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(71) Applicant (for all designated States except US): **THE GOVERNMENT OF THE UNITED STATES OF AMERICA, AS REPRESENTED BY THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES, CENTERS FOR DISEASE CONTROL AND PREVENTION** [US/US]; Technology Transfer Office, 4770 Buford Highway, MS K79, Atlanta, GA 30341 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **ANDERSON, Larry J.** [US/US]; 4770 Buford Highway, Atlanta, GA (US).

HAYNES, Lia M. [US/US]; Centers for Disease Control and Prevention, Technology Transfer Office, 4770 Buford Highway, MS K79, Atlanta, GA 30341 (US). **TRIPP, Ralph A.** [US/US]; 759 N. Superior Ave., Decatur, GA 30033 (US).

(74) Agents: **GOLDSTEIN, Avery N.** et al.; Gifford, Krass, Sprinkle, Anderson & Citkowski, P.C., 2701 Troy Center Drive, Suite 330, Post Office Box 7021, Troy, MI 48007-7021 (US).

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(54) Title: ANTI-RSV IMMUNOGENS AND METHODS OF IMMUNIZATION

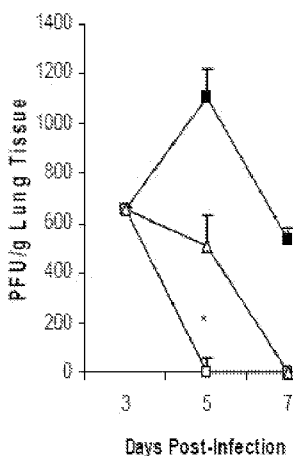


FIG. 2

Solid squares represent control antibody treated mice. Open triangles represent anti-RSV G antibody treated (low-dose) mice. The open squares represent anti-RSV G antibody treated (high-dose) treated mice.

(57) Abstract: Immunogenic polypeptides corresponding to one or more RSV G glycoproteins, or analogues thereof, are provided as components of vaccines. The inventive compositions are useful as both a prophylactic and therapeutic for the prevention and treatment of RSV infections and associated pulmonary or other diseases. The inventive immunogens include regions of the RSV G protein, specifically, amino acid residues 164-176 of RSV G A2 protein or analogues thereof. This inventive immunogen is operable alone or in combination with other polypeptides such as the RSV G protein amino acid residues 155-206, or other vaccines such as live RSV vaccines, or inactivated RSV vaccines or immunogenic analogues thereof.



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ANTI-RSV IMMUNOGENS AND METHODS OF IMMUNIZATION

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 61/231,162 filed August 4, 2009 and U.S. Provisional Application No. 61/333,496 filed May 11, 2010, the entire contents of both are incorporated herein by reference.

FIELD OF THE INVENTION

[0002] The invention relates generally to poly-amino acid immunogens. More specifically, the invention relates to polypeptides related to RSV-G glycoprotein that induces an immune response in a subject. The peptides promote immunity to RSV infection while preventing undesirable immunological responses in a subject.

BACKGROUND OF THE INVENTION

[0003] Respiratory syncytial virus (RSV) is the most common cause of serious lower respiratory illness in infants and young children worldwide. Immunoprotection is incomplete after a subject has been exposed to RSV such that repeat infections occur throughout life often with serious complications in the elderly and immune compromised patients. Unfortunately, while numerous vaccine candidates have been proposed and studied, there is no safe and effective vaccine available and treatment options are limited.

[0004] RSV displays two major surface viral proteins, the G (attachment) and the F (fusion) glycoproteins. The RSV F glycoprotein is much more effective in inducing protective immunity in animal model systems. Consequently, efforts to treat and prevent RSV infection have often focused on anti-RSV F protein antibodies, antiviral drugs that affect the F protein, and live virus or F protein-based vaccines. For example, prophylactic treatment with a palivizumab (a neutralizing, anti-RSV F protein monoclonal antibody) decreases the incidence of serious RSV induced infection in high-risk infants and young children. Palivizumab is not effective in treating active infection and as such is limited in its use. Similarly, other treatments for acute RSV infection also proved ineffective or minimally effective. For example, the antiviral Ribavirin has limited efficacy and is seldom used except for treatment of infection in immunocompromised patients. Without being limited to a single hypothesis, one possible explanation for the ineffectiveness of existing RSV treatments is that the virus-induced host inflammatory response is important to disease pathogenesis and this inflammatory response is

only partially responsive to antiviral therapy once infection is established. Consequently, effective treatment may require both anti-inflammatory and antiviral components.

[0005] The G protein of RSV may play a role in inducing and modulating the host immune response to infection. Intact RSV G protein expression in the infecting virus is associated with a lower frequency of IFN γ expressing cells and a higher frequency of IL-4 expressing cells. RSV G protein has also been associated with increased pulmonary eosinophilia after RSV challenge in formalin-inactivated RSV vaccinated mice, increased pulmonary levels of substance P in RSV challenged mice, and decreased respiratory rates associated with its administration to mice.

[0006] Efforts to make a safe and effective vaccine have failed to date. One problem in developing a vaccine is the fact that a formalin inactivated vaccine led to more serious disease with later RSV infection when administered to young children, leading to concerns that any non-live RSV vaccine may be unsafe in young children. Another problem with prior art vaccines is an inability to induce a highly effective protective immune response. This problem is highlighted by the fact that natural infection provides only limited protection from reinfection and disease.

[0007] As such, there is a need for a safe and effective vaccine for RSV.

SUMMARY OF THE INVENTION

[0008] The present invention provides a single or multi-component vaccine that has one or more isolated immunogens related to the RSV G protein. Inventive vaccines include one or more immunogens corresponding to fragments of RSV G protein including residues 164-176, residues 163-190, and residues 155-206 of SEQ ID NOs 1, 7, 8, or analogues thereof. Optionally, the immunogens are amino acid sequences corresponding to one or more of SEQ ID NOs: 2-6, or analogues thereof. The immunogens produce antibodies or otherwise produce an immune response in an immunized subject. One or more immunogens optionally include a tag to assist in isolation of the immunogen. An immunogen is optionally recombinant.

[0009] In a multi-component vaccine (i.e. at least a first and a second immunogen) the immunogens are optionally associated by a linker. Association is optionally covalent. A linker is optionally an intermediate polypeptide fragment that may be excisable. In some embodiments the immunogens of a multi-component vaccine are the polypeptides of SEQ ID NOs: 3, 4, 5, 6, or analogues thereof.

[0010] Inventive vaccines optionally contain an adjuvant illustratively dimethyl dioctadecylammonium bromide (DDA); monophosphoryl lipid A (MPL); LTK63, lipophilic quaternary

ammonium salt-DDA, DDA-MPL, aluminum salts, aluminum hydroxyide, aluminum phosphate, potassium aluminum phosphate, Montanide ISA-51, ISA-720, microparticles, immunostimulatory complexes, liposomes, virosomes, virus-like particles, CpG oligonucleotides, cholera toxin, heat-labile toxin from *E. coli*, lipoproteins, dendritic cells, IL-12, GM-CSF, nanoparticles illustratively including calcium phosphate nanoparticles, combination of soybean oil, emulsifying agents, and ethanol to form a nanoemulsion; AS04, ZADAXIN, or combinations thereof.

[0011] The inventive vaccines optionally contain an emulsification agent illustratively supramolecular biovectors (SMBV), nanoparticles, liposomes, or combinations thereof.

10 [0012] The invention also provides a process of creating an immune response in a subject. Optionally, the process includes administering an inventive immunogen or polypeptide to a subject. Administration is by any effective method known in the art illustratively subcutaneous, intramuscular, intranasal, oral, intravaginal, intravenous, intramucosal, or combinations thereof.

[0013] More than one vaccine is optionally administered to a subject. A second vaccine is 15 optionally administered prior to, simultaneously with, or subsequent to administration of a first vaccine. It is appreciated that a second vaccine is optionally an amino acid sequence corresponding to amino acid position 155 to amino acid position 206 of SEQ ID NO: 1 or an analogue thereof; or an amino acid sequence corresponding to amino acid position 163 to amino acid position 190 of SEQ ID NOs: 1, 7, 8, or an analogue thereof; or a combination thereof.

20 [0014] Optionally, the present invention is administered to make another RSV vaccine safer by inducing antibodies that protect from an aberrant or enhanced response to otherwise induced by the different type of RSV vaccine.

[0015] Also provided is a pharmaceutical package including a vaccine with one or more immunogens of the subject invention, an emulsification agent and an adjuvant.

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BRIEF DESCRIPTION OF THE DRAWINGS

[0016] FIG. 1 represents the relative levels of IL-4 (A) or IFN γ (B) in mice challenged with RSV that were either treated or not treated with antibodies that bind the G protein similar to those induced by the inventive immunogens where the black bars represent control antibody treated mice, the open bars represent anti-RSV G antibody treated (low-dose or 150 μ g) mice, and the 30 gray bars represent anti-RSV G antibody treated (high-dose or 300 μ g) treated mice;

[0017] FIG. 2 represents virus titer reductions in mice challenged with RSV that were treated or not treated with antibodies that bind the G protein similar to those induced by the inventive

immunogens after RSV infection where solid squares represent control antibody treated mice, open triangles represent anti-RSV G antibody treated (low-dose) mice, and the open squares represent anti-RSV G antibody treated (high-dose) treated mice;

5 [0018] FIG. 3 represents reduction in pulmonary eosinophilia in formalin-inactivated-RSV A2 (FI-A2) vaccinated mice challenged with RSV following prophylactic treatment, or not, with antibodies that bind the G protein similar to those induced by the inventive immunogen after RSV challenge ;

10 [0019] FIG. 4 represents the total cell content of BAL fluid (A), eosinophil levels in BAL (B), IL-4 content in BAL fluid (C) and weight loss in mice challenged with RSV following immunization with FI-A2 and vaccines from RSVGA-CH17 (SEQ ID NO.5);

[0020] FIG. 5 represents the total cell content of BAL fluid (A), eosinophil levels in BAL (B), IL-4 content in BAL fluid (C) and weight loss in mice following RSV challenge after being immunized with FI-A2 and vaccines derived from RSVGA-A2 (SEQ ID NO. 4).

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

15 [0021] The present invention may be understood more readily by reference to the following detailed description of the preferred embodiments of the invention and the Examples included herein.

[0022] Before the present compounds and methods are disclosed and described, it is to be understood that this invention is not limited to specific proteins, specific methods, or specific
20 nucleic acids, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

[0023] It must be noted that, as used in the specification and the appended claims, the
25 singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise.

[0024] The present invention both results in an effective and protective immune response, but also does not suffer the propensity to induce more serious disease similar to the formalin inactivated respiratory syncytial virus (RSV) vaccines of the prior art. As such, the present invention has utility as an immunogen and method of immunization against infection by RSV.

30 [0025] An aspect of this invention provides non-live RSV vaccines which are produced, or non-live RSV vaccines which are improved, by selecting regions of the RSV G glycoprotein so that when the vaccines are administered to a human or animal higher titers of antibodies are

produced that block the biological action of polypeptide portions of the G glycoproteins of subsequently-infecting RSV viruses or promote removal of viruses or infected cells.

[0026] In another aspect of this invention vaccines are provided which when administered to a human or animal, induce an immune response such as the production of antibodies that block or otherwise alter the biological function of the G glycoprotein of subsequently-infecting RSV viruses. Alternatively, the vaccine includes one or more G glycoprotein peptides or polypeptides, or analogues thereof, from different RSV strains having the foregoing ability. RSV strains illustratively include those expressing RSVGA, RSVGA2, RSVGA_CH17 or RSVB G glycoprotein.

10 [0027] Also provided is a method for improving or identifying drugs, antibodies, peptides, polypeptides or other blocking molecules that can be used to treat RSV disease and/or to be used as vaccines to prevent RSV disease.

[0028] The present invention employs a polypeptide that includes a 13 amino acid sequence of the RSV G protein (aa 164 to 176 in the A2 strain of RSV) or an immunogenic analogue thereof, the RSV G protein (RSVGA_CH17 synthetic aa 163-190), the RSVB G protein sequence RSVGB (synthetic aa 155-206) or two polypeptides one each for the RSV group A and B strain specific and conserved regions (illustratively amino acids 163 to 190 for group A and amino acids 155 to 206 for group B strains of RSV) that effectively induce immunoprotective antibodies in a subject. In addition, the 13 aa region is substantially conserved among RSV strains and the combination of the group A and B specific regions are present on all strains such that the inventive vaccines provide broad-based protection.

20 [0029] An immunogen illustratively includes amino acid residues corresponding to amino acids 164 to 176 of SEQ ID NO: 1, or an analogue thereof. Optionally, the immunogen is the amino acid sequence of SEQ ID NO: 2.

