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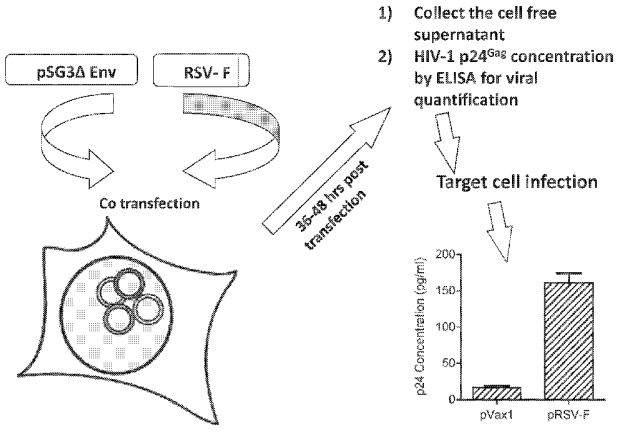
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(54) **Title**: HUMAN RESPIRATORY SYNCYTIAL VIRUS CONCENSUS ANTIGENS, NUCLEIC ACID CONSTRUCTS AND VACCINES MADE THEREFORM, AND METHODS OF USING SAME

Figure 12
RSV-Fusion Pseudo viral production in 293T cells



(57) **Abstract**: Nucleic acid molecules and compositions comprising one or more nucleic acid sequences that encode an RSV immunogen are disclosed. Nucleic acid are disclosed that comprise the sequences that encodes consensus RSV F protein or immunogenic fragment thereof, sequences that encodes an RSV G(A) protein or immunogenic fragment thereof and sequences that encodes an RSV G(B) protein or immunogenic fragment thereof. Compositions comprising one, combinations of two or all three sequences are disclosed. The coding sequences optionally include operable linked coding sequence that encode a signal peptide. Nucleic acid molecules and compositions comprising the chemokine CC20 and/or a consensus RSV M2-1 protein or immunogenic fragment thereof are also disclosed. Immunomodulatory methods and methods of inducing an immune response against RSV are disclosed. Method of preventing RSV infection and methods of treating individuals infected with RSV are disclosed.

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HUMAN RESPIRATORY SYNCYTIAL VIRUS CONCENSUS ANTIGENS, NUCLEIC ACID CONSTRUCTS AND VACCINES MADE THEREFORM, AND METHODS OF USING SAME

FIELD OF THE INVENTION

The present invention relates to consensus antigenic respiratory syncytia virus proteins and nucleic acid molecules which encode the same; improved respiratory syncytia virus vaccines including such proteins and/or nucleic acid molecules; and methods for using the vaccines for inducing immune responses and preventing respiratory syncytia virus infection and/or treating individuals infected with respiratory syncytia virus.

BACKGROUND OF THE INVENTION

This application claims priority to U.S. Provisional Application No. 61/622,279, which is incorporated herein, in its entirety, by reference.

Human Respiratory Syncytial Virus (RSV) is the most common cause of upper and lower respiratory tract infections among infants and young children (premature infants especially prone to disease). World-wide, RSV is the leading cause of serious lower respiratory infections in infants (especially among those born prematurely and having chronic lung diseases or congenital heart diseases) and young children worldwide, and is responsible for a variety of illnesses, including 20-25% of pneumonia cases and 45-50% of bronchiolitis cases among hospitalized children. In the U.S., RSV invention results in approximately 120,000 hospitalization and 500+ deaths each year. In the population of <1 year old, RSV is the leading cause of infant viral death and mortality among this population is 10 times greater than mortality due to influenza infection. Global annual morbidity and mortality estimated to be 64 million and 160,000 deaths respectively. RSV-related medical costs estimated to be >$650 million/year.

While essentially all children experience an infection by two years of age, the peak age for serious RSV infection is at 2-6 months of age. The majority of infections resolve uneventfully. In some children, however, infection may predispose children for asthma and

airway hyper-responsiveness later in life. Natural RSV infection does not confer lifetime immunity, and therefore, individuals may be repeatedly infected.

RSV infection raises significant issue among the elderly and other vulnerable populations. Among the elderly, RSV is the second leading cause of viral death. Transplant recipients and other immunocompromised populations as well as individuals suffering from cystic fibrosis are vulnerable to serious health consequences due to infection.

Respiratory Syncytial Virus (RSV) is an enveloped, negative-sense, single-stranded RNA virus of the family *Paramyxoviridae,*a family which includes common respiratory viruses such as influenza and those causing mumps and measles. In all, RSV has ten genes encoding eleven different proteins. The eleven RSV proteins include: proteins 1) protein "NS1" and 2) protein "NS2", which inhibit type I interferon activity; 3) protein "N", the nucleocapsid protein which associates with RNA forming nucleocapsid; 4) protein "P", which is a cofactor for protein L; 5) protein "M", the matrix protein which required for viral assembly; 6) protein "SH", which is expressed on the viral surface forms the viral coat with protein G and protein F; 7) protein "G", which is highly glycosylated, expressed on the surface, involved in viral attachment and binds glycosaminoglycans (GAGs); 8) protein "F", which is expressed on the surface, viral-cell membrane fusion and mediating fusion to allow entry of virus into the cell cytoplasm; 9) protein "M2-1", which is a matrix protein and elongation factor; 10) protein "M2-2", which is a matrix protein and transcription factor;. and, 11) protein "L", which is RNA polymerase. The M2 gene encodes both protein M2-1 and protein M2-2 in overlapping open reading frames. The primary CD8 T cell epitope is encoded by the M2 gene. There are two major subtypes of human RSV - A and B. The major difference between the two subtypes resides within the G protein.

RSV infection often results both immune mediated pathoglogy and virus mediated pathology. Primary RSV infection often results in acute bronchiolitis that leads to inflammation-induced airway obstruction. RSV F binding has been shown to induce apoptosis resulting in the sloughing of ciliated epithelial cells, compromised pulmonary clearance, and consequent secondary infections.

Unfortunately, despite the immense effort, there are still no effective vaccines available for RSV. In 1966-1967, first RSV vaccine candidate, a formalin-inactivated alum-precipitated

RSV preparation (FI-RSV vaccine) resulted in enhanced disease in vaccinated children upon subsequent natural infection.. Histological analysis of lungs of children who died from enhanced disease caused by infection after vaccination revealed extensive mononuclear cell infiltration including pulmonary eosinophilia. The FI-RSV vaccine generated only binding antibodies without neutralizing activity because of denatured F protein and did not induce CTL activity.

Subsequent experiments have suggested that this enhanced pulmonary disease is associated with an exaggerated Th2-type cytokine response by CD4 T cells, a poor cytolytic response by CD8 T cells, and a weak neutralizing antibody response. RSV infection of FI-RSV vaccinated BALB/c and C57BL/6 mice resulted in enhanced disease observed in FI-RSV vaccinated children. Characteristic of a Th2-mediated immune response suggested immunized children were primed for Th2 immune response by vaccine. Increased levels of Th2-associated cytokines IL-5, IL-4, and IL-13 and chemokineeotaxin with a decrease in Th1-associated cytokine IL-12 were exhibited. Depletion of IL-4, IL-10, or IL-13 resulted in significant decrease in enhanced disease after RSV challenge.

Live attenuated and inactivated whole virus vaccines have also failed to protect. The candidate vaccines were either insufficiently attenuated or demonstrated the potential for enhanced disease. In 1982, a live attenuated RSV vaccine was found to be safe, but not effective for prevention of RSV illness.

In 1983, a Native American infant, "Baby Moose", who was thought to have B streptococcal disease but who actually was infected with RSV, serendipitously improved when he received IGIV. This result prompted study of IGIV for RSV disease. In the mid 1980s through 1990 studies of standard IGIV for treatment and prevention of RSV illness validated the role of antibodies in prevention of RSV disease.

In the early 1990s, RSV vaccine studies were re-initiated using various subunit varieties. These trials failed to show significant protection from disease.

From mid 1990s to early 2002, clinical trials of palivizumab (monoclonal antibody specific for RSV-F) for prevention of serious respiratory tract disease caused by RSV produced positive results. In September 2003, palivizumab was approved for prevention of RSV-associated disease in high-risk children. Prophylactic treatment with palivizumab is effective in

reducing the severity of disease, but is only recommended for high-risk patients due to the high cost involved in the treatment.

Although past studies have failed to yield in effective RSV vaccines, they have convincingly demonstrated the importance of immune responses in providing a thorough protection against RSV infection. Studies have found evidence supporting the importance of humoral responses and antibodies in protection against RSV-mediated disease. The presence of IgG antibodies in the lung directly correlates with reduced viral load and children with less severe RSV disease often have significantly higher anti-RSV antibody titers before infection Antibody to fusion protein is an important correlate of immunity. Infants who did not become infected with RSV had higher mean titers of IgG than infected infants and were born to mothers who had significantly higher maternal RSV-specific IgG antibody levels than the mothers of infants who became infected. The importance of antibody in mediating protection helps explain why premature infants are at such high risk for serious illness after RSV infection. Maternal IgG is not efficiently transferred to the fetus until the third trimester of pregnancy.

Similarly, the importance of cellular immune responses in providing a thorough protection against RSV infection has also been demonstrated. Children with T cell deficiency have difficulty clearing the virus and are more susceptible to subsequent RSV infection. In animal studies, depletion of CD8 T cells alone in mice does not result in chronic infection, but does result in delayed viral clearance. Clearance requires IFN-γ, FasL, TNF-α. Deficiency in any of these results in delayed viral clearance. Further, C57BL/6 mice immunized with VV-G do not develop RSV-associated enhanced disease, but depletion of CD8 T cells before immunization results in disease.

There remains a need for an RSV vaccine, including an immunogenic antigen, that induces long-term protection against RSV. There is also a need for a cost-effective delivery system to enable mass prophylactic vaccination against RSV. There is also a need for additional therapeutic agents to treat individuals infected with RSV.

SUMMARY OF THE INVENTION

Nucleic acid molecules are provided which comprising one or more nucleic acid sequences that encode one o more consensus RSV immunogens. The RSV immunogens include the consensus RSV F immunogen, the consensus RSV-G(A) immunogen, and the consensus RSV G(B) immunogen.

Compositions are provided which comprise one or more nucleic acid sequences that encode an RSV immunogen. The nucleic acids are selected from the group consisting of: a) a nucleic acid coding sequence that encodes an RSV F immunogen, b) a nucleic acid coding sequence that encodes an RSV Ga immunogen, and c) a nucleic acid coding sequence that encodes an RSV Gb immunogen.

In some embodiments, the nucleic acid coding sequence that encodes an RSV F immunogen encodes an RSV F immunogen selected from the group consisting of: SEQ ID NO:2, a fragment of SEQ ID NO:2, a protein that is at least 98% homologous to SEQ ID NO:2 and a fragment of a protein that is at least 98% homologous to SEQ ID NO:2. In each instance, the nucleic acid coding sequence that encodes an RSV F immunogen may optionally further comprise coding sequence that encodes a signal peptide operably linked to the sequence.

In some embodiments, the nucleic acid coding sequence that encodes an RSV Ga immunogen encodes an RSV Ga immunogen selected from the group consisting of: SEQ ID NO:6, a fragment of SEQ ID NO:6, a protein that is at least 98% homologous to SEQ ID NO:6 and a fragment of a protein that is at least 98% homologous to SEQ ID NO:6. In each instance, the nucleic acid coding sequence that encodes an RSV Ga immunogen may optionally further comprise coding sequence that encodes a signal peptide operably linked to the sequence.

In some embodiments, the nucleic acid coding sequence that encodes an RSV Gb immunogen encodes an RSV Gb immunogen selected from the group consisting of: SEQ ID NO:8, a fragment of SEQ ID NO:8, a protein that is at least 98% homologous to SEQ ID NO:8 and a fragment of a protein that is at least 98% homologous to SEQ ID NO:8. In each instance, the nucleic acid coding sequence that encodes an RSV Gb immunogen may optionally further comprise coding sequence that encodes a signal peptide operably linked to the sequence.

In some embodiments, the nucleic acid coding sequence that encodes an RSV F immunogen is selected from the group consisting of: nucleic acid sequences comprising SEQ ID NO:1, nucleic acid sequences comprising a fragment of SEQ ID NO:1, nucleic acid sequences comprising sequences that is at least 98% homologous to SEQ ID NO:1, and nucleic acid sequences comprising a fragment of a sequence that is at least 98% homologous to SEQ ID NO:1. In each instance, the nucleic acid coding sequence that encodes an RSV F immunogen may optionally further comprise coding sequence that encodes a signal peptide operably linked to the sequence. Nucleic acid sequences comprising sequences that is at least 98% homologous to SEQ ID NO:1 preferable encode a protein that is at least 98% homologous to SEQ ID NO:2. Nucleic acid sequences comprising a fragment of a sequence that is at least 98% homologous to SEQ ID NO:1 preferable encode a fragment of a protein that is at least 98% homologous to SEQ ID NO:2.

In some embodiments, the nucleic acid coding sequence that encodes an RSV Ga immunogen is selected from the group consisting of: nucleic acid sequences comprising SEQ ID NO:5, nucleic acid sequences comprising a fragment of SEQ ID NO:5, nucleic acid sequences comprising sequences that is at least 98% homologous to SEQ ID NO:5, and nucleic acid sequences comprising a fragment of a sequence that is at least 98% homologous to SEQ ID NO:5. In each instance, the nucleic acid coding sequence that encodes an RSV Ga immunogen may optionally further comprise coding sequence that encodes a signal peptide operably linked to the sequence. Nucleic acid sequences comprising sequences that is at least 98% homologous to SEQ ID NO:5 preferable encode a protein that is at least 98% homologous to SEQ ID NO:6. Nucleic acid sequences comprising a fragment of a sequence that is at least 98% homologous to SEQ ID NO:5 preferable encode a fragment of a protein that is at least 98% homologous to SEQ ID NO:6.

In some embodiments, the nucleic acid coding sequence that encodes an RSV Gb immunogen is selected from the group consisting of: nucleic acid sequences comprising SEQ ID NO:7, nucleic acid sequences comprising a fragment of SEQ ID NO:7, nucleic acid sequences comprising sequences that is at least 98% homologous to SEQ ID NO:7, and nucleic acid sequences comprising a fragment of a sequence that is at least 98% homologous to SEQ ID

NO:7. In each instance, the nucleic acid coding sequence that encodes an RSV Gb immunogen may optionally further comprise coding sequence that encodes a signal peptide operably linked to the sequence. Nucleic acid sequences comprising sequences that is at least 98% homologous to SEQ ID NO:7 preferable encode a protein that is at least 98% homologous to SEQ ID NO:8. Nucleic acid sequences comprising a fragment of a sequence that is at least 98% homologous to SEQ ID NO:7 preferable encode a fragment of a protein that is at least 98% homologous to SEQ ID NO:8.

In some embodiments, the compositions comprises one or more nucleic acid sequences selected from the group consisting of: a nucleic acid sequence that encodes SEQ ID NO:10; a nucleic acid sequence that encodes SEQ ID NO:14; and a nucleic acid sequence that encodes SEQ ID NO:16.

In some embodiments, the compositions comprise one or more nucleic acid sequences selected from the group consisting of: a nucleic acid sequence comprising SEQ ID NO:9; a nucleic acid sequence comprising SEQ ID NO:13; and a nucleic acid sequence comprising SEQ ID NO:15.

In some embodiments, the compositions compriss nucleic acid sequences selected from the group consisting of:

a nucleic acid coding sequence that encodes an RSV F immunogen,

a nucleic acid coding sequence that encodes an RSV Ga immunogen,

a nucleic acid coding sequence that encodes an RSV Gb immunogen,

a nucleic acid coding sequence that encodes an RSV F immunogen and a nucleic acid coding sequence that encodes an RSV Ga immunogen,

a nucleic acid coding sequence that encodes an RSV F immunogen and a nucleic acid coding sequence that encodes an RSV Gb immunogen,

a nucleic acid coding sequence that encodes an RSV Ga immunogen and a nucleic acid coding sequence that encodes an RSV Gb immunogen,

a nucleic acid coding sequence that encodes an RSV F immunogen, a nucleic acid coding sequence that encodes an RSV Ga immunogen and a nucleic acid coding sequence that encodes an RSV Gb immunogen,

The composition may further comprise a nucleic acid coding sequence that encodes CCL20 and/or be formulated for delivery to an individual using electroporation and/or further comprising nucleic acid sequences that encode one or more proteins selected from the group consisting of: IL-12, IL-15 and IL-28.

In some embodiments, one or more nucleic acid coding sequences are part of one or more plasmids. In some embodiments, one or more nucleic acid coding sequences are each incorporated into a separate plasmid.

Method are provided for inducing an immune response against RSV comprising administering a composition to an individual in an amount effective to induce an immune response in said individual.

Method are provided for treating an individual who has been diagnosed with RSV comprising administering a therapeutically effective amount of a composition to an individual.

Method are provided for preventing RSV infection an individual comprising administering a prophylactically effective amount of the composition to an individual.

Novel "RSV F immunogen", "RSV Ga immunogen", and "RSV Gb immunogen" are provided. In some embodiments, proteins selected from the group consisting of: SEQ ID NO:2; SEQ ID NO:6; SEQ ID NO:8; SEQ ID NO:10; SEQ ID NO:14; and SEQ ID NO:16 are provided.

Methods of modulating immune responses using a nucleic acid coding sequence that encodes the chemokine CCL20 are also provided.

In addition, provided herein is a nucleic acid coding sequence that encodes a consensus RSV M2-1 immunogen. In some embodiments, the nucleic acid coding sequence that encodes an RSV M2-1 immunogen encodes an RSV M2-1 immunogen selected from the group consisting of: SEQ ID NO:4, a fragment of SEQ ID NO:4, a protein that is at least 98% homologous to SEQ ID NO:4 and a fragment of a protein that is at least 98% homologous to SEQ ID NO:4. In each instance, the nucleic acid coding sequence that encodes an RSV M2-1 immunogen may optionally further comprise coding sequence that encodes a signal peptide operably linked to the sequence.

In some embodiments, the nucleic acid coding sequence that encodes an RSV M2-1 immunogen is selected from the group consisting of: nucleic acid sequences comprising SEQ ID NO:3, nucleic acid sequences comprising a fragment of SEQ ID NO:3, nucleic acid sequences comprising sequences that is at least 98% homologous to SEQ ID NO:3, and nucleic acid sequences comprising a fragment of a sequence that is at least 98% homologous to SEQ ID NO:3. In each instance, the nucleic acid coding sequence that encodes an RSV M2-1 immunogen may optionally further comprise coding sequence that encodes a signal peptide operably linked to the sequence. Nucleic acid sequences comprising sequences that is at least 98% homologous to SEQ ID NO:3 preferable encode a protein that is at least 98% homologous to SEQ ID NO:4. Nucleic acid sequences comprising a fragment of a sequence that is at least 98% homologous to SEQ ID NO:3 preferable encode a fragment of a protein that is at least 98% homologous to SEQ ID NO:4.

In some embodiments, compositions are provided which comprises one or more nucleic acid sequences including a nucleic acid sequence that encodes SEQ ID NO:12.

In some embodiments, the compositions are provided which comprise one or more nucleic acid sequences including a nucleic acid sequence comprising SEQ ID NO:11.

In some embodiments, the compositions compriss nucleic acid sequences selected from the group consisting of: a nucleic acid coding sequence that encodes an RSV M2-1 immunogen, a nucleic acid coding sequence that encodes an RSV F immunogen and a nucleic acid coding sequence that encodes an RSV M2-1 immunogen, a nucleic acid coding sequence that encodes an RSV M2-1 immunogen and a nucleic acid coding sequence that encodes an RSV Ga immunogen, a nucleic acid coding sequence that encodes an RSV M2-1 immunogen and a nucleic acid coding sequence that encodes an RSV Gb immunogen, a nucleic acid coding sequence that encodes an RSV F immunogen, a nucleic acid coding sequence that encodes an RSV M2-1 immunogen and a nucleic acid coding sequence that encodes an RSV Ga immunogen, a nucleic acid coding sequence that encodes an RSV F immunogen, a nucleic acid coding sequence that encodes an RSV M2-1 immunogen and a nucleic acid coding sequence that encodes an RSV Gb immunogen, a nucleic acid coding sequence that encodes an RSV M2-1 immunogen, a nucleic acid coding sequence that encodes an RSV Ga immunogen and a nucleic

acid coding sequence that encodes an RSV Gb immunogen, and a nucleic acid coding sequence that encodes an RSV F immunogen, a nucleic acid coding sequence that encodes an RSV M2-1 immunogen, a nucleic acid coding sequence that encodes an RSV Ga immunogen and a nucleic acid coding sequence that encodes an RSV Gb immunogen.

Novel " "RSV M2-1 immunogen is also provided. In some embodiments, proteins selected from the group consisting of: SEQ ID NO:4 and SEQ ID NO:12 are provided.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the phylogenetic tree of RSV-F Protein, the consensus RSV F protein amino acid sequence is set forth in SEQ ID NO:2.

Figure 2 shows the phylogenetic tree of RSV M2-1 Protein, the consensus RSV M2-1 protein amino acid sequence is set forth in SEQ ID NO:4.

Figure 3 shows results anti-RSV F protein IgG at various time points following immunization with RSV F vaccine.

Figure 4 shows results anti-RSV F protein IgA at various time points following immunization with RSV F vaccine.

Figure 5 shows results from a comparison of IFN-γ production at different dosage of RSV-F vaccine approximately 8 weeks after last immunization.

Figure 6 shows results from a comparison of IFN-γ+ CD8 and CD4 T cells in spleen after RSV-F +/- chemokine immunization in the spleen.

Figure 7 shows results from a comparison of IFN-γ+ CD8 and CD4 T cells in spleen after RSV-F +/- chemokine immunization in the lung.

Figure 8 shows results from a comparison of IgG Endpoint Titer in Sera and IgG subtypes (IgG1 vs IgG2a) in Sera.

Figure 9 shows a comparison of IgG2a/IgG1 ratio.

Figure 10 depicts Env-Pseudotyped viruses in TZM-BL Cells/ "ON".

Figure 11 depicts Env-Pseudotyped viruses in TZM-BL Cells/ "OFF".

Figure 12 depicts RSV-Fusion Pseudo viral production in 293T cells.

Figure 13 shows the immunization protocol used for a RSV-F Neutralization Study using the assay.

Figure 14 shows data from quantification of RSV-F mediated infection in different target cells.

Figure 15 shows data from quantification of RSV-F neutralization activity in TZM-bl HeLa cells.

Figure 16 shows data from quantification of cell death activity by RSV-F pseudo virus in HeLa cells.

Figure 17 shows the immunization schedule for RSV-M2 dosage study that was undertaken.

Figure 18 shows data collected from that study including a comparison of IFN-g production at different dosages of RSV-M2 vaccine 1 week after last immunization.

Figure 19 depicts an immunization schedule for evaluation of the combination of RSV-F vaccine with CCL20.

Figure 20 shows data from the study including data from a comparison of IFN-g production in each of spleen, liver and lung with different dosages of CCL20 at 1 week after last immunization.

Figure 21 shows data of a comparison of IgG subtypes (IgG1 vs IgG2a) in Sera.

Figures 22A-22C show plasmid designs and construction of RSV-F, RSV-G(A), and RSV-G(B) constructs. Figures 22A shows plasmid design and construction of RSV-F construct. Figures 22B shows plasmid design and construction of RSV-G(A) construct. Figures 22C shows plasmid design and construction of RSV-G(B) construct.

Figure 23 shows results confirming in vitro expression of RSV-F, RSV-G(A), and RSV-G(B) constructs.

Figure 24 shows the immunization schedule for an RSV-F Dosage Study.

Figure 25 shows IgG data from RSV-F Dosage Study.

Figure 26 shows IFN-γ data from RSV-F Dosage Study.

Figure 27 shows the immunization schedule for a RSV-F PSEUDO Neutralization Study.

Figure 28 shows data from the RSV-F PSEUDO Neutralization Study.

Figure 29 shows data from two comparisons to determine inhibition of apoptotic cell death activity by RSV-F pseudovirus in HeLa cells.

Figure 30 shows the immunization schedule for an RSV-G(A) and RSV-(B) Dosage Study.

Figure 31 shows IgG data from RSV-G(A) from the RSV-G(A) and RSV-(B) Dosage Study.

Figure 32 shows IgG data from RSV-G(A) from the RSV-G(A) and RSV-(B) Dosage Study.

Figure 33 is a chart showing a summary of RSV Preliminary Neutralization results using Mono Vaccines in Mouse Studies.

Figure 34 shows the RSV Rabbit Study Design that was followed.

Figure 35 shows data from the RSV Rabbit Study of measured humoral immunity against RSV-F.

Figure 36 shows data from the RSV Rabbit Study of measured humoral immunity against RSV-G.

Figure 37 shows the RSV Non-Human Primate Study Design that was followed.

Figure 38A-38D show data from the RSV Non-Human Primate Study of measured humoral immunity against RSV-F. Figure 38A shows data from measured humoral immunity against RSV-F in naïve animals. Figure 38B shows data from measured humoral immunity against RSV-F in animals who received the cocktail vaccine IM at one site. Figure 38C shows data from measured humoral immunity against RSV-F in animals who received the cocktail vaccine plus rhMEC construct IM at one site. Figure 38D shows data from measured humoral immunity against RSV-F in animals who received the cocktail vaccine ID at three sites.

Figure 39A-39D show data from the RSV Non-Human Primate Study of measured humoral immunity against RSV-G. Figure 39A shows data from measured humoral immunity against RSV-G in naïve animals. Figure 39B shows data from measured humoral immunity against RSV-G in animals who received the cocktail vaccine IM at one site. Figure 39C shows data from measured humoral immunity against RSV-G in animals who received the cocktail

vaccine plus rhMEC construct IM at one site. Figure 39D shows data from measured humoral immunity against RSV-G in animals who received the cocktail vaccine ID at three sites.

Figure 40 is a chart showing a summary of RSV Preliminary Neutralization results using cocktail vaccines in Rabbit Non-Human Primate Studies.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

In one aspect of the invention, it is desired that the consensus antigen provides for improved transcription and translation, including having one or more of the following: low GC content leader sequence to increase transcription; mRNA stability and codon optimization; eliminating to the extent possible cis-acting sequence motifs (i.e., internal TATA-boxes).

In some aspects of the invention, it is desired to generate a consensus antigen that generates a broad immune response across multiple strains, including having one or more of the following: incorporate all available full-length sequences; computer generate sequences that utilize the most commonly occurring amino acid at each position; and increase cross-reactivity between strains.

The attached Sequence Listing includes SEQ ID NOs:1-16 which are various RSV sequences and embodiments.

SEQ ID NO:1 shows a nucleotide sequence encoding an RSV F without start codon (ATG) or signal peptide.

SEQ ID NO:2 shows an amino sequence encoding an RSV F without Met encoded by a start codon (ATG) or signal peptide.

SEQ ID NO:3 shows a nucleotide sequence encoding an RSV M2-1 without start codon (ATG) or signal peptide.

SEQ ID NO:4 shows an amino sequence encoding an RSV M2-1 without Met encoded by a start codon (ATG) or signal peptide.

SEQ ID NO:5 shows a nucleotide sequence encoding an RSV Ga without start codon (ATG) or signal peptide.

SEQ ID NO:6 shows an amino sequence encoding RSV Ga without Met encoded by a start codon (ATG) or signal peptide.

SEQ ID NO:7 shows a nucleotide sequence encoding an RSV Gb without start codon (ATG) or signal peptide.

SEQ ID NO:8 shows an amino sequence encoding an RSV Gb without Met encoded by a start codon (ATG) or signal peptide.

SEQ ID NO:9 shows a nucleotide sequence encoding an RSV F including start codon (ATG)/sequences encoding IgE signal peptide.

SEQ ID NO:10 shows an amino sequence encoding an RSV F with Met encoded by start codon/IgE signal peptide.

SEQ ID NO:11 shows a nucleotide sequence encoding an RSV M2-1 including start codon (ATG)/sequences encoding IgE signal peptide.

SEQ ID NO:12 shows an amino sequence encoding an RSV M2-1 with Met encoded by start codon/IgE signal peptide.

SEQ ID NO:13 shows a nucleotide sequence encoding an RSV Ga including start codon (ATG)/sequences encoding IgE signal peptide.

SEQ ID NO:14 shows an amino sequence encoding an RSV Ga with Met encoded by start codon/IgE signal peptide.

SEQ ID NO:15 shows a nucleotide sequence encoding an RSV Gb including start codon (ATG)/sequences encoding IgE signal peptide.

SEQ ID NO:16 shows an amino sequence encoding an RSV Gb with Met encoded by start codon/IgE signal peptide.

The attached Sequence Listing includes SEQ ID NO:17 which is the amino acid sequence of the IgE signal peptide.

SEQ ID NO:17 shows and amino acid sequence of an IgE signal peptide.

The attached Sequence Listing includes SEQ ID NOs:18-21 which are various CCL20 sequences and embodiments.

SEQ ID NO:18 shows a nucleotide sequence encoding an CCL20 without start codon (ATG) or signal peptide

SEQ ID NO:19 CCL20 without Met encoded by a start codon (ATG) or signal peptide

SEQ ID NO:20 shows a nucleotide sequence encoding an CCL20 including start codon (ATG)/sequences encoding IgE signal peptide

SEQ ID NO:21 CCL20 with Met encoded by start codon/IgE signal peptide

The attached Sequence Listing includes SEQ ID NO:22-31 which are amino acid sequences of derived from RSV immunogens.

SEQ ID NO:22 is a T cell epitope of RSV F Immunogen.

SEQ ID NO:23 is a T cell epitope of RSV F Immunogen.

SEQ ID NO:24 is a T cell epitope of RSV F Immunogen.

SEQ ID NO:25 is a T cell epitope of RSV F Immunogen.

SEQ ID NO:26 is a RSV F Immunogen protease cleavage site.

SEQ ID NO:27 is a RSV F Immunogen protease cleavage site.

SEQ ID NO:28 is a RSV F Immunogen fusion peptide.

SEQ ID NO:29 is a RSV F Immunogen binding site for palivizumab.

SEQ ID NO:30 is a RSV M2-1 Immunodominant T cell epitope.

SEQ ID NO:31is a RSV M2-1 Subdominant T cell epitope.

In some aspects of the invention, RSV F immunogen and sequences that encode the RSV F immunogen are provided. In some aspects of the invention, RSV Ga immunogen and sequences that encode the RSV Ga immunogen are provided. In some aspects of the invention, RSV Gb immunogen and sequences that encode the RSV Gb immunogen are provided. Compositions comprising combinations sequences that encode the RSV F immunogen, sequences that encode the RSV Ga immunogen and sequences that encode the RSV Gb immunogen are provided, including each of sequences that encode the RSV F immunogen, sequences that encode the RSV Ga immunogen and sequences that encode the RSV Gb immunogen.

RSV M2-1 immunogen and sequences that encode the RSV M2-1 immunogen are also provided and my be used alone or in combination with other proteins and/or nucleic acid sequences.

1. Definitions.

The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting. As used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise.

For recitation of numeric ranges herein, each intervening number there between with the same degree of precision is explicitly contemplated. For example, for the range of 6-9, the numbers 7 and 8 are contemplated in addition to 6 and 9, and for the range 6.0-7.0, the numbers 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6,9, and 7.0 are explicitly contemplated.

a. Adjuvant

"Adjuvant" as used herein may mean any molecule added to the DNA plasmid vaccines described herein to enhance antigenicity of the one or more Respiratory Syncytial Virus (RSV.) antigens encoded by the DNA plasmids and encoding nucleic acid sequences described hereinafter.

b. Antibody

"Antibody" may mean an antibody of classes IgG, IgM, IgA, IgD or IgE, or fragments, fragments or derivatives thereof, including Fab, F(ab')2, Fd, and single chain antibodies, diabodies, bispecific antibodies, bifunctional antibodies and derivatives thereof. The antibody may be an antibody isolated from the serum sample of mammal, a polyclonal antibody, affinity purified antibody, or mixtures thereof which exhibits sufficient binding specificity to a desired epitope or a sequence derived therefrom.

c. Coding Sequence

"Coding sequence" or "encoding nucleic acid" as used herein may mean refers to the nucleic acid (RNA or DNA molecule) that comprise a nucleotide sequence which encodes a protein. The coding sequence may further include initiation and termination signals operably linked to regulatory elements including a promoter and polyadenylation signal capable of directing expression in the cells of an individual or mammal to whom the nucleic acid is administered.

In some embodiments, the coding sequence for the amino acid sequence of consensus RSV F immunogen, which is set forth in SEQ ID NO:2, is SEQ ID NO:1. Such coding sequence may optionally further comprise a start codon that encodes an N terminal methionine or a signal peptide such as an IgE or IgG signal peptide. SEQ ID NO:10 corresponds to SEQ ID NO:2 with an IgE signal peptide (SEQ ID NO:17). SEQ ID NO:9 encodes SEQ ID NO:10.

In some embodiments, the coding sequence for the amino acid sequence of consensus RSV Ga immunogen, which is set forth in SEQ ID NO:6, is SEQ ID NO:5. Such coding sequence may optionally further comprise a start codon that encodes an N terminal methionine or a signal peptide such as an IgE or IgG signal peptide. SEQ ID NO:14 corresponds to SEQ ID NO:6 with an IgE signal peptide (SEQ ID NO:17). SEQ ID NO:13 encodes SEQ ID NO:14.

In some embodiments, the coding sequence for the amino acid sequence of consensus RSV Gb immunogen, which is set forth in SEQ ID NO:8, is SEQ ID NO:7. Such coding sequence may optionally further comprise a start codon that encodes an N terminal methionine or a signal peptide such as an IgE or IgG signal peptide. SEQ ID NO:16 corresponds to SEQ ID NO:8 with an IgE signal peptide (SEQ ID NO:17). SEQ ID NO:15 encodes SEQ ID NO:16.

Also provides is a coding sequence, SEQ ID NO:3, which encodes the amino acid sequence of consensus RSV M2-1 immunogen, which is set forth in SEQ ID NO:4. Such coding sequence may optionally further comprise a start codon that encodes an N terminal methionine or a signal peptide such as an IgE or IgG signal peptide. SEQ ID NO:12 corresponds to SEQ ID NO:4 with an IgE signal peptide (SEQ ID NO:17). SEQ ID NO:11 encodes SEQ ID NO:12.

d. Complement

"Complement" or "complementary" as used herein may mean a nucleic acid may mean Watson-Crick (*e.g.*, A-T/U and C-G) or Hoogsteen base pairing between nucleotides or nucleotide analogs of nucleic acid molecules.

e. Consensus or Consensus Sequence

"Consensus" or "consensus sequence" as used herein may mean a synthetic nucleic acid sequence, or corresponding polypeptide sequence, constructed based on analysis of an alignment of multiple subtypes of a particular RSV antigen, that can be used to induce broad immunity against multiple subtypes or serotypes of a particular RSV antigen. Consensus RSV antigens

may include consensus amino acid sequences of protein F, protein G and protein M2. Nucleotide sequences that encode the consensus amino acid sequences are also provided. Also, synthetic antigens such as fusion proteins may be manipulated to include consensus sequences (or consensus antigens).

The amino acid sequence of consensus RSV F immunogen is set forth in SEQ ID NO:2. Such sequence may optionally comprise an N terminal methionine or a signal peptide such as an IgE or IgG signal peptide. SEQ ID NO:10 corresponds to SEQ ID NO:2 with an IgE signal peptide (SEQ ID NO:17).

The amino acid sequence of consensus RSV Ga immunogen is set forth in SEQ ID NO:6. Such sequence may optionally comprise an N terminal methionine or a signal peptide such as an IgE or IgG signal peptide. SEQ ID NO:14 corresponds to SEQ ID NO:6 with an IgE signal peptide (SEQ ID NO:17).

The amino acid sequence of consensus RSV Gb immunogen is set forth in SEQ ID NO:8. Such sequence may optionally comprise an N terminal methionine or a signal peptide such as an IgE or IgG signal peptide. SEQ ID NO:16 corresponds to SEQ ID NO:8 with an IgE signal peptide (SEQ ID NO:17).

Also provided is the amino acid sequence of consensus RSV M2-1 immunogen which is set forth in SEQ ID NO:4. Such sequence may optionally comprise an N terminal methionine or a signal peptide such as an IgE or IgG signal peptide. SEQ ID NO:12 corresponds to SEQ ID NO:4 with an IgE signal peptide (SEQ ID NO:17).

f. Constant Current

"Constant current" as used herein to define a current that is received or experienced by a tissue, or cells defining said tissue, over the duration of an electrical pulse delivered to same tissue. The electrical pulse is delivered from the electroporation devices described herein. This current remains at a constant amperage in said tissue over the life of an electrical pulse because the electroporation device provided herein has a feedback element, preferably having instantaneous feedback. The feedback element can measure the resistance of the tissue (or cells) throughout the duration of the pulse and cause the electroporation device to alter its electrical energy output (e.g., increase voltage) so current in same tissue remains constant throughout the

electrical pulse (on the order of microseconds), and from pulse to pulse. In some embodiments, the feedback element comprises a controller.

g. Current Feedback or Feedback

"Current feedback" or "feedback" as used herein may be used interchangeably and may mean the active response of the provided electroporation devices, which comprises measuring the current in tissue between electrodes and altering the energy output delivered by the EP device accordingly in order to maintain the current at a constant level. This constant level is preset by a user prior to initiation of a pulse sequence or electrical treatment. The feedback may be accomplished by the electroporation component, e.g., controller, of the electroporation device, as the electrical circuit therein is able to continuously monitor the current in tissue between electrodes and compare that monitored current (or current within tissue) to a preset current and continuously make energy-output adjustments to maintain the monitored current at preset levels. The feedback loop may be instantaneous as it is an analog closed-loop feedback.

h. Decentralized Current

"Decentralized current" as used herein may mean the pattern of electrical currents delivered from the various needle electrode arrays of the electroporation devices described herein, wherein the patterns minimize, or preferably eliminate, the occurrence of electroporation related heat stress on any area of tissue being electroporated.

i. Electroporation

"Electroporation," "electro-permeabilization," or "electro-kinetic enhancement" ("EP") as used interchangeably herein may refer to the use of a transmembrane electric field pulse to induce microscopic pathways (pores) in a bio-membrane; their presence allows biomolecules such as plasmids, oligonucleotides, siRNA, drugs, ions, and water to pass from one side of the cellular membrane to the other.

j. Feedback Mechanism

"Feedback mechanism" as used herein may refer to a process performed by either software or hardware (or firmware), which process receives and compares the impedance of the desired tissue (before, during, and/or after the delivery of pulse of energy) with a present value,

preferably current, and adjusts the pulse of energy delivered to achieve the preset value. A feedback mechanism may be performed by an analog closed loop circuit.

k. Fragment

"Fragment" may mean a polypeptide fragment of an RSV Immunogen that is capable of eliciting an immune response in a mammal against RSV by recognizing the particular RSV antigen. An RSV Immunogen is refers to the consensus sequences set forth herein for RSV F Immunogen, RSV M2-1 Immunogen, RSV Ga Immunogen, RSV Gb Immunogen, in each case with or without signal peptides and/or a methionine at position 1, proteins 98% or more homologous to the consensus sequences set forth herein, proteins 99% or more homologous to the consensus sequences set forth herein, and proteins 100% identical to the consensus sequences set forth herein, in each case with or without signal peptides and/or a methionine at position 1. A fragment may or may not for example comprise a fragments of an RSV Immunogen linked to a signal peptide such as an immunoglobulin signal peptide for example IgE signal peptide (SEQ ID NO:17) or IgG signal peptide.

A fragment of RSV F consensus Immunogen may be a fragment of SEQ ID NO:2, of SEQ ID NO:10, of a protein 98% or more homologous to the RSV F consensus Immunogen sequences set forth herein, of a protein 99% or more homologous to the RSV F consensus Immunogen sequences set forth herein, or of RSV F consensus Immunogen 100% identical to the consensus sequences set forth herein, in each case with or without signal peptides and/or a methionine at position 1. Such fragments may comprise 20% or more, 25% or more, 30% or more, 35% or more, 40% or more, 45% or more, 50% or more, 55% or more, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 91% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, 99% or more percent of the length of the particular full length RSV F consensus Immunogen sequence set forth herein, excluding any non-RSV signal peptide added. Fragments refer to fragments of a polypeptide that is 98% or more, or 99% or more homologous to the sequences of the RSV F consensus Immunogen set forth herein and 100% identical to such sequence, in each case with or without signal peptides and/or a methionine at position 1. The fragment may comprise a fragment of a polypeptide that is 98% or more homologous, 99% or

more homologous, or 100% identical to the RSV F consensus Immunogen and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. In some embodiments, a fragment of RSV F Immunogen may comprise a fragment of a SEQ ID NO:2. The fragment may comprise a fragments of SEQ ID NO:2 linked to a signal peptide such as an immunoglobulin signal peptide for example IgE signal peptide (SEQ ID NO:17) or IgG signal peptide. Thus in some embodiments, the fragment is a fragment of SEQ ID NO:10 which comprises SEQ ID NO:17. The fragment may comprise fragments of SEQ ID NO:2 linked to an N terminal methionine. Fragments also refer to fragments of a polypeptide that is 98% or more, or 99% or more homologous to SEQ ID NO:2 . The fragment may comprise a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:2 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may comprise a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:2 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may comprise a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:2 and additionally comprise an N terminal methionine which is not included when calculating percent homology. The fragment may comprise a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:2 and additionally comprise an N terminal methionine which is not included when calculating percent homology. The RSV F Immunogen is 575 amino acids. In some embodiments, the fragments thereof may be 115 or more amino acids in length, 163 or more, 230 or more, 288 or more, 345 or more, 403 or more, 460 or more, 515 or more, 520 or more, 525 or more, 530 or more, 535 or more, 540 or more, 545 or more, 550 or more, 555 or more, 560 or more, 565 or more, 570 or more, 574 or more in length. Polypeptide fragments may be fewer than 140 amino acids, fewer than 190, fewer than 250, fewer than 283, fewer than 300, fewer than 365, fewer than 425, fewer than 495, fewer than 518, fewer than 523, fewer than 528, fewer than 533, fewer than 547, fewer than 552, fewer than 563, or fewer than 572 in length. Fragments may further comprise an N terminal methionine and/or a signal peptide such as an immunoglobulin signal peptide, for example an IgE or IgG signal peptide. The N terminal methionine and/or signal peptide may be linked to a

574 amino acid or smaller fragment thereof. The N terminal methionine and/or signal peptide may be linked to a polypeptide that is 98% homologous to the 574 amino acid or to a smaller fragment of a polypeptide that is 98% homologous to the 574 amino acid sequence. The N terminal methionine and/or signal peptide may be linked to a polypeptide that is 99% homologous to the 574 amino acid sequence or to a smaller fragment of a polypeptide that is 99% homologous to the 574 amino acid sequence. In calculating degrees of homology a polypeptide has to SEQ ID NO:2 or a fragment thereof, an N terminal methionine and/or any signal peptide is not included in such calculation. The sequences of the signal peptide are not used in determining homology. Thus, for example, although SEQ ID NO:10 comprises SEQ ID NO:2 operably linked to a signal peptide SEQ ID NO:17, SEQ ID NO:10 comprises a fragment of SEQ ID NO:2, that is 100% homologous to a fragment of SEQ ID NO:2, notwithstanding the signal peptide which is absent in SEQ ID NO:2. Thus, proteins which comprise fragments of a polypeptide that is at least 98% homologous to a fragment of SEQ ID NO:2 intended to refer to proteins which fragments of a polypeptide that is at least 98% homologous to a fragment of SEQ ID NO:2 that are at least 115 amino acids and may optionally be linked to a, for example, a signal peptide. SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, and SEQ ID NO:25 correspond to T cell epitopes of RSV F immunogen set forth in SEQ ID NO:2. In some embodiments, fragments comprise all or part of one or more of these sequences or sequences 98% or more or 99% or more homologous to them. SEQ ID NO:26 and SEQ ID NO:27 are RSV F Immunogen protease cleavage sites. SEQ ID NO:28 is an RSV F Immunogen fusion peptide. SEQ ID NO:29 is the RSV F Immunogen binding site for palivizumab.

A fragment of RSV Ga consensus Immunogen may be a fragment of SEQ ID NO:6, of SEQ ID NO:14, of a protein 98% or more homologous to the RSV Ga consensus Immunogen sequences set forth herein, of a protein 99% or more homologous to the RSV Ga consensus Immunogen sequences set forth herein, or of RSV Ga consensus Immunogen 100% identical to the consensus sequences set forth herein, in each case with or without signal peptides and/or a methionine at position 1. Such fragments may comprise may comprise 20% or more, 25% or more, 30% or more, 35% or more, 40% or more, 45% or more, 50% or more, 55% or more, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more,

91% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, 99% or more percent of the length of the particular full length RSV Ga consensus Immunogen sequence set forth herein, excluding any non-RSV signal peptide added. Fragments refer to fragments of a polypeptide that is 98% or more, or 99% or more homologous to the sequences of the RSV Ga consensus Immunogen set forth herein and 100% identical to such sequence, in each case with or without signal peptides and/or a methionine at position 1. The fragment may comprise a fragment of a polypeptide that is 98% or more homologous, 99% or more homologous, or 100% identical to the RSV Ga consensus Immunogen and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. In some embodiments, a fragment of RSV Ga Immunogen may comprise a fragment of SEQ ID NO:6. The fragment may comprise a fragments of SEQ ID NO:6 linked to a signal peptide such as an immunoglobulin signal peptide for example IgE signal peptide (SEQ ID NO:17) or IgG signal peptide. Thus in some embodiments, the fragment is a fragment of SEQ ID NO:14 which comprises SEQ ID NO:17. The fragment may comprise fragments of SEQ ID NO:6 linked to an N terminal methionine. Fragments also refer to fragments of a polypeptide that is 98% or more, or 99% or more homologous to SEQ ID NO:6. The fragment may comprise a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:6 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may comprise a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:6 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may comprise a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:6 and additionally comprise an N terminal methionine which is not included when calculating percent homology. The fragment may comprise a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:6 and additionally comprise an N terminal methionine which is not included when calculating percent homology. The RSV Ga Immunogen is 297 amino acids excluding an N terminal methionine and/or signal peptide. In some embodiments, the fragments thereof may be 125 or more amino acids in length, 130 or more, 140 or more, 150 or more, 160 or more, 170 or more, 180 or more, 190 or more,

200 or more, 210 or more, 220 or more, 230 or more, 240 or more, 250 or more, 260 or more, 270 or more, 280 or more, 285 or more, 290 or more, 295 or more in length. Polypeptide fragments may be fewer than 135 amino acids, fewer than 145, fewer than 155, fewer than 165, fewer than 175, fewer than 185, fewer than 195, fewer than 205, fewer than 215, fewer than 225, fewer than 235, fewer than 245, fewer than 255, fewer than 265, fewer than 275, fewer than 285, fewer than 288, fewer than 292, or fewer than 296 in length. Fragments may further comprise an N terminal methionine and/or a signal peptide such as an immunoglobulin signal peptide, for example an IgE or IgG signal peptide. The N terminal methionine and/or signal peptide may be linked to a 296 amino acid or smaller fragment thereof. The N terminal methionine and/or signal peptide may be linked to a polypeptide that is 98% homologous to the 296 amino acid or to a smaller fragment of a polypeptide that is 98% homologous to the 296 amino acid sequence. The N terminal methionine and/or signal peptide may be linked to a polypeptide that is 99% homologous to the 296 amino acid sequence or to a smaller fragment of a polypeptide that is 99% homologous to the 296 amino acid sequence. In calculating degrees of homology a polypeptide has to SEQ ID NO:6 or a fragment thereof, an N terminal methionine and/or any signal peptide is not included in such calculation. The sequences of the signal peptide are not used in determining homology. Thus, for example, although SEQ ID NO:14 comprises SEQ ID NO:6 operably linked to a signal peptide SEQ ID NO:17, SEQ ID NO:14 comprises a fragment of SEQ ID NO:6, that is 100% homologous to a fragment of SEQ ID NO:6, notwithstanding the signal peptide which is absent in SEQ ID NO:6. Thus, proteins which comprise fragments of a polypeptide that is at least 98% homologous to a fragment of SEQ ID NO:6 intended to refer to proteins which fragments of a polypeptide that is at least 98% homologous to a fragment of SEQ ID NO:6 that are at least 125 amino acids and may optionally be linked to a, for example, a signal peptide.

A fragment of RSV Gb consensus Immunogen such as that which is disclosed in SEQ ID NO:8, SEQ ID NO:16, proteins 98% or more homologous to the RSV Gb consensus Immunogen sequences set forth herein, proteins 99% or more homologous to the RSV Gb consensus Immunogen sequences set forth herein, and RSV Gb consensus Immunogen 100% identical to the consensus sequences set forth herein, in each case with or without signal peptides and/or a

methionine at position 1, may comprise 20% or more, 25% or more, 30% or more, 35% or more, 40% or more, 45% or more, 50% or more, 55% or more, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 91% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, 99% or more percent of the length of the particular full length RSV Gb consensus Immunogen sequence set forth herein, excluding any non-RSV signal peptide added. Fragments refer to fragments of a polypeptide that is 98% or more, or 99% or more homologous to the sequences of the RSV Gb consensus Immunogen set forth herein and 100% identical to such sequence, in each case with or without signal peptides and/or a methionine at position 1. The fragment may comprise a fragment of a polypeptide that is 98% or more homologous, 99% or more homologous, or 100% identical to the RSV Gb consensus Immunogen and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. In some embodiments, a fragment of RSV Gb Immunogen may comprise a fragment of SEQ ID NO:8. The fragment may comprise a fragments of SEQ ID NO:8 linked to a signal peptide such as an immunoglobulin signal peptide for example IgE signal peptide (SEQ ID NO:17) or IgG signal peptide. Thus in some embodiments, the fragment is a fragment of SEQ ID NO:16 which comprises SEQ ID NO:17. The fragment may comprise fragments of SEQ ID NO:8 linked to an N terminal methionine. Fragments also refer to fragments of a polypeptide that is 98% or more, or 99% or more homologous to SEQ ID NO:8. The fragment may comprise a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:8 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may comprise a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:8 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may comprise a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:8 and additionally comprise an N terminal methionine which is not included when calculating percent homology. The fragment may comprise a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:8 and additionally comprise an N terminal methionine which is not included when calculating percent homology. The RSV Gb Immunogen is 291

amino acids excluding an N terminal methionine and/or signal peptide. In some embodiments, the fragments thereof may be 125 or more amino acids in length, 130 or more, 140 or more, 150 or more, 160 or more, 170 or more, 180 or more, 190 or more, 200 or more, 210 or more, 220 or more, 230 or more, 240 or more, 250 or more, 260 or more, 270 or more, 280 or more, 285 or more, 288 or more in length. Polypeptide fragments may be fewer than 135 amino acids, fewer than 145, fewer than 155, fewer than 165, fewer than 175, fewer than 185, fewer than 195, fewer than 205, fewer than 215, fewer than 225, fewer than 235, fewer than 245, fewer than 255, fewer than 265, fewer than 275, fewer than 285, fewer than 288, fewer than 290 in length. Fragments may further comprise an N terminal methionine and/or a signal peptide such as an immunoglobulin signal peptide, for example an IgE or IgG signal peptide. The N terminal methionine and/or signal peptide may be linked to a 290 amino acid or smaller fragment thereof. The N terminal methionine and/or signal peptide may be linked to a polypeptide that is 98% homologous to the 290 amino acid or to a smaller fragment of a polypeptide that is 98% homologous to the 290 amino acid sequence. The N terminal methionine and/or signal peptide may be linked to a polypeptide that is 99% homologous to the 290 amino acid sequence or to a smaller fragment of a polypeptide that is 99% homologous to the 290 amino acid sequence. In calculating degrees of homology a polypeptide has to SEQ ID NO:6 or a fragment thereof, an N terminal methionine and/or any signal peptide is not included in such calculation. The sequences of the signal peptide are not used in determining homology. Thus, for example, although SEQ ID NO:16 comprises SEQ ID NO:8 operably linked to a signal peptide SEQ ID NO:17, SEQ ID NO:16 comprises a fragment of SEQ ID NO:8, that is 100% homologous to a fragment of SEQ ID NO:8, notwithstanding the signal peptide which is absent in SEQ ID NO:8. Thus, proteins which comprise fragments of a polypeptide that is at least 98% homologous to a fragment of SEQ ID NO:8 intended to refer to proteins which fragments of a polypeptide that is at least 98% homologous to a fragment of SEQ ID NO:8 that are at least 125 amino acids and may optionally be linked to a, for example, a signal peptide.

In addition, a fragment of RSV M2-1 consensus Immunogen may be a fragment of SEQ ID NO:4, of SEQ ID NO:12, of a protein 98% or more homologous to the RSV M2-1 consensus Immunogen sequences set forth herein, of a protein 99% or more homologous to the RSV M2-1

consensus Immunogen sequences set forth herein, or of RSV M2-1 consensus Immunogen 100% identical to the consensus sequences set forth herein, in each case with or without signal peptides and/or a methionine at position 1. Such fragments may comprise may comprise 20% or more, 25% or more, 30% or more, 35% or more, 40% or more, 45% or more, 50% or more, 55% or more, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 91% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, 99% or more percent of the length of the particular full length RSV M2-1 consensus Immunogen sequence set forth herein, excluding any non-RSV signal peptide added. Fragments refer to fragments of a polypeptide that is 98% or more, or 99% or more homologous to the sequences of the RSV M2-1 consensus Immunogen set forth herein and 100% identical to such sequence, in each case with or without signal peptides and/or a methionine at position 1. The fragment may comprise a fragment of a polypeptide that is 98% or more homologous, 99% or more homologous, or 100% identical to the RSV M2-1 consensus Immunogen and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. In some embodiments, a fragment of RSV M2-1 Immunogen may comprise a fragment of a SEQ ID NO:4. The fragment may comprise a fragments of SEQ ID NO:4 linked to a signal peptide such as an immunoglobulin signal peptide for example IgE signal peptide (SEQ ID NO:17) or IgG signal peptide. Thus in some embodiments, the fragment is a fragment of SEQ ID NO:12 which comprises SEQ ID NO:17. The fragment may comprise fragments of SEQ ID NO:4 linked to an N terminal methionine. Fragments also refer to fragments of a polypeptide that is 98% or more, or 99% or more homologous to SEQ ID NO:4. The fragment may comprise a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:4 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may comprise a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:4 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may comprise a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:4 and additionally comprise an N terminal methionine which is not included when

calculating percent homology. The fragment may comprise a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:4 and additionally comprise an N terminal methionine which is not included when calculating percent homology. The RSV M2-1 Immunogen is 195 amino acids. In some embodiments, the fragments thereof may be 25 or more amino acids in length, 30 or more, 40 or more, 50 or more, 60 or more, 70 or more, 80 or more, 90 or more, 100 or more, 110 or more, 120 or more, 130 or more, 140 or more, 150 or more, 160 or more, 170 or more, 180 or more, 184 or more, 190 or more, 193 or more in length. Polypeptide fragments may be fewer than 35 amino acids, fewer than 45, fewer than 55, fewer than 65, fewer than 75, fewer than 85, fewer than 95, fewer than 105, fewer than 115, fewer than 125, fewer than 135, fewer than 145, fewer than 155, fewer than 165, fewer than 175, fewer than 185, fewer than 188, fewer than 192, or fewer than 194 in length. Fragments may further comprise an N terminal methionine and/or a signal peptide such as an immunoglobulin signal peptide, for example an IgE or IgG signal peptide. The N terminal methionine and/or signal peptide may be linked to a 194 amino acid or smaller fragment thereof. The N terminal methionine and/or signal peptide may be linked to a polypeptide that is 98% homologous to the 194 amino acid or to a smaller fragment of a polypeptide that is 98% homologous to the 194 amino acid sequence. The N terminal methionine and/or signal peptide may be linked to a polypeptide that is 99% homologous to the 194 amino acid sequence or to a smaller fragment of a polypeptide that is 99% homologous to the 194 amino acid sequence. In calculating degrees of homology a polypeptide has to SEQ ID NO:4 or a fragment thereof, an N terminal methionine and/or any signal peptide is not included in such calculation. The sequences of the signal peptide are not used in determining homology. Thus, for example, although SEQ ID NO:12 comprises SEQ ID NO:4 operably linked to a signal peptide SEQ ID NO:17, SEQ ID NO:12 comprises a fragment of SEQ ID NO:4, that is 100% homologous to a fragment of SEQ ID NO:4, notwithstanding the signal peptide which is absent in SEQ ID NO:4. Thus, proteins which comprise fragments of a polypeptide that is at least 98% homologous to a fragment of SEQ ID NO:4 intended to refer to proteins which fragments of a polypeptide that is at least 98% homologous to a fragment of SEQ ID NO:4 that are at least 25 amino acids and may optionally be linked to a, for example, a signal peptide. SEQ ID NO:30 corresponds to an immunodominant T cell epitope of RSV M2-1

immunogen set forth in SEQ ID NO:4. SEQ ID NO:31 refers to a subdominant T cell epitope corresponds to an immunodominant T cell epitope of RSV M2-1 immunogen set forth in SEQ ID NO:4. In some embodiments, fragments comprise all or part of one or more of these sequences or sequences 98% or more or 99% or more homologous to them.

"Fragment" may also mean a nucleic acid fragment of that encodes an RSV Immunogen fragment set forth above, i.e. RSV F Immunogen, RSV M2-1 Immunogen, RSV Ga Immunogen, RSV Gb Immunogen, in each case with or without signal peptides and/or a methionine at position 1, proteins 98% or more homologous to the consensus sequences set forth herein, proteins 99% or more homologous to the consensus sequences set forth herein, and proteins 100% identical to the consensus sequences set forth herein, in each case with or without signal peptides and/or a methionine at position 1. A fragment may or may not for example comprise a fragments of nucleic acid sequences that encode an RSV Immunogen linked to coding sequence for an N terminal methionine (i.e. a start codon) or coding sequences encoding a signal peptide such as an immunoglobulin signal peptide for example IgE signal peptide (SEQ ID NO:17) or IgG signal peptide. A fragment of a coding sequence that encodes an RSV Immunogen may comprise 20% or more, 25% or more, 30% or more, 35% or more, 40% or more, 45% or more, 50% or more, 55% or more, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 91% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, 99% or more percent of the length of coding sequence of the particular full length RSV Immunogen sequence set forth herein, excluding any coding sequence that encodes non-RSV signal peptide added. Fragments also refer to fragments of coding sequences which encode a polypeptide that is 98% or more, or 99% or more homologous to the sequences of the RSV Immunogen set forth herein. The fragment may comprise a fragment of a polypeptide that is 98% or more homologous to and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology.

