiment, the invention provides a kit that may contain packaging material, and an antibody that binds to a SARS-CoV S polypeptide, for example, an S polypeptide having SEQ ID NO: 1, 3, 4, 6, 7 or a fragment of SEQ ID NO: 1, or a conservative variant thereof that is formulated for administration to an animal, such as a human. Such a kit may optionally contain a syringe to allow for injection of the antibody contained within the kit into an animal, such as a human.

The invention also provides a kit which contains packaging material and DNA vaccine having a DNA molecule or expression vector encoding a polypeptide with an amino acid sequence as set forth in SEQ ID NO: 1, 3, 4, 6, 7, or a fragment of SEQ ID NO: 1, or a conservative variant thereof. The kit may also contain a device for administering the DNA vaccine (e.g. a syringe or gene gun) to allow for administration of the vaccine contained within the kit into an animal, such as a human.

The invention also provides a kit which contains packaging material and immunogenic composition or a vaccine composition that includes a polypeptide with an amino acid sequence as set forth in SEQ ID NO: 1, 3, 4, 6, 7, or a fragment of SEQ ID NO: 1, or a conservative variant thereof. The kit may also contain a device for administering the composition or vaccine (e.g. a syringe) to
allow for administration of the vaccine contained within the kit into an animal, such as a human.

The invention also provides a kit for detecting SARS-CoV infection, which contains packaging material and a polypeptide with an amino acid sequence as set forth in SEQ ID NO: 1, 3, 4, 6, 7 or a fragment of SEQ ID NO: 1, or a conservative variant thereof. The polypeptide(s) can be immobilized onto a solid support. Such a kit may be used for detection of antibodies directed against the SARS-CoV in the serum of infected animals or humans. The kit can also contain a means for detecting binding of such antibodies to the S polypeptide(s).

The invention also provides a kit for detecting SARS-CoV infection, which contains packaging material and an antibody that can bind a SARS-CoV S polypeptide as set forth in SEQ ID NO: 1, 3, 4, 6, 7 or a fragment of SEQ ID NO: 1, or a conservative variant thereof. The antibodies can be immobilized onto a solid support. Such a kit may be used for detection of SARS viruses or SARS S polypeptides in the serum of infected animals or humans. The kit can also contain a means for detecting binding of such S polypeptide(s) by the antibodies.

The invention is further illustrated by the following non-limiting Examples.

**EXAMPLE 1: Recombinant MVA Encoding SARS-CoV Spike Polypeptides Effectively Immunizes Animals Against SARS-CoV Infection**

This Example shows that a full-length Spike (S) polypeptide of SARS-CoV, expressed by MVA, induces formation of neutralizing antibodies. Such an immunogenic composition of this recombinant MVA-SARS-CoV S poxvirus protectively immunizes mice against a subsequent infection with SARS-CoV.

**Materials and Methods**

**Viruses and Cells.** Primary chicken embryo fibroblast cells (CEF) prepared from 10-day old embryos were grown in minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS) and used to propagate and titer MVA and recombinant MVA strains.

**Spike Nucleic Acid Isolation and Recombinant Virus Construction.** The 3,768-nucleotide open reading frame encoding the SARS-CoV S of the
Urbani strain was copied and amplified from SARS-CoV virion RNA by RT-PCR, and cloned and sequenced. A clone was identified that exactly matched the published sequence (Gene Bank accession number AY278741). Two poxvirus transcription termination motifs (TTTTTNT) in S were altered using the QuickChange Multi Site-Directed Mutagenesis kit (Invitrogen). After mutagenesis, the entire S gene was PCR amplified with or without an influenza virus hemagglutinin (HA) epitope tag and inserted into the XmaI site of the pLW44 transfer vector (provided by L. Wyatt) bringing it under the control of the early/late modified vaccinia virus H5 early late promoter (Wyatt et al. (1996) Vaccine 14, 1451-1458) and adjacent to the gene encoding enhanced green fluorescent protein (GFP) regulated by the vaccinia virus P11 late promoter. The correct sequence of the entire S DNA insert was confirmed and recombinant MVAs were made by transfecting transfer plasmids into CEF that were infected with 0.05 plaque forming units (PFU) of MVA per cell. Florescent plaques were cloned by six successive rounds of plaque isolation, propagated in CEF, and purified by sedimentation through a sucrose cushion as described by Earl et al. (1998) in Current Protocols in Molecular Biology, eds. Ausubel et al. (Greene Publishing Associates & Wiley Interscience, New York), Vol. 2, pp. 16.17.1-16.17.19. Titers of MVA/S and MVA/S-HA were determined by staining plaques with anti-vaccinia virus rabbit and anti-HA mouse antibodies, respectively.

**Western Blotting.** CEF and HeLa cells were infected with 5 PFU of recombinant MVA for 18 h. Infected cells were lysed in ice-cold RIPA buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% Triton X-100, 0.1% SDS, 0.5% sodium deoxycholate] supplemented with protease inhibitor cocktail (Sigma). Lysates were kept on ice for 10 min, centrifuged and resolved by SDS polyacrylamide gel electrophoresis (PAGE) on a bis-Tris 4 -12% polyacrylamide gel. Proteins were transferred to a nitrocellulose membrane, blocked with 5% skimmed milk in phosphate buffered saline (PBS), and incubated for 1 h at room temperature with anti-HA mouse mAb (Covance) or anti-SARS-CoV S rabbit polyclonal antibody (IMG-541, Imgenex) diluted 1:1000 or 1:500 in blocking buffer, respectively. The membrane was washed in PBS containing Tween-20 (0.1%) and incubated for 1 h with horseradish peroxidase-conjugated anti-mouse and anti-rabbit secondary antibody (Calbiochem) diluted 1:2000. The membrane
was washed and proteins were visualized with the Super Signal chemiluminescence substrate (Pierce).

**Endoglycosidase (endo) H and Peptide N-Glycosidase (PNGase) F**

*Treatments.* Cleared cell lysates were incubated with 20 µl of anti-HA affinity matrix (Roche) overnight at 4°C. The agarose beads were washed and incubated with endo H and PNGase F (New England Biolabs) according to manufacturer’s instructions and the proteins were analyzed by western blotting using peroxidase-conjugated anti-HA mouse mAb (Roche).

**Pulse-Chase Analysis.** HeLa cells were mock infected or infected with 5 PFU per cell of MVA or MVA/S-HA and 18 h later were incubated for 30 min in Dulbecco’s Modified Eagle’s medium lacking methionine and cysteine, labeled with 100 µCi of [35S]methionine and [35S]cysteine per ml of medium for 10 min, washed and chased with medium supplemented with 2 mM methionine and 2 mM cysteine. At each time, cells were harvested, lysed in ice-cold RIPA buffer, and clarified lysates were incubated with 20 µl of anti-HA affinity matrix overnight at 4°C as above. Washed agarose beads were treated with endo H and the samples were resolved by SDS-PAGE and detected by autoradiography.

**Confocal Microscopy.** CEF or HeLa cells on coverslips were infected with 5 PFU per cell of MVA, MVA/S or MVA/S-HA, incubated for 18 h, and either left unfixed and unpermeabilized or fixed with cold 4% paraformaldehyde in PBS for 20 min at room temperature and permeabilized with 2.5% digitonin in PBS for 5 min on ice. The coverslips were washed and incubated with anti-SARS mouse serum kindly provided by Larry Anderson (CDC, Atlanta) or anti-HA mouse mAb for 1 h at room temperature, washed again, and incubated with Alexa 594-conjugated-anti-mouse IgG (Molecular Probes) diluted in PBS containing 10% FBS for 30 min at room temperature. Coverslips were mounted in 20% glycerol and examined with an inverted confocal microscope.

**Enzyme-linked Immunosorbent Assay (ELISA).** A 96-well plate was coated overnight at 4°C with 50 ng per well of soluble recombinant protein containing the S1 domain of SARS-CoV S made in insect cells, blocked with 5% skimmed milk in PBS containing 0.2% Tween-20 for 1 h at 37°C and incubated with two-fold dilutions of serum from unimmunized or immunized mice for 1 h at 37°C. After extensive washes, the plate was incubated for 1 h with horse
radish peroxidase-conjugated secondary anti-mouse antibody (Roche) diluted in blocking buffer, washed again and incubated with substrate solution (3,3'-5,5'-tetramethylbenzidine, Roche). The difference in absorbance at 370 and 492 nm was determined, readings from wells lacking antigen were subtracted and endpoint titers were calculated when the absorbance difference was <0.1.

**Neutralization Assay.** Neutralizing antibody was determined by the inhibition of cytopathic effects mediated by SARS-CoV on Vero cell monolayers as described by Subbarao et al. (2004) *J. Virol.* 78, 3572-3577. The dilution of serum that completely prevented cytopathic effect in 50% of the wells was calculated (Reed, L. J. & Muench, H. (1938) *Am. J. Hyg.* 27, 493-497).

**Animal Challenge Experiments.** Groups of 8 BALB/c mice were inoculated intranasally (IN) or intramuscularly (IM) with $10^7$ PFU of MVA or MVA/S at 0 and 4 weeks. Four weeks after the second immunization, animals were challenged IN with $10^4$ tissue culture infectious doses$_{50}$ (TCID$_{50}$) of SARS-CoV as described (14). Two days later the lungs and nasal turbinates of 4 animals in each group were removed and the SARS-CoV titers were determined as described by Subbarao et al. (2004) *J. Virol.* 78, 3572-3577.

To obtain serum for passive protection studies, two groups of 8 BALB/c mice received MVA/S or MVA IM at 0 and 4 weeks. Three weeks after the last immunization, sera were collected and pooled. Undiluted or diluted MVA/S or MVA serum in a total volume of 0.4 ml was injected intraperitoneally (IP) to 2 to 4 naïve mice. Mice were bled the following day to determine their levels of SARS-CoV specific neutralizing antibody and then each was challenged with $10^5$ TCID$_{50}$ of SARS-CoV and analyzed as above.

**Results**

**Characterization of SARS-CoV S Expressed by Recombinant MVA.** A cDNA clone containing the entire open reading frame encoding SARS-CoV S was modified by introducing silent mutations that eliminated two poxvirus transcription termination signals and was placed under the control of an early/late vaccinia virus promoter (mH5) and inserted by homologous recombination into the site of an existing deletion (del III) within the MVA genome to produce MVA/S (FIG. 1A). A second recombinant virus, MVA/S-
HA, was also constructed with a 9-amino acid HA epitope tag coding sequence at the end of the S open reading frame. In each case, the gene encoding GFP regulated by a vaccinia virus promoter was co-inserted into the MVA genome in order to facilitate the screening and isolation of recombinant viruses by repeated plaque purifications. Both viruses replicated well in CEF and the SARS-CoV S insert was genetically stable as assayed by plaque immunostaining with S-specific antibodies.

A protein doublet with an estimated mass of approximately 200 kDa, significantly higher than the value of 135 kDa for the unmodified protein predicted from the nucleotide sequence, was detected by SDS-PAGE of lysates of cells infected with MVA/S and MVA/S-HA and Western blotting with polyclonal antibody to S or a mAb to HA (FIG. 1B; data for MVA/S not shown). In addition, some S was trapped near the top of the gel, presumably due to aggregates or oligomers that were not dissociated by treatment with SDS and reducing agent at 100°C.

The SARS-CoV S has 23 potential N-linked glycosylation sites (Rota et al. (2003) Science 300, 1394-1399), the presence of which could contribute to the mass of the protein determined by SDS-PAGE. To evaluate this possibility, S expressed in HeLa cells was treated with PNGase F, which hydrolyzes all types of N-glycan chains. PNGase F treatment converted the 200-kDa doublet to a single sharp band of approximately 160 kDa (FIG. 2A), which was still greater than the 135 kDa estimated from the gene sequence. However, differences of this magnitude between the theoretical mass and the mass estimated by SDS-PAGE are commonly found, and this discrepancy does not necessarily indicate that S contains additional post-translational modifications.

Further experiments were carried out using endo H, which digests the N-linked high-mannose carbohydrate side chains of glycoproteins that are synthesized in the endoplasmic reticulum (ER), but not after conversion to a more complex form in the medial Golgi apparatus. Only a subpopulation of S was digested, since both the original size protein and a faster migrating one were detected (FIG. 2A). The latter had a slightly higher mass than the PNGase F-treated protein, consistent with N-acetylglucosamine residues remaining after hydrolysis by endo H. To determine the kinetics of acquisition of endo H resistance, cells infected with MVA/S-HA were pulse labeled for 10 min with
[35S]methionine and [35S]cysteine and then chased in medium containing unlabeled amino acids. At each time point, the epitope tagged S protein was isolated using an HA mAb affinity matrix; one portion was analyzed directly by SDS-PAGE and autoradiography and an equal portion was first digested with endo H. Immediately after the pulse, a sharp 200-kDa band was detected that became more diffuse during the chase and was resolved as a doublet by 60 min in the absence of endo H treatment (FIG. 2B). The pulse-labeled S was completely digested to a 160-kDa species by endo H (FIG. 2B). A faint endo H-resistant band appeared by 40 min of chase (seen as a diffuse band in this particular experiment) indicating that a small fraction of S had become resistant to digestion (FIG. 2B). Even after 80 min, however, there was still considerable endo H-sensitive S.

Cellular Localization of S. The glycosylation and partial resistance to endo H was consistent with trafficking of the SARS-CoV S through the ER to the Golgi compartment. To determine whether S was expressed at the cell surface, unpermeabilized CEF that had been infected with MVA/S were stained with antibody to S followed by Alexa 594-conjugated-anti-mouse IgG. Whereas the fluorescence due to co-expressed GFP was present throughout the cytoplasm, the labeling of S was restricted to the cell surface (FIG. 3, row 2). Moreover no labeling occurred in cells infected with the MVA vector (FIG. 3, row 1) or uninfected cells (not shown). Experiments were also carried out with cells infected with MVA/S-HA except that antibody to the epitope tag was used. The absence of staining of unpermeabilized cells (FIG. 3, row 3) was consistent with the S protein having a type 1 topology with the tagged C-terminus in the cytoplasm. After permeabilization of the plasma membrane with digitonin, both plasma membrane and juxtanuclear staining were evident (FIG. 3, row 4). Similar patterns were found when infected HeLa cells were examined by confocal microscopy (not shown).