25 [0030] In some embodiments an analogue of an immunogen is used as an immunogen. An analog is optionally an immunogen with one or more mutations such as amino acid substitutions, insertions, alterations, modifications, or other amino acid changes that increase, decrease, or not alter the ability of the protein to elicit an immune response in a subject, bind to CX3CR1, or both relative to a wild-type protein. It is appreciated that the CX3C motif is immune modulatory. Altering the amino acid of the motif, or alterations in regions near the motif in a wild type immunogen improves safety while still producing antibodies that block the binding of an immunogen to the receptor CX3CR1. Therefore, it is also appreciated that amino acid insertions, illustratively to produce a CX1C, CX2C, CX4C, CX5C, CX6C to CX10C inclusive, are

performed. Optionally, one or more of the cysteine residues in the chemokine motif, CX3C, is altered such as by deletion, substitution, or other modification to the cysteine residue(s) itself. It is appreciated that any combination of the alterations to a wild-type immunogen sequence is envisioned.

5 [0031] Several post-translational modifications are similarly envisioned as within the scope of the present inventions illustratively including incorporation of a non-naturally occurring amino acid(s), phosphorylation, glycosylation, addition of pendent groups such as biotinylation, fluorophores, lumiphores, radioactive groups, antigens, or other molecules all of which are encompassed by the term analogue. An analogue of an immunogen is optionally a fragment of
10 an immunogen. An analogue of an immunogen is a polypeptide that has some level of activity of inducing an immune response in a subject toward a region of the RSV G glycoprotein. An analogue optionally has between 0.1% and 200% the activity of a wild-type immunogen. An analogue of an immunogen is optionally a wild-type RSV G glycoprotein sequence that is altered in at least property relative to the wild-type protein sequence. Such properties illustratively
15 include immunogenicity, thermal stability, pH/stability profile, stability towards oxidation, solubility, or combinations thereof. Methods of synthesizing an immunogen, modifying an immunogen, testing for immunogenic activity or a property of an immunogen analogue illustratively receptor binding, or expressing an immunogen or analogue thereof are achievable by methods ordinarily practiced in the art illustratively by methods disclosed in Molecular
20 Cloning: A Laboratory Manual, 2nd ed., vol. 1-3, ed. Sambrook et al., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989; Current Protocols in Molecular Biology, ed. Ausubel et al., Greene Publishing and Wiley-Interscience, New York, 1992 (with periodic updates); and Short Protocols in Molecular Biology, ed. Ausubel et al., 52 ed., Wiley-Interscience, New York, 2002, the contents of each of which are incorporated herein by
25 reference.

[0032] An immunogen illustratively includes amino acid residues corresponding to amino acids 163 to 190 of SEQ ID NOs: 1, 8, or analogues thereof. Optionally, the immunogen is the amino acid sequence of SEQ ID NOs: 4 or 5.

[0033] An immunogen illustratively includes amino acid residues corresponding to amino
30 acids 155 to 206 of SEQ ID NO: 7 or an analogue thereof. Optionally, the immunogen is the amino acid sequence of SEQ ID NO: 6.

[0034] The term "polypeptide" "protein" or are used interchangeably herein and are illustratively a chain of two or more amino acid residues. An immunogen is illustratively a

polypeptide that has some level of immunogenic activity, i.e. the ability to induce the production of antibodies in an organism. In some embodiments, a polypeptide is the amino acid sequence for RSV G protein or analogues thereof used alone or combined with other peptide or otherwise immunogenic sequence(s) or therapeutics. Illustrative examples of RSV G protein sequences are found at GenBank accession number P03423 and SEQ ID NO. 1, as well as accession number P20896 and SEQ ID NO: 7 and SEQ ID NO: 8.

[0035] Immunogens are optionally recombinant and obtained by methods known in the art. Illustratively, a nucleotide sequence is cloned into a plasmid which is transfected into *E. coli* and expressed. To ease purification procedures the expressed polypeptides optionally include a tag.

10 Illustrative examples of include poly-histidine, CBP, CYD (covalent yet dissociable NorpD peptide), strep-2, FLAG, HPC or heavy chain of protein C peptide tag, or GST and MBP protein fusion tag systems. It is appreciated that other tag systems are similarly operable. In some embodiments recombinant polypeptides are expressed in *E. coli* and purified using an affinity tag system followed by enzymatic cleavage of the tag such as by incorporating a factor Xa, thrombin, or other enzyme cleavage site in the expressed polypeptide. Methods of tag cleavage are known in the art and any effective method is appreciated to be suitable for use in the instant invention.

[0036] An isolated RSV G protein immunogen or analogue thereof is provided. Optionally, an inventive RSV G protein has the sequence represented by SEQ ID NOs: 1, 8, or analogues thereof illustratively, SEQ ID NOs: 2, 3, or 4, or analogues thereof. Optionally, RSV G protein fragment is the sequence represented by SEQ ID NOs: 5 or 6 or analogues thereof. Optionally, RSV G protein has the sequence represented by SEQ ID NO: 7 or an analogue thereof.

[0037] RSV G protein is optionally recombinant. However, it is also envisioned that naturally occurring RSV G protein may be isolated from at least a portion of the sample material for which the wild type sequence is normally found. Methods for purification of protein from organism derived samples are known and are within the level of skill in the art.

[0038] It is recognized that numerous variants, analogues, or homologues are within the scope of the present invention including amino acid substitutions, alterations, modifications, or other amino acid changes that increase, decrease, or do not alter the function or immunogenic propensity of the inventive immunogen or vaccine. It is further appreciated that the inventive sequences of SEQ ID NOs: 1-8 are optionally modified by the addition of one or more amino acids, sugars, nucleotides, pendent groups, fluorophores, lumiphores, radioactive molecules,

lipids, fatty acids, derivatives thereof, or other groups known in the art. Illustratively, an inventive immunogen is conjugated to a protein.

[0039] An immunogen is optionally conjugated to a protein that promotes the immunogenicity of an immunogen, illustratively keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA), or modifications thereof illustratively BLUE CARRIER immunogenic protein from Thermo Scientific, Rockford, IL. Other sources of natural or artificial immunogenic protein conjugates are known in the art. Optionally, an immunogen is conjugated to an antibody. Optionally, an immunogen is conjugated to other regions of G-protein that may or may not also contain epitopes.

10 [0040] Several post-translational modifications are similarly envisioned as within the scope of the present invention illustratively including incorporation of a non-naturally occurring amino acid(s), phosphorylation, glycosylation, sulfation, and addition of pendent groups such as biotinylation, fluorophores, lumiphores, radioactive groups, antigens, or other molecules. Such modifications are also appreciated as encompassed by the term analogue.

15 [0041] It is appreciated that the inventive polypeptides, immunogens, or vaccines of the present invention are phosphorylated or unphosphorylated. RSV G protein is a naturally phosphorylated and glycosylated protein. Optionally, an immunogen is disulfide bonded. Disulfide bonds can be to amino acid residues within the sequence or to a second polypeptide or molecule.

20 [0042] RSV G protein fragments optionally have a single or multiple phosphorylations therein or other posttranslational modifications depending on particular amino acid substitutions. Methods of expressing and purifying natural or recombinant phosphoproteins or glycoproteins are known in the art. Illustratively, phosphoproteins and glycoproteins are recombinantly expressed in eukaryotic cells. Exemplary eukaryotic cells include yeast, HeLa cells, 293 cells, 25 COS cells, chinese hamster ovary cells (CHO), and many other cell types known in the art. Both eukaryotic and prokaryotic expression systems and cells are available illustratively from Invitrogen Corp., Carlsbad, CA. It is appreciated that cell-free expression systems are similarly operable.

[0043] Optionally, the polypeptide is produced without glycosylation or phosphorylation. 30 In a non-limiting example, polypeptides are synthesized in *E. coli* which is recognized in the art as incapable of properly glycosylating an expressed protein. It is appreciated that other synthetic means that do not glycosylate the polypeptide are similarly suitable. Thus, in this embodiment protein(s) are free from glycosylation.

[0044] Modifications and changes can be made in the structure of the polypeptides that are the subject of the application and still obtain a molecule having similar or improved characteristics as the wild-type sequence (e.g., a conservative amino acid substitution). For example, certain amino acids can be substituted for other amino acids in a sequence without appreciable loss of immunogenic activity. Because it is the interactive capacity and nature of a polypeptide that defines that polypeptide's biological functional activity, certain amino acid sequence substitutions can be made in a polypeptide sequence and nevertheless obtain a polypeptide with like or improved properties. Optionally, a polypeptide is used that has less or more immunogenic activity compared to the wild-type sequence.

[0045] In making such changes, the hydrophobic index of amino acids can be considered. The importance of the hydrophobic amino acid index in conferring interactive biologic function on a polypeptide is generally understood in the art. It is known that certain amino acids can be substituted for other amino acids having a similar hydrophobic index or score and still result in a polypeptide with similar biological activity. Each amino acid has been assigned a hydrophobic index on the basis of its hydrophobicity and charge characteristics. Those indices are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cysteine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

[0046] It is believed that the relative hydrophobic character of the amino acid determines the secondary structure of the resultant polypeptide, which in turn defines the interaction of the polypeptide with other molecules, such as enzymes, substrates, receptors, antibodies, antigens, and the like. It is known in the art that an amino acid can be substituted by another amino acid having a similar hydrophobic index and still obtain a functionally equivalent polypeptide. In such changes, the substitution of amino acids whose hydrophobic indices are within ± 2 is optionally used, those within ± 1 are optionally used, and those within ± 0.5 are optionally used.

[0047] Substitution of like amino acids can also be made on the basis of hydrophilicity, particularly, where the biological functional equivalent polypeptide or peptide thereby created is intended for use in immunological embodiments. The following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0 \pm 1); glutamate (+3.0 \pm 1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); proline (-0.5 \pm 1); threonine (-0.4); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4). It is

understood that an amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent, and in particular, an immunologically equivalent polypeptide. In such changes, the substitution of amino acids whose hydrophilicity values are within ± 2 are optionally used, those within ± 1 are optionally used, and those within ± 0.5 are optionally used.

5 [0048] Amino acid substitutions are generally based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions that take various of the foregoing characteristics into consideration are well known to those of skill in the art and include (original residue: exemplary
10 substitution): (Ala: Gly, Ser), (Arg: Lys), (Asn: Gln, His), (Asp: Glu, Cys, Ser), (Gln: Asn), (Glu: Asp), (Gly: Ala), (His: Asn, Gln), (Ile: Leu, Val), (Leu: Ile, Val), (Lys: Arg), (Met: Leu, Tyr), (Ser: Thr), (Thr: Ser), (Tyr: Trp, Phe), and (Val: Ile, Leu). Embodiments of this disclosure thus contemplate functional or biological equivalents of a polypeptide as set forth above. In particular, embodiments of the polypeptides can include variants having about 50%,
15 60%, 70%, 80%, 90%, and 95% sequence identity to the polypeptide of interest.

[0049] Some embodiments of the present invention are compositions containing RSV G protein nucleic acid that can be expressed as encoded polypeptides or proteins. Optionally, nucleic acid sequences are those that encode polypeptides of SEQ ID NOs: 1-8. As the genetic code is degenerate and a person having ordinary skill in the art understands how to construct,
20 isolate or otherwise identify a nucleic acid sequence that when expressed will produce a particular polypeptide sequence, any nucleic acid sequence that will produce the inventive polypeptides is within the scope of the present invention. The engineering of DNA segment(s) for expression in a prokaryotic or eukaryotic system may be performed by techniques generally known to those of skill in recombinant expression. It is believed that virtually any expression
25 system may be employed in the expression of the claimed nucleic and amino sequences. An exemplary nucleic acid sequence encoding RSV G protein is found within the gene sequence of accession number P03423. A person of ordinary skill in the art recognizes how to identify portions of the P03423 sequence that give rise to polypeptides of SEQ ID NOs: 1-4. Similarly, an exemplary nucleic acid sequence encoding RSV G protein is found within the gene sequence
30 of accession number P20896. A person of ordinary skill in the art recognizes how to identify portions of the P20896 sequence that give rise to polypeptides of SEQ ID NOs: 6-7.

[0050] A nucleic acid as used herein refers to single- or double-stranded molecules which may be DNA, comprised of the nucleotide bases A, T, C and G, or RNA, comprised of the bases

A, U (substitutes for T), C, and G. The nucleic acid may represent a coding strand or its complement. Nucleic acids may be identical in sequence to the sequence which is naturally occurring or may include alternative codons which encode the same amino acid as that which is found in the naturally occurring sequence. Furthermore, nucleic acids may include codons which represent conservative substitutions of amino acids as are well known in the art.