In some embodiments, the fragments are fragments of a nucleic acid sequence 98% or more homologous to SEQ ID NO1. In some embodiments, the fragments are fragments of a nucleic acid sequence 99% or more homologous to SEQ ID NO:1. In some embodiments, the

fragments are fragments of SEQ ID NO:1. It is intended that nucleic acid sequences encoding the polypeptide fragments described herein are expressly disclosed, i.e. fragments of nucleic acid sequences that encode the proteins having the size and homology of the peptide fragments. In some embodiments, fragments of nucleic acid sequences that encode the proteins having the size and homology of the peptide fragments are fragments of SEQ ID NO:1. A fragment of a coding sequence that encodes RSV F consensus Immunogen such as that which is disclosed in SEQ ID NO:2, SEQ ID NO:10, proteins 98% or more homologous to the RSV F consensus Immunogen sequences set forth herein, proteins 99% or more homologous to the RSV F consensus Immunogen sequences set forth herein, and RSV F consensus Immunogen 100% identical to the consensus sequences set forth herein, in each case with or without signal peptides and/or a methionine at position 1, such as for example SEQ ID NO:1 or SEQ ID NO:9, may comprise 20% or more, 25% or more, 30% or more, 35% or more, 40% or more, 45% or more, 50% or more, 55% or more, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 91% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, 99% or more percent of the length of the particular full length RSV F consensus Immunogen sequence set forth herein, excluding any non-RSV signal peptide added. Fragments may encode fragments of a polypeptide that is 98% or more, or 99% or more homologous to the sequences of the RSV F consensus Immunogen set forth herein and 100% identical to such sequence, in each case with or without signal peptides and/or a methionine at position 1. The fragment may encode a fragment of a polypeptide that is 98% or more homologous, 99% or more homologous, or 100% identical to the RSV F consensus Immunogen and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. In some embodiments, a fragment that encodes RSV F Immunogen may encode a fragment of a SEQ ID NO:2 and comprise a fragment of SEQ ID NO:1. The fragment may encode fragments of SEQ ID NO:2 linked to a signal peptide such as an immunoglobulin signal peptide for example IgE signal peptide (SEQ ID NO:17) or IgG signal peptide. Thus in some embodiments, the fragment is a fragment of SEQ ID NO:10 which comprises SEQ ID NO:17 and may be encoded by SEQ ID NO:9. The fragment may comprise fragments of SEQ ID NO:1 linked to start codon. Fragments

also encode fragments of a polypeptide that is 98% or more, or 99% or more homologous to SEQ ID NO:2. The fragment may encode a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:2 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may encode a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:2 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may encode a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:2 and additionally comprise an N terminal methionine which is not included when calculating percent homology. The fragment may encode a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:2 and additionally comprise an N terminal methionine which is not included when calculating percent homology. Fragments may be 98% or more, or 99% or more homologous to SEQ ID NO:1. The fragment may be 98% or more homologous to SEQ ID NO:1 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may be 99% or more homologous to SEQ ID NO:1 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may be 98% or more homologous to SEQ ID NO:1 and additionally comprise an N terminal methionine which is not included when calculating percent homology. The fragment may be 99% or more homologous to SEQ ID NO:1 and additionally comprise an N terminal methionine which is not included when calculating percent homology. Fragments of SEQ ID NO:1, fragments of nucleic acid sequence 98% or more homologous to SEQ ID NO:1, and fragments of nucleic acid sequence 99% or more homologous to SEQ ID NO:1, in each case with or without start codons or coding sequences that encode signal peptides may encode fragments of RSV F immunogen that may be 115 or more amino acids in length, 163 or more, 230 or more, 288 or more, 345 or more, 403 or more, 460 or more, 515 or more, 520 or more, 525 or more, 530 or more, 535 or more, 540 or more, 545 or more, 550 or more, 555 or more, 560 or more, 565 or more, 570 or more, 574 or more in length. Polypeptide fragments may be fewer than 140 amino acids, fewer than 190, fewer than 250, fewer than 283, fewer than 300, fewer than 365, fewer than 425, fewer than 495,

fewer than 518, fewer than 523, fewer than 528, fewer than 533, fewer than 547, fewer than 552, fewer than 563, or fewer than 572 in length. Fragments may further encode an N terminal methionine and/or a signal peptide such as an immunoglobulin signal peptide, for example an IgE or IgG signal peptide. The N terminal methionine and/or signal peptide may be linked to sequences that encode a 574 amino acid or smaller fragment thereof. The fragment may encode an N terminal methionine and/or signal peptide linked to a polypeptide that is 98% homologous to the 574 amino acid or to a smaller fragment of a polypeptide that is 98% homologous to the 574 amino acid sequence. The fragment may encode an N terminal methionine and/or signal peptide linked to a polypeptide that is 99% homologous to the 574 amino acid sequence or to a smaller fragment of a polypeptide that is 99% homologous to the 574 amino acid sequence. In calculating degrees of homology a polypeptide has to SEQ ID NO:2 or a fragment thereof, an N terminal methionine and/or any signal peptide is not included in such calculation. The sequences of the signal peptide are not used in determining homology. Thus, for example, although SEQ ID NO:7 comprises SEQ ID NO:1 operably linked to a coding sequences that encode signal peptide SEQ ID NO:17, SEQ ID NO:7 comprises a fragment of SEQ ID NO:1 that is 100% homologous to a fragment of SEQ ID NO:1, notwithstanding the signal peptide coding sequence which is absent in SEQ ID NO:1. SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, and SEQ ID NO:25 correspond to T cell epitopes of RSV F immunogen set forth in SEQ ID NO:2. In some embodiments, fragments comprise coding sequences that encode all or part of one or more of these sequences or sequences 98% or more or 99% or more homologous to them including fragments of SEQ ID NO:1 or sequences that are 98% or more or 99% or more homologous to SEQ ID NO:1.

In some embodiments, the fragments are fragments of a nucleic acid sequence 98% or more homologous to SEQ ID NO:5. In some embodiments, the fragments are fragments of a nucleic acid sequence 99% or more homologous to SEQ ID NO:5. In some embodiments, the fragments are fragments of SEQ ID NO:5. It is intended that nucleic acid sequences encoding the polypeptide fragments described herein are expressly disclosed, i.e. fragments of nucleic acid sequences that encode the proteins having the size and homology of the peptide fragments. In some embodiments, fragments of nucleic acid sequences that encode the proteins having the size

and homology of the peptide fragments are fragments of SEQ ID NO:5. A fragment of a coding sequence that encodes RSV Ga consensus Immunogen such as that which is disclosed in SEQ ID NO:6, SEQ ID NO:14, proteins 98% or more homologous to the RSV Ga consensus Immunogen sequences set forth herein, proteins 99% or more homologous to the RSV Ga consensus Immunogen sequences set forth herein, and RSV Ga consensus Immunogen 100% identical to the consensus sequences set forth herein, in each case with or without signal peptides and/or a methionine at position 1, such as for example SEQ ID NO:5 or SEQ ID NO:13, may comprise 20% or more, 25% or more, 30% or more, 35% or more, 40% or more, 45% or more, 50% or more, 55% or more, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 91% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, 99% or more percent of the length of the particular full length RSV Ga consensus Immunogen sequence set forth herein, excluding any non-RSV signal peptide added. Fragments may encode fragments of a polypeptide that is 98% or more, or 99% or more homologous to the sequences of the RSV Ga consensus Immunogen set forth herein and 100% identical to such sequence, in each case with or without signal peptides and/or a methionine at position 1. The fragment may encode a fragment of a polypeptide that is 98% or more homologous, 99% or more homologous, or 100% identical to the RSV Ga consensus Immunogen and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. In some embodiments, a fragment that encodes RSV Ga Immunogen may encode a fragment of a SEQ ID NO:6 and comprise a fragment of SEQ ID NO:5. The fragment may encode fragments of SEQ ID NO:6 linked to a signal peptide such as an immunoglobulin signal peptide for example IgE signal peptide (SEQ ID NO:17) or IgG signal peptide. Thus in some embodiments, the fragment is a fragment of SEQ ID NO:14 which comprises SEQ ID NO:17 and may be encoded by SEQ ID NO:13. The fragment may comprise fragments of SEQ ID NO:5 linked to start codon. Fragments also encode fragments of a polypeptide that is 98% or more, or 99% or more homologous to SEQ ID NO:6. The fragment may encode a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:6 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The

fragment may encode a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:6 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may encode a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:6 and additionally comprise an N terminal methionine which is not included when calculating percent homology. The fragment may encode a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:6 and additionally comprise an N terminal methionine which is not included when calculating percent homology. Fragments may be 98% or more, or 99% or more homologous to SEQ ID NO:5. The fragment may be 98% or more homologous to SEQ ID NO:5 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may be 99% or more homologous to SEQ ID NO:5 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may be 98% or more homologous to SEQ ID NO:5 and additionally comprise an N terminal methionine which is not included when calculating percent homology. The fragment may be 99% or more homologous to SEQ ID NO:5 and additionally comprise an N terminal methionine which is not included when calculating percent homology. Fragments of SEQ ID NO:5, fragments of nucleic acid sequence 98% or more homologous to SEQ ID NO:5, and fragments of nucleic acid sequence 99% or more homologous to SEQ ID NO:5, in each case with or without start codons or coding sequences that encode signal peptides may encode fragments of RSV Ga immunogen that may be In some embodiments, the fragments thereof may be 125 or more amino acids in length, 130 or more, 140 or more, 150 or more, 160 or more, 170 or more, 180 or more, 190 or more, 200 or more, 210 or more, 220 or more, 230 or more, 240 or more, 250 or more, 260 or more, 270 or more, 280 or more, 285 or more, 290 or more, 295 or more in length. Polypeptide fragments may be fewer than 135 amino acids, fewer than 145, fewer than 155, fewer than 165, fewer than 175, fewer than 185, fewer than 195, fewer than 205, fewer than 215, fewer than 225, fewer than 235, fewer than 245, fewer than 255, fewer than 265, fewer than 275, fewer than 285, fewer than 288, fewer than 292, or fewer than 296 in length. Fragments may further encode an N terminal methionine and/or a signal peptide such as an immunoglobulin signal peptide, for example an

IgE or IgG signal peptide. The N terminal methionine and/or signal peptide may be linked to sequences that encode a 296 amino acid or smaller fragment thereof. The fragment may encode an N terminal methionine and/or signal peptide linked to a polypeptide that is 98% homologous to the 296 amino acid or to a smaller fragment of a polypeptide that is 98% homologous to the 296 amino acid sequence. The fragment may encode an N terminal methionine and/or signal peptide linked to a polypeptide that is 99% homologous to the 296 amino acid sequence or to a smaller fragment of a polypeptide that is 99% homologous to the 296 amino acid sequence. In calculating degrees of homology a polypeptide has to SEQ ID NO:6 or a fragment thereof, an N terminal methionine and/or any signal peptide is not included in such calculation. The sequences of the signal peptide are not used in determining homology. Thus, for example, although SEQ ID NO:13 comprises SEQ ID NO:5 operably linked to a coding sequences that encode signal peptide SEQ ID NO:17, SEQ ID NO:13 comprises a fragment of SEQ ID NO:5 that is 100% homologous to a fragment of SEQ ID NO:5, notwithstanding the signal peptide coding sequence which is absent in SEQ ID NO:5.

In some embodiments, the fragments are fragments of a nucleic acid sequence 98% or more homologous to SEQ ID NO:7. In some embodiments, the fragments are fragments of a nucleic acid sequence 99% or more homologous to SEQ ID NO:7. In some embodiments, the fragments are fragments of SEQ ID NO:7. It is intended that nucleic acid sequences encoding the polypeptide fragments described herein are expressly disclosed, i.e. fragments of nucleic acid sequences that encode the proteins having the size and homology of the peptide fragments. In some embodiments, fragments of nucleic acid sequences that encode the proteins having the size and homology of the peptide fragments are fragments of SEQ ID NO:7. A fragment of a coding sequence that encodes RSV Gb consensus Immunogen such as that which is disclosed in SEQ ID NO:8, SEQ ID NO:16, proteins 98% or more homologous to the RSV Gb consensus Immunogen sequences set forth herein, proteins 99% or more homologous to the RSV Gb consensus Immunogen sequences set forth herein, and RSV Gb consensus Immunogen 100% identical to the consensus sequences set forth herein, in each case with or without signal peptides and/or a methionine at position 1, such as for example SEQ ID NO:7 or SEQ ID NO:15, may comprise 20% or more, 25% or more, 30% or more, 35% or more, 40% or more, 45% or more, 50% or

more, 55% or more, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 91% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, 99% or more percent of the length of the particular full length RSV Gb consensus Immunogen sequence set forth herein, excluding any non-RSV signal peptide added. Fragments may encode fragments of a polypeptide that is 98% or more, or 99% or more homologous to the sequences of the RSV Gb consensus Immunogen set forth herein and 100% identical to such sequence, in each case with or without signal peptides and/or a methionine at position 1. The fragment may encode a fragment of a polypeptide that is 98% or more homologous, 99% or more homologous, or 100% identical to the RSV Gb consensus Immunogen and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. In some embodiments, a fragment that encodes RSV Gb Immunogen may encode a fragment of a SEQ ID NO:8 and comprise a fragment of SEQ ID NO:7. The fragment may encode fragments of SEQ ID NO:8 linked to a signal peptide such as an immunoglobulin signal peptide for example IgE signal peptide (SEQ ID NO:17) or IgG signal peptide. Thus in some embodiments, the fragment is a fragment of SEQ ID NO:16 which comprises SEQ ID NO:17 and may be encoded by SEQ ID NO:15. The fragment may comprise fragments of SEQ ID NO:7 linked to start codon. Fragments also encode fragments of a polypeptide that is 98% or more, or 99% or more homologous to SEQ ID NO:8. The fragment may encode a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:8 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may encode a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:8 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may encode a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:8 and additionally comprise an N terminal methionine which is not included when calculating percent homology. The fragment may encode a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:8 and additionally comprise an N terminal methionine which is not included when calculating percent homology. Fragments may be 98% or more, or 99% or more homologous to SEQ ID NO:7. The

fragment may be 98% or more homologous to SEQ ID NO:7 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may be 99% or more homologous to SEQ ID NO:7 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may be 98% or more homologous to SEQ ID NO:7 and additionally comprise an N terminal methionine which is not included when calculating percent homology. The fragment may be 99% or more homologous to SEQ ID NO:7 and additionally comprise an N terminal methionine which is not included when calculating percent homology. Fragments of SEQ ID NO:7, fragments of nucleic acid sequence 98% or more homologous to SEQ ID NO:7, and fragments of nucleic acid sequence 99% or more homologous to SEQ ID NO:7, in each case with or without start codons or coding sequences that encode signal peptides may encode fragments of RSV Gb immunogen that may be In some embodiments, the fragments thereof may be 125 or more amino acids in length, 130 or more, 140 or more, 150 or more, 160 or more, 170 or more, 180 or more, 190 or more, 200 or more, 210 or more, 220 or more, 230 or more, 240 or more, 250 or more, 260 or more, 270 or more, 280 or more, 285 or more, 288 or more in length. Polypeptide fragments may be fewer than 135 amino acids, fewer than 145, fewer than 155, fewer than 165, fewer than 175, fewer than 185, fewer than 195, fewer than 205, fewer than 215, fewer than 225, fewer than 235, fewer than 245, fewer than 255, fewer than 265, fewer than 275, fewer than 285, fewer than 288, fewer than 290 in length. Fragments may further encode an N terminal methionine and/or a signal peptide such as an immunoglobulin signal peptide, for example an IgE or IgG signal peptide. The N terminal methionine and/or signal peptide may be linked to sequences that encode a 290 amino acid or smaller fragment thereof. The fragment may encode an N terminal methionine and/or signal peptide linked to a polypeptide that is 98% homologous to the 290 amino acid or to a smaller fragment of a polypeptide that is 98% homologous to the 290 amino acid sequence. The fragment may encode an N terminal methionine and/or signal peptide linked to a polypeptide that is 99% homologous to the 290 amino acid sequence or to a smaller fragment of a polypeptide that is 99% homologous to the 290 amino acid sequence. In calculating degrees of homology a polypeptide has to SEQ ID NO:8 or a fragment thereof, an N terminal methionine

and/or any signal peptide is not included in such calculation. The sequences of the signal peptide are not used in determining homology. Thus, for example, although SEQ ID NO:15 comprises SEQ ID NO:7 operably linked to a coding sequences that encode signal peptide SEQ ID NO:17, SEQ ID NO:15 comprises a fragment of SEQ ID NO:7 that is 100% homologous to a fragment of SEQ ID NO:7, notwithstanding the signal peptide coding sequence which is absent in SEQ ID NO:7.

Also provides are sequences related to RSV M2-1 including fragments of a nucleic acid sequence 98% or more homologous to SEQ ID NO:3. In some embodiments, the fragments are fragments of a nucleic acid sequence 99% or more homologous to SEQ ID NO:3. In some embodiments, the fragments are fragments of SEQ ID NO:3. It is intended that nucleic acid sequences encoding the polypeptide fragments described herein are expressly disclosed, i.e. fragments of nucleic acid sequences that encode the proteins having the size and homology of the peptide fragments. In some embodiments, fragments of nucleic acid sequences that encode the proteins having the size and homology of the peptide fragments are fragments of SEQ ID NO:3. A fragment of a coding sequence that encodes RSV M2-1 consensus Immunogen such as that which is disclosed in SEQ ID NO:4, SEQ ID NO:12, proteins 98% or more homologous to the RSV M2-1 consensus Immunogen sequences set forth herein, proteins 99% or more homologous to the RSV M2-1 consensus Immunogen sequences set forth herein, and RSV M2-1 consensus Immunogen 100% identical to the consensus sequences set forth herein, in each case with or without signal peptides and/or a methionine at position 1, such as for example SEQ ID NO:3 or SEQ ID NO:11, may comprise 20% or more, 25% or more, 30% or more, 35% or more, 40% or more, 45% or more, 50% or more, 55% or more, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 91% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, 99% or more percent of the length of the particular full length RSV M2-1 consensus Immunogen sequence set forth herein, excluding any non-RSV signal peptide added. Fragments may encode fragments of a polypeptide that is 98% or more, or 99% or more homologous to the sequences of the RSV M2-1 consensus Immunogen set forth herein and 100% identical to such sequence, in each case with or without signal peptides and/or a methionine at position 1. The fragment may encode a

fragment of a polypeptide that is 98% or more homologous, 99% or more homologous, or 100% identical to the RSV M2-1 consensus Immunogen and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. In some embodiments, a fragment that encodes RSV M2-1 Immunogen may encode a fragment of a SEQ ID NO:4 and comprise a fragment of SEQ ID NO:3. The fragment may encode fragments of SEQ ID NO:4 linked to a signal peptide such as an immunoglobulin signal peptide for example IgE signal peptide (SEQ ID NO:17) or IgG signal peptide. Thus in some embodiments, the fragment is a fragment of SEQ ID NO:12 which comprises SEQ ID NO:17 and may be encoded by SEQ ID NO:11. The fragment may comprise fragments of SEQ ID NO:3 linked to start codon. Fragments also encode fragments of a polypeptide that is 98% or more, or 99% or more homologous to SEQ ID NO:4. The fragment may encode a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:4 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may encode a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:4 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may encode a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:4 and additionally comprise an N terminal methionine which is not included when calculating percent homology. The fragment may encode a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:4 and additionally comprise an N terminal methionine which is not included when calculating percent homology. Fragments may be 98% or more, or 99% or more homologous to SEQ ID NO:3. The fragment may be 98% or more homologous to SEQ ID NO:3 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may be 99% or more homologous to SEQ ID NO:3 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may be 98% or more homologous to SEQ ID NO:3 and additionally comprise an N terminal methionine which is not included when calculating percent homology. The fragment may be 99% or more homologous to SEQ ID NO:3 and additionally comprise an N terminal

methionine which is not included when calculating percent homology. Fragments of SEQ ID NO:3, fragments of nucleic acid sequence 98% or more homologous to SEQ ID NO:3, and fragments of nucleic acid sequence 99% or more homologous to SEQ ID NO:3, in each case with or without start codons or coding sequences that encode signal peptides may encode fragments of RSV M2-1 immunogen that may be In some embodiments, the fragments thereof may be 25 or more amino acids in length, 30 or more, 40 or more, 50 or more, 60 or more, 70 or more, 80 or more, 90 or more, 100 or more, 110 or more, 120 or more, 130 or more, 140 or more, 150 or more, 160 or more, 170 or more, 180 or more, 184 or more, 190 or more, 193 or more in length. Polypeptide fragments may be fewer than 35 amino acids, fewer than 45, fewer than 55, fewer than 65, fewer than 75, fewer than 85, fewer than 95, fewer than 105, fewer than 115, fewer than 125, fewer than 135, fewer than 145, fewer than 155, fewer than 165, fewer than 175, fewer than 185, fewer than 188, fewer than 192, or fewer than 194 in length. Fragments may further encode an N terminal methionine and/or a signal peptide such as an immunoglobulin signal peptide, for example an IgE or IgG signal peptide. The N terminal methionine and/or signal peptide may be linked to sequences that encode a 194 amino acid or smaller fragment thereof. The fragment may encode an N terminal methionine and/or signal peptide linked to a polypeptide that is 98% homologous to the 194 amino acid or to a smaller fragment of a polypeptide that is 98% homologous to the 194 amino acid sequence. The fragment may encode an N terminal methionine and/or signal peptide linked to a polypeptide that is 99% homologous to the 194 amino acid sequence or to a smaller fragment of a polypeptide that is 99% homologous to the 194 amino acid sequence. In calculating degrees of homology a polypeptide has to SEQ ID NO:4 or a fragment thereof, an N terminal methionine and/or any signal peptide is not included in such calculation. The sequences of the signal peptide are not used in determining homology. Thus, for example, although SEQ ID NO:9 comprises SEQ ID NO:3 operably linked to a coding sequences that encode signal peptide SEQ ID NO:17, SEQ ID NO:9 comprises a fragment of SEQ ID NO:3 that is 100% homologous to a fragment of SEQ ID NO:3, notwithstanding the signal peptide coding sequence which is absent in SEQ ID NO:3. SEQ ID NO:30 corresponds to an immunodominant T cell epitope of RSV M2-1 immunogen set forth in SEQ ID NO:4. SEQ ID NO:31 refers to a subdominant T cell epitope corresponds to an

immunodominant T cell epitope of RSV M2-1 immunogen set forth in SEQ ID NO:4. In some embodiments, fragments comprise coding sequences that encode all or part of one or more of these sequences or sequences 98% or more or 99% or more homologous to them including fragments of SEQ ID NO:3 or sequences that are 98% or more or 99% or more homologous to SEQ ID NO:3.

l. Identical

"Identical" or "identity" as used herein in the context of two or more nucleic acids or polypeptide sequences, may mean that the sequences have a specified percentage of residues that are the same over a specified region. The percentage may be calculated by optimally aligning the two sequences, comparing the two sequences over the specified region, determining the number of positions at which the identical residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the specified region, and multiplying the result by 100 to yield the percentage of sequence identity. In cases where the two sequences are of different lengths or the alignment produces one or more staggered ends and the specified region of comparison includes only a single sequence, the residues of single sequence are included in the denominator but not the numerator of the calculation. When comparing DNA and RNA, thymine (T) and uracil (U) may be considered equivalent. Identity may be performed manually or by using a computer sequence algorithm such as BLAST or BLAST 2.0.

m. Impedance

"Impedance" as used herein may be used when discussing the feedback mechanism and can be converted to a current value according to Ohm's law, thus enabling comparisons with the preset current.

n. Immune Response

"Immune response" as used herein may mean the activation of a host's immune system, e.g., that of a mammal, in response to the introduction of one or more RSV consensus antigen via the provided DNA plasmid vaccines. The immune response can be in the form of a cellular or humoral response, or both.

o. Nucleic Acid

"Nucleic acid" or "oligonucleotide" or "polynucleotide" as used herein may mean at least two nucleotides covalently linked together. The depiction of a single strand also defines the sequence of the complementary strand. Thus, a nucleic acid also encompasses the complementary strand of a depicted single strand. Many variants of a nucleic acid may be used for the same purpose as a given nucleic acid. Thus, a nucleic acid also encompasses substantially identical nucleic acids and complements thereof. A single strand provides a probe that may hybridize to a target sequence under stringent hybridization conditions. Thus, a nucleic acid also encompasses a probe that hybridizes under stringent hybridization conditions.

Nucleic acids may be single stranded or double stranded, or may contain portions of both double stranded and single stranded sequence. The nucleic acid may be DNA, both genomic and cDNA, RNA, or a hybrid, where the nucleic acid may contain combinations of deoxyribo- and ribo-nucleotides, and combinations of bases including uracil, adenine, thymine, cytosine, guanine, inosine, xanthine hypoxanthine, isocytosine and isoguanine. Nucleic acids may be obtained by chemical synthesis methods or by recombinant methods.

p. Operably Linked

"Operably linked" as used herein may mean that expression of a gene is under the control of a promoter with which it is spatially connected. A promoter may be positioned 5' (upstream) or 3' (downstream) of a gene under its control. The distance between the promoter and a gene may be approximately the same as the distance between that promoter and the gene it controls in the gene from which the promoter is derived. As is known in the art, variation in this distance may be accommodated without loss of promoter function.

q. Promoter

"Promoter" as used herein may mean a synthetic or naturally-derived molecule which is capable of conferring, activating or enhancing expression of a nucleic acid in a cell. A promoter may comprise one or more specific transcriptional regulatory sequences to further enhance expression and/or to alter the spatial expression and/or temporal expression of same. A promoter may also comprise distal enhancer or repressor elements, which can be located as much as several thousand base pairs from the start site of transcription. A promoter may be derived from

sources including viral, bacterial, fungal, plants, insects, and animals. A promoter may regulate the expression of a gene component constitutively, or differentially with respect to cell, the tissue or organ in which expression occurs or, with respect to the developmental stage at which expression occurs, or in response to external stimuli such as physiological stresses, pathogens, metal ions, or inducing agents. Representative examples of promoters include the bacteriophage T7 promoter, bacteriophage T3 promoter, SP6 promoter, lac operator-promoter, tac promoter, SV40 late promoter, SV40 early promoter, RSV-LTR promoter, CMV IE promoter, SV40 early promoter or SV40 late promoter and the CMV IE promoter.

r. Stringent Hybridization Conditions

"Stringent hybridization conditions" as used herein may mean conditions under which a first nucleic acid sequence (e.g., probe) will hybridize to a second nucleic acid sequence (e.g., target), such as in a complex mixture of nucleic acids. Stringent conditions are sequence-dependent and will be different in different circumstances. Stringent conditions may be selected to be about 5-10°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength pH. The T_m may be the temperature (under defined ionic strength, pH, and nucleic concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at T_m, 50% of the probes are occupied at equilibrium). Stringent conditions may be those in which the salt concentration is less than about 1.0 M sodium ion, such as about 0.01-1.0 M sodium ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (e.g., about 10-50 nucleotides) and at least about 60°C for long probes (e.g., greater than about 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. For selective or specific hybridization, a positive signal may be at least 2 to 10 times background hybridization. Exemplary stringent hybridization conditions include the following: 50% formamide, 5x SSC, and 1% SDS, incubating at 42°C, or, 5x SSC, 1% SDS, incubating at 65°C, with wash in 0.2x SSC, and 0.1% SDS at 65°C.

s. Substantially Complementary

"Substantially complementary" as used herein may mean that a first sequence is at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98% or 99% identical to the complement of

a second sequence over a region of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more nucleotides or amino acids, or that the two sequences hybridize under stringent hybridization conditions.

t. Substantially Identical

"Substantially identical" as used herein may mean that a first and second sequence are at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98% or 99% identical over a region of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more nucleotides or amino acids, or with respect to nucleic acids, if the first sequence is substantially complementary to the complement of the second sequence.

u. Variant

"Variant" used herein with respect to a nucleic acid may mean (i) a portion or fragment of a referenced nucleotide sequence; (ii) the complement of a referenced nucleotide sequence or portion thereof; (iii) a nucleic acid that is substantially identical to a referenced nucleic acid or the complement thereof; or (iv) a nucleic acid that hybridizes under stringent conditions to the referenced nucleic acid, complement thereof, or a sequences substantially identical thereto.

"Variant" with respect to a peptide or polypeptide that differs in amino acid sequence by the insertion, deletion, or conservative substitution of amino acids, but retain at least one biological activity. Variant may also mean a protein with an amino acid sequence that is substantially identical to a referenced protein with an amino acid sequence that retains at least one biological activity. A conservative substitution of an amino acid, i.e., replacing an amino acid with a different amino acid of similar properties (e.g., hydrophilicity, degree and distribution of charged regions) is recognized in the art as typically involving a minor change. These minor changes can be identified, in part, by considering the hydropathic index of amino acids, as understood in the art. Kyte et al., J. Mol. Biol. 157:105-132 (1982). The hydropathic index of an amino acid is based on a consideration of its hydrophobicity and charge. It is known in the art that amino acids of similar hydropathic indexes can be substituted and still retain protein function. In one aspect, amino acids having hydropathic indexes of ±2 are substituted. The hydrophilicity of amino acids can also be used to reveal substitutions that would result in proteins retaining biological function. A consideration of the hydrophilicity of amino acids in

the context of a peptide permits calculation of the greatest local average hydrophilicity of that peptide, a useful measure that has been reported to correlate well with antigenicity and immunogenicity. U.S. Patent No. 4,554,101, incorporated fully herein by reference. Substitution of amino acids having similar hydrophilicity values can result in peptides retaining biological activity, for example immunogenicity, as is understood in the art. Substitutions may be performed with amino acids having hydrophilicity values within ±2 of each other. Both the hyrophobicity index and the hydrophilicity value of amino acids are influenced by the particular side chain of that amino acid. Consistent with that observation, amino acid substitutions that are compatible with biological function are understood to depend on the relative similarity of the amino acids, and particularly the side chains of those amino acids, as revealed by the hydrophobicity, hydrophilicity, charge, size, and other properties.

v. Vector

"Vector" used herein may mean a nucleic acid sequence containing an origin of replication. A vector may be a plasmid, bacteriophage, bacterial artificial chromosome or yeast artificial chromosome. A vector may be a DNA or RNA vector. A vector may be either a self-replicating extrachromosomal vector or a vector which integrates into a host genome.

2. RSV Proteins

Provided herein are RSV proteins, also referred to herein as "RSV immunogens", are consensus antigens capable of eliciting an immune response against RSV. Three of the consensus RSV immunogens are: RSV F immunogen, and two forms of RSV G immunogen, referred to herein as RSV Ga immunogen and RSV Gb immunogen. Two RSV G immunogen forms are provided due to the diversity among RSV G proteins. In some embodiments, the RSV immunogens may comprise a signal peptide from a different protein such as an immunoglobulin protein, for example an IgE signal peptide (SEQ ID NO:17) or an IgG signal peptide. RSV immunogen include RSV F immunogen, RSV Ga immunogen and RSV Gb immunogen. Also provided herein is the "RSV immunogens" that is a consensus antigens capable of eliciting an immune response against RSV M2-1, i.e. RSV M2-1 immunogen which may optionally comprise a signal peptide from a different protein such as an immunoglobulin protein, for example an IgE signal peptide (SEQ ID NO:17) or an IgG signal peptide.

"RSV F immunogens" refer to proteins comprising immunogenic fragments of proteins that are at least 98% homologous to SEQ ID NO:2. The RSV F immunogen may comprise immunogenic fragments of proteins that are at least 99% homologous to SEQ ID NO:2. The RSV F immunogen may comprise immunogenic fragments of SEQ ID NO:2. In each instance the immunogenic fragments may optionally further comprise a signal peptide such as SEQ ID NO:17. The RSV F immunogen that comprises an immunogenic fragment of SEQ ID NO:2 and signal peptide SEQ ID NO:17 may be an immunogenic fragments of SEQ ID NO:10 that comprises SEQ ID NO:17. RSV F immunogen may comprise a protein that is at least 98% homologous to SEQ ID NO:2, and in some embodiments may comprise a protein that is at least 99% homologous to SEQ ID NO:2. The RSV F immunogen may comprise SEQ ID NO:2. In each instance the RSV F immunogen may optionally further comprise a signal peptide such as SEQ ID NO:17. The RSV F immunogen that comprises SEQ ID NO:2 and signal peptide SEQ ID NO:17 may comprise SEQ ID NO:10.

"RSV Ga immunogens" refer to proteins comprising immunogenic fragments of proteins that are at least 98% homologous to SEQ ID NO:6. The RSV Ga immunogen may comprise immunogenic fragments of proteins that are at least 99% homologous to SEQ ID NO:6. The RSV Ga immunogen may comprise immunogenic fragments of SEQ ID NO:6. In each instance the immunogenic fragments may optionally further comprise a signal peptide such as SEQ ID NO:17. The RSV Ga immunogen that comprises an immunogenic fragment of SEQ ID NO:6 and signal peptide SEQ ID NO:17 may be an immunogenic fragments of SEQ ID NO:14 that comprises SEQ ID NO:17. RSV Ga immunogen may comprise a protein that is at least 98% homologous to SEQ ID NO:6, and in some embodiments may comprise a protein that is at least 99% homologous to SEQ ID NO:6. The RSV Ga immunogen may comprise SEQ ID NO:6. In each instance the RSV Ga immunogen may optionally further comprise a signal peptide such as SEQ ID NO:17. The RSV Ga immunogen that comprises SEQ ID NO:6 and signal peptide SEQ ID NO:17 may comprise SEQ ID NO:14.

"RSV Gb immunogens" refer to proteins comprising immunogenic fragments of proteins that are at least 98% homologous to SEQ ID NO:8. The RSV Gb immunogen may comprise immunogenic fragments of proteins that are at least 99% homologous to SEQ ID NO:8. The

RSV Gb immunogen may comprise immunogenic fragments of SEQ ID NO:8. In each instance the immunogenic fragments may optionally further comprise a signal peptide such as SEQ ID NO:17. The RSV Gb immunogen that comprises an immunogenic fragment of SEQ ID NO:8 and signal peptide SEQ ID NO:17 may be an immunogenic fragments of SEQ ID NO:16 that comprises SEQ ID NO:17. RSV Gb immunogen may comprise a protein that is at least 98% homologous to SEQ ID NO:8, and in some embodiments may comprise a protein that is at least 99% homologous to SEQ ID NO:8. The RSV Gb immunogen may comprise SEQ ID NO:8. In each instance the RSV Gb immunogen may optionally further comprise a signal peptide such as SEQ ID NO:17. The RSV Gb immunogen that comprises SEQ ID NO:8 and signal peptide SEQ ID NO:17 may comprise SEQ ID NO:16.

In some embodiments, fusion proteins are provided which comprise a combination of two or more of the RSV proteins set forth herein. For example, fusion proteins may comprise: SEQ ID NO:2 or an immunogenic fragment thereof and SEQ ID NO:6 or an immunogenic fragment thereof; SEQ ID NO:2 or an immunogenic fragment thereof and SEQ ID NO:8 or an immunogenic fragment thereof; or SEQ ID NO:6 or an immunogenic fragment thereof and SEQ ID NO:8 or an immunogenic fragment thereof. Fusion proteins may comprise: SEQ ID NO:2 or an immunogenic fragment thereof, SEQ ID NO:6 or an immunogenic fragment thereof and SEQ ID NO:8 or an immunogenic fragment thereof. Alternatively one or more of the fusion proteins above may comprise a sequence at least 98% or at least 99% homologous to SEQ ID NO:2 or an immunogenic fragment thereof in place of the portion of the fusion protein described above corresponding to SEQ ID NO:2 or an immunogenic fragment. Likewise, one or more of the fusion proteins above may comprise a sequence at least 98% or at least 99% homologous to SEQ ID NO:6 or an immunogenic fragment thereof in place of the portion of the fusion protein described above corresponding to SEQ ID NO:6 or an immunogenic fragment; and/or one or more of the fusion proteins above may comprise a sequence at least 98% or at least 99% homologous to SEQ ID NO:8 or an immunogenic fragment thereof in place of the portion of the fusion protein described above corresponding to SEQ ID NO:8 or an immunogenic fragment. Fusion proteins may include combinations of consensus proteins and/or fragments of consensus protein and/or variants of consensus protein and/or fragments of variants consensus proteins.

The consensus proteins and/or fragments of consensus protein and/or variants of consensus protein and/or fragments of variants consensus proteins may be linked directly adjacent to each other or linked with a spacer or one more amino acids in between. In some embodiments, the space may be a proteolyic cleavage site. In some embodiments, the space may be a proteolyic cleavage site recognized by a protease found in cells to which the vaccine is intended to be administered and/or taken up.

"RSV M2-1 immunogens" refer to proteins comprising immunogenic fragments of proteins that are at least 98% homologous to SEQ ID NO:4. The RSV M2-1 immunogen may comprise immunogenic fragments of proteins that are at least 99% homologous to SEQ ID NO:4. The RSV M2-1 immunogen may comprise immunogenic fragments of SEQ ID NO:4. In each instance the immunogenic fragments may optionally further comprise a signal peptide such as SEQ ID NO:17. The RSV M2-1 immunogen that comprises an immunogenic fragment of SEQ ID NO:4 and signal peptide SEQ ID NO:17 may be an immunogenic fragments of SEQ ID NO:12 that comprises SEQ ID NO:17. RSV M2-1 immunogen may comprise a protein that is at least 98% homologous to SEQ ID NO:4, and in some embodiments may comprise a protein that is at least 99% homologous to SEQ ID NO:4. The RSV M2-1 immunogen may comprise SEQ ID NO:4. In each instance the RSV M2-1 immunogen may optionally further comprise a signal peptide such as SEQ ID NO:17. The RSV M2-1 immunogen that comprises SEQ ID NO:4 and signal peptide SEQ ID NO:17 may comprise SEQ ID NO:12. In some embodiments, fusion proteins are provided which comprise a combination of RSV M2-1 and another RSV immunogen proteins set forth herein. For example, fusion proteins may comprise: SEQ ID NO:2 or an immunogenic fragment thereof and SEQ ID NO:4 or an immunogenic fragment thereof; SEQ ID NO:4 or an immunogenic fragment thereof and SEQ ID NO:6 or an immunogenic fragment thereof, SEQ ID NO:4 or an immunogenic fragment thereof and SEQ ID NO:8 or an immunogenic fragment thereof; or SEQ ID NO:6 or an immunogenic fragment thereof and SEQ ID NO:8 or an immunogenic fragment thereof. Fusion proteins may comprise: SEQ ID NO:4 or an immunogenic fragment thereof. Alternatively one or more of the fusion proteins above may comprise a sequence at least 98% or at least 99% homologous to SEQ ID NO:4 or an

immunogenic fragment thereof in place of the portion of the fusion protein described above corresponding to SEQ ID NO:4 or an immunogenic fragment.

3. Coding sequences encoding RSV Proteins

Provided herein are nucleic acid sequences that encode the "RSV immunogens" set forth herein. Administration of nucleic acid molecules comprising the nucleic acid sequences when taken up and expressed by cells results in a broad immune response against RSV. Coding sequences for RSV immunogens are provided, i.e. nucleic acid sequences that encode RSV F immunogen, nucleic acid sequences that encode RSV Ga immunogen and nucleic acid sequences that encode RSV Gb immunogen as well as for nucleic acid sequences that encode RSV M2-1 immunogen. In some embodiments, the nucleic acid sequences that encode RSV immunogens may comprise nucleic acid sequences that encode a signal peptide from a different protein such as an immunoglobulin protein, for example an IgE signal peptide (SEQ ID NO:17) or an IgG signal peptide.

Nucleic acid sequences that encode RSV F immunogens may encode proteins comprising immunogenic fragments of proteins that are at least 98% homologous to SEQ ID NO:2. Nucleic acid sequences that encode RSV F immunogens may encode proteins that comprise immunogenic fragments of proteins that are at least 99% homologous to SEQ ID NO:2. Nucleic acid sequences that encode RSV F immunogens may encode proteins that comprise immunogenic fragments of SEQ ID NO:2. In each instance the nucleic acid sequences that encode proteins that comprise the immunogenic fragments may optionally further comprise nucleic acid sequences that encode a signal peptide such as nucleic acid sequences that encode SEQ ID NO:17. Nucleic acid sequences that encode RSV F immunogens may encode proteins that comprises an immunogenic fragment of SEQ ID NO:2 and signal peptide SEQ ID NO:17. Nucleic acid sequences that encode RSV F immunogens may encode proteins that may be an immunogenic fragment of SEQ ID NO:10 that comprises SEQ ID NO:17. Nucleic acid sequences that encode RSV F immunogens may encode proteins that comprise a protein that is at least 98% homologous to SEQ ID NO:2, and in some embodiments may encode a protein that is at least 99% homologous to SEQ ID NO:2. Nucleic acid sequences that encode RSV F immunogens may encode proteins that comprise SEQ ID NO:2. In each instance nucleic acid

sequences that encode RSV F immunogens may optionally further comprise nucleic acid sequences that encode a signal peptide such as SEQ ID NO:17. Nucleic acid sequences that encode RSV F immunogens may encode a protein that comprises SEQ ID NO:2 and signal peptide SEQ ID NO:17. Nucleic acid sequences that encode RSV F immunogens may encode a protein that comprises SEQ ID NO:10.

Nucleic acid sequences that encode RSV F immunogens may comprise fragments of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:1. The nucleic acid sequence that is at least 98% homologous to SEQ ID NO:1 encodes a protein that is at least 98% homologous to SEQ ID NO:2. The fragment encodes an immunogenic fragment of the protein that is at least 98% homologous to SEQ ID NO:2. In some embodiments, the nucleic acid sequences that encodes the RSV F immunogen may comprise fragments of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:1 wherein the nucleic acid sequence encodes a protein that is at least 99% homologous to SEQ ID NO:2, in some embodiments nucleic acid sequence encodes a protein that comprises SEQ ID NO:2, and the fragment of the nucleic acid sequence encodes an immunogenic fragment of the protein that is at least 99% homologous to SEQ ID NO:2 such as an immunogenic fragment SEQ ID NO:2. In some such embodiments, the nucleic acid sequences that encodes the RSV F immunogen may comprise fragments of a nucleic acid sequence that is at least 99% homologous to SEQ ID NO:1 and in some embodiments, fragments of SEQ ID NO:1. In some embodiments, the nucleic acid sequences that encode RSV F immunogens may optionally further comprise nucleic acid sequences that encode a signal peptide such as SEQ ID NO:17. Nucleic acid sequences that encode RSV F immunogens may comprise fragments of SEQ ID NO:2 and coding sequence encoding signal peptide SEQ ID NO:17. Nucleic acid sequences that encode RSV F immunogens may comprise fragments of SEQ ID NO:9 that include coding sequence encoding signal peptide SEQ ID NO:17.

Nucleic acid sequences that encode RSV Ga immunogens may encode proteins comprising immunogenic fragments of proteins that are at least 98% homologous to SEQ ID NO:6. Nucleic acid sequences that encode RSV Ga immunogens may encode proteins that comprise immunogenic fragments of proteins that are at least 99% homologous to SEQ ID NO:6.

Nucleic acid sequences that encode RSV Ga immunogens may encode proteins that comprise immunogenic fragments of SEQ ID NO:6. In each instance the nucleic acid sequences that encode proteins that comprise the immunogenic fragments may optionally further comprise nucleic acid sequences that encode a signal peptide such as nucleic acid sequences that encode SEQ ID NO:17. Nucleic acid sequences that encode RSV Ga immunogens may encode proteins that comprises an immunogenic fragment of SEQ ID NO:6 and signal peptide SEQ ID NO:17. Nucleic acid sequences that encode RSV Ga immunogens may encode proteins that may be an immunogenic fragment of SEQ ID NO:14 that comprises SEQ ID NO:17. Nucleic acid sequences that encode RSV Ga immunogens may encode proteins that comprise a protein that is at least 98% homologous to SEQ ID NO:6, and in some embodiments may encode a protein that is at least 99% homologous to SEQ ID NO:6. Nucleic acid sequences that encode RSV Ga immunogens may encode proteins that comprise SEQ ID NO:6. In each instance nucleic acid sequences that encode RSV Ga immunogens may optionally further comprise nucleic acid sequences that encode a signal peptide such as SEQ ID NO:17. Nucleic acid sequences that encode RSV Ga immunogens may encode a protein that comprises SEQ ID NO:6 and signal peptide SEQ ID NO:17. Nucleic acid sequences that encode RSV Ga immunogens may encode a protein that comprises SEQ ID NO:14.

Nucleic acid sequences that encode RSV Ga immunogens may comprise fragments of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:5. The nucleic acid sequence that is at least 98% homologous to SEQ ID NO:5 encodes a protein that is at least 98% homologous to SEQ ID NO:6. The fragment encodes an immunogenic fragment of the protein that is at least 98% homologous to SEQ ID NO:6. In some embodiments, the nucleic acid sequences that encodes the RSV Ga immunogen may comprise fragments of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:5 wherein the nucleic acid sequence encodes a protein that is at least 99% homologous to SEQ ID NO:6, in some embodiments nucleic acid sequence encodes a protein that comprises SEQ ID NO:6, and the fragment of the nucleic acid sequence encodes an immunogenic fragment of the protein that is at least 99% homologous to SEQ ID NO:6 such as an immunogenic fragment SEQ ID NO:6. In some such embodiments, the nucleic acid sequences that encodes the RSV Ga immunogen may comprise

fragments of a nucleic acid sequence that is at least 99% homologous to SEQ ID NO:5 and in some embodiments, fragments of SEQ ID NO:5. In some embodiments, the nucleic acid sequences that encode RSV Ga immunogens may optionally further comprise nucleic acid sequences that encode a signal peptide such as SEQ ID NO:17. Nucleic acid sequences that encode RSV Ga immunogens may comprise fragments of SEQ ID NO:6 and coding sequence encoding signal peptide SEQ ID NO:17. Nucleic acid sequences that encode RSV Ga immunogens may comprise fragments of SEQ ID NO:13 that include coding sequence encoding signal peptide SEQ ID NO:17.

Nucleic acid sequences that encode RSV Gb immunogens may encode proteins comprising immunogenic fragments of proteins that are at least 98% homologous to SEQ ID NO:8. Nucleic acid sequences that encode RSV Gb immunogens may encode proteins that comprise immunogenic fragments of proteins that are at least 99% homologous to SEQ ID NO:8. Nucleic acid sequences that encode RSV Gb immunogens may encode proteins that comprise immunogenic fragments of SEQ ID NO:8. In each instance the nucleic acid sequences that encode proteins that comprise the immunogenic fragments may optionally further comprise nucleic acid sequences that encode a signal peptide such as nucleic acid sequences that encode SEQ ID NO:17. Nucleic acid sequences that encode RSV Gb immunogens may encode proteins that comprises an immunogenic fragment of SEQ ID NO:8 and signal peptide SEQ ID NO:17. Nucleic acid sequences that encode RSV Gb immunogens may encode proteins that may be an immunogenic fragment of SEQ ID NO:16 that comprises SEQ ID NO:17. Nucleic acid sequences that encode RSV Gb immunogens may encode proteins that comprise a protein that is at least 98% homologous to SEQ ID NO:8, and in some embodiments may encode a protein that is at least 99% homologous to SEQ ID NO:8. Nucleic acid sequences that encode RSV Gb immunogens may encode proteins that comprise SEQ ID NO:8. In each instance nucleic acid sequences that encode RSV Gb immunogens may optionally further comprise nucleic acid sequences that encode a signal peptide such as SEQ ID NO:17. Nucleic acid sequences that encode RSV Gb immunogens may encode a protein that comprises SEQ ID NO:8 and signal peptide SEQ ID NO:17. Nucleic acid sequences that encode RSV Gb immunogens may encode a protein that comprises SEQ ID NO:16.

Nucleic acid sequences that encode RSV Gb immunogens may comprise fragments of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:7. The nucleic acid sequence that is at least 98% homologous to SEQ ID NO:7 encodes a protein that is at least 98% homologous to SEQ ID NO:8. The fragment encodes an immunogenic fragment of the protein that is at least 98% homologous to SEQ ID NO:8. In some embodiments, the nucleic acid sequences that encodes the RSV Gb immunogen may comprise fragments of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:7 wherein the nucleic acid sequence encodes a protein that is at least 99% homologous to SEQ ID NO:8, in some embodiments nucleic acid sequence encodes a protein that comprises SEQ ID NO:8, and the fragment of the nucleic acid sequence encodes an immunogenic fragment of the protein that is at least 99% homologous to SEQ ID NO:8 such as an immunogenic fragment SEQ ID NO:8. In some such embodiments, the nucleic acid sequences that encodes the RSV Gb immunogen may comprise fragments of a nucleic acid sequence that is at least 99% homologous to SEQ ID NO:7 and in some embodiments, fragments of SEQ ID NO:7. In some embodiments, the nucleic acid sequences that encode RSV Gb immunogens may optionally further comprise nucleic acid sequences that encode a signal peptide such as SEQ ID NO:17. Nucleic acid sequences that encode RSV Gb immunogens may comprise fragments of SEQ ID NO:8 and coding sequence encoding signal peptide SEQ ID NO:17. Nucleic acid sequences that encode RSV Gb immunogens may comprise fragments of SEQ ID NO:15 that include coding sequence encoding signal peptide SEQ ID NO:17.

Nucleic acid sequences that encode RSV M2-1 immunogens may encode proteins comprising immunogenic fragments of proteins that are at least 98% homologous to SEQ ID NO:4. Nucleic acid sequences that encode RSV M2-1 immunogens may encode proteins that comprise immunogenic fragments of proteins that are at least 99% homologous to SEQ ID NO:4. Nucleic acid sequences that encode RSV M2-1 immunogens may encode proteins that comprise immunogenic fragments of SEQ ID NO:4. In each instance the nucleic acid sequences that encode proteins that comprise the immunogenic fragments may optionally further comprise nucleic acid sequences that encode a signal peptide such as nucleic acid sequences that encode SEQ ID NO:17. Nucleic acid sequences that encode RSV M2-1 immunogens may encode

proteins that comprises an immunogenic fragment of SEQ ID NO:4 and signal peptide SEQ ID NO:17. Nucleic acid sequences that encode RSV F immunogens may encode proteins that may be an immunogenic fragment of SEQ ID NO:12 that comprises SEQ ID NO:17. Nucleic acid sequences that encode RSV M2-1 immunogens may encode proteins that comprise a protein that is at least 98% homologous to SEQ ID NO:4, and in some embodiments may encodes a protein that is at least 99% homologous to SEQ ID NO:4. Nucleic acid sequences that encode RSV M2-1 immunogens may encode proteins that comprise SEQ ID NO:4. In each instance nucleic acid sequences that encode RSV M2-1 immunogens may optionally further comprise nucleic acid sequences that encode a signal peptide such as SEQ ID NO:17. Nucleic acid sequences that encode RSV M2-1 immunogens may encode a protein that comprises SEQ ID NO:4 and signal peptide SEQ ID NO:17. Nucleic acid sequences that encode RSV M2-1 immunogens may encode a protein that comprises SEQ ID NO:12.

Nucleic acid sequences that encode RSV M2-1 immunogens may comprise fragments of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:3. The nucleic acid sequence that is at least 98% homologous to SEQ ID NO:3 encodes a protein that is at least 98% homologous to SEQ ID NO:4. The fragment encodes an immunogenic fragment of the protein that is at least 98% homologous to SEQ ID NO:4. In some embodiments, the nucleic acid sequences that encodes the RSV M2-1 immunogen may comprise fragments of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:3 wherein the nucleic acid sequence encodes a protein that is at least 99% homologous to SEQ ID NO:4, in some embodiments nucleic acid sequence encodes a protein that comprises SEQ ID NO:4, and the fragment of the nucleic acid sequence encodes an immunogenic fragment of the protein that is at least 99% homologous to SEQ ID NO:4 such as an immunogenic fragment SEQ ID NO:4. In some such embodiments, the nucleic acid sequences that encodes the RSV M2-1 immunogen may comprise fragments of a nucleic acid sequence that is at least 99% homologous to SEQ ID NO:3 and in some embodiments, fragments of SEQ ID NO:3. In some embodiments, the nucleic acid sequences that encode RSV M2-1 immunogens may optionally further comprise nucleic acid sequences that encode a signal peptide such as SEQ ID NO:17. Nucleic acid sequences that encode RSV M2-1 immunogens may comprise fragments of SEQ ID NO:4 and coding sequence

encoding signal peptide SEQ ID NO:17. Nucleic acid sequences that encode RSV M2-1 immunogens may comprise fragments of SEQ ID NO:11 that include coding sequence encoding signal peptide SEQ ID NO:17.

Coding sequences may encode fusion proteins which comprise a combination of two or more of the RSV proteins set forth herein. Coding sequences may encode fusion proteins that include combinations of consensus proteins and/or fragments of consensus protein and/or variants of consensus protein and/or fragments of variants consensus proteins. The consensus proteins and/or fragments of consensus protein and/or variants of consensus protein and/or fragments of variants consensus proteins may be linked directly adjacent to each other or linked with a spacer or one more amino acids in between. In some embodiments, the space may be a proteolyic cleavage site. In some embodiments, the space may be a proteolyic cleavage site recognized by a protease found in cells to which the vaccine is intended to be administered and/or taken up.

In some embodiments, coding sequences encode fusion proteins which comprise a combination of two or more of the RSV proteins set forth herein. For example, coding sequences may encode fusion proteins that comprise: SEQ ID NO:2 or an immunogenic fragment thereof and SEQ ID NO:4 or an immunogenic fragment thereof, such as SEQ ID NO:1 or an immunogenic fragment-encoding fragment thereof and SEQ ID NO:3 or an immunogenic fragment thereof-encoding fragment thereof; SEQ ID NO:2 or an immunogenic fragment thereof and SEQ ID NO:6 or an immunogenic fragment thereof, such as SEQ ID NO:1 or an immunogenic fragment-encoding fragment thereof and SEQ ID NO:5 or an immunogenic fragment thereof-encoding fragment thereof; SEQ ID NO:2 or an immunogenic fragment thereof and SEQ ID NO:8 or an immunogenic fragment thereof, such as SEQ ID NO:1 or an immunogenic fragment-encoding fragment thereof and SEQ ID NO:7 or an immunogenic fragment thereof-encoding fragment thereof; SEQ ID NO:4 or an immunogenic fragment thereof and SEQ ID NO:6 or an immunogenic fragment thereof, such as SEQ ID NO:3 or an immunogenic fragment-encoding fragment thereof and SEQ ID NO:5 or an immunogenic fragment thereof-encoding fragment thereof; SEQ ID NO:4 or an immunogenic fragment thereof and SEQ ID NO:8 or an immunogenic fragment thereof, such as SEQ ID NO:3 or an

immunogenic fragment-encoding fragment thereof and SEQ ID NO:5 or an immunogenic fragment thereof-encoding fragment thereof; and SEQ ID NO:6 or an immunogenic fragment thereof and SEQ ID NO:8 or an immunogenic fragment thereof, such as SEQ ID NO:5 or an immunogenic fragment-encoding fragment thereof and SEQ ID NO:7 or an immunogenic fragment thereof-encoding fragment thereof. Coding sequences may encode fusion proteins which comprise a combination of three RSV proteins set forth herein. For example, coding sequences may encode fusion proteins that comprise: SEQ ID NO:2 or an immunogenic fragment thereof , SEQ ID NO:4 or an immunogenic fragment thereof and SEQ ID NO:6 or an immunogenic fragment thereof, such as SEQ ID NO:1 or an immunogenic fragment-encoding fragment thereof, SEQ ID NO:3 or an immunogenic fragment thereof-encoding fragment thereof and SEQ ID NO:5 or an immunogenic fragment-encoding fragment thereof; SEQ ID NO:2 or an immunogenic fragment thereof , SEQ ID NO:4 or an immunogenic fragment thereof and SEQ ID NO:8 or an immunogenic fragment thereof, such as SEQ ID NO:1 or an immunogenic fragment-encoding fragment thereof, SEQ ID NO:3 or an immunogenic fragment thereof-encoding fragment thereof and SEQ ID NO:7 or an immunogenic fragment-encoding fragment thereof; and SEQ ID NO:4 or an immunogenic fragment thereof , SEQ ID NO:6 or an immunogenic fragment thereof and SEQ ID NO:8 or an immunogenic fragment thereof, such as SEQ ID NO:3 or an immunogenic fragment-encoding fragment thereof, SEQ ID NO:5 or an immunogenic fragment thereof-encoding fragment thereof and SEQ ID NO:7 or an immunogenic fragment-encoding fragment thereof. Coding sequences may encode fusion proteins which comprise a combination of the four RSV proteins set forth herein such as coding sequences that encode SEQ ID NO:2 or an immunogenic fragment thereof , SEQ ID NO:4 or an immunogenic fragment thereof, SEQ ID NO:6 or an immunogenic fragment thereof, and SEQ ID NO:8 or an immunogenic fragment thereof , such as SEQ ID NO:1 or an immunogenic fragment-encoding fragment thereof, SEQ ID NO:3 or an immunogenic fragment thereof-encoding fragment thereof, SEQ ID NO:5 or an immunogenic fragment-encoding fragment thereof and SEQ ID NO:7 or an immunogenic fragment-encoding fragment thereof. Alternatively coding sequence encode one or more of the fusion proteins above may comprise a sequence that encodes a protein that is at least 98% or at least 99% homologous to SEQ ID NO:2 or an immunogenic fragment

thereof in place of the portion of the fusion protein described above corresponding to SEQ ID NO:2 or an immunogenic fragment. Likewise, coding sequences for one or more of the fusion proteins above comprise coding sequence at least 98% or at least 99% homologous to SEQ ID NO:4 or an immunogenic fragment thereof in place of the portion of the fusion protein described above corresponding to SEQ ID NO:4 or an immunogenic fragment; one or more of the fusion proteins above may comprise a sequence at least 98% or at least 99% homologous to SEQ ID NO:6 or an immunogenic fragment thereof in place of the portion of the fusion protein described above corresponding to SEQ ID NO:6 or an immunogenic fragment; and/or one or more of the fusion proteins above may comprise a sequence at least 98% or at least 99% homologous to SEQ ID NO:8 or an immunogenic fragment thereof in place of the portion of the fusion protein described above corresponding to SEQ ID NO:8 or an immunogenic fragment. In some embodiments, coding sequence encode one or more of the fusion proteins above may comprise a sequence that e is at least 98% or at least 99% homologous to SEQ ID NO:1 or fragment thereof that is at least 98% or at least 99% homologous to an immunogenic fragment of SEQ ID NO:2 in place of the portion of the coding sequence of the fusion protein described above corresponding to portion that encodes SEQ ID NO:2 or an immunogenic fragment. Coding sequence encode one or more of the fusion proteins above may comprise a sequence that is at least 98% or at least 99% homologous to SEQ ID NO:3 or fragment thereof that is at least 98% or at least 99% homologous to an immunogenic fragment of SEQ ID NO:4 in place of the portion of the coding sequence of the fusion protein described above corresponding to portion that encodes SEQ ID NO:4 or an immunogenic fragment. Coding sequence encode one or more of the fusion proteins above may comprise a sequence that is at least 98% or at least 99% homologous to SEQ ID NO:5 or fragment thereof that is at least 98% or at least 99% homologous to an immunogenic fragment of SEQ ID NO:6 in place of the portion of the coding sequence of the fusion protein described above corresponding to portion that encodes SEQ ID NO:6 or an immunogenic fragment. Coding sequence encode one or more of the fusion proteins above may comprise a sequence that is at least 98% or at least 99% homologous to SEQ ID NO:7 or fragment thereof that is at least 98% or at least 99% homologous to an immunogenic fragment of SEQ ID NO:8 in place of the portion of the coding sequence of the fusion protein described above corresponding

to portion that encodes SEQ ID NO:8 or an immunogenic fragment. Fusion proteins may include combinations of consensus proteins and/or fragments of consensus protein and/or variants of consensus protein and/or fragments of variants consensus proteins. The consensus proteins and/or fragments of consensus protein and/or variants of consensus protein and/or fragments of variants consensus proteins may be linked directly adjacent to each other or linked with a spacer or one more amino acids in between. In some embodiments, the space may be a proteolytic cleavage site. In some embodiments, the space may be a proteolytic cleavage site recognized by a protease found in cells to which the vaccine is intended to be administered and/or taken up.

4. Plasmid

Provided herein is a vector that is capable of expressing one or more RSV proteins in the cell of a mammal in a quantity effective to elicit an immune response in the mammal. The vector may comprise heterologous nucleic acid encoding the one or more RSV antigens. The vector may be a plasmid. The plasmid may be useful for transfecting cells with nucleic acid encoding an RSV antigen, which the transformed host cell is cultured and maintained under conditions wherein expression of the RSV antigen takes place.

The plasmid may comprise a nucleic acid sequence that encodes one or more of the various RSV proteins disclosed above including coding sequences that encode synthetic, consensus antigen capable of eliciting an immune response against RSV are provided for each of the following three proteins: F, Ga and Gb, fragments of such proteins, variants of such proteins, fragments of variants or fusion proteins which are made up of combinations of consensus proteins and/or fragments of consensus protein and/or variants of consensus protein and/or fragments of variants consensus proteins. The plasmid may comprise a nucleic acid sequence that encodes RSV M2-1 proteins disclosed above including coding sequences that encode synthetic, consensus antigen capable of eliciting an immune response against RSV M2-1, fragments of such proteins, variants of such proteins, fragments of variants or fusion proteins which are made up of combinations of consensus proteins and/or fragments of consensus protein and/or variants of consensus protein and/or fragments of variants consensus proteins.