Immunogenicity of MVA/S in mice. Mice were inoculated IN or IM with 10^7 PFU of MVA/S at 0 time and again at 4 weeks. Antibody was determined by an endpoint ELISA using a recombinant protein consisting of the S1 domain of SARS-CoV made in insect cells and purified by affinity chromatography. Antibody was detected at 4 weeks and peaked at 6 weeks (FIG. 4A). Similar titers were obtained after either route of inoculation. The titers
began to decline with time and were not boosted at two weeks after the SARS-CoV challenge described in the next section (FIG. 4A).

The ability of sera to neutralize SARS CoV infectivity for VERO cells was determined as described by Subbarao et al., (2004) J. Virol. 78, 3572-3577. Neutralizing antibody was detected after the second immunization by either the IN or IM route (FIG. 4B).

Protection of Mice Immunized with MVA/S. Previous studies demonstrated that mice inoculated IN with SARS-CoV exhibit no overt signs of disease but have elevated virus titers in the respiratory tract that peak within 2 days and are cleared by 7 days (Subbarao et al., (2004) J. Virol. 78, 3572-3577). The present study employed three control and two experimental groups. The controls were mice that were uninoculated or that had received the MVA vector IM or IN. When these mice were challenged with $10^4$ TCID$_{50}$ of SARS-CoV, approximately $10^5$ TCID$_{50}$ of SARS-CoV per g of lung was recovered on day 2 (FIG. 5). By contrast, the titers of SARS-CoV from the lungs of mice immunized with MVA/S either IM or IN were reduced by about $10^4$ to levels that were barely above the limit of detection (FIG. 5). About $10^3$ TCID$_{50}$ per g of SARS-CoV were recovered from the nasal turbinates of control mice, but this too was significantly reduced in the immunized animals (FIG. 5). The severe reduction in SARS-CoV replication may explain the absence of an amnestic ELISA antibody response to S following challenge (FIG. 4A). Neutralizing titers to SARS CoV were not measured after challenge.

Passive Protection Mediated by Serum from MVA/S Immunized Mice. MVA can induce both humoral and cell mediated immune responses. To determine a role for antibody, sera were pooled that were obtained from mice that had been immunized IM with $10^7$ PFU of MVA/S or MVA on day 0 and 28 and bled three weeks later. The ELISA titer to S was about 1:25,000 and the mean neutralizing titer was 1:284. Undiluted or diluted serum (0.4 ml) was administered IP to naïve mice to evaluate the protective role of antibody. As a positive control, hyperimmune SARS-CoV serum was administered to two mice (Subbarao et al., (2004) J. Virol. 78, 3572-3577). On the next day, the mice received an intranasal challenge of $10^5$ TCID$_{50}$ of SARS-CoV, and two days later, their nasal turbinates and lungs were removed to measure the virus titers.
As shown in Table 3, administration of undiluted MVA/S serum reduced the lung titers by $10^{5.1}$ compared to recipients of MVA control serum. These data indicate that antibodies to SARS-CoV S polypeptide conferred the observed protection. Protection was observed despite a neutralization titer of only 1:35 in recipient mice. Replication of SARS-CoV increased as the quantity of passively transferred serum decreased, but significant reductions in lung virus titers still occurred at sera dilutions of 1:4, 1:16 and 1:64. The absence of detectable neutralizing antibody in mice receiving these dilutions of passively transferred serum probably reflects a low sensitivity of the in vitro neutralization assay as indicated by the fact that the ELISA titers to S were more than 100-fold higher than the neutralization titers (FIG. 4). The recovery of SARS-CoV from the nasal turbinates was also reduced, but to a relatively lesser extent than from the lungs.
### Table 3

<table>
<thead>
<tr>
<th>Passively Transferred Antibody*</th>
<th>Neutralizing titer of antibody administered</th>
<th>Geometric mean neutralizing titer in recipient mice</th>
<th>Virus Replication in Challenged Mice†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nasal Turbinates</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean (±SE) virus titer²</td>
</tr>
</tbody>
</table>
| MVA/S undiluted                 | 1:284                                       | 1:35                                           | 2.9 ± 0.57                             | 2/3                                | 0.08$ | 1.7 ± 0.17               | 1/3                  | 0.03$
| MVA/S 1:4                       | 1:71                                        | <1:8                                           | 2.4 ± 0.32                             | 2/4                                | 0.02 | 3.2 ± 0.58               | ¾                   | 0.02$
| MVA/S 1:16                      | 1:18                                        | <1:8                                           | 2.5 ± 0.39                             | 2/4                                | 0.02 | 5.5 ± 0.20               | 4/4                  | 0.03$
| MVA/S 1:64                      | <1:16                                       | <1:8                                           | 3.4 ± 0.58                             | 4/4                                | 0.19 | 6.0 ± 0.10               | 4/4                  | 0.02$
| MVA/S 1:128                     | <1:16                                       | <1:8                                           | 3.4 ± 0.36                             | 4/4                                | 0.11 | 6.5 ± 0.25               | 4/4                  | 0.25$
| MVA/S undiluted                 | <1:16                                       | <1:8                                           | 4.0 ± 0.20                             | 4/4                                |      | 6.8 ± 0.25               | 4/4                  |      $
| SARS-CoV 1:4                    | 1:512                                       | 1:17                                           | ≤1.8 ± 0$†                            | 0/2                                | 0.06$ | ≤1.5 ± 0†                | 0/2                  | 0.06$

*The indicated dilutions of antibody in 400 μl were administered to recipient mice by intraperitoneal injection.

†Mice were challenged with $10^5$ TCID$_{50}$ SARA-CoV intranasally (IN).

‡Virus titers are expressed as log$_{10}$ TCID$_{50}$/g tissue.

§P values comparing titers with those seen in mice that received undiluted MVA control antibody in a Mann Whitney U non-parametric analysis.

$Small sample size affected statistical significance.

Virus not detected; the lower limit of detection of infectious virus in a 5% w/v suspension of nasal turbinate was 1.8 log$_{10}$ TCID$_{50}$/g tissue and in a 10% w/v suspension of lung homogenate the detection limit was 1.5 log$_{10}$ TCID$_{50}$/g tissue.

As illustrated above the SARS-CoV S polypeptide can be expressed in a native conformation and can induce antibodies that neutralize SARS-CoV.

The secretory pathway of a cell has an important quality control function and the trafficking of a protein from the ER to the plasma membrane is a sign of proper folding. The N-linked oligosaccharide pathway is frequently used for tracking protein movement. Addition of N-linked oligosaccharides occurs in the ER and the conversion of the high mannose form to complex endo H-resistant N-linked chains occurs upon transport from the cis to the medial Golgi compartment.
The S open reading frame of SARS-CoV was expressed by recombinant MVA as a protein of approximately 200 kDa, which was reduced to 160 kDa by a glycosidase specific for N-linked carbohydrates. Trafficking of S to the medial Golgi apparatus was indicated by acquisition of endo H resistance by a subpopulation of molecules within 40 min after pulse labeling. The staining of the surface of unpermeabilized cells infected with MVA/S by S-specific antibody provided direct evidence for insertion into the plasma membrane. Furthermore, the inability of antibody to a C-terminal epitope tag to stain cells unless they were permeabilized indicated that S has a type 1 topology in the membrane.

S1 and S2 cleavage products were not detected, as found for group 2 but not group 1 CoV S proteins (Gallagher, T. M. & Buchmeier, M. J. (2001) *Virology* **279**, 371-374). Xiao and co-workers ((2003) *Biochem. Biophys. Res. Commun.* **312**, 1159-1164) expressed full length S by transfection and detected low amounts of several smaller than full-length S fragments, which they suggested might include specific cleavage products, though no evidence to support this was presented. The characterization studies provided herein strongly suggested that MVA expressed a properly folded form of S.

The MVA/S construct was then tested to determine whether it would elicit neutralizing antibodies. The ability of intramuscular (IM) or intranasal (IN) inoculation of a recombinant MVA to prevent upper and lower respiratory infections has previously been observed using a rodent model of parainfluenza virus 3 (Wyatt, L. S., Shors, S. T., Murphy, B. R. & Moss, B. (1996) *Vaccine* **14**, 1451-1458). Mice immunized with MVA/S by IN or IM routes developed antibodies that bound to the S1 domain of S and neutralized SARS-CoV in vitro. Furthermore, mice immunized IM or IN exhibited little or no replication of SARS CoV in the upper and lower respiratory tracts following an IN inoculation. Control mice vaccinated with the MVA vector by IN or IM routes were unprotected, indicating that the effect was specific for the expressed S protein and was not due enhanced non-specific immunity.

Previous studies showed that IP inoculation of hyperimmune serum from mice inoculated twice with SARS CoV provided protection against SARS-CoV in the lower respiratory tract and to a lesser extent in the upper respiratory tract (Subbarao et al. (2004) *J. Virol.* **78**, 3572-3577). Protection with serum from
MVA/S-immunized animals was demonstrated in the present study. Because serum from animals inoculated with the MVA vector had no effect, the protection was likely due to S-specific antibodies. These results indicated that the S of SARS CoV, like that of other CoV, is an important target of neutralizing antibodies both in vitro and in vivo.

No enhanced virus replication or obvious disease was found in mice that were immunized with MVA/S prior to challenge with SARS-CoV, as has been found after immunization with a vaccinia virus vector expressing S from feline infectious peritonitis virus and challenge with the corresponding virus (Vennema et al. (1990) *J. Virol.* 64, 1407-1409). The latter effect is thought to be due to S antibody-dependent enhanced infection of macrophages. See Corapi et al. (1992) *J. Virol.* 66, 6695-6705; Olsen et al. (1992) *J. Virol.* 66, 956-965.

Thus, the present study provides encouraging results for the development of SARS-CoV vaccines based on the highly attenuated MVA vector expressing S.

**EXAMPLE 2: Immunogenic Spike Polypeptides Protect Against SARS Infection**

This Example illustrates expression of a secreted, glycosylated polypeptide including amino acids 14 to 762 of the SARS coronavirus (SARS-CoV) spike protein and a polyhistidine tag in recombinant baculovirus-infected insect cells. Mice that received the affinity-purified protein with either a saponin (QS21) or a Ribi (MPL + TDM) adjuvant subcutaneously and were challenged intranasally with SARS-CoV, produced neutralizing antibodies and protection against SARS-CoV intranasal infection. The best results were obtained with QS21 and protein, which provided the highest antibody as well as complete protection of the upper and lower respiratory tract.

**Materials and Methods**

*Vector construction.* A cDNA encoding amino acids 14 to 762 of the SARS-CoV (Urbani strain) S protein (GenBank accession no. AY278741) with 6 histidine residues appended to the C-terminus was inserted into the BamHI and EcoRI sites of the baculovirus transfer vector pMelBacB (Invitrogen) so that the honeybee melittin signal peptide was in frame with the S protein. The plasmid
and linearized *Autographa californica* multiple nuclear polyhedrosis virus DNA were transfected into SF9 and a recombinant baculovirus was clonally purified following the Bac-N-Blue system protocol (Invitrogen).

In particular, the recombinant baculoviruses were constructed to express the substantially full length SARS-CoV spike protein, or N- or C-terminal fragments of the SARS-CoV spike protein (nS or cS). In all cases, native signal sequences as well as transmembrane and cytoplasmic regions (ATM+CT) were deleted. For the full length S(ATOM+CT) polypeptide, a cDNA encoding amino acids 14 to 1195 of the SARS-CoV (Urbani Strain) S protein was used (see GenBank accession no. AY278741, starting at nucleotide 21531) with a sequence for 6 histidine residues attached to its 3’end. The sequences of the S(ATOM+CT) polypeptide (14-1195AA, SEQ ID NO:4) and cDNA (SEQ ID NO:5) are shown in FIG. 6 and are provided hereinabove.

This S(ATOM+CT) cDNA was cloned into the BamHI and EcoRI sites of the baculovirus transfer vector pMelBacB (Invitrogen) in frame with the honeybee melittin signal peptide under a strong polyhedrin promoter. N- (nS) and C- (cS) terminal fragments encoding gene sequences were cloned in a similar way.

A spike polypeptide encoding the N-terminal 14-762 amino acids was selected on the basis of hydrophilicity and secondary structure predictions using Kyte and Doolittle and Chou Fasman algorithms (McVector 7.2) and also because it encompasses the receptor binding region as well as the region corresponding to S1 of other coronaviruses. The sequence of this N-terminal 14-762 amino acid spike polypeptide is as follows (SEQ ID NO:6).

<p>| | | | | | | | | | | | | | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>SDLRCT</td>
<td>TFDDVQAPNY</td>
<td>TQHTSSMRGV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>41</td>
<td>YYPDEIFRSD</td>
<td>TLYLTQDLFL</td>
<td>PFYSNVTFGH</td>
<td>TINHTFGNPV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>81</td>
<td>IFPKDGIIYFA</td>
<td>ATEKSNVVRG</td>
<td>WVFGSTMNKK</td>
<td>SQSVIINNS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>121</td>
<td>TNVIRACNF</td>
<td>ELCNPFPAV</td>
<td>SKPMGTQHTH</td>
<td>MIFDNAFNCT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>161</td>
<td>FEYISDAFSL</td>
<td>DVSEKSGNFK</td>
<td>HLREFVFKNK</td>
<td>DGFLYYVKGY</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>201</td>
<td>QPIDVVRDLPL</td>
<td>SGFNTLKPFF</td>
<td>KLFPGINITN</td>
<td>FRAITALFSP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>241</td>
<td>AQDITGTSAA</td>
<td>AYFVGYLKPT</td>
<td>TFMLKYDENG</td>
<td>TITDADVDCQ</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>281</td>
<td>NPLAEKCSV</td>
<td>KSFEIDKGIY</td>
<td>QTSNFRVVF</td>
<td>GDVVRFPNIT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>321</td>
<td>NLCPPGFEN</td>
<td>ATKFPSVYAYW</td>
<td>ERKKAISNCA</td>
<td>DXSYSVLNSTF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>361</td>
<td>FSTFKCYVGS</td>
<td>ATKLNDCFS</td>
<td>NVYADSFVVK</td>
<td>GDDVRQIAPG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>401</td>
<td>QTGVIADYN</td>
<td>YKLPDDFMGCV</td>
<td>LAWNTRNIDA</td>
<td>TSTGNYNKY</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>441</td>
<td>RYLHGBKLRP</td>
<td>FERDISNVFF</td>
<td>SPDGKPCTPP</td>
<td>ALNCYWPLND</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>481</td>
<td>YQFYTTGIG</td>
<td>YQPYFRVVVLS</td>
<td>FELLNAPATV</td>
<td>CGFPLSTDL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>521</td>
<td>KNQCVN FN</td>
<td>GLTGTGVLTP</td>
<td>SSKRFQFQQ</td>
<td>FGRDVSDFTD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The C-terminal fragment employed consisted of the remaining 763-1195 amino acid residues of the spike protein. The sequence for this C-terminal 763-1195 amino acid spike polypeptide is as follows (SEQ ID NO:7).

```
763 VFAQVKQM YKTPTLYKGY GFNFSQILPD PLKPTKRSFI
801 EDLNPKVLTL ADAGFMKQYG ECLGDINARD LICAQKFNGL
841 TVLPLLTDI MIAAYTAALV SGATAGWTF GAGAALQIPF
881 AMQNYARFNG IGTQNVLYE NQKQIANQFN KAIQIQESL
921 TTSTALGKL QDVVNQQA LTLVKLSS NFGAISSVLN
961 DILSRLDKVE AEVQIIDRLIT GRLQSLQTVY TQQLIRAAMRT
1001 RASANLAATK MSECVLGQSK RVDFCGKGYH LMSFPQAAPH
1041 GVVFLHTVY FSQHRNFATTA PAICHEGKAY FPREVGVFNF
1081 GTSWFITQRN FFQPQITTDD NTFVSGNCDV VIGIINNTVY
1121 DPLQPELDSF KEELDKYFKN HTSPDVLDGD ISGINASVNVN
1161 IQKEIDRLNE VAKNLNESLI DLQELGKYEQ YIKWPHHHHH
1201 H
```

Recombinant plasmids encoding the spike polypeptides and the linearized Autographa californica multiple nuclear polyhedrosis virus DNA were transfected into Sf9 insect cells. Recombinant baculoviruses were purified following the Bac-N-Blue system protocol (Invitrogen). The expression was checked by western blotting that showed ~110 kDa band of nS, ~200 kDa band of S(ATM+CT) and ~50 kDa band of cS. nS has been purified further on large scale with a yield of 10mg/l of culture supernatant.

**Expression and purification of recombinant nS protein.** High Five cells were infected with recombinant baculovirus at a multiplicity of infection of 10 for 120 h. The culture supernatant was concentrated five fold with a Millipore Labscale transverse flow filter system and was clarified by centrifugation in a Sorvall H6000A rotor at 3000 rpm for 30 min at 4 °C. The supernatant was dialyzed against phosphate pH 7.4 buffered saline (PBS) and then incubated with a 50% (wt/vol) slurry of nickel-nitrilotriacetic acid (Ni-NTA) agarose (Qiagen) for 3-4 h at 4 °C. The mixture was loaded into a column that was washed with 10 bed-volumes of wash buffer (50 mM phosphate pH 8 buffer/300 mM NaCl/10 mM imidazole/1 mM phenyl methyl sulfonyl fluoride), 10 bed-volumes of wash
buffer containing 25 mM imidazole, 2 bed-volumes of wash buffer containing 40 mM imidazole, and 3 bed-volumes of wash buffer containing 200 mM imidazole. The pooled 200 mM imidazole eluate containing nS was dialyzed against PBS and concentrated using a Millipore Amicon ultra filter. Protein samples were analyzed on a 4-12% bis-Tris polyacrylamide gel (Invitrogen) and stained with GelCode Blue stain reagent (Pierce) and with Silver Stain Plus kit (BioRad). Where indicated, N-glycosidase F treatment was carried out as described in Bisht et al. (2004) Proc. Natl. Acad. Sci. USA 101, 6641-6646.

**Immunological assays.** Western blotting was carried out using standard procedures and an anti-His mouse mAb (Qiagen) or anti-SARS-CoV S rabbit polyclonal antibody (IMG-541, Imgenex, San Diego) diluted 1:1000 and 1:500 in blocking buffer respectively. ELISA and confocal microscopy were carried out as described in Bisht et al. (2004) Proc. Natl. Acad. Sci. USA 101, 6641-6646.

**Immunization protocol and SARS-CoV challenge.** Groups of seven female 6-week BALB/c mice were injected subcutaneously with 10 μg of nS protein or with an unrelated vaccinia virus protein LIR on days 0, 28 and 56. Approximately four weeks after the third immunization, mice were intranasally challenged with $10^5$ TCID<sub>50</sub> of SARS-CoV in 50 μl. Two days later, their lungs and nasal turbinates were removed and SARS-CoV titers were determined as described in Subbarao et al. (2004) *J. Virol.* 78, 3572-3577. A non-parametric Mann-Whitney U test was used for statistical analysis.

**Results**

A baculovirus/insect cell system was used to express an N-terminal fragment of S (nS) as a secreted glycosylated protein that could be readily purified under native conditions. The N-terminal 762 amino acids of the S protein was selected on the basis of hydrophilicity and secondary structure predictions using Kyte and Dolittle and Chou Fasman algorithms (McVector 7.2) and because it includes the region corresponding to S1 of other coronaviruses. A transfer vector was constructed in which the polyhedrin promoter regulates expression of an nS protein comprised of amino acids 14 to 762 of S preceded by the honeybee melittin signal peptide and followed by six histidines (FIG. 7A). A baculovirus expressing nS was derived by
recombination in insect cells. The yield of secreted and affinity purified nS was approximately 10 mg/l of culture supernatant, and a single major band of ~110 kDa was seen by SDS-polyacrylamide gel electrophoresis after staining with Coomassie Blue (FIG. 7B, lane 1) or silver nitrate (FIG. 7B, lane 2). Upon western blotting, the same 110-kDa band was recognized by antibodies to the polyhistidine tag and SARS-CoV S protein (FIG. 7B, lanes 3 and 4). Treatment with peptide N-glycosidase F reduced the mobility of the protein to ~85 kDa, demonstrating that the higher than expected apparent mass was due to N-glycosylation (FIG. 7C).

To analyze immunogenicity, nS protein mixed with MPL + TDM or QS21 adjuvant was injected subcutaneously into BALB/c mice on days 0, 28, and 56. Control mice were immunized with adjuvant and a secreted form of the vaccinia virus membrane protein L1R that was also produced in the baculovirus system and purified by affinity chromatography (Fogg et al., 2004) J. Virol. 78, 10230-10237). As an initial evaluation of immunogenicity, sera from the mice were tested for antibodies that recognize S protein expressed on the surface of cells by recombinant modified vaccinia virus Ankara (MVA/S) (Bisht et al. 2004) Proc. Natl. Acad. Sci. USA 101, 6641-6646. Because the endoplasmic reticulum acts as a filter for misfolded proteins, S present on the cell surface is likely to be correctly folded. Although SARS-CoV-infected cells could be used for the same purpose, considerably higher containment levels would be required. Uninfected HeLa cells or HeLa cells infected with non-recombinant MVA or MVA/S were fixed and stained with pooled mouse serum followed by Alexa 594-conjugated-anti-mouse IgG and analyzed by confocal microscopy. The serum obtained from mice immunized with nS in QS21 or MPL + TDM adjuvant stained the surface of cells infected with MVA/S but did not detectably stain uninfected cells or cells infected with non-recombinant MVA (FIG. 8). In contrast, serum from control mice that were immunized with the vaccinia virus L1R protein stained cells infected with non-recombinant and MVA/S equally (not shown). These data indicated that the antibodies produced by nS were able to bind to the membrane-associated form of full length S.

The relative binding activity of pooled serum from mice immunized with nS and QS21 or MPL + TDL adjuvant were analyzed using nS as the capture antigen. Antibody was detected after the primary inoculation of nS with QS21
and the reciprocal ELISA titer was boosted to 1:409,600 after two more inoculations (FIG. 9A). With MPL + TDM adjuvant, the antibody response to nS was detected only after boosting but subsequently reached approximately 25% of the level achieved with QS21. The IgG2a/IgG1 ratio is an indicator of Th1 help. The specific IgG2a/IgG1 titers from mice immunized with QS21 and MPL + TDM were 0.25 and 0.03 respectively, suggesting a greater Th1 response with the former adjuvant. A determining effect of adjuvant on helper T cell responses has been noted (Cribbs et al. (2003) Int. Immunol. 15, 505-514; Santos et al. (2002) Vaccine 21, 30-43). For comparative purposes, we also determined the IgG2a/IgG1 ratio of serum previously obtained from mice immunized with MVA/S. Although the overall IgG titers were lower in mice immunized with MVA/S (Bisht et al. (2004) Proc. Natl. Acad. Sci. USA 101, 6641-6646) than with the nS protein, the IgG2a/IgG1 ratios were higher with values of 2 and 4 for pooled sera of mice immunized intranasally and intramuscularly, respectively.

The high titer of nS-binding antibody and its recognition of full-length membrane-bound S encouraged us to evaluate the ability of the immune sera to neutralize the infectivity of SARS-CoV. Significant neutralizing activity was observed after the second inoculation of nS with either adjuvant (FIG. 9B). However, the mean neutralizing titer of 1:1269 achieved with QS21 was 4.6-fold higher than that obtained with MPL + TDM. Thus there was good correspondence between the relative binding and neutralizing activities of sera obtained with QS21 and MPL + TDM adjuvants.

Subbarao et al. (J. Virol. 78, 3572-3577 (2004)) demonstrated that SARS-CoV replicates in the respiratory tract of BALB/C mice and that replication was reduced following passive administration of neutralizing antibody. In this model, peak titers were reached within 1 to 2 days depending on the dose and clearing occurred by 7 days. Two days after the intranasal administration of 10^5 TCID_{50} of SARS-CoV, 10^8 TCID_{50} of virus per g of lung was recovered in control mice immunized with the vaccinia virus L1R protein in either adjuvant (FIG. 10A). By contrast, there was at least a 10^6-fold reduction in viral load in the lungs of mice immunized with nS regardless of the adjuvant (FIG. 4A). The difference was highly significant (p=0.0017) as determined using the Mann-Whitney non-parametric statistical method. Indeed, virus was detected in only one mouse out of seven in each of the test groups.
The virus titers in the nasal turbinates showed a $10^3$-fold reduction relative to controls when nS was administered with MPL + TDM adjuvant and $>10^4$-fold reduction when nS was given with QS21 (FIG. 10B). The effect of vaccination with either adjuvant was highly significant when compared with controls ($p=0.0017$) determined as above. Virus was detected in the nasal turbinates of 4 of 7 test mice immunized with nS and the MPL + TDM adjuvant whereas the titers were uniformly below detection in the turbinates of mice immunized with nS and QS21. The better protection obtained with the QS21 adjuvant was also statistically significant ($p=0.0250$), using the Mann-Whitney non-parametric statistical method corrected for ties, consistent with the higher binding and neutralizing antibody titers. The failure of the nS antibody response to be boosted after challenge (FIG. 9A) was also consistent with the absence of virus replication.

Thus, a recombinant polypeptide containing amino acids 14 to 762 of the SARS-CoV S protein that was administered with adjuvant induced neutralizing antibody and protectively immunized mice against upper and lower respiratory infections with SARS-CoV. Although the ability of a protein vaccine to protectively immunize against SARS-CoV has not previously been reported, recent studies have shown that the protein segment used herein contains the angiotensin-converting enzyme 2 receptor-binding region (Babcock et al. (2004) J. Virol. 78, 4552-4560; Wong et al. (2004) J. Biol. Chem. 279, 3197-3201; Xiao et al. (2003) Biophys. Res. Commun. 312, 1159-1164).

The protein vaccine described herein induced higher neutralizing antibody and complete protection against an intranasal SARS-CoV challenge than that achieved by inoculation of mice with live SARS-CoV (Subbarao et al. (2004) J. Virol. 78, 3572-3577), MVA expressing the full length S (Bisht et al., Proc. Natl. Acad. Sci. USA 101, 6641-6646 (2004)), or DNA expressing full length S or S lacking the transmembrane and cytoplasmic domains (Yang et al., Nature 428, 561-564 (2004)). The better protection achieved in this study is correlated with the higher antibody response. Although nS with either QS21 or MPL + TDM was effective, the former adjuvant induced higher binding and neutralizing antibody and better protection of the upper respiratory tract. Vaccination with QS21 also induced a more balanced helper T-cell response than MPL + TDM as indicated by the higher IgG2a/IgG1 ratio. However, we
attribute the greater protection with QS21 adjuvant to the higher overall antibody response since MVA/S induced a considerably higher IgG2a/IgG1 ratio but was less protective than nS with QS21.

5 References


an attenuated parainfluenza virus expressing the SARS coronavirus spike protein for the prevention of SARS. Lancet 363, 2122-2127.


neutralizing antibodies: implication for developing subunit vaccine.


Rota, P. A., Oberste, M. S., Monroe, S. S., Nix, W. A., Campagnoli, R.,

Icenogle, J. P., Penaranda, S., Bankamp, B., Maher, K., Chen, M. H.,


Sanchez, A., Liffick, S., Holloway, B., Limor, J., McCaustland, K.,

Olsen-Rasmussen, M., Fouchier, R., Gunther, S., Osterhaus, A. D.,


Subbarao, K., McAuliffe, J., Vogel, L. K., Faile, G., Fischer, S., Tatti, K.,

Packard, M., Shieh, W.-J., Zaki, S., Murphy, B., 2004. Prior infection and passive transfer of neutralizing antibody prevents replication of
SARS coronavirus in the respiratory tract of mice. J. Virol. 78, 3572-3577.