[0051] As used herein, the term "isolated nucleic acid" means a nucleic acid separated or substantially free from at least some of the other components of the naturally occurring organism, for example, the cell structural components commonly found associated with nucleic acids in a cellular environment and/or other nucleic acids. The isolation of nucleic acids can therefore be accomplished by techniques such as cell lysis followed by phenol plus chloroform extraction, followed by ethanol precipitation of the nucleic acids. The nucleic acids of this invention can be isolated from cells according to methods well known in the art for isolating nucleic acids. Alternatively, the nucleic acids of the present invention can be synthesized according to standard protocols well described in the literature for synthesizing nucleic acids.

Modifications to the nucleic acids of the invention are also contemplated, provided that the essential structure and function of the peptide or polypeptide encoded by the nucleic acid are maintained.

[0052] The nucleic acid encoding the peptide or polypeptide of this invention can be part of a recombinant nucleic acid construct comprising any combination of restriction sites and/or functional elements as are well known in the art which facilitate molecular cloning and other recombinant DNA manipulations. Thus, the present invention further provides a recombinant nucleic acid construct comprising a nucleic acid encoding a peptide and/or polypeptide of this invention.

[0053] Generally speaking, it may be more convenient to employ as the recombinant polynucleotide a cDNA version of the polynucleotide. It is believed that the use of a cDNA version will provide advantages in that the size of the gene will generally be much smaller and more readily employed to transfect the targeted cell than will a genomic gene, which will typically be up to an order of magnitude larger than the cDNA gene. However, the inventor does not exclude the possibility of employing a genomic version of a particular gene where desired.

[0054] As used herein, the terms "engineered cells" and "recombinant cells" are synonymous with "host cells" and are intended to refer to a cell into which an exogenous DNA segment or gene, such as a cDNA or gene has been introduced. Therefore, engineered cells are distinguishable from naturally occurring cells which do not contain a recombinantly introduced

exogenous DNA segment or gene. A host cell is optionally a naturally occurring cell that is transformed with an exogenous DNA segment or gene or a cell that is not modified. A host cell optionally does not possess a naturally occurring gene encoding RSV G protein. Engineered cells are thus cells having a gene or genes introduced through the hand of man. Recombinant cells include those having an introduced cDNA or genomic DNA, and also include genes positioned adjacent to a promoter not naturally associated with the particular introduced gene.

5 [0055] To express a recombinant encoded polypeptide in accordance with the present invention one would illustratively prepare an expression vector that comprises a polynucleotide under the control of one or more promoters. To bring a coding sequence "under the control of" a promoter, one positions the 5' end of the translational initiation site of the reading frame generally between about 1 and 50 nucleotides "downstream" of (i.e., 3' of) the chosen promoter. The "upstream" promoter stimulates transcription of the inserted DNA and promotes expression of the encoded recombinant protein. This is the meaning of "recombinant expression" in the context used here.

15 [0056] Many standard techniques are available to construct expression vectors containing the appropriate nucleic acids and transcriptional/translational control sequences in order to achieve protein or peptide expression in a variety of host-expression systems. Cell types available for expression include, but are not limited to, bacteria, such as *E. coli* and *B. subtilis* transformed with recombinant phage DNA, plasmid DNA or cosmid DNA expression vectors.

20 [0057] Certain examples of prokaryotic hosts are *E. coli* strain RR1, *E. coli* LE392, *E. coli* B, *E. coli* .chi. 1776 (ATCC No. 31537) as well as *E. coli* W3110 (F-, lambda-, prototrophic, ATCC No. 273325); bacilli such as *Bacillus subtilis*; and other enterobacteria such as *Salmonella typhimurium*, *Serratia marcescens*, and various *Pseudomonas* species.

25 [0058] In general, plasmid vectors containing replicon and control sequences that are derived from species compatible with the host cell are used in connection with these hosts. The vector ordinarily carries a replication site, as well as marking sequences that are capable of providing phenotypic selection in transformed cells. For example, *E. coli* is often transformed using pBR322, a plasmid derived from an *E. coli* species. Plasmid pBR322 contains genes for ampicillin and tetracycline resistance and thus provides easy means for identifying transformed cells. The pBR322 plasmid, or other microbial plasmid or phage may also contain, or be modified to contain, promoters that can be used by the microbial organism for expression of its own proteins.

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[0059] In addition, phage vectors containing replicon and control sequences that are compatible with the host microorganism can be used as transforming vectors in connection with these hosts. For example, the phage lambda may be utilized in making a recombinant phage vector that can be used to transform host cells, such as *E. coli* LE392.

5 [0060] Further useful vectors include pIN vectors and pGEX vectors, for use in generating glutathione S-transferase (GST) soluble fusion proteins for later purification and separation or cleavage. Other suitable fusion proteins are those with β -galactosidase, ubiquitin, or the like.

[0061] Promoters that are most commonly used in recombinant DNA construction include the β -lactamase (penicillinase), lactose and tryptophan (*trp*) promoter systems. While these are
10 the most commonly used, other microbial promoters have been discovered and utilized, and details concerning their nucleotide sequences have been published, enabling those of skill in the art to ligate them functionally with plasmid vectors.

[0062] For expression in *Saccharomyces*, the plasmid YRp7, for example, is commonly used. This plasmid contains the *trp1* gene, which provides a selection marker for a mutant strain
15 of yeast lacking the ability to grow in tryptophan, for example ATCC No. 44076 or PEP4-1. The presence of the *trp1* lesion as a characteristic of the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan.

[0063] Suitable promoting sequences in yeast vectors include the promoters for 3-phosphoglycerate kinase or other glycolytic enzymes, such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase. In constructing suitable expression plasmids, the
20 termination sequences associated with these genes are also ligated into the expression vector 3' of the sequence desired to be expressed to provide polyadenylation of the mRNA and
25 termination.

[0064] Other suitable promoters, which have the additional advantage of transcription controlled by growth conditions, include the promoter region for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, and the aforementioned glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible
30 for maltose and galactose utilization.

[0065] In addition to microorganisms, cultures of cells derived from multicellular organisms may also be used as hosts. In principle, any such cell culture is operable, whether from vertebrate or invertebrate culture. In addition to mammalian cells, these include insect cell

systems infected with recombinant virus expression vectors (e.g., baculovirus); and plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing one or more coding sequences.

5 [0066] In a useful insect system, *Autographica californica nuclear polyhedrosis* virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The isolated nucleic acid coding sequences are cloned into non-essential regions (for example the polyhedron gene) of the virus and placed under control of an AcNPV promoter (for example, the polyhedron promoter). Successful insertion of the coding sequences results in the
10 inactivation of the polyhedron gene and production of non-occluded recombinant virus (i.e., virus lacking the proteinaceous coat coded for by the polyhedron gene). These recombinant viruses are then used to infect *Spodoptera frugiperda* cells in which the inserted gene is expressed (e.g., U.S. Patent No. 4,215,051).

[0067] Examples of useful mammalian host cell lines are VERO and HeLa cells, Chinese
15 hamster ovary (CHO) cell lines, W138, BHK, COS-7, 293, HepG2, Hep2, NIH3T3, RIN, Calu-3 and MDCK cell lines. In addition, a host cell may be chosen that modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the encoded protein.

20 [0068] Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. Expression vectors for use in mammalian cells ordinarily include an origin of replication (as necessary), a promoter located in front of the gene to be expressed, along with any necessary
25 ribosome binding sites, RNA splice sites, polyadenylation site, and transcriptional terminator sequences. The origin of replication may be provided either by construction of the vector to include an exogenous origin, such as may be derived from SV40 or other viral (e.g., Polyoma, Adeno, VSV, BPV) source, or may be provided by the host cell chromosomal replication mechanism. If the vector is integrated into the host cell chromosome, the latter is often
30 sufficient.

[0069] The promoters may be derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter). Further, it is also possible, and may be desirable, to utilize

promoter or control sequences normally associated with the desired gene sequence, provided such control sequences are compatible with the host cell systems.

[0070] A number of viral based expression systems may be utilized, for example, commonly used promoters are derived from polyoma, Adenovirus 2, Adenovirus 5, cytomegalovirus and Simian Virus 40 (SV40). The early and late promoters of SV40 virus are useful because both are obtained easily from the virus as a fragment which also contains the SV40 viral origin of replication. Smaller or larger SV40 fragments may also be used, provided there is included the approximately 250 bp sequence extending from the *HindIII* site toward the *BglII* site located in the viral origin of replication.

10 [0071] In cases where an adenovirus is used as an expression vector, the coding sequences may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of
15 expressing proteins in infected hosts.

[0072] Specific initiation signals may also be required for efficient translation of the claimed isolated nucleic acid coding sequences. These signals include the ATG initiation codon and adjacent sequences. Exogenous translational control signals, including the ATG initiation codon, may additionally need to be provided. One of ordinary skill in the art would readily be capable of determining this need and providing the necessary signals. It is well known that the initiation codon must be in-frame (or in-phase) with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements or
20 transcription terminators.

[0073] In eukaryotic expression, one will also typically desire to incorporate into the transcriptional unit an appropriate polyadenylation site if one was not contained within the original cloned segment. Typically, the poly A addition site is placed about 30 to 2000 nucleotides "downstream" of the termination site of the protein at a position prior to transcription
25 termination.

[0074] For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines that stably express constructs encoding proteins may be engineered. Rather than using expression vectors that contain viral origins of replication, host
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cells can be transformed with vectors controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched medium, and then are switched to a selective medium. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci, which in turn can be cloned and expanded into cell lines.

5 [0075] A number of selection systems may be used, including, but not limited, to the herpes simplex virus thymidine kinase, hypoxanthine-guanine phosphoribosyltransferase and adenine phosphoribosyltransferase genes, in tk⁻, hgprt⁻ or aprt⁻ cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for dhfr, which confers resistance to methotrexate; gpt, which confers resistance to mycophenolic acid; neo, which confers resistance to the aminoglycoside G-418; and hygromycin. It is appreciated that numerous other selection systems are known in the art that are similarly operable in the present invention.

15 [0076] The nucleic acids encoding the peptides and polypeptides of this invention can also be administered as nucleic acid vaccines. For the purposes of vaccine delivery, a nucleic acid encoding a peptide or polypeptide of this invention can be in an expression vector that can comprise viral nucleic acid including, but not limited to, vaccinia virus, adenovirus, retrovirus and/or adeno-associated virus nucleic acid. The nucleic acid or vector of this invention can also be in a liposome or a delivery vehicle which can be taken up by a cell via receptor-mediated or other type of endocytosis. The nucleic acid vaccines of this invention can be in a pharmaceutically acceptable carrier or administered with an adjuvant. The nucleic acids encoding the peptides and polypeptides of this invention can also be administered to cells *in vivo* or *ex vivo*.

25 [0077] It is contemplated that the isolated nucleic acids or polypeptides of the disclosure may be "overexpressed", i.e., expressed in increased levels relative to its natural expression in cells of its indigenous organism, or even relative to the expression of other proteins in the recombinant host cell. Such overexpression may be assessed by a variety of methods, including radio-labeling and/or protein purification. However, simple and direct methods are preferred, for example, those involving SDS/PAGE and protein staining or immunoblotting, followed by quantitative analyses, such as densitometric scanning of the resultant gel or blot. A specific increase in the level of the recombinant protein or peptide in comparison to the level in natural in

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transfected cells is indicative of overexpression, as is a relative abundance of the specific protein in relation to the other proteins produced by the host cell and, e.g., visible on a gel.

[0078] Further aspects of the present disclosure concern the isolation such as the purification, and in particular embodiments, the substantial purification, of an encoded isolated polypeptide or immunogen. The terms peptide or protein as used herein are synonymous with polypeptide or immunogen. The term "purified" or "isolated" polypeptide, peptide or protein as used herein, is intended to refer to a composition, isolatable from other components, wherein the protein or peptide is purified to any degree relative to its naturally-obtainable state, or its state as expressed in a cell, i.e., in this case, relative to its purity within a cell. An isolated protein or peptide, therefore, also refers to a protein or peptide, free from the environment in which it may naturally occur.

[0079] Generally, "purified" or "isolated" will refer to a protein or peptide composition that has been subjected to fractionation to remove various other components, and which composition substantially retains its expressed biological activity. Where the term "substantially" purified is used, this designation will refer to a composition in which the protein or peptide forms the major component of the composition, such as constituting about 50% or more of the proteins in the composition.