A single plasmid may contain coding sequence for a single RSV Immunogen, coding sequence for two RSV Immunogens, or coding sequence for three RSV Immunogens. In some embodiments, a coding sequence for a fourth RSV Immunogen ay be provided. A single plasmid may contain coding sequence for RSV F Immunogen. A single plasmid may contain coding sequence for RSV Ga Immunogen. A single plasmid may contain coding sequence for RSV Gb Immunogen. A single plasmid may contain coding sequence for RSV F Immunogen and coding sequence for RSV Ga Immunogen. A single plasmid may contain coding sequence for RSV F Immunogen and coding sequence for RSV Gb Immunogen. A single plasmid may contain coding sequence for RSV Ga Immunogen and coding sequence for RSV Gb Immunogen. A single plasmid may contain coding sequence for RSV F Immunogen, coding sequence for RSV Ga Immunogen and coding sequence for RSV Gb Immunogen.

If RSV M2-1 coding sequence is provided, a single plasmid may contain coding sequence for RSV M2-1 Immunogen. A single plasmid may contain coding sequence for RSV F Immunogen and coding sequence for RSV M2-1 Immunogen. A single plasmid may contain coding sequence for RSV M2-1 Immunogen and coding sequence for RSV Ga Immunogen. A single plasmid may contain coding sequence for RSV M2-1 Immunogen and coding sequence for RSV Gb Immunogen. A single plasmid may contain coding sequence for RSV F Immunogen, coding sequence for RSV M2-1 Immunogen and coding sequence for RSV Ga Immunogen. A single plasmid may contain coding sequence for RSV F Immunogen, coding sequence for RSV M2-1 Immunogen and coding sequence for RSV Gb Immunogen. A single plasmid may contain coding sequence for RSV M2-1 Immunogen, coding sequence for RSV Ga Immunogen and coding sequence for RSV Gb Immunogen. A single plasmid may contain coding sequence for RSV F Immunogen, coding sequence for RSV M2-1 Immunogen, coding sequence for RSV Ga Immunogen and coding sequence for RSV Gb Immunogen.

In some embodiments, a plasmid may comprise coding sequence that encodes CCR20 alone or as part of one these plasmids. Similarly, plasmids may further comprise coding sequences for IL-12, IL-15 and/or IL-28.

The plasmid may further comprise an initiation codon, which may be upstream of the coding sequence, and a stop codon, which may be downstream of the coding sequence. The initiation and termination codon may be in frame with the coding sequence.

The plasmid may also comprise a promoter that is operably linked to the coding sequence The promoter operably linked to the coding sequence may be a promoter from simian virus 40 (SV40), a mouse mammary tumor virus (MMTV) promoter, a human immunodeficiency virus (HIV) promoter such as the bovine immunodeficiency virus (BIV) long terminal repeat (LTR) promoter, a Moloney virus promoter, an avian leukosis virus (ALV) promoter, a cytomegalovirus (CMV) promoter such as the CMV immediate early promoter, Epstein Barr virus (EBV) promoter, or a Rous sarcoma virus (RSV) promoter. The promoter may also be a promoter from a human gene such as human actin, human myosin, human hemoglobin, human muscle creatine, or human metalothionein. The promoter may also be a tissue specific promoter, such as a muscle or skin specific promoter, natural or synthetic. Examples of such promoters are described in US patent application publication no. US20040175727, the contents of which are incorporated herein in its entirety.

The plasmid may also comprise a polyadenylation signal, which may be downstream of the coding sequence. The polyadenylation signal may be a SV40 polyadenylation signal, LTR polyadenylation signal, bovine growth hormone (bGH) polyadenylation signal, human growth hormone (hGH) polyadenylation signal, or human β-globin polyadenylation signal. The SV40 polyadenylation signal may be a polyadenylation signal from a pCEP4 plasmid (Invitrogen, San Diego, CA).

The plasmid may also comprise an enhancer upstream of the coding sequence. The enhancer may be human actin, human myosin, human hemoglobin, human muscle creatine or a viral enhancer such as one from CMV, FMDV, RSV or EBV. Polynucleotide function enhances are described in U.S. Patent Nos. 5,593,972, 5,962,428, and WO94/016737, the contents of each are fully incorporated by reference.

The plasmid may also comprise a mammalian origin of replication in order to maintain the plasmid extrachromosomally and produce multiple copies of the plasmid in a cell. The plasmid may be pVAX1, pCEP4 or pREP4 from Invitrogen (San Diego, CA), which may

comprise the Epstein Barr virus origin of replication and nuclear antigen EBNA-1 coding region, which may produce high copy episomal replication without integration. The backbone of the plasmid may be pAV0242. The plasmid may be a replication defective adenovirus type 5 (Ad5) plasmid.

The plasmid may also comprise a regulatory sequence, which may be well suited for gene expression in a cell into which the plasmid is administered. The coding sequence may comprise a codon that may allow more efficient transcription of the coding sequence in the host cell.

The coding sequence may also comprise an Ig leader sequence. The leader sequence may be 5' of the coding sequence. The consensus antigens encoded by this sequence may comprise an N-terminal Ig leader followed by a consensus antigen protein. The N-terminal Ig leader may be IgE or IgG.

The plasmid may be pSE420 (Invitrogen, San Diego, Calif.), which may be used for protein production in *Escherichia coli* (E.coli). The plasmid may also be pYES2 (Invitrogen, San Diego, Calif.), which may be used for protein production in Saccharomyces cerevisiae strains of yeast. The plasmid may also be of the MAXBAC™ complete baculovirus expression system (Invitrogen, San Diego, Calif.), which may be used for protein production in insect cells. The plasmid may also be pcDNA I or pcDNA3 (Invitrogen, San Diego, Calif.), which may be used for protein production in mammalian cells such as Chinese hamster ovary (CHO) cells.

5. Compositions

Compositions are provided which comprise nucleic acid molecules. The compositions may comprise a plurality of copies of a single nucleic acid molecule such a single plasmid, a plurality of copies of a two or more different nucleic acid molecules such as two or more different plasmids. For example a compositions may comprise plurality of two, three, four, five, six, seven, eight, nine or ten or more different nucleic acid molecules. Such a compositions may comprise plurality of two, three, four, five, six, seven, eight, nine or ten or more different plasmids.

Compositions may comprise nucleic acid molecules, such as plasmids, that collectively contain coding sequence for a single RSV Immunogen, coding sequence for two RSV

Immunogens, or coding sequence for three RSV Immunogens. In some instances, coding sequence for a fourth RSV Immunogen may be provided.

Compositions comprising coding sequence of two RSV Immunogens may be on a single nucleic acid molecule such as a single plasmid or the compositions may comprise two different nucleic acid molecule such as two different plasmids wherein one nucleic acid molecule comprises the coding sequence one RSV Immunogen and the other nucleic acid molecule comprises the coding sequence different RSV Immunogen. Similarly, compositions comprising coding sequence three RSV Immunogens may comprise a single nucleic acid molecule such as a single plasmid, two different nucleic acid molecules or three different nucleic acid molecules. Likewise, compositions comprising coding sequence four RSV Immunogens may comprise a single nucleic acid molecule such as a single plasmid, two different nucleic acid molecules, three different nucleic acid molecules or four different nucleic acid molecule.

In some embodiments, a composition comprises a plurality single nucleic acid molecule encoding one RSV Immunogen such as RSV F Immunogen, RSV Ga Immunogen or RSV Gb Immunogen. In some embodiments, a composition comprises a plurality single nucleic acid molecule, such a single plasmid encoding two RSV Immunogen such as RSV F Immunogen and RSV Ga Immunogen, RSV F Immunogen and RSV Gb Immunogen, and RSV Ga Immunogen and RSV Gb Immunogen. In some embodiments, a composition comprises a plurality single nucleic acid molecule, such a single plasmid encoding three RSV Immunogen such as RSV Ga Immunogen, RSV F Immunogen and RSV Gb Immunogen.

In some embodiments, a composition comprises a plurality two different nucleic acid molecules, such as two plasmids, each different nucleic acid molecule comprising a single different coding sequence for a different RSV Immunogen wherein pairs of different nucleic acid molecule comprise RSV F Immunogen and RSV Ga Immunogen, RSV F Immunogen and RSV Gb Immunogen, and RSV Ga Immunogen and RSV Gb Immunogen.

In some embodiments, a composition comprises a plurality two different nucleic acid molecules, such as two plasmids, which collectively comprising coding sequence for three different RSV Immunogen. In some embodiments, one encodes RSV F Immunogen and RSV Ga Immunogen, and the second encodes RSV Gb Immunogen. In some embodiments, one

encodes RSV F Immunogen and RSV Gb Immunogen, and the second encodes RSV Ga Immunogen. In some embodiments, one encodes Ga Immunogen and RSV Gb Immunogen, and the second encodes RSV F Immunogen.

In some embodiments, a composition comprises a plurality single nucleic acid molecule encoding RSV M2-1 Immunogen.

In some embodiments in which coding sequences for RSV M2-1 are provided, a composition comprises a plurality single nucleic acid molecules, such a single plasmid encoding two RSV Immunogen such as RSV F Immunogen and RSV M2-1 Immunogen, RSV M2-1 Immunogen and RSV Ga Immunogen, and RSV M2-1 Immunogen and RSV Gb Immunogen.

In some embodiments in which coding sequences for RSV M2-1 are provided, a plurality single nucleic acid molecules, such a single plasmid encoding three RSV Immunogen such as RSV F Immunogen, RSV M2-1 Immunogen and RSV Ga Immunogen, RSV F Immunogen, RSV M2-1 Immunogen and RSV Gb Immunogen, RSV M2-1 Immunogen, RSV Ga Immunogen and RSV Gb Immunogen. In some embodiments, a composition comprises a plurality single nucleic acid molecule, such a single plasmid encoding three RSV Immunogen such as RSV F Immunogen, RSV M2-1 Immunogen, RSV Ga Immunogen and RSV Gb Immunogen.

In some embodiments in which coding sequences for RSV M2-1 are provided, a composition comprises a plurality two different nucleic acid molecules, such as two plasmids, each different nucleic acid molecule comprising a single different coding sequence for a different RSV Immunogen wherein pairs of different nucleic acid molecule comprise RSV F Immunogen and RSV M2-1 Immunogen, RSV M2-1 Immunogen and RSV Ga Immunogen, and RSV M2-1 Immunogen and RSV Gb Immunogen.

In some embodiments in which coding sequences for RSV M2-1 are provided, a composition comprises a plurality two different nucleic acid molecules, such as two plasmids, which collectively comprising coding sequence for three different RSV Immunogen or which one is RSV M2-1. Embodiments may be selected from the group consisting of: one nucleic acid molecule encodes RSV F Immunogen and RSV M2-1 Immunogen and the second encodes RSV Ga Immunogen; one nucleic acid molecule encodes RSV F Immunogen and RSV M2-1 Immunogen and the second encodes RSV Gb Immunogen; one encodes RSV F Immunogen and

RSV Ga Immunogen, and the second encodes RSV M2-1 Immunogen; one encodes RSV F Immunogen and RSV Gb Immunogen, and the second encodes RSV M2-1 Immunogen; one encodes RSV M2-1 Immunogen and RSV Ga Immunogen, and the second encodes RSV F Immunogen; one encodes RSV M2-1 Immunogen and RSV Ga Immunogen, and the second encodes RSV Gb Immunogen; one encodes RSV M2-1 Immunogen and RSV Gb Immunogen, and the second encodes RSV F Immunogen; one encodes RSV M2-1 Immunogen and RSV Gb Immunogen, and the second encodes RSV Ga Immunogen; and one encodes Ga Immunogen and RSV Gb Immunogen, and the second encodes RSV M2-1 Immunogen.

In some embodiments in which coding sequences for RSV M2-1 are provided, a composition comprises a plurality two different nucleic acid molecules, such as two plasmids, which collectively comprising coding sequence for four different RSV Immunogen. In some embodiments: one nucleic acid molecule encodes RSV F Immunogen and the second encodes RSV M2-1 Immunogen, RSV Ga Immunogen and RSV Gb Immunogen; one nucleic acid molecule encodes RSV M2-1 Immunogen and the second encodes RSV F Immunogen, RSV Ga Immunogen and RSV Gb Immunogen; one encodes RSV Ga Immunogen and the second encodes RSV F Immunogen, RSV M2-1 Immunogen and RSV Gb Immunogen, one encodes RSV Gb Immunogen and the second encodes RSV F Immunogen, RSV M2-1 Immunogen and RSV Ga Immunogen, one encodes RSV F and RSV M2-1 Immunogen the second encodes RSV Ga Immunogen and RSV Gb Immunogen, one encodes RSV F and RSV Ga Immunogen the second encodes RSV M2-1 Immunogen and RSV Gb Immunogen, and one encodes RSV F and RSV Gb Immunogen the second encodes RSV M2-1 Immunogen and RSV Ga Immunogen. In some embodiments, a composition comprises a plurality three different nucleic acid molecules, such as three plasmids, which collectively comprising coding sequence for three different RSV Immunogen. In some embodiments: one nucleic acid molecule encodes RSV F Immunogen, one encodes RSV M2-1 Immunogen and the third second encodes RSV Ga Immunogen; one nucleic acid molecule encodes RSV F Immunogen, one encodes RSV M2-1 Immunogen and the third second encodes RSV Gb Immunogen; one nucleic acid molecule encodes RSV F Immunogen, one encodes RSV Ga Immunogen and the third second encodes RSV Gb Immunogen; and one nucleic acid molecule encodes RSV M2-1 Immunogen, one encodes RSV Ga Immunogen and

the third second encodes RSV Gb Immunogen. In some embodiments, a composition comprises a plurality three different nucleic acid molecules, such as three plasmids, which collectively comprising coding sequence for four different RSV Immunogens. In some embodiments: one nucleic acid molecule encodes RSV F Immunogen, the second encodes RSV M2-1 Immunogen and the third encodes RSV Ga Immunogen and RSV Gb Immunogen; one nucleic acid molecule encodes RSV F Immunogen, the second encodes RSV Ga Immunogen and the third encodes RSV M2-1 Immunogen and RSV Gb Immunogen; one nucleic acid molecule encodes RSV F Immunogen, the second encodes RSV Gb Immunogen and the third encodes RSV M2-1 Immunogen and RSV Ga Immunogen; one nucleic acid molecule encodes RSV M2-1 Immunogen, the second encodes RSV Ga Immunogen and the third encodes RSV F Immunogen and RSV Gb Immunogen; one nucleic acid molecule encodes RSV M2-1 Immunogen, the second encodes RSV Gb Immunogen, and the third encodes RSV F Immunogen and RSV Ga Immunogen; and one encodes RSV Ga Immunogen, the second encodes RSV Gb Immunogen, and the third encodes RSV F Immunogen and RSV M2-1. In some embodiments, a composition comprises a plurality four different nucleic acid molecules, such as four plasmids, which collectively comprising coding sequence for four different RSV Immunogens, i.e. one nucleic acid molecule encodes RSV F Immunogen, the second encodes RSV M2-1 Immunogen, the third encodes RSV Ga Immunogen and the fourth encodes RSV Gb Immunogen.

In some embodiments, a composition further comprises coding sequence for chemokine CCL20, IL-12, IL-15 and/or IL-28. Coding sequence for chemokine CCL20, IL-12, IL-15 and/or IL-28 may be included on one or more nucleic acid molecules that comprise .coding sequence for one or more RSV Immunogens. Coding sequence for chemokine CCL20, IL-12, IL-15 and/or IL-28 may be included on a separate nucleic acid molecules such as a separate plasmid.

6. Vaccine

Provided herein is a vaccine capable of generating in a mammal an immune response against RSV. The vaccine may comprise each plasmid as discussed above. The vaccine may comprise a plurality of the plasmids, or combinations thereof. The vaccine may be provided to induce a therapeutic or prophylactic immune response.

In some embodiments, the vaccine is preferably Thl biased so as not to play into prior Th2 related pathogenesis and it should be able to function in a neutralization sensitive environment. Preferably, it should be non-live.

Vaccines may be used to deliver one or more immunogenic targets selected from the group consisting of: F, Ga and Gb, fragments of such proteins, variants of such proteins, fragments of variants. In the case of delivery of multiple targets, vaccines may include multiple compositions or a single compositions. Plasmids may be used which encode multiple proteins on a single plasmid or compositions which comprise different plasmids that encode different proteins. In some embodiments, vaccines may be used to deliver M2, fragments thereof , variants of M2-1, fragments of variants, alone or in combination with one or more immunogenic targets selected from the group consisting of: F, Ga and Gb, fragments of such proteins, variants of such proteins, fragments of variants.. In the case of delivery of multiple targets, vaccines may include multiple compositions or a single compositions. Plasmids may be used which encode multiple proteins on a single plasmid or compositions which comprise different plasmids that encode different proteins.

The vaccine may further comprise a pharmaceutically acceptable excipient. The pharmaceutically acceptable excipient may be functional molecules as vehicles, adjuvants, carriers, or diluents. The pharmaceutically acceptable excipient may be a transfection facilitating agent, which may include surface active agents, such as immune-stimulating complexes (ISCOMS), Freunds incomplete adjuvant, LPS analog including monophosphoryl lipid A, muramyl peptides, quinone analogs, vesicles such as squalene and squalene, hyaluronic acid, lipids, liposomes, calcium ions, viral proteins, polyanions, polycations, or nanoparticles, or other known transfection facilitating agents.

The transfection facilitating agent is a polyanion, polycation, including poly-L-glutamate (LGS), or lipid. The transfection facilitating agent is poly-L-glutamate, and more preferably, the poly-L-glutamate is present in the vaccine at a concentration less than 6 mg/ml. The transfection facilitating agent may also include surface active agents such as immune-stimulating complexes (ISCOMS), Freunds incomplete adjuvant, LPS analog including monophosphoryl lipid A, muramyl peptides, quinone analogs and vesicles such as squalene and squalene, and hyaluronic

acid may also be used administered in conjunction with the genetic construct. In some embodiments, the DNA plasmid vaccines may also include a transfection facilitating agent such as lipids, liposomes, including lecithin liposomes or other liposomes known in the art, as a DNA-liposome mixture (see for example W09324640), calcium ions, viral proteins, polyanions, polycations, or nanoparticles, or other known transfection facilitating agents. Preferably, the transfection facilitating agent is a polyanion, polycation, including poly-L-glutamate (LGS), or lipid. Concentration of the transfection agent in the vaccine is less than 4 mg/ml, less than 2 mg/ml, less than 1 mg/ml, less than 0.750 mg/ml, less than 0.500 mg/ml, less than 0.250 mg/ml, less than 0.100 mg/ml, less than 0.050 mg/ml, or less than 0.010 mg/ml.

The pharmaceutically acceptable excipient may be one or more adjuvants. An adjuvant may be other genes that are expressed from the same or from an alternative plasmid or are delivered as proteins in combination with the plasmid above in the vaccine. The one or more adjuvants may be proteins and/or nucleic acid molecules that encode proteins selected from the group consisting of: CCL20, α-interferon (IFN- α), β-interferon (IFN-β), γ-interferon, platelet derived growth factor (PDGF), TNFα, TNFβ, GM-CSF, epidermal growth factor (EGF), cutaneous T cell-attracting chemokine (CTACK), epithelial thymus-expressed chemokine (TECK), mucosae-associated epithelial chemokine (MEC), IL-12, IL-15 including IL-15 having the signal sequence or coding sequence that encodes the signal sequence deleted and optionally including a different signal peptide such as that from IgE or coding sequence that encodes a difference signal peptide such as that from IgE, IL-28, MHC, CD80, CD86, IL-1, IL-2, IL-4, IL-5, IL-6, IL-10, IL-18, MCP-1, MIP-lα, MIP-1β, IL-8, L-selectin, P-selectin, E-selectin, CD34, GlyCAM-1, MadCAM-1, LFA-1, VLA-1, Mac-1, pl50.95, PECAM, ICAM-1, ICAM-2, ICAM-3, CD2, LFA-3, M-CSF, G-CSF, mutant forms of IL-18, CD40, CD40L, vascular growth factor, fibroblast growth factor, IL-7, nerve growth factor, vascular endothelial growth factor, Fas, TNF receptor, Flt, Apo-1, p55, WSL-1, DR3, TRAMP, Apo-3, AIR, LARD, NGRF, DR4, DR5, KILLER, TRAIL-R2, TRICK2, DR6, Caspase ICE, Fos, c-jun, Sp-1, Ap-1, Ap-2, p38, p65Rel, MyD88, IRAK, TRAF6, IkB, Inactive NIK, SAP K, SAP-1, JNK, interferon response genes, NFkB, Bax, TRAIL, TRAILrec, TRAILrecDRC5, TRAIL-R3, TRAIL-R4, RANK, RANK LIGAND, Ox40, Ox40 LIGAND, NKG2D, MICA, MICB, NKG2A, NKG2B, NKG2C, NKG2E,

NKG2F, TAP1, TAP2 and functional fragments thereof. or a combination thereof. In some embodiments adjuvant may be one or more proteins and/or nucleic acid molecules that encode proteins selected from the group consisting of: CCL-20, IL-12, IL-15, IL-28, CTACK, TECK, MEC or RANTES. Examples of IL-12 constructs and sequences are disclosed in PCT application no. PCT/US1997/019502 and corresponding US Application Serial No. 08/956,865, and U.S. Provisional Application Serial No 61/569600 filed December 12, 2011, which are each incorporated herein by reference. Examples of IL-15 constructs and sequences are disclosed in PCT application no. PCT/US04/18962 and corresponding US Application Serial No. 10/560,650, and in PCT application no. PCT/US07/00886 and corresponding U.S. Application Serial No. 12/160,766, and in PCT application no. PCT/US10/048827, which are each incorporated herein by reference. Examples of IL-28 constructs and sequences are disclosed in PCT application no. PCT/US09/039648 and corresponding U.S. Application Serial No. 12/936,192, which are each incorporated herein by reference. Examples of RANTES and other constructs and sequences are disclosed in PCT application no. PCT/US1999/004332 and corresponding U.S. Application Serial No. and 09/622452, which are each incorporated herein by reference. Other examples of RANTES constructs and sequences are disclosed in PCT application no. PCT/US11/024098, which is incorporated herein by reference. Examples of RANTES and other constructs and sequences are disclosed in PCT application no. PCT/US1999/004332 and corresponding U.S. Application Serial No. 09/622452, which are each incorporated herein by reference. Other examples of RANTES constructs and sequences are disclosed in PCT application no. PCT/US11/024098, which is incorporated herein by reference. Examples of chemokines CTACK, TECK and MEC constructs and sequences are disclosed in PCT application no. PCT/US2005/042231 and corresponding U.S. Application Serial No. 11/719,646, which are each incorporated herein by reference. Examples of OX40 and other immunomodulators are disclosed in U.S. Application Serial No. 10/560,653, which is incorporated herein by reference. Examples of DR5 and other immunomodulators are disclosed in U.S. Application Serial No. 09/622452, which is incorporated herein by reference.

The vaccine may further comprise a genetic vaccine facilitator agent as described in U.S. Serial No. 021,579 filed April 1, 1994, which is fully incorporated by reference.

The vaccine may comprise the consensus antigens and plasmids at quantities of from about 1 nanogram to 100 milligrams; about 1 microgram to about 10 milligrams; or preferably about 0.1 microgram to about 10 milligrams; or more preferably about 1 milligram to about 2 milligram. In some preferred embodiments, pharmaceutical compositions according to the present invention comprise about 5 nanogram to about 1000 micrograms of DNA. In some preferred embodiments, the pharmaceutical compositions contain about 10 nanograms to about 800 micrograms of DNA. In some preferred embodiments, the pharmaceutical compositions contain about 0.1 to about 500 micrograms of DNA. In some preferred embodiments, the pharmaceutical compositions contain about 1 to about 350 micrograms of DNA. In some preferred embodiments, the pharmaceutical compositions contain about 25 to about 250 micrograms, from about 100 to about 200 microgram, from about 1 nanogram to 100 milligrams; from about 1 microgram to about 10 milligrams; from about 0.1 microgram to about 10 milligrams; from about 1 milligram to about 2 milligram, from about 5 nanogram to about 1000 micrograms, from about 10 nanograms to about 800 micrograms, from about 0.1 to about 500 micrograms, from about 1 to about 350 micrograms, from about 25 to about 250 micrograms, from about 100 to about 200 microgram of the consensus antigen or plasmid thereof.

The vaccine may be formulated according to the mode of administration to be used. An injectable vaccine pharmaceutical composition may be sterile, pyrogen free and particulate free. An isotonic formulation or solution may be used. Additives for isotonicity may include sodium chloride, dextrose, mannitol, sorbitol, and lactose. The vaccine may comprise a vasoconstriction agent. The isotonic solutions may include phosphate buffered saline. Vaccine may further comprise stabilizers including gelatin and albumin. The stabilizing may allow the formulation to be stable at room or ambient temperature for extended periods of time such as LGS or polycations or polyanions to the vaccine formulation.

7. Methods of Delivery the Vaccine

Provided herein is a method for delivering the vaccine for providing genetic constructs and proteins of the consensus antigen which comprise epitopes that make them particular effective against inmmunogens of RSV against which an immune response can be induced. The method of delivering the vaccine or vaccination may be provided to induce a therapeutic and

prophylactic immune response. The vaccination process may generate in the mammal an immune response against RSV. The vaccine may be delivered to an individual to modulate modulate the activity of the mammal's immune system and enhance the immune response. The delivery of the vaccine may be the transfection of the consensus antigen as a nucleic acid molecule that is expressed in the cell and delivered to the surface of the cell upon which the immune system recognized and induces a cellular, humoral, or cellular and humoral response. The delivery of the vaccine may be used to induce or elicit and immune response in mammals against RSV by administering to the mammals the vaccine as discussed above.

Upon delivery of the vaccine and plasmid into the cells of the mammal, the transfected cells will express and secrete consensus antigens for each of the plasmids injected from the vaccine. These proteins will be recognized as foreign by the immune system and antibodies will be made against them. These antibodies will be maintained by the immune system and allow for an effective response to subsequent RSV infections.

The vaccine may be administered to a mammal to elicit an immune response in a mammal. The mammal may be human, primate, non-human primate, cow, cattle, sheep, goat, antelope, bison, water buffalo, bison, bovids, deer, hedgehogs, elephants, llama, alpaca, mice, rats, and chicken.

a. Combination Treatments

The vaccine may be administered in combination with other proteins and/or genes encoding CCL20, α-interferon, γ-interferon, platelet derived growth factor (PDGF), TNFα, TNFβ, GM-CSF, epidermal growth factor (EGF), cutaneous T cell-attracting chemokine (CTACK), epithelial thymus-expressed chemokine (TECK), mucosae-associated epithelial chemokine (MEC), IL-12, IL-15 including IL-15 having the signal sequence deleted and optionally including the different signal peptide such as the IgE signal peptide, MHC, CD80, CD86, IL-28, IL-1, IL-2, IL-4, IL-5, IL-6, IL-10, IL-18, MCP-1, MIP-lα, MIP-1β, IL-8, RANTES, L-selectin, P-selectin, E-selectin, CD34, GlyCAM-1, MadCAM-1, LFA-1, VLA-1, Mac-1, pl50.95, PECAM, ICAM-1, ICAM-2, ICAM-3, CD2, LFA-3, M-CSF, G-CSF, mutant forms of IL-18, CD40, CD40L, vascular growth factor, fibroblast growth factor, IL-7, nerve growth factor, vascular endothelial growth factor, Fas, TNF receptor, Flt, Apo-1, p55, WSL-1,

DR3, TRAMP, Apo-3, AIR, LARD, NGRF, DR4, DR5, KILLER, TRAIL-R2, TRICK2, DR6, Caspase ICE, Fos, c-jun, Sp-1, Ap-1, Ap-2, p38, p65Rel, MyD88, IRAK, TRAF6, IkB, Inactive NIK, SAP K, SAP-1, JNK, interferon response genes, NFkB, Bax, TRAIL, TRAILrec, TRAILrecDRC5, TRAIL-R3, TRAIL-R4, RANK, RANK LIGAND, Ox40, Ox40 LIGAND, NKG2D, MICA, MICB, NKG2A, NKG2B, NKG2C, NKG2E, NKG2F, TAP1, TAP2 and functional fragments thereof or combinations thereof. In some embodiments, the vaccine is administered in combination with one or more of the following nucleic acid molecules and/or proteins: nucleic acid molecules selected from the group consisting of nucleic acid molecules comprising coding sequence that encode one or more of CCL20, IL-12, IL-15, IL-28, CTACK, TECK, MEC and RANTES or functional fragments thereof, and proteins selected from the group consisting of: CCL02, IL-12 protein, IL-15 protein, IL-28 protein, CTACK protein, TECK protein, MEC protein or RANTES protein or functional fragments thereof.

The vaccine may be administered by different routes including orally, parenterally, sublingually, transdermally, rectally, transmucosally, topically, via inhalation, via buccal administration, intrapleurally, intravenous, intraarterial, intraperitoneal, subcutaneous, intramuscular, intranasal intrathecal, and intraarticular or combinations thereof. For veterinary use, the composition may be administered as a suitably acceptable formulation in accordance with normal veterinary practice. The veterinarian can readily determine the dosing regimen and route of administration that is most appropriate for a particular animal.. The vaccine may be administered by traditional syringes, needleless injection devices, "microprojectile bombardment gone guns", or other physical methods such as electroporation ("EP"), "hydrodynamic method", or ultrasound.

The plasmid of the vaccine may be delivered to the mammal by several well known technologies including DNA injection (also referred to as DNA vaccination) with and without in vivo electroporation, liposome mediated, nanoparticle facilitated, recombinant vectors such as recombinant adenovirus, recombinant adenovirus associated virus and recombinant vaccinia. The consensus antigen may be delivered via DNA injection and along with in vivo electroporation.

b. Electroporation

Administration of the vaccine via electroporation of the plasmids of the vaccine may be accomplished using electroporation devices that can be configured to deliver to a desired tissue of a mammal a pulse of energy effective to cause reversible pores to form in cell membranes, and preferable the pulse of energy is a constant current similar to a preset current input by a user. The electroporation device may comprise an electroporation component and an electrode assembly or handle assembly. The electroporation component may include and incorporate one or more of the various elements of the electroporation devices, including: controller, current waveform generator, impedance tester, waveform logger, input element, status reporting element, communication port, memory component, power source, and power switch. The electroporation may be accomplished using an in vivo electroporation device, for example CELLECTRA EP system (VGX Pharmaceuticals, Blue Bell, PA) or Elgen electroporator (Genetronics, San Diego, CA) to facilitate transfection of cells by the plasmid.

The electroporation component may function as one element of the electroporation devices, and the other elements are separate elements (or components) in communication with the electroporation component. The electroporation component may function as more than one element of the electroporation devices, which may be in communication with still other elements of the electroporation devices separate from the electroporation component. The elements of the electroporation devices existing as parts of one electromechanical or mechanical device may not limited as the elements can function as one device or as separate elements in communication with one another. The electroporation component may be capable of delivering the pulse of energy that produces the constant current in the desired tissue, and includes a feedback mechanism. The electrode assembly may include an electrode array having a plurality of electrodes in a spatial arrangement, wherein the electrode assembly receives the pulse of energy from the electroporation component and delivers same to the desired tissue through the electrodes. At least one of the plurality of electrodes is neutral during delivery of the pulse of energy and measures impedance in the desired tissue and communicates the impedance to the electroporation component. The feedback mechanism may receive the measured impedance and

can adjust the pulse of energy delivered by the electroporation component to maintain the constant current.

A plurality of electrodes may deliver the pulse of energy in a decentralized pattern. The plurality of electrodes may deliver the pulse of energy in the decentralized pattern through the control of the electrodes under a programmed sequence, and the programmed sequence is input by a user to the electroporation component. The programmed sequence may comprise a plurality of pulses delivered in sequence, wherein each pulse of the plurality of pulses is delivered by at least two active electrodes with one neutral electrode that measures impedance, and wherein a subsequent pulse of the plurality of pulses is delivered by a different one of at least two active electrodes with one neutral electrode that measures impedance.

The feedback mechanism may be performed by either hardware or software. The feedback mechanism may be performed by an analog closed-loop circuit. The feedback occurs every 50 μs, 20 μs, 10 μs or 1 μs, but is preferably a real-time feedback or instantaneous (i.e., substantially instantaneous as determined by available techniques for determining response time). The neutral electrode may measure the impedance in the desired tissue and communicates the impedance to the feedback mechanism, and the feedback mechanism responds to the impedance and adjusts the pulse of energy to maintain the constant current at a value similar to the preset current. The feedback mechanism may maintain the constant current continuously and instantaneously during the delivery of the pulse of energy.

Examples of electroporation devices and electroporation methods that may facilitate delivery of the DNA vaccines of the present invention, include those described in U.S. Patent No. 7,245,963 by Draghia-Akli, et al., U.S. Patent Pub. 2005/0052630 submitted by Smith, et al., the contents of which are hereby incorporated by reference in their entirety. Other electroporation devices and electroporation methods that may be used for facilitating delivery of the DNA vaccines include those provided in co-pending and co-owned U.S. Patent Application, Serial No. 11/874072, filed October 17, 2007, which claims the benefit under 35 USC 119(e) to U.S. Provisional Applications Ser. Nos. 60/852,149, filed October 17, 2006, and 60/978,982, filed October 10, 2007, all of which are hereby incorporated in their entirety.

U.S. Patent No. 7,245,963 by Draghia-Akli, et al. describes modular electrode systems and their use for facilitating the introduction of a biomolecule into cells of a selected tissue in a body or plant. The modular electrode systems may comprise a plurality of needle electrodes; a hypodermic needle; an electrical connector that provides a conductive link from a programmable constant-current pulse controller to the plurality of needle electrodes; and a power source. An operator can grasp the plurality of needle electrodes that are mounted on a support structure and firmly insert them into the selected tissue in a body or plant. The biomolecules are then delivered via the hypodermic needle into the selected tissue. The programmable constant-current pulse controller is activated and constant-current electrical pulse is applied to the plurality of needle electrodes. The applied constant-current electrical pulse facilitates the introduction of the biomolecule into the cell between the plurality of electrodes. The entire content of U.S. Patent No. 7,245,963 is hereby incorporated by reference.

U.S. Patent Pub. 2005/0052630 submitted by Smith, et al. describes an electroporation device which may be used to effectively facilitate the introduction of a biomolecule into cells of a selected tissue in a body or plant. The electroporation device comprises an electro-kinetic device ("EKD device") whose operation is specified by software or firmware. The EKD device produces a series of programmable constant-current pulse patterns between electrodes in an array based on user control and input of the pulse parameters, and allows the storage and acquisition of current waveform data. The electroporation device also comprises a replaceable electrode disk having an array of needle electrodes, a central injection channel for an injection needle, and a removable guide disk. The entire content of U.S. Patent Pub. 2005/0052630 is hereby incorporated by reference.

The electrode arrays and methods described in U.S. Patent No. 7,245,963 and U.S. Patent Pub. 2005/0052630 may be adapted for deep penetration into not only tissues such as muscle, but also other tissues or organs. Because of the configuration of the electrode array, the injection needle (to deliver the biomolecule of choice) is also inserted completely into the target organ, and the injection is administered perpendicular to the target issue, in the area that is pre-delineated by the electrodes The electrodes described in U.S. Patent No. 7,245,963 and U.S. Patent Pub. 2005/005263 are preferably 20 mm long and 21 gauge.

Additionally, contemplated in some embodiments that incorporate electroporation devices and uses thereof, there are electroporation devices that are those described in the following patents: US Patent 5,273,525 issued December 28, 1993, US Patents 6,110,161 issued August 29, 2000, 6,261,281 issued July 17, 2001, and 6,958,060 issued October 25, 2005, and US patent 6,939,862 issued September 6, 2005. Furthermore, patents covering subject matter provided in US patent 6,697,669 issued February 24, 2004, which concerns delivery of DNA using any of a variety of devices, and US patent 7,328,064 issued February 5, 2008, drawn to method of injecting DNA are contemplated herein. The above-patents are incorporated by reference in their entirety.

c. Method of Preparing DNA Plasmids

Provided herein is methods for preparing the DNA plasmids that comprise the DNA vaccines discussed herein. The DNA plasmids, after the final subcloning step into the mammalian expression plasmid, can be used to inoculate a cell culture in a large scale fermentation tank, using known methods in the art.

The DNA plasmids for use with the EP devices of the present invention can be formulated or manufactured using a combination of known devices and techniques, but preferably they are manufactured using an optimized plasmid manufacturing technique that is described in a licensed, co-pending U.S. provisional application U.S. Serial No. 60/939,792, which was filed on May 23, 2007. In some examples, the DNA plasmids used in these studies can be formulated at concentrations greater than or equal to 10 mg/mL. The manufacturing techniques also include or incorporate various devices and protocols that are commonly known to those of ordinary skill in the art, in addition to those described in U.S. Serial No. 60/939792, including those described in a licensed patent, US Patent No. 7,238,522, which issued on July 3, 2007. The above-referenced application and patent, US Serial No. 60/939,792 and US Patent No. 7,238,522, respectively, are hereby incorporated in their entirety.

EXAMPLES

The present invention is further illustrated in the following Examples. It should be understood that these Examples, while indicating preferred embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, various modifications of the invention in addition to those shown and described herein will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims

Example 1

With expected continued increases in the number of individuals at high risk for RSV infection in the coming years (*e.g.*, young infants and the elderly), there is a dire need for the development of an efficacious and financially manageable treatment for RSV infection. DNA vaccines, small bacterial plasmids genetically engineered to express an encoded protein of interest upon immunization, offer several significant advantages that may help in circumventing many of the hurdles involved in designing an efficacious RSV vaccine. For instance, to prevent clinically significant illness and re-infection, the vaccine would have to confer greater and longer lasting immunity than that which occurs from natural RSV infection. DNA vaccines not only elicit both humoral (*e.g.*, neutralization antibody) and cellular (*e.g.*, cytotoxic T lymphocytes) immune response, but can also elicit lifelong immunity against many viruses. In addition, the primary targets for RSV vaccination are neonates and young infants. Neonatal immune system is functionally immature and thus, develops only a weak immune response to conventional vaccines (*e.g.*, live attenuated or inactivated virus). DNA vaccines, on the other hand, have been shown to be safe and immunogenic in neonatal animal models. Furthermore, the presence of maternal antibody at the time of immunization has been known to suppress or inhibit immune responses to a variety of conventional vaccines. With DNA vaccine, however, the immunizing protein is not present in the vaccine preparation and therefore, should not be susceptible to direct inactivation by maternal antibodies.

Currently, there are three main RSV vaccines in or near early stages of clinical trials: 1) live attenuated RSV (MedImmune, Philadelphia, PA); 2) Sendai virus expressing the RSV F and/or G proteins (AmVac, Switzerland); and 3) virus-like particles containing the G protein of RSV (Novavax, Rockville, MD) (http://www.clinicaltrials.gov/ct2/results?term=RSV&pg=l). Although these approaches have shown some degree of efficacy in the prevention of RSV infection, there are other inherent disadvantages associated with such vaccine designs. For instance, the biggest issues with live attenuated virus are under-attenuation or mutations leading to reversion to virulence and therefore, should not be administered to immunocompromised individuals. A common drawback to all viral vectors is the host immune response as recognition of viral proteins leads to the generation of neutralizing antibodies that may significantly reduce the efficacy of the vaccine upon subsequent immunization. Lastly, the G protein of RSV is highly variable between the two subtypes of RSV and therefore, any vaccine targeting the G protein is not likely to provide cross protection.

In contrast, DNA vaccines have been shown to be highly immunogenic and lack many of the disadvantages associated with the above described vaccine approach . When delivered via electroporation, DNA vaccine immunogenicity further improves. DNA vaccines contain less than a complete set of viral components and like protein subunit vaccines have no risk for virulence reversion or infection of the host making them safe to use in both infants and in immunocompromised individuals.

Using consensus sequences of both subtype A and B of RSV provides the basis to obtain broader protection against both strains of the virus. Such advantages make consensus RSV DNA vaccine much more beneficial and potentially more efficacious than any other approaches suggested in the past

Major RSV proteins - fusion (F), glycoprotein (Ga), and glycoprotein (Ga) were chosen as targets for new RSV vaccines. Alsochosen for evaluation was the second matrix protein (M2). As mentioned above, F and G proteins are expressed on the surface and are ideal targets for neutralizing antibodies. M2 protein contains the major immunodominant CD8 T cell epitope. For each of these RSV proteins, a single consensus sequence was generated based on the amino acid sequences provided by PUBMED for both subtype A and B of RSV (Figure 1 shows the

phylogenetic tree of RSV-F Protein, the consensus RSV F protein amino acid sequence is set forth in SEQ ID NO:2; Figure 2 shows the phylogenetic tree of RSV M2-1 Protein, the consensus RSV M2-1 protein amino acid sequence is set forth in SEQ ID NO:4. In a preferred embodiments, codon and RNA optimized DNA sequences that encode the consensus protein sequences are produced and used to make DNA vaccines which may be delivered using electroporation.

Example 2

Successful expression of RSV consensus F protein was confirmed through immunofluorescence microscopy and/or western blot analysis. Constructs were used in immunization studies.

Mice were immunized with varying dosages of the different RSV DNA vaccine constructs. One group received the DNA vaccine backbone plasmid pVAX as a control. One group received 5 μg dose of plasmid RSV-F, one group received 15 μg dose of plasmid RSV-F; one group received 30 μg dose of plasmid RSV-F; and one group received 60 μg dose of plasmid RSV-F.

Mice were immunized 2 weeks apart for a total of 3 immunizations (weeks 0, 2 and 4) intramuscularly with electroporation. Mice were bled at weeks 2, 4, 6 and 12 after each immunization and the total RSV F protein specific IgG and IgA antibody was measured in the sera. Figure 3 shows anti-RSV F protein IgG after immunization 1 (i.e. at week 0), immunization 2 (i.e. week 2) and after immunizations 3 (i.e. week 4). Figure 4 shows anti-RSV F protein IgA after immunization 1 (i.e. at week 0), immunization 2 (i.e. week 2) and after immunizations 3 (i.e. week 4). Mice were sacrificed at week 12. In addition to comparisons of total IgG in sera and of total IgA in sera, a comparison of IFN-γ production at different dosage of RSV-F vaccine approximately 8 weeks after last immunization was done.

RSV-F DNA vaccine elicited high levels of both IgG and IgA in sera. Responses were detectable as early as 2 weeks after 2nd immunization for all dosage studied. The antibody responses induced appear to be strongly Thl biased, thus avoiding prior issues of the side effects of Th2 immune responses to RSV. RSV-F specific CD8 T cells were observed even after 8

weeks after the last immunization. Figure 5 shows results from a comparison of IFN-γ production at different dosage of RSV-F vaccine approximately 8 weeks after last immunization.

Example 3

Chemokines are a family of small proteins secreted by cells with ability to induce directed chemotaxis toward a higher concentration gradient in nearby responsive cells. Various groups have investigated the feasibility of using chemokines as immune adjuvants.

CCL28/MEC (mucosa-associated epithelial chemokine) which binds CCR10 is expressed on epithelial cells in the gut, lung, breast, and the salivary glands. Use of mucosal chemokines in previous studies showed their utility as immune adjuvants, inducing better immune response in the mucosal tissues after systemic immunization.

Experiments were performed to test the use of a CCL28 genetic construct as an adjuvant for the consensus RSV F protein DNA vaccine construct. A genetic construct that comprised nucleotide sequence encoding CCL20, also referred to a liver activation regulated chemokine (LARC) or Macrophage Inflammatory Protein-3 (MIP3A), was also tested as a possible adjuvant for the consensus RSV F protein DNA vaccine construct.

The immunization schedule for RSV-F plus chemokine experiments utilized three groups of mice: one receiving RSV-F construct only; one receiving RSV-F construct plus CCL20 construct; and one receiving RSV-F construct plus CCL28 construct. Mice were immunized a total of three times: at week 0, at week 2 and at week 4. Mice were bled at week 2, week 4 and week 5. At week 5, mice underwent vaginal wash to collect vaginal samples and were sacrificed after which lungs and spleens were collected.

Comparisons were made of IFN-γ+ CD8 and CD4 T cells in spleen after RSV-F +/- chemokine immunization in the spleen (Figure 6) and in lung (Figure 7) after RSV-F +/- chemokine immunization.

IgG Endpoint Titer in Sera was measured and IgG subtypes (IgG1 vs IgG2a) in Sera were compared (Figure 8). A comparison of IgG2a/IgG1 ratio was also done (Figure 9).

CCL20 and CCL28 chemokines did not appear to enhance CD8 and CD4 T cell immune responses after RSV-F DNA vaccination in either the spleen or the lung (based on IFN- γ ICS) although it is unclear if the dosage of chemokine construct was insufficient.

Animals receiving the CCL20 immune adjuvant exhibited a heavy bias towards a Th1 immune response (primarily IgG2a) after RSV-F DNA vaccination. Driving immune response away from Th2 and towards Th1 may be useful in order to avoid vaccine-associated enhanced disease after RSV infection.

Example 4

As noted above, administration of coding sequences that encode CCL20 led to a preferential Th1 immune response and away from Th2 immune responses. This property of CCL20 administration may be useful in the context of both an immunotherapeutic as well as a vaccine adjuvant for other targets in which a shift increasing Th1 immune responses and decreasing Th2 immune responses is desirable.

For example, CCL20 or administration of nucleic acid molecules that encode CCL20 may be useful to treat individuals who have autoimmune diseases or inflammatory conditions characterized by Th2 immune responses. The ability to decrease Th2 immune responses as shown herein can be used to reduce Th2 immune responses associated with certain autoimmune diseases or inflammatory conditions including for example.

In addition, CCL20 may be used as a vaccine adjuvant as described herein with vaccines targeting other infection diseases as well as cancer. The Th1 increasing/Th2 decreasing properties associated with the use of CCL20 as a vaccine adjuvant may be useful in vaccines. SEQ ID NO:18 and SEQ ID NO:20 contains high expression coding sequences for CCL20 with and without coding sequences encoding the IgE signal peptide. In some embodiments, SEQ ID NO:18 further comprises a start codon. SEQ ID NO:19 and SEQ ID NO:21 contains consensus sequences for CCL20 with and without coding sequences encoding the IgE signal peptide. In some embodiments, SEQ ID NO:19 further comprises an N terminal; methionine

Example 5

The Luciferase Reporter Gene Assay in TZM-bl Cells is based on Single-Round Infection with Molecularly Cloned Env-Pseudotyped Viruses. TZM-bl (JC53-bl) is a genetically

engineered HeLa cell line that expresses CD4, CXCR4 and CCR5 and contains Tat-inducible Luc and β-Gal reporter genes. The assay provides high success rate in single-round infections, increased assay capacity (2-day assay), increased precision (accurately measure 50% neutralization), improved level of standardization (stable cell line), and an optimized and validated assay platform. Montefiori, D.C. (2004) Current Protocols in Immunology, John Wiley & Sons, 12.11.1-12.11.15, which is incorporated herein by reference, described the assay.

Figure 10 depicts Env-Pseudotyped viruses in TZM-BL Cells/ "ON".

Figure 11 depicts Env-Pseudotyped viruses in TZM-BL Cells/ "OFF".

Figure 12 depicts RSV-Fusion Pseudo viral production in 293T cells.

Figure 13 shows the immunization protocol used for a RSV-F Neutralization Study using the assay.

Figure 14 shows data from quantification of RSV-F mediated infection in different target cells. Infectivity of pseudotypes bearing RSV-F proteins. Pseudotype viruses were pelleted, and the particle amounts were estimated by quantifying HIV p24 antigen. Cells were infected with pseudotype viruses or no envelope bearing a panel of diverse cell lines containing 10ng of particulate HIV p24 antigen. At 72 h postinfection, cells supernatant and assayed for p24 antigen. Values are the means of triplicate wells with the standard deviations.

Figure 15 shows data from quantification of RSV-F neutralization activity in TZM-bl HeLa cells. The data show results of a comparison of neutralizing ability to RSV-F using luciferase assay from RSV-F immunized serum samples from DNA vaccinated animals that can block the HIV-Luc/FMDV pseudovirus from entering and infecting target cells. The titer is defined as the reciprocal of the dilution of plasma that produces 50% inhibition of virus. The ability of the serum sample from the vaccinated mice to neutralize and block the infectivity of HIV-1 Luc/RSV-F the absence of such blocking activity in normal mouse serum (NMS).

Figure 16 shows data from quantification of cell death activity by RSV-F pseudo virus in HeLa cells. The results show that consensus RSV-F DNA plasmid elicits potent humoral and cellular immune responses in mice after 3 vaccinations with electroporation. A high level of RSV-F specific IgG in sera was observed as early as 1 week post 2nd immunization with just 5 ug DNA. Cell-mediated immune response were detectable as far out as 8 weeks post 3rd

immunization suggesting the long-term persistence of immune response after RSV-F DNA vaccination. Increase in RSV-F DNA concentration did not result in increased immune response. Antibodies generated after 3 vaccinations with RSV-F DNA plus electroporation were capable of neutralizing infection as measured by pseudo-virus neutralization assay. Results also showed mitigated RSV-F mediated apoptosis of target cells.

Example 6

Figure 17 shows the immunization schedule for RSV-M2 dosage study that was undertaken. Data collected from that study includes a comparison of IFN-g production at different dosages of RSV-M2 vaccine 1 week after last immunization (Figure 18). The results showed that a consensus-based RSV-M2 DNA plasmid elicited significant cellular immune responses in mice after 3 vaccinations with electroporation. High level of IFN-g+ T cells specific for either the immunodominant or subdominant epitopes of RSV-M2 in both spleen and in lung were observed. Maximum immune response was observed with 60 ug DNA (however, response not much greater than that observed with 10 ug DNA).

Example 7

Further study was undertaken to evaluate CCL20 as potential immune adjuvant to enhance mucosal immune response after DNA immunization. CCL20 (liver activation regulated chemokine – LARC; macrophage inflammatory protein 3 – MIP3a) binds to CCR6 and is expressed in lymph nodes, liver, appendix, fetal lung (lower levels in thymus, testis, prostate, and gut).

Figure 19 depicts an immunization schedule for evaluation of the combination of RSV-F vaccine with CCL20.

Figure 20 shows data from the study including data from a comparison of IFN-g production in each of spleen, liver and lung with different dosages of CCL20 at 1 week after last immunization.

Figure 21 shows data of a comparison of IgG subtypes (IgG1 vs IgG2a) in Sera.

Results show that co-immunization with CCL20 does not enhance cell-mediated immune response in both the lung and the liver after RSV-F DNA immunization. CCL20 appeared to bias RSV-F specific IgG antibodies generated after DNA immunization towards a Th1-isotype.

Less IgG1 and more IgG2a were observed in sera after CCL20 + RSV-F co-immunization compared to RSV-F immunization alone.

Thus, provided are improved vaccines, improved methods for inducing immune such responses, including mucosal immune responses, and for prophylactically and/or therapeutically immunizing individuals against immunogens, particularly those associated with pathogens and other agents which enter the host via mucosal tissue. For examples, infectious agents commonly enter the host across a mucosal tissue such as the oral mucosa and other mucosa of the alimentary canal, the respiratory tract including olfactory and conjunctival mucosa, the mammary glands, and the genitourinary tract.

Composition comprising nucleic acid sequences that encodes an immunogen in combination with nucleic acid sequences that encodes CCL20 and functional fragments thereof are provided. The composition may comprise an isolated nucleic acid molecule that encodes both an immunogen and CCL20 and functional fragments thereof and/or compositions comprising an isolated nucleic acid molecule that encodes an immunogen in combination with an isolated nucleic acid molecule that encodes CCL20 and functional fragments thereof. Such compositions may be provided as injectable pharmaceutical.

The compositions may be used in methods of inducing an immune response in an individual against an immunogen, comprising administering to the individual such a composition.

Recombinant vaccines comprising a nucleotide sequence that encodes an immunogen operably linked to regulatory elements and a nucleotide sequences that CCL20 or functional fragments thereof are provided as are methods of inducing an immune response, including methods of inducing a mucosal immune response, in an individual against an immunogen comprising administering such a recombinant vaccine to an individual.

Live attenuated pathogenscomprising a nucleotide sequence that CCL20 or functional fragments thereof, and to methods of inducing an immune response, including methods of inducing a mucosal immune response, in an individual against a pathogen comprising administering the live attenuated pathogen to an individual are also provided.

Methods of inducing an immune response in an individual against an immunogen comprising administering to said individual CCL20 protein or a functional fragment thereof in combination with an isolated nucleic acid molecule that encodes an immunogen; and/or a recombinant vaccine that encodes an immunogen and/or a subunit vaccine that comprises an immunogen and/or a live attenuated vaccine and/or a killed vaccine. Compositions comprising CCL20 protein or a functional fragments thereof in combination with one or more of an isolated nucleic acid molecule that encodes an immunogen; a recombinant vaccine comprising a nucleotide sequence that encodes an immunogen operably linked to regulatory elements; a live attenuated pathogen; and a killed pathogen are provided. As used herein, "functional fragment of CCL20" is meant to refer to a fragment of an iCCl20protein that, when delivered in conjunction with an immunogen, provides an modified immune response compared to the immune response that is induced when the immunogen is delivered without the fragment. Fragments are generally 10 or more amino acids in length. In some embodiments, functional fragments of CCL20 may comprise 20% or more, 25% or more, 30% or more, 35% or more, 40% or more, 45% or more, 50% or more, 55% or more, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 91% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, 99% or more percent of the length of the particular full length CCL20 protein.

When delivered in combination with an immunogen, CCL20 and functional fragments thereof, and combinations thereof modulates immune responses. Accordingly, a combination of these proteins may be delivered as components of a DNA or protein based vaccine in order to induce a therapeutic or prophylactic immune response or in compositions useful to induce an immune response. In some embodiments, the means to deliver the immunogen is a DNA vaccine, a recombinant vaccine, a protein subunit vaccine, an attenuated vaccine or a killed vaccine. In some embodiments, the means to deliver one or more of CCL20 and functional fragments thereof is by expression of coding sequences included in a DNA vaccine, a recombinant vaccine or an attenuated vaccine.

Immune responses result in the production of antigen specific antibodies and/or antigen specific T- and B-cells. Antigen specific antibodies and/or cells provide the means to protect

against infection, to reduce or to clear existing infection. They can also be isolated from the individual and used in other applications such as passive immunity protocols, immunocolumns or as reagents.

In some embodiments, CCL20 is useful to induce mucosal immune responses, particularly increased Th1 responses characterized by increased IgG2a responses and descreased IgG1 responses, even in protocols where the composition is delivered systemically. Co-immunization with an immunogen such as by DNA vaccine or other means plasmid plus one or more of these chemokines, such as in a DNA vaccine or part of the coding sequence of another type of vaccine, will provide a unique adjuvanting property.

CCL20 protein and nucleotides which encode it can be obtained from many sources, natural and sythentic. In some embodiments for which protein is used, for example, one having ordinary skill in the art can, using well known techniques, isolates CCL20 protein from natural sources using, for example, immuno columns which contain antibodies that specifically bind to the protein. Alternatively, the protein may be separated using electrophoresis, isolated from the electrophoresis matrix and purified by for example dialysis to yield essentially pure protein. Other well known protein purification technologies can be employed to produce isolated, essentially pure protein. In some embodiments for which protein is used, for example, one having ordinary skill in the art can, using well known techniques, inserts DNA molecules that encode any of CCL20 and functional fragments thereof into a commercially available expression vector for use in well known expression systems. In addition to isolating proteins from natural sources or producing proteins by recombinant techniques, automated peptide synthesizers may also be employed to produce isolated, essentially pure protein.

According to some aspects of the present invention, compositions and methods are provided which prophylactically and/or therapeutically immunize an individual against an immunogen such as an allergen, a pathogen or abnormal, disease-related cells. The vaccine may be any type of vaccine such as, a live attenuated vaccine, a cell vaccine, a recombinant vaccine or a nucleic, acid or DNA vaccine. By delivering nucleic acid molecules that encode an immunogen and one or more of CL20 and functional fragments thereof the immune response induced by the vaccine may be modulated.

Compositions for delivering immunogens and CCL20 are provided, particularly those comprising one or more nucleic acid molecules that comprise a nucleotide sequence that encodes one or more of CCL20 and functional fragments thereof operably linked to regulatory elements in combination with a nucleotide sequence that encodes an immunogen operably linked to regulatory elements. The nucleic acid sequences encoding CCL20 and the immunogen may be one the same molecule or on separate molecules.

The nucleic acid molecules may be delivered using any of several well known technologies including DNA injection (also referred to as DNA vaccination), recombinant vectors such as recombinant adenovirus, recombinant adenovirus associated virus and recombinant vaccinia. In some embodiments, DNA vaccines are constructed as described herein and delivered using electroporation as described herein. The descriptions for RSV vacicnes set forth herein, for example, may be applied to the use of CCL20 with other immunogens. DNA vaccines are described in U.S. Pat. Nos. 5,593,972, 5,739,118, 5,817,637, 5,830,876, 5,962,428, 5,981,505, 5,580,859, 5,703,055, 5,676,594, and the priority applications cited therein, which are each incorporated herein by reference. In addition to the delivery protocols described in those applications, alternative methods of delivering DNA are described in U.S. Pat. Nos. 4,945,050 and 5,036,006, which are both incorporated herein by reference. Routes of administration include, but are not limited to, intramuscular, intranasally, intraperitoneal, intradermal, subcutaneous, intravenous, intraarterially, intraocularly and oral as well as topically, transdermally, by inhalation or suppository or to mucosal tissue such as by lavage to vaginal, rectal, urethral, buccal and sublingual tissue. Preferred routes of administration include intramuscular, intraperitoneal, intradermal and subcutaneous injection. In some embodiments, nucleotide sequences that encode CCL20 or functional fragments thereof may be linked to IgE signal peptide. In some embodiments, the immunogen is a pathogen antigen, a cancer-associated antigen or an antigen linked to cells associated with autoimmune diseases. The present invention may be used to immunize an individual against all pathogens such as viruses, prokaryote and pathogenic eukaryotic organisms such as unicellular pathogenic organisms and multicellular parasites. The present invention is particularly useful to immunize an individual against those pathogens which infect cells and which are not encapsulated such as viruses, and prokaryote

such as gonorrhea, listeria and shigella. In addition, the present invention is also useful to immunize an individual against protozoan pathogens that include a stage in the life cycle where they are intracellular pathogens. Examples of pathogens include the following. Viruses in the Picornavirus Family Genera such as rhinoviruses (responsible for - 50% cases of the common cold), Etheroviruses, (include polioviruses, coxsackieviruses, echoviruses, and human enteroviruses such as hepatitis A virus), and Apthoviruses (Veterinary; target antigens include: VP1, VP2, VP3, VP4, VPG). Viruses in the Calcivirus Family Genera include Norwalk Group of Viruses (causative agent of epidemic gastroenteritis). Viruses of the Togavirus Family Genera include Alphaviruses: (examples include Senilis viruses, RossRiver virus and Eastern & Western, Equine encephalitis). Reovirus include Rubella virus. Flariviridue Family Examples include dengue, yellow fever, Japanese encephalitis, St. Louis encephalitis and tick borne encephalitis viruses. West Nile virus (Genbank NC001563, AF533540, AF404757, AF404756, AF404755, AF404754, AF404753, AF481864, M12294, AF317203, AF196835, AF260969, AF260968, AF260967, AF206518 and AF202541) Representative Target antigens: E NS5 C. Hepatitis C Virus. Coronavirus Family include infectious bronchitis virus. porcine transmissible gastroenteric virus, Porcine hemaglutinating encephalomyelitis virus, Feline infectious peritonitis virus, Feline enteric coronavirus, Canine coronavirus, SARS associated coronavirus, human respiratory coronaviruses cause ~40 cases of common cold. EX. 224E, OC43. Target antigens: E1 - also called M or matrix protein E2 - also called S or Spike protein E3 - also called BE or hemagglutin- elterose glycoprotein N – nucleocapsid. Rhabdovirus Family Genera include Vesiliovirus Lyssavirus such as rabies. Target antigen: G protein N protein. Filoviridue Family includes Hemorrhagic fever viruses such as (Medical) Marburg and Ebola virus. Paramyxovirus Genera includes paramyxovirus: Family such as Mumps virus, New Castle disease virus, Morbillivirus such as Measles, canine distemper, Pneuminvirus such as Respiratory syncytial virus, Orthomyxovirus. The Influenza virus Family includes Bungavirus Family Genera such as bungavirus, California encephalitis, LA Crosse, Phlebovirus such as Rift Valley Fever, Hantavirus such as puremala, a hemahagin fever virus, Nairvirus, unassigned bungaviruses Arenavirus Family LCM, and Lassi fever virus. Reovirus Family Genera includes Reovirus, Rotavirus, Orbiviruses such as Colorado Tick fever, Lebombo, equine encephalosis, blue tongue.