All patents and publications referenced or mentioned herein are indicative of the levels of skill of those skilled in the art to which the invention pertains, and each such referenced patent or publication is hereby incorporated by reference to the same extent as if it had been incorporated by reference in its entirety individually or set forth herein in its entirety. Applicants reserve the right to physically incorporate into this specification any and all materials and information from any such cited patents or publications.

The specific methods and compositions described herein are representative of preferred embodiments and are exemplary and not intended as limitations on the scope of the invention. Other objects, aspects, and embodiments will occur to those skilled in the art upon consideration of this specification, and are encompassed within the spirit of the invention as defined by the scope of the claims. It will be readily apparent to one skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention. The invention illustratively described herein suitably may be practiced in the absence of any element or elements, or limitation or limitations, which is not specifically disclosed herein as essential. The methods and processes illustratively described herein suitably may be practiced in differing orders of
steps, and that they are not necessarily restricted to the orders of steps indicated herein or in the claims. As used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "a host cell" includes a plurality (for example, a culture or population) of such host cells, and so forth. Under no circumstances may the patent be interpreted to be limited to the specific examples or embodiments or methods specifically disclosed herein. Under no circumstances may the patent be interpreted to be limited by any statement made by any Examiner or any other official or employee of the Patent and Trademark Office unless such statement is specifically and without qualification or reservation expressly adopted in a responsive writing by Applicants.

The terms and expressions that have been employed are used as terms of description and not of limitation, and there is no intent in the use of such terms and expressions to exclude any equivalent of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention as claimed. Thus, it will be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

Other embodiments are within the following claims. In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.
WHAT IS CLAIMED:

1. An isolated polypeptide consisting essentially of SEQ ID NO:4, 6 or 7.

2. An isolated nucleic acid encoding a polypeptide consisting essentially of SEQ ID NO:4, 6 or 7.

3. The nucleic acid of claim 2, wherein the nucleic acid comprises SEQ ID NO:2 or 5.

4. An antibody that can bind to a SARS Coronavirus polypeptide consisting essentially of SEQ ID NO:4, 6 or 7.

5. A recombinant attenuated poxvirus comprising a genome with a nucleic acid insertion that encodes a SARS Coronavirus polypeptide consisting essentially of SEQ ID NO:1, 3, 4, 6 or 7.

6. The recombinant attenuated poxvirus of claim 5, wherein the nucleic acid insertion comprises SEQ ID NO:2 or 5.

7. The recombinant attenuated poxvirus of claim 5, wherein the poxvirus is a modified MVA virus.

8. A recombinant attenuated baculovirus comprising a nucleic acid encoding a SARS Coronavirus polypeptide consisting essentially of SEQ ID NO:4, 6 or 7.

9. The recombinant attenuated baculovirus of claim 5, wherein the nucleic acid comprises SEQ ID NO:2 or 5.

10. A DNA vaccine comprising a pharmaceutically acceptable carrier and a vector encoding a SARS Coronavirus polypeptide consisting essentially of SEQ ID NO:1, 3, 4, 6 or 7.

11. A composition comprising a carrier and an effective amount of SARS Coronavirus polypeptide consisting essentially of SEQ ID NO:4, 6, 7, or a combination thereof.

12. The composition of claim 11, wherein the amount is effective for generating antibody production in an animal.

13. A composition comprising a carrier and an effective amount of a recombinant attenuated poxvirus comprising a genome with a nucleic acid insertion that encodes a SARS Coronavirus polypeptide consisting essentially of SEQ ID NO:1, 3, 4, 6 or 7.

14. The composition of claim 13, wherein the amount is effective for generating antibody production in an animal.
15. A composition comprising a carrier and an effective amount of antibody that can bind to a SARS Coronavirus polypeptide consisting essentially of SEQ ID NO: 4, 6 or 7.

16. The composition of claim 15, wherein the amount is effective to inhibit SARS Coronavirus replication in the animal.

17. A method for generating an immune response in an animal against a SARS Coronavirus S polypeptide comprising: administering to the animal an immunologically effective amount of the composition of any one of claims 11 or 13.

18. A method for inhibiting SARS Coronavirus infection in an animal comprising: administering to the animal an immunologically effective amount of the composition of any one of claims 11, 13 or 15.

19. A method for treating SARS Coronavirus infection in an animal comprising: administering to the animal an effective amount of the composition of claim 15.

20. The method of claim 19, wherein the amount is effective to inhibit SARS Coronavirus replication in the animal.

21. A diagnostic kit for detection of a SARS Coronavirus infection in a mammal comprising packaging material, an antibody that can bind to a SARS Coronavirus polypeptide consisting essentially of SEQ ID NO: 4, 6 or 7, and instructions for detection of a SARS Coronavirus infection in a mammal.

22. A diagnostic kit for detection of a SARS Coronavirus infection in a mammal comprising packaging material, a SARS Coronavirus polypeptide consisting essentially of SEQ ID NO: 4, 6 or 7, and instructions for detection of a SARS Coronavirus infection in a mammal.
FIG. 1

A

MVA → P11 → mH5 → GFP → S → del III

B

HeLa control  MVA  MVA/S-HA  HeLa control  MVA  MVA/S-HA

220—
97—
66—
45—
30—
1 2 3 4 5 6
FIG. 2

A

<table>
<thead>
<tr>
<th></th>
<th>Endo H</th>
<th>PNGase F</th>
</tr>
</thead>
<tbody>
<tr>
<td>HeLa control</td>
<td>- - +</td>
<td>- - +</td>
</tr>
<tr>
<td>MVA</td>
<td>- - +</td>
<td>- - +</td>
</tr>
<tr>
<td>MVA/S-HA</td>
<td>- - +</td>
<td>- - +</td>
</tr>
</tbody>
</table>

220\(^{-}\)

97\(^{-}\)

1 2 3 4 5 6 7 8

B

<table>
<thead>
<tr>
<th>Chase (min)</th>
<th>0</th>
<th>20</th>
<th>40</th>
<th>60</th>
<th>80</th>
</tr>
</thead>
<tbody>
<tr>
<td>EndoH</td>
<td>- +</td>
<td>- +</td>
<td>- +</td>
<td>- +</td>
<td>- +</td>
</tr>
</tbody>
</table>

220\(^{-}\)

97\(^{-}\)
FIG. 4

A

Reciprocal ELISA titers

100000

10000

1000

10

1

Prebleed 4 6 8 10

Week of study

IM

IN

B

Neutralizing titers

1000

100

10

1

Prebleed 4 6 8

Week of study

IM

IN
FIG. 5

![Bar graph showing virus titer (log_{10} TCID₅₀/g) for different immunogens.](image-url)
<table>
<thead>
<tr>
<th>Translation of S 14-1195AA [A]</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRP-1-1</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>110</td>
</tr>
<tr>
<td>210</td>
</tr>
<tr>
<td>S</td>
</tr>
<tr>
<td>Q</td>
</tr>
<tr>
<td>A</td>
</tr>
</tbody>
</table>

**FIGURE 6**

The figure contains a sequence of nucleotide or amino acid codes, possibly from a genetic or molecular biology context, with a focus on translation and sequence alignment.
TTGTCGCTTGGTGGTTTTTGTCTACCATAAGCACAAGTCACAGTCTGGC
AACAGGCAACCCAAAACGAGATGCCATCTGTGTCTAGTGTCAGC

TRANSLATION OF S 14-1195AA [A]

GTGATTATTATTACAAATTTGACTAAATATGTTCTACCGACATGTAACCTT
CCTAATAATAATGTTTACAGATTACACCCAAATATGCTCGTACATTTGAA

TRANSLATION OF S 14-1195AA [A]

TGAATTTGTTGACACACCTTTTCTTGCTTCTAAACCATGCTACACTT
ACTTACACACTTGTTGGAAAGACACAAAGATTTACAGTGTTACCCATGTC

TRANSLATION OF S 14-1195AA [A]

AGACACATACTATGATATTGATTATGCTATTAAATTGACTCTTTCCAGTAC
TCTGTTGATGACTAATAGCTATTAGTAAATTACAGGAAAGCTCATG

TRANSLATION OF S 14-1195AA [A]

ATATCAGATTACCGTTTTTCGCTTTGATTTTCAGAAAAAGTGCAAGTAAATTAA
TATAGACTACCGGAAAAGCGCGCAGTAGACAAAGTCTTTTCCAGTCCGTTAAAATT

TRANSLATION OF S 14-1195AA [A]
ACACTTACGAGTTTTGTTTTAAAAATAAAAGATGGTGTCTCTATGTT
TGTGAAATGCTCTCACAACACAATTTTTATTTCTACCACAAGAGATCAA
H L R E F V F K N K D G F L Y V>
_________TRANSLATION OF S 14-1195AA [A]__________>

ATAAGGGCTTATCAACCATATGGATGCGGTTGATCTACCTTTCGTTTT
TATTCCCGATAGTTGGATATATCTACATCAAGCAGACTAGATGGAAGACCAA
Y K G Y Q P I D V V R D L P S G F>
_________TRANSLATION OF S 14-1195AA [A]__________>

AACACTTTGAAACTATTTTTAAGTGGCTCTTGGTATAAACATTACAA
TTGTGAAACTTTTGAGATAAATAATTCAACGGAAGACCATATAATGTGAATGTT
N T L K P I F K L P L G I N I T N>
_________TRANSLATION OF S 14-1195AA [A]__________>

TTTTAGAGCCATTCTCTAGCCTTTTTCACCTGCTCAAGACACATTGGGCA
AAATCTCGGAAGATGGTTGAAGCTGGAAGAGTGGACGATTCTGTGAAACCCG
F R A I L T A F S P A Q D I W G>
_________TRANSLATION OF S 14-1195AA [A]__________>

CGTTCGGTGAGCTATTTTGGTTTGGCTATTTAAGCCACTACATTTATG
GCAGTCGACGTCGATCAAACAACCCGATAAATTTTGGTGATGTAATAAC
T S A A A A Y F V G Y L K P T T F M>
_________TRANSLATION OF S 14-1195AA [A]__________>
CTCAAGTATGAGTAAATTTGTACAATCAGATGCTGTGATTGTTTCTCA
GAGTTCATACCTACCTTTTTACATGTAGTGTCTACCAGACAATTACAAAGGT
LKYDENGITIDAVDCSQ>

TRANSLATION OF S 14-1195AA [A]>

810 820 830 840 850
AAATCCACTTGGTCAATCTCAAATGCTGTTAAGACCTTTGAGATTGACA
TTTAGGTTGAAGCTGTAGTTTACGAGACATTTCTCAGAAACTCTAATGT
NPLAELKCSVKSFEID>

TRANSLATION OF S 14-1195AA [A]>

860 870 880 890 900
AAGGAATTTACCAGACCTCTCTAATTTTCAAGGGTTTCCCTAGGAGATTT
TTCCCTAAATGCTCGAGGATGAATCTGCCCACAAGGGAGCTCTCTACCA
KGIYQTSNFRTVVPSSGV>

TRANSLATION OF S 14-1195AA [A]>

910 920 930 940 950
GTGAGATTCCCTAAATTTACAAACTTGCTCCTTTTGAGAGGTTTTTAA
CACTCTAAGGGATTATAATGTGTTGGAACACAGAACAATTCTCTCCAAAATT
VRFPPNITNLCFPEGEVFN>

TRANSLATION OF S 14-1195AA [A]>

960 970 980 990 1000
TGCTACTAAATTCCCCTCGTCTATGCTAGGGAGGAAAAAATTTCTA
ACGATGATTGAGGAAAAAGACGGATGACCCTCTCTCTTTTAAAAAGAT
AKTFPSVYAWERKKIS>

TRANSLATION OF S 14-1195AA [A]>

1010 1020 1030 1040 1050
ATTGTGTTGCTAGTTACTCTGTGCTCTACACATTTTTTTCAACC
1810 1820 1830 1840 1850
CACTCACACCAGCTTGGCCATATATATCTGAAATCTGATATCCAG
GTGGACTGTTGTGCTGACAGCAGGCTTTTCTATAGCTAAGAC
QLTPAWRIVYSTGNNVFQ>
TRANSLATION OF S14-1195AA [A]__________>

1860 1870 1880 1890 1900
GACTCAAGCAGGCTGTTTATAGAGCTGAGCATGTGCACACTCTCTATG
CTGAGTTCGTCCGAAGAAATATCATCCCGACTCTCGTACAGCTGTAAGAATAC
TQAGCLIGAEHVDTSY>
TRANSLATION OF S14-1195AA [A]__________>

1910 1920 1930 1940 1950
AGTGCGACATCCCTATTTGAGCTGGCATTTTGTGCTTAGTTACCAACTACAGTT
TCAGCGTGAAGGATAAACCTCGAACCCGATAACAGATCAATGTATGTCAG
ECDIGAGICAASYHTV>
TRANSLATION OF S14-1195AA [A]__________>

TCTTTATTACGTAGTACACCCAAATCTATTGTGCTTATATCAGACG
AGAATAATGCACTATGAGTGGTTTATAGATAACACCAGATATGATACAG
SLRSTSQKSIVAYTMS>
TRANSLATION OF S14-1195AA [A]__________>

2010 2020 2030 2040 2050
TTTACGCTGATAGTTCAATTTGCTCTCTGAAATACCCAGATTATGCTGATAGT
AAATCAGCAGTATGCAAGTTAGGATATTTGTGTAACAGATAG
LGADSSIAYSNNNTIAI>
TRANSLATION OF S14-1195AA [A]__________>
2060 2070 2080 2090 2100
CTACTAACTTTTCAATTAGCATTACTACAGAGTAATGCCTGTTTCTATG
GATGATTGAAAGTTATCGTAATATGCTGCTTCTATTACGGACAAGATAC
PTNFSPISITTEVMPSM>
TRANSLATION OF S14-1195AA [A]>

2110 2120 2130 2140 2150
GCTAAAACCTCCTGGTAGATTGTAATATGTCATCTCTGCGGAGATTCTACTGA
CGATTTTTGGAGGCGATCTAACCATTATACATGTAGACGCCTCTCAAAGATGACT
AKTSVDNMYICGDS>
TRANSLATION OF S14-1195AA [A]>