[0080] Various methods for quantifying the degree of isolation of the protein or peptide will be known to those of skill in the art in light of the present disclosure and based on common knowledge in the art. These include, for example, determining the specific activity of an active fraction, or assessing the number of polypeptides within a fraction by SDS/PAGE analysis. A optional method for assessing the purity of a fraction is to calculate the specific activity of the fraction, to compare it to the specific activity of the initial extract, and to thus calculate the degree of purity, herein assessed by a "-fold purification number". The actual units used to represent the amount of purity will, of course, be dependent upon the particular assay technique chosen to follow the purification and whether or not the expressed protein or peptide exhibits a detectable activity or is otherwise recognizable such as via an ELISA assay.

[0081] Various techniques suitable for use in protein isolation will be well known to those of skill in the art. These include, for example, precipitation with ammonium sulphate, polyethylene glycol, antibodies and the like or by heat denaturation, followed by centrifugation; chromatography steps such as ion exchange, gel filtration, reverse phase, hydroxyapatite and affinity chromatography; isoelectric focusing; gel electrophoresis; and combinations of such and other techniques. As is generally known in the art, it is believed that the order of conducting the

various purification steps may be changed, or that certain steps may be omitted, and still result in a suitable method for the preparation of a substantially isolated protein or peptide.

[0082] There is no general requirement that the protein or peptide always be provided in their most isolated state. Indeed, it is contemplated that less substantially isolated products will have utility in certain embodiments. Partial isolation may be accomplished by using fewer purification steps in combination, or by utilizing different forms of the same general purification scheme. For example, it is appreciated that a cation-exchange column chromatography performed utilizing an HPLC apparatus will generally result in a greater-fold purification than the same technique utilizing a low pressure chromatography system. Methods exhibiting a lower degree of relative isolation may have advantages in total recovery of protein product, or in maintaining the activity of an expressed protein.

[0083] It is known that the migration of a polypeptide can vary, sometimes significantly, with different conditions of SDS/PAGE (Capaldi et al., *Biochem. Biophys. Res. Comm.*, 76:425, 1977). It will therefore be appreciated that under differing electrophoresis conditions, the apparent molecular weights of purified or partially purified expression products may vary.

[0084] The inventive method also illustratively includes isolation of inventive polypeptide from a host cell or host cell medium. Methods of protein isolation illustratively include column chromatography, affinity chromatography, gel electrophoresis, filtration, or other methods known in the art. An immunogen is optionally expressed with a tag operable for affinity purification. A tag is optionally a 6x His tag. A 6x His tagged inventive immunogen is illustratively isolated by Ni-NTA column chromatography or using an anti-6x His tag antibody fused to a solid support. (Geneway Biotech, San Diego, CA) Other tags and purification systems are similarly operable.

[0085] It is appreciated that an immunogen is optionally not tagged. In some embodiments isolation is optionally achieved by methods known in the art illustratively including ion-exchange chromatography, affinity chromatography, precipitation with salt such as ammonium sulfate, streptomycin sulfate, or protamine sulfate, reverse phase chromatography, size exclusion chromatography such as gel exclusion chromatography, HPLC, immobilized metal chelate chromatography, or other methods known in the art. One of skill in the art may select the most appropriate isolation and purification techniques without departing from the scope of this invention.

[0086] An immunogen or analogue thereof is optionally chemically synthesized. Methods of chemical synthesis have produced proteins greater than 600 amino acids in length with or

without the inclusion of modifications such as glycosylation and phosphorylation. Methods of chemical protein and peptide synthesis illustratively include solid phase protein chemical synthesis and solution phase peptide synthesis or by the method of Hackeng, TM, et al., *Proc Natl Acad Sci U S A*, 1997; 94(15):7845-50, the contents of which are incorporated herein by reference. Illustrative methods of chemical protein synthesis are reviewed by Miranda, LP, *Peptide Science*, 2000, 55:217-26 and Kochendoerfer GG, *Curr Opin Drug Discov Devel.* 2001; 4(2):205-14, the contents of which are incorporated herein by reference.

[0087] Immunogens are optionally characterized by measurements including, without limitation, western blot, macromolecular mass determinations by biophysical determinations, SDS-PAGE/staining, HPLC, ELISA, mass spectrometry, antibody recognition assays, cell viability assays, apoptosis assays, and assays to infer immune protection or immune pathology by adoptive transfer of cells, proteins or antibodies.

[0088] An immunogen is optionally modified to increase its immunogenicity. In a non-limiting example, the immunogen is coupled to chemical compounds or immunogenic carriers, provided that the coupling does not interfere with the desired biological activity of either the antigen or the carrier. Coupling is optionally by covalent association. For a review of some general considerations in coupling strategies, see *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory, ed. E. Harlow and D. Lane (1988), the contents of which are incorporated herein by reference. Useful immunogenic carriers known in the art, include, without limitation, keyhole limpet hemocyanin (KLH); bovine serum albumin (BSA), ovalbumin, PPD (purified protein derivative of tuberculin); red blood cells; tetanus toxoid; cholera toxoid; agarose beads; activated carbon; or bentonite. Useful chemical compounds for coupling include, without limitation, dinitrophenol groups and arsonilic acid.

[0089] An immunogen may also be modified by other techniques, illustratively including denaturation with heat and/or SDS.

[0090] An immunogen of the present invention may also be used in the form of a pharmaceutically acceptable salt. Suitable acids and bases which are capable of forming salts with the immunogens of the present invention are well known to those of skill in the art, and include inorganic and organic acids and bases.

[0091] In another aspect, the invention provides a multi-component vaccine. Optionally, a multi-component vaccine contains more than one immunogen. An inventive vaccine optionally contains 2, 3, 4, 5, 6, 7, 8, 9, 10, or more immunogens in a single vaccine. Optionally, a first immunogen is a polypeptide corresponding to amino acid position 164 to amino acid position

176 of SEQ ID NO: 1 (RSVA2 strain), or an analogue thereof. Optionally, a first immunogen is a polypeptide corresponding to amino acid position 163 to amino acid position 190 of SEQ ID NOs: 1, 7 (RSV B_18537), 8 (RSVGA_CH17), or analogues thereof. It is appreciated that any of the aforementioned modifications, mutations, or alterations stated herein or otherwise known in the art are operable as to the inventive immunogens of the present invention. Optionally, a first immunogen is a polypeptide of SEQ ID NO: 2 (RSVGA_A2).

[0092] An optional second immunogen is a polypeptide corresponding to amino acid position 155 to amino acid position 206 of SEQ ID NO: 1 or an analogue thereof or to amino acid position 163 to amino acid position 190 of SEQ ID NOs: 1, 7, 8, or an analogue thereof.

10 Optionally, the first and the second immunogens differ by at least one atom. Optionally, the second immunogen is a longer or shorter polypeptide than the first immunogen. Optionally, the first and second immunogens confer unique but possibly overlapping immunogenic characteristics.

[0093] Optionally, the second immunogen is a polypeptide of SEQ ID NOs: 3 (RSVGA_A2), 4 (RSVGA_A2), 5 (RSVGA_CH17), or 6 (RSVGB). Alternatively, the second immunogen is an analogue of SEQ ID NOs: 3, 4, 5, or 6.

[0094] An inventive vaccine is optionally a multicomponent vaccine of two immunogens such as the immunogen of amino acid position 163 to amino acid position 190 of SEQ ID NOs: 1 or 7, or an analogue thereof and a second immunogen corresponding to amino acid position 155 to amino acid position 206 of SEQ ID NO: 1 or an analogue thereof.

[0095] It is further appreciated that the first, second, or first and second immunogens, or other polypeptides of the present invention are optionally within a vaccine that contains other immunogens. Illustratively, other immunogens include the immunogens of SEQ ID NOs. 2, 3, 4, 5, 6, or the RSV F protein or an analogue thereof. The entire RSV F protein sequence need not be present. A fragment of the RSV F protein operable to induce an immune response is operable as a component of a multi-component vaccine. It is appreciated that the immunogen related to the F protein sequence may be an analogue thereof. It is appreciated that in some embodiments a vaccine is free of RSV F protein or an analogue thereof, e.g. RSV F protein or an analogue thereof is optionally absent from a vaccine.

30 [0096] The first and second immunogens are optionally separately expressed, isolated and formulated as separate polypeptides and subsequently combined to form as single vaccine for administration by a single dose or other dosing schedule operable to induce a protective immune response in a subject. Alternatively, the first and second immunogen are co-expressed in the

same host cell. Co-expression is optionally by expression of individual polypeptide sequences on the same or different expression vectors. The polypeptides are optionally co-expressed by simultaneous expression in a host cell, or are differentially expressed by exposing the host cell to different conditions to express one polypeptide while maintaining the coding sequence for the other in a non-expressive state. The resulting expressed polypeptides are simultaneously or differentially purified or otherwise made suitable for administration.

5 [0097] Optionally, the first and second immunogens are present in the same expression system. The first and second immunogens are optionally associated by a linker. A linker is optionally an intermediate polypeptide fragment. Optionally, the nucleic acid sequence encoding the first and second immunogens are linked by a ribosome binding site. As such each gene may be present on a single plasmid or other expression system and be simultaneously or differentially expressed.

10 [0098] Where the first and second immunogens are linked by a linker that is a polypeptide sequence or partial polypeptide sequence, the intermediate sequence is optionally excisable. Intermediate or terminal protein or peptide excision is performed by methods known in the art. Illustratively, excision is performed by a protease cleavage. A protease is optionally, factor Xa, thrombin, or other substantially specific protease known in the art. A protease optionally recognizes a single or two sites within a linker.

15 [0099] Optionally, an inventive vaccine contains an adjuvant. Suitable adjuvants illustratively include dimethyl dioctadecyl-ammonium bromide (DDA); monophosphoryl lipid A (MPL); LTK63, lipophilic quaternary ammonium salt-DDA, DDA-MPL, aluminum salts, aluminum hydroxide, aluminum phosphate, potassium aluminum phosphate, Montanide ISA-51, ISA-720, microparticles, immunostimulatory complexes, liposomes, virosomes, virus-like particles, CpG oligonucleotides, cholera toxin, heat-labile toxin from *E. coli*, lipoproteins, dendritic cells, IL-12, GM-CSF, nanoparticles illustratively including calcium phosphate nanoparticles, combination of soybean oil, emulsifying agents, and ethanol to form a nanoemulsion; AS04, ZADAXIN, or combinations thereof.

20 [00100] An immunogen is optionally delivered as naked polypeptide, naked nucleotide, in aqueous solution, in an emulsion, or in other suitable delivery compositions. In some embodiments an immunogen is delivered as a vaccine or as a vaccine component of a pharmaceutical package. Optionally, a vaccine (or multiple vaccines) is present in an emulsion comprised of suitable emulsification agents. Optionally, a multicomponent vaccine is emulsified. Optionally, a single subunit vaccine is emulsified. Suitable emulsification agents

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illustratively include supramolecular biovectors (SMBV), nanoparticles such as described by Major, M, et al, *Biochim. Biophys. Acta*, 1997; 1327:32-40, De Migel, I, et al, *Pharm. Res.*, 2000; 17:817-824, US Patent Nos 6,017,513, 7,097,849, 7,041,705, 6,979,456, 6,846,917, 6,663,861, 6,544,646, 6,541,030, 6,368,602, Castignolles, N., et al, *Vaccine*, 1996; 14:1353-1360, Prieur, E., et al, *Vaccine*, 1996; 14:511-520, Baudner B, et al, *Infect Immun*, 2002; 70:4785-4790; Liposomes such as described by El Guink et al., *Vaccine*, 1989; 7:147-151, and in U.S. Pat. No. 4,196,191; or other agents known in the art. Agents suitable for use are generally available from Sigma-Aldrich, St. Louis, MO. The emulsification agent is optionally a dimethyl dioctadecyl -ammonium bromide. Optionally, the adjuvant is monophosphoryl lipid A.

5 [00101] The proteins and nucleic acid sequences of the invention, alone or in combination with other antigens, antibodies or nucleic acid sequences may further be used in therapeutic compositions and in methods for treating humans and/or animals with RSV infection. For example, one such therapeutic composition may be formulated to contain a carrier or diluent and one or more RSV immunogens of the invention. The therapeutic composition illustratively
15 contains an inventive RSV protein, nucleic acid sequence, or analogue thereof as described herein and a suitable pharmaceutical carrier. Optionally, a therapeutic composition contains an RSV G protein fragment of SEQ ID NOs: 1-7. Optionally, a therapeutic composition is a multi-component vaccine. A multicomponent vaccine optionally contains an immunogen with the polypeptide sequence of SEQ ID NOs: 2, 4, 5, or 6 and a second immunogen of polypeptide
20 sequence SEQ ID NO: 3.