Retrovirus Family Sub-Family includes oncorivirinal such as leukemia virus, HTLVI and HTLVII, Lentivirinal such as HIV, feline immunodeficiency virus, equine infections, and anemia virus. Spumavirinal Papovavirus Family Sub-Family includes Polyomaviruses such as BKU and JCU viruses, Sub-Family Papillomavirus. Adenovirus such as EX AD7, ARD., O.B., 275. Parvovirus Family includes Feline parvovirus, Feline panleucopeniavirus, Canine parvovirus, Porcine parvovirus. Herpesvirus Family Sub-Family includes alpha-Genera Simplexvirus herpesviridue HSVI (Genbank X14112, NC001806), HSVII (NC001798) Varicellovinis, pseudorabies, varicella zoster. Sub-Family - beta- incoudes Genera Cytomegalovirus such as herpesviridue HCMV. Muromegalovirus Sub-Family includes Genera: Lymphocryptovirus, Gamma- EBV - (Burkitts lympho), herpesviridue. Rhadinovirus Poxvirus Family Sub-Family Genera Variola includes (Smallpox) Chordopoxviridue Vaccinia (Cowpox) (Parapoxivirus) Auipoxvirus, Capripoxvirus Leporipoxvirus. Suipoxviru's Sub-Family includes Entemopoxviridue Hepadnavirus. Hepatitis B virus Family Unclassified Hepatitis delta virus. Bacterial pathogens include pathogenic gram-positive cocci such as pneurnococcal; staphylococcal; and streptococcal. Pathogenic gram-negative cocci include: meningococcal; and gonococcal. Pathogenic enteric gram-negative bacilli include: enterobacteriaceae; pseudomonas, acinetobacteria and eikenella, melioidosis;, sahnonella; shigellosis; hemophilus; chancroid; brucellosis; tularemia; yersinia (pasteurella); streptobacillus mortiliformis and spirillum; listeria monocytogenes; erysipelothrix rhusiopathiae; diphtheria, cholera, anthrax; donovanosis (granuloma inguinale); and bartonellosis. Pathogenic anaerobic bacteria include: tetanus; botulism; other clostridia; tuberculosis; leprosy; and other mycobacteria. Pathogenic spirochetal diseases include: syphilis; - treponematoses: yaws, pinta and endemic syphilis; and leptospirosis. Other infections caused by higher pathogen bacteria and pathogenic fungi include: actinomycosis; .nocardiosis; cryptococcosis, blastomycosis, histoplasmosis and coccidioidomycosis; candidiasis, aspergillosis, and mucormycosis; sporotrichosis; paracoccidiodomycosis, petriellidiosis, torulopsosis, mycetoma, and chromomycosis; and dermatophytosis. Rickettsial infections include rickettsial and rickettsioses. Examples of mycoplasma and chlarnydial infections include: mycoplasma pneurnoniae; lymphogranuloma venereum; psittacosis; and perinatal chlamydial infections. Pathogenic eukaryotes Pathogenic

protozoans and helminths and infections thereby include: amebiasis; malaria; leishmaniasis; trypanosomiasis; toxoplasmosis; pneurnocystis carinii; babesiosis; giardiasis; trichinosis; filariasis; schistosomiasis; nematodes; trematodes or flukes; and cestode (tapeworm) infections. Vaccines can be made such as DNA constructs that comprise DNA sequences that encode the peptides that comprise at least an epitope identical or substantially similar to an epitope displayed on a pathogen antigen. In some embodiments, the methods of immunizing an individual against a pathogen are directed against RSV, HIV, HSV, HCV, WNV or HBV.

Methods of conferring a protective immune response against hyperproliferating cells utilize as immunogens proteins that are characteristic in hyperproliferative diseases. These are useful in vaccines and methods of treating individuals suffering from hyperproliferative diseases. Examples of hyperproliferative diseases include all forms of cancer and psoriasis. Examples of target proteins against hyperproliferative diseases include those which are proteins encoded by oncogenes such as myb, myc, fyn, and the translocation gene bcr/abl, ras, src, P53, neu, trk and EGRF. In addition to oncogene products as target antigens, target proteins for anti-cancer treatments and protective regimens include variable regions of antibodies made by B cell lymphomas and variable regions of T cell receptors of T cell lymphomas which, in some embodiments, are also used target antigens for autoimmune disease. Other tumor-associated proteins can be used as target proteins such as proteins that are found at higher levels in tumor cells including the protein recognized by monoclonal antibody 17-IA and folate binding proteins or PSA. Methods of treating individuals suffering from autoimmune diseases and disorders by conferring a broad based protective immune response against targets that are associated with autoimmunity including cell receptors and cells which produce "self"-directed antibodies are also provided. T cell mediated autoimmune diseases include Rheumatoid arthritis (RA), multiple sclerosis (MS), Sjogren's syndrome, sarcoidosis, insulin dependent diabetes mellitus (1DDM), autoimmune thyroiditis, reactive arthritis, ankylosing spondylitis, scleroderma, polymyositis, dermatomyositis, psoriasis, vasculitis, Wegener's granulomatosis, Crohn's disease and ulcerative colitis. Each of these diseases is characterized by T cell receptors that bind to endogenous antigens and initiate the inflammatory cascade associated with autoimmune diseases. Vaccination against the variable region of the T cells would elicit an immune response including

CTLs to eliminate those T cells. In RA, several specific variable regions of T cell receptors (TCRs) that are involved in the disease have been characterized. These TCRs include V.beta.-3, V.beta.-14, 20 V.beta.-17 and V.alpha.-17. Thus, vaccination with a DNA construct that encodes at least one of these proteins will elicit an immune response that will target T cells involved in RA. See: Howell, M. D., et al., 1991 Proc. Nat. Acad. Sci. USA 88:10921-10925; Piliard, X., et al, 1991 Science 253:325-329; Williams, W. V., et al., 1992 J Clin. Invest. 90:326-333; each of which is incorporated herein by reference. In MS, several specific variable regions of TCRs that are involved in the disease have been characterized. These TCRs include VfP and V.alpha.-10. Thus, vaccination with a DNA construct that encodes at least one of these proteins will elicit an immune response that will target T cells involved in MS. See: Wucherpfennig, K. W., et al., 1990 Science 248:1016-1019; Oksenberg, J. R., et al, 1990 Nature 345:344-346; each of which is incorporated herein by reference. In scleroderma, several specific variable regions of TCRs that are involved in the disease have been characterized. These TCRs include V.beta.-6, V.beta.-g, V.beta.-14 and V.alpha.-16, V.alpha.-3C, V.alpha.-7, V.alpha.-14, V.alpha.-15, V.alpha.-16, V.alpha.-28 and V.alpha.-12. Thus, vaccination with a DNA construct that encodes at least one of these proteins will elicit an immune response that will target T cells involved in scleroderma. In order to treat patients suffering from a T cell mediated autoimmune disease, particularly those for which the variable region of the TCR has yet to be characterized, a synovial biopsy can be performed. Samples of the T cells present can be taken and the variable region of those TCRs identified using standard techniques. Genetic vaccines can be prepared using this information. B cell mediated autoimmune diseases include Lupus (SLE), Grave's disease, myasthenia gravis, autoimmune hemolytic anemia, autoimmune thrombocytopenia, asthma, cryoglobulineinia, primary biliary sclerosis and pernicious anemia. Each of these diseases is characterized by antibodies that bind to endogenous antigens and initiate the inflammatory cascade associated with autoimmune diseases. Vaccination against the variable region of antibodies would elicit an immune response including CTLs to eliminate those B cells that produce the antibody.

In order to treat patients suffering from a B cell mediated autoimmune disease, the variable region of the antibodies involved in the autoimmune activity must be identified. A biopsy can be performed and samples of the antibodies present at a site of inflammation can be

taken. The variable region of those antibodies can be identified using standard techniques. Genetic vaccines can be prepared using this information. In the case of SLE, one antigen is believed to be DNA. Thus, in patients to be immunized against SLE, their sera can be screened for anti-DNA antibodies and a vaccine can be prepared which includes DNA constructs that encode the variable region of such anti-DNA antibodies found in the sera. Common structural features among the variable regions of both TCRs and antibodies are well known. The DNA sequence encoding a particular TCR or antibody can generally be found following well known methods such as those described in Kabat, et al 1987 Sequence of Proteins of Immunological Interest U.S. Department of Health and Human Services, Bethesda Md., which is incorporated herein by reference. In addition, a general method for cloning functional variable regions from antibodies can be found in Chaudhary, V. K., et al, 1990 Proc. Natl. Acad Sci. USA 87:1066, which is incorporated herein by reference.

In addition to using expressible forms of CCL20 protein coding sequence to improve DNA vaccines, improved attenuated live vaccines, improved killed vaccines and improved vaccines that use recombinant vectors to deliver foreign genes that encode antigens and well as subunit and glycoprotein vaccines are provided which comprise CCL20 protein or a functional fragment thereof and/or coding sequences that encode CCL20 or a functional fragment thereof. Examples of attenuated live vaccines, those using recombinant vectors to deliver foreign antigens, subunit vaccines and glycoprotein vaccines are described in U.S. Pat. Nos. 4,510,245; 4,797,368; 4,722,848; 4,790,987; 4,920,209; 5,017,487; 5,077,044; 5,110,587; 5,112,749; 5,174,993; 5,223,424; 5,225,336; 5,240,703; 5,242,829; 5,294,441; 5,294,548; 5,310,668; 5,387,744; 5,389,368; 5,424,065; 5,451,499; 5,453,364; 5,462,734; 5,470,734; 5,474,935; 5,482,713; 5,591,439; 5,643,579; 5,650,309; 5,698,202; 5,955,088; 6,034,298; 6,042,836; 6,156,319 and 6,589,529, which are each incorporated herein by reference.

Example 8

Using consensus sequences of RSV immunogens F, G(A) and G(B) provides the basis to obtain neutiralizing immune responses. Such advantages make consensus RSV DNA vaccine much more beneficial and potentially more efficacious than any other approaches suggested in the past

Major RSV proteins - fusion (F), glycoprotein (Ga), and glycoprotein (Ga) were chosen as targets for new RSV vaccines. As mentioned above, F and G proteins are expressed on the surface and are ideal targets for neutralizing antibodies. A cocktail DNA vaccine was prepared comprising plasmids that have sequence which encode and express consensus RSV-F, plasmids that have sequence which encode and express consensus RSV-G(A) and plasmids that have sequence which encode and express consensus RSV-G(B). The individual plasmids as well as the cocktail were used in studies. The cocktail vaccine was used in studies with rabbits and non-human primates and the results of those studies show that high levels of neutralizing immune response are induced, indicating that the cocktail vaccine can be used in both prophylactic and therapeutic applications with particular value in protecting against RSV infection.

As shown in Figures 22A, Figures 22B and Figures 22C, single insert plasmids for RSV-F, RSV-G(A), and RSV-G(B), respectively, were constructed using the pVAX vector. Consensus sequences for each respective plasmid were cloned into the pVAX vector between the BamHI and XhoI sites. The resulting plasmids were: pRSV-F, which comprises an 1813 base pair insert that encodes consensus RSV-F cloned into pVAX to produce a plasmid having 4812 base pairs; pRSV-G(A), which comprises a 945 base pair insert that encodes consensus RSV-G(A) cloned into pVAX to produce a plasmid having 3944 base pairs; and pRSV-G(B), which comprises a 930 base pair insert that encodes consensus RSV-G(B) cloned into pVAX to produce a plasmid having 3929 base pairs.

To confirm expression of RSV-F, RSV-G(A), and RSV-G(B) constructs, RD cells were transfected with pRSV-F, pRSV-G(A) and pRSV-G(B), respectively. Immunofluorescent evaluation was performed using immunized, pooled rabbit sera. RSV Immunogens were visualized by immunofluorescence by binding anti-RSV immunogen antibodies chemically conjugated with fluorescein isothiocyanate (FITC). DAPI was used as a nuclear counterstain. Results in Figure 23 show RD cells transfected with pVAX showed no immunostaining by FITC labeled anti-RSV immunogen antibodies.

An RSV-F Dosage Study was done using mice. The immunization schedule is shown in Figure 24. Five groups of mice were used. Group 1 were control injected with pVAX. Group 2 were injected with 5μg pRSV-F construct. Group 3 were injected with 15μg pRSV-F construct.

Group 4 were injected with 30μg pRSV-F construct. Group 5 were injected with 60μg pRSV-F construct. Animals were immunized a total of three times using electroporation: first immunization at Week 0, second immunization at Week 2 and third immunization at Week 4. Sera was collected and IgG measured a total of four times: first collection at Week 2, second collection at Week 4, third collection at Week 6 and fourth collection at Week 12. Animals were sacrificed at Week 12, spleens were harvested and IFN-gamma was measured via ELISPOT.

Figure 25 shows data from the RSV-F Dosage comparing total RSV-F IgG in Sera. Data is shown from sera collected after immunization #1, after immunization #2, and after immunization #3. Anti-RSV-F IgG was measured at reciprocal serum dilutions of 10, 100, 1,000 and 10,000. Data show pVAX did not induce antibodies after any immunization. Data show pRSV-F construct did not induce antibodies after first immunization. Data show pRSV-F construct induced antibodies after second and third immunization.

Figure 26 shows data from the RSV-F Dosage comparing IFN-γ production induced by RSV-F vaccination approximately eight weeks after last immunization. Data is shown comparing IFN-γ production (SFU/106 splenocytes) from animals injected with pVAX and 15 μg average dose pRSV-F construct.

An RSV-F PSEUDO Neutralization Study was done using mice. The immunization schedule is shown in Figure 27. Mice were immunized with 15μg pRSV-F construct. Animals were immunized a total of three times using electroporation: first immunization at Week 0, second immunization at Week 2 and third immunization at Week 4. Sera was collected and neutralization assay performed at Week 5.

Data from the RSV-F PSEUDO Neutralization Study is shown in Figure 28. RSV-F pseudo-type neutralization activity in TZM-bl HeLa cells was quantified. A comparison of neutralizing ability of RSV-F using luciferase assay from RSV-F immunized serum samples from DNA vaccinated animals that can block the HIV-Luc/RSV-F pseudovirus from entering and infecting target cells. The titer is defined as the reciprocal of the dilution of plasma that produces 50% inhibition of virus. Note the ability of the serum sample from the vaccinated mice to neutralize and block infectivity of HIV-Luc/RSV-F in the absence of such blocking activity in normal mouse serum (NMS).

As shown in Figure 29, two comparison were done using sera from two animals vaccinated with pRSV-F (RSV-F2 and RSV-F5) versus sera from pVAX vaccinated animals to determine inhibition of apoptotic cell death activity by RSV-F pseudovirus in HeLa cells. For each comparison, sera was diluted 1:1, 1:10, 1:100 and 1:1000. The level of cell death expressed as a percentage was determined showing that sera from animals vaccinated with pRSV-F inhibited apoptotic cell death of HeLa cell exposed to RSV-F pseudovirus in the presence of sera.

An RSV-G(A) and RSV-(B) Dosage Study was done using mice. The immunization schedule is shown in Figure 30. Six groups of mice were used. Group 1 were control injected with pVAX. Group 2 were injected with 1μg pRSV-G(A) or pRSV-G(B) construct. Group 3 were injected with 5μg pRSV-G(A) or pRSV-G(B) construct. Group 4 were injected with 10μg pRSV-G(A) or pRSV-G(B) construct. Group 5 were injected with 20μg pRSV-G(A) or pRSV-G(B) construct. Group 6 were injected with 40μg pRSV-G(A) or pRSV-G(B) construct. Animals were immunized a total of three times using electroporation: first immunization at Week 0, second immunization at Week 2 and third immunization at Week 4. Sera was collected and IgG measured a total of two times: first collection at Week 4, and the second collection at Week 6.

Figure 31 shows data for IgG in sera after RSV-G(A) DNA immunization in the RSV(A) or RSV-G(B) Dosage Study. Total anti-RSV-G(A) IgG in Sera was measured from the Dosage Study. Data is shown from sera collected at various doses as set out in the x-axis of the graph in Figure 31. Anti-RSV-G(A) IgG was measured at serum dilution of 1:100.

Figure 32 shows data for IgG in sera after RSV-G(B) DNA immunization in the RSV(A) or RSV-G(B) Dosage Study. Total anti-RSV-G(B) IgG in Sera was measured from the Dosage Study. Data is shown from sera collected at various doses as set out in the x-axis of the graph in Figure 31. Anti-RSV-G(B) IgG was measured at serum dilution of 1:100.

Figure 33 is a chart showing s summary of RSV Preliminary Neutralization results using Mono Vaccines in Mouse Studies. Neutralization titers from sera collected 3 days post immunization were measured. Negative serum was used as a negative control. Positive controls included an anti-RSV monoclonal antibody and anti-RSV polyclonal serum. Neutralization

titers were measured for controls and for sera from two mice vaccinated with pRSV-F, two mice vaccinated with pRSV-G(A) and one mouse vaccinated with pRSV-G(B).

An RSV Rabbit Study was designed and undertaken. The RSV Rabbit Study Design is shown in Figure 34. Four female New Zealand white (NZW) rabbits were immunized with a cocktail containing pRSV-F, pRSV-G(A), and pRSV-G(B). Each animal received 200 μg/plasmid in a final 100 μl volume. Animals were immunized a total of four times using intramuscular/electroporation immunization. The first immunization was done at Week 0, the second immunization at Week 3, the third immunization at Week 6 and the fourth at Week 9. Blood was collected from animals six times: Week -2 (Pre-bleed - 2 weeks prior to first immunization), Week 0 (Pre-bleed prior to first immunization), Week 2, Week 5, Week 8 and Week 13. Blood was analyzed using ELISA and virus neutralization assays.

Data from the Rabbit Study is shown in Figure 35. Humoral immunity against RSV-F was measured. Anti-RSV-F IgG from pre-bleed samples taken at Weeks -2 and 0 and from samples taken at Week 2, at Week 5, at Week 8 and at Week 13. At Week 2, animals from which samples were collected had been immunized one time and the sample was collected 2 weeks post immunization. At Week 5, animals from which samples were collected had been immunized two times and the sample was collected 2 weeks after the second immunization. At Week 8, animals from which samples were collected had been immunized three times and the sample was collected 2 weeks after the third immunization. At Week 13, animals from which samples were collected had been immunized four times and the sample was collected 4 weeks after the fourth immunization.

Data from the Rabbit Study is shown in Figure 36. Humoral immunity against RSV-G was measured. Anti-RSV-G IgG from pre-bleed samples taken at Weeks -2 and 0 and from samples taken at Week 2, at Week 5, at Week 8 and at Week 13. At Week 2, animals from which samples were collected had been immunized one time and the sample was collected 2 weeks post immunization. At Week 5, animals from which samples were collected had been immunized two times and the sample was collected 2 weeks after the second immunization. At Week 8, animals from which samples were collected had been immunized three times and the sample was collected 2 weeks after the third immunization. At Week 13, animals from which

samples were collected had been immunized four times and the sample was collected 4 weeks after the fourth immunization.

An RSV Non-human Primate Study was undertaken. The purpose of the study was to evaluate the humoral immunogenicity of the single-insert pDNA RSV vaccines administered as a cocktail in the non-human primate (NHP). Also, the study was used to evaluate and determine whether factors such as multiple site delivery of the vaccine, electroporation method, and delivery with an adjuvant would increase the breadth of the humoral responses. The experiments were performed to determine if the synthetic DNA vaccines against RSV-F, RSV-G(A), and RSV-G(B) stimulate high titer of anti-RSV antibodies, to analyze the specific immune responses in animals vaccinated with the RSV cocktail and to evaluate in the effect of adjuvant and/or multiple site delivery on the immune responses generated. The RSV Non-Human Primate Study Design is shown in Figure 37. The non-human primate used were Rhesus macaques. In the study, 15 Rhesus macaques were divided into 4 groups: three groups contained four animals each and one group contained three animals. The groups in which n = 4 were treated groups, i.e. they were vaccinated. The group in which n = 3 was the untreated group which served as a control. The groups were treated as follows.

Group 1 (n=4): each animal was administered a cocktail of 1 mg RSV-F, 1 mg RSV-G(A), 1 mg RSV-G(B), mixed and delivered IM in 1 site.

Group 2 (n=4): each animal was administered a cocktail of 1 mg RSV-F, 1 mg RSV-G(A), 1 mg RSV-G(B), 1 mg rhMEC, mixed and delivered IM in 1 site.

Group 3 (n=4): each animal was administered a cocktail of 1 mg RSV-F, 1 mg RSV-G(A), 1 mg RSV-G(B), 1 mixed and delivered ID in 3 sites.

Group 4 (n=3): each animal was untreated.

Animals were immunized a total of three times using electroporation. The first immunization was done at Week 1, the second immunization at Week 5, and the third immunization at Week 13. Blood was collected from animals six times: Week 0 (Pre-bleed prior to first immunization),

Week 3, Week 7, Week 15, Week 17 and Week 18. Blood was analyzed using ELISA and virus neutralization assays.

Figure 38A-38D show data from the RSV Non-Human Primate Study of measured humoral immunity against RSV-F. Figure 38A shows data from measured humoral immunity against RSV-F in naïve animals. Figure 38B shows data from measured humoral immunity against RSV-F in animals who received the cocktail vaccine IM at one site. Figure 38C shows data from measured humoral immunity against RSV-F in animals who received the cocktail vaccine plus rhMEC construct IM at one site. Figure 38D shows data from measured humoral immunity against RSV-F in animals who received the cocktail vaccine ID at three sites. Anti-RSV-F IgG from pre-bleed samples taken at Week 0 and from samples taken at Week 3, at Week 15 and at Week 18. At Week 3, animals from which samples were collected had been immunized one time and the sample was collected 2 weeks post immunization. At Week 15, animals from which samples were collected had been immunized three times and the sample was collected 2 weeks after the third immunization. At Week 18, animals from which samples were collected had been immunized three times and the sample was collected 5 weeks after the third immunization.

Figure 39A-39D show data from the RSV Non-Human Primate Study of measured humoral immunity against RSV-G. Figure 39A shows data from measured humoral immunity against RSV-G in naïve animals. Figure 39B shows data from measured humoral immunity against RSV-G in animals who received the cocktail vaccine IM at one site. Figure 39C shows data from measured humoral immunity against RSV-G in animals who received the cocktail vaccine plus rhMEC construct IM at one site. Figure 39D shows data from measured humoral immunity against RSV-G in animals who received the cocktail vaccine ID at three sites. Anti-RSV-G IgG from pre-bleed samples taken at Week 0 and from samples taken at Week 3, at Week 15 and at Week 18. At Week 3, animals from which samples were collected had been immunized one time and the sample was collected 2 weeks post immunization. At Week 15, animals from which samples were collected had been immunized three times and the sample was collected 2 weeks after the third immunization. At Week 18, animals from which samples were

collected had been immunized three times and the sample was collected 5 weeks after the third immunization.

Figure 40 is a chart showing a summary of RSV Preliminary Neutralization results using a cocktail vaccines in Rabbit Non-Human Primate Studies. Neutralization titers from sera collected 3 days post immunization were measured. Negative serum was used as a negative control. Positive controls included an anti-RSV monoclonal antibody and anti-RSV polyclonal serum. Neutralization titers were measured for controls and for sera from two rabbits vaccinated with the cocktail vaccine and two non-human primates vaccinated with the cocktail vaccine. Data from pre-bleeds shows that neutralization titers in the rabbits and the non-human primates were generated post vaccination.

CLAIMS

1. A composition comprising one or more nucleic acid sequences that encode an RSV immunogen selected from the group consisting of:

a) a nucleic acid coding sequence that encodes an RSV F immunogen, wherein the nucleic acid coding sequence that encodes the RSV F immunogen is selected from the group consisting of:

a nucleic acid sequence encoding SEQ ID NO:2 and optionally further comprising coding sequence that encodes a signal peptide operably linked to the sequence that encodes SEQ ID NO:2;

a nucleic acid sequence that encodes a fragment of SEQ ID NO:2 that is at least 115 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to the sequence that encodes the fragment of SEQ ID NO:2;

a nucleic acid sequence that encodes a protein that is at least 98% homologous to SEQ ID NO:2 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence that encodes that protein that is at least 98% homologous to SEQ ID NO:2; and

a nucleic acid sequence that encodes a fragment of a protein that is at least 98% homologous to SEQ ID NO:2 that is at least 115 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to the sequence that encodes the fragment of a protein that is at least 98% homologous to SEQ ID NO:2;

b) a nucleic acid coding sequence that encodes an RSV Ga immunogen, wherein the nucleic acid coding sequence that encodes the RSV Ga immunogen is selected from the group consisting of:

a nucleic acid sequence encoding SEQ ID NO:6 and optionally further comprising coding sequence that encodes a signal peptide operably linked to the sequence that encodes SEQ ID NO:6;

a nucleic acid sequence that encodes a fragment of SEQ ID NO:6 that is at least 125 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to the sequence that encodes the fragment of SEQ ID NO:6;

a nucleic acid sequence that encodes a protein that is at least 98% homologous to SEQ ID NO:6 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence that encodes that protein that is at least 98% homologous to SEQ ID NO:6; and

a nucleic acid sequence that encodes a fragment of a protein that is at least 98% homologous to SEQ ID NO:6 that is at least 125 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to the sequence that encodes the fragment of a protein that is at least 98% homologous to SEQ ID NO:6;

and

c) a nucleic acid coding sequence that encodes an RSV Gb immunogen, wherein the nucleic acid coding sequence that encodes the RSV Gb immunogen is selected from the group consisting of:

a nucleic acid sequence encoding SEQ ID NO:8 and optionally further comprising coding sequence that encodes a signal peptide operably linked to the sequence that encodes SEQ ID NO:8;

a nucleic acid sequence that encodes a fragment of SEQ ID NO:8 that is at least 125 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to the sequence that encodes the fragment of SEQ ID NO:8;

a nucleic acid sequence that encodes a protein that is at least 98% homologous to SEQ ID NO:8 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence that encodes that protein that is at least 98% homologous to SEQ ID NO:8; and

a nucleic acid sequence that encodes a fragment of a protein that is at least 98% homologous to SEQ ID NO:8 that is at least 125 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to the sequence that encodes the fragment of a protein that is at least 98% homologous to SEQ ID NO:8.

2. The composition of claim 1 comprising one or more nucleic acid sequences that encode an RSV immunogen selected from the group consisting of:

a) a nucleic acid coding sequence that encodes an RSV F immunogen, wherein the nucleic acid coding sequence that encodes the RSV F immunogen is selected from the group consisting of:

a nucleic acid sequence comprising SEQ ID NO:1 and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:1;

a nucleic acid sequence comprising a fragment of SEQ ID NO:1 encoding at least 115 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:1;

a nucleic acid sequence at least 98% homologous to SEQ ID NO:1 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence at least 98% homologous to SEQ ID NO:1; and

a fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:1 encoding at least 115 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:1;

b) a nucleic acid coding sequence that encodes an RSV Ga immunogen, wherein the nucleic acid coding sequence that encodes the RSV Ga immunogen is selected from the group consisting of:

a nucleic acid sequence comprising SEQ ID NO:5 and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:5;

a nucleic acid sequence comprising a fragment of SEQ ID NO:5 encoding at least 125 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:5;

a nucleic acid sequence at least 98% homologous to SEQ ID NO:5 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence at least 98% homologous to SEQ ID NO:5; and

a fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:5 encoding at least 125 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:5; and

c) a nucleic acid coding sequence that encodes an RSV Gb immunogen, wherein the nucleic acid coding sequence that encodes the RSV Gb immunogen is selected from the group consisting of:

a nucleic acid sequence comprising SEQ ID NO:7 and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:7;

a nucleic acid sequence comprising a fragment of SEQ ID NO:7 encoding at least 125 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:7;

a nucleic acid sequence at least 98% homologous to SEQ ID NO:7 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence at least 98% homologous to SEQ ID NO:7; and

a fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:7 encoding at least 125 or more amino acids and optionally further comprises coding

sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:7.

3. The composition of claim 1 comprising one or more nucleic acid sequences selected from the group consisting of:

a) a nucleic acid coding sequence that encodes an RSV F immunogen comprising SEQ ID NO:2 and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:2;

b) a nucleic acid coding sequence that encodes an RSV Ga immunogen comprising SEQ ID NO:6 and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:6;

and

c) a nucleic acid coding sequence that encodes an RSV Gb immunogen comprising SEQ ID NO:8 and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:8.

4. The composition of claim 3 comprising one or more nucleic acid sequences selected from the group consisting of:

a) a nucleic acid coding sequence that encodes an RSV F immunogen, the coding sequence comprising SEQ ID NO:1 and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:1;

b) a nucleic acid coding sequence that encodes an RSV Ga immunogen, the coding sequence comprising SEQ ID NO:5 and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:5;

and

c) a nucleic acid coding sequence that encodes an RSV Gb immunogen, the coding sequence comprising SEQ ID NO:7 and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:7.

5. The composition of claim 3 comprising one or more nucleic acid sequences selected from the group consisting of:

a) a nucleic acid sequence that encodes SEQ ID NO:10;

b) a nucleic acid sequence that encodes SEQ ID NO:14;

and

c) a nucleic acid sequence that encodes SEQ ID NO:16;

6. The composition of claim 3 comprising one or more nucleic acid sequences selected from the group consisting of:

a) a nucleic acid sequence comprising SEQ ID NO:9;

b) a nucleic acid sequence comprising SEQ ID NO:13;

and

c) a nucleic acid sequence comprising SEQ ID NO:15.

7. The composition of claim 1 comprising one or more nucleic acid sequences selected from the group consisting of:

a) a nucleic acid coding sequence that encodes an RSV F immunogen comprising a fragment of SEQ ID NO:2 having at least 345 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:2, or a fragment of a protein that is at least 98% homologous to SEQ ID NO:2 having at least 345 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that encodes a protein least 98% homologous to SEQ ID NO:2;

b) a nucleic acid coding sequence that encodes an RSV Ga immunogen comprising a fragment of SEQ ID NO:6 having at least 200 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:6, or a fragment of a protein that is at least 98% homologous to SEQ ID NO:6 having at least 200 or more amino acids and optionally further comprises coding sequence that encodes a signal

peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that encodes a protein least 98% homologous to SEQ ID NO:6;
and

c) a nucleic acid coding sequence that encodes an RSV Gb immunogen comprising a fragment of SEQ ID NO:8 having at least 200 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:8, or a fragment of a protein that is at least 98% homologous to SEQ ID NO:8 having at least 200 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that encodes a protein least 98% homologous to SEQ ID NO:8.

8. The composition of claim 7 comprising one or more nucleic acid sequences selected from the group consisting of:

a) a nucleic acid coding sequence that encodes an RSV F immunogen comprising a nucleic acid sequence comprising a fragment of SEQ ID NO:1 encoding at least 345 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:1, or a fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:1 encoding at least 345 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:1;

b) a nucleic acid coding sequence that encodes an RSV Ga immunogen, comprising a fragment of SEQ ID NO:5 encoding at least 200 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:5, or a fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:5 encoding at least 125 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:5;
and

c) a nucleic acid coding sequence that encodes an RSV Gb immunogen comprising a fragment of SEQ ID NO:7 encoding at least 200 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:7, or a fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:7 encoding at least 200 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:7.

9. The composition of claim 1 comprising one or more nucleic acid sequences selected from the group consisting of:

a) a nucleic acid coding sequence that encodes an RSV F immunogen comprising a fragment of SEQ ID NO:2 having at least 550 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:2, or a fragment of a protein that is at least 98% homologous to SEQ ID NO:2 having at least 550 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that encodes a protein least 98% homologous to SEQ ID NO:2;

b) a nucleic acid coding sequence that encodes an RSV Ga immunogen comprising a fragment of SEQ ID NO:6 having at least 270 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:6, or a fragment of a protein that is at least 98% homologous to SEQ ID NO:6 having at least 270 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that encodes a protein least 98% homologous to SEQ ID NO:6;

and

c) a nucleic acid coding sequence that encodes an RSV Gb immunogen comprising a fragment of SEQ ID NO:8 having at least 270 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:8, or a fragment of a protein that is at least 98% homologous to SEQ ID NO:8 having at least 270 or

more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that encodes a protein least 98% homologous to SEQ ID NO:8.

10. The composition of claim 9 comprising one or more nucleic acid sequences selected from the group consisting of:

a) a nucleic acid coding sequence that encodes an RSV F immunogen comprising a nucleic acid sequence comprising a fragment of SEQ ID NO:1 encoding at least 550 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:1, or a fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:1 encoding at least 550 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:1;

b) a nucleic acid coding sequence that encodes an RSV Ga immunogen, comprising a fragment of SEQ ID NO:5 encoding at least 270 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:5, or a fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:5 encoding at least 270 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:5;

and

c) a nucleic acid coding sequence that encodes an RSV Gb immunogen comprising a fragment of SEQ ID NO:7 encoding at least 270 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:7, or a fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:7 encoding at least 270 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:7.

11. The composition of claim 1 comprising one or more nucleic acid sequences selected from the group consisting of:

a) a nucleic acid coding sequence that encodes an RSV F immunogen at least 99% homologous to SEQ ID NO:2 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence at least 99% homologous to SEQ ID NO:2;

b) a nucleic acid coding sequence that encodes an RSV Ga immunogen at least 99% homologous to SEQ ID NO:6 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence at least 98% homologous to SEQ ID NO:6; and

c) a nucleic acid coding sequence that encodes an RSV Gb immunogen at least 99% homologous to SEQ ID NO:8 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence at least 98% homologous to SEQ ID NO:8.

12. The composition of claim 11 comprising one or more nucleic acid sequences selected from the group consisting of:

a) a nucleic acid coding sequence that encodes an RSV F immunogen, the coding sequence being at least 99% homologous to SEQ ID NO:1 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence at least 99% homologous to SEQ ID NO:1;

b) a nucleic acid coding sequence that encodes an RSV Ga immunogen, the coding sequence being at least 99% homologous to SEQ ID NO:5 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence at least 98% homologous to SEQ ID NO:5;

and

c) a nucleic acid coding sequence that encodes an RSV Gb immunogen, the coding sequence being at least 99% homologous to SEQ ID NO:7 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence at least 98% homologous to SEQ ID NO:7.

13. A composition of claim 1 comprising nucleic acid sequences selected from the group consisting of:

a nucleic acid coding sequence that encodes an RSV F immunogen,

a nucleic acid coding sequence that encodes an RSV Ga immunogen,

a nucleic acid coding sequence that encodes an RSV Gb immunogen,

a nucleic acid coding sequence that encodes an RSV F immunogen and a nucleic acid coding sequence that encodes an RSV Ga immunogen,

a nucleic acid coding sequence that encodes an RSV F immunogen and a nucleic acid coding sequence that encodes an RSV Gb immunogen,

a nucleic acid coding sequence that encodes an RSV Ga immunogen and a nucleic acid coding sequence that encodes an RSV Gb immunogen, and

a nucleic acid coding sequence that encodes an RSV F immunogen, a nucleic acid coding sequence that encodes an RSV Ga immunogen and a nucleic acid coding sequence that encodes an RSV Gb immunogen.

14. The composition of any of claims 1-13 further comprising a nucleic acid coding sequence that encodes CCL20.

15. The composition of any of claims 1-13 formulated for delivery to an individual using electroporation.

16. The composition of any of claims 1-13 further comprising nucleic acid sequences that encode one or more proteins selected from the group consisting of: IL-12, IL-15 and IL-28.

17. The composition of any of claims 1-13 wherein said one or more nucleic acid coding sequences are part of one or more plasmids.

18. The composition of any of claims 1-13 wherein each of the one or more nucleic acid coding sequences are part of incorporated into a separate plasmid.

19. A method of inducing an immune response against RSV comprising administering the composition of any of claims 1-13 to an individual in an amount effective to induce an immune response in said individual.

20. A method of treating an individual who has been diagnosed with RSV comprising administering a therapeutically effective amount of the composition of any of claims 1-13 to an individual.

21. A method of preventing RSV infection an individual comprising administering a prophylactically effective amount of the composition of any of claims 1-13 to an individual.

22. A protein selected from the group consisting of: SEQ ID NO:2; SEQ ID NO:6; SEQ ID NO:8; SEQ ID NO:10; SEQ ID NO:14; and SEQ ID NO:16.

23. A composition comprising a nucleic acid coding sequence that encodes an RSV M2-1 immunogen, wherein the nucleic acid coding sequence that encodes the RSV M2-1 immunogen is selected from the group consisting of:

a nucleic acid sequence encoding SEQ ID NO:4 and optionally further comprising coding sequence that encodes a signal peptide operably linked to the sequence that encodes SEQ ID NO:4;

a nucleic acid sequence that encodes a fragment of SEQ ID NO:4 that is at least 25 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to the sequence that encodes the fragment of SEQ ID NO:4;

a nucleic acid sequence that encodes a protein that is at least 98% homologous to SEQ ID NO:4 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence that encodes that protein that is at least 98% homologous to SEQ ID NO:4; and

a nucleic acid sequence that encodes a fragment of a protein that is at least 98% homologous to SEQ ID NO:4 that is at least 25 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to the sequence that encodes the fragment of a protein that is at least 98% homologous to SEQ ID NO:4.

24. The composition of claim 23 comprising a nucleic acid coding sequence that encodes an RSV M2-1 immunogen, wherein the nucleic acid coding sequence that encodes the RSV M2-1 immunogen is selected from the group consisting of:

a nucleic acid sequence comprising SEQ ID NO:3 and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:3;

a nucleic acid sequence comprising a fragment of SEQ ID NO:3 encoding at least 25 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:3;

a nucleic acid sequence at least 98% homologous to SEQ ID NO:3 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence at least 98% homologous to SEQ ID NO:3; and

a fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:3 encoding at least 25 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:3.

25. The composition of claim 23 comprising a nucleic acid coding sequence that encodes an RSV M2-1 immunogen comprising SEQ ID NO:4 and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:4.

26. The composition of claim 25 comprising a nucleic acid coding sequence that encodes an RSV M2-1 immunogen, the coding sequence comprising SEQ ID NO:3 and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:3.

27. The composition of claim 25 comprising a nucleic acid sequence that encodes SEQ ID NO:12.

28. The composition of claim 25 comprising a nucleic acid sequence comprising SEQ ID NO:11.

29. The composition of claim 23 comprising a nucleic acid coding sequence that encodes an RSV M2-1 immunogen comprising a fragment of SEQ ID NO:4 having at least 130 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:4, or a fragment of a protein that is at least 98% homologous to SEQ ID NO:4 having at least 130 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that encodes a protein least 98% homologous to SEQ ID NO:4.

30. The composition of claim 29 comprising a nucleic acid coding sequence that encodes an RSV M2-1 immunogen, comprising a fragment of SEQ ID NO:3 encoding at least 130 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:3, or a fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:3 encoding at least 130 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:3.

31. The composition of claim 23 comprising a nucleic acid coding sequence that encodes an RSV M2-1 immunogen comprising a fragment of SEQ ID NO:4 having at least 165 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide

operably linked to SEQ ID NO:4, or a fragment of a protein that is at least 98% homologous to SEQ ID NO:4 having at least 165 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that encodes a protein least 98% homologous to SEQ ID NO:4.

32. The composition of claim 31 comprising a nucleic acid coding sequence that encodes an RSV M2-1 immunogen, comprising a fragment of SEQ ID NO:3 encoding at least 165 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:3, or a fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:3 encoding at least 165 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:3.

33. The composition of claim 23 comprising a nucleic acid coding sequence that encodes the RSV M2-1 immunogen at least 99% homologous to SEQ ID NO:4 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence at least 99% homologous to SEQ ID NO:4.

34. The composition of claim 33 comprising nucleic acid coding sequence that encodes the RSV M2-1 immunogen, the coding sequence being at least 99% homologous to SEQ ID NO:3 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence at least 99% homologous to SEQ ID NO:3.

35. A composition of claim 23 comprising nucleic acid sequences selected from the group consisting of:

a nucleic acid coding sequence that encodes an RSV M2-1 immunogen,

a nucleic acid coding sequence that encodes an RSV F immunogen and a nucleic acid coding sequence that encodes an RSV M2-1 immunogen,

a nucleic acid coding sequence that encodes an RSV M2-1 immunogen and a nucleic acid coding sequence that encodes an RSV Ga immunogen,

a nucleic acid coding sequence that encodes an RSV M2-1 immunogen and a nucleic acid coding sequence that encodes an RSV Gb immunogen,

a nucleic acid coding sequence that encodes an RSV F immunogen, a nucleic acid coding sequence that encodes an RSV M2-1 immunogen and a nucleic acid coding sequence that encodes an RSV Ga immunogen,

a nucleic acid coding sequence that encodes an RSV F immunogen, a nucleic acid coding sequence that encodes an RSV M2-1 immunogen and a nucleic acid coding sequence that encodes an RSV Gb immunogen,

a nucleic acid coding sequence that encodes an RSV M2-1 immunogen, a nucleic acid coding sequence that encodes an RSV Ga immunogen and a nucleic acid coding sequence that encodes an RSV Gb immunogen, and

a nucleic acid coding sequence that encodes an RSV F immunogen, a nucleic acid coding sequence that encodes an RSV M2-1 immunogen, a nucleic acid coding sequence that encodes an RSV Ga immunogen and a nucleic acid coding sequence that encodes an RSV Gb immunogen.

36. The composition of any of claims 23-35 further comprising a nucleic acid coding sequence that encodes CCL20.

37. The composition of any of claims 23-35 formulated for delivery to an individual using electroporation.

38. The composition of any of claims 23-35 further comprising nucleic acid sequences that encode one or more proteins selected from the group consisting of: IL-12, IL-15 and IL-28.

39. The composition of any of claims 23-35 wherein said one or more nucleic acid coding sequences are part of one or more plasmids.

40. The composition of any of claims 23-35 wherein each of the one or more nucleic acid coding sequences are part of incorporated into a separate plasmid.

41. A method of inducing an immune response against RSV comprising administering the composition of any of claims 23-35 to an individual in an amount effective to induce an immune response in said individual.

42. A method of treating an individual who has been diagnosed with RSV comprising administering a therapeutically effective amount of the composition of any of claims 23-35 to an individual.

43. A method of preventing RSV infection an individual comprising administering a prophylactically effective amount of the composition of any of claims 23-35 to an individual.

44. A protein selected from the group consisting of: SEQ ID NO:4 and SEQ ID NO:12.

Figure 1

Phylogenetic Tree of RSV-F Protein

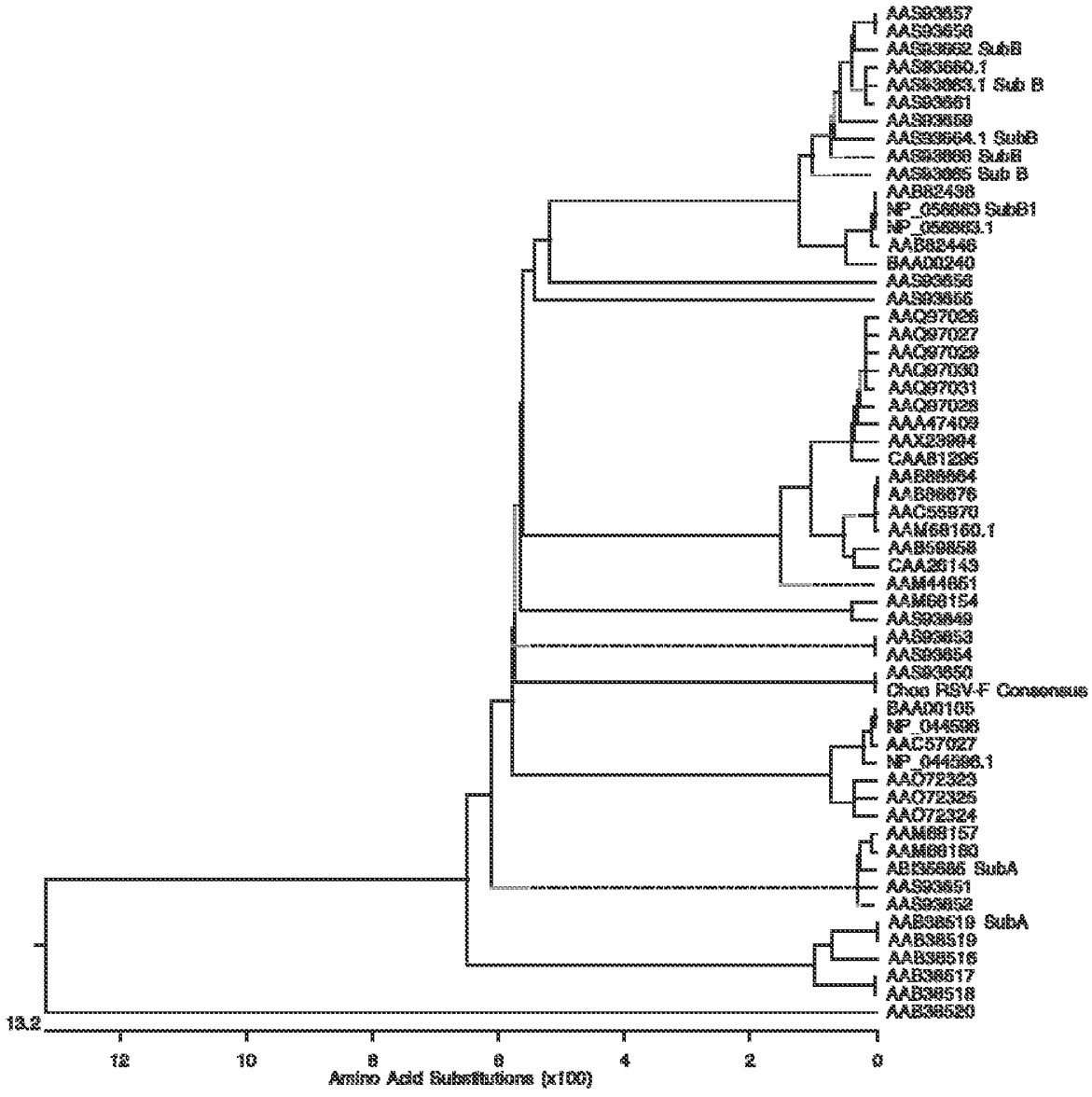


Figure 2

Phylogenetic Tree of RSV-M2 Protein

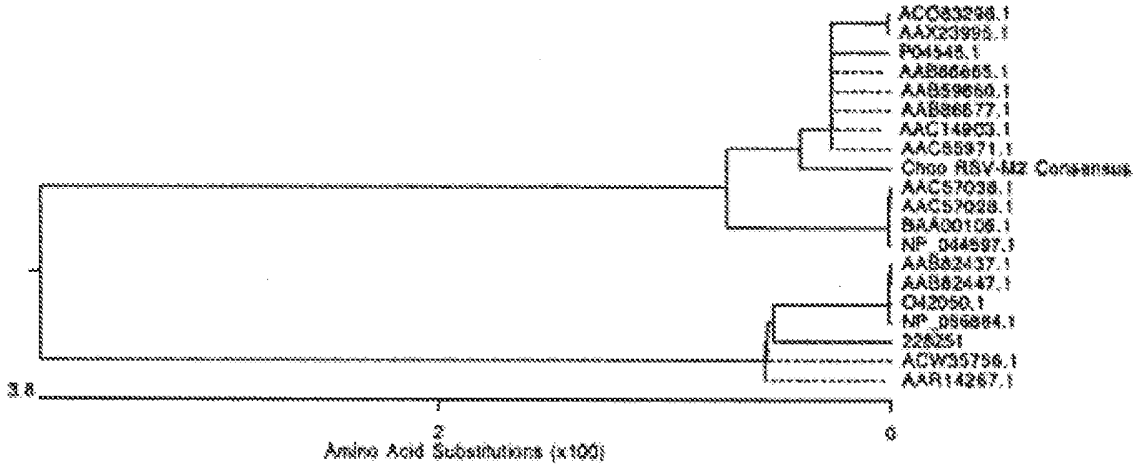


Figure 3

Comparison of total anti-RSV-F Protein IgG in Sera

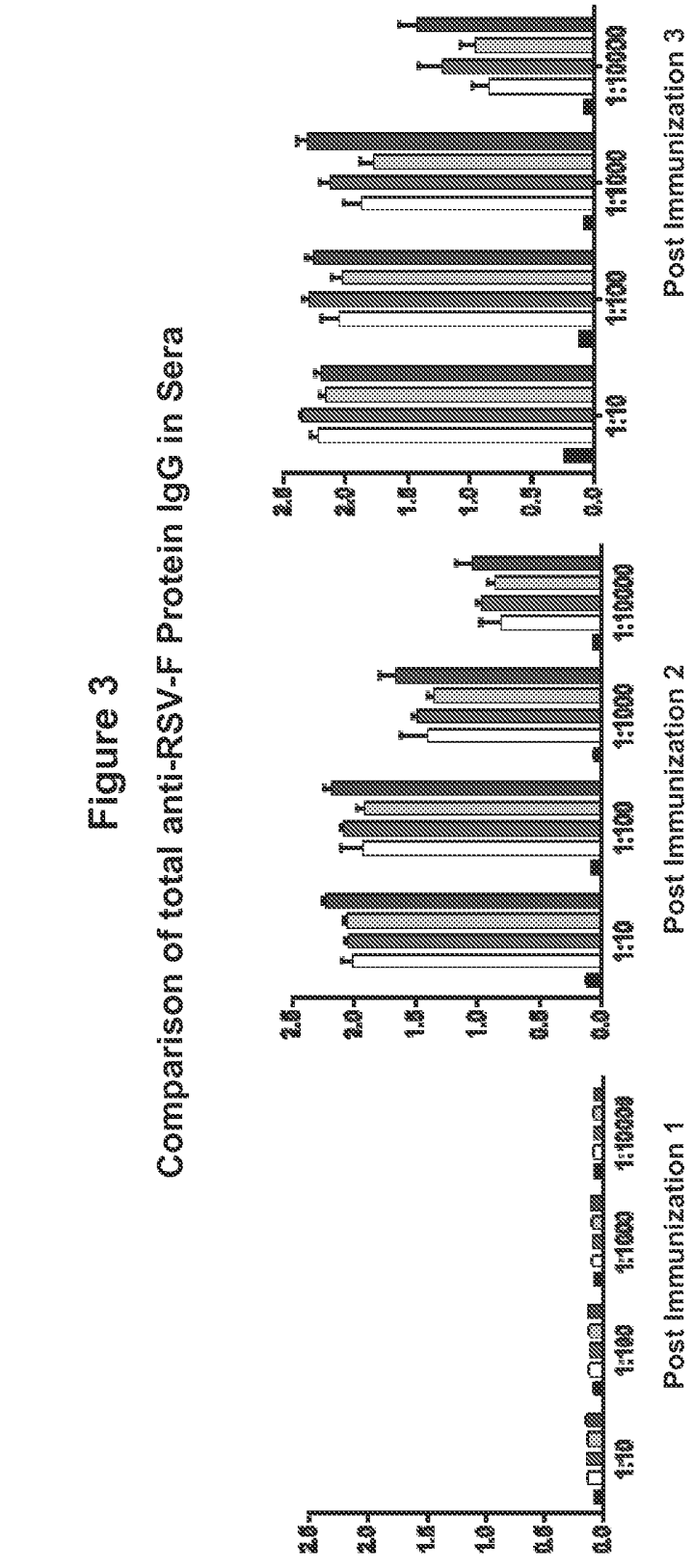


Figure 4

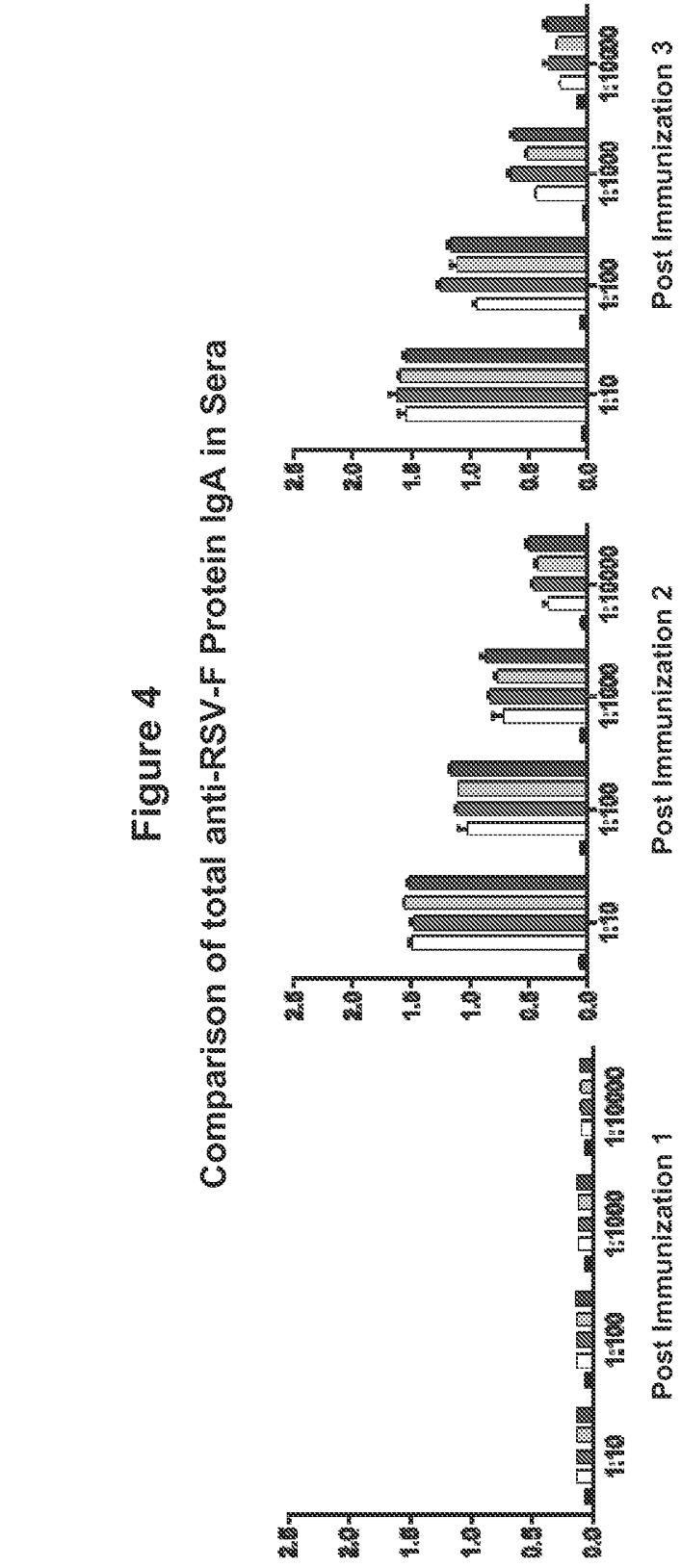

Comparison of total anti-RSV-F Protein IgA in Sera
2.5
2.0
1.5
1.0
0.5
0.0
1:10
1:100
1:1000
1:10000
Post Immunization 1
Post Immunization 2
Post Immunization 3

Figure 5

Comparison of IFN-g production at different dosage of RSV-F vaccine approximately 8 weeks after last immunization

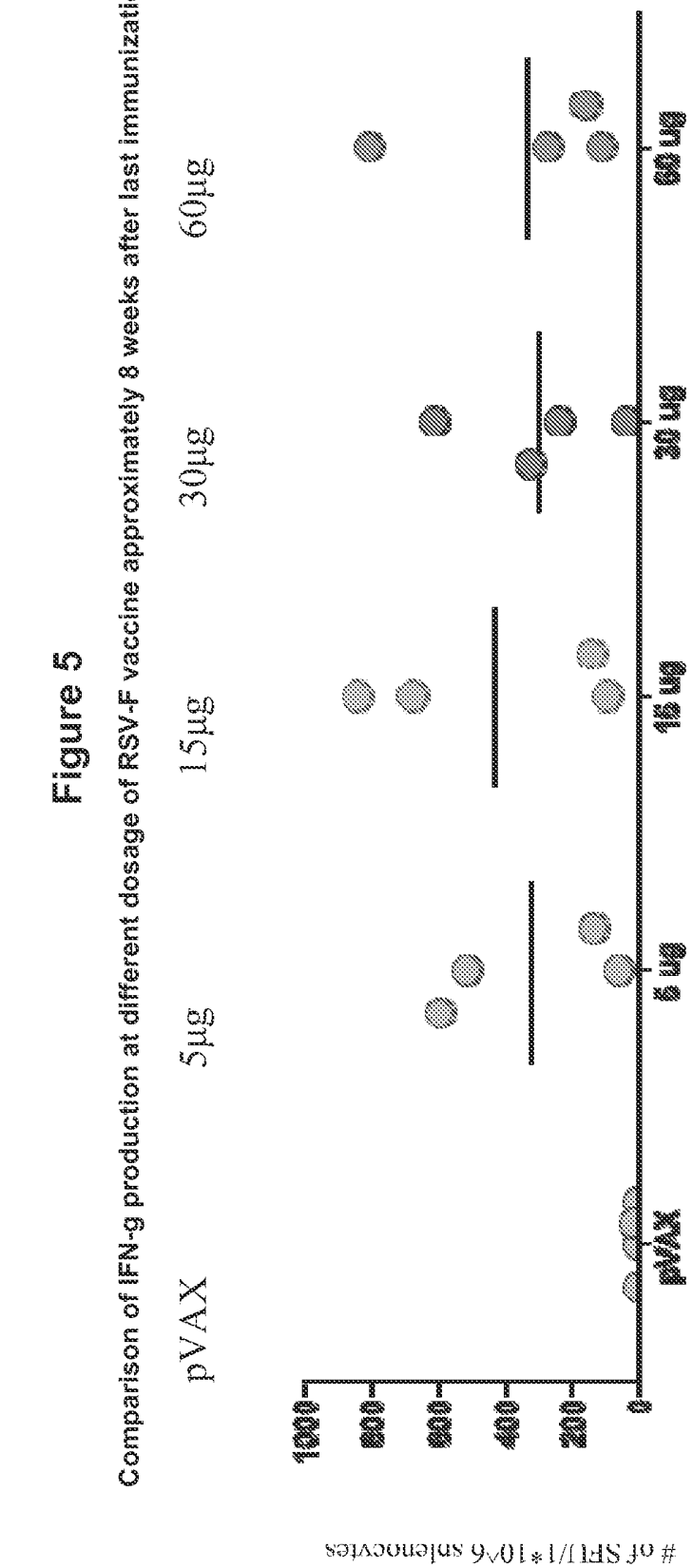


Figure 6

Comparison of IFN-γ+ CD8 or CD4 T cells in spleen after RSV-F +/- chemokine immunization