2160 2170 2180 2190 2200
ATGTGCTAAATTGCTTCCATTCAATATGCTGTGTTTTGCAACAAACTAATC
TACACGATTTAACAGAAGAGTTAACATGAGAAACTGTGTGGATTTAG
CANLQLQYGSFCTQLN>
TRANSLATION OF S14-1195AA [A]>

2210 2220 2230 2240 2250
GTCGACTCTCTAGGTATTTGCTGTGAAACAGGATCAGAACACACGTAAGTG
CAGGTGAGAGTCATTACAGACCTTGCTTTGTTGTGCTACTTAC
RALSGIAAEQDRNTREV>
TRANSLATION OF S14-1195AA [A]>

2260 2270 2280 2290 2300
TTGCTCTAAAGTCAAACCATTGTAATATGCTGCTTCTATTACGGACAAGATAC
FAQVKQMYKTPTLKYPG>
TRANSLATION OF S14-1195AA [A]>

2310 2320 2330 2340 2350
TGGTTTATAATTTCACAAATATTTACCTGACCCTCTAAAGCCAACTAAGA
ACCAAATTTACAAAGTTTTATATATGACTGGGAGATTTGCTGCTGATTCT
GFNFSQILPDPPLKPDK>_________TRANSLATION OF S 14-1195AA [A]_________>

2360 2370 2380 2390 2400
GGTCTTTTTATGAGGACTTGCTCTTTTATAAGGTGACACTGCTGATGCT
CCAGAAAATATACCTCCTGAGAGAGAAATTATTCCACTGTGAGCGACTAGA
RSFIEDLLFLNKVALDAD>_________TRANSLATION OF S 14-1195AA [A]_________>

2410 2420 2430 2440 2450
GGCTTCATGAAGCAATATGGGGAATGCGCTAGGTAATATAATGCTAGAGA
CCGAAGTACTCTTCTTTAACCCTACGGAATCCACTATAATTACGATCCTCT
GFMKQYGECLGDIANRD>_________TRANSLATION OF S 14-1195AA [A]_________>

2460 2470 2480 2490 2500
TTCATTTCGTGCGCAGAAGTTTCAATTGAGACTTACAGTGTTGGCCACTCTGC
AGGTAAACACGGTCTTCAAGTGTAATCTCAGCTACACGGGTGGAGACG
LIQAKFKGGLTVLLPL>_________TRANSLATION OF S 14-1195AA [A]_________>

2510 2520 2530 2540 2550
TCACTGATGATATGATTTGCTGCTACACTGCTGCTATTAGTGTTGGTACT
AGTGACTCATATACTACACACGAGATGTAGCAGACAGATCAAATCACCAGA
LTDMDMIAAYTAAALVSGT>_________TRANSLATION OF S 14-1195AA [A]_________>

2560 2570 2580 2590 2600
GCCACTGCTGGATGAGACATTGTGCTGCTGGCGCTGCTCTCAATACCTTTT
CGTGCAGACCTACTGTAAACCAACACGACCGGAGAGAATTTATG
ATAGWTFGAGAAALQIPF>

TRANSLATION OF S14-1195AA [A]>

2610 2620 2630 2640 2650
TGCTATGCAATTGCCATATAGGTTTCAATGGCATTGAACCCAAATG
ACGATACGTTTAACCCTATATCCAAAGTACCCTAACCTAATGGGT
AMQMYFNGIGVQN>

TRANSLATION OF S14-1195AA [A]>

2660 2670 2680 2690 2700
TTCTCTATGACGCACACAACAAATCGCCACCTTAAACTAAAGGCGATT
AAGAGATACTCTTGGTGTGGTTAGCGGTTGTTAATGTTGGTCGCTAA
VLKENQKQIANQFNKAI>

TRANSLATION OF S14-1195AA [A]>

2710 2720 2730 2740 2750
AGTCACAAATTCAAGAATCTCAGCTACAACACATCAACTGCAATGGGGCAAGCT
TCAGTTTAAGTGTTTGATGTGTTGATGTACGTAACGCACGTTGAGA>
SIQIESLTTTSTALGKL>

TRANSLATION OF S14-1195AA [A]>

2760 2770 2780 2790 2800
GCAAGACGTTGTAACCAAGATGCTCAAGCATTAAACACACTTGTAAAC
CGTTCTGCACAATTGGTCTACGAGTTCGTAATTGTGTTGAAACAAATTG
QDVNVNQAALNTLKV>

TRANSLATION OF S14-1195AA [A]>

2810 2820 2830 2840 2850
AATTTGCTCTAAATTTGGTGCAATTTCTAAGTGCTAATGAGATATCCTT
TTGAAATCGAGATTAACAAACACGTAAAAGTTCACACGATTACTATGGAA
QLSSNFGAISSVLNDIL

TRANSLATION OF S14-1195AA [A]

2860 2870 2880 2890 2900
TCGGCAGCTTTGATAAAAGTGCGAGGAGGTACAATAATTGACAGGTTAATTAC
AGCGCTGAACATTTTCAGCTCCGCTCCATTTAATGCTGCAATATAATG
SRILDKEAVEQIDRLIT

TRANSLATION OF S14-1195AA [A]

2910 2920 2930 2940 2950
AGGCAGACTTCAAGCTTCATATGTAACACAAACAACTATAATCAGG3
TCCGCTGAAAGTTCGCGATACATTGTTGTTGTATATTGCC
GRLQSLQTYVTQQLIR

TRANSLATION OF S14-1195AA [A]

2960 2970 2980 2990 3000
CTGCTGAAATACGGCTTCGCTCAATTTGCTGCTACTAAATGTCTGAG
GACGACTTTAGTCCGAAGACGATAGAAGAGGCGATGATTTTACAGACTC
AAEIRASANLAAATKMS

TRANSLATION OF S14-1195AA [A]

3010 3020 3030 3040 3050
TGTGTTCTGACAAATCAAAAGAGTGGCACTTTTTGTGAAAGGCTACCA
ACACAAAGAACTCTGTAGTMTTTTCTCATCAAATGAAACACCTTTCCGATG
CVLQGSKRVDFCGKGYH

TRANSLATION OF S14-1195AA [A]

3060 3070 3080 3090 3100
CCTTATGCTCTTCACAAAGCAGCCGCGCATGTTGTTTCTCTACTATG
GGAAATACAGGAAGGCTGCTGCGGGCGTGACACACAGAAGGATG
LMSFPQAAPHGVVFLH
3360 3370 3380 3390 3400
CAAGAAGAGCTGGACAGTGATCTTCGTCGTTACCATACATCCACCAAGATGTTG
GTTCCTTGACCTCTGTTCAAGTTTTTATAATGATTAGTAGTGCATCCTACAAC
KEELDKYFKNHTS<SPD V>
____________TRANSLATION OF S 14-1195AA [A]____________>

3410 3420 3430 3440 3450
ATCTGGCGACATTTCCAGCCTATACGCTTCTGTGTCGTAACATTCAAAAA
TAGAACCCTGCAGAAGTTCGTAAGTTCGGAAGACAGCAGTTGTAAGTTTTT
DLGISGINASVVPNQK>
____________TRANSLATION OF S 14-1195AA [A]____________>

3460 3470 3480 3490 3500
GAAATGGACCGCTCAATGAGGTCGCTAAAAATTAAATGAAATCACTCAT
CTTAACTGCGGAGTTACTCCAGCGATTTTAAATTTACTTAGTGAGTA
BDRLNVEVAKLNESLIG>
____________TRANSLATION OF S 14-1195AA [A]____________>

3510 3520 3530 3540 3550
TGACCTCTCAAGAATGGGAAATATGAGCATATATATATAGATGCCCTCAG
ACTGGAAAGTTCTTAAACCCTTTATACAGTTATATATATTTACCGGAGTAG
DLQELGKYEQYIKWP>
____________TRANSLATION OF S 14-1195AA [A]____________>

3560
ATCCACCTACACCATTGA
TATGCGTAGGTAACT
HHHHH*
__________TRANSLATION__>
FIG. 7

A

B

C

<table>
<thead>
<tr>
<th></th>
<th>Anti-His</th>
<th>Anti-S</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNGaseF</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>220</td>
<td></td>
<td></td>
</tr>
<tr>
<td>97</td>
<td></td>
<td></td>
</tr>
<tr>
<td>66</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Glycosylated

Unglycosylated
FIG. 8
FIG. 10

A

![Graph A showing mean virus titer ± SE (log_{10} TCID_{50}/g) for different immunization groups.]

B

![Graph B showing mean virus titer ± SE (log_{10} TCID_{50}/g) for different immunization groups.]

Immunization Groups

nS+MPL  L1R+MPL  nS+QS21  L1R+QS21
<110> National Institutes of Health
5  Moss, Bernard
       Bisht, Himani
       Wyatt, Linda S.

<120> Soluble Fragments of the SARS-CoV Spike Glycoprotein
10
<130> 1662.041WO1

<150> US 60/558,995
<151> 2004-04-05
15
<160> 7

<170> FastSEQ for Windows Version 4.0

20<210> 1
211> 1255
212> PRT
213> Urbani strain of SARS-CoV

25<400> 1
    Met Phe Ile Phe Leu Leu Phe Leu Thr Leu Thr Ser Gly Ser Asp Leu
1  5  10  15
    Asp Arg Cys Thr Thr Phe Asp Asp Val Gln Ala Pro Asn Tyr Thr Gln
20 25 30
30 His Thr Ser Ser Met Arg Gly Val Tyr Tyr Pro Asp Glu Ile Phe Arg
35 40 45
    Ser Asp Thr Leu Tyr Leu Thr Gln Asp Leu Phe Leu Pro Phe Tyr Ser
50 55 60
    Asn Val Thr Gly Phe His Thr Ile Asn His Thr Phe Gly Asn Pro Val
3565 70 75 80
    Ile Pro Phe Lys Asp Gly Ile Tyr Phe Ala Ala Thr Glu Lys Ser Asn
85 90 95
    Val Val Arg Gly Trp Val Phe Gly Ser Thr Met Asn Asn Lys Ser Gln
100 105 110
40 Ser Val Ile Ile Ile Asn Asn Ser Thr Asn Val Val Ile Arg Ala Cys
115 120 125
    Asn Phe Glu Leu Cys Asp Asn Pro Phe Phe Ala Val Ser Lys Pro Met
130 135 140
Gly Thr Gln Thr His Thr Met Ile Phe Asp Asn Ala Phe Asn Cys Thr
145 150 155 160
Phe Glu Tyr Ile Ser Asp Ala Phe Ser Leu Asp Val Ser Glu Lys Ser
165 170 175
Gly Asn Phe Lys His Leu Arg Glu Phe Val Phe Lys Asn Lys Asp Gly
180 185 190
Phe Leu Tyr Val Tyr Lys Gly Tyr Gln Pro Ile Asp Val Val Arg Asp
195 200 205
Leu Pro Ser Gly Phe Asn Thr Leu Lys Pro Ile Phe Lys Leu Pro Leu
10 210 215 220
Gly Ile Asn Ile Thr Asn Phe Arg Ala Ile Leu Thr Ala Phe Ser Pro
225 230 235 240
Ala Gln Asp Ile Trp Gly Thr Ser Ala Ala Ala Tyr Phe Val Gly Tyr
245 250 255
Leu Lys Pro Thr Thr Phe Met Leu Lys Tyr Asp Glu Asn Gly Thr Ile
260 265 270
Thr Asp Ala Val Asp Cys Ser Gln Asn Pro Leu Ala Glu Leu Lys Cys
275 280 285
Ser Val Lys Ser Phe Glu Ile Asp Lys Gly Ile Tyr Gln Thr Ser Asn
290 295 300
Phe Arg Val Val Pro Ser Gly Asp Val Val Arg Phe Pro Asn Ile Thr
305 310 315 320
Asn Leu Cys Pro Phe Gln Asp Ala Thr Lys Phe Pro Ser
325 330 335
Val Tyr Ala Trp Glu Arg Lys Lys Ile Ser Asn Cys Val Ala Asp Tyr
340 345 350
Ser Val Leu Tyr Asn Ser Thr Phe Phe Ser Thr Phe Lys Cys Tyr Gly
355 360 365
Val Ser Ala Thr Lys Leu Asn Phe Asp Leu Cys Phe Ser Asn Val Tyr Ala
370 375 380
Asp Ser Phe Val Val Lys Gly Asp Asp Val Arg Glu Ile Ala Pro Gly
385 390 395 400
Gln Thr Gly Val Ile Ala Asp Tyr Asn Tyr Lys Leu Pro Asp Asp Phe
405 410 415
Met Gly Cys Val Leu Ala Trp Asn Thr Arg Asn Ile Asp Ala Thr Ser
420 425 430
Thr Gly Asn Tyr Tyr Lys Tyr Arg Tyr Leu Arg His Gly Lys Leu
435 440 445
Arg Pro Phe Glu Arg Asp Ile Ser Asn Val Pro Phe Ser Pro Asp Gly
450 455 460
Lys Pro Cys Thr Pro Pro Ala Leu Asn Cys Tyr Trp Pro Leu Asn Asp
465 470 475 480
Tyr Gly Phe Tyr Thr Thr Thr Gly Ile Gly Tyr Gln Pro Tyr Arg Val
485        490        495
Val Val Leu Ser Phe Glu Leu Leu Asn Ala Pro Ala Thr Val Cys Gly
500        505        510
5Pro Lys Leu Ser Thr Asp Leu Ile Lys Asn Gln Cys Val Asn Phe Asn
515        520        525
Phe Asn Gly Leu Thr Gly Thr Gly Val Leu Thr Pro Ser Ser Lys Arg
530        535        540
Phe Gln Pro Phe Gln Gln Phe Gly Arg Asp Val Ser Asp Phe Thr Asp
10545       550        555        560
Ser Val Arg Asp Pro Lys Thr Ser Glu Ile Leu Asp Ile Ser Pro Cys
565        570        575
Ser Phe Gly Gly Val Ser Val Ile Thr Pro Gly Thr Asn Ala Ser Ser
580        585        590
15Glu Val Ala Val Leu Tyr Gln Asp Val Asn Cys Thr Asp Val Ser Thr
595        600        605
Ala Ile His Ala Asp Gln Leu Thr Pro Ala Trp Arg Ile Tyr Ser Thr
610        615        620
Gly Asn Asn Val Phe Gln Thr Gln Ala Gly Cys Leu Ile Gly Ala Glu
20625       630        635        640
His Val Asp Thr Ser Tyr Glu Cys Asp Ile Pro Ile Gly Ala Gly Ile
645        650        655
Cys Ala Ser Tyr His Thr Val Ser Leu Leu Arg Ser Thr Ser Gln Lys
660        665        670
25Ser Ile Val Ala Tyr Thr Met Ser Leu Gly Ala Asp Ser Ser Ile Ala
675        680        685
Tyr Ser Asn Thr Ile Ala Ile Pro Thr Asn Phe Ser Ile Ser Ile
690        695        700
Thr Thr Glu Val Met Pro Val Ser Met Ala Lys Thr Ser Val Asp Cys
30705       710        715        720
Asn Met Tyr Ile Cys Gly Asp Ser Thr Glu Cys Ala Asn Leu Leu Leu
725        730        735
Gln Tyr Gly Ser Phe Cys Thr Gln Leu Asn Arg Ala Leu Ser Gly Ile
740        745        750
35Ala Ala Glu Gln Asp Arg Asn Thr Arg Glu Val Phe Ala Gln Val Lys
755        760        765
Gln Met Tyr Lys Thr Pro Thr Leu Lys Tyr Phe Gly Phe Asn Phe
770        775        780
Ser Gln Ile Leu Pro Asp Pro Leu Lys Pro Thr Lys Arg Ser Phe Ile
40785       790        795        800
Glu Asp Leu Leu Phe Asn Lys Val Thr Leu Ala Asp Ala Gly Phe Met
805        810        815
Gly Ile Asn Ala Ser Val Val Asn Ile Gln Lys Glu Ile Asp Arg Leu
1155 1160 1165
Asn Glu Val Ala Lys Asn Leu Asn Glu Ser Leu Ile Asp Leu Gln Glu
1170 1175 1180
5Leu Gly Lys Tyr Glu Gln Tyr Ile Lys Trp Pro Trp Tyr Val Trp Leu
1185 1190 1195 1200
Gly Phe Ile Ala Gly Leu Ile Ala Ile Val Met Val Thr Ile Leu Leu
1205 1210 1215
Cys Cys Met Thr Ser Cys Cys Ser Cys Leu Lys Gly Ala Cys Ser Cys
1220 1225 1230
Gly Ser Cys Cys Lys Phe Asp Glu Asp Asp Ser Glu Pro Val Leu Lys
1235 1240 1245
Gly Val Lys Leu His Tyr Thr
1250 1255