[00102] Suitable pharmaceutically acceptable carriers facilitate administration of the proteins but are physiologically inert and/or non-harmful. Carriers may be selected by one of skill in the art. Exemplary carriers include sterile water or saline, lactose, sucrose, calcium phosphate, gelatin, dextran, agar, pectin, peanut oil, olive oil, sesame oil, and water. Additionally, the carrier
25 or diluent may include a time delay material, such as glycerol monostearate or glycerol distearate alone or with a wax. In addition, slow release polymer formulations can be used.

[00103] Optionally, the inventive composition contains conventional pharmaceutical ingredients, such as preservatives, or chemical stabilizers. Suitable ingredients operable herein include, for example, casamino acids, sucrose, gelatin, phenol red, N-Z amine, monopotassium
30 diphosphate, lactose, lactalbumin hydrolysate, and dried milk.

[00104] Alternatively, or in addition to the RSV immunogens of the present invention, other agents useful in treating RSV infection, e.g., antivirals or immunostimulatory agents are expected to be useful in reducing or eliminating disease symptoms. Agents operable herein

optionally act to assist the natural immunity of the infected human or animal. Thus, such agents may operate in concert with the therapeutic compositions of this invention. The development of therapeutic compositions containing these agents is within the skill of one in the art and in view of the teachings of this invention.

5 [00105] Immunological compositions and other pharmaceutical compositions containing the immunogen(s) described herein are included within the scope of the present invention. One or more of these compositions can be formulated and packaged, alone or in combination, using methods and materials known to those skilled in the art for vaccines. The immunological response may be therapeutic or prophylactic and may provide antibody immunity or cellular
10 immunity such as that produced by T lymphocytes such as cytotoxic T lymphocytes or CD4⁺T lymphocytes.

[00106] To enhance immunogenicity, the inventive vaccines or immunogens can be conjugated to a carrier molecule. Suitable immunogenic carriers include proteins, polypeptides or peptides such as albumin, hemocyanin, thyroglobulin and derivatives thereof, particularly
15 bovine serum albumin (BSA) and keyhole limpet hemocyanin (KLH), polysaccharides, carbohydrates, polymers, and solid phases. Other protein derived or non-protein derived substances are known to those skilled in the art. An immunogenic carrier typically has a molecular weight of at least 1,000 Daltons, and optionally of greater than 10,000 Daltons. Carrier molecules often contain a reactive group to facilitate covalent conjugation to a hapten.
20 The carboxylic acid group or amine group of amino acids or the sugar groups of glycoproteins are often used in this manner. Carriers lacking such groups can often be reacted with appropriate chemicals to produce them. Optionally, an immune response is produced when the immunogen is injected into animals such as humans, primates, mice, rabbits, rats, goats, sheep, guinea pigs, chickens, and other animals, optionally mice and humans. Alternatively, a multiple antigenic
25 peptide comprising multiple copies of an immunogen, or an antigenically or immunologically equivalent immunogen may be sufficiently antigenic to improve immunogenicity without the use of a carrier.

[00107] The inventive vaccines and immunogens are optionally administered with an adjuvant. Optionally an adjuvant is alum (aluminum phosphate or aluminum hydroxide).
30 Chemically defined preparations such as muramyl dipeptide, monophosphoryl lipid A, phospholipid conjugates, encapsulation of the conjugate within a proteoliposome, and encapsulation of the protein in lipid vesicles are also operable with the present invention.

[00108] Methods for treating individuals diagnosed with RSV disease and conditions associated with RSV infection, such as bronchiolitis, upper respiratory infection, and lower respiratory infection by administering the compositions described herein are also provided in this invention.

5 [00109] Also provided are methods creating an immune response in a subject or methods of treating or preventing an RSV infection in a subject comprising administering to the subject an effective amount of vaccine, immunogen, molecule, antibody or peptide-encoding nucleic acid of the invention. Optionally, a vaccine includes the immunogen of SEQ ID NOs: 2, 3, 4, 5, or 6, or a combination of multiple polypeptides of SEQ ID NOs: 2, 3, 4, 5, or 6.

10 [00110] As used herein the term "immune response" means the increase or decrease in a physiological response to an antigen in a subject. Illustrative immune responses include, but are not limited to reduction in leukocyte trafficking, activation of T-cells, activation of cytotoxic T lymphocytes, modulating populations of CD4⁺ cells, CD11b⁺ cells, NK cells, DX5⁺ cells, B220⁺ cells, or RB6-8C5⁺ PMN cells, altering virus clearance rate, altering leukocyte trafficking,
15 altering interferon gamma (IFN- γ) production, decreasing virus replication rate, or other immune responses recognized in the art. An immune response is a protective immune response if any immune response prevents infection by RSV, provides RSV clearance, prevents RSV entry into a cell, or other prevention recognized in the art.

[00111] The inventive method also provides administering a second vaccine. A second
20 vaccine optionally includes a polypeptide corresponding to amino acid position 155 to amino acid position 206 of SEQ ID NO: 1 or an analogue thereof. Optionally, a second vaccine includes an immunogen with the amino acid sequence of SEQ ID NOs: 2-6. Optionally, a second vaccine is a multi-component vaccine that has two or more immunogens. Optionally, a second vaccine includes the immunogens of any of SEQ ID NOs: 3-6. Optionally, a first or
25 second vaccine is RSV. Optionally, a first or second vaccine is formalin inactivated RSV A2 or other inactivated strain. Optionally a first or second vaccine or immunogen is not a virus

[00112] The second vaccine is optionally administered simultaneous with a first vaccine. Optionally, the second vaccine is administered prior to or subsequent to the first vaccine.

[00113] Prior vaccines for RSV suffered numerous negative side effects including an
30 increase in the severity of RSV associated disease subsequent to administration of a formalin inactivated vaccine in young children. Associated with enhanced disease after prior attenuated RSV vaccine administration is the increase in eosinophils in pulmonary tissue. Haynes, L., et al., *J Virol*, 2003; 77:9831-9844. Antibodies directed toward epitope regions in RSV G-protein or

developed following vaccination with the inventive vaccine(s) decrease pulmonary eosinophilia after RSV infection in subjects suggesting improved safety and efficacy when combined with traditional formalin attenuated RSV vaccine. (e.g. FIG. 4 or 5) Thus, in some embodiments of the present invention is prior, simultaneous, or subsequent administration of the inventive vaccine(s) with other RSV vaccines.

[00114] The invention is optionally delivered with, prior to, or subsequent to an anti-viral composition. Anti-viral compositions illustratively include small drug-like molecule inhibitors of RSV replication and infection, nucleoside analogs such as ribavarin, EICAR, Pyrazofurin, 3-deazaguanine, GR92938X and LY253963. These inhibitors are targeted to inhibit inosine monophosphate dehydrogenase (IMPDH). Inhibitors targeted to inhibit virus adsorption and entry are also useful herein. Illustratively among this class are polyoxometalates and CL387626 (Wyeth-Ayerst, Pearl River, NY). Other examples of polyoxometalates are T118, Trimeris' benzathrone, BABIM and RD30028. Antisense oligonucleotide inhibitors of RSV are also useful, such as V590, an inhibitor that targets residues in RSV NS1/NS2 genes.

[00115] Immunogens are optionally incorporated into a pharmaceutical carrier such as saline, dextrose, water, glycerol, ethanol, other therapeutic compounds, and combinations thereof. The formulation should be appropriate for the mode of administration and may include other immune modifiers such as heparin. The composition may also contain other additional biologically inert ingredients such as flavorants, fillers, etc.

[00116] Suitable methods of administration include, but are not limited to intramuscular, intravenous, intranasal, mucosal, oral, parenteral, intravaginal, transdermal, via aerosol delivery or by any route that produces the desired biological effect or immune response.

[00117] A vaccine of the invention is optionally packaged in a single dosage for immunization by parenteral (i.e., intramuscular, intradermal or subcutaneous) administration or nasopharyngeal (i.e., intranasal) administration. The vaccine is optionally delivered by inhalation. The vaccine is optionally combined with a pharmaceutically acceptable carrier to facilitate administration. The carrier is usually water or a buffered saline, with or without a preservative. The vaccine may be lyophilized for resuspension at the time of administration or in solution.

[00118] Optional microencapsulation of the inventive vaccine will also provide a controlled release. A number of factors contribute to the selection of a particular polymer for microencapsulation. The reproducibility of polymer synthesis and the microencapsulation process, the cost of the microencapsulation materials and process, the toxicological profile, the

requirements for variable release kinetics and the physicochemical compatibility of the polymer and the antigens are all factors that may be considered. Examples of useful polymers illustratively include polycarbonates, polyesters, polyurethanes, polyorthoesters polyamides, poly (d,l-lactide-co-glycolide) (PLGA) and other biodegradable polymers.

5 [00119] The inventive vaccine may additionally contain stabilizers such as thimerosal (ethyl(2-mercaptobenzoate-S)mercury sodium salt) (Sigma Chemical Company, St. Louis, MO) or physiologically acceptable preservatives.

[00120] According to the method of the invention, a human or an animal may be treated for for RSV, other viral infection or bacterial infection by administering an effective amount of an
10 inventive vaccine. Optionally, a vaccine is administered prophylactically. An "effective amount" is an amount that will induce an immune response in a subject. Illustratively, an effective amount of the compositions of this invention ranges from nanogram/kg to milligram/kg amounts for young children and adults. Equivalent dosages for lighter or heavier body weights can readily be determined. The dose should be adjusted to suit the individual to whom the
15 composition is administered and will vary with age, weight and metabolism of the individual. The exact amount of the composition required will vary from subject to subject, depending on the species, age, weight and general condition of the subject, the particular peptide or polypeptide used, its mode of administration and the like. An appropriate amount can be determined by one of ordinary skill in the art using only routine experimentation given the
20 teachings herein. One skilled in the art will realize that dosages are best optimized by the practicing physician or veterinarian and methods for determining dose amounts and regimens and preparing dosage forms are described, for example, in *Remington's Pharmaceutical Sciences*, (Martin, E. W., ed., latest edition), Mack Publishing Co., Easton, PA. Optionally, a single administration is operable to induce an immune response.

25 [00121] Methods involving conventional biological techniques are described herein. Such techniques are generally known in the art and are described in detail in methodology treatises such as *Molecular Cloning: A Laboratory Manual*, 3rd ed., vol. 1-3, ed. Sambrook et al., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 2001; *Current Protocols in Molecular Biology*, ed. Ausubel et al., Greene Publishing and Wiley-Interscience, New York,
30 1992 (with periodic updates); and *Short Protocols in Molecular Biology*, ed. Ausubel et al., 52 ed., Wiley-Interscience, New York, 2002. Immunological methods (e.g., preparation of antigen-specific antibodies, immunoprecipitation, and immunoblotting) are described, e.g., in *Current*

Protocols in Immunology, ed. Coligan et al., John Wiley & Sons, New York, 1991; and Methods of Immunological Analysis, ed. Masseyeff et al., John Wiley & Sons, New York, 1992.

[00122] Additional protocols such as PCR Protocols can be found in A Guide to Methods and Applications Academic Press, NY. Methods for protein purification include such methods as ammonium sulfate precipitation, column chromatography, electrophoresis, centrifugation, crystallization, and others. See, e.g., Ausubel, et al. (1987 and periodic supplements); Deutscher (1990) "Guide to Protein Purification," Methods in Enzymology vol. 182, and other volumes in this series; Current Protocols in Protein Science, John Wiley and Sons, New York, NY; and manufacturer's literature on use of protein purification products known to those of skill in the art.

5 [00123] Assays for neural cell biological activities are described, e.g., in Wouterlood (ed. 1995) Neuroscience Protocols modules 10, Elsevier; Methods in Neurosciences Academic Press; and Neuromethods Humana Press, Totowa, NJ.

[00124] FACS analyses are illustratively described in Melamed, et al. (1990) Flow Cytometry and Sorting Wiley-Liss, Inc., New York, NY; Shapiro (1988) Practical Flow Cytometry Liss, New York, NY; and Robinson, et al. (1993) Handbook of Flow Cytometry Methods Wiley-Liss, New York, NY.

[00125] Various aspects of the present invention are illustrated by the following non-limiting examples. The examples are for illustrative purposes and are not a limitation on any practice of the present invention. It will be understood that variations and modifications can be made without departing from the spirit and scope of the invention. While the examples are generally directed to mammalian tissue, specifically, analyses of mouse tissue, a person having ordinary skill in the art recognizes that similar techniques and other techniques known in the art readily translate the examples to other mammals such as humans. Reagents illustrated herein are commonly cross reactive between mammalian species or alternative reagents with similar properties are commercially available, and a person of ordinary skill in the art readily understands where such reagents may be obtained. Variations within the concepts of the invention are apparent to those skilled in the art. Additional reagents, and preparation of proteins such as immunogens, cells, etc, are illustratively found in WO 97/27299 and U.S. Publication No. 2006-0018925, the contents of each of which are incorporated herein by reference.