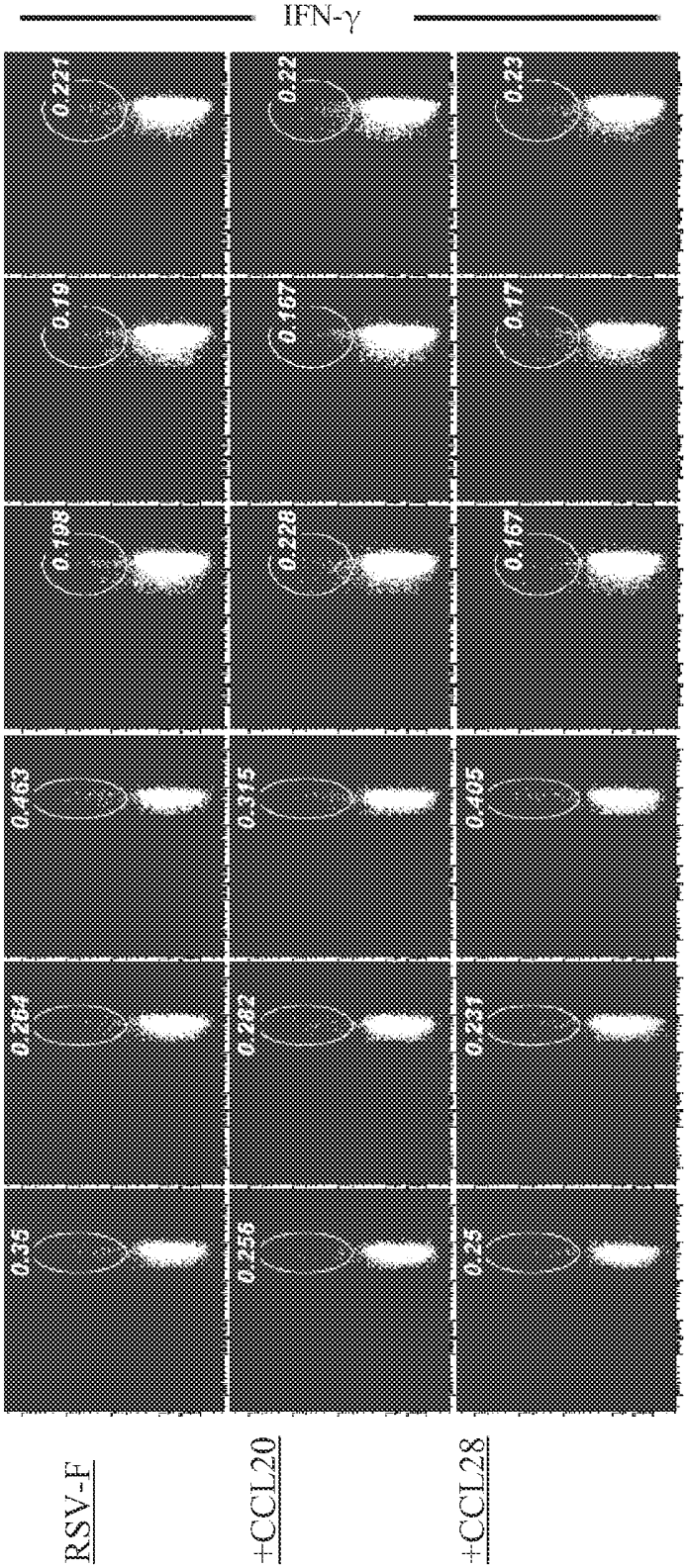


Figure 7

Comparison of IFN-γ+ CD8 or CD4 T cells in lung after RSV-F +/- chemokine immunization

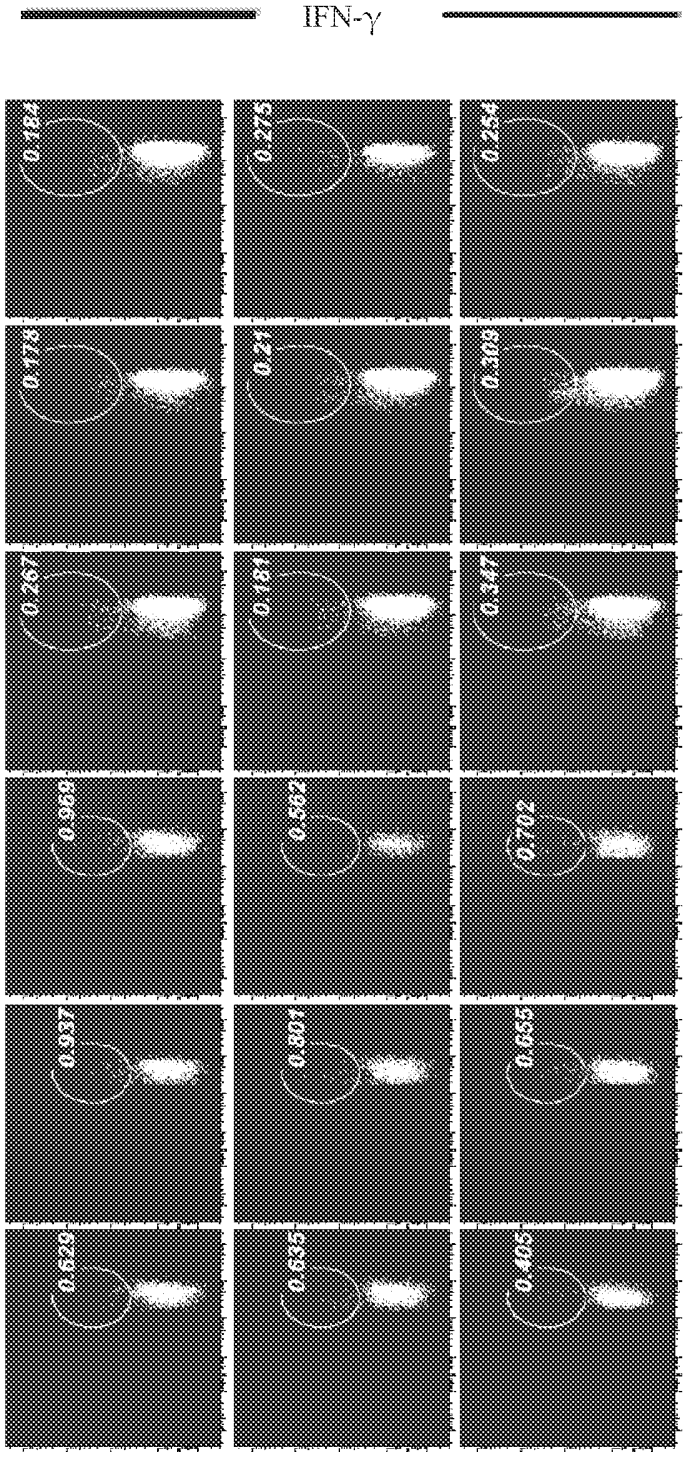


Figure 8
Comparison of IgG subtypes (IgG1 vs IgG2a) in Sera

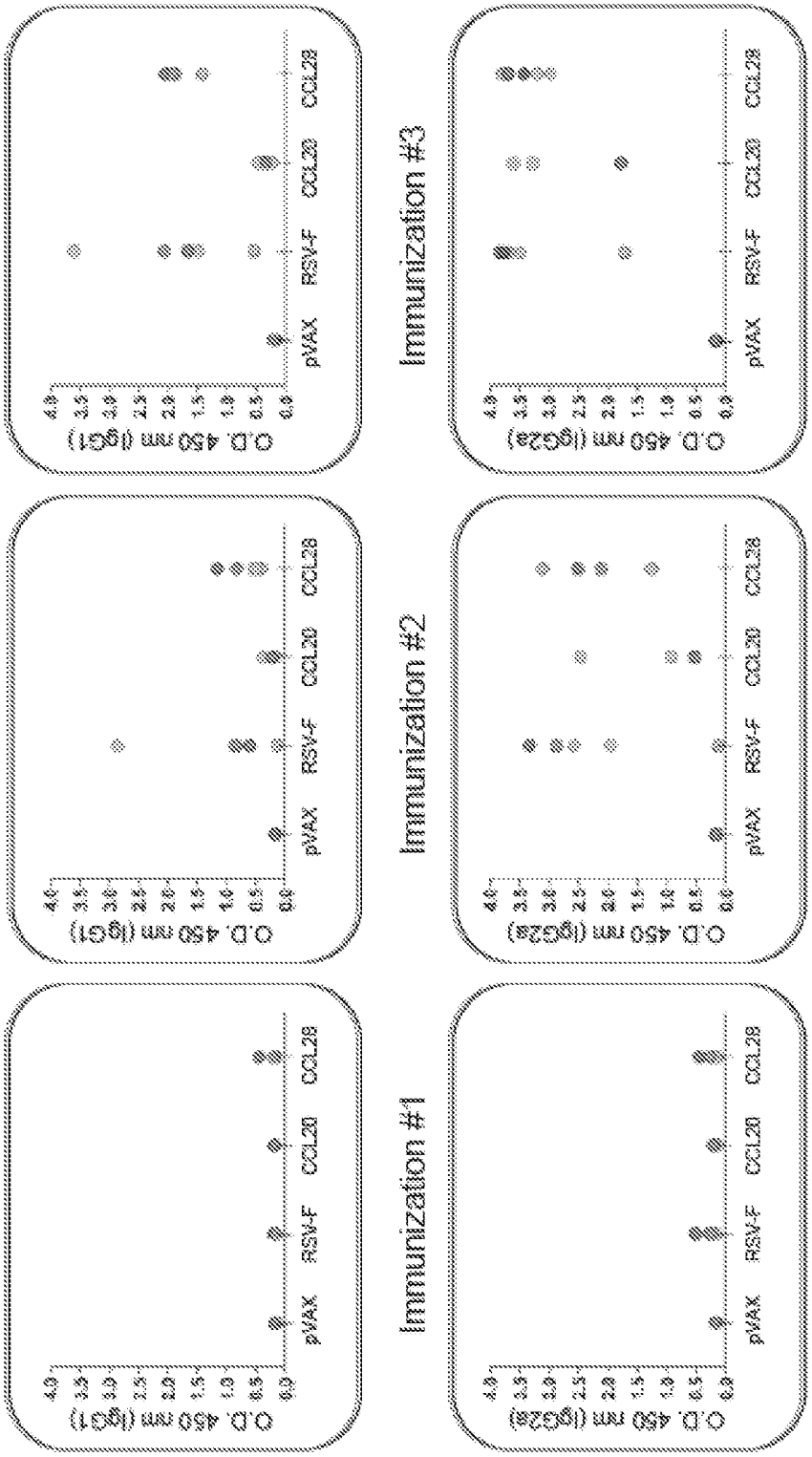


Figure 9

Comparison of IgG2a/IgG Ratio

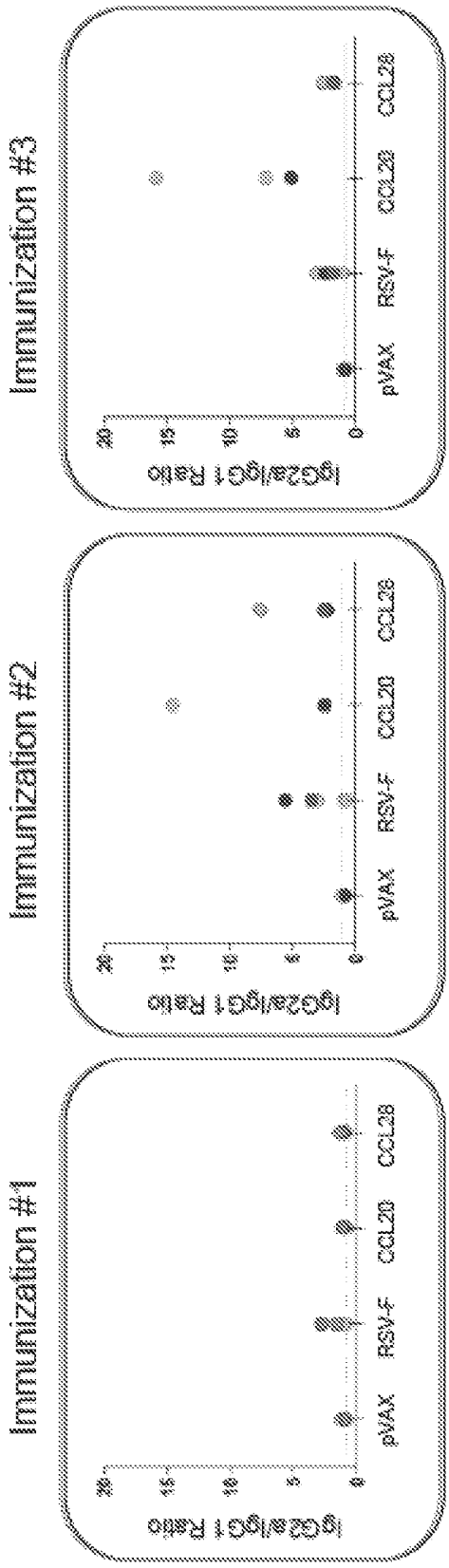


Figure 10

Env-Pseudotyped viruses in TZM-BL Cells/ "ON"

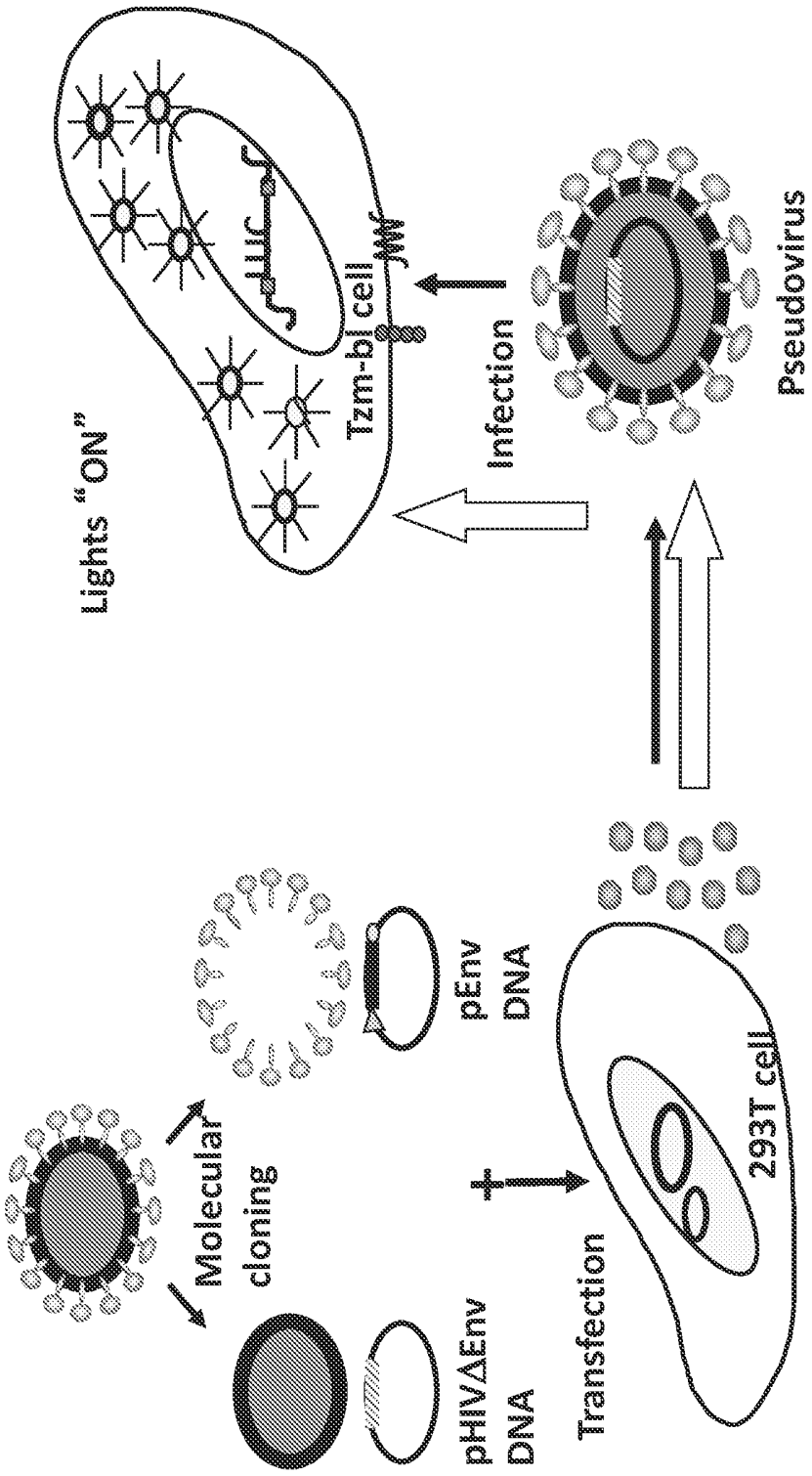


Figure 11

Env-Pseudotyped viruses in TZM-BL Cells/ "OFF"

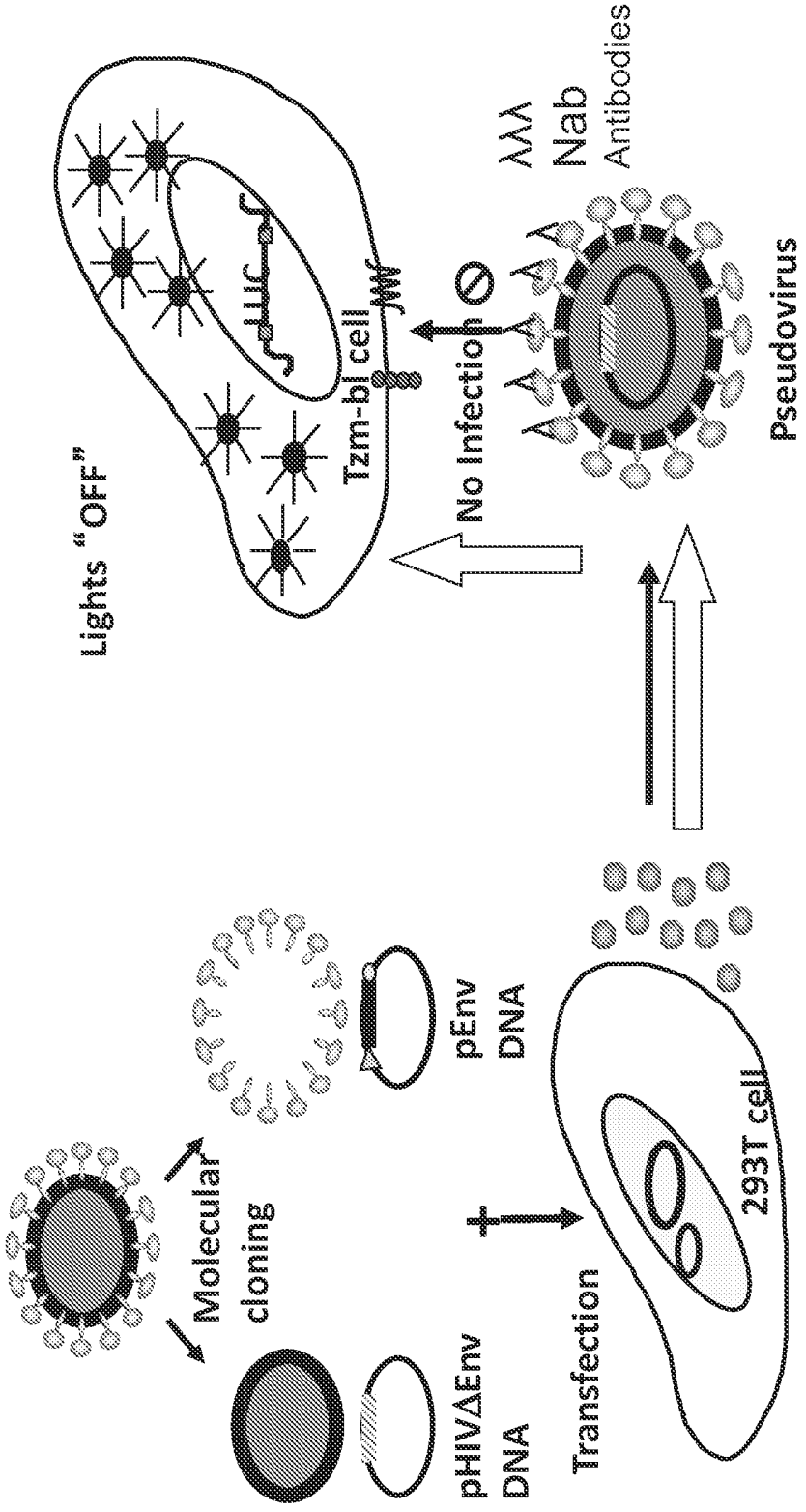


Figure 12

RSV-Fusion Pseudo viral production in 293T cells

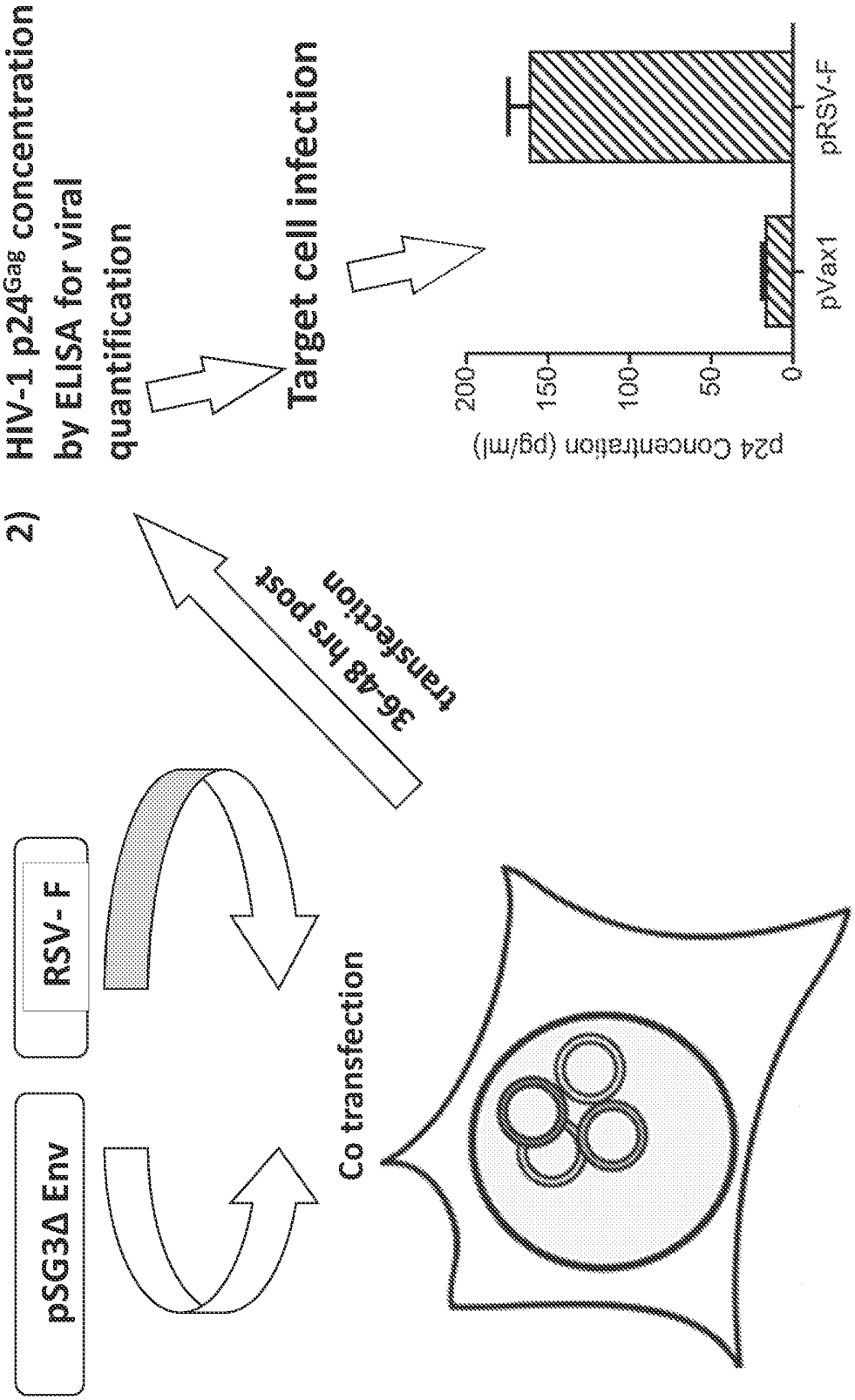


Figure 13
Immunization Schedule for RSV-F Neutralization Study

Figure 14

Quantification of RSV-F mediated infection in different target cells

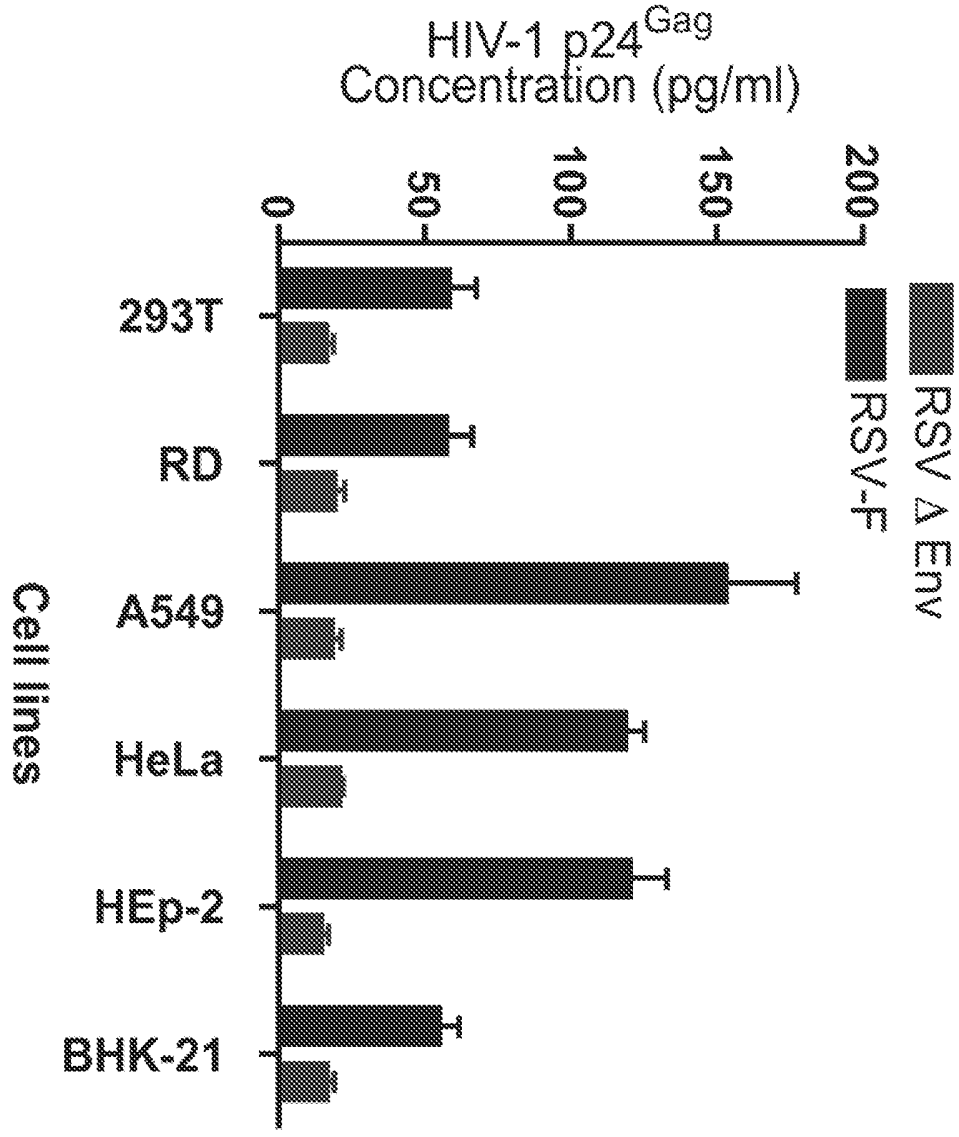


Figure 15

Quantification of RSV-F neutralization activity in TZM-bl HeLa cells

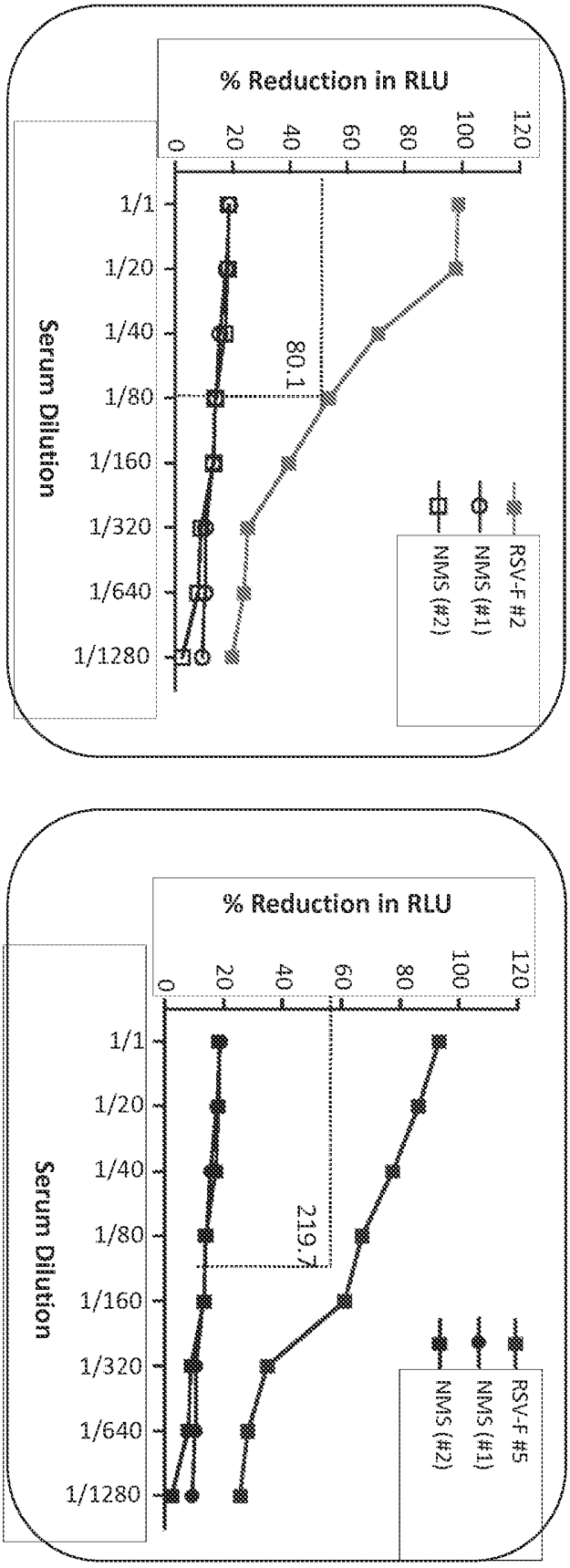


Figure 16
Quantification of cell death activity by RSV-F pseudo virus in HeLa cells

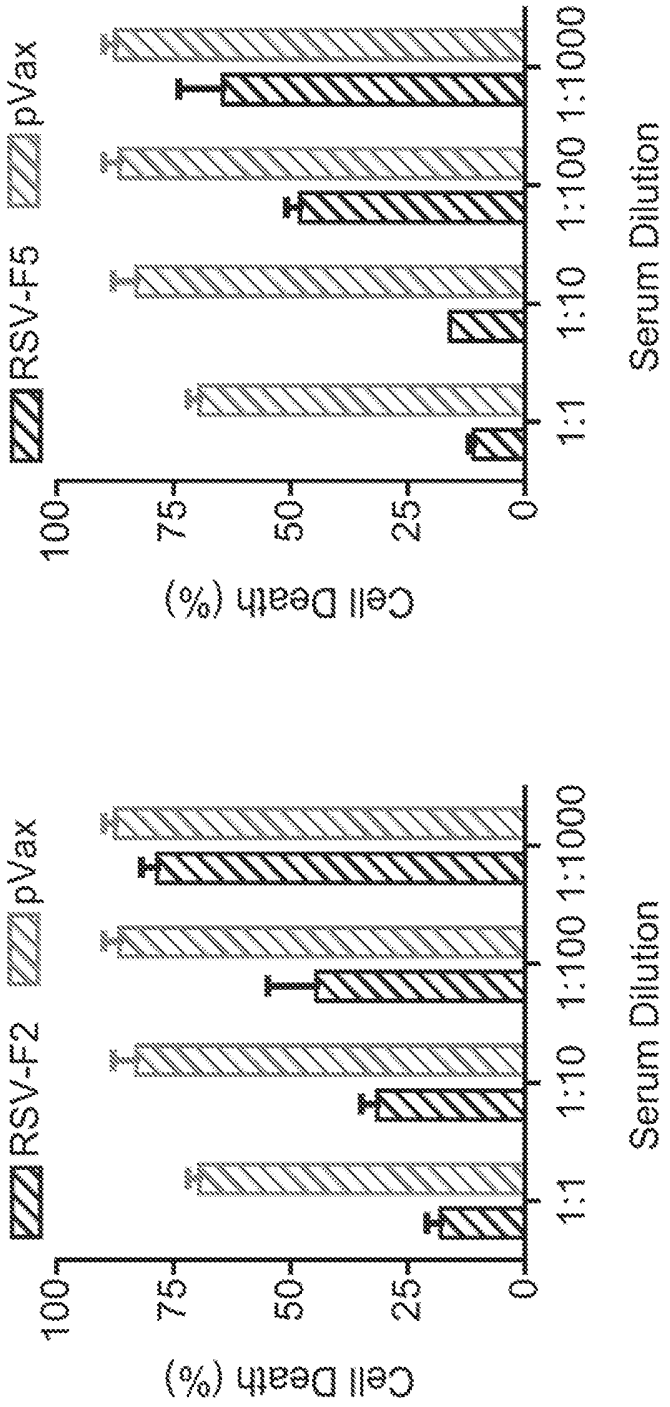


Figure 17
Immunization Schedule for RSV-M2 Dosage Study

Figure 18
Comparison of IFN-g production at different dosages of RSV-M2 vaccine 1 week after last immunization

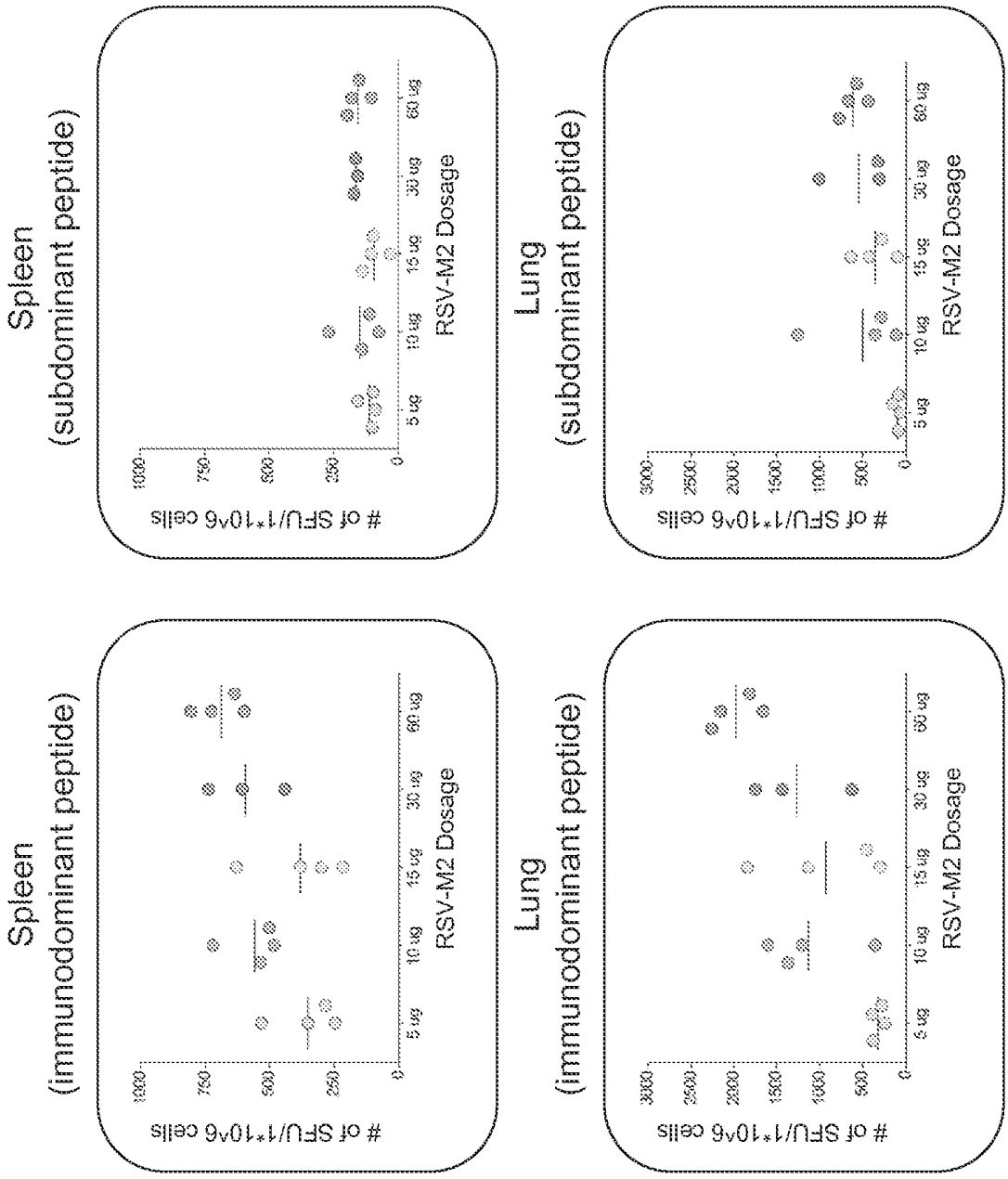
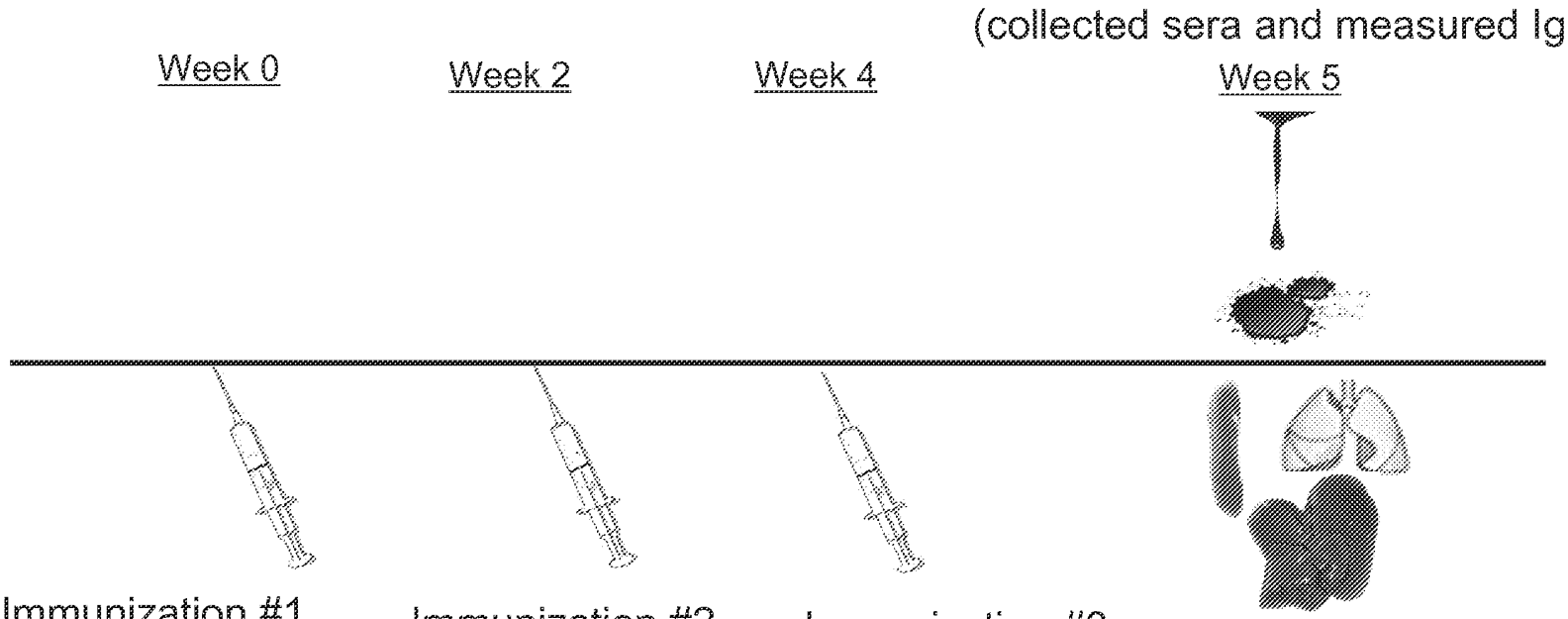
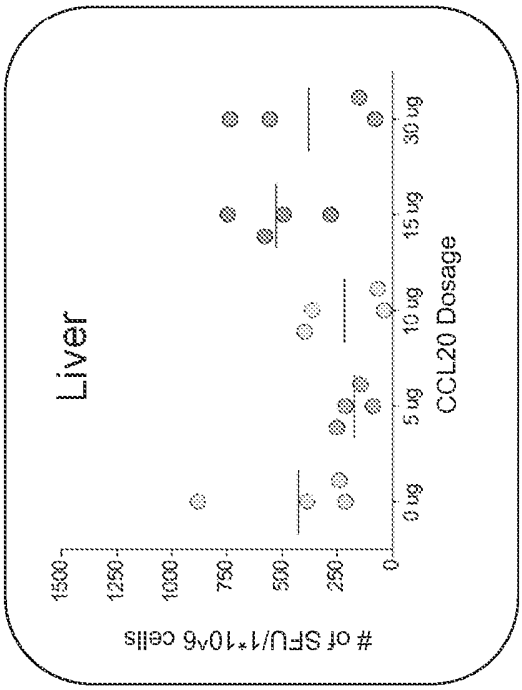


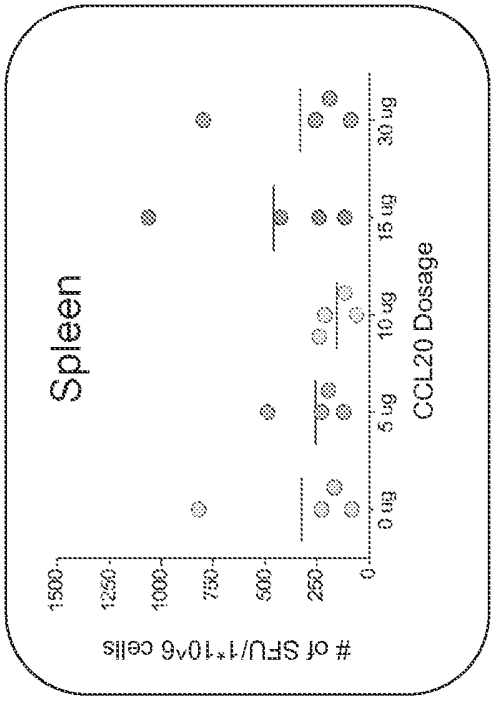
Figure 19
Immunization Schedule for RSV-F + CCL20 Study



Groups:
1.RSV-F (5 ug) alone
2.RSV-F (5 ug) + CCL20 (5 ug)
3.RSV-F (5 ug) + CCL20 (10 ug)
4.RSV-F (5 ug) + CCL20 (15 ug)
5.RSV-F (5 ug) + CCL20 (30 ug)

Figure 20
Comparison of IFN-g production with different dosages of CCL20 at 1 week after last immunization





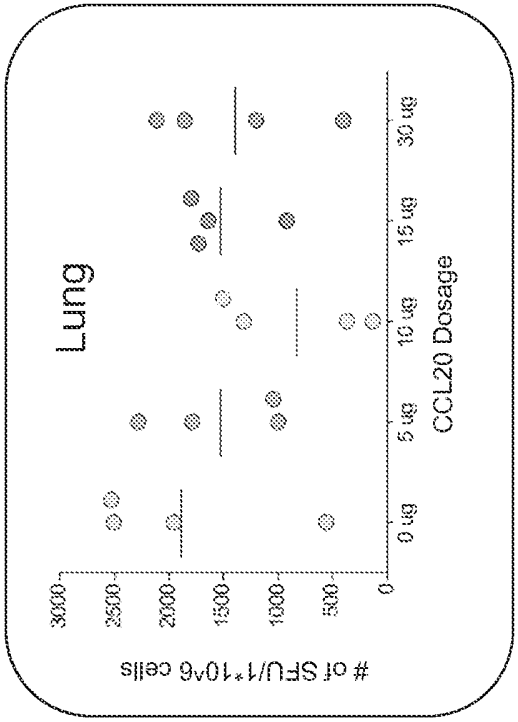


Figure 21
Comparison of IgG subtypes (IgG1 vs IgG2a) in Sera

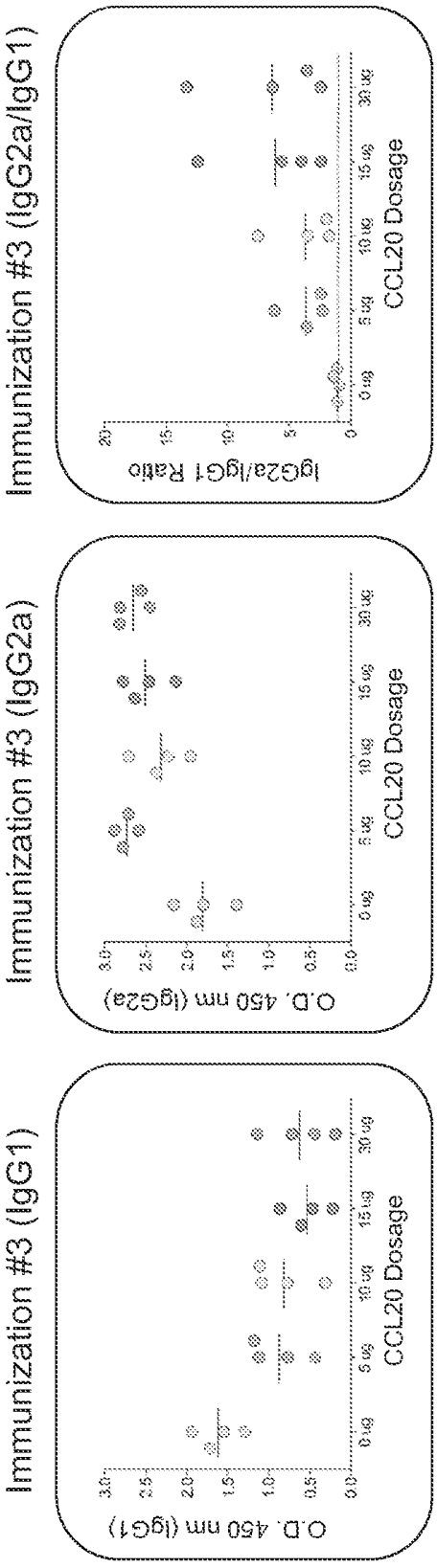


Figure 22A

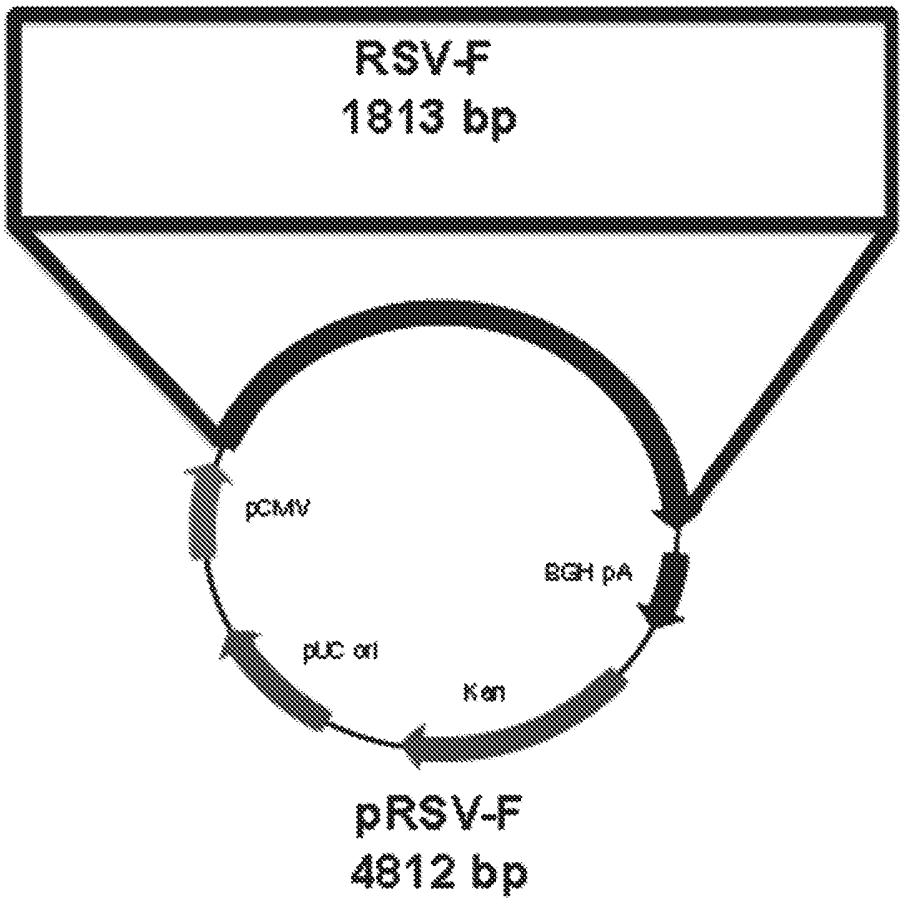
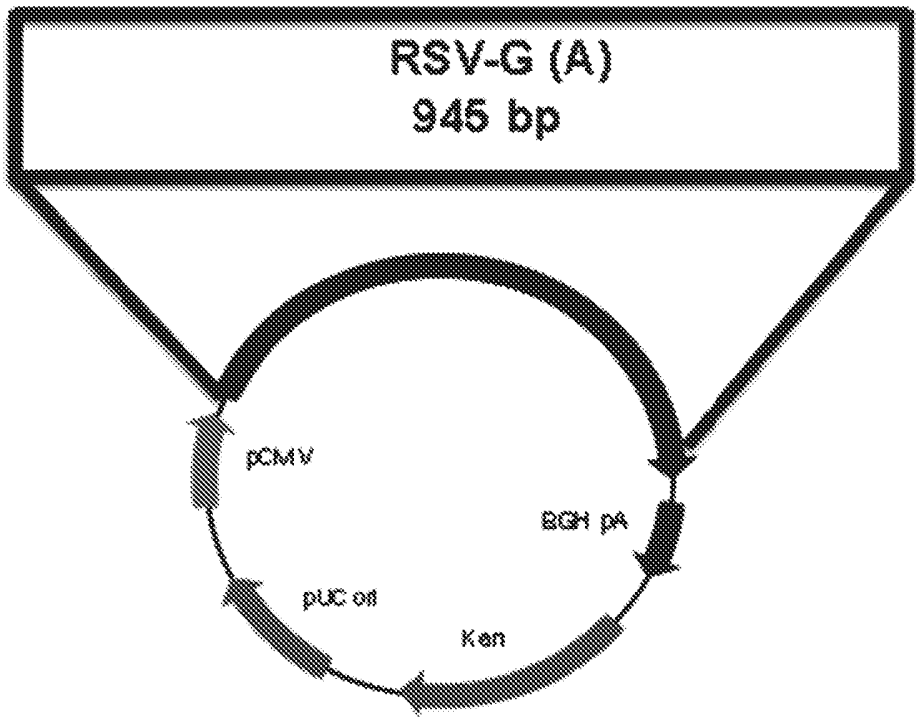
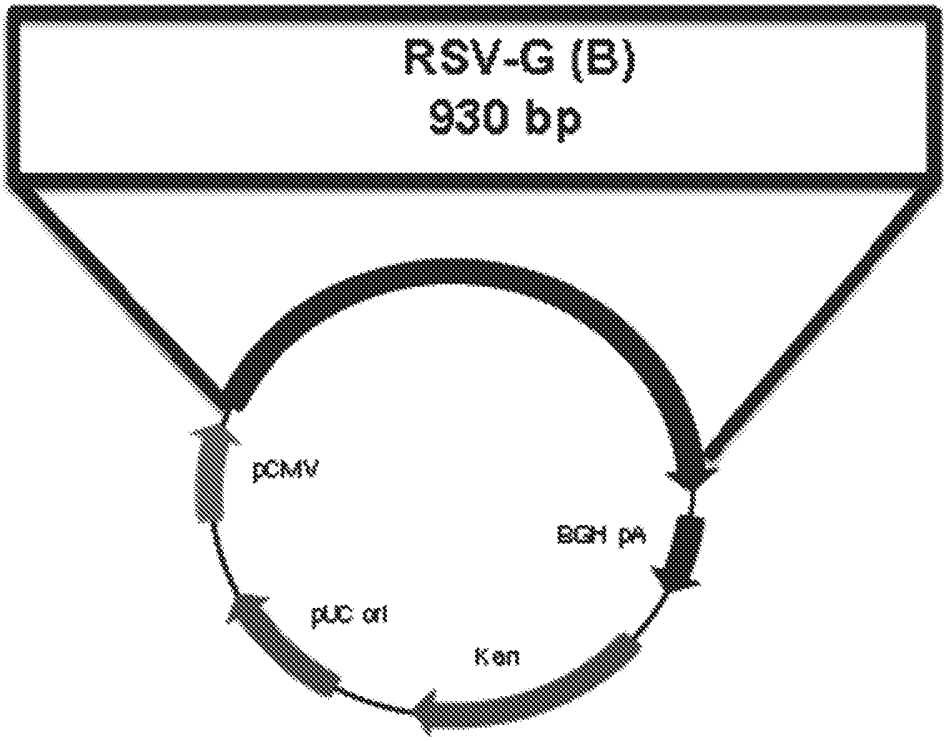


Figure 22B



pRSV-G (subtype A)
3944 bp

Figure 22C



pRSV-G (subtype B)
3929 bp

Figure 23

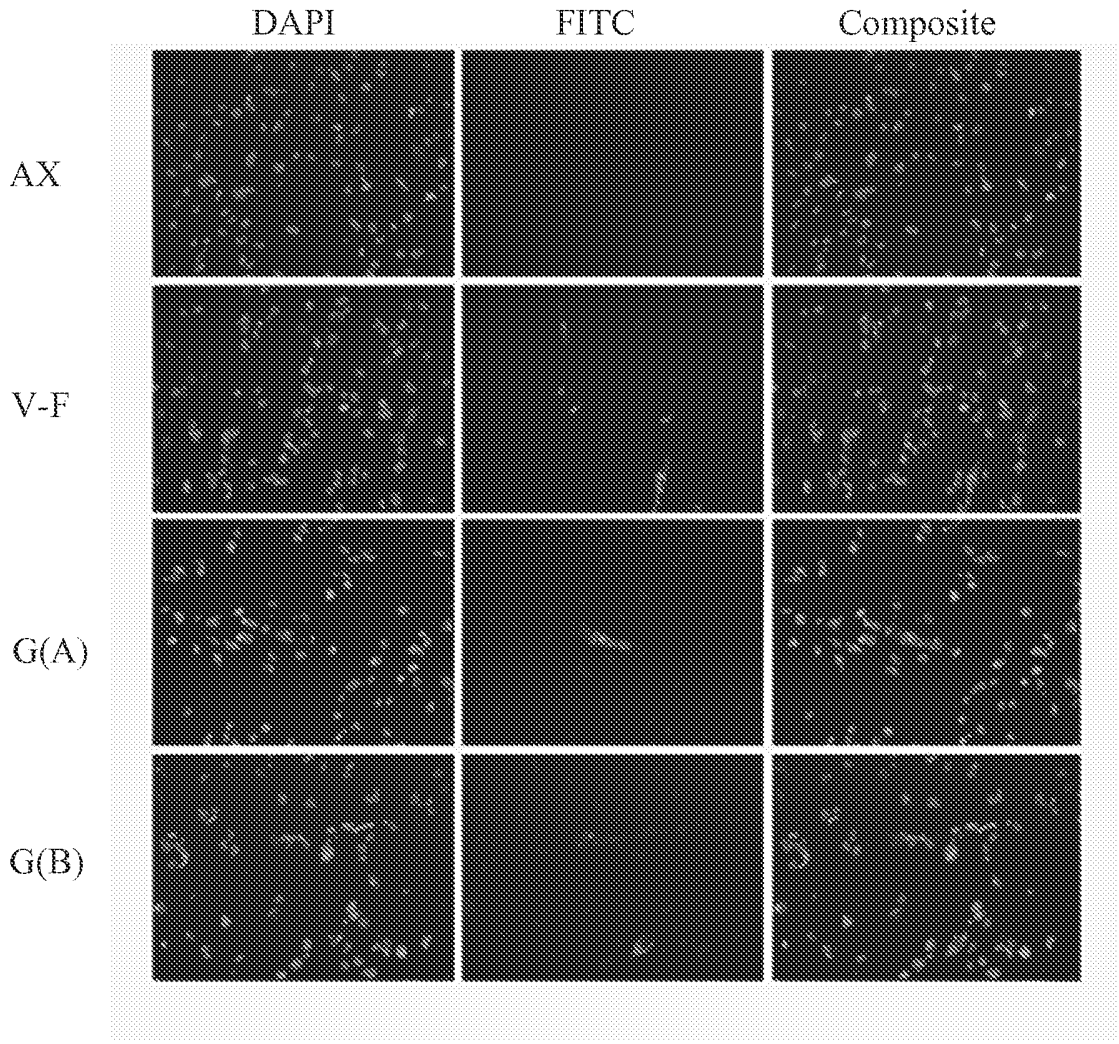


Figure 24

Immunization Schedule for RSV-F Dosage Study

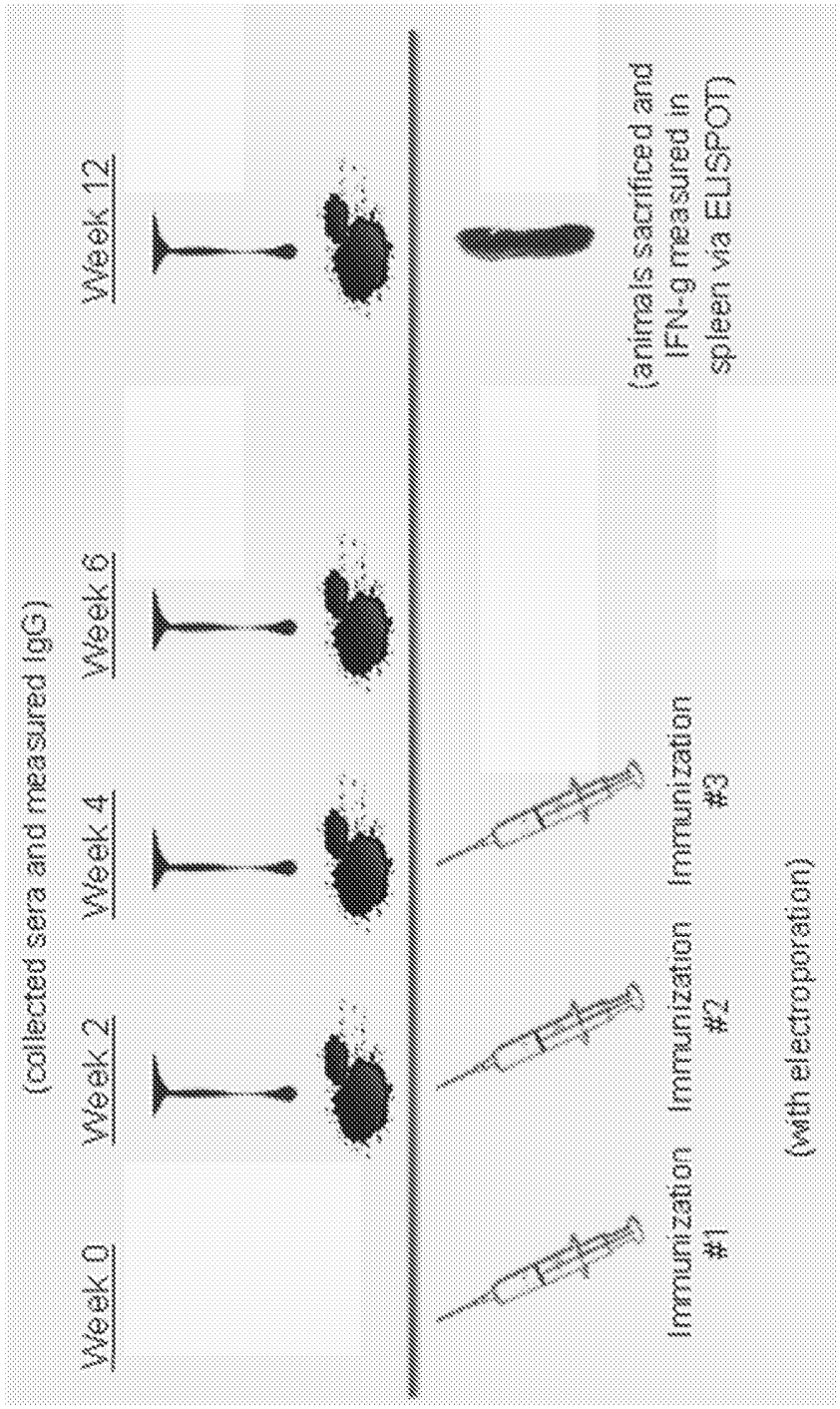
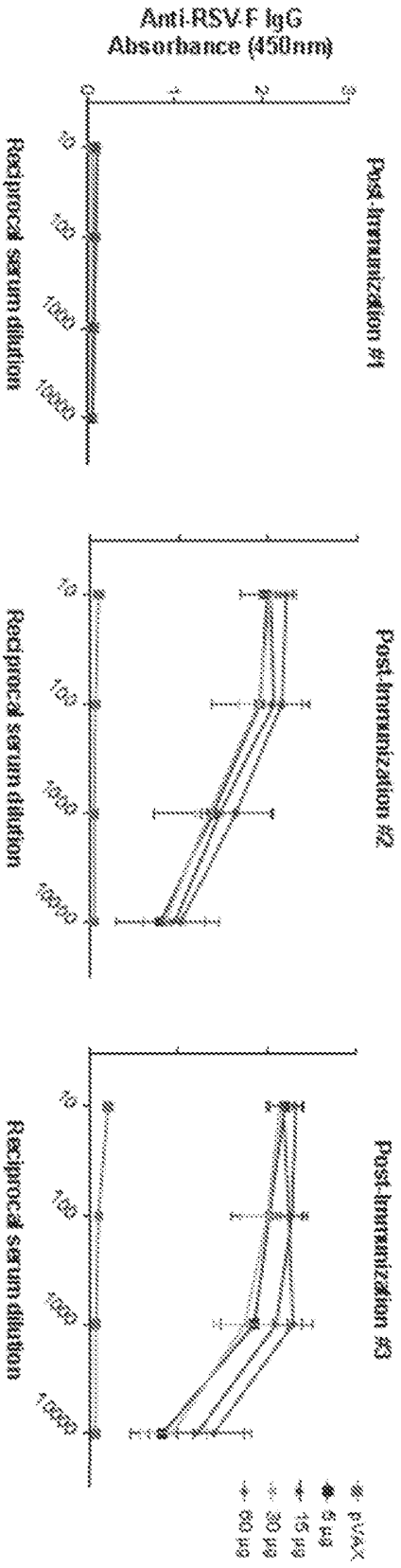