<210> 2
<211> 3768
<212> DNA
<213> Urbani strain of SARS-CoV

20

<400> 2
atgttttatctctttatatcttactctcactagtggtatgcggagccctgctcacc
1
acattttgtagatgtcagttcttacatacactagcatctctcatctctcatctctcatcatctctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctcatctctca
cttgcttgga atacagtagg aacatgatgct acttcaactg gtaattataa ttataaatat
aggtatctta gagctgggca gctattggc ttttgagag agaattatca cttgcctctcc
ccctcgtatg gcaacacttt gacccacctt gcatctttta ctatttatgcc attaataat
atatgcattt tggctgacag gcagctctct cactcactcta gatcttggtct gttatcactct
cttccaaaga gatttccaaacc attttcaaca aacagcgggt cttgctgtgtg ttttccactaat
tccgctgag atcctcataa atctgacatt taatcctgct cctctgcatc ttttggttct

gttgcaggt aagcaccagc ttttcctctt ataatttactt gctgctctgc gcaacagagt
ttatttataa cttgcagctc ctcattttca tattcttgcc attaattttc gatgtgctga

10 ttaaacagca ggttgctgacag gcaacagagt cttcactctt atgtgctgta cttttaatttt
catcctcattt gacccactct cttcttgcc ctttgctgtg tttttcttttaa aacccagaag
ctatttttaa gctgccaactgc ggctggtgct gcgcagctct ccctttttttt

15 ttmgtggttc atggctgttg ataattttttttt gttgctgttg ataattttttttt

19 atgactgactgt gcagctgactg atgtgctgct gcagctgactg atgtgctgct gctgtgctgct

gctgtgctgct gcagctgactg atgtgctgct gcagctgactg atgtgctgct
gctgtgctgct gcagctgactg atgtgctgct gcagctgactg atgtgctgct
gctgtgctgct gcagctgactg atgtgctgct gcagctgactg atgtgctgct
<210> 3
<211> 1255
<212> PRT
<213> NS-1 strain of SARS-CoV
5

Met Phe Ile Phe Leu Leu Phe Leu Thr Leu Thr Ser Gly Ser Asp Leu
1 5 10 15
Asp Arg Cys Thr Thr Phe Asp Asp Val Gln Ala Pro Asn Tyr Thr Gln
10 20 25 30
His Thr Ser Ser Met Arg Gly Val Tyr Tyr Pro Asp Glu Ile Phe Arg
35 40 45
Ser Asp Thr Leu Tyr Leu Thr Gln Asp Leu Phe Leu Pro Phe Tyr Ser
50 55 60
15Asn Val Thr Gly Phe His Thr Ile Asn His Thr Phe Gly Asn Pro Val
65 70 75 80
Ile Pro Phe Lys Asp Gly Ile Tyr Phe Ala Ala Thr Glu Lys Ser Asn
85 90 95
Val Val Arg Gly Trp Val Phe Gly Ser Thr Met Asn Asn Lys Ser Gln
20 100 105 110
Ser Val Ile Ile Asn Ser Thr Asn Val Val Ile Arg Ala Cys
115 120 125
Asn Phe Glu Leu Cys Asp Asn Pro Phe Phe Ala Val Ser Lys Pro Met
130 135 140
25Gly Thr Gln Thr His Thr Met Ile Phe Asp Asn Ala Phe Asn Cys Thr
145 150 155 160
Phe Glu Tyr Ile Ser Asp Ala Phe Ser Leu Asp Val Ser Glu Lys Ser
165 170 175
Gly Asn Phe Lys His Leu Arg Glu Phe Val Phe Lys Asn Lys Asp Gly
30 180 185 190
Phe Leu Tyr Val Tyr Lys Gly Tyr Glu Pro Ile Asp Val Val Arg Asp
195 200 205
Leu Pro Ser Gly Phe Asn Thr Leu Lys Pro Ile Phe Lys Leu Pro Leu
210 215 220
35Gly Ile Asn Ile Thr Asn Phe Arg Ala Ile Leu Thr Ala Phe Ser Pro
225 230 235 240
Ala Gln Asp Thr Trp Gly Thr Ser Ala Ala Ala Tyr Phe Val Gly Tyr
245 250 255
Leu Lys Pro Thr Thr Phe Met Leu Lys Tyr Asp Glu Asn Gly Thr Ile
40 260 265 270
Thr Asp Ala Val Asp Cys Ser Gln Asn Pro Leu Ala Glu Leu Lys Cys
275 280 285
Ser Val Lys Ser Phe Glu Ile Asp Lys Gly Ile Tyr Gln Thr Ser Asn
290 295 300
Phe Arg Val Val Pro Ser Gly Asp Val Val Arg Phe Pro Asn Ile Thr
305 310 315 320
5Asn Leu Cys Pro Phe Gly Glu Val Phe Asn Ala Thr Lys Phe Pro Ser
325 330 335
Val Tyr Ala Trp Glu Arg Lys Lys Ile Ser Asn Cys Val Ala Asp Tyr
340 345 350
Ser Val Leu Tyr Asn Ser Thr Phe Phe Ser Thr Phe Lys Cys Tyr Gly
10 355 360 365
Val Ser Ala Thr Lys Leu Asn Asp Leu Cys Phe Ser Asn Val Tyr Ala
370 375 380
Asp Ser Phe Val Val Lys Gly Asp Val Arg Gln Ile Ala Pro Gly
385 390 395 400
15Gln Thr Gly Val Ile Ala Asp Tyr Asn Tyr Lys Leu Pro Asp Asp Phe
405 410 415
Met Gly Cys Val Leu Ala Trp Asn Thr Arg Asn Ile Asp Ala Thr Ser
420 425 430
Thr Gly Asn Tyr Asn Tyr Lys Tyr Arg Tyr Leu Arg His Gly Lys Leu
20 435 440 445
Arg Pro Phe Glu Arg Asp Ile Ser Asn Val Pro Phe Ser Pro Asp Gly
450 455 460
Lys Pro Cys Thr Pro Pro Ala Leu Asn Cys Tyr Trp Pro Leu Asn Asp
465 470 475 480
25Tyr Gly Phe Tyr Thr Thr Thr Gly Ile Gly Tyr Gln Pro Tyr Arg Val
485 490 495
Val Val Leu Ser Phe Glu Leu Leu Asn Ala Pro Ala Thr Val Cys Gly
500 505 510
Pro Lys Leu Ser Thr Asp Leu Ile Lys Asn Gln Cys Val Asn Phe Asn
30 515 520 525
Phe Asn Gly Leu Thr Gly Thr Gly Val Leu Thr Pro Ser Ser Lys Arg
530 535 540
Phe Glu Pro Phe Glu Phe Gly Arg Asp Val Ser Asp Phe Thr Asp
545 550 555 560
35Ser Val Arg Asp Pro Lys Thr Ser Glu Ile Leu Asp Ile Ser Pro Cys
565 570 575
Ser Phe Gly Val Ser Val Ile Thr Pro Gly Thr Asn Ala Ser Ser
580 585 590
Glu Val Ala Val Leu Tyr Gln Asp Val Asn Cys Thr Asp Val Ser Thr
40 595 600 605
Ala Ile His Ala Asp Gln Leu Thr Pro Ala Trp Arg Ile Tyr Ser Thr
610 615 620
Gly Asn Asn Val Phe Gln Thr Gln Ala Gly Cys Leu Ile Gly Ala Glu
625 630 635 640
His Val Asp Thr Ser Tyr Glu Cys Asp Ile Pro Ile Gly Ala Gly Ile
645 650 655
5 Cys Ala Ser Tyr His Thr Val Ser Leu Leu Arg Ser Thr Ser Gln Lys
660 665 670
Ser Ile Val Ala Tyr Thr Met Ser Leu Gly Ala Asp Ser Ser Ile Ala
675 680 685
Tyr Ser Asn Asn Thr Ile Ala Ile Pro Thr Asn Phe Ser Ile Ser Ile
10 690 695 700
Thr Thr Glu Val Met Pro Val Ser Met Ala Lys Thr Ser Val Asp Cys
705 710 715 720
Asn Met Tyr Ile Cys Gly Asp Ser Thr Glu Cys Ala Asn Leu Leu Leu
725 730 735
15 Gln Tyr Gly Ser Phe Cys Thr Gln Leu Asn Arg Ala Leu Ser Gly Ile
740 745 750
 Ala Ala Glu Gln Asp Arg Asn Thr Arg Glu Val Phe Ala Gln Val Lys
755 760 765
 Gln Met Tyr Lys Thr Pro Thr Leu Lys Tyr Phe Gly Gly Phe Asn Phe
20 770 775 780
 Ser Gln Ile Leu Pro Asp Pro Leu Lys Pro Thr Lys Arg Ser Phe Ile
785 790 795 800
 Glu Asp Leu Leu Phe Asn Lys Val Thr Leu Ala Asp Ala Gly Phe Met
805 810 815
25 Lys Gln Tyr Gly Glu Cys Leu Gly Asp Ile Asn Ala Arg Asp Leu Ile
820 825 830
 Cys Ala Gln Lys Phe Asn Gly Leu Thr Val Leu Pro Pro Leu Thr Leu
835 840 845
 Asp Asp Met Ile Ala Ala Tyr Thr Ala Ala Leu Val Ser Gly Thr Ala
30 850 855 860
 Thr Ala Gly Trp Thr Phe Gly Ala Gly Ala Leu Gln Ile Pro Phe
865 870 875 880
 Ala Met Gln Met Ala Tyr Arg Phe Asn Gly Ile Gly Val Thr Gln Asn
885 890 895
35 Val Leu Tyr Glu Asn Gln Lys Gln Ile Ala Asn Gln Phe Asn Lys Ala
900 905 910
 Ile Ser Gln Ile Gln Glu Ser Leu Thr Thr Thr Ser Thr Ala Leu Gly
915 920 925
 Lys Leu Gln Asp Val Val Asn Gln Asn Ala Gln Ala Leu Asn Thr Leu
40 930 935 940
 Val Lys Gln Leu Ser Ser Asn Phe Gly Ala Ile Ser Ser Val Leu Asn
945 950 955 960
Asp Ile Leu Ser Arg Leu Asp Lys Val Glu Ala Glu Val Gln Ile Asp
965 970 975
Arg Leu Ile Thr Gly Arg Leu Gln Ser Leu Gln Thr Tyr Val Thr Gln
980 985 990
5Gln Leu Ile Arg Ala Ala Glu Ile Met Ala Ser Ala Asn Leu Ala Ala
995 1000 1005
Thr Lys Met Ser Glu Cys Val Leu Gly Gln Ser Lys Arg Val Asp Phe
1010 1015 1020
Cys Gly Lys Gly Tyr His Leu Met Ser Phe Pro Gln Ala Ala Pro His
101025 1030 1035 1040
Gly Val Val Phe Leu His Val Thr Tyr Val Pro Ser Gln Glu Arg Asn
1045 1050 1055
Phe Thr Thr Ala Pro Ala Ile Cys His Glu Gly Lys Ala Tyr Phe Pro
1060 1065 1070
15Arg Glu Gly Val Phe Val Phe Asn Gly Thr Ser Trp Phe Ile Thr Gln
1075 1080 1085
Arg Asn Phe Phe Ser Pro Gln Ile Ile Thr Thr Asp Asn Thr Phe Val
1090 1095 1100
Ser Gly Asn Cys Asp Val Val Ile Gly Ile Ile Asn Asn Thr Val Tyr
201105 1110 1115 1120
Asp Pro Leu Gln Pro Glu Leu Asp Ser Phe Lys Glu Leu Asp Lys
1125 1130 1135
Tyr Phe Lys Asn His Thr Ser Pro Asp Val Asp Leu Gly Asp Ile Ser
1140 1145 1150
25Gly Ile Asn Ala Ser Val Val Asn Ile Gln Lys Glu Ile Asp Arg Leu
1155 1160 1165
Asn Glu Val Ala Lys Asn Leu Asn Glu Ser Leu Ile Asp Leu Gln Glu
1170 1175 1180
Leu Gly Lys Tyr Glu Gln Tyr Ile Lys Trp Pro Trp Tyr Val Trp Leu
301185 1190 1195 1200
Gly Phe Ile Ala Gly Leu Ile Ala Ile Val Met Val Thr Ile Leu Leu
1205 1210 1215
Cys Cys Met Thr Ser Cys Cys Ser Cys Leu Lys Gly Ala Cys Ser Cys
1220 1225 1230
35Gly Ser Cys Lys Phe Asp Glu Asp Asp Ser Glu Pro Val Leu Lys
1235 1240 1245
Gly Val Lys Leu His Tyr Thr
1250 1255
A synthetic polypeptide