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25
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Example 1: Preparation of immunogens

[00126] RSV G protein immunogens are prepared as pharmaceutical compositions with a pharmaceutically acceptable carrier, excipient or diluent. For example, the RSV G protein

sequences described herein and encoded by nucleic acid contained in modified pET-32-LIC plasmids are expressed as thioredoxin (Trx)-fusion proteins in transformed *E. coli* BL21/DE3 cells following induction with IPTG. All Trx-fusion proteins are recovered from transformed cell pellets by extraction with 8M urea, followed by affinity purification using TALON® (Clontech, Palo Alto, Calif.) and dialysis against PBS. Purified Trx-RSV G polypeptides are freed from contaminating endotoxin by treatment with polymyxin B beads (BioRad, Hercules, CA). Details and modifications of this procedure are well known to those of ordinary skill in the art. Upon use for immunization, immunogens are further combined or admixed with an adjuvant, such as alum or a proteosome-based adjuvant.

10 [00127] Alternatively, immunogens are synthesized using a simultaneous, multiple solid-phase peptide synthesis method on a peptide synthesizer (Perkin-Elmer Applied Biosystems, Berkeley, CA), and tested for homogeneity by reverse-phase liquid chromatography and capillary electrophoresis.

[00128] Immunogens are assayed for their ability to bind CX3C receptor types by determination of cell adherence. A pcDNA 3.1 plasmid containing the CX3CR1 gene and a neomycin resistance gene is stably transfected into human embryonic kidney (HEK) cells. Neomycin resistance is used for selection and maintenance of plasmid transfection of human embryonic kidney cells. To confirm transfection, lysates of CX3CR1-transfected HEK cells are stained with anti-CX3CR1 antibody in western blot analysis or intact transfected cells are examined by flow cytometry. Purified ¹²⁵I-G glycoprotein is examined for binding to 293-CX3CR1 cells essentially as described in Combadiere, C., et al., *J. Biol. Chem.*, 1998; 273:23799-23804, the contents of which are incorporated herein by reference. Purified ¹²⁵I-G glycoprotein in binding buffer (Hanks' buffered saline solution with 1 mg/ml bovine serum albumin and 0.01% azide, pH 7.4, final volume 200 μl) is incubated with 10⁶ CX3CR1-transfected HEK cells in the absence of or presence of varying concentrations of unlabeled immunogen of either a wild-type sequence or analogue sequence. After 2 hours at room temperature the cells are washed with one ml of binding medium. The presence of wild-type G-glycoprotein immunogen sequence effectively competes with ¹²⁵I-G glycoprotein binding to cells as revealed by reduced gamma emissions from the cell pellet following washing. The presence of analogue sequences shows altered competition indicating alteration in CX3C receptor binding by the analogue relative to the wild-type sequence.

Example 2: Preparation of RSV

[00129] The A2 strain of RSV is propagated in Vero cells (ATCC CCL 881) essentially as described in Tripp, RA, et al, *J. Virol.* 1999; 73:7099-107. Briefly, Vero cells are maintained in DMEM (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 2% heat-inactivated (56°C) fetal bovine serum (FBS; HyClone Laboratories, Salt Lake City, Utah), 1% L-glutamine, and 1% antibiotic-antimycotic (all from GIBCO) (TCM). Upon detectable cytopathic effect, the TCM is decanted and replaced with a minimal volume of Dulbecco's modified phosphate-buffered saline (D-PBS) and frozen at -70°C. The flask is thawed, and the loosely adherent cell monolayer is scraped off with a cell scraper (Costar, Cambridge, Mass.) and collected. The cells and supernatant are frozen at -70°C, thawed, and then centrifuged at 2,000 × g for 15 min at 4°C. The virus titer is determined by methylcellulose plaque assay on Vero cells.

Example 3: Subject Anti-RSV G Monoclonal Antibody Treatment

[00130] Eight- to ten-week old, specific-pathogen-free, female BALB/c mice (The Jackson Laboratories) are used as subjects for studies. Mice are housed in micro-isolator cages and fed sterilized water and food *ad libitum*. All studies are performed in accordance with the guidelines of the Institutional Animal Care and Use Committee.

[00131] Mice are anesthetized by intraperitoneal (i.p.) administration of Avertin (2% 2,2,2-tribromoethanol, 2% tert-amyl-alcohol, 180-250 mg/kg), and intranasal (i.n.) challenged with 10⁶ PFU of RSV in serum-free DMEM (50 µl volume). Three days post-infection (p.i.) mice are given by i.p. administration 150 µg (low dose) - 300 µg (high dose) per mouse of anti-G glycoprotein (131-2G, endotoxin <5EU/mg) monoclonal antibody or control normal mouse IgG (Pierce). No fewer than three mice per treatment are examined per time point.

Example 4: Anti-RSV G mAb treatment reduces pulmonary leukocyte infiltration.

[00132] RSV infected mice are treated (as described in Example 3) with either the control normal mouse IgG or anti-RSV G monoclonal antibody are analyzed for pulmonary leukocyte trafficking as described by Haynes LM, et al, *J. Infect. Dis.*, 2009, 200(3):439-447. Briefly, mice are anesthetized with Avertin (2,2,2 tribromoethanol) and exsanguinated by severing the right brachial artery. Bronchoalveolar leukocyte cells are harvested by lavaging the lungs three times with 1 ml Dulbecco's PBS. BAL cells are washed in Dulbecco's PBS (GIBCO) containing 1% bovine serum albumin (Sigma-Aldrich, St. Louis, MO) and then stained (4°C, 30 min) with an appropriate dilution of fluorescein isothiocyanate (FITC)-, allophycocyanin (APC)-, or phycoerythrin (PE)-conjugated anti-CD3ε (145-2C11), anti-CD45R/B220 (RA3-6B2), anti-CD8

(Ly-2), anti-neutrophil (PMN) (RB6-8C5), anti-CD11b (M1/70) antibodies or isotype antibody controls (BDBiosciences, Inc.).

[00133] Anti-G antibody treatment correlates with significant reduction in pulmonary leukocyte trafficking and titer of virus in the lung compared to control treated mice by day 5 post-infection. By day 7 post-infection, the level of pulmonary infiltration in treated mice continues to exhibit significantly reduced pulmonary cell infiltration.

[00134] The alveolar inflammatory cell types affected by anti-G monoclonal antibody treatment and the bronchoalveolar lavage (BAL) cell subsets are determined at several time points post-treatment (Table 1). Immunization is linked to a marked reduction in CD4⁺ and CD11b⁺ cell trafficking, and with modest decreases in CD8⁺, DX5⁺ NK, and RB6-8C5⁺ PMN cells in the BAL cell population on day 5 p.i. By day 7 p.i., immunization decreases the numbers of all cell subsets examined.

[00135] Table 1: nIg represents control normal mouse IgG treated mice and anti-G represents mice treated with anti-RSV G antibodies. p.i. represents days post infection.

Day p.i.	Phenotype ^b	nIg	anti-G (300 ug)	% Reduction
5	CD8	5632 ± 667	3240 ± 437	42
	CD4	4347 ± 514	1520 ± 205*	65
	B220	1778 ± 210	880 ± 118*	51
	DX5	4644 ± 549	2760 ± 373	41
	CD11b	5138 ± 608	1840 ± 248*	64
	PMN	2470 ± 292	1080 ± 145*	56
7	CD8	7054 ± 879	2415 ± 445*	66
	CD4	4023 ± 501	1120 ± 206*	72
	B220	3790 ± 472	1190 ± 219*	69
	DX5	6355 ± 791	2275 ± 419*	64
	CD11b	8512 ± 1060	2975 ± 548*	65
	PMN	1224 ± 153	630 ± 116	49

15 [00136]

Example 5 Anti-G antibody treatment reduces post-infection IFN γ levels in the lung.

[00137] Mouse lungs are analyzed for IL-4 and IFN γ levels in cell-free bronchoalveolar lavage (BAL) fluid using a capture enzyme-linked immunoassay kit per manufacturer's instructions (eBiosciences, San Diego, CA). Antibody treatment is not associated with differences in the low levels of IL-4 (FIG. 1A). In contrast, antibody treatment markedly decreases IFN γ levels in cell-free BAL supernatants (FIG. 1B).

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Example 6: RSV levels are reduced by antibodies given following RSV infection.

[00138] Serum from individual mice is collected 14 days after the second of two subcutaneous administrations of an immunogen in alum, as described in Example 3. Aliquots of pre-titrated RSV are mixed with serially diluted samples of individual mouse sera and incubated for 1 hr at room temperature and assayed for RSV neutralizing antibodies by plaque reduction assay. Mixtures are applied in duplicate to 24-well plates containing 60-80% confluent monolayers of HEp-2 or Vero cells, adsorbed for 90 minutes at 4° C, followed by washing and incubation of the plates for 40 h at 37° C in 1 ml of RPMI medium supplemented with 1% fetal calf serum. After incubation, the monolayers are fixed with 15% formaldehyde or 80:20 acetone:PBS and stained with 0.01% crystal violet or immunostaining for visualization of viral plaques. Plaque reduction is calculated as the plaque reduction neutralization titer₅₀ (PRNT₅₀), which is the reciprocal dilution of sera required to neutralize 50% of RSV plaques on a sub-confluent monolayer of HEp-2 or Vero cells. Immunization with either the 164-176 RSV G region polypeptide or the multicomponent vaccine incorporating the 163-190 RSV G region polypeptide along with the 155-206 RSV G region polypeptide demonstrate strong neutralizing antibody responses.

Example 7: RSV titer reduction in antibody treated mice.

[00139] Virus titers in the lungs of RSV-infected mice are determined as previously described by Sullender, W, *Virology*, 1995; 209:70-79. Briefly, lungs are aseptically removed from 3-4 mice per group at days 3, 5, 7 or 11 p.i. and stored at -70°C until assay. Lungs are weighed and individual lung samples homogenized in 1 ml of cold, sterile Dulbecco's PBS (GIBCO), and ten-fold serial dilutions (in serum free DMEM) of the lung homogenates are added to confluent Vero cell monolayers in 24 well plates. Following adsorption for 2 hours at 37°C, cell monolayers are overlaid with tissue culture media DMEM (GIBCO) containing 10% FBS (Hyclone, Logan, UT), incubated at 37°C for 3-4 days and then enumerated by immunostaining with monoclonal antibodies against the G and F proteins (232-1F, 130-6D and 131-2A, respectively).

[00140] On day 5 post-infection, which corresponds to the peak of viral replication in this model, there is a marked decrease ($P < 0.016$) in virus titers in immunized mice, and by day 7 the levels of virus are below the limits of detection (FIG. 2).

Example 8: Pulmonary eosinophilia reduction in anti-RSV G monoclonal antibody treated FI-A2 immunized mice.

[00141] Mice (6-8 wks old) are immunized with 10^6 PFU equivalents of formalin-inactivated RSV/A2 (FI-A2) in the superficial gluteal muscle and rested for four weeks. One day prior to challenge with 10^6 PFU live RSV, mice are given by i.p. administration 300 μ g per mouse of anti-G glycoprotein (131-2G, endotoxin <5EU/mg) monoclonal antibody or control normal mouse IgG (Pierce) (as described in Example 3). Induction of eosinophilia is determined according to procedures described in Mader et al. *Vaccine* 18:1110, 2000. Treatment with anti-RSV G monoclonal antibody (131-2G) protects mice against RSV challenge. Interestingly, at the peak of inflammation (day 5 p.i.), the levels of pulmonary eosinophilia are much reduced compared to control antibody treated mice (FIG. 3).

Example 9: G-protein immunization prevents FI-A2 induced toxicity

[00142] Eight- to ten-week old, specific-pathogen-free, female BALB/c mice (The Jackson Laboratories) are used as subjects for immunization. Mice are housed in micro-isolator cages and fed sterilized water and food *ad libitum*. All studies are performed in accordance with the guidelines of the Institutional Animal Care and Use Committee.