Figure 25


Anti-RSV F IgG
Absorbance (450nm)
Post-immunization #1
Post-immunization #2
Post-immunization #3
Reciprocal serum dilution
40
100
400
1600
pVAX
15 ug
30 ug
60 ug

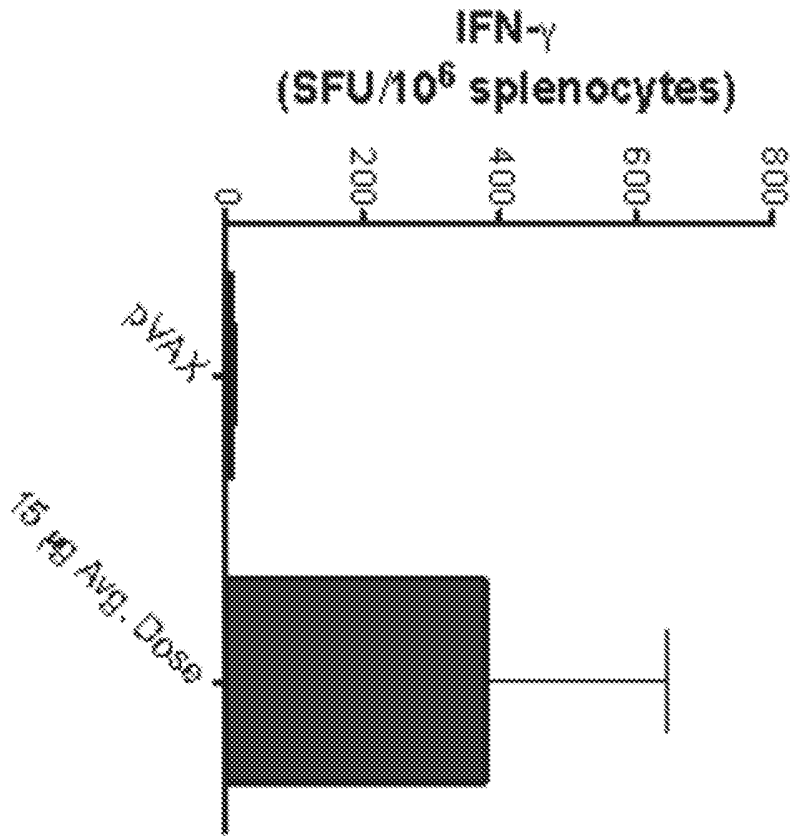


Figure 26

Figure 27

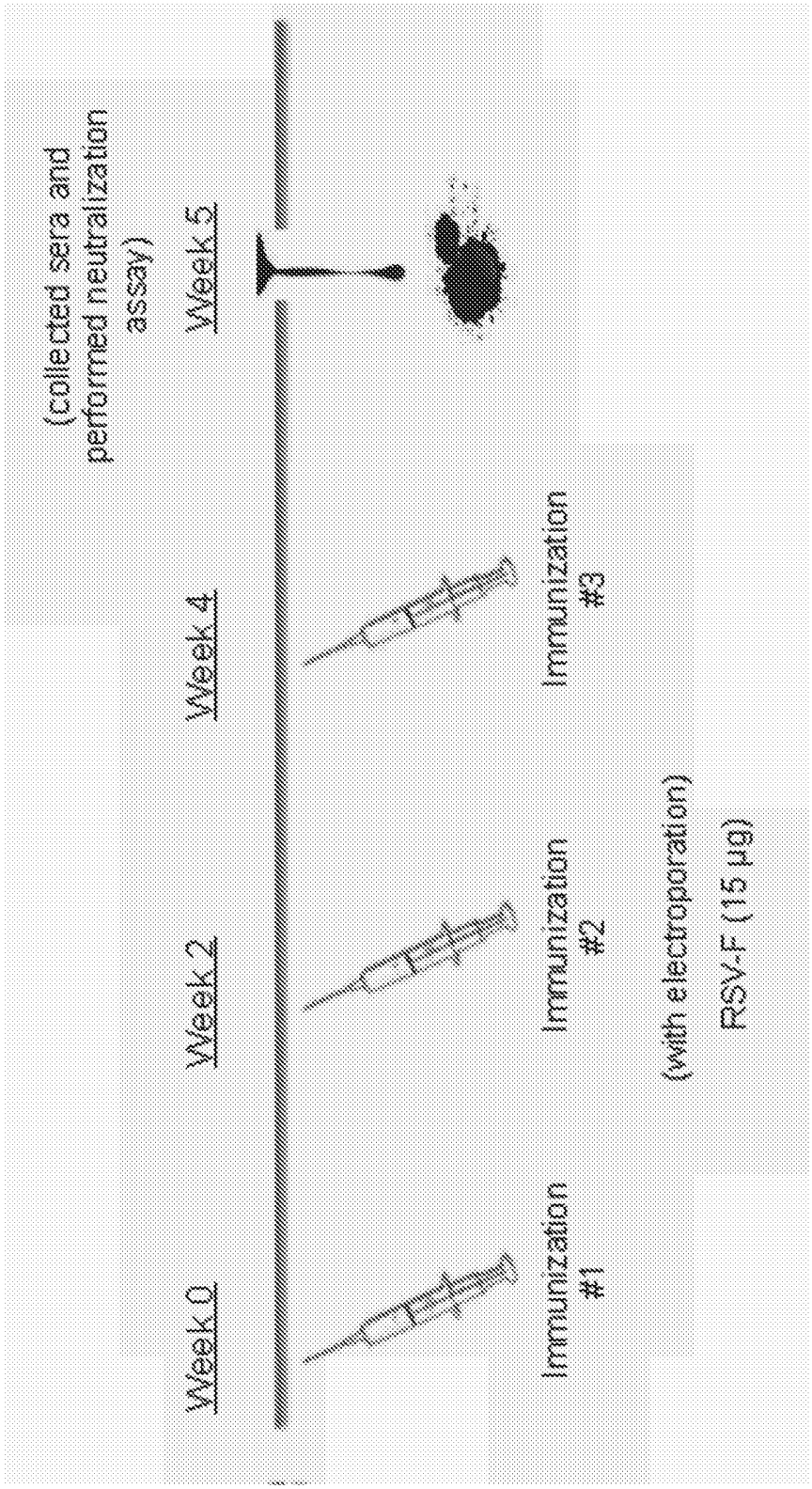

Week 0
Week 2
Week 4
Week 5
(collected sera and performed neutralization assay)
Immunization #1
Immunization #2
Immunization #3
(with electroporation)
RSV-F (15 µg)

Figure 28

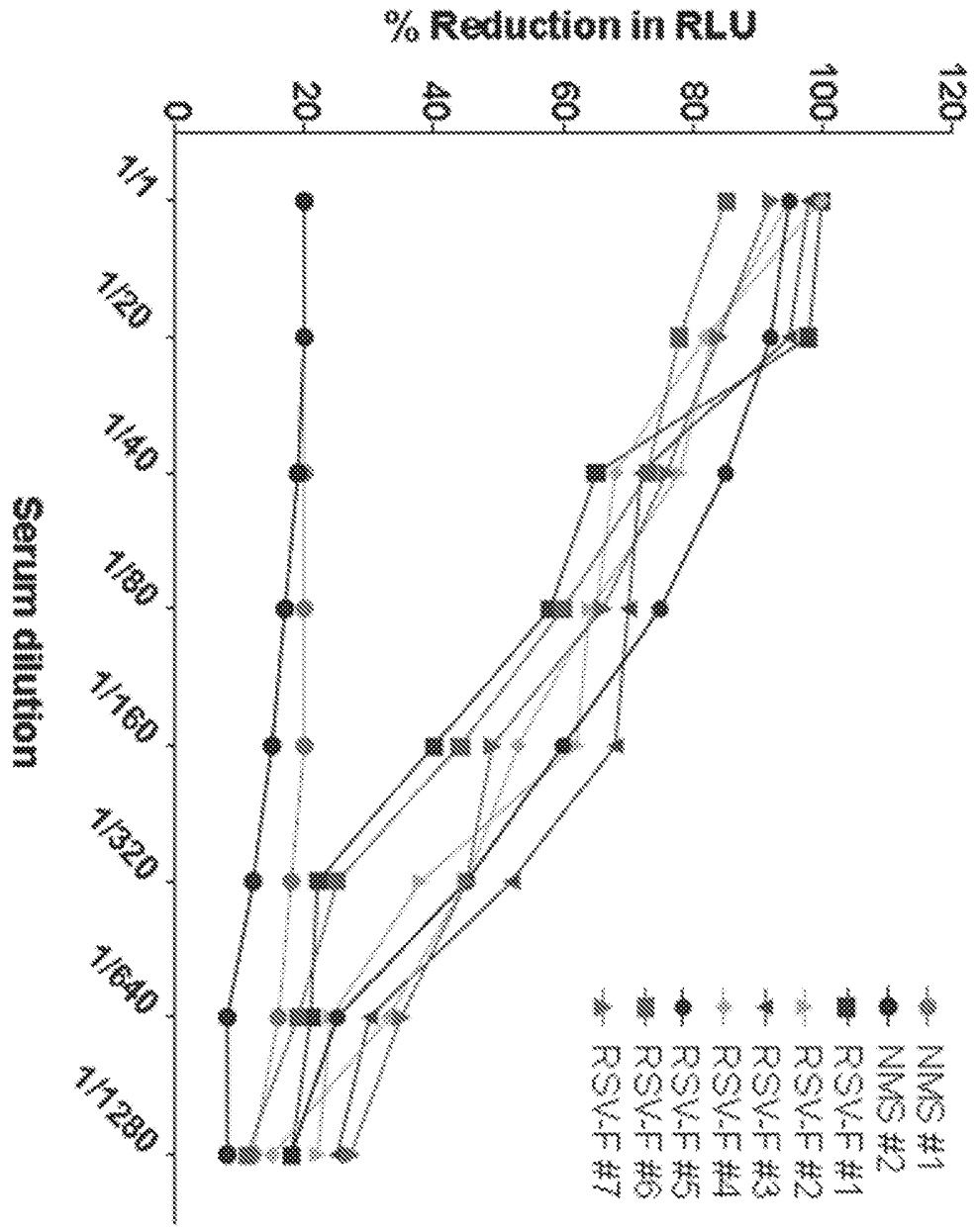

% Reduction in RLU
0
20
40
60
80
100
120
Serum dilution
1/1
1/20
1/40
1/80
1/160
1/320
1/640
1/1280
NMS #1
NMS #2
RSV-F #1
RSV-F #2
RSV-F #3
RSV-F #4
RSV-F #5
RSV-F #6
RSV-F #7

Figure 29

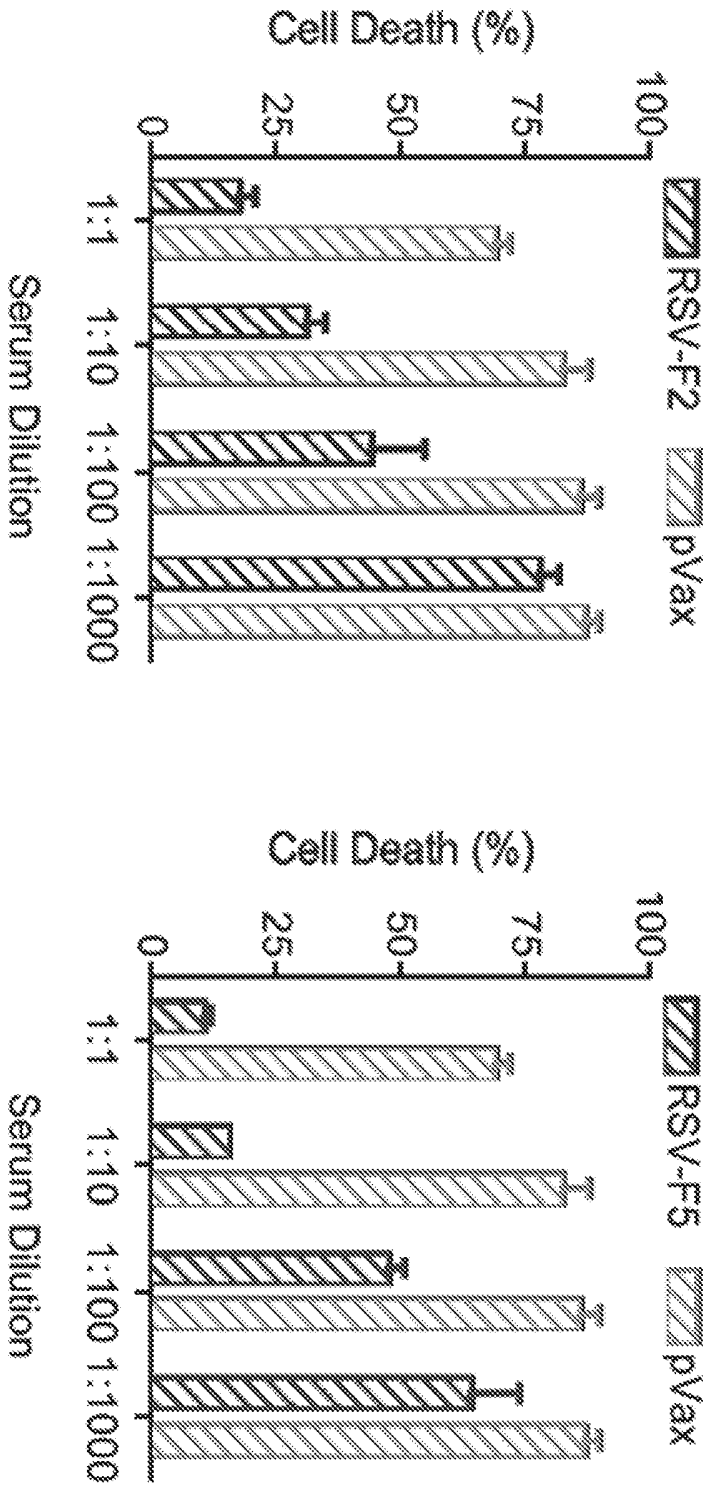

Cell Death (%)
0
25
50
75
100
Serum Dilution
1:1
1:10
1:100
1:1000
RSV-F2
pVax
Cell Death (%)
0
25
50
75
100
Serum Dilution
1:1
1:10
1:100
1:1000
RSV-F5
pVax

Figure 30

Immunization Schedule for RSV-G(A) and RSV-(B) Dosage Study

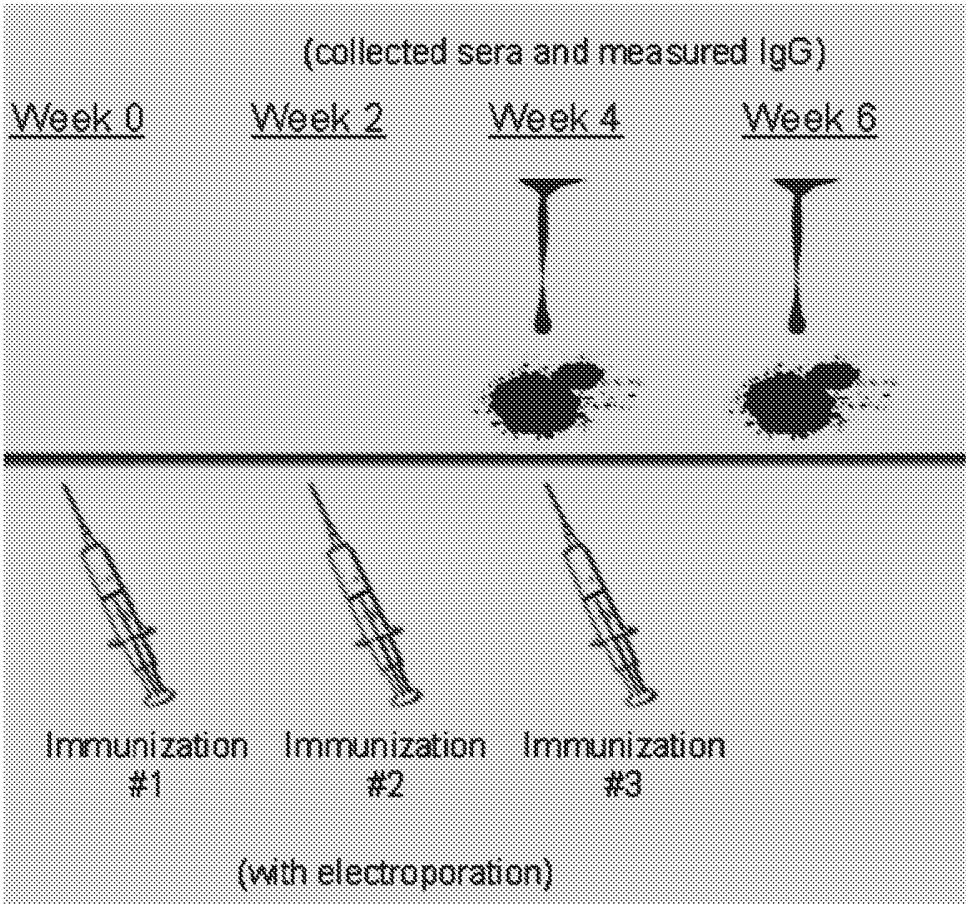


Figure 31

Total IgG in Sera after RSV-G(A) DNA immunization

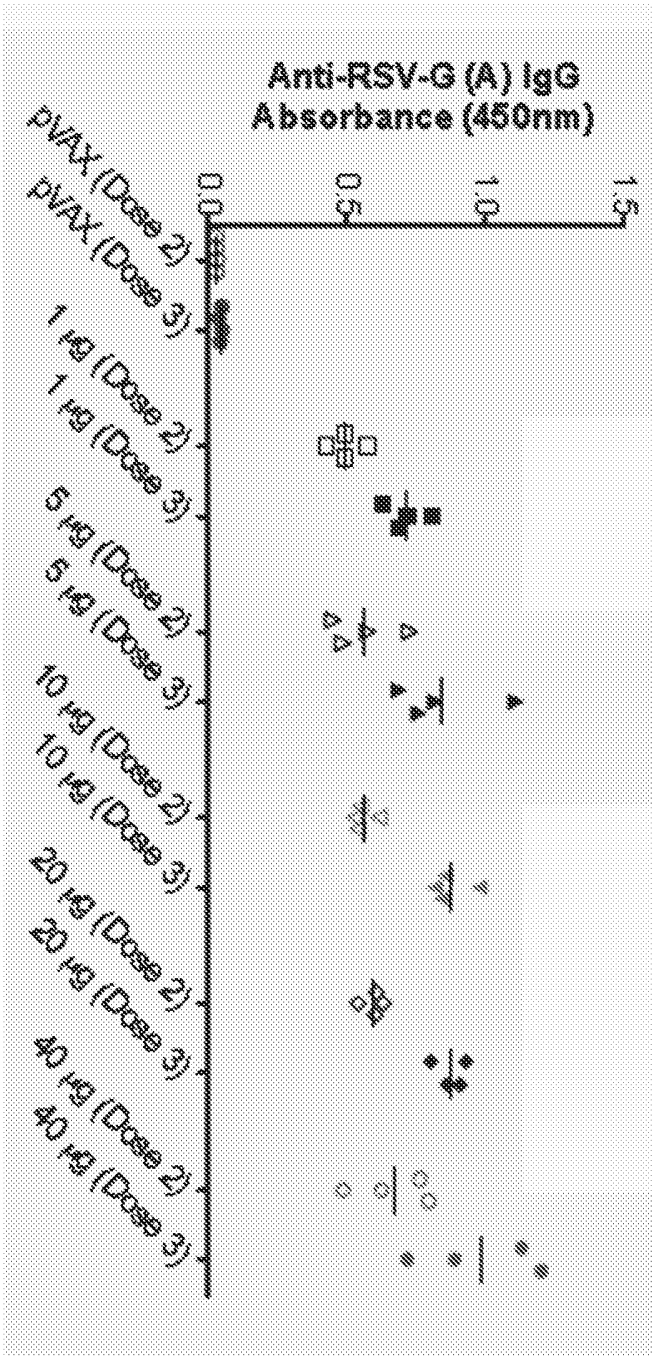


Figure 32

Total IgG in Sera after RSV-G(B) DNA immunization

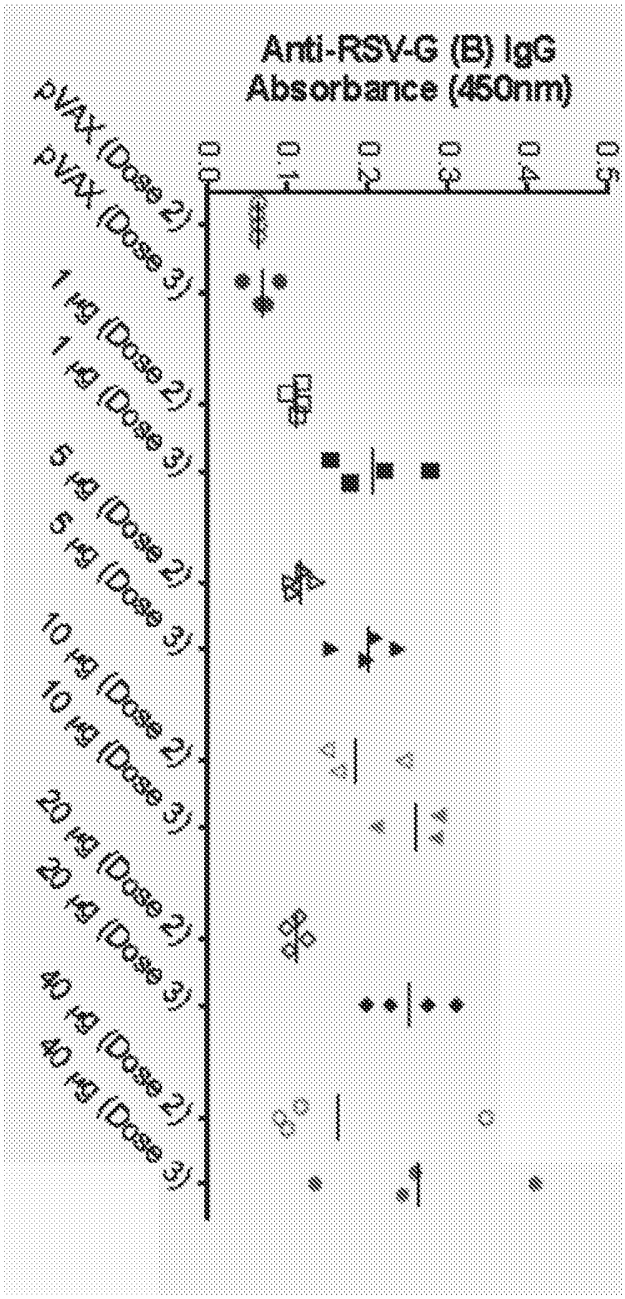


Figure 33

Preliminary Neutralization Results from Mono Vaccine Mouse Studies

Sample Tested	Neut Titer Post 3rd Imm
Negative serum	0
Mab anti RSV	40
Polyclonal Serum	160
Mouse 1-F Ag	320
Mouse 2-F Ag	160
Mouse 3- GA	80
Mouse 4 - GA	320
Mouse 14 - GB	160

Figure 34

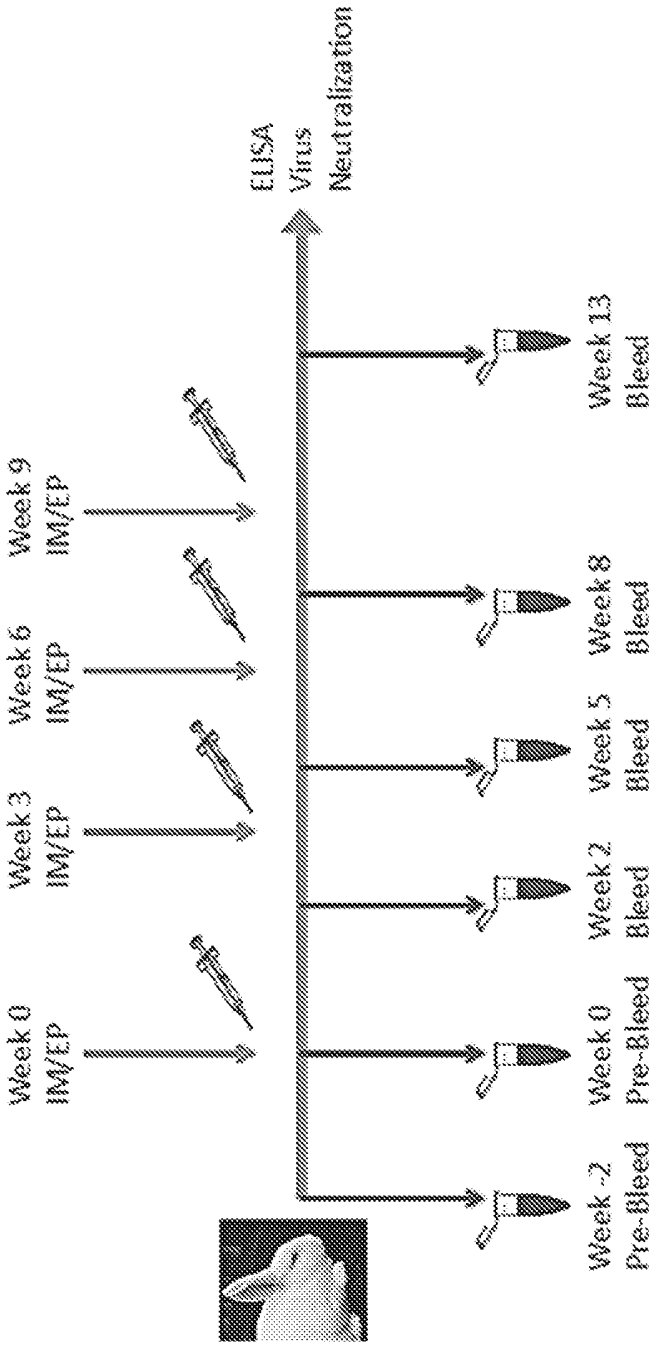

Week 0
IM/EP
Week 3
IM/EP
Week 6
IM/EP
Week 9
IM/EP
Week -2
Pre-Bleed
Week 0
Pre-Bleed
Week 2
Bleed
Week 5
Bleed
Week 8
Bleed
Week 13
Bleed
ELISA
Virus
Neutralization

Figure 35

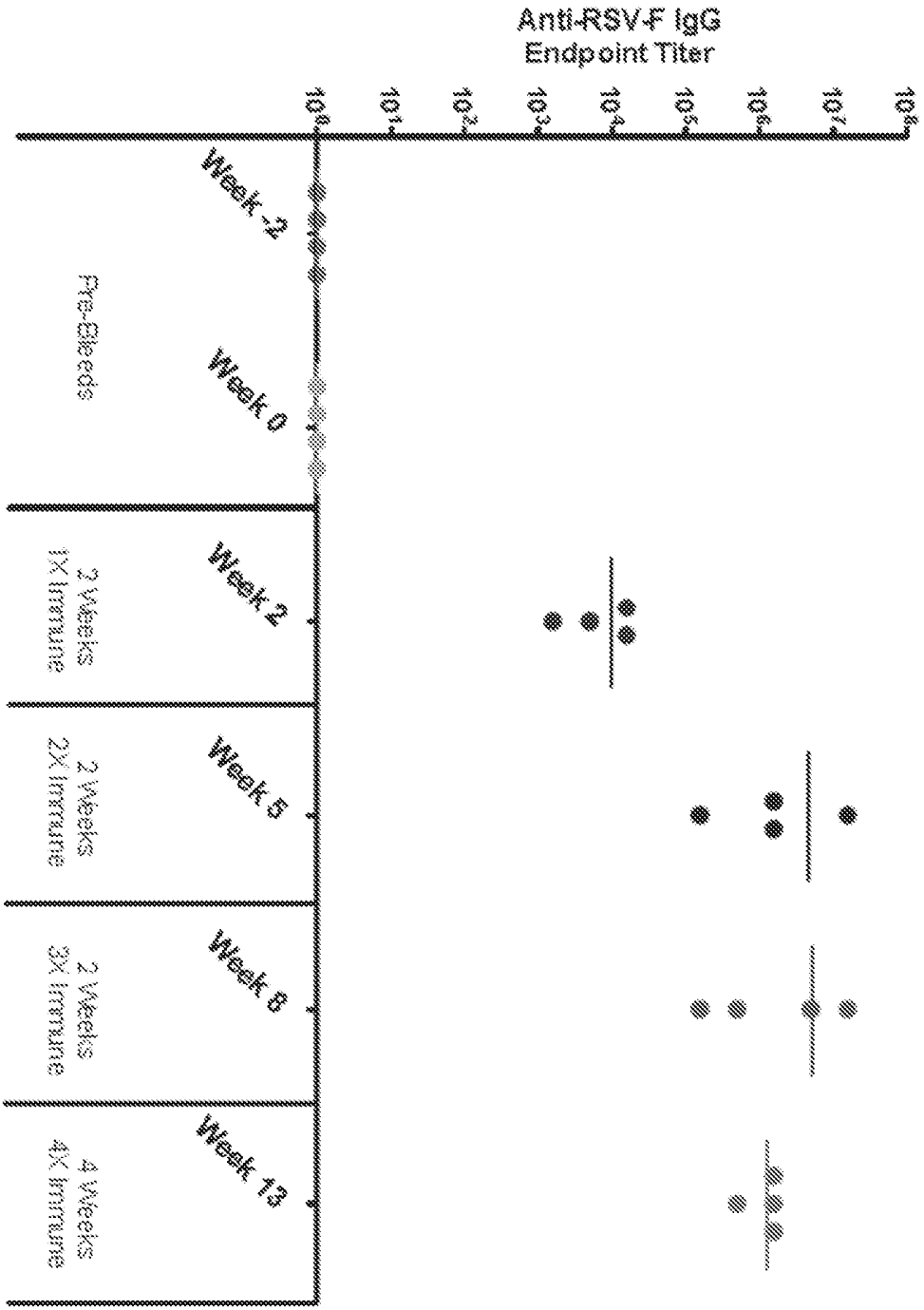

Anti-RSV-F IgG
Endpoint Titer
10^0
10^1
10^2
10^3
10^4
10^5
10^6
10^7
10^8
Week -2
Week 0
Week 2
Week 5
Week 6
Week 13
Pre-Bleeds
2 Weeks
1X Immune
2 Weeks
2X Immune
2 Weeks
3X Immune
4 Weeks
4X Immune

Figure 36

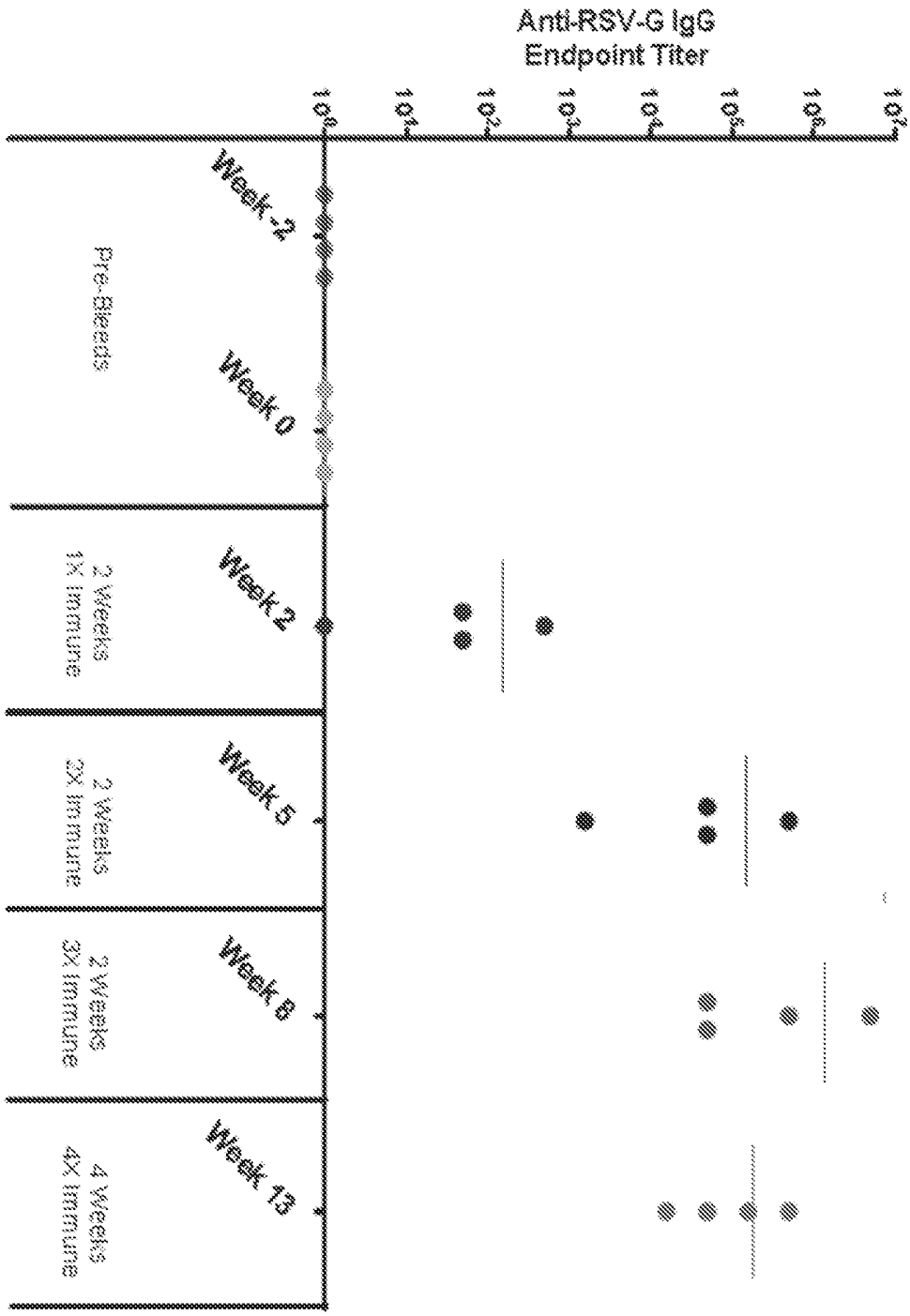

Anti-RSV-G IgG
Endpoint Titer
10^0
10^1
10^2
10^3
10^4
10^5
10^6
10^7
Week -2
Week 0
Week 2
Week 5
Week 8
Week 13
Pre-Bleeds
2 Weeks
1X Immune
2 Weeks
2X Immune
2 Weeks
3X Immune
4 Weeks
4X Immune

Figure 37

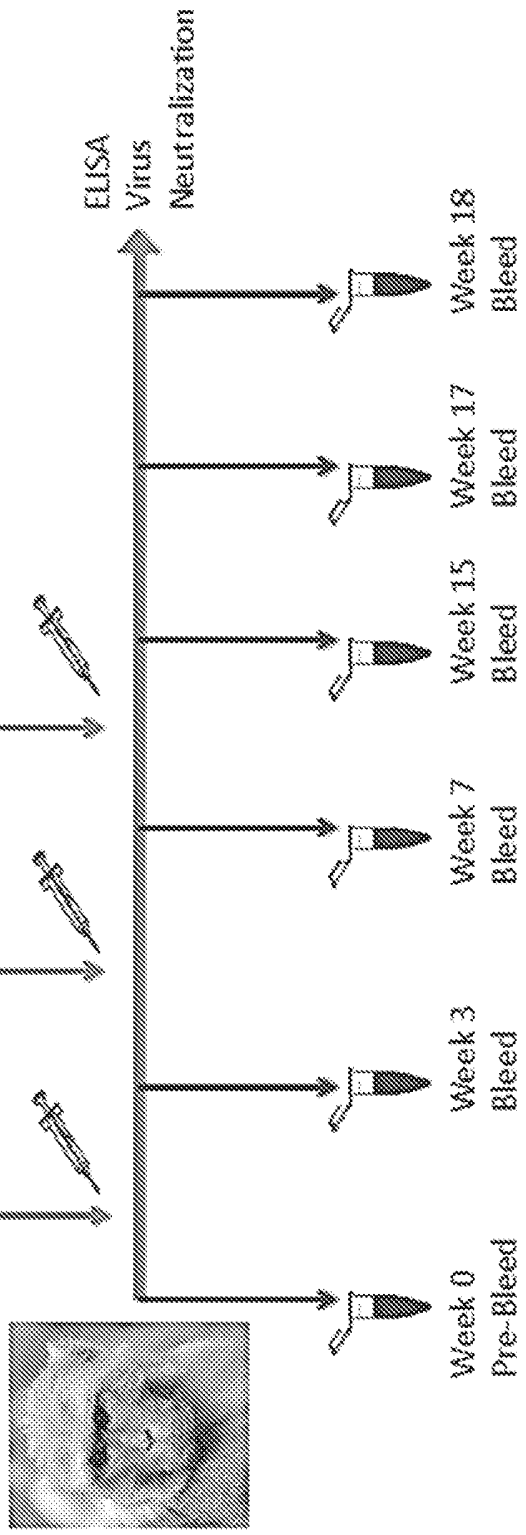

Week 1
Immunize/EP
Week 5
Immunize/EP
Week 13
Immunize/EP
ELISA
Virus
Neutralization
Week 0
Pre-Bleed
Week 3
Bleed
Week 7
Bleed
Week 15
Bleed
Week 17
Bleed
Week 18
Bleed

Figure 38A

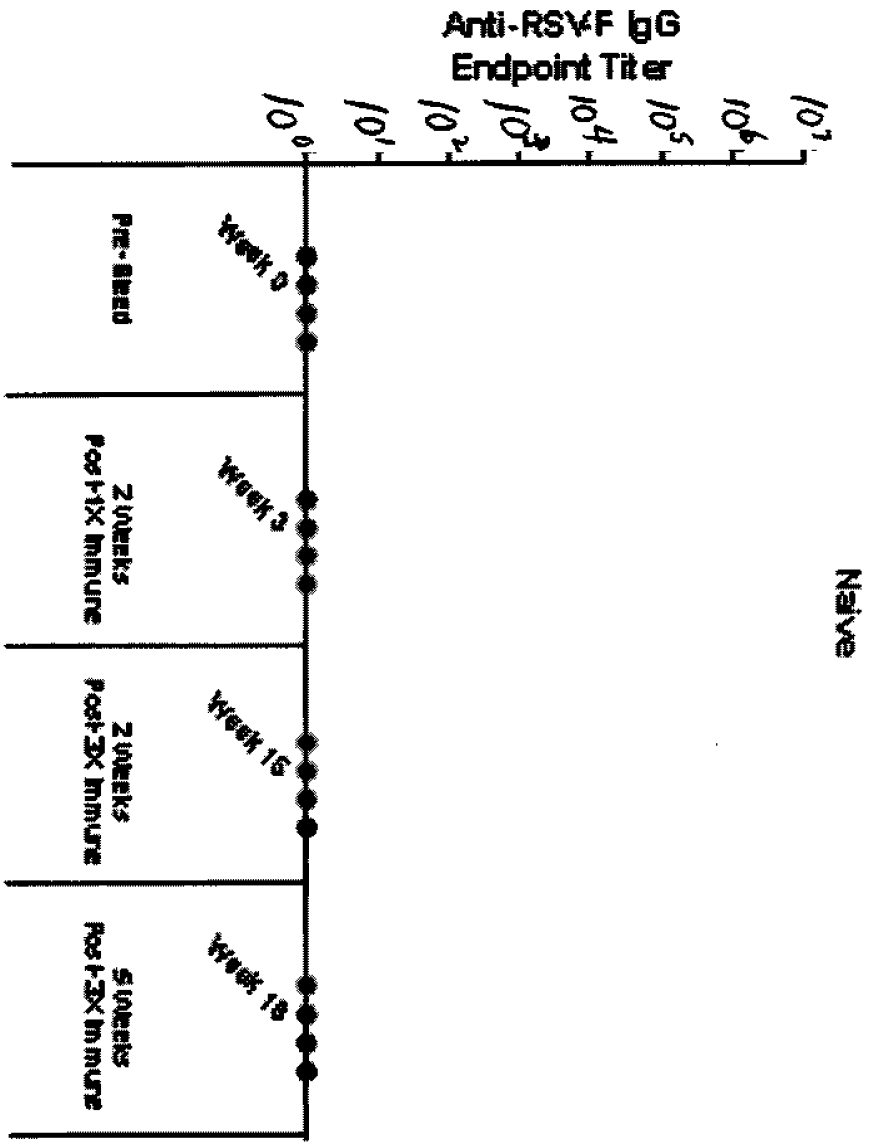

Anti-RSVF IgG
Endpoint Titer
10^0
10^1
10^2
10^3
10^4
10^5
10^6
10^7
Pre-Bleed
Week 0
2 Weeks Post 1X Immune
Week 3
2 Weeks Post 2X Immune
Week 16
5 Weeks Post 2X Immune
Week 18
NAIVE

Figure 38B

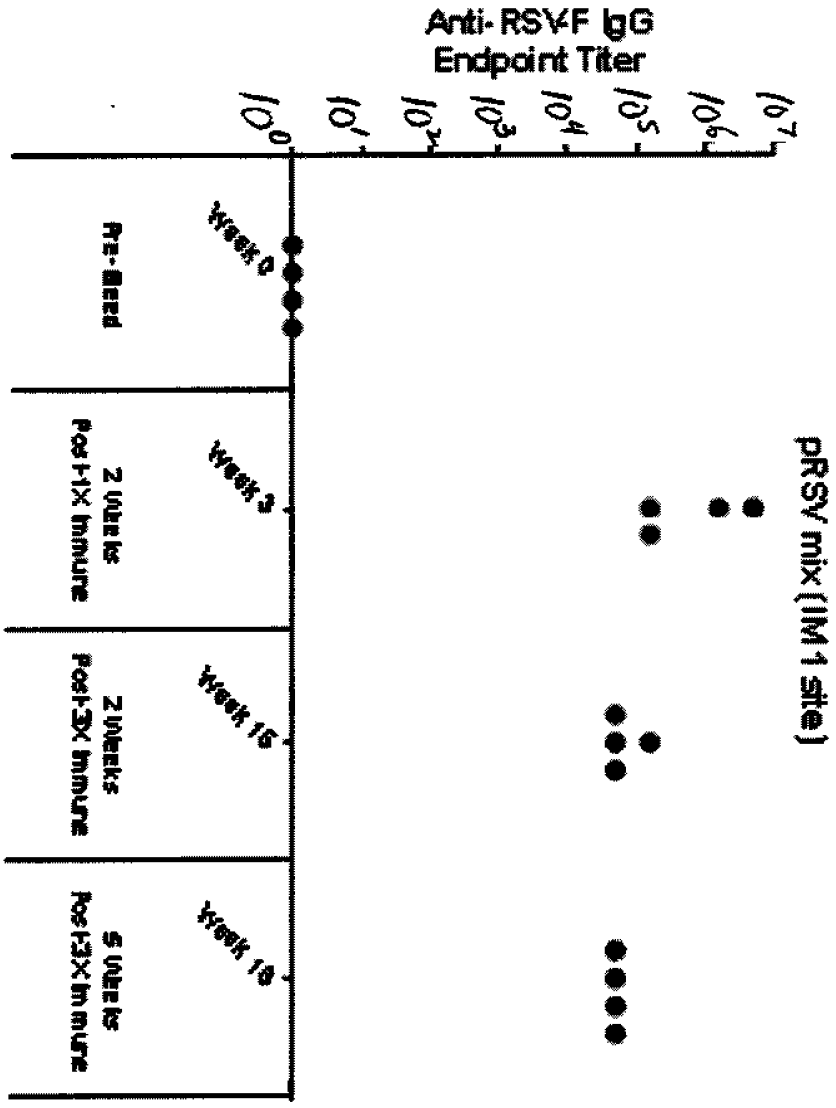

Anti-RSV-F IgG
Endpoint Titer
10^0
10^1
10^2
10^3
10^4
10^5
10^6
10^7
Week 0
Week 3
Week 15
Week 19
Pre-Bleed
2 Weeks
Post-1X Immune
2 Weeks
Post-2X Immune
5 Weeks
Post-3X Immune
pRSV mix (IM 1 site)

Figure 38C

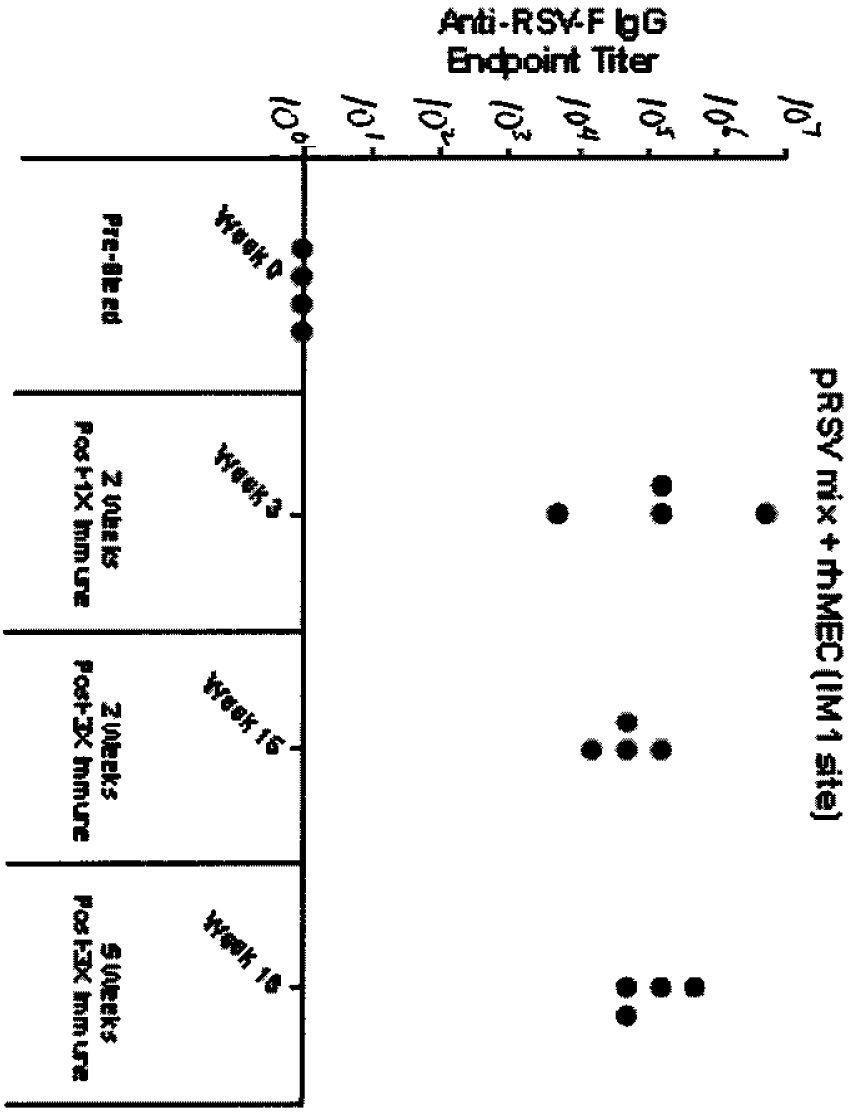

Anti-RSV-F IgG
Endpoint Titer
10^0
10^1
10^2
10^3
10^4
10^5
10^6
10^7
Pre-Bleed
Week 0
2 Weeks Post-1X Immune
Week 3
2 Weeks Post-2X Immune
Week 15
5 Weeks Post-3X Immune
Week 18
PRSV mix + rhMEC (IM 1 site)

Figure 38D

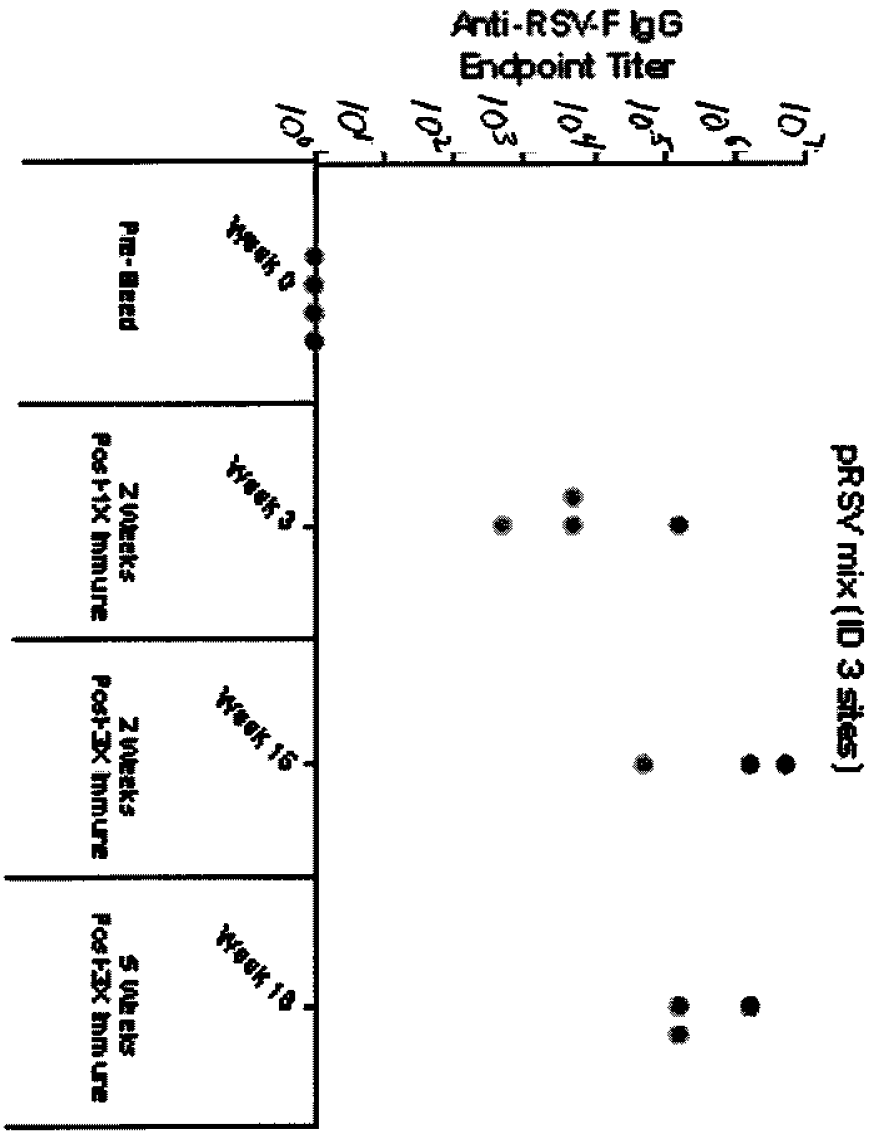

Anti-RSV-F IgG
Endpoint Titer
10^6
10^1
10^2
10^3
10^4
10^5
10^6
10^7
Week 0
Week 3
Week 16
Week 18
Pre-Bleed
2 Weeks Post-1X Immune
2 Weeks Post-2X Immune
5 Weeks Post-2X Immune
pRSV mix (ID 3 sites)

Figure 39A

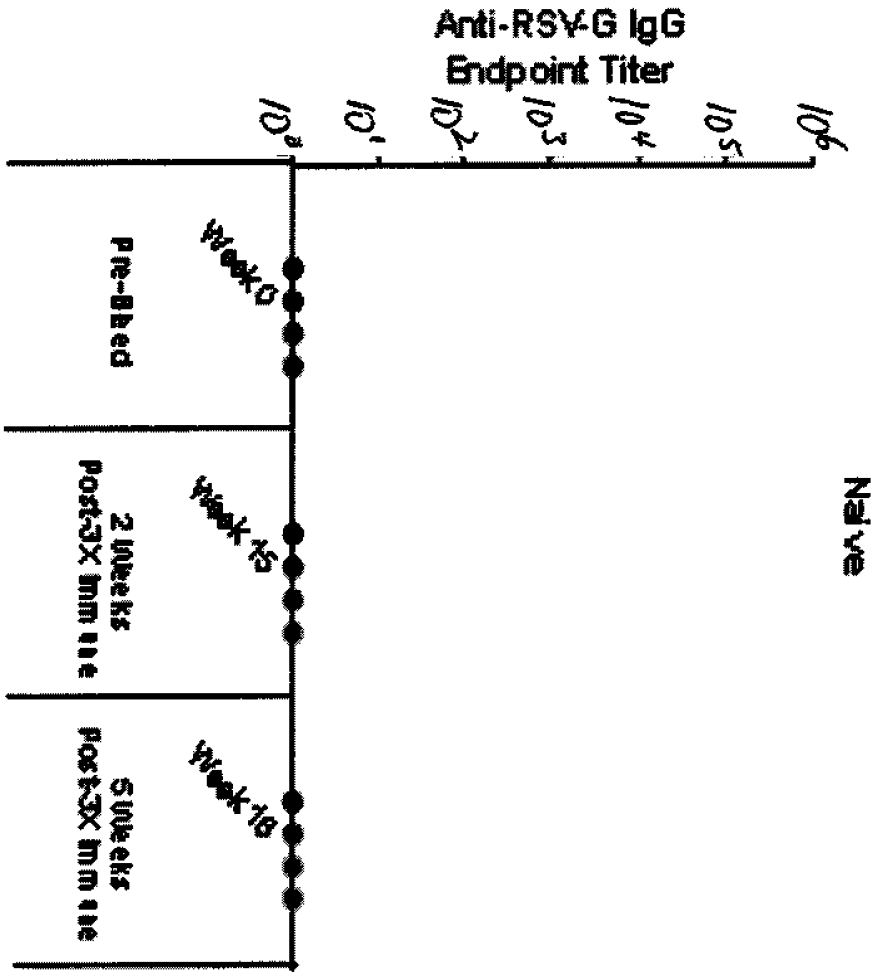

Anti-RSV-G IgG
Endpoint Titer
10^0
10^1
10^2
10^3
10^4
10^5
10^6
Week 0
Week 28
Week 76
Pre-Bleed
2 Weeks
Post-3X Immune
5 Weeks
Post-3X Immune
Naïve

Figure 39B

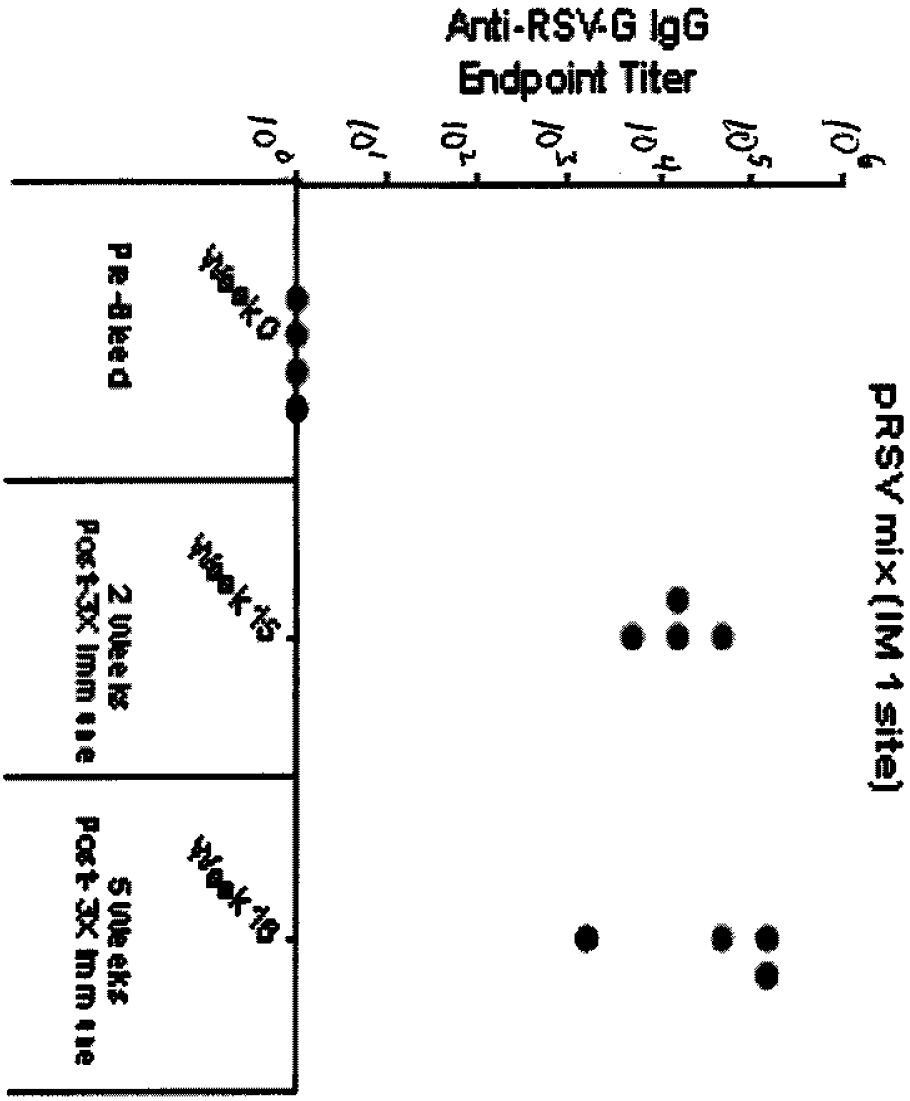

Anti-RSV-G IgG
Endpoint Titer
10^0
10^1
10^2
10^3
10^4
10^5
10^6
Pre-Bleed
Week 0
2 Weeks Post-3X Immune
Week 16
5 Weeks Post-3X Immune
Week 19
pRSV mix (IM 1 site)