<table>
<thead>
<tr>
<th>Amida</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ser Asp Leu Asp Arg Cys Thr Thr Phe Asp Asp Val Gln Ala Pro Asn</td>
<td>1</td>
</tr>
<tr>
<td>Tyr Thr Gln His Thr Ser Ser Met Arg Gly Val Tyr Tyr Pro Asp Glu</td>
<td>5</td>
</tr>
<tr>
<td>Ile Phe Arg Ser Thr Leu Tyr Leu Thr Gln Asp Leu Phe Leu Pro</td>
<td>10</td>
</tr>
<tr>
<td>Phe Tyr Ser Asn Val Thr Gly Phe His Thr Ile Asn His Thr Phe Gly</td>
<td>15</td>
</tr>
<tr>
<td>Asn Pro Val Ile Pro Phe Lys Asp Gly Ile Tyr Phe Ala Ala Thr Glu</td>
<td>20</td>
</tr>
<tr>
<td>Lys Ser Asn Val Val Arg Gly Trp Val Phe Gly Ser Thr Met Asn Asn</td>
<td>25</td>
</tr>
<tr>
<td>Lys Ser Gln Ser Val Ile Ile Ile Asn Asn Ser Thr Asn Val Val Ile</td>
<td>30</td>
</tr>
<tr>
<td>Arg Ala Cys Asn Phe Glu Leu Cys Asp Asn Pro Phe Phe Ala Val Ser</td>
<td>35</td>
</tr>
<tr>
<td>Lys Pro Met Gly Thr Gln Thr His Thr Met Ile Phe Asp Asn Ala Phe</td>
<td>40</td>
</tr>
<tr>
<td>Asn Cys Thr Phe Glu Tyr Ile Ser Asp Ala Phe Ser Leu Asp Val Ser</td>
<td>45</td>
</tr>
<tr>
<td>Glu Lys Ser Gly Asn Phe Lys His Leu Arg Glu Phe Val Phe Lys Asn</td>
<td>50</td>
</tr>
<tr>
<td>Lys Asp Gly Phe Leu Tyr Val Tyr Lys Gly Tyr Glu Pro Ile Asp Val</td>
<td>55</td>
</tr>
<tr>
<td>Val Arg Asp Leu Pro Ser Gly Phe Asn Thr Leu Lys Pro Ile Phe Lys</td>
<td>60</td>
</tr>
<tr>
<td>Leu Pro Leu Gly Ile Asn Ile Thr Asn Phe Arg Ala Ile Leu Thr Ala</td>
<td>65</td>
</tr>
<tr>
<td>Phe Ser Pro Ala Gly Ile Thr Gly Thr Ser Ala Ala Ala Tyr Phe</td>
<td>70</td>
</tr>
<tr>
<td>Val Gly Tyr Leu Lys Pro Thr Thr Phe Met Leu Lys Tyr Asp Glu Asn</td>
<td>75</td>
</tr>
</tbody>
</table>
Gly Thr Ile Thr Asp Ala Val Asp Cys Ser Gln Asn Pro Leu Ala Glu
260 265 270
Leu Lys Cys Ser Val Lys Ser Phe Glu Ile Asp Lys Gly Ile Tyr Gln
275 280 285
5Thr Ser Asn Phe Arg Val Val Pro Ser Gly Asp Val Val Arg Phe Pro
290 295 300
Asn Ile Thr Asn Leu Cys Pro Phe Gly Glu Val Phe Asn Ala Thr Lys
305 310 315 320
Phe Pro Ser Val Tyr Ala Trp Glu Arg Lys Lys Ile Ser Asn Cys Val
330 335
10 Ala Asp Tyr Ser Val Leu Tyr Asn Ser Thr Phe Phe Ser Thr Phe Lys
340 345 350
Cys Tyr Gly Val Ser Ala Thr Lys Leu Asn Asp Leu Cys Phe Ser Asn
355 360 365
15Val Tyr Ala Asp Ser Phe Val Val Lys Gly Asp Asp Val Arg Gln Ile
370 375 380
Ala Pro Gly Gln Thr Gly Val Ile Ala Asp Tyr Asn Tyr Lys Leu Pro
385 390 395 400
Asp Asp Phe Met Gly Cys Val Leu Ala Trp Asn Thr Arg Asn Ile Asp
405 410 415
20 Ala Thr Ser Thr Gly Asn Tyr Asn Tyr Lys Tyr Arg Tyr Leu Arg His
420 425 430
Gly Lys Leu Arg Pro Phe Glu Arg Asp Ile Ser Asn Val Pro Phe Ser
435 440 445
25Pro Asp Gly Lys Pro Cys Thr Pro Pro Ala Leu Asn Cys Tyr Trp Pro
450 455 460
Leu Asn Asp Tyr Gly Phe Tyr Thr Thr Gly Ile Gly Tyr Gln Pro
465 470 475 480
Tyr Arg Val Val Leu Ser Phe Glu Leu Leu Asn Ala Pro Ala Thr
30 485 490 495
30 Val Cys Gly Pro Lys Leu Ser Thr Asp Leu Ile Lys Asn Gln Cys Val
500 505 510
Asn Phe Asn Phe Asn Gly Leu Thr Gly Thr Gly Val Leu Thr Pro Ser
515 520 525
35Ser Lys Arg Phe Gln Pro Phe Gln Gln Phe Gly Arg Asp Val Ser Asp
530 535 540
Phe Thr Asp Ser Val Arg Asp Pro Lys Thr Ser Glu Ile Leu Asp Ile
545 550 555 560
Ser Pro Cys Ser Phe Gly Gly Val Ser Val Ile Thr Pro Gly Thr Asn
565 570 575
40 Ala Ser Ser Glu Val Ala Val Leu Tyr Gln Asp Val Asn Cys Thr Asp
580 585 590
Val Ser Thr Ala Ile His Ala Asp Gln Leu Thr Pro Ala Trp Arg Ile
595  600  605
Tyr Ser Thr Gly Asn Asn Val Phe Gln Thr Gln Ala Gly Cys Leu Ile
610  615  620
5Gly Ala Glu His Val Asp Thr Ser Tyr Glu Cys Asp Ile Pro Ile Gly
625  630  635  640
Ala Gly Ile Cys Ala Ser Tyr His Thr Val Ser Leu Leu Arg Ser Thr
645  650  655
Ser Gln Lys Ser Ile Val Ala Tyr Thr Met Ser Leu Gly Ala Asp Ser
10  660  665  670
Ser Ile Ala Tyr Ser Asn Thr Ile Ala Ile Pro Thr Asn Phe Ser
675  680  685
Ile Ser Ile Thr Thr Glu Val Met Pro Val Ser Met Ala Lys Thr Ser
690  695  700
15Val Asp Cys Asn Met Tyr Ile Cys Gly Asp Ser Thr Glu Cys Ala Asn
705  710  715  720
Leu Leu Leu Glu Tyr Gly Ser Phe Cys Thr Gln Leu Asn Arg Ala Leu
725  730  735
Ser Gly Ile Ala Ala Glu Gln Asp Arg Asn Thr Arg Glu Val Phe Ala
20  740  745  750
Gln Val Lys Gln Met Tyr Lys Thr Pro Thr Leu Lys Tyr Phe Gly Gly
755  760  765
Phe Asn Phe Ser Gln Ile Leu Pro Asp Pro Leu Lys Pro Thr Lys Arg
770  775  780
25Ser Phe Ile Glu Asp Leu Leu Phe Asn Lys Val Thr Leu Ala Asp Ala
785  790  795  800
Gly Phe Met Lys Gln Tyr Gly Glu Cys Leu Gly Asp Ile Asn Ala Arg
805  810  815
Asp Leu Ile Cys Ala Glu Lys Phe Asn Gly Leu Thr Val Leu Pro Pro
30  820  825  830
Leu Leu Thr Asp Met Ile Ala Ala Tyr Thr Ala Ala Leu Val Ser
835  840  845
Gly Thr Ala Thr Ala Gly Trp Thr Phe Gly Ala Gly Ala Leu Gln
850  855  860
35Ile Pro Phe Ala Met Gln Met Ala Tyr Arg Phe Asn Gly Ile Gly Val
865  870  875  880
Thr Gln Asn Val Leu Tyr Glu Asn Gln Lys Gln Ile Ala Asn Gln Phe
885  890  895
Asn Lys Ala Ile Ser Gln Ile Glu Glu Ser Leu Thr Thr Thr Ser Thr
40  900  905  910
Ala Leu Gly Lys Leu Gln Asp Val Val Asn Gln Asn Ala Gln Ala Leu
915  920  925
Asn Thr Leu Val Lys Gln Leu Ser Ser Asn Phe Gly Ala Ile Ser Ser 930 935 940
Val Leu Asn Asp Ile Leu Ser Ser Arg Leu Asp Lys Val Glu Ala Glu Val 945 950 955 960
5Gln Ile Asp Arg Leu Ile Thr Gly Arg Leu Gln Ser Leu Gln Thr Tyr 965 970 975
Val Thr Gln Gln Leu Ile Arg Ala Ala Glu Ile Arg Ala Ser Ala Asn 980 985 990
Leu Ala Ala Thr Lys Met Ser Glu Cys Val Leu Gly Gln Ser Lys Arg 10 995 1000 1005
Val Asp Phe Cys Gly Lys Gly Tyr His Leu Met Ser Phe Pro Gln Ala 1010 1015 1020
Ala Pro His Gly Val Val Phe Leu His Val Thr Tyr Val Pro Ser Gln 1025 1030 1035 1040
15Glu Arg Asn Phe Thr Thr Ala Pro Ala Ile Cys His Glu Gly Lys Ala 1045 1050 1055
Tyr Phe Pro Arg Glu Gly Val Phe Val Phe Asn Gly Thr Ser Trp Phe 1060 1065 1070
Ile Thr Gln Arg Asn Phe Phe Ser Pro Gln Ile Ile Thr Thr Asp Asn 20 1075 1080 1085
Thr Phe Val Ser Gly Asn Asp Val Val Ile Gly Ile Ile Asn Asn 1090 1095 1100
Thr Val Tyr Asp Pro Leu Gln Pro Glu Leu Asp Ser Phe Lys Glu Glu 1105 1110 1115 1120
25Leu Asp Lys Tyr Phe Lys Asn His Thr Ser Pro Asp Val Asp Leu Gly 1125 1130 1135
Asp Ile Ser Gly Ile Asn Ala Ser Val Val Asn Ile Gln Lys Glu Ile 1140 1145 1150
Asp Arg Leu Asn Glu Val Ala Lys Asn Leu Asn Glu Ser Leu Ile Asp 30 1155 1160 1165
Leu Gln Glu Leu Gly Lys Tyr Glu Gln Tyr Ile Lys Trp Pro His His 1170 1175 1180
His His His His 1185