[00143] Immunization with this G polypeptide will induce anti-G antibodies that act similarly to anti-G monoclonal antibodies as described in Example 3 and inhibit pulmonary disease associated with FI-RSV immunization. Mice (6-8 wks old) are 1) immunized with 10^6 PFU equivalents of formalin-inactivated RSV/A2 (FI-A2) in the superficial gluteal muscle, rested for 24 hrs and then 1) subcutaneously immunized on the hind limb with KLH conjugated RSV CH17 peptide (aa163-190; SEQ ID NO. 5) (FI-A2+KLH-CH17-1 day) (10 μ g protein in a volume of 50 μ l PBS/Titermax); 2) rested for four weeks and then immunized with KLH-RSV CH17 peptide (FI-A2+KLH-CH17-4wks) (10 μ g protein in a volume of 50 μ l PBS/Titermax); or 3) boosted at equivalent times with PBS control (50 μ L). Two to three weeks after peptide immunization, both peptide boosted groups are intraperitoneally boosted with KLH-CH17 in PBS.

[00144] Two weeks following the second peptide boost, groups are challenged intranasally with 10^6 PFU live RSV A2 in serum-free DMEM (50 μ l volume), and total bronchoalveolar lavage (BAL) cells (FIG. 4A), eosinophil infiltration (FIG. 4B), IL-4 cytokine production (FIG.

4C) and body weights (FIG. 4D) are determined. No fewer than three mice per treatment are examined per time point.

[00145] Mice boosted either at day 1 or four weeks following immunization with F1-A2 show significantly reduced total cell counts in lavage fluid (FIG. 4A) at 3, 5, and 7 days post RSV challenge. Correspondingly, eosinophilia is statistically significantly reduced at all three analyses days (FIG. 4B). IL-4 levels trend toward reduced levels with statistical significance observed at day 5 in the four week boost group (FIG. 4C). An important marker for disease severity is observed in FIG. 4D where FI-A2 alone immunized mice demonstrate as much as 10% weight loss in by 5 days post-challenge. Boosting at either day 1 or four weeks following FI-A2, prevents weight loss in the same time period.

Example 10: A second G-protein sequence prevents F1-A2 induced toxicity.

[00146] Mice (6-8 wks old) are 1) immunized with 10^6 PFU equivalents of formalin-inactivated RSV/A2 (FI-A2) in the superficial gluteal muscle, rested for 24 hrs and then 1) subcutaneously immunized on the hind limb with KLH conjugated RSV A2peptide (SEQ ID NO: 4) (FI-A2+KLH-A2-1day) (10 μ g protein in a volume of 50 μ l PBS/alum); 2) rested for four weeks and then immunized with KLH-RSV A2peptide (FI-A2+KLH-A2-4wks) (10 μ g protein in a volume of 50 μ l PBS/alum); or 3) boosted at equivalent times with PBS control (50 μ L). Two to three weeks after peptide immunization, both peptide boosted groups are intraperitoneally boosted with KLH-A2 in PBS.

[00147] Two weeks following the second peptide boost, groups are challenged intranasally with 10^6 PFU live RSV A2 in serum-free DMEM (50 μ l volume), and total bronchoalveolar lavage (BAL) cells (FIG. 4A), eosinophil infiltration (FIG. 4B), IL-4 cytokine production (FIG. 4C) and body weights (FIG. 4D) are determined. No fewer than three mice per treatment are examined per time point.

[00148] Similar to the results observed following boosting with CH17 (SEQ ID NO: 5) of Example 9, mice boosted either at day 1 or four weeks with KLH-A2 (SEQ ID NO: 4) following immunization with F1-A2 show significantly reduced total cell counts in lavage fluid (FIG. 5A) at 3 and 5 days post RSV challenge. Correspondingly, eosinophilia is statistically significantly reduced at all three analyses days (FIG. 5B). IL-4 levels trend toward reduced levels (FIG. 5C). Organismal disease severity is reduced relative to the weight loss observed in the F1-A2 immunized group where boosting at four weeks following FI-A2 produced no weight loss in

days 3-7. Mice boosted at day 1 showed mild weight loss at day 3 that reversed to modest weight gain by day 7 indicating more rapid recovery.

Example 11: Immunization with RSV B strain G-protein peptide aa155-206 induces decreased disease severity with reduced F1-A2 induced toxicity.

5 [00149] Mice are immunized as in Example 9 with B strain G-protein peptide aa155-206 (SEQ ID NO: 6) and challenged with RSV-A2 strain as in Example 4 or RSV-B1 strain. Although the RSV B G-protein has a unique sequence relative to the A strains, immunization with the peptide of SEQ ID NO: 6 demonstrates reduced levels of pulmonary eosinophilia compared to prior immunization attempts with alternate vaccines. In measurements of disease
10 severity as in Example 8 and 9, immunization with SEQ ID NO: 6 also reduces weight loss and IL-4 levels relative to control mice. Also, similar to immunization with SEQ ID NO: 4, mice immunized with the peptide corresponding to the B strain (SEQ ID NO: 6) demonstrate similar reductions in inflammatory bronchoalveolar lavage (BAL) cell subsets determined at several time points post-treatment. Immunization is linked to a marked reduction in CD4⁺ and CD11b⁺
15 cell trafficking, and with modest decreases in CD8⁺, DX5⁺ NK, and RB6-8C5⁺ PMN cells in the BAL cell population on day 5 p.i. By day 7 p.i., immunization decreases the numbers of all cell subsets examined.

Example 12: Boosting with RSV B G-protein fragments protects against F1-A2 induced
20 toxicity.

[00150] Mice are immunized as in Example 9 and boosted with KLH conjugated peptide SEQ ID NO: 6 either at one day following immunization or four weeks following immunization. Similar results to those of both the A2 and CH17 peptides are observed with SEQ ID NO: 6. Mice boosted either at day 1 or four weeks following immunization with F1-A2 show reduced
25 total cell counts in lavage fluid at 3, 5, and 7 days post RSV challenge. Correspondingly, eosinophilia is reduced at all three analyses days. IL-4 levels trend toward reduced levels. Finally, boosting mice with SEQ ID NO: 6 at either day 1 or four weeks produces no post-challenge weight loss in the same time period.

Example 13: RSV levels are reduced by antibodies produced following immunization.

[00151] Serum from individual mice is collected 14 days after the second of two subcutaneous administrations of an immunogen in alum, as described in Example 3. Aliquots of pre-titered RSV are mixed with serially diluted samples of individual mouse sera and incubated for 1 hr at room temperature and assayed for RSV neutralizing antibodies by plaque reduction assay. Mixtures are applied in duplicate to 24-well plates containing 60-80% confluent monolayers of Vero cells, adsorbed for 90 minutes at 4° C, followed by washing and incubation of the plates for 40 h at 37° C in 1 ml of DMEM medium supplemented with 1% fetal calf serum. After incubation, the monolayers are fixed with acetone-methonal mixture and plaques enumerated by immunostaining with monoclonal anti-RSV antibodies. Plaque reduction is calculated as the plaque reduction neutralization titer₅₀ (PRNT₅₀), which is the reciprocal dilution of sera required to neutralize 50% of RSV plaques on a sub-confluent monolayer of Vero cells. Immunization with peptide SEQ ID NOs: 4-6 or the multicomponent vaccine incorporating the 163-190 RSV G A region polypeptide along with the 155-206 RSV G B region polypeptide demonstrate strong neutralizing antibody responses.

[00152] Patents and publications mentioned in the specification are indicative of the levels of those skilled in the art to which the invention pertains. These patents and publications are incorporated herein by reference to the same extent as if each individual application or publication was specifically and individually expressed explicitly in detail herein.

[00153] The foregoing description is illustrative of particular embodiments of the invention, but is not meant to be a limitation upon the practice thereof. The following claims, including all equivalents thereof, are intended to define the scope of the invention.

CLAIMS

1. A respiratory syncytial virus vaccine comprising an isolated immunogen that elicits a protective immune response, said immunogen comprising an amino acid sequence that is:

5 (i) amino acid position 164 to amino acid position 176 of SEQ ID NO: 1 or an analogue thereof;

(ii) amino acid position 163 to amino acid position 190 of SEQ ID NO: 1, amino acid position 163 to amino acid position 190 of SEQ ID NO. 7, amino acid position 163 to amino acid position 190 of SEQ ID NO. 8, or an analogue thereof; or

10 (iii) amino acid position 155 to amino acid position 206 of SEQ ID NO: 1 or an analogue thereof.

2. The vaccine of claim 1 wherein said immunogen is SEQ ID NO: 2.

3. The vaccine of claim 1 wherein said immunogen is SEQ ID NO: 4.

4. The vaccine of claim 1 wherein said immunogen is SEQ ID NO: 5.

15 5. The vaccine of claim 1 wherein said immunogen is SEQ ID NO: 6.

6. The vaccine of claims 1 to 5 wherein said immunogen is recombinant.

7. The vaccine of claims 1 to 5 wherein said immunogen further comprises a tag suitable for isolation of the immunogen.

8. A vaccine comprising a first immunogen and a second immunogen;
20 said first immunogen is an isolated amino acid sequence comprising amino acid position 163 to amino acid position 190 of SEQ ID NOs: 1, 7, 8, or an analogue thereof; and
said second immunogen is an isolated amino acid sequence comprising amino acid position 155 to amino acid position 206 of SEQ ID NOs: 1 or 7, or an analogue thereof.

9. The vaccine of claim 8 wherein said first immunogen and said second
25 immunogen are covalently associated by a linker.

10. The vaccine of claim 9 wherein said linker is an excisable intermediate polypeptide fragment.

11. The vaccine of claims 8 to 10 wherein said first immunogen is a polypeptide of SEQ ID NOs: 4 or 5.

12. The vaccine of claims 8 to 10 wherein said second immunogen is a polypeptide of SEQ ID NOs: 3 or 6.

5 13. The vaccine of claims 1 to 5 or claims 8 to 10 further comprising an adjuvant.

14. The vaccine of claim 13 wherein said adjuvant is dimethyl dioctadecylammonium bromide (DDA); monophosphoryl lipid A (MPL); LTK63, lipophilic quaternary ammonium salt-DDA, DDA-MPL, aluminum salts, aluminum hydroxide, aluminum phosphate, potassium aluminum phosphate, Montanide ISA-51, ISA-720, microparticles,
10 immunostimulatory complexes, liposomes, virosomes, virus-like particles, CpG oligonucleotides, cholera toxin, heat-labile toxin from *E. coli*, lipoproteins, dendritic cells, IL-12, GM-CSF, nanoparticles illustratively including calcium phosphate nanoparticles, combination of soybean oil, emulsifying agents, and ethanol to form a nanoemulsion; AS04, ZADAXIN, or combinations thereof.

15 15. The vaccine of claims 1 to 5 or claims 8 to 10 further comprising an emulsification agent.

16. The vaccine of claim 15 wherein said emulsification agent is supramolecular biovectors (SMBV), nanoparticles, liposomes, or combinations thereof.

17. A process of creating an immune response in a subject comprising:
20 administering to a subject a first vaccine, said vaccine comprising the immunogen of claims 1 to 5 or claims 8 to 10; and
allowing sufficient time for the subject to develop the immune response.

18. The process of claim 17 wherein said administering is through the route selected from the group comprising: subcutaneous, intramuscular, intranasal, oral, intravaginal,
25 intravenous, intramucosal, or combinations thereof.

19. The process of claim 17 further comprising administering to a subject a second vaccine.

20. The process of claim 19 wherein said second vaccine is an amino acid sequence corresponding to amino acid position 155 to amino acid position 206 of SEQ ID NO: 1 or an analogue thereof; or an amino acid sequence corresponding to amino acid position 163 to amino acid position 190 of SEQ ID NOs: 1,7, 8, or an analogue thereof; or a combination thereof.

5 21. The process of claim 19 wherein administering said first vaccine is

- (i) prior to administering said second vaccine;
- (ii) subsequent to administering said second vaccine; or
- (iii) simultaneous with said second vaccine.

22. A pharmaceutical package comprising:

- 10 a vaccine selected from the group comprising the immunogen of claims 1 to 5, the vaccine of claims 8 to 10, or combinations thereof;
- an emulsification agent; and
 - an adjuvant.

23. The pharmaceutical package of claim 22 wherein said adjuvant is selected from
15 the group comprising: dimethyl dioctadecyl-ammonium bromide (DDA); monophosphoryl lipid A (MPL); LTK63, lipophilic quaternary ammonium salt-DDA, DDA-MPL, aluminum salts, aluminum hydroxide, aluminum phosphate, potassium aluminum phosphate, Montanide ISA-51, ISA-720, microparticles, immunostimulatory complexes, liposomes, virosomes, virus-like particles, CpG oligonucleotides, cholera toxin, heat-labile toxin from *E. coli*, lipoproteins,
20 dendritic cells, IL-12, GM-CSF, nanoparticles illustratively including calcium phosphate nanoparticles, combination of soybean oil, emulsifying agents, and ethanol to form a nanoemulsion; AS04, ZADAXIN, or combinations thereof.