Figure 39C

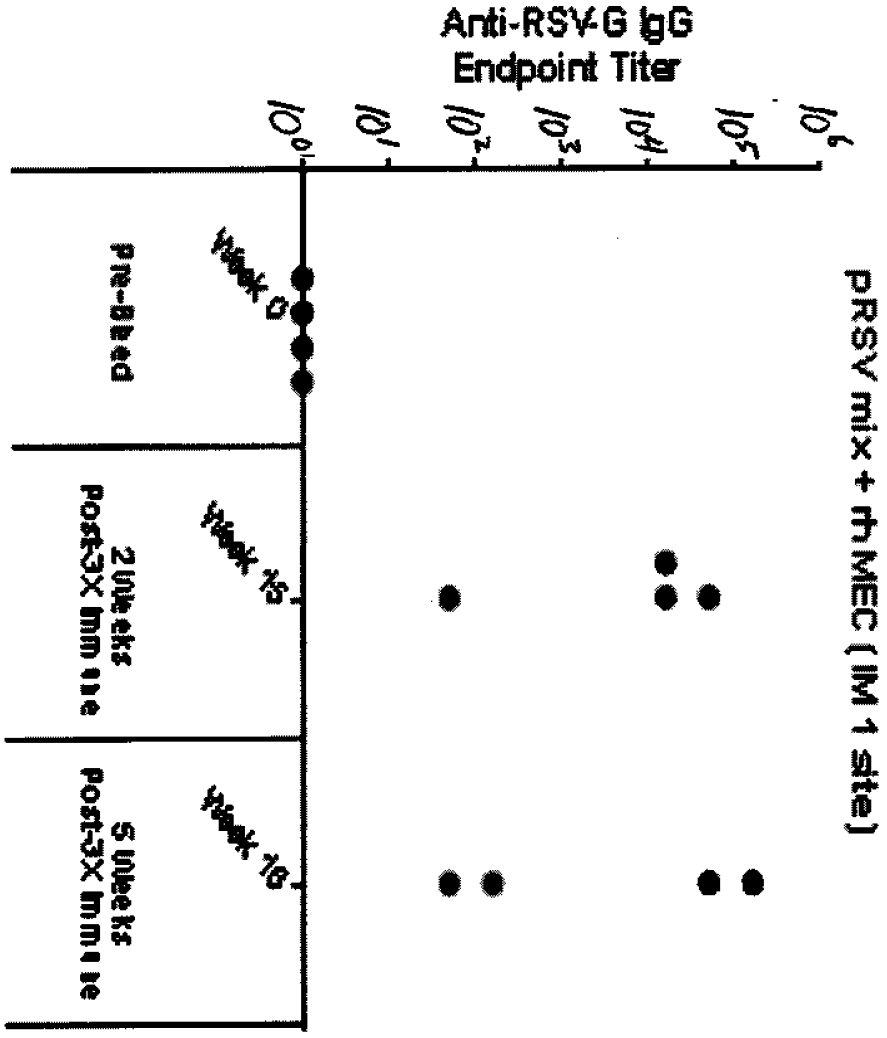

Anti-RSV-G IgG
Endpoint Titer
10^0
10^1
10^2
10^3
10^4
10^5
10^6
Week 0
Week 26
Week 78
Pre-Bleed
2 Weeks
Post-3X Immune
5 Weeks
Post-3X Immune
PRSV mix + rhMEC (IM 1 site)

Figure 39D

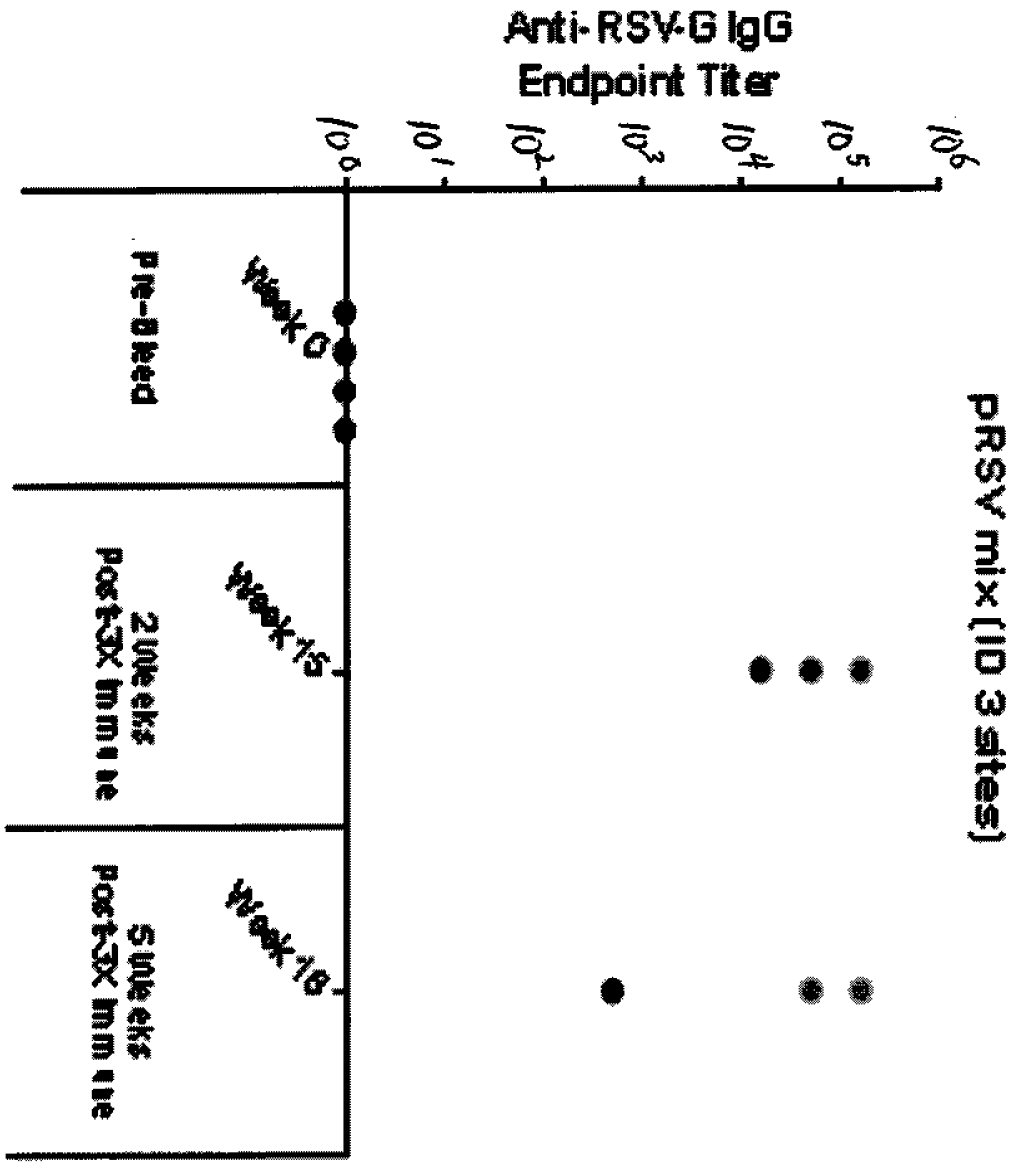

Anti-RSV-G IgG
Endpoint Titer
10^0
10^1
10^2
10^3
10^4
10^5
10^6
Week 0
Week 15
Week 19
Pre-Bleed
2 Weeks Post-3X Immune
5 Weeks Post-3X Immune
PRSV mix (ID 3 sites)

Figure 40

Preliminary Neutralization Results from Cocktail Vaccine in Rabbit and Non-Human Primate Studies

Sample Tested	Preadsorbed	Neut Titer Post-3d Imm
Negative serum		0
Mab anti RSV		40
Polyclonal Serum		160
Rabbit 71	0	640
Rabbit 72	0	320
NHP IM-1	0	640
NHP IM-2	0	320

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/036008

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C07K 14/135 (2013.01)
USPC - 424/211.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - A61K 39/12, 39/155; A61P 31/14, 37/04; C07K 14/115, 14/135 (2013.01)
USPC - 424/186.1, 199.1, 211.1; 435/69.1; 530/350

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

CPC: A61K 39/00, 39/155, 2039/5256; C07K 14/005, 14/135, 16/1027; C12N 2760/18522, 2760/18534

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Patbase, Google Patents, Google, Pubmed

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2008/114149 A2 (BLAIS et al) 25 September 2008 (25.09.2008) entire document	1-22
A	US 2011/0014220 A1 (CHOW et al) 20 January 2011 (20.01.2011) entire document	1-22
A	WO 2009/039178 A1 (KALERGIS et al) 26 March 2009 (26.03.2009) entire document	1-22
A	WO 2004/010935 A2 (YOUNG et al) 05 February 2004 (05.02.2004) entire document This document can be viewed by entering the doc number at the following url: http://worldwide.espacenet.com/numberSearch?locale=en_EP	1-22
P, Y	US 2012/0276142 A1 (WEINER et al) 01 November 2012 (01.11.2012) entire document	1-22

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 September 2013	20 SEP 2013
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/036008

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

   a. (means)

   ☐ on paper

   ☒ in electronic form

   b. (time)

   ☒ in the international application as filed

   ☐ together with the international application in electronic form

   ☐ subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

Specifically, SEQ ID NOs:1, 2, 9, and 10 were searched.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/036008

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See Extra Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-22, limited to a composition comprising an RSV F immunogen, wherein the RSV F immunogen is selected to be SEQ ID NO:2, further comprising nucleic acid sequence SEQ ID NO:1 that encodes for SEQ ID NO:2; the composition further comprising the RSV F immunogen of SEQ ID NO:2 operably linked to a signal peptide, wherein the RSV F immunogen and signal peptide fusion protein is selected to be SEQ ID NO:10, encoded by nucleic acid sequence SEQ ID NO:9.

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

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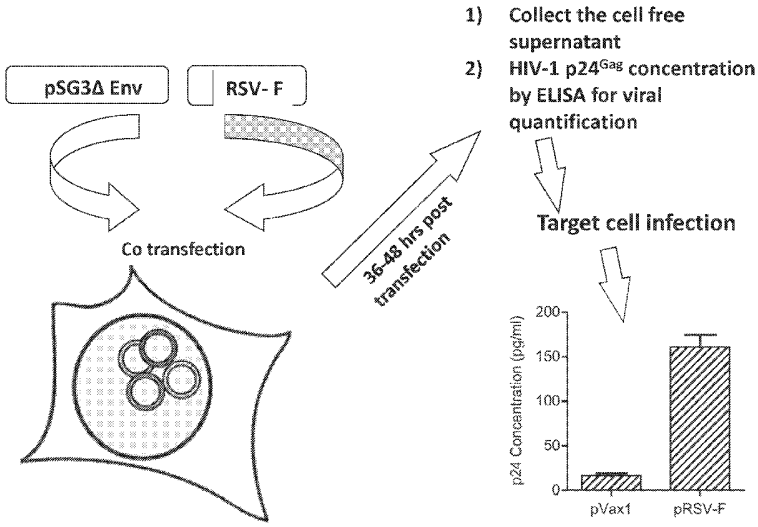
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[Continued on next page]

(54) Title: HUMAN RESPIRATORY SYNCYTIAL VIRUS CONSENSUS ANTIGENS, NUCLEIC ACID CONSTRUCTS AND VACCINES MADE THEREFROM, AND METHODS OF USING SAME

Figure 12
RSV-Fusion Pseudo viral production in 293T cells



(57) Abstract: Nucleic acid molecules and compositions comprising one or more nucleic acid sequences that encode an RSV immunogen are disclosed. Nucleic acid are disclosed that comprise the sequences that encodes consensus RSV F protein or immunogenic fragment thereof, sequences that encodes an RSV G(A) protein or immunogenic fragment thereof and sequences that encodes an RSV G(B) protein or immunogenic fragment thereof. Compositions comprising one, combinations of two or all three sequences are disclosed. The coding sequences optionally include operable linked coding sequence that encode a signal peptide. Nucleic acid molecules and compositions comprising the chemokine CC20 and/or a consensus RSV M2-1 protein or immunogenic fragment thereof are also disclosed. Immunomodulatory methods and methods of inducing an immune response against RSV are disclosed. Method of preventing RSV infection and methods of treating individuals infected with RSV are disclosed.

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HUMAN RESPIRATORY SYNCYTIAL VIRUS CONSENSUS ANTIGENS, NUCLEIC ACID CONSTRUCTS AND VACCINES MADE THEREFROM, AND METHODS OF USING SAME

FIELD OF THE INVENTION

The present invention relates to consensus antigenic respiratory syncytia virus proteins and nucleic acid molecules which encode the same; improved respiratory syncytia virus vaccines including such proteins and/or nucleic acid molecules; and methods for using the vaccines for inducing immune responses and preventing respiratory syncytia virus infection and/or treating individuals infected with respiratory syncytia virus.

BACKGROUND OF THE INVENTION

This application claims priority to U.S. Provisional Application No. 61/622,279, which is incorporated herein, in its entirety, by reference.

Human Respiratory Syncytial Virus (RSV) is the most common cause of upper and lower respiratory tract infections among infants and young children (premature infants especially prone to disease). World-wide, RSV is the leading cause of serious lower respiratory infections in infants (especially among those born prematurely and having chronic lung diseases or congenital heart diseases) and young children worldwide, and is responsible for a variety of illnesses, including 20-25% of pneumonia cases and 45-50% of bronchiolitis cases among hospitalized children. In the U.S., RSV invention results in approximately 120,000 hospitalization and 500+ deaths each year. In the population of <1 year old, RSV is the leading cause of infant viral death and mortality among this population is 10 times greater than mortality due to influenza infection. Global annual morbidity and mortality estimated to be 64 million and 160,000 deaths respectively. RSV-related medical costs estimated to be >$650 million/year.

While essentially all children experience an infection by two years of age, the peak age for serious RSV infection is at 2-6 months of age. The majority of infections resolve uneventfully. In some children, however, infection may predispose children for asthma and

airway hyper-responsiveness later in life. Natural RSV infection does not confer lifetime immunity, and therefore, individuals may be repeatedly infected.

RSV infection raises significant issue among the elderly and other vulnerable populations. Among the elderly, RSV is the second leading cause of viral death. Transplant recipients and other immunocompromised populations as well as individuals suffering from cystic fibrosis are vulnerable to serious health consequences due to infection.

Respiratory Syncytial Virus (RSV) is an enveloped, negative-sense, single-stranded RNA virus of the family *Paramyxoviridae,*a family which includes common respiratory viruses such as influenza and those causing mumps and measles. In all, RSV has ten genes encoding eleven different proteins. The eleven RSV proteins include: proteins 1) protein "NS1" and 2) protein "NS2", which inhibit type I interferon activity; 3) protein "N", the nucleocapsid protein which associates with RNA forming nucleocapsid; 4) protein "P", which is a cofactor for protein L; 5) protein "M", the matrix protein which required for viral assembly; 6) protein "SH", which is expressed on the viral surface forms the viral coat with protein G and protein F; 7) protein "G", which is highly glycosylated, expressed on the surface, involved in viral attachment and binds glycosaminoglycans (GAGs); 8) protein "F", which is expressed on the surface, viral-cell membrane fusion and mediating fusion to allow entry of virus into the cell cytoplasm; 9) protein "M2-1", which is a matrix protein and elongation factor; 10) protein "M2-2", which is a matrix protein and transcription factor;. and, 11) protein "L", which is RNA polymerase. The M2 gene encodes both protein M2-1 and protein M2-2 in overlapping open reading frames. The primary CD8 T cell epitope is encoded by the M2 gene. There are two major subtypes of human RSV - A and B. The major difference between the two subtypes resides within the G protein.

RSV infection often results both immune mediated pathoglogy and virus mediated pathology. Primary RSV infection often results in acute bronchiolitis that leads to inflammation-induced airway obstruction. RSV F binding has been shown to induce apoptosis resulting in the sloughing of ciliated epithelial cells, compromised pulmonary clearance, and consequent secondary infections.

Unfortunately, despite the immense effort, there are still no effective vaccines available for RSV. In 1966-1967, first RSV vaccine candidate, a formalin-inactivated alum-precipitated

RSV preparation (FI-RSV vaccine) resulted in enhanced disease in vaccinated children upon subsequent natural infection.. Histological analysis of lungs of children who died from enhanced disease caused by infection after vaccination revealed extensive mononuclear cell infiltration including pulmonary eosinophilia. The FI-RSV vaccine generated only binding antibodies without neutralizing activity because of denatured F protein and did not induce CTL activity.

Subsequent experiments have suggested that this enhanced pulmonary disease is associated with an exaggerated Th2-type cytokine response by CD4 T cells, a poor cytolytic response by CD8 T cells, and a weak neutralizing antibody response. RSV infection of FI-RSV vaccinated BALB/c and C57BL/6 mice resulted in enhanced disease observed in FI-RSV vaccinated children. Characteristic of a Th2-mediated immune response suggested immunized children were primed for Th2 immune response by vaccine. Increased levels of Th2-associated cytokines IL-5, IL-4, and IL-13 and chemokineeotaxin with a decrease in Th1-associated cytokine IL-12 were exhibited. Depletion of IL-4, IL-10, or IL-13 resulted in significant decrease in enhanced disease after RSV challenge.

Live attenuated and inactivated whole virus vaccines have also failed to protect. The candidate vaccines were either insufficiently attenuated or demonstrated the potential for enhanced disease. In 1982, a live attenuated RSV vaccine was found to be safe, but not effective for prevention of RSV illness.

In 1983, a Native American infant, "Baby Moose", who was thought to have B streptococcal disease but who actually was infected with RSV, serendipitously improved when he received IGIV. This result prompted study of IGIV for RSV disease. In the mid 1980s through 1990 studies of standard IGIV for treatment and prevention of RSV illness validated the role of antibodies in prevention of RSV disease.

In the early 1990s, RSV vaccine studies were re-initiated using various subunit varieties. These trials failed to show significant protection from disease.

From mid 1990s to early 2002, clinical trials of palivizumab (monoclonal antibody specific for RSV-F) for prevention of serious respiratory tract disease caused by RSV produced positive results. In September 2003, palivizumab was approved for prevention of RSV-associated disease in high-risk children. Prophylactic treatment with palivizumab is effective in

reducing the severity of disease, but is only recommended for high-risk patients due to the high cost involved in the treatment.

Although past studies have failed to yield in effective RSV vaccines, they have convincingly demonstrated the importance of immune responses in providing a thorough protection against RSV infection. Studies have found evidence supporting the importance of humoral responses and antibodies in protection against RSV-mediated disease. The presence of IgG antibodies in the lung directly correlates with reduced viral load and children with less severe RSV disease often have significantly higher anti-RSV antibody titers before infection Antibody to fusion protein is an important correlate of immunity. Infants who did not become infected with RSV had higher mean titers of IgG than infected infants and were born to mothers who had significantly higher maternal RSV-specific IgG antibody levels than the mothers of infants who became infected. The importance of antibody in mediating protection helps explain why premature infants are at such high risk for serious illness after RSV infection. Maternal IgG is not efficiently transferred to the fetus until the third trimester of pregnancy.

Similarly, the importance of cellular immune responses in providing a thorough protection against RSV infection has also been demonstrated. Children with T cell deficiency have difficulty clearing the virus and are more susceptible to subsequent RSV infection. In animal studies, depletion of CD8 T cells alone in mice does not result in chronic infection, but does result in delayed viral clearance. Clearance requires IFN-γ, FasL, TNF-α. Deficiency in any of these results in delayed viral clearance. Further, C57BL/6 mice immunized with VV-G do not develop RSV-associated enhanced disease, but depletion of CD8 T cells before immunization results in disease.

There remains a need for an RSV vaccine, including an immunogenic antigen, that induces long-term protection against RSV. There is also a need for a cost-effective delivery system to enable mass prophylactic vaccination against RSV. There is also a need for additional therapeutic agents to treat individuals infected with RSV.

SUMMARY OF THE INVENTION

Nucleic acid molecules are provided which comprising one or more nucleic acid sequences that encode one o more consensus RSV immunogens. The RSV immunogens include the consensus RSV F immunogen, the consensus RSV-G(A) immunogen, and the consensus RSV G(B) immunogen.

Compositions are provided which comprise one or more nucleic acid sequences that encode an RSV immunogen. The nucleic acids are selected from the group consisting of: a) a nucleic acid coding sequence that encodes an RSV F immunogen, b) a nucleic acid coding sequence that encodes an RSV Ga immunogen, and c) a nucleic acid coding sequence that encodes an RSV Gb immunogen.

In some embodiments, the nucleic acid coding sequence that encodes an RSV F immunogen encodes an RSV F immunogen selected from the group consisting of: SEQ ID NO:2, a fragment of SEQ ID NO:2, a protein that is at least 98% homologous to SEQ ID NO:2 and a fragment of a protein that is at least 98% homologous to SEQ ID NO:2. In each instance, the nucleic acid coding sequence that encodes an RSV F immunogen may optionally further comprise coding sequence that encodes a signal peptide operably linked to the sequence.

In some embodiments, the nucleic acid coding sequence that encodes an RSV Ga immunogen encodes an RSV Ga immunogen selected from the group consisting of: SEQ ID NO:6, a fragment of SEQ ID NO:6, a protein that is at least 98% homologous to SEQ ID NO:6 and a fragment of a protein that is at least 98% homologous to SEQ ID NO:6. In each instance, the nucleic acid coding sequence that encodes an RSV Ga immunogen may optionally further comprise coding sequence that encodes a signal peptide operably linked to the sequence.

In some embodiments, the nucleic acid coding sequence that encodes an RSV Gb immunogen encodes an RSV Gb immunogen selected from the group consisting of: SEQ ID NO:8, a fragment of SEQ ID NO:8, a protein that is at least 98% homologous to SEQ ID NO:8 and a fragment of a protein that is at least 98% homologous to SEQ ID NO:8. In each instance, the nucleic acid coding sequence that encodes an RSV Gb immunogen may optionally further comprise coding sequence that encodes a signal peptide operably linked to the sequence.

In some embodiments, the nucleic acid coding sequence that encodes an RSV F immunogen is selected from the group consisting of: nucleic acid sequences comprising SEQ ID NO:1, nucleic acid sequences comprising a fragment of SEQ ID NO:1, nucleic acid sequences comprising sequences that is at least 98% homologous to SEQ ID NO:1, and nucleic acid sequences comprising a fragment of a sequence that is at least 98% homologous to SEQ ID NO:1. In each instance, the nucleic acid coding sequence that encodes an RSV F immunogen may optionally further comprise coding sequence that encodes a signal peptide operably linked to the sequence. Nucleic acid sequences comprising sequences that is at least 98% homologous to SEQ ID NO:1 preferable encode a protein that is at least 98% homologous to SEQ ID NO:2. Nucleic acid sequences comprising a fragment of a sequence that is at least 98% homologous to SEQ ID NO:1 preferable encode a fragment of a protein that is at least 98% homologous to SEQ ID NO:2.

In some embodiments, the nucleic acid coding sequence that encodes an RSV Ga immunogen is selected from the group consisting of: nucleic acid sequences comprising SEQ ID NO:5, nucleic acid sequences comprising a fragment of SEQ ID NO:5, nucleic acid sequences comprising sequences that is at least 98% homologous to SEQ ID NO:5, and nucleic acid sequences comprising a fragment of a sequence that is at least 98% homologous to SEQ ID NO:5. In each instance, the nucleic acid coding sequence that encodes an RSV Ga immunogen may optionally further comprise coding sequence that encodes a signal peptide operably linked to the sequence. Nucleic acid sequences comprising sequences that is at least 98% homologous to SEQ ID NO:5 preferable encode a protein that is at least 98% homologous to SEQ ID NO:6. Nucleic acid sequences comprising a fragment of a sequence that is at least 98% homologous to SEQ ID NO:5 preferable encode a fragment of a protein that is at least 98% homologous to SEQ ID NO:6.

In some embodiments, the nucleic acid coding sequence that encodes an RSV Gb immunogen is selected from the group consisting of: nucleic acid sequences comprising SEQ ID NO:7, nucleic acid sequences comprising a fragment of SEQ ID NO:7, nucleic acid sequences comprising sequences that is at least 98% homologous to SEQ ID NO:7, and nucleic acid sequences comprising a fragment of a sequence that is at least 98% homologous to SEQ ID

NO:7. In each instance, the nucleic acid coding sequence that encodes an RSV Gb immunogen may optionally further comprise coding sequence that encodes a signal peptide operably linked to the sequence. Nucleic acid sequences comprising sequences that is at least 98% homologous to SEQ ID NO:7 preferable encode a protein that is at least 98% homologous to SEQ ID NO:8. Nucleic acid sequences comprising a fragment of a sequence that is at least 98% homologous to SEQ ID NO:7 preferable encode a fragment of a protein that is at least 98% homologous to SEQ ID NO:8.

In some embodiments, the compositions comprises one or more nucleic acid sequences selected from the group consisting of: a nucleic acid sequence that encodes SEQ ID NO:10; a nucleic acid sequence that encodes SEQ ID NO:14; and a nucleic acid sequence that encodes SEQ ID NO:16.

In some embodiments, the compositions comprise one or more nucleic acid sequences selected from the group consisting of: a nucleic acid sequence comprising SEQ ID NO:9; a nucleic acid sequence comprising SEQ ID NO:13; and a nucleic acid sequence comprising SEQ ID NO:15.

In some embodiments, the compositions compriss nucleic acid sequences selected from the group consisting of:

a nucleic acid coding sequence that encodes an RSV F immunogen,

a nucleic acid coding sequence that encodes an RSV Ga immunogen,

a nucleic acid coding sequence that encodes an RSV Gb immunogen,

a nucleic acid coding sequence that encodes an RSV F immunogen and a nucleic acid coding sequence that encodes an RSV Ga immunogen,

a nucleic acid coding sequence that encodes an RSV F immunogen and a nucleic acid coding sequence that encodes an RSV Gb immunogen,

a nucleic acid coding sequence that encodes an RSV Ga immunogen and a nucleic acid coding sequence that encodes an RSV Gb immunogen,

a nucleic acid coding sequence that encodes an RSV F immunogen, a nucleic acid coding sequence that encodes an RSV Ga immunogen and a nucleic acid coding sequence that encodes an RSV Gb immunogen,

The composition may further comprise a nucleic acid coding sequence that encodes CCL20 and/or be formulated for delivery to an individual using electroporation and/or further comprising nucleic acid sequences that encode one or more proteins selected from the group consisting of: IL-12, IL-15 and IL-28.

In some embodiments, one or more nucleic acid coding sequences are part of one or more plasmids. In some embodiments, one or more nucleic acid coding sequences are each incorporated into a separate plasmid.

Method are provided for inducing an immune response against RSV comprising administering a composition to an individual in an amount effective to induce an immune response in said individual.

Method are provided for treating an individual who has been diagnosed with RSV comprising administering a therapeutically effective amount of a composition to an individual.

Method are provided for preventing RSV infection an individual comprising administering a prophylactically effective amount of the composition to an individual.

Novel "RSV F immunogen", "RSV Ga immunogen", and "RSV Gb immunogen" are provided. In some embodiments, proteins selected from the group consisting of: SEQ ID NO:2; SEQ ID NO:6; SEQ ID NO:8; SEQ ID NO:10; SEQ ID NO:14; and SEQ ID NO:16 are provided.

Methods of modulating immune responses using a nucleic acid coding sequence that encodes the chemokine CCL20 are also provided.

In addition, provided herein is a nucleic acid coding sequence that encodes a consensus RSV M2-1 immunogen. In some embodiments, the nucleic acid coding sequence that encodes an RSV M2-1 immunogen encodes an RSV M2-1 immunogen selected from the group consisting of: SEQ ID NO:4, a fragment of SEQ ID NO:4, a protein that is at least 98% homologous to SEQ ID NO:4 and a fragment of a protein that is at least 98% homologous to SEQ ID NO:4. In each instance, the nucleic acid coding sequence that encodes an RSV M2-1 immunogen may optionally further comprise coding sequence that encodes a signal peptide operably linked to the sequence.

In some embodiments, the nucleic acid coding sequence that encodes an RSV M2-1 immunogen is selected from the group consisting of: nucleic acid sequences comprising SEQ ID NO:3, nucleic acid sequences comprising a fragment of SEQ ID NO:3, nucleic acid sequences comprising sequences that is at least 98% homologous to SEQ ID NO:3, and nucleic acid sequences comprising a fragment of a sequence that is at least 98% homologous to SEQ ID NO:3. In each instance, the nucleic acid coding sequence that encodes an RSV M2-1 immunogen may optionally further comprise coding sequence that encodes a signal peptide operably linked to the sequence. Nucleic acid sequences comprising sequences that is at least 98% homologous to SEQ ID NO:3 preferable encode a protein that is at least 98% homologous to SEQ ID NO:4. Nucleic acid sequences comprising a fragment of a sequence that is at least 98% homologous to SEQ ID NO:3 preferable encode a fragment of a protein that is at least 98% homologous to SEQ ID NO:4.

In some embodiments, compositions are provided which comprises one or more nucleic acid sequences including a nucleic acid sequence that encodes SEQ ID NO:12.

In some embodiments, the compositions are provided which comprise one or more nucleic acid sequences including a nucleic acid sequence comprising SEQ ID NO:11.

In some embodiments, the compositions compriss nucleic acid sequences selected from the group consisting of: a nucleic acid coding sequence that encodes an RSV M2-1 immunogen, a nucleic acid coding sequence that encodes an RSV F immunogen and a nucleic acid coding sequence that encodes an RSV M2-1 immunogen, a nucleic acid coding sequence that encodes an RSV M2-1 immunogen and a nucleic acid coding sequence that encodes an RSV Ga immunogen, a nucleic acid coding sequence that encodes an RSV M2-1 immunogen and a nucleic acid coding sequence that encodes an RSV Gb immunogen, a nucleic acid coding sequence that encodes an RSV F immunogen, a nucleic acid coding sequence that encodes an RSV M2-1 immunogen and a nucleic acid coding sequence that encodes an RSV Ga immunogen, a nucleic acid coding sequence that encodes an RSV F immunogen, a nucleic acid coding sequence that encodes an RSV M2-1 immunogen and a nucleic acid coding sequence that encodes an RSV Gb immunogen, a nucleic acid coding sequence that encodes an RSV M2-1 immunogen, a nucleic acid coding sequence that encodes an RSV Ga immunogen and a nucleic

acid coding sequence that encodes an RSV Gb immunogen, and a nucleic acid coding sequence that encodes an RSV F immunogen, a nucleic acid coding sequence that encodes an RSV M2-1 immunogen, a nucleic acid coding sequence that encodes an RSV Ga immunogen and a nucleic acid coding sequence that encodes an RSV Gb immunogen.

Novel " "RSV M2-1 immunogen is also provided. In some embodiments, proteins selected from the group consisting of: SEQ ID NO:4 and SEQ ID NO:12 are provided.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the phylogenetic tree of RSV-F Protein, the consensus RSV F protein amino acid sequence is set forth in SEQ ID NO:2.

Figure 2 shows the phylogenetic tree of RSV M2-1 Protein, the consensus RSV M2-1 protein amino acid sequence is set forth in SEQ ID NO:4.

Figure 3 shows results anti-RSV F protein IgG at various time points following immunization with RSV F vaccine.

Figure 4 shows results anti-RSV F protein IgA at various time points following immunization with RSV F vaccine.

Figure 5 shows results from a comparison of IFN-γ production at different dosage of RSV-F vaccine approximately 8 weeks after last immunization.

Figure 6 shows results from a comparison of IFN-γ+ CD8 and CD4 T cells in spleen after RSV-F +/- chemokine immunization in the spleen.

Figure 7 shows results from a comparison of IFN-γ+ CD8 and CD4 T cells in spleen after RSV-F +/- chemokine immunization in the lung.

Figure 8 shows results from a comparison of IgG Endpoint Titer in Sera and IgG subtypes (IgG1 vs IgG2a) in Sera.

Figure 9 shows a comparison of IgG2a/IgG1 ratio.

Figure 10 depicts Env-Pseudotyped viruses in TZM-BL Cells/ "ON".

Figure 11 depicts Env-Pseudotyped viruses in TZM-BL Cells/ "OFF".

Figure 12 depicts RSV-Fusion Pseudo viral production in 293T cells.

Figure 13 shows the immunization protocol used for a RSV-F Neutralization Study using the assay.

Figure 14 shows data from quantification of RSV-F mediated infection in different target cells.

Figure 15 shows data from quantification of RSV-F neutralization activity in TZM-bl HeLa cells.

Figure 16 shows data from quantification of cell death activity by RSV-F pseudo virus in HeLa cells.

Figure 17 shows the immunization schedule for RSV-M2 dosage study that was undertaken.

Figure 18 shows data collected from that study including a comparison of IFN-g production at different dosages of RSV-M2 vaccine 1 week after last immunization.

Figure 19 depicts an immunization schedule for evaluation of the combination of RSV-F vaccine with CCL20.

Figure 20 shows data from the study including data from a comparison of IFN-g production in each of spleen, liver and lung with different dosages of CCL20 at 1 week after last immunization.

Figure 21 shows data of a comparison of IgG subtypes (IgG1 vs IgG2a) in Sera.

Figures 22A-22C show plasmid designs and construction of RSV-F, RSV-G(A), and RSV-G(B) constructs. Figures 22A shows plasmid design and construction of RSV-F construct. Figures 22B shows plasmid design and construction of RSV-G(A) construct. Figures 22C shows plasmid design and construction of RSV-G(B) construct.

Figure 23 shows results confirming in vitro expression of RSV-F, RSV-G(A), and RSV-G(B) constructs.

Figure 24 shows the immunization schedule for an RSV-F Dosage Study.

Figure 25 shows IgG data from RSV-F Dosage Study.

Figure 26 shows IFN-γ data from RSV-F Dosage Study.

Figure 27 shows the immunization schedule for a RSV-F PSEUDO Neutralization Study.

Figure 28 shows data from the RSV-F PSEUDO Neutralization Study.

Figure 29 shows data from two comparisons to determine inhibition of apoptotic cell death activity by RSV-F pseudovirus in HeLa cells.

Figure 30 shows the immunization schedule for an RSV-G(A) and RSV-(B) Dosage Study.

Figure 31 shows IgG data from RSV-G(A) from the RSV-G(A) and RSV-(B) Dosage Study.

Figure 32 shows IgG data from RSV-G(A) from the RSV-G(A) and RSV-(B) Dosage Study.

Figure 33 is a chart showing a summary of RSV Preliminary Neutralization results using Mono Vaccines in Mouse Studies.

Figure 34 shows the RSV Rabbit Study Design that was followed.

Figure 35 shows data from the RSV Rabbit Study of measured humoral immunity against RSV-F.

Figure 36 shows data from the RSV Rabbit Study of measured humoral immunity against RSV-G.

Figure 37 shows the RSV Non-Human Primate Study Design that was followed.

Figure 38A-38D show data from the RSV Non-Human Primate Study of measured humoral immunity against RSV-F. Figure 38A shows data from measured humoral immunity against RSV-F in naïve animals. Figure 38B shows data from measured humoral immunity against RSV-F in animals who received the cocktail vaccine IM at one site. Figure 38C shows data from measured humoral immunity against RSV-F in animals who received the cocktail vaccine plus rhMEC construct IM at one site. Figure 38D shows data from measured humoral immunity against RSV-F in animals who received the cocktail vaccine ID at three sites.

Figure 39A-39D show data from the RSV Non-Human Primate Study of measured humoral immunity against RSV-G. Figure 39A shows data from measured humoral immunity against RSV-G in naïve animals. Figure 39B shows data from measured humoral immunity against RSV-G in animals who received the cocktail vaccine IM at one site. Figure 39C shows data from measured humoral immunity against RSV-G in animals who received the cocktail

vaccine plus rhMEC construct IM at one site. Figure 39D shows data from measured humoral immunity against RSV-G in animals who received the cocktail vaccine ID at three sites.

Figure 40 is a chart showing a summary of RSV Preliminary Neutralization results using cocktail vaccines in Rabbit Non-Human Primate Studies.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

In one aspect of the invention, it is desired that the consensus antigen provides for improved transcription and translation, including having one or more of the following: low GC content leader sequence to increase transcription; mRNA stability and codon optimization; eliminating to the extent possible cis-acting sequence motifs (i.e., internal TATA-boxes).

In some aspects of the invention, it is desired to generate a consensus antigen that generates a broad immune response across multiple strains, including having one or more of the following: incorporate all available full-length sequences; computer generate sequences that utilize the most commonly occurring amino acid at each position; and increase cross-reactivity between strains.

The attached Sequence Listing includes SEQ ID NOs:1-16 which are various RSV sequences and embodiments.

SEQ ID NO:1 shows a nucleotide sequence encoding an RSV F without start codon (ATG) or signal peptide.

SEQ ID NO:2 shows an amino sequence encoding an RSV F without Met encoded by a start codon (ATG) or signal peptide.

SEQ ID NO:3 shows a nucleotide sequence encoding an RSV M2-1 without start codon (ATG) or signal peptide.

SEQ ID NO:4 shows an amino sequence encoding an RSV M2-1 without Met encoded by a start codon (ATG) or signal peptide.

SEQ ID NO:5 shows a nucleotide sequence encoding an RSV Ga without start codon (ATG) or signal peptide.

SEQ ID NO:6 shows an amino sequence encoding RSV Ga without Met encoded by a start codon (ATG) or signal peptide.

SEQ ID NO:7 shows a nucleotide sequence encoding an RSV Gb without start codon (ATG) or signal peptide.

SEQ ID NO:8 shows an amino sequence encoding an RSV Gb without Met encoded by a start codon (ATG) or signal peptide.

SEQ ID NO:9 shows a nucleotide sequence encoding an RSV F including start codon (ATG)/sequences encoding IgE signal peptide.

SEQ ID NO:10 shows an amino sequence encoding an RSV F with Met encoded by start codon/IgE signal peptide.

SEQ ID NO:11 shows a nucleotide sequence encoding an RSV M2-1 including start codon (ATG)/sequences encoding IgE signal peptide.

SEQ ID NO:12 shows an amino sequence encoding an RSV M2-1 with Met encoded by start codon/IgE signal peptide.

SEQ ID NO:13 shows a nucleotide sequence encoding an RSV Ga including start codon (ATG)/sequences encoding IgE signal peptide.

SEQ ID NO:14 shows an amino sequence encoding an RSV Ga with Met encoded by start codon/IgE signal peptide.

SEQ ID NO:15 shows a nucleotide sequence encoding an RSV Gb including start codon (ATG)/sequences encoding IgE signal peptide.

SEQ ID NO:16 shows an amino sequence encoding an RSV Gb with Met encoded by start codon/IgE signal peptide.

The attached Sequence Listing includes SEQ ID NO:17 which is the amino acid sequence of the IgE signal peptide.

SEQ ID NO:17 shows and amino acid sequence of an IgE signal peptide.

The attached Sequence Listing includes SEQ ID NOs:18-21 which are various CCL20 sequences and embodiments.

SEQ ID NO:18 shows a nucleotide sequence encoding an CCL20 without start codon (ATG) or signal peptide

SEQ ID NO:19 CCL20 without Met encoded by a start codon (ATG) or signal peptide

SEQ ID NO:20 shows a nucleotide sequence encoding an CCL20 including start codon (ATG)/sequences encoding IgE signal peptide

SEQ ID NO:21 CCL20 with Met encoded by start codon/IgE signal peptide

The attached Sequence Listing includes SEQ ID NO:22-31 which are amino acid sequences of derived from RSV immunogens.

SEQ ID NO:22 is a T cell epitope of RSV F Immunogen.

SEQ ID NO:23 is a T cell epitope of RSV F Immunogen.

SEQ ID NO:24 is a T cell epitope of RSV F Immunogen.

SEQ ID NO:25 is a T cell epitope of RSV F Immunogen.

SEQ ID NO:26 is a RSV F Immunogen protease cleavage site.

SEQ ID NO:27 is a RSV F Immunogen protease cleavage site.

SEQ ID NO:28 is a RSV F Immunogen fusion peptide.

SEQ ID NO:29 is a RSV F Immunogen binding site for palivizumab.

SEQ ID NO:30 is a RSV M2-1 Immunodominant T cell epitope.

SEQ ID NO:31is a RSV M2-1 Subdominant T cell epitope.

In some aspects of the invention, RSV F immunogen and sequences that encode the RSV F immunogen are provided. In some aspects of the invention, RSV Ga immunogen and sequences that encode the RSV Ga immunogen are provided. In some aspects of the invention, RSV Gb immunogen and sequences that encode the RSV Gb immunogen are provided. Compositions comprising combinations sequences that encode the RSV F immunogen, sequences that encode the RSV Ga immunogen and sequences that encode the RSV Gb immunogen are provided, including each of sequences that encode the RSV F immunogen, sequences that encode the RSV Ga immunogen and sequences that encode the RSV Gb immunogen.

RSV M2-1 immunogen and sequences that encode the RSV M2-1 immunogen are also provided and my be used alone or in combination with other proteins and/or nucleic acid sequences.

1. Definitions.

The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting. As used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise.

For recitation of numeric ranges herein, each intervening number there between with the same degree of precision is explicitly contemplated. For example, for the range of 6-9, the numbers 7 and 8 are contemplated in addition to 6 and 9, and for the range 6.0-7.0, the numbers 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6,9, and 7.0 are explicitly contemplated.

a. Adjuvant

"Adjuvant" as used herein may mean any molecule added to the DNA plasmid vaccines described herein to enhance antigenicity of the one or more Respiratory Syncytial Virus (RSV.) antigens encoded by the DNA plasmids and encoding nucleic acid sequences described hereinafter.

b. Antibody

"Antibody" may mean an antibody of classes IgG, IgM, IgA, IgD or IgE, or fragments, fragments or derivatives thereof, including Fab, F(ab')2, Fd, and single chain antibodies, diabodies, bispecific antibodies, bifunctional antibodies and derivatives thereof. The antibody may be an antibody isolated from the serum sample of mammal, a polyclonal antibody, affinity purified antibody, or mixtures thereof which exhibits sufficient binding specificity to a desired epitope or a sequence derived therefrom.

c. Coding Sequence

"Coding sequence" or "encoding nucleic acid" as used herein may mean refers to the nucleic acid (RNA or DNA molecule) that comprise a nucleotide sequence which encodes a protein. The coding sequence may further include initiation and termination signals operably linked to regulatory elements including a promoter and polyadenylation signal capable of directing expression in the cells of an individual or mammal to whom the nucleic acid is administered.

In some embodiments, the coding sequence for the amino acid sequence of consensus RSV F immunogen, which is set forth in SEQ ID NO:2, is SEQ ID NO:1. Such coding sequence may optionally further comprise a start codon that encodes an N terminal methionine or a signal peptide such as an IgE or IgG signal peptide. SEQ ID NO:10 corresponds to SEQ ID NO:2 with an IgE signal peptide (SEQ ID NO:17). SEQ ID NO:9 encodes SEQ ID NO:10.

In some embodiments, the coding sequence for the amino acid sequence of consensus RSV Ga immunogen, which is set forth in SEQ ID NO:6, is SEQ ID NO:5. Such coding sequence may optionally further comprise a start codon that encodes an N terminal methionine or a signal peptide such as an IgE or IgG signal peptide. SEQ ID NO:14 corresponds to SEQ ID NO:6 with an IgE signal peptide (SEQ ID NO:17). SEQ ID NO:13 encodes SEQ ID NO:14.

In some embodiments, the coding sequence for the amino acid sequence of consensus RSV Gb immunogen, which is set forth in SEQ ID NO:8, is SEQ ID NO:7. Such coding sequence may optionally further comprise a start codon that encodes an N terminal methionine or a signal peptide such as an IgE or IgG signal peptide. SEQ ID NO:16 corresponds to SEQ ID NO:8 with an IgE signal peptide (SEQ ID NO:17). SEQ ID NO:15 encodes SEQ ID NO:16.

Also provides is a coding sequence, SEQ ID NO:3, which encodes the amino acid sequence of consensus RSV M2-1 immunogen, which is set forth in SEQ ID NO:4. Such coding sequence may optionally further comprise a start codon that encodes an N terminal methionine or a signal peptide such as an IgE or IgG signal peptide. SEQ ID NO:12 corresponds to SEQ ID NO:4 with an IgE signal peptide (SEQ ID NO:17). SEQ ID NO:11 encodes SEQ ID NO:12.

d. Complement

"Complement" or "complementary" as used herein may mean a nucleic acid may mean Watson-Crick (*e.g.*, A-T/U and C-G) or Hoogsteen base pairing between nucleotides or nucleotide analogs of nucleic acid molecules.

e. Consensus or Consensus Sequence

"Consensus" or "consensus sequence" as used herein may mean a synthetic nucleic acid sequence, or corresponding polypeptide sequence, constructed based on analysis of an alignment of multiple subtypes of a particular RSV antigen, that can be used to induce broad immunity against multiple subtypes or serotypes of a particular RSV antigen. Consensus RSV antigens

may include consensus amino acid sequences of protein F, protein G and protein M2. Nucleotide sequences that encode the consensus amino acid sequences are also provided. Also, synthetic antigens such as fusion proteins may be manipulated to include consensus sequences (or consensus antigens).

The amino acid sequence of consensus RSV F immunogen is set forth in SEQ ID NO:2. Such sequence may optionally comprise an N terminal methionine or a signal peptide such as an IgE or IgG signal peptide. SEQ ID NO:10 corresponds to SEQ ID NO:2 with an IgE signal peptide (SEQ ID NO:17).

The amino acid sequence of consensus RSV Ga immunogen is set forth in SEQ ID NO:6. Such sequence may optionally comprise an N terminal methionine or a signal peptide such as an IgE or IgG signal peptide. SEQ ID NO:14 corresponds to SEQ ID NO:6 with an IgE signal peptide (SEQ ID NO:17).

The amino acid sequence of consensus RSV Gb immunogen is set forth in SEQ ID NO:8. Such sequence may optionally comprise an N terminal methionine or a signal peptide such as an IgE or IgG signal peptide. SEQ ID NO:16 corresponds to SEQ ID NO:8 with an IgE signal peptide (SEQ ID NO:17).

Also provided is the amino acid sequence of consensus RSV M2-1 immunogen which is set forth in SEQ ID NO:4. Such sequence may optionally comprise an N terminal methionine or a signal peptide such as an IgE or IgG signal peptide. SEQ ID NO:12 corresponds to SEQ ID NO:4 with an IgE signal peptide (SEQ ID NO:17).

f. Constant Current

"Constant current" as used herein to define a current that is received or experienced by a tissue, or cells defining said tissue, over the duration of an electrical pulse delivered to same tissue. The electrical pulse is delivered from the electroporation devices described herein. This current remains at a constant amperage in said tissue over the life of an electrical pulse because the electroporation device provided herein has a feedback element, preferably having instantaneous feedback. The feedback element can measure the resistance of the tissue (or cells) throughout the duration of the pulse and cause the electroporation device to alter its electrical energy output (e.g., increase voltage) so current in same tissue remains constant throughout the

electrical pulse (on the order of microseconds), and from pulse to pulse. In some embodiments, the feedback element comprises a controller.

g. Current Feedback or Feedback

"Current feedback" or "feedback" as used herein may be used interchangeably and may mean the active response of the provided electroporation devices, which comprises measuring the current in tissue between electrodes and altering the energy output delivered by the EP device accordingly in order to maintain the current at a constant level. This constant level is preset by a user prior to initiation of a pulse sequence or electrical treatment. The feedback may be accomplished by the electroporation component, e.g., controller, of the electroporation device, as the electrical circuit therein is able to continuously monitor the current in tissue between electrodes and compare that monitored current (or current within tissue) to a preset current and continuously make energy-output adjustments to maintain the monitored current at preset levels. The feedback loop may be instantaneous as it is an analog closed-loop feedback.

h. Decentralized Current

"Decentralized current" as used herein may mean the pattern of electrical currents delivered from the various needle electrode arrays of the electroporation devices described herein, wherein the patterns minimize, or preferably eliminate, the occurrence of electroporation related heat stress on any area of tissue being electroporated.

i. Electroporation

"Electroporation," "electro-permeabilization," or "electro-kinetic enhancement" ("EP") as used interchangeably herein may refer to the use of a transmembrane electric field pulse to induce microscopic pathways (pores) in a bio-membrane; their presence allows biomolecules such as plasmids, oligonucleotides, siRNA, drugs, ions, and water to pass from one side of the cellular membrane to the other.

j. Feedback Mechanism

"Feedback mechanism" as used herein may refer to a process performed by either software or hardware (or firmware), which process receives and compares the impedance of the desired tissue (before, during, and/or after the delivery of pulse of energy) with a present value,

preferably current, and adjusts the pulse of energy delivered to achieve the preset value. A feedback mechanism may be performed by an analog closed loop circuit.

k. Fragment

"Fragment" may mean a polypeptide fragment of an RSV Immunogen that is capable of eliciting an immune response in a mammal against RSV by recognizing the particular RSV antigen. An RSV Immunogen is refers to the consensus sequences set forth herein for RSV F Immunogen, RSV M2-1 Immunogen, RSV Ga Immunogen, RSV Gb Immunogen, in each case with or without signal peptides and/or a methionine at position 1, proteins 98% or more homologous to the consensus sequences set forth herein, proteins 99% or more homologous to the consensus sequences set forth herein, and proteins 100% identical to the consensus sequences set forth herein, in each case with or without signal peptides and/or a methionine at position 1. A fragment may or may not for example comprise a fragments of an RSV Immunogen linked to a signal peptide such as an immunoglobulin signal peptide for example IgE signal peptide (SEQ ID NO:17) or IgG signal peptide.

A fragment of RSV F consensus Immunogen may be a fragment of SEQ ID NO:2, of SEQ ID NO:10, of a protein 98% or more homologous to the RSV F consensus Immunogen sequences set forth herein, of a protein 99% or more homologous to the RSV F consensus Immunogen sequences set forth herein, or of RSV F consensus Immunogen 100% identical to the consensus sequences set forth herein, in each case with or without signal peptides and/or a methionine at position 1. Such fragments may comprise 20% or more, 25% or more, 30% or more, 35% or more, 40% or more, 45% or more, 50% or more, 55% or more, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 91% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, 99% or more percent of the length of the particular full length RSV F consensus Immunogen sequence set forth herein, excluding any non-RSV signal peptide added. Fragments refer to fragments of a polypeptide that is 98% or more, or 99% or more homologous to the sequences of the RSV F consensus Immunogen set forth herein and 100% identical to such sequence, in each case with or without signal peptides and/or a methionine at position 1. The fragment may comprise a fragment of a polypeptide that is 98% or more homologous, 99% or

more homologous, or 100% identical to the RSV F consensus Immunogen and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. In some embodiments, a fragment of RSV F Immunogen may comprise a fragment of a SEQ ID NO:2. The fragment may comprise a fragments of SEQ ID NO:2 linked to a signal peptide such as an immunoglobulin signal peptide for example IgE signal peptide (SEQ ID NO:17) or IgG signal peptide. Thus in some embodiments, the fragment is a fragment of SEQ ID NO:10 which comprises SEQ ID NO:17. The fragment may comprise fragments of SEQ ID NO:2 linked to an N terminal methionine. Fragments also refer to fragments of a polypeptide that is 98% or more, or 99% or more homologous to SEQ ID NO:2 . The fragment may comprise a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:2 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may comprise a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:2 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may comprise a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:2 and additionally comprise an N terminal methionine which is not included when calculating percent homology. The fragment may comprise a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:2 and additionally comprise an N terminal methionine which is not included when calculating percent homology. The RSV F Immunogen is 575 amino acids. In some embodiments, the fragments thereof may be 115 or more amino acids in length, 163 or more, 230 or more, 288 or more, 345 or more, 403 or more, 460 or more, 515 or more, 520 or more, 525 or more, 530 or more, 535 or more, 540 or more, 545 or more, 550 or more, 555 or more, 560 or more, 565 or more, 570 or more, 574 or more in length. Polypeptide fragments may be fewer than 140 amino acids, fewer than 190, fewer than 250, fewer than 283, fewer than 300, fewer than 365, fewer than 425, fewer than 495, fewer than 518, fewer than 523, fewer than 528, fewer than 533, fewer than 547, fewer than 552, fewer than 563, or fewer than 572 in length. Fragments may further comprise an N terminal methionine and/or a signal peptide such as an immunoglobulin signal peptide, for example an IgE or IgG signal peptide. The N terminal methionine and/or signal peptide may be linked to a

574 amino acid or smaller fragment thereof. The N terminal methionine and/or signal peptide may be linked to a polypeptide that is 98% homologous to the 574 amino acid or to a smaller fragment of a polypeptide that is 98% homologous to the 574 amino acid sequence. The N terminal methionine and/or signal peptide may be linked to a polypeptide that is 99% homologous to the 574 amino acid sequence or to a smaller fragment of a polypeptide that is 99% homologous to the 574 amino acid sequence. In calculating degrees of homology a polypeptide has to SEQ ID NO:2 or a fragment thereof, an N terminal methionine and/or any signal peptide is not included in such calculation. The sequences of the signal peptide are not used in determining homology. Thus, for example, although SEQ ID NO:10 comprises SEQ ID NO:2 operably linked to a signal peptide SEQ ID NO:17, SEQ ID NO:10 comprises a fragment of SEQ ID NO:2, that is 100% homologous to a fragment of SEQ ID NO:2, notwithstanding the signal peptide which is absent in SEQ ID NO:2. Thus, proteins which comprise fragments of a polypeptide that is at least 98% homologous to a fragment of SEQ ID NO:2 intended to refer to proteins which fragments of a polypeptide that is at least 98% homologous to a fragment of SEQ ID NO:2 that are at least 115 amino acids and may optionally be linked to a, for example, a signal peptide. SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, and SEQ ID NO:25 correspond to T cell epitopes of RSV F immunogen set forth in SEQ ID NO:2. In some embodiments, fragments comprise all or part of one or more of these sequences or sequences 98% or more or 99% or more homologous to them. SEQ ID NO:26 and SEQ ID NO:27 are RSV F Immunogen protease cleavage sites. SEQ ID NO:28 is an RSV F Immunogen fusion peptide. SEQ ID NO:29 is the RSV F Immunogen binding site for palivizumab.

A fragment of RSV Ga consensus Immunogen may be a fragment of SEQ ID NO:6, of SEQ ID NO:14, of a protein 98% or more homologous to the RSV Ga consensus Immunogen sequences set forth herein, of a protein 99% or more homologous to the RSV Ga consensus Immunogen sequences set forth herein, or of RSV Ga consensus Immunogen 100% identical to the consensus sequences set forth herein, in each case with or without signal peptides and/or a methionine at position 1. Such fragments may comprise may comprise 20% or more, 25% or more, 30% or more, 35% or more, 40% or more, 45% or more, 50% or more, 55% or more, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more,

91% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, 99% or more percent of the length of the particular full length RSV Ga consensus Immunogen sequence set forth herein, excluding any non-RSV signal peptide added. Fragments refer to fragments of a polypeptide that is 98% or more, or 99% or more homologous to the sequences of the RSV Ga consensus Immunogen set forth herein and 100% identical to such sequence, in each case with or without signal peptides and/or a methionine at position 1. The fragment may comprise a fragment of a polypeptide that is 98% or more homologous, 99% or more homologous, or 100% identical to the RSV Ga consensus Immunogen and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. In some embodiments, a fragment of RSV Ga Immunogen may comprise a fragment of SEQ ID NO:6. The fragment may comprise a fragments of SEQ ID NO:6 linked to a signal peptide such as an immunoglobulin signal peptide for example IgE signal peptide (SEQ ID NO:17) or IgG signal peptide. Thus in some embodiments, the fragment is a fragment of SEQ ID NO:14 which comprises SEQ ID NO:17. The fragment may comprise fragments of SEQ ID NO:6 linked to an N terminal methionine. Fragments also refer to fragments of a polypeptide that is 98% or more, or 99% or more homologous to SEQ ID NO:6. The fragment may comprise a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:6 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may comprise a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:6 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may comprise a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:6 and additionally comprise an N terminal methionine which is not included when calculating percent homology. The fragment may comprise a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:6 and additionally comprise an N terminal methionine which is not included when calculating percent homology. The RSV Ga Immunogen is 297 amino acids excluding an N terminal methionine and/or signal peptide. In some embodiments, the fragments thereof may be 125 or more amino acids in length, 130 or more, 140 or more, 150 or more, 160 or more, 170 or more, 180 or more, 190 or more,

200 or more, 210 or more, 220 or more, 230 or more, 240 or more, 250 or more, 260 or more, 270 or more, 280 or more, 285 or more, 290 or more, 295 or more in length. Polypeptide fragments may be fewer than 135 amino acids, fewer than 145, fewer than 155, fewer than 165, fewer than 175, fewer than 185, fewer than 195, fewer than 205, fewer than 215, fewer than 225, fewer than 235, fewer than 245, fewer than 255, fewer than 265, fewer than 275, fewer than 285, fewer than 288, fewer than 292, or fewer than 296 in length. Fragments may further comprise an N terminal methionine and/or a signal peptide such as an immunoglobulin signal peptide, for example an IgE or IgG signal peptide. The N terminal methionine and/or signal peptide may be linked to a 296 amino acid or smaller fragment thereof. The N terminal methionine and/or signal peptide may be linked to a polypeptide that is 98% homologous to the 296 amino acid or to a smaller fragment of a polypeptide that is 98% homologous to the 296 amino acid sequence. The N terminal methionine and/or signal peptide may be linked to a polypeptide that is 99% homologous to the 296 amino acid sequence or to a smaller fragment of a polypeptide that is 99% homologous to the 296 amino acid sequence. In calculating degrees of homology a polypeptide has to SEQ ID NO:6 or a fragment thereof, an N terminal methionine and/or any signal peptide is not included in such calculation. The sequences of the signal peptide are not used in determining homology. Thus, for example, although SEQ ID NO:14 comprises SEQ ID NO:6 operably linked to a signal peptide SEQ ID NO:17, SEQ ID NO:14 comprises a fragment of SEQ ID NO:6, that is 100% homologous to a fragment of SEQ ID NO:6, notwithstanding the signal peptide which is absent in SEQ ID NO:6. Thus, proteins which comprise fragments of a polypeptide that is at least 98% homologous to a fragment of SEQ ID NO:6 intended to refer to proteins which fragments of a polypeptide that is at least 98% homologous to a fragment of SEQ ID NO:6 that are at least 125 amino acids and may optionally be linked to a, for example, a signal peptide.