<210> 5
<211> 3567
<212> DNA
<213> Artificial Sequence

40

<220>
<223> A synthetic oligonucleotide
<400> 5
agtgacccctg accgggtc cacctttttgt gatggttcaag ccctctaatta cactcaacact 60
actccatcta tgaggggggt ttactatctc gatggaatttt tttagactca gaccttttat 120
ctcaacag ctatttctt tccattattt ttactaatga caggttgttca tactattaat 180
5catacgctgtg gcaacccggtc cattcttttt aagagtggtta tttatattgc tgccacacag 240
aatcaataag ttggtcctgtg ttgggttttt gttggtccac tgaacaaacaa gtcacagtgc 300
gtattatatt taaccaaatc tcataatgtg gtttacgtag catgtaacct tgaatttgggt 360
gacaaccttt tttgtgtctgt ttctaaaccc atgggtacac acagacatatc tagtatatcc 420
gataattgcat tttattgcaac tttctgatagc atattctgatg ctttctgtct gtgtgtttca 480
10gaaaggtcag gttaatttaaa ttaaataaa gaagttggttt 540
cctattgttt attaagggtta tcaacattata gatgtagttc gtgatctacc ttctgtgttt 600
aacacctttgaa acacatttttt taagttgtcct cttgtgtata acattcaaca 660
atttctacac ctttttccac ggtctcaagac atttggggga caatgctagg cagccatttt 720
gttgcctatt taagacccac tacatattatg cttcaagtattg atgaaaatgg tacaatcaca 780
15gtatttgcttg attttgcttca aatactccct gctgaaactca aatgtctgtg ttaagctttt 840
zagatggaca aaggaatatta ccgaacacttt aattttcaggg ttggttctctc agagatgttt 900
gttagatctc ctatataattac aaactcttgtg ctttttggag agttttttta tgcatacaca 960
ctctctctgt ctcatgcatat ggcagaaaaa aaatctttta atttggtgttt tcagactcatt 1020
gtctctctca acaaccaattttcttaaccct ttaagtgtcct atgggcttttc tgccactaag 1080
20tttagaagattaa ttttgccttctt caaagtctatc gccagaattt tttagttgcaa gggatgatg 1140
gtaaagacagag taccggccac acacacttgt atttttagtgct atataaaa attattggca 1200
agataattca tgggtctgtg ctggatgtgg gatattaggaa acattgatgc acctctacat 1260
ggtaattatt aattattaaa tagttatctt agacatgcca aaccttggcc ctttggagaga 1320
gcataattca atggcctttc cttctcatgat gcacacacac gccacccccc acgctctctaa 1380
25tgattatttggtc cattaacttaga ttattggtttt tacacactct cttggacctt gcaccaacct 1440
tacagaggttag ttaacttcttg ttttggacttc ttaaatgcac ccgcacaggt ttggtgacca 1500
agatatcaca cttgatcctta taagacaccg ttggatcaatt ttaatttttaa tggactctact 1560
gtactgttgg tgttaacctctc ttttcaccag agatattcaca atttggccttt gcgctctctc 1620
agttttatgct atttacactgac ttttccttggc agracattata caatgacaatg attaacact 1680
30tcactctttct cttttttgggg tttgggttaata taacaccttg gaacaaatgc ttcacatcga 1740
gtgctgttctc tatataacag tgtttactgtg actgtatgttt ttaacctgac catgtaga 1800
caacctcag cagcttgggc catatattctg actggaacaa acttatatttc gacacacgat 1860
ggctgctttta tagagctgtg catgtgacaag acctttattag aagttggtacat tcctatttga 1920
gcgccaggcat gttgcttttt tcaccttggg gcgtatacaag ccaaaactct 1980
35atttgctttct atatctagttgc tttagggtgc atgtaggtca tttgctctct catataacc 2040
attgctatactc tcaacactcct ctaattacag attatacagag aagtaagctc tttttctatat 2100
gctaaactaatcg ctagtgagctt tataattgctg atcctctgag atgtgcttat 2160
ttgcctcttc acatattttg tttttgaccac caactattaatc gtcacactctc aagattttggc 2220
gcgtgacagct atcgccacac agttgaagtt ttggctcaag tcaacaaatgtgacaaa 2280
40caactttgag aatctttttg ttttttttaaat ttttcataacgtt ccctcttaat 2340
ciaacataagag gtctttttatg ttaggaacctt gttttattatg agtgtagacg ccttggtcagc 2400
ggctctcatga agcaatattgg cgataatgtcac ggtcatatatta actgtacag ttcattttgt 2460
gcgcagaagt tcaatgcttg tcaagctctgc tcactgtgta tagatgaggct 2520
gcctacactgt tgaagtggtact gccactgcttg gatggacatt tgggtgtgagc 2580
gctgtctctc aaataaccttt tgcctatgca atggtcatata ggttcaatgg cattggagt 2640
acccaaatg ttctctctga gaacaacaa caaatgcgc aaccatattaa caaggccatt 2700
5agtcacatc aagatcacta tcaacacact tcaactgtcat tggcgaaagct gcacagcttg 2760
gtaaccaga atgtctcaagc attaacaacaca cctgtaaacc aacttagctc taatgtagttgt 2820
gcataattc gaattgcttac tgcgtagctt gctggcactt caaagtcgca ggcggaggtga 2880
caattgaca gggaaccttc caaacgccctc aacactctggt aacacacaacaa 2940
cataacagg tctgtgaaat cagggctctc gctaactcttg ctgtcatctaa aagtttgtcag 3000
10gttgtctcttg gcaacatcaaa aagagttgcaag ttggtggaa aaggtctaca cctgtgtctcc 3060
ttcacacaag cagccccgca tgggtgttgtc ttcttactatg tcacgtagtg gcccattccag 3120
gagagagact tcaacacagc gcagcagcaaat tgtcatgaag gcnaaaccata cttccctcgt 3180
gaaggggttt ttggtgatttc tggcaaccttg gtttttatatt tggcagagaa cttctttttctt 3240
ccaacacat ttcctacaga caatacattt gtttcaagag aatgtgattgt cgttatggtc 3300
15atctttaca accacagttta tctactctgt ccaactgtacg tcagacacat ccaagaaaaag 3360
cggaaagcgt actcctgcaat tcctctttgct tgtgtgtact ggtttggttg cttctgctcagc 3420
attcagcagg cttgcctcgaat cattcacaac gcataatgg gctggctgtcta aatattaatt 3480
aatcttaag ttcacatcac tgcctctcacg caattgaggg aatagttgca taattatatc 3540
tggcctctcga ttctcctcagc ccattga
20
210> 749
212> PRT
213> SARS-CoV
25
400> 6
Ser Asp Leu Asp Arg Cys Thr Thr Phe Asp Asp Val Gln Ala Pro Asn
1 5 10 15
Tyr Thr Gln His Thr Ser Ser Met Arg Gly Val Tyr Tyr Pro Asp Glu
30 20 25 30
Ile Phe Arg Ser Asp Thr Leu Tyr Leu Thr Gln Asp Leu Phe Leu Pro
35 40 45
Phe Tyr Ser Asn Val Thr Gly Phe His Thr Ile Asn His Thr Phe Gly
50 55 60
35Asn Pro Val Ile Pro Phe Lys Asp Gly Ile Tyr Phe Ala Ala Thr Glu
65 70 75 80
Lys Ser Asn Val Val Arg Gly Trp Val Phe Gly Ser Thr Met Asn Asn
85 90 95
Lys Ser Gln Ser Val Ile Ile Ile Asn Asn Ser Thr Asn Val Val Ile
40 100 105 110
Arg Ala Cys Asn Phe Glu Leu Cys Asp Asn Pro Phe Phe Ala Val Ser
115 120 125
Lys Pro Met Gly Thr Glu Thr His Thr Met Ile Phe Asp Asn Ala Phe
130 135 140
Asn Cys Thr Phe Glu Tyr Ile Ser Asp Ala Phe Ser Leu Asp Val Ser
145 150 155 160
Glu Lys Ser Gly Phe Lys His Leu Arg Glu Phe Val Phe Lys Asn
165 170 175
Lys Asp Gly Phe Leu Tyr Val Tyr Lys Gly Tyr Gln Pro Ile Asp Val
180 185 190
Val Arg Asp Leu Pro Ser Gly Phe Asn Thr Leu Lys Pro Ile Phe Lys
190 195 200 205
Leu Pro Leu Gly Ile Asn Ile Thr Asn Phe Arg Ala Ile Leu Thr Ala
210 215 220
Phe Ser Pro Ala Gln Asp Ile Trp Gly Thr Ser Ala Ala Ala Tyr Phe
225 230 235 240
Val Gly Tyr Leu Lys Pro Thr Thr Phe Met Leu Lys Tyr Asp Glu Asn
245 250 255
Gly Thr Ile Thr Asp Ala Val Asp Cys Ser Glu Asn Pro Leu Ala Glu
260 265 270
Leu Lys Cys Ser Val Lys Ser Phe Glu Ile Asp Lys Gly Ile Tyr Gln
275 280 285
Thr Ser Asn Phe Arg Val Val Pro Ser Gly Asp Val Val Arg Phe Pro
290 295 300
Asn Ile Thr Asn Leu Cys Pro Phe Gly Glu Val Phe Asn Ala Thr Lys
305 310 315 320
Phe Pro Ser Val Tyr Ala Trp Glu Arg Lys Ile Ser Asn Cys Val
325 330 335
Ala Asp Tyr Ser Val Leu Tyr Asn Ser Thr Phe Ser Thr Phe Lys
340 345 350
Cys Tyr Gly Val Ser Ala Thr Lys Leu Asn Asp Leu Cys Phe Ser Asn
355 360 365
Pro Val Tyr Ala Asp Ser Phe Val Val Lys Gly Asp Asp Val Arg Glu Ile
370 375 380
Asp Val Gln Thr Gly Val Ile Ala Asp Tyr Asn Tyr Lys Leu Pro
385 390 395 400
Asp Thr Ser Val Thr Leu Asn Tyr Tyry Tyr Leu Arg His
405 410 415
Gly Lys Val Arg Pro Phe Glu Arg Asp Ile Ser Asn Val Pro Phe Ser
420 425 430
Pro Asp Gly Lys Pro Cys Thr Pro Pro Ala Leu Asn Cys Tyr Trp Pro
435 440 445
Pro 450 455 460
Leu Asn Asp Tyr Gly Phe Tyr Thr Thr Thr Gly Ile Gly Tyr Gln Pro
465 470 475 480
tyr Arg Val Val Leu Ser Phe Glu Leu Leu Asn Ala Pro Ala Thr
485 490 495
Val Cys Gly Pro Lys Leu Ser Thr Asp Leu Ile Lys Asn Gln Cys Val
500 505 510
Asn Phe Asn Phe Asn Gly Leu Thr Gly Thr Gly Val Leu Thr Pro Ser
515 520 525
Ser Lys Arg Phe Gln Pro Phe Gln Gln Phe Gly Arg Asp Val Ser Asp
530 535 540
Phe Thr Asp Ser Val Arg Asp Pro Lys Thr Ser Glu Ile Leu Asp Ile
545 550 555 560
Ser Pro Cys Ser Phe Gly Gly Val Ser Val Ile Thr Pro Gly Thr Asn
565 570 575
Ala Ser Ser Glu Val Ala Val Leu Tyr Gln Asp Val Asn Cys Thr Asp
580 585 590
Val Ser Thr Ala Ile His Ala Asp Gln Leu Thr Pro Ala Trp Arg Ile
595 600 605
Tyr Ser Thr Gly Asn Asn Val Phe Gln Thr Gln Ala Gly Cys Leu Ile
610 615 620
Gly Ala Glu His Val Asp Thr Ser Tyr Glu Cys Asp Ile Pro Ile Gly
625 630 635 640
Ala Gly Ile Cys Ala Ser Tyr His Thr Val Ser Leu Leu Arg Ser Thr
645 650 655
Ser Gln Lys Ser Ile Val Ala Tyr Thr Met Ser Leu Gly Ala Asp Ser
660 665 670
Ser Ile Ala Tyr Ser Asn Asn Thr Ile Ala Ile Pro Thr Asn Phe Ser
675 680 685
Ile Ser Ile Thr Thr Glu Val Met Pro Val Ser Met Ala Lys Thr Ser
690 695 700
Val Asp Cys Asn Met Tyr Ile Cys Gly Asp Ser Thr Glu Cys Ala Asn
705 710 715 720
Leu Leu Leu Gly Tyr Gly Ser Phe Cys Thr Gln Leu Asn Arg Ala Leu
725 730 735
Ser Gly Ile Ala Ala Glu Gln Asp Arg Asn Thr Arg Glu
740 745

<210> 7
<211> 439
40<212> PRT
<213> SARS-CoV
Val Phe Ala Gln Val Lys Gln Met Tyr Lys Thr Pro Thr Leu Lys Tyr
1 5 10 15
Phe Gly Gly Phe Asn Phe Ser Gln Ile Leu Pro Asp Pro Leu Lys Pro
5 20 25 30
Thr Lys Arg Ser Phe Ile Glu Asp Leu Leu Phe Asn Lys Val Thr Leu
35 40 45
Ala Asp Ala Gly Phe Met Lys Gln Tyr Gly Glu Cys Leu Gly Asp Ile
50 55 60
Asn Ala Arg Asp Leu Ile Cys Ala Gln Lys Phe Asn Gly Leu Thr Val
65 70 75 80
Leu Pro Pro Leu Leu Thr Asp Asp Met Ile Ala Ala Tyr Thr Ala Ala
85 90 95
Leu Val Ser Gly Thr Ala Thr Ala Gly Trp Thr Phe Gly Ala Gly Ala
100 105 110
Ala Leu Gln Ile Pro Phe Ala Met Gln Met Ala Tyr Arg Phe Asn Gly
115 120 125
Ile Gly Val Thr Gln Asn Val Leu Tyr Glu Asn Gln Lys Gln Ile Ala
130 135 140
Asn Gln Phe Asn Lys Ala Ile Ser Gln Ile Gln Glu Ser Leu Thr Thr
145 150 155 160
Thr Ser Thr Ala Leu Gly Lys Leu Gln Asp Val Val Asn Gln Asn Ala
165 170 175
Gln Ala Leu Asn Thr Leu Val Lys Gln Leu Ser Ser Asn Phe Gly Ala
180 185 190
Ile Ser Ser Val Leu Asn Asp Ile Leu Ser Arg Leu Asp Lys Val Glu
195 200 205
Ala Glu Val Gln Ile Asp Arg Leu Ile Thr Gly Arg Leu Gln Ser Leu
210 215 220
Gln Thr Tyr Val Thr Gln Gln Leu Ile Arg Ala Ala Ala Glu Ile Arg Ala
225 230 235 240
Ser Ala Asn Leu Ala Ala Thr Lys Met Ser Glu Cys Val Leu Gln Gln
245 250 255
Ser Lys Arg Val Asp Phe Cys Gly Lys Gly Tyr His Leu Met Ser Phe
260 265 270
Pro Gln Ala Ala Pro His Gly Val Val Phe Leu His Val Thr Tyr Val
275 280 285
Pro Ser Gln Glu Arg Asn Phe Thr Thr Ala Pro Ala Ile Cys His Glu
290 295 300
Gly Lys Ala Tyr Phe Pro Arg Glu Gly Val Phe Val Phe Asn Gly Thr
305 310 315 320
Ser Trp Phe Ile Thr Gln Arg Asn Phe Phe Ser Pro Gln Ile Ile Thr
325 330 335
Thr Asp Asn Thr Phe Val Ser Gly Asn Cys Asp Val Val Ile Gly Ile
340 345 350
5 Ile Asn Asn Thr Val Tyr Asp Pro Leu Gln Pro Glu Leu Asp Ser Phe
355 360 365
Lys Glu Glu Leu Asp Lys Tyr Phe Lys Asn His Thr Ser Pro Asp Val
370 375 380
Asp Leu Gly Asp Ile Ser Gly Ile Asn Ala Ser Val Val Asn Ile Gln
10385 390 395 400
Lys Glu Ile Asp Arg Leu Asn Glu Val Ala Lys Asn Leu Asn Glu Ser
405 410 415
Leu Ile Asp Leu Gln Glu Leu Gly Lys Tyr Glu Gln Tyr Ile Lys Trp
420 425 430
15 Pro His His His His His
435