24. The pharmaceutical package of claim 22 wherein said emulsification agent is
25 selected from the group comprising: supramolecular biovectors (SMBV), nanoparticles, liposomes, or combinations thereof.

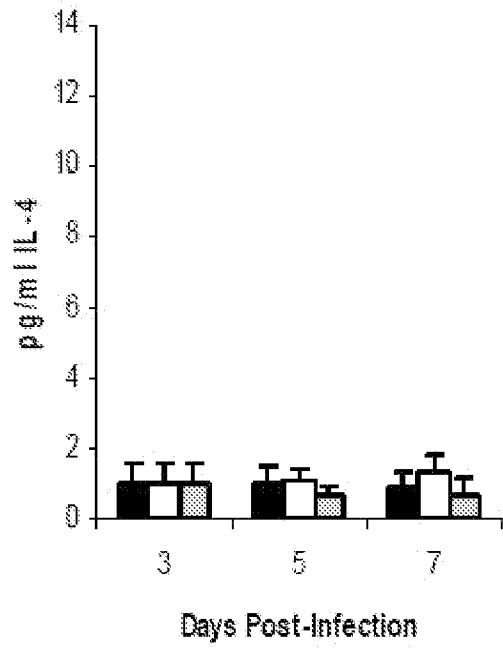


FIG. 1A

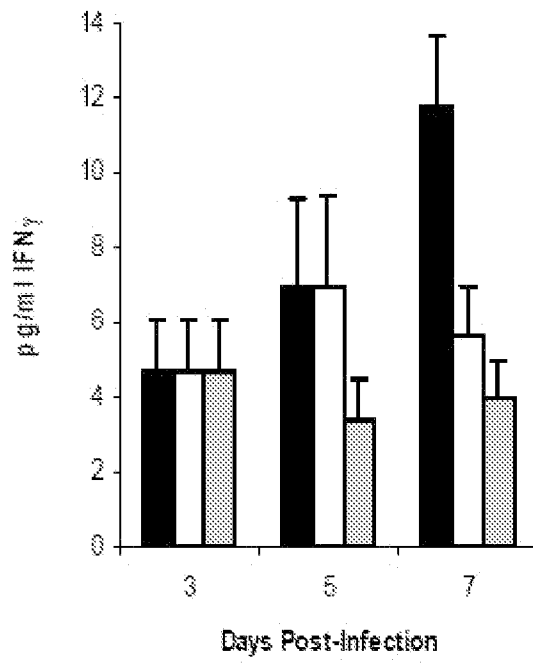


FIG. 1B

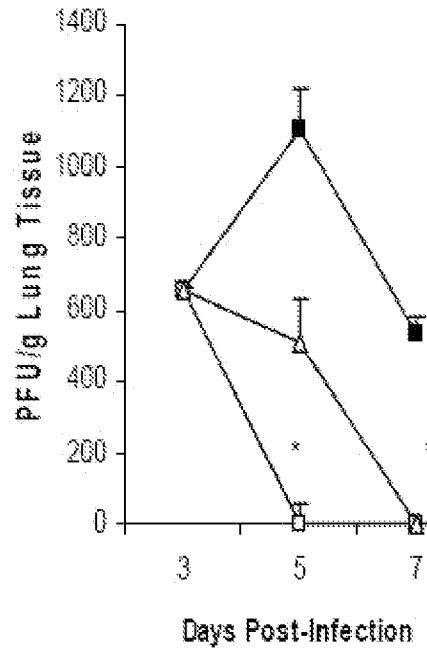


FIG. 2

Solid squares represent control antibody treated mice. Open triangles represent anti-RSV G antibody treated (low-dose) mice. The open squares represent anti-RSV G antibody treated (high-dose) treated mice.

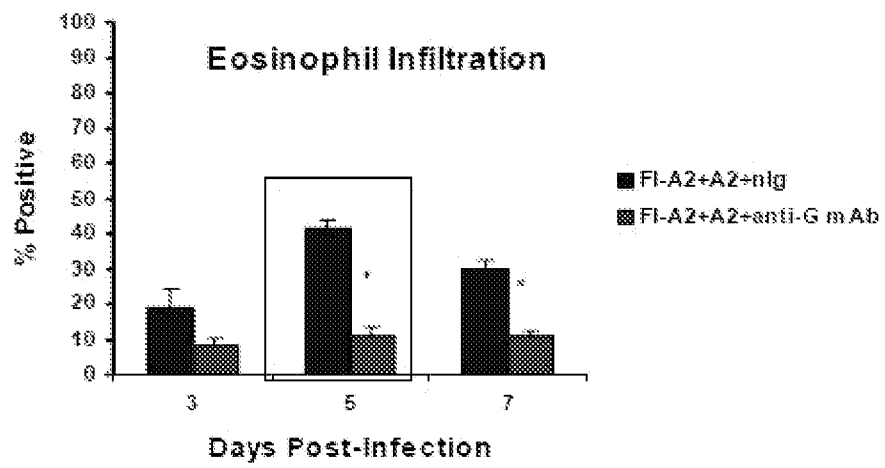


FIG. 3

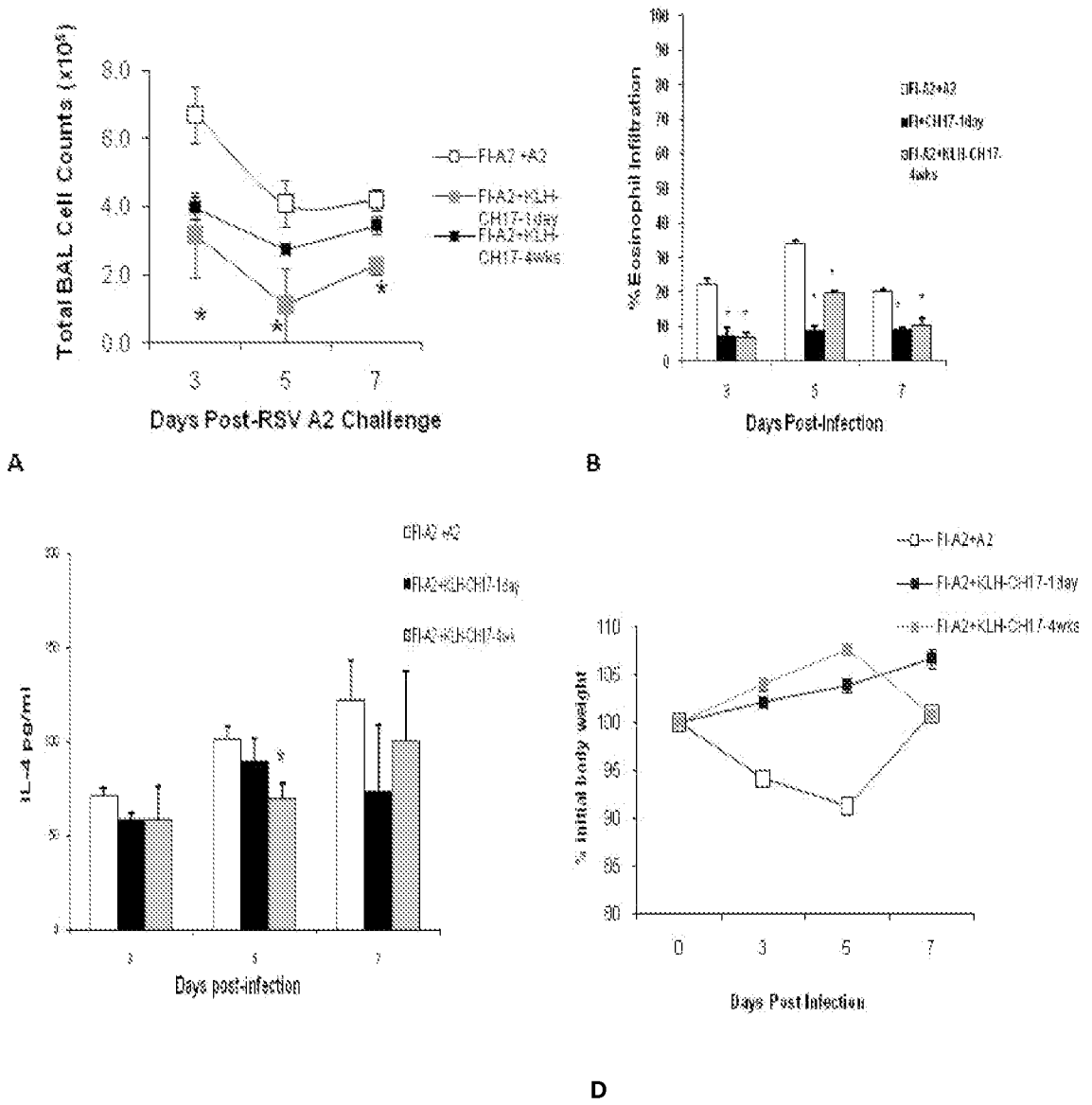


FIG. 4

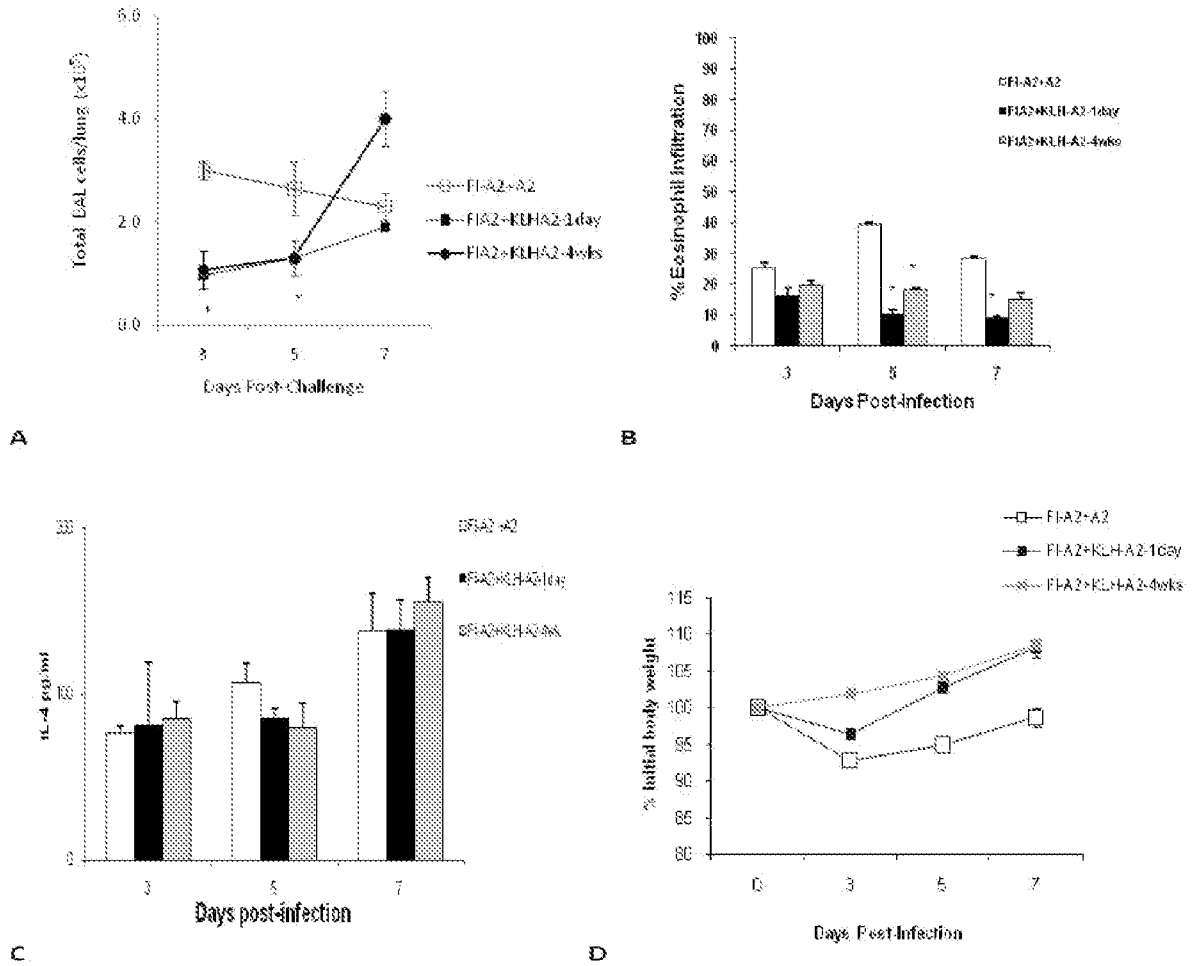


FIG. 5