A fragment of RSV Gb consensus Immunogen such as that which is disclosed in SEQ ID NO:8, SEQ ID NO:16, proteins 98% or more homologous to the RSV Gb consensus Immunogen sequences set forth herein, proteins 99% or more homologous to the RSV Gb consensus Immunogen sequences set forth herein, and RSV Gb consensus Immunogen 100% identical to the consensus sequences set forth herein, in each case with or without signal peptides and/or a

methionine at position 1, may comprise 20% or more, 25% or more, 30% or more, 35% or more, 40% or more, 45% or more, 50% or more, 55% or more, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 91% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, 99% or more percent of the length of the particular full length RSV Gb consensus Immunogen sequence set forth herein, excluding any non-RSV signal peptide added. Fragments refer to fragments of a polypeptide that is 98% or more, or 99% or more homologous to the sequences of the RSV Gb consensus Immunogen set forth herein and 100% identical to such sequence, in each case with or without signal peptides and/or a methionine at position 1. The fragment may comprise a fragment of a polypeptide that is 98% or more homologous, 99% or more homologous, or 100% identical to the RSV Gb consensus Immunogen and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. In some embodiments, a fragment of RSV Gb Immunogen may comprise a fragment of SEQ ID NO:8. The fragment may comprise a fragments of SEQ ID NO:8 linked to a signal peptide such as an immunoglobulin signal peptide for example IgE signal peptide (SEQ ID NO:17) or IgG signal peptide. Thus in some embodiments, the fragment is a fragment of SEQ ID NO:16 which comprises SEQ ID NO:17. The fragment may comprise fragments of SEQ ID NO:8 linked to an N terminal methionine. Fragments also refer to fragments of a polypeptide that is 98% or more, or 99% or more homologous to SEQ ID NO:8. The fragment may comprise a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:8 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may comprise a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:8 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may comprise a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:8 and additionally comprise an N terminal methionine which is not included when calculating percent homology. The fragment may comprise a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:8 and additionally comprise an N terminal methionine which is not included when calculating percent homology. The RSV Gb Immunogen is 291

amino acids excluding an N terminal methionine and/or signal peptide. In some embodiments, the fragments thereof may be 125 or more amino acids in length, 130 or more, 140 or more, 150 or more, 160 or more, 170 or more, 180 or more, 190 or more, 200 or more, 210 or more, 220 or more, 230 or more, 240 or more, 250 or more, 260 or more, 270 or more, 280 or more, 285 or more, 288 or more in length. Polypeptide fragments may be fewer than 135 amino acids, fewer than 145, fewer than 155, fewer than 165, fewer than 175, fewer than 185, fewer than 195, fewer than 205, fewer than 215, fewer than 225, fewer than 235, fewer than 245, fewer than 255, fewer than 265, fewer than 275, fewer than 285, fewer than 288, fewer than 290 in length. Fragments may further comprise an N terminal methionine and/or a signal peptide such as an immunoglobulin signal peptide, for example an IgE or IgG signal peptide. The N terminal methionine and/or signal peptide may be linked to a 290 amino acid or smaller fragment thereof. The N terminal methionine and/or signal peptide may be linked to a polypeptide that is 98% homologous to the 290 amino acid or to a smaller fragment of a polypeptide that is 98% homologous to the 290 amino acid sequence. The N terminal methionine and/or signal peptide may be linked to a polypeptide that is 99% homologous to the 290 amino acid sequence or to a smaller fragment of a polypeptide that is 99% homologous to the 290 amino acid sequence. In calculating degrees of homology a polypeptide has to SEQ ID NO:6 or a fragment thereof, an N terminal methionine and/or any signal peptide is not included in such calculation. The sequences of the signal peptide are not used in determining homology. Thus, for example, although SEQ ID NO:16 comprises SEQ ID NO:8 operably linked to a signal peptide SEQ ID NO:17, SEQ ID NO:16 comprises a fragment of SEQ ID NO:8, that is 100% homologous to a fragment of SEQ ID NO:8, notwithstanding the signal peptide which is absent in SEQ ID NO:8. Thus, proteins which comprise fragments of a polypeptide that is at least 98% homologous to a fragment of SEQ ID NO:8 intended to refer to proteins which fragments of a polypeptide that is at least 98% homologous to a fragment of SEQ ID NO:8 that are at least 125 amino acids and may optionally be linked to a, for example, a signal peptide.

In addition, a fragment of RSV M2-1 consensus Immunogen may be a fragment of SEQ ID NO:4, of SEQ ID NO:12, of a protein 98% or more homologous to the RSV M2-1 consensus Immunogen sequences set forth herein, of a protein 99% or more homologous to the RSV M2-1

consensus Immunogen sequences set forth herein, or of RSV M2-1 consensus Immunogen 100% identical to the consensus sequences set forth herein, in each case with or without signal peptides and/or a methionine at position 1. Such fragments may comprise may comprise 20% or more, 25% or more, 30% or more, 35% or more, 40% or more, 45% or more, 50% or more, 55% or more, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 91% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, 99% or more percent of the length of the particular full length RSV M2-1 consensus Immunogen sequence set forth herein, excluding any non-RSV signal peptide added. Fragments refer to fragments of a polypeptide that is 98% or more, or 99% or more homologous to the sequences of the RSV M2-1 consensus Immunogen set forth herein and 100% identical to such sequence, in each case with or without signal peptides and/or a methionine at position 1. The fragment may comprise a fragment of a polypeptide that is 98% or more homologous, 99% or more homologous, or 100% identical to the RSV M2-1 consensus Immunogen and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. In some embodiments, a fragment of RSV M2-1 Immunogen may comprise a fragment of a SEQ ID NO:4. The fragment may comprise a fragments of SEQ ID NO:4 linked to a signal peptide such as an immunoglobulin signal peptide for example IgE signal peptide (SEQ ID NO:17) or IgG signal peptide. Thus in some embodiments, the fragment is a fragment of SEQ ID NO:12 which comprises SEQ ID NO:17. The fragment may comprise fragments of SEQ ID NO:4 linked to an N terminal methionine. Fragments also refer to fragments of a polypeptide that is 98% or more, or 99% or more homologous to SEQ ID NO:4. The fragment may comprise a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:4 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may comprise a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:4 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may comprise a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:4 and additionally comprise an N terminal methionine which is not included when

calculating percent homology. The fragment may comprise a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:4 and additionally comprise an N terminal methionine which is not included when calculating percent homology. The RSV M2-1 Immunogen is 195 amino acids. In some embodiments, the fragments thereof may be 25 or more amino acids in length, 30 or more, 40 or more, 50 or more, 60 or more, 70 or more, 80 or more, 90 or more, 100 or more, 110 or more, 120 or more, 130 or more, 140 or more, 150 or more, 160 or more, 170 or more, 180 or more, 184 or more, 190 or more, 193 or more in length. Polypeptide fragments may be fewer than 35 amino acids, fewer than 45, fewer than 55, fewer than 65, fewer than 75, fewer than 85, fewer than 95, fewer than 105, fewer than 115, fewer than 125, fewer than 135, fewer than 145, fewer than 155, fewer than 165, fewer than 175, fewer than 185, fewer than 188, fewer than 192, or fewer than 194 in length. Fragments may further comprise an N terminal methionine and/or a signal peptide such as an immunoglobulin signal peptide, for example an IgE or IgG signal peptide. The N terminal methionine and/or signal peptide may be linked to a 194 amino acid or smaller fragment thereof. The N terminal methionine and/or signal peptide may be linked to a polypeptide that is 98% homologous to the 194 amino acid or to a smaller fragment of a polypeptide that is 98% homologous to the 194 amino acid sequence. The N terminal methionine and/or signal peptide may be linked to a polypeptide that is 99% homologous to the 194 amino acid sequence or to a smaller fragment of a polypeptide that is 99% homologous to the 194 amino acid sequence. In calculating degrees of homology a polypeptide has to SEQ ID NO:4 or a fragment thereof, an N terminal methionine and/or any signal peptide is not included in such calculation. The sequences of the signal peptide are not used in determining homology. Thus, for example, although SEQ ID NO:12 comprises SEQ ID NO:4 operably linked to a signal peptide SEQ ID NO:17, SEQ ID NO:12 comprises a fragment of SEQ ID NO:4, that is 100% homologous to a fragment of SEQ ID NO:4, notwithstanding the signal peptide which is absent in SEQ ID NO:4. Thus, proteins which comprise fragments of a polypeptide that is at least 98% homologous to a fragment of SEQ ID NO:4 intended to refer to proteins which fragments of a polypeptide that is at least 98% homologous to a fragment of SEQ ID NO:4 that are at least 25 amino acids and may optionally be linked to a, for example, a signal peptide. SEQ ID NO:30 corresponds to an immunodominant T cell epitope of RSV M2-1

immunogen set forth in SEQ ID NO:4. SEQ ID NO:31 refers to a subdominant T cell epitope corresponds to an immunodominant T cell epitope of RSV M2-1 immunogen set forth in SEQ ID NO:4. In some embodiments, fragments comprise all or part of one or more of these sequences or sequences 98% or more or 99% or more homologous to them.

"Fragment" may also mean a nucleic acid fragment of that encodes an RSV Immunogen fragment set forth above, i.e. RSV F Immunogen, RSV M2-1 Immunogen, RSV Ga Immunogen, RSV Gb Immunogen, in each case with or without signal peptides and/or a methionine at position 1, proteins 98% or more homologous to the consensus sequences set forth herein, proteins 99% or more homologous to the consensus sequences set forth herein, and proteins 100% identical to the consensus sequences set forth herein, in each case with or without signal peptides and/or a methionine at position 1. A fragment may or may not for example comprise a fragments of nucleic acid sequences that encode an RSV Immunogen linked to coding sequence for an N terminal methionine (i.e. a start codon) or coding sequences encoding a signal peptide such as an immunoglobulin signal peptide for example IgE signal peptide (SEQ ID NO:17) or IgG signal peptide. A fragment of a coding sequence that encodes an RSV Immunogen may comprise 20% or more, 25% or more, 30% or more, 35% or more, 40% or more, 45% or more, 50% or more, 55% or more, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 91% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, 99% or more percent of the length of coding sequence of the particular full length RSV Immunogen sequence set forth herein, excluding any coding sequence that encodes non-RSV signal peptide added. Fragments also refer to fragments of coding sequences which encode a polypeptide that is 98% or more, or 99% or more homologous to the sequences of the RSV Immunogen set forth herein. The fragment may comprise a fragment of a polypeptide that is 98% or more homologous to and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology.

In some embodiments, the fragments are fragments of a nucleic acid sequence 98% or more homologous to SEQ ID NO1. In some embodiments, the fragments are fragments of a nucleic acid sequence 99% or more homologous to SEQ ID NO:1. In some embodiments, the

fragments are fragments of SEQ ID NO:1. It is intended that nucleic acid sequences encoding the polypeptide fragments described herein are expressly disclosed, i.e. fragments of nucleic acid sequences that encode the proteins having the size and homology of the peptide fragments. In some embodiments, fragments of nucleic acid sequences that encode the proteins having the size and homology of the peptide fragments are fragments of SEQ ID NO:1. A fragment of a coding sequence that encodes RSV F consensus Immunogen such as that which is disclosed in SEQ ID NO:2, SEQ ID NO:10, proteins 98% or more homologous to the RSV F consensus Immunogen sequences set forth herein, proteins 99% or more homologous to the RSV F consensus Immunogen sequences set forth herein, and RSV F consensus Immunogen 100% identical to the consensus sequences set forth herein, in each case with or without signal peptides and/or a methionine at position 1, such as for example SEQ ID NO:1 or SEQ ID NO:9, may comprise 20% or more, 25% or more, 30% or more, 35% or more, 40% or more, 45% or more, 50% or more, 55% or more, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 91% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, 99% or more percent of the length of the particular full length RSV F consensus Immunogen sequence set forth herein, excluding any non-RSV signal peptide added. Fragments may encode fragments of a polypeptide that is 98% or more, or 99% or more homologous to the sequences of the RSV F consensus Immunogen set forth herein and 100% identical to such sequence, in each case with or without signal peptides and/or a methionine at position 1. The fragment may encode a fragment of a polypeptide that is 98% or more homologous, 99% or more homologous, or 100% identical to the RSV F consensus Immunogen and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. In some embodiments, a fragment that encodes RSV F Immunogen may encode a fragment of a SEQ ID NO:2 and comprise a fragment of SEQ ID NO:1. The fragment may encode fragments of SEQ ID NO:2 linked to a signal peptide such as an immunoglobulin signal peptide for example IgE signal peptide (SEQ ID NO:17) or IgG signal peptide. Thus in some embodiments, the fragment is a fragment of SEQ ID NO:10 which comprises SEQ ID NO:17 and may be encoded by SEQ ID NO:9. The fragment may comprise fragments of SEQ ID NO:1 linked to start codon. Fragments

also encode fragments of a polypeptide that is 98% or more, or 99% or more homologous to SEQ ID NO:2. The fragment may encode a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:2 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may encode a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:2 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may encode a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:2 and additionally comprise an N terminal methionine which is not included when calculating percent homology. The fragment may encode a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:2 and additionally comprise an N terminal methionine which is not included when calculating percent homology. Fragments may be 98% or more, or 99% or more homologous to SEQ ID NO:1. The fragment may be 98% or more homologous to SEQ ID NO:1 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may be 99% or more homologous to SEQ ID NO:1 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may be 98% or more homologous to SEQ ID NO:1 and additionally comprise an N terminal methionine which is not included when calculating percent homology. The fragment may be 99% or more homologous to SEQ ID NO:1 and additionally comprise an N terminal methionine which is not included when calculating percent homology. Fragments of SEQ ID NO:1, fragments of nucleic acid sequence 98% or more homologous to SEQ ID NO:1, and fragments of nucleic acid sequence 99% or more homologous to SEQ ID NO:1, in each case with or without start codons or coding sequences that encode signal peptides may encode fragments of RSV F immunogen that may be 115 or more amino acids in length, 163 or more, 230 or more, 288 or more, 345 or more, 403 or more, 460 or more, 515 or more, 520 or more, 525 or more, 530 or more, 535 or more, 540 or more, 545 or more, 550 or more, 555 or more, 560 or more, 565 or more, 570 or more, 574 or more in length. Polypeptide fragments may be fewer than 140 amino acids, fewer than 190, fewer than 250, fewer than 283, fewer than 300, fewer than 365, fewer than 425, fewer than 495,

fewer than 518, fewer than 523, fewer than 528, fewer than 533, fewer than 547, fewer than 552, fewer than 563, or fewer than 572 in length. Fragments may further encode an N terminal methionine and/or a signal peptide such as an immunoglobulin signal peptide, for example an IgE or IgG signal peptide. The N terminal methionine and/or signal peptide may be linked to sequences that encode a 574 amino acid or smaller fragment thereof. The fragment may encode an N terminal methionine and/or signal peptide linked to a polypeptide that is 98% homologous to the 574 amino acid or to a smaller fragment of a polypeptide that is 98% homologous to the 574 amino acid sequence. The fragment may encode an N terminal methionine and/or signal peptide linked to a polypeptide that is 99% homologous to the 574 amino acid sequence or to a smaller fragment of a polypeptide that is 99% homologous to the 574 amino acid sequence. In calculating degrees of homology a polypeptide has to SEQ ID NO:2 or a fragment thereof, an N terminal methionine and/or any signal peptide is not included in such calculation. The sequences of the signal peptide are not used in determining homology. Thus, for example, although SEQ ID NO:7 comprises SEQ ID NO:1 operably linked to a coding sequences that encode signal peptide SEQ ID NO:17, SEQ ID NO:7 comprises a fragment of SEQ ID NO:1 that is 100% homologous to a fragment of SEQ ID NO:1, notwithstanding the signal peptide coding sequence which is absent in SEQ ID NO:1. SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, and SEQ ID NO:25 correspond to T cell epitopes of RSV F immunogen set forth in SEQ ID NO:2. In some embodiments, fragments comprise coding sequences that encode all or part of one or more of these sequences or sequences 98% or more or 99% or more homologous to them including fragments of SEQ ID NO:1 or sequences that are 98% or more or 99% or more homologous to SEQ ID NO:1.

In some embodiments, the fragments are fragments of a nucleic acid sequence 98% or more homologous to SEQ ID NO:5. In some embodiments, the fragments are fragments of a nucleic acid sequence 99% or more homologous to SEQ ID NO:5. In some embodiments, the fragments are fragments of SEQ ID NO:5. It is intended that nucleic acid sequences encoding the polypeptide fragments described herein are expressly disclosed, i.e. fragments of nucleic acid sequences that encode the proteins having the size and homology of the peptide fragments. In some embodiments, fragments of nucleic acid sequences that encode the proteins having the size

and homology of the peptide fragments are fragments of SEQ ID NO:5. A fragment of a coding sequence that encodes RSV Ga consensus Immunogen such as that which is disclosed in SEQ ID NO:6, SEQ ID NO:14, proteins 98% or more homologous to the RSV Ga consensus Immunogen sequences set forth herein, proteins 99% or more homologous to the RSV Ga consensus Immunogen sequences set forth herein, and RSV Ga consensus Immunogen 100% identical to the consensus sequences set forth herein, in each case with or without signal peptides and/or a methionine at position 1, such as for example SEQ ID NO:5 or SEQ ID NO:13, may comprise 20% or more, 25% or more, 30% or more, 35% or more, 40% or more, 45% or more, 50% or more, 55% or more, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 91% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, 99% or more percent of the length of the particular full length RSV Ga consensus Immunogen sequence set forth herein, excluding any non-RSV signal peptide added. Fragments may encode fragments of a polypeptide that is 98% or more, or 99% or more homologous to the sequences of the RSV Ga consensus Immunogen set forth herein and 100% identical to such sequence, in each case with or without signal peptides and/or a methionine at position 1. The fragment may encode a fragment of a polypeptide that is 98% or more homologous, 99% or more homologous, or 100% identical to the RSV Ga consensus Immunogen and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. In some embodiments, a fragment that encodes RSV Ga Immunogen may encode a fragment of a SEQ ID NO:6 and comprise a fragment of SEQ ID NO:5. The fragment may encode fragments of SEQ ID NO:6 linked to a signal peptide such as an immunoglobulin signal peptide for example IgE signal peptide (SEQ ID NO:17) or IgG signal peptide. Thus in some embodiments, the fragment is a fragment of SEQ ID NO:14 which comprises SEQ ID NO:17 and may be encoded by SEQ ID NO:13. The fragment may comprise fragments of SEQ ID NO:5 linked to start codon. Fragments also encode fragments of a polypeptide that is 98% or more, or 99% or more homologous to SEQ ID NO:6. The fragment may encode a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:6 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The

fragment may encode a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:6 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may encode a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:6 and additionally comprise an N terminal methionine which is not included when calculating percent homology. The fragment may encode a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:6 and additionally comprise an N terminal methionine which is not included when calculating percent homology. Fragments may be 98% or more, or 99% or more homologous to SEQ ID NO:5. The fragment may be 98% or more homologous to SEQ ID NO:5 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may be 99% or more homologous to SEQ ID NO:5 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may be 98% or more homologous to SEQ ID NO:5 and additionally comprise an N terminal methionine which is not included when calculating percent homology. The fragment may be 99% or more homologous to SEQ ID NO:5 and additionally comprise an N terminal methionine which is not included when calculating percent homology. Fragments of SEQ ID NO:5, fragments of nucleic acid sequence 98% or more homologous to SEQ ID NO:5, and fragments of nucleic acid sequence 99% or more homologous to SEQ ID NO:5, in each case with or without start codons or coding sequences that encode signal peptides may encode fragments of RSV Ga immunogen that may be In some embodiments, the fragments thereof may be 125 or more amino acids in length, 130 or more, 140 or more, 150 or more, 160 or more, 170 or more, 180 or more, 190 or more, 200 or more, 210 or more, 220 or more, 230 or more, 240 or more, 250 or more, 260 or more, 270 or more, 280 or more, 285 or more, 290 or more, 295 or more in length. Polypeptide fragments may be fewer than 135 amino acids, fewer than 145, fewer than 155, fewer than 165, fewer than 175, fewer than 185, fewer than 195, fewer than 205, fewer than 215, fewer than 225, fewer than 235, fewer than 245, fewer than 255, fewer than 265, fewer than 275, fewer than 285, fewer than 288, fewer than 292, or fewer than 296 in length. Fragments may further encode an N terminal methionine and/or a signal peptide such as an immunoglobulin signal peptide, for example an

IgE or IgG signal peptide. The N terminal methionine and/or signal peptide may be linked to sequences that encode a 296 amino acid or smaller fragment thereof. The fragment may encode an N terminal methionine and/or signal peptide linked to a polypeptide that is 98% homologous to the 296 amino acid or to a smaller fragment of a polypeptide that is 98% homologous to the 296 amino acid sequence. The fragment may encode an N terminal methionine and/or signal peptide linked to a polypeptide that is 99% homologous to the 296 amino acid sequence or to a smaller fragment of a polypeptide that is 99% homologous to the 296 amino acid sequence. In calculating degrees of homology a polypeptide has to SEQ ID NO:6 or a fragment thereof, an N terminal methionine and/or any signal peptide is not included in such calculation. The sequences of the signal peptide are not used in determining homology. Thus, for example, although SEQ ID NO:13 comprises SEQ ID NO:5 operably linked to a coding sequences that encode signal peptide SEQ ID NO:17, SEQ ID NO:13 comprises a fragment of SEQ ID NO:5 that is 100% homologous to a fragment of SEQ ID NO:5, notwithstanding the signal peptide coding sequence which is absent in SEQ ID NO:5.

In some embodiments, the fragments are fragments of a nucleic acid sequence 98% or more homologous to SEQ ID NO:7. In some embodiments, the fragments are fragments of a nucleic acid sequence 99% or more homologous to SEQ ID NO:7. In some embodiments, the fragments are fragments of SEQ ID NO:7. It is intended that nucleic acid sequences encoding the polypeptide fragments described herein are expressly disclosed, i.e. fragments of nucleic acid sequences that encode the proteins having the size and homology of the peptide fragments. In some embodiments, fragments of nucleic acid sequences that encode the proteins having the size and homology of the peptide fragments are fragments of SEQ ID NO:7. A fragment of a coding sequence that encodes RSV Gb consensus Immunogen such as that which is disclosed in SEQ ID NO:8, SEQ ID NO:16, proteins 98% or more homologous to the RSV Gb consensus Immunogen sequences set forth herein, proteins 99% or more homologous to the RSV Gb consensus Immunogen sequences set forth herein, and RSV Gb consensus Immunogen 100% identical to the consensus sequences set forth herein, in each case with or without signal peptides and/or a methionine at position 1, such as for example SEQ ID NO:7 or SEQ ID NO:15, may comprise 20% or more, 25% or more, 30% or more, 35% or more, 40% or more, 45% or more, 50% or

more, 55% or more, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 91% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, 99% or more percent of the length of the particular full length RSV Gb consensus Immunogen sequence set forth herein, excluding any non-RSV signal peptide added. Fragments may encode fragments of a polypeptide that is 98% or more, or 99% or more homologous to the sequences of the RSV Gb consensus Immunogen set forth herein and 100% identical to such sequence, in each case with or without signal peptides and/or a methionine at position 1. The fragment may encode a fragment of a polypeptide that is 98% or more homologous, 99% or more homologous, or 100% identical to the RSV Gb consensus Immunogen and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. In some embodiments, a fragment that encodes RSV Gb Immunogen may encode a fragment of a SEQ ID NO:8 and comprise a fragment of SEQ ID NO:7. The fragment may encode fragments of SEQ ID NO:8 linked to a signal peptide such as an immunoglobulin signal peptide for example IgE signal peptide (SEQ ID NO:17) or IgG signal peptide. Thus in some embodiments, the fragment is a fragment of SEQ ID NO:16 which comprises SEQ ID NO:17 and may be encoded by SEQ ID NO:15. The fragment may comprise fragments of SEQ ID NO:7 linked to start codon. Fragments also encode fragments of a polypeptide that is 98% or more, or 99% or more homologous to SEQ ID NO:8. The fragment may encode a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:8 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may encode a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:8 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may encode a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:8 and additionally comprise an N terminal methionine which is not included when calculating percent homology. The fragment may encode a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:8 and additionally comprise an N terminal methionine which is not included when calculating percent homology. Fragments may be 98% or more, or 99% or more homologous to SEQ ID NO:7. The

fragment may be 98% or more homologous to SEQ ID NO:7 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may be 99% or more homologous to SEQ ID NO:7 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may be 98% or more homologous to SEQ ID NO:7 and additionally comprise an N terminal methionine which is not included when calculating percent homology. The fragment may be 99% or more homologous to SEQ ID NO:7 and additionally comprise an N terminal methionine which is not included when calculating percent homology. Fragments of SEQ ID NO:7, fragments of nucleic acid sequence 98% or more homologous to SEQ ID NO:7, and fragments of nucleic acid sequence 99% or more homologous to SEQ ID NO:7, in each case with or without start codons or coding sequences that encode signal peptides may encode fragments of RSV Gb immunogen that may be In some embodiments, the fragments thereof may be 125 or more amino acids in length, 130 or more, 140 or more, 150 or more, 160 or more, 170 or more, 180 or more, 190 or more, 200 or more, 210 or more, 220 or more, 230 or more, 240 or more, 250 or more, 260 or more, 270 or more, 280 or more, 285 or more, 288 or more in length. Polypeptide fragments may be fewer than 135 amino acids, fewer than 145, fewer than 155, fewer than 165, fewer than 175, fewer than 185, fewer than 195, fewer than 205, fewer than 215, fewer than 225, fewer than 235, fewer than 245, fewer than 255, fewer than 265, fewer than 275, fewer than 285, fewer than 288, fewer than 290 in length. Fragments may further encode an N terminal methionine and/or a signal peptide such as an immunoglobulin signal peptide, for example an IgE or IgG signal peptide. The N terminal methionine and/or signal peptide may be linked to sequences that encode a 290 amino acid or smaller fragment thereof. The fragment may encode an N terminal methionine and/or signal peptide linked to a polypeptide that is 98% homologous to the 290 amino acid or to a smaller fragment of a polypeptide that is 98% homologous to the 290 amino acid sequence. The fragment may encode an N terminal methionine and/or signal peptide linked to a polypeptide that is 99% homologous to the 290 amino acid sequence or to a smaller fragment of a polypeptide that is 99% homologous to the 290 amino acid sequence. In calculating degrees of homology a polypeptide has to SEQ ID NO:8 or a fragment thereof, an N terminal methionine

and/or any signal peptide is not included in such calculation. The sequences of the signal peptide are not used in determining homology. Thus, for example, although SEQ ID NO:15 comprises SEQ ID NO:7 operably linked to a coding sequences that encode signal peptide SEQ ID NO:17, SEQ ID NO:15 comprises a fragment of SEQ ID NO:7 that is 100% homologous to a fragment of SEQ ID NO:7, notwithstanding the signal peptide coding sequence which is absent in SEQ ID NO:7.

Also provides are sequences related to RSV M2-1 including fragments of a nucleic acid sequence 98% or more homologous to SEQ ID NO:3. In some embodiments, the fragments are fragments of a nucleic acid sequence 99% or more homologous to SEQ ID NO:3. In some embodiments, the fragments are fragments of SEQ ID NO:3. It is intended that nucleic acid sequences encoding the polypeptide fragments described herein are expressly disclosed, i.e. fragments of nucleic acid sequences that encode the proteins having the size and homology of the peptide fragments. In some embodiments, fragments of nucleic acid sequences that encode the proteins having the size and homology of the peptide fragments are fragments of SEQ ID NO:3. A fragment of a coding sequence that encodes RSV M2-1 consensus Immunogen such as that which is disclosed in SEQ ID NO:4, SEQ ID NO:12, proteins 98% or more homologous to the RSV M2-1 consensus Immunogen sequences set forth herein, proteins 99% or more homologous to the RSV M2-1 consensus Immunogen sequences set forth herein, and RSV M2-1 consensus Immunogen 100% identical to the consensus sequences set forth herein, in each case with or without signal peptides and/or a methionine at position 1, such as for example SEQ ID NO:3 or SEQ ID NO:11, may comprise 20% or more, 25% or more, 30% or more, 35% or more, 40% or more, 45% or more, 50% or more, 55% or more, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 91% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, 99% or more percent of the length of the particular full length RSV M2-1 consensus Immunogen sequence set forth herein, excluding any non-RSV signal peptide added. Fragments may encode fragments of a polypeptide that is 98% or more, or 99% or more homologous to the sequences of the RSV M2-1 consensus Immunogen set forth herein and 100% identical to such sequence, in each case with or without signal peptides and/or a methionine at position 1. The fragment may encode a

fragment of a polypeptide that is 98% or more homologous, 99% or more homologous, or 100% identical to the RSV M2-1 consensus Immunogen and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. In some embodiments, a fragment that encodes RSV M2-1 Immunogen may encode a fragment of a SEQ ID NO:4 and comprise a fragment of SEQ ID NO:3. The fragment may encode fragments of SEQ ID NO:4 linked to a signal peptide such as an immunoglobulin signal peptide for example IgE signal peptide (SEQ ID NO:17) or IgG signal peptide. Thus in some embodiments, the fragment is a fragment of SEQ ID NO:12 which comprises SEQ ID NO:17 and may be encoded by SEQ ID NO:11. The fragment may comprise fragments of SEQ ID NO:3 linked to start codon. Fragments also encode fragments of a polypeptide that is 98% or more, or 99% or more homologous to SEQ ID NO:4. The fragment may encode a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:4 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may encode a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:4 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may encode a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:4 and additionally comprise an N terminal methionine which is not included when calculating percent homology. The fragment may encode a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:4 and additionally comprise an N terminal methionine which is not included when calculating percent homology. Fragments may be 98% or more, or 99% or more homologous to SEQ ID NO:3. The fragment may be 98% or more homologous to SEQ ID NO:3 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may be 99% or more homologous to SEQ ID NO:3 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may be 98% or more homologous to SEQ ID NO:3 and additionally comprise an N terminal methionine which is not included when calculating percent homology. The fragment may be 99% or more homologous to SEQ ID NO:3 and additionally comprise an N terminal

methionine which is not included when calculating percent homology. Fragments of SEQ ID NO:3, fragments of nucleic acid sequence 98% or more homologous to SEQ ID NO:3, and fragments of nucleic acid sequence 99% or more homologous to SEQ ID NO:3, in each case with or without start codons or coding sequences that encode signal peptides may encode fragments of RSV M2-1 immunogen that may be In some embodiments, the fragments thereof may be 25 or more amino acids in length, 30 or more, 40 or more, 50 or more, 60 or more, 70 or more, 80 or more, 90 or more, 100 or more, 110 or more, 120 or more, 130 or more, 140 or more, 150 or more, 160 or more, 170 or more, 180 or more, 184 or more, 190 or more, 193 or more in length. Polypeptide fragments may be fewer than 35 amino acids, fewer than 45, fewer than 55, fewer than 65, fewer than 75, fewer than 85, fewer than 95, fewer than 105, fewer than 115, fewer than 125, fewer than 135, fewer than 145, fewer than 155, fewer than 165, fewer than 175, fewer than 185, fewer than 188, fewer than 192, or fewer than 194 in length. Fragments may further encode an N terminal methionine and/or a signal peptide such as an immunoglobulin signal peptide, for example an IgE or IgG signal peptide. The N terminal methionine and/or signal peptide may be linked to sequences that encode a 194 amino acid or smaller fragment thereof. The fragment may encode an N terminal methionine and/or signal peptide linked to a polypeptide that is 98% homologous to the 194 amino acid or to a smaller fragment of a polypeptide that is 98% homologous to the 194 amino acid sequence. The fragment may encode an N terminal methionine and/or signal peptide linked to a polypeptide that is 99% homologous to the 194 amino acid sequence or to a smaller fragment of a polypeptide that is 99% homologous to the 194 amino acid sequence. In calculating degrees of homology a polypeptide has to SEQ ID NO:4 or a fragment thereof, an N terminal methionine and/or any signal peptide is not included in such calculation. The sequences of the signal peptide are not used in determining homology. Thus, for example, although SEQ ID NO:9 comprises SEQ ID NO:3 operably linked to a coding sequences that encode signal peptide SEQ ID NO:17, SEQ ID NO:9 comprises a fragment of SEQ ID NO:3 that is 100% homologous to a fragment of SEQ ID NO:3, notwithstanding the signal peptide coding sequence which is absent in SEQ ID NO:3. SEQ ID NO:30 corresponds to an immunodominant T cell epitope of RSV M2-1 immunogen set forth in SEQ ID NO:4. SEQ ID NO:31 refers to a subdominant T cell epitope corresponds to an

immunodominant T cell epitope of RSV M2-1 immunogen set forth in SEQ ID NO:4. In some embodiments, fragments comprise coding sequences that encode all or part of one or more of these sequences or sequences 98% or more or 99% or more homologous to them including fragments of SEQ ID NO:3 or sequences that are 98% or more or 99% or more homologous to SEQ ID NO:3.

l. Identical

"Identical" or "identity" as used herein in the context of two or more nucleic acids or polypeptide sequences, may mean that the sequences have a specified percentage of residues that are the same over a specified region. The percentage may be calculated by optimally aligning the two sequences, comparing the two sequences over the specified region, determining the number of positions at which the identical residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the specified region, and multiplying the result by 100 to yield the percentage of sequence identity. In cases where the two sequences are of different lengths or the alignment produces one or more staggered ends and the specified region of comparison includes only a single sequence, the residues of single sequence are included in the denominator but not the numerator of the calculation. When comparing DNA and RNA, thymine (T) and uracil (U) may be considered equivalent. Identity may be performed manually or by using a computer sequence algorithm such as BLAST or BLAST 2.0.

m. Impedance

"Impedance" as used herein may be used when discussing the feedback mechanism and can be converted to a current value according to Ohm's law, thus enabling comparisons with the preset current.

n. Immune Response

"Immune response" as used herein may mean the activation of a host's immune system, e.g., that of a mammal, in response to the introduction of one or more RSV consensus antigen via the provided DNA plasmid vaccines. The immune response can be in the form of a cellular or humoral response, or both.

o. Nucleic Acid

"Nucleic acid" or "oligonucleotide" or "polynucleotide" as used herein may mean at least two nucleotides covalently linked together. The depiction of a single strand also defines the sequence of the complementary strand. Thus, a nucleic acid also encompasses the complementary strand of a depicted single strand. Many variants of a nucleic acid may be used for the same purpose as a given nucleic acid. Thus, a nucleic acid also encompasses substantially identical nucleic acids and complements thereof. A single strand provides a probe that may hybridize to a target sequence under stringent hybridization conditions. Thus, a nucleic acid also encompasses a probe that hybridizes under stringent hybridization conditions.

Nucleic acids may be single stranded or double stranded, or may contain portions of both double stranded and single stranded sequence. The nucleic acid may be DNA, both genomic and cDNA, RNA, or a hybrid, where the nucleic acid may contain combinations of deoxyribo- and ribo-nucleotides, and combinations of bases including uracil, adenine, thymine, cytosine, guanine, inosine, xanthine hypoxanthine, isocytosine and isoguanine. Nucleic acids may be obtained by chemical synthesis methods or by recombinant methods.

p. Operably Linked

"Operably linked" as used herein may mean that expression of a gene is under the control of a promoter with which it is spatially connected. A promoter may be positioned 5' (upstream) or 3' (downstream) of a gene under its control. The distance between the promoter and a gene may be approximately the same as the distance between that promoter and the gene it controls in the gene from which the promoter is derived. As is known in the art, variation in this distance may be accommodated without loss of promoter function.

q. Promoter

"Promoter" as used herein may mean a synthetic or naturally-derived molecule which is capable of conferring, activating or enhancing expression of a nucleic acid in a cell. A promoter may comprise one or more specific transcriptional regulatory sequences to further enhance expression and/or to alter the spatial expression and/or temporal expression of same. A promoter may also comprise distal enhancer or repressor elements, which can be located as much as several thousand base pairs from the start site of transcription. A promoter may be derived from

sources including viral, bacterial, fungal, plants, insects, and animals. A promoter may regulate the expression of a gene component constitutively, or differentially with respect to cell, the tissue or organ in which expression occurs or, with respect to the developmental stage at which expression occurs, or in response to external stimuli such as physiological stresses, pathogens, metal ions, or inducing agents. Representative examples of promoters include the bacteriophage T7 promoter, bacteriophage T3 promoter, SP6 promoter, lac operator-promoter, tac promoter, SV40 late promoter, SV40 early promoter, RSV-LTR promoter, CMV IE promoter, SV40 early promoter or SV40 late promoter and the CMV IE promoter.

r. Stringent Hybridization Conditions

"Stringent hybridization conditions" as used herein may mean conditions under which a first nucleic acid sequence (e.g., probe) will hybridize to a second nucleic acid sequence (e.g., target), such as in a complex mixture of nucleic acids. Stringent conditions are sequence-dependent and will be different in different circumstances. Stringent conditions may be selected to be about 5-10°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength pH. The T_m may be the temperature (under defined ionic strength, pH, and nucleic concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at T_m, 50% of the probes are occupied at equilibrium). Stringent conditions may be those in which the salt concentration is less than about 1.0 M sodium ion, such as about 0.01-1.0 M sodium ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (e.g., about 10-50 nucleotides) and at least about 60°C for long probes (e.g., greater than about 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. For selective or specific hybridization, a positive signal may be at least 2 to 10 times background hybridization. Exemplary stringent hybridization conditions include the following: 50% formamide, 5x SSC, and 1% SDS, incubating at 42°C, or, 5x SSC, 1% SDS, incubating at 65°C, with wash in 0.2x SSC, and 0.1% SDS at 65°C.

s. Substantially Complementary

"Substantially complementary" as used herein may mean that a first sequence is at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98% or 99% identical to the complement of

a second sequence over a region of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more nucleotides or amino acids, or that the two sequences hybridize under stringent hybridization conditions.

t. Substantially Identical

"Substantially identical" as used herein may mean that a first and second sequence are at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98% or 99% identical over a region of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more nucleotides or amino acids, or with respect to nucleic acids, if the first sequence is substantially complementary to the complement of the second sequence.

u. Variant

"Variant" used herein with respect to a nucleic acid may mean (i) a portion or fragment of a referenced nucleotide sequence; (ii) the complement of a referenced nucleotide sequence or portion thereof; (iii) a nucleic acid that is substantially identical to a referenced nucleic acid or the complement thereof; or (iv) a nucleic acid that hybridizes under stringent conditions to the referenced nucleic acid, complement thereof, or a sequences substantially identical thereto.

"Variant" with respect to a peptide or polypeptide that differs in amino acid sequence by the insertion, deletion, or conservative substitution of amino acids, but retain at least one biological activity. Variant may also mean a protein with an amino acid sequence that is substantially identical to a referenced protein with an amino acid sequence that retains at least one biological activity. A conservative substitution of an amino acid, i.e., replacing an amino acid with a different amino acid of similar properties (e.g., hydrophilicity, degree and distribution of charged regions) is recognized in the art as typically involving a minor change. These minor changes can be identified, in part, by considering the hydropathic index of amino acids, as understood in the art. Kyte et al., J. Mol. Biol. 157:105-132 (1982). The hydropathic index of an amino acid is based on a consideration of its hydrophobicity and charge. It is known in the art that amino acids of similar hydropathic indexes can be substituted and still retain protein function. In one aspect, amino acids having hydropathic indexes of ±2 are substituted. The hydrophilicity of amino acids can also be used to reveal substitutions that would result in proteins retaining biological function. A consideration of the hydrophilicity of amino acids in

the context of a peptide permits calculation of the greatest local average hydrophilicity of that peptide, a useful measure that has been reported to correlate well with antigenicity and immunogenicity. U.S. Patent No. 4,554,101, incorporated fully herein by reference. Substitution of amino acids having similar hydrophilicity values can result in peptides retaining biological activity, for example immunogenicity, as is understood in the art. Substitutions may be performed with amino acids having hydrophilicity values within ±2 of each other. Both the hyrophobicity index and the hydrophilicity value of amino acids are influenced by the particular side chain of that amino acid. Consistent with that observation, amino acid substitutions that are compatible with biological function are understood to depend on the relative similarity of the amino acids, and particularly the side chains of those amino acids, as revealed by the hydrophobicity, hydrophilicity, charge, size, and other properties.

v. Vector

"Vector" used herein may mean a nucleic acid sequence containing an origin of replication. A vector may be a plasmid, bacteriophage, bacterial artificial chromosome or yeast artificial chromosome. A vector may be a DNA or RNA vector. A vector may be either a self-replicating extrachromosomal vector or a vector which integrates into a host genome.

2. RSV Proteins

Provided herein are RSV proteins, also referred to herein as "RSV immunogens", are consensus antigens capable of eliciting an immune response against RSV. Three of the consensus RSV immunogens are: RSV F immunogen, and two forms of RSV G immunogen, referred to herein as RSV Ga immunogen and RSV Gb immunogen. Two RSV G immunogen forms are provided due to the diversity among RSV G proteins. In some embodiments, the RSV immunogens may comprise a signal peptide from a different protein such as an immunoglobulin protein, for example an IgE signal peptide (SEQ ID NO:17) or an IgG signal peptide. RSV immunogen include RSV F immunogen, RSV Ga immunogen and RSV Gb immunogen. Also provided herein is the "RSV immunogens" that is a consensus antigens capable of eliciting an immune response against RSV M2-1, i.e. RSV M2-1 immunogen which may optionally comprise a signal peptide from a different protein such as an immunoglobulin protein, for example an IgE signal peptide (SEQ ID NO:17) or an IgG signal peptide.

"RSV F immunogens" refer to proteins comprising immunogenic fragments of proteins that are at least 98% homologous to SEQ ID NO:2. The RSV F immunogen may comprise immunogenic fragments of proteins that are at least 99% homologous to SEQ ID NO:2. The RSV F immunogen may comprise immunogenic fragments of SEQ ID NO:2. In each instance the immunogenic fragments may optionally further comprise a signal peptide such as SEQ ID NO:17. The RSV F immunogen that comprises an immunogenic fragment of SEQ ID NO:2 and signal peptide SEQ ID NO:17 may be an immunogenic fragments of SEQ ID NO:10 that comprises SEQ ID NO:17. RSV F immunogen may comprise a protein that is at least 98% homologous to SEQ ID NO:2, and in some embodiments may comprise a protein that is at least 99% homologous to SEQ ID NO:2. The RSV F immunogen may comprise SEQ ID NO:2. In each instance the RSV F immunogen may optionally further comprise a signal peptide such as SEQ ID NO:17. The RSV F immunogen that comprises SEQ ID NO:2 and signal peptide SEQ ID NO:17 may comprise SEQ ID NO:10.

"RSV Ga immunogens" refer to proteins comprising immunogenic fragments of proteins that are at least 98% homologous to SEQ ID NO:6. The RSV Ga immunogen may comprise immunogenic fragments of proteins that are at least 99% homologous to SEQ ID NO:6. The RSV Ga immunogen may comprise immunogenic fragments of SEQ ID NO:6. In each instance the immunogenic fragments may optionally further comprise a signal peptide such as SEQ ID NO:17. The RSV Ga immunogen that comprises an immunogenic fragment of SEQ ID NO:6 and signal peptide SEQ ID NO:17 may be an immunogenic fragments of SEQ ID NO:14 that comprises SEQ ID NO:17. RSV Ga immunogen may comprise a protein that is at least 98% homologous to SEQ ID NO:6, and in some embodiments may comprise a protein that is at least 99% homologous to SEQ ID NO:6. The RSV Ga immunogen may comprise SEQ ID NO:6. In each instance the RSV Ga immunogen may optionally further comprise a signal peptide such as SEQ ID NO:17. The RSV Ga immunogen that comprises SEQ ID NO:6 and signal peptide SEQ ID NO:17 may comprise SEQ ID NO:14.

"RSV Gb immunogens" refer to proteins comprising immunogenic fragments of proteins that are at least 98% homologous to SEQ ID NO:8. The RSV Gb immunogen may comprise immunogenic fragments of proteins that are at least 99% homologous to SEQ ID NO:8. The

RSV Gb immunogen may comprise immunogenic fragments of SEQ ID NO:8. In each instance the immunogenic fragments may optionally further comprise a signal peptide such as SEQ ID NO:17. The RSV Gb immunogen that comprises an immunogenic fragment of SEQ ID NO:8 and signal peptide SEQ ID NO:17 may be an immunogenic fragments of SEQ ID NO:16 that comprises SEQ ID NO:17. RSV Gb immunogen may comprise a protein that is at least 98% homologous to SEQ ID NO:8, and in some embodiments may comprise a protein that is at least 99% homologous to SEQ ID NO:8. The RSV Gb immunogen may comprise SEQ ID NO:8. In each instance the RSV Gb immunogen may optionally further comprise a signal peptide such as SEQ ID NO:17. The RSV Gb immunogen that comprises SEQ ID NO:8 and signal peptide SEQ ID NO:17 may comprise SEQ ID NO:16.

In some embodiments, fusion proteins are provided which comprise a combination of two or more of the RSV proteins set forth herein. For example, fusion proteins may comprise: SEQ ID NO:2 or an immunogenic fragment thereof and SEQ ID NO:6 or an immunogenic fragment thereof; SEQ ID NO:2 or an immunogenic fragment thereof and SEQ ID NO:8 or an immunogenic fragment thereof; or SEQ ID NO:6 or an immunogenic fragment thereof and SEQ ID NO:8 or an immunogenic fragment thereof. Fusion proteins may comprise: SEQ ID NO:2 or an immunogenic fragment thereof, SEQ ID NO:6 or an immunogenic fragment thereof and SEQ ID NO:8 or an immunogenic fragment thereof. Alternatively one or more of the fusion proteins above may comprise a sequence at least 98% or at least 99% homologous to SEQ ID NO:2 or an immunogenic fragment thereof in place of the portion of the fusion protein described above corresponding to SEQ ID NO:2 or an immunogenic fragment. Likewise, one or more of the fusion proteins above may comprise a sequence at least 98% or at least 99% homologous to SEQ ID NO:6 or an immunogenic fragment thereof in place of the portion of the fusion protein described above corresponding to SEQ ID NO:6 or an immunogenic fragment; and/or one or more of the fusion proteins above may comprise a sequence at least 98% or at least 99% homologous to SEQ ID NO:8 or an immunogenic fragment thereof in place of the portion of the fusion protein described above corresponding to SEQ ID NO:8 or an immunogenic fragment. Fusion proteins may include combinations of consensus proteins and/or fragments of consensus protein and/or variants of consensus protein and/or fragments of variants consensus proteins.

The consensus proteins and/or fragments of consensus protein and/or variants of consensus protein and/or fragments of variants consensus proteins may be linked directly adjacent to each other or linked with a spacer or one more amino acids in between. In some embodiments, the space may be a proteolyic cleavage site. In some embodiments, the space may be a proteolyic cleavage site recognized by a protease found in cells to which the vaccine is intended to be administered and/or taken up.

"RSV M2-1 immunogens" refer to proteins comprising immunogenic fragments of proteins that are at least 98% homologous to SEQ ID NO:4. The RSV M2-1 immunogen may comprise immunogenic fragments of proteins that are at least 99% homologous to SEQ ID NO:4. The RSV M2-1 immunogen may comprise immunogenic fragments of SEQ ID NO:4. In each instance the immunogenic fragments may optionally further comprise a signal peptide such as SEQ ID NO:17. The RSV M2-1 immunogen that comprises an immunogenic fragment of SEQ ID NO:4 and signal peptide SEQ ID NO:17 may be an immunogenic fragments of SEQ ID NO:12 that comprises SEQ ID NO:17. RSV M2-1 immunogen may comprise a protein that is at least 98% homologous to SEQ ID NO:4, and in some embodiments may comprise a protein that is at least 99% homologous to SEQ ID NO:4. The RSV M2-1 immunogen may comprise SEQ ID NO:4. In each instance the RSV M2-1 immunogen may optionally further comprise a signal peptide such as SEQ ID NO:17. The RSV M2-1 immunogen that comprises SEQ ID NO:4 and signal peptide SEQ ID NO:17 may comprise SEQ ID NO:12. In some embodiments, fusion proteins are provided which comprise a combination of RSV M2-1 and another RSV immunogen proteins set forth herein. For example, fusion proteins may comprise: SEQ ID NO:2 or an immunogenic fragment thereof and SEQ ID NO:4 or an immunogenic fragment thereof; SEQ ID NO:4 or an immunogenic fragment thereof and SEQ ID NO:6 or an immunogenic fragment thereof, SEQ ID NO:4 or an immunogenic fragment thereof and SEQ ID NO:8 or an immunogenic fragment thereof; or SEQ ID NO:6 or an immunogenic fragment thereof and SEQ ID NO:8 or an immunogenic fragment thereof. Fusion proteins may comprise: SEQ ID NO:4 or an immunogenic fragment thereof. Alternatively one or more of the fusion proteins above may comprise a sequence at least 98% or at least 99% homologous to SEQ ID NO:4 or an

immunogenic fragment thereof in place of the portion of the fusion protein described above corresponding to SEQ ID NO:4 or an immunogenic fragment.

3. Coding sequences encoding RSV Proteins

Provided herein are nucleic acid sequences that encode the "RSV immunogens" set forth herein. Administration of nucleic acid molecules comprising the nucleic acid sequences when taken up and expressed by cells results in a broad immune response against RSV. Coding sequences for RSV immunogens are provided, i.e. nucleic acid sequences that encode RSV F immunogen, nucleic acid sequences that encode RSV Ga immunogen and nucleic acid sequences that encode RSV Gb immunogen as well as for nucleic acid sequences that encode RSV M2-1 immunogen. In some embodiments, the nucleic acid sequences that encode RSV immunogens may comprise nucleic acid sequences that encode a signal peptide from a different protein such as an immunoglobulin protein, for example an IgE signal peptide (SEQ ID NO:17) or an IgG signal peptide.

Nucleic acid sequences that encode RSV F immunogens may encode proteins comprising immunogenic fragments of proteins that are at least 98% homologous to SEQ ID NO:2. Nucleic acid sequences that encode RSV F immunogens may encode proteins that comprise immunogenic fragments of proteins that are at least 99% homologous to SEQ ID NO:2. Nucleic acid sequences that encode RSV F immunogens may encode proteins that comprise immunogenic fragments of SEQ ID NO:2. In each instance the nucleic acid sequences that encode proteins that comprise the immunogenic fragments may optionally further comprise nucleic acid sequences that encode a signal peptide such as nucleic acid sequences that encode SEQ ID NO:17. Nucleic acid sequences that encode RSV F immunogens may encode proteins that comprises an immunogenic fragment of SEQ ID NO:2 and signal peptide SEQ ID NO:17. Nucleic acid sequences that encode RSV F immunogens may encode proteins that may be an immunogenic fragment of SEQ ID NO:10 that comprises SEQ ID NO:17. Nucleic acid sequences that encode RSV F immunogens may encode proteins that comprise a protein that is at least 98% homologous to SEQ ID NO:2, and in some embodiments may encode a protein that is at least 99% homologous to SEQ ID NO:2. Nucleic acid sequences that encode RSV F immunogens may encode proteins that comprise SEQ ID NO:2. In each instance nucleic acid

sequences that encode RSV F immunogens may optionally further comprise nucleic acid sequences that encode a signal peptide such as SEQ ID NO:17. Nucleic acid sequences that encode RSV F immunogens may encode a protein that comprises SEQ ID NO:2 and signal peptide SEQ ID NO:17. Nucleic acid sequences that encode RSV F immunogens may encode a protein that comprises SEQ ID NO:10.

Nucleic acid sequences that encode RSV F immunogens may comprise fragments of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:1. The nucleic acid sequence that is at least 98% homologous to SEQ ID NO:1 encodes a protein that is at least 98% homologous to SEQ ID NO:2. The fragment encodes an immunogenic fragment of the protein that is at least 98% homologous to SEQ ID NO:2. In some embodiments, the nucleic acid sequences that encodes the RSV F immunogen may comprise fragments of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:1 wherein the nucleic acid sequence encodes a protein that is at least 99% homologous to SEQ ID NO:2, in some embodiments nucleic acid sequence encodes a protein that comprises SEQ ID NO:2, and the fragment of the nucleic acid sequence encodes an immunogenic fragment of the protein that is at least 99% homologous to SEQ ID NO:2 such as an immunogenic fragment SEQ ID NO:2. In some such embodiments, the nucleic acid sequences that encodes the RSV F immunogen may comprise fragments of a nucleic acid sequence that is at least 99% homologous to SEQ ID NO:1 and in some embodiments, fragments of SEQ ID NO:1. In some embodiments, the nucleic acid sequences that encode RSV F immunogens may optionally further comprise nucleic acid sequences that encode a signal peptide such as SEQ ID NO:17. Nucleic acid sequences that encode RSV F immunogens may comprise fragments of SEQ ID NO:2 and coding sequence encoding signal peptide SEQ ID NO:17. Nucleic acid sequences that encode RSV F immunogens may comprise fragments of SEQ ID NO:9 that include coding sequence encoding signal peptide SEQ ID NO:17.

Nucleic acid sequences that encode RSV Ga immunogens may encode proteins comprising immunogenic fragments of proteins that are at least 98% homologous to SEQ ID NO:6. Nucleic acid sequences that encode RSV Ga immunogens may encode proteins that comprise immunogenic fragments of proteins that are at least 99% homologous to SEQ ID NO:6.

Nucleic acid sequences that encode RSV Ga immunogens may encode proteins that comprise immunogenic fragments of SEQ ID NO:6. In each instance the nucleic acid sequences that encode proteins that comprise the immunogenic fragments may optionally further comprise nucleic acid sequences that encode a signal peptide such as nucleic acid sequences that encode SEQ ID NO:17. Nucleic acid sequences that encode RSV Ga immunogens may encode proteins that comprises an immunogenic fragment of SEQ ID NO:6 and signal peptide SEQ ID NO:17. Nucleic acid sequences that encode RSV Ga immunogens may encode proteins that may be an immunogenic fragment of SEQ ID NO:14 that comprises SEQ ID NO:17. Nucleic acid sequences that encode RSV Ga immunogens may encode proteins that comprise a protein that is at least 98% homologous to SEQ ID NO:6, and in some embodiments may encode a protein that is at least 99% homologous to SEQ ID NO:6. Nucleic acid sequences that encode RSV Ga immunogens may encode proteins that comprise SEQ ID NO:6. In each instance nucleic acid sequences that encode RSV Ga immunogens may optionally further comprise nucleic acid sequences that encode a signal peptide such as SEQ ID NO:17. Nucleic acid sequences that encode RSV Ga immunogens may encode a protein that comprises SEQ ID NO:6 and signal peptide SEQ ID NO:17. Nucleic acid sequences that encode RSV Ga immunogens may encode a protein that comprises SEQ ID NO:14.

Nucleic acid sequences that encode RSV Ga immunogens may comprise fragments of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:5. The nucleic acid sequence that is at least 98% homologous to SEQ ID NO:5 encodes a protein that is at least 98% homologous to SEQ ID NO:6. The fragment encodes an immunogenic fragment of the protein that is at least 98% homologous to SEQ ID NO:6. In some embodiments, the nucleic acid sequences that encodes the RSV Ga immunogen may comprise fragments of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:5 wherein the nucleic acid sequence encodes a protein that is at least 99% homologous to SEQ ID NO:6, in some embodiments nucleic acid sequence encodes a protein that comprises SEQ ID NO:6, and the fragment of the nucleic acid sequence encodes an immunogenic fragment of the protein that is at least 99% homologous to SEQ ID NO:6 such as an immunogenic fragment SEQ ID NO:6. In some such embodiments, the nucleic acid sequences that encodes the RSV Ga immunogen may comprise

fragments of a nucleic acid sequence that is at least 99% homologous to SEQ ID NO:5 and in some embodiments, fragments of SEQ ID NO:5. In some embodiments, the nucleic acid sequences that encode RSV Ga immunogens may optionally further comprise nucleic acid sequences that encode a signal peptide such as SEQ ID NO:17. Nucleic acid sequences that encode RSV Ga immunogens may comprise fragments of SEQ ID NO:6 and coding sequence encoding signal peptide SEQ ID NO:17. Nucleic acid sequences that encode RSV Ga immunogens may comprise fragments of SEQ ID NO:13 that include coding sequence encoding signal peptide SEQ ID NO:17.

Nucleic acid sequences that encode RSV Gb immunogens may encode proteins comprising immunogenic fragments of proteins that are at least 98% homologous to SEQ ID NO:8. Nucleic acid sequences that encode RSV Gb immunogens may encode proteins that comprise immunogenic fragments of proteins that are at least 99% homologous to SEQ ID NO:8. Nucleic acid sequences that encode RSV Gb immunogens may encode proteins that comprise immunogenic fragments of SEQ ID NO:8. In each instance the nucleic acid sequences that encode proteins that comprise the immunogenic fragments may optionally further comprise nucleic acid sequences that encode a signal peptide such as nucleic acid sequences that encode SEQ ID NO:17. Nucleic acid sequences that encode RSV Gb immunogens may encode proteins that comprises an immunogenic fragment of SEQ ID NO:8 and signal peptide SEQ ID NO:17. Nucleic acid sequences that encode RSV Gb immunogens may encode proteins that may be an immunogenic fragment of SEQ ID NO:16 that comprises SEQ ID NO:17. Nucleic acid sequences that encode RSV Gb immunogens may encode proteins that comprise a protein that is at least 98% homologous to SEQ ID NO:8, and in some embodiments may encode a protein that is at least 99% homologous to SEQ ID NO:8. Nucleic acid sequences that encode RSV Gb immunogens may encode proteins that comprise SEQ ID NO:8. In each instance nucleic acid sequences that encode RSV Gb immunogens may optionally further comprise nucleic acid sequences that encode a signal peptide such as SEQ ID NO:17. Nucleic acid sequences that encode RSV Gb immunogens may encode a protein that comprises SEQ ID NO:8 and signal peptide SEQ ID NO:17. Nucleic acid sequences that encode RSV Gb immunogens may encode a protein that comprises SEQ ID NO:16.

Nucleic acid sequences that encode RSV Gb immunogens may comprise fragments of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:7. The nucleic acid sequence that is at least 98% homologous to SEQ ID NO:7 encodes a protein that is at least 98% homologous to SEQ ID NO:8. The fragment encodes an immunogenic fragment of the protein that is at least 98% homologous to SEQ ID NO:8. In some embodiments, the nucleic acid sequences that encodes the RSV Gb immunogen may comprise fragments of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:7 wherein the nucleic acid sequence encodes a protein that is at least 99% homologous to SEQ ID NO:8, in some embodiments nucleic acid sequence encodes a protein that comprises SEQ ID NO:8, and the fragment of the nucleic acid sequence encodes an immunogenic fragment of the protein that is at least 99% homologous to SEQ ID NO:8 such as an immunogenic fragment SEQ ID NO:8. In some such embodiments, the nucleic acid sequences that encodes the RSV Gb immunogen may comprise fragments of a nucleic acid sequence that is at least 99% homologous to SEQ ID NO:7 and in some embodiments, fragments of SEQ ID NO:7. In some embodiments, the nucleic acid sequences that encode RSV Gb immunogens may optionally further comprise nucleic acid sequences that encode a signal peptide such as SEQ ID NO:17. Nucleic acid sequences that encode RSV Gb immunogens may comprise fragments of SEQ ID NO:8 and coding sequence encoding signal peptide SEQ ID NO:17. Nucleic acid sequences that encode RSV Gb immunogens may comprise fragments of SEQ ID NO:15 that include coding sequence encoding signal peptide SEQ ID NO:17.

Nucleic acid sequences that encode RSV M2-1 immunogens may encode proteins comprising immunogenic fragments of proteins that are at least 98% homologous to SEQ ID NO:4. Nucleic acid sequences that encode RSV M2-1 immunogens may encode proteins that comprise immunogenic fragments of proteins that are at least 99% homologous to SEQ ID NO:4. Nucleic acid sequences that encode RSV M2-1 immunogens may encode proteins that comprise immunogenic fragments of SEQ ID NO:4. In each instance the nucleic acid sequences that encode proteins that comprise the immunogenic fragments may optionally further comprise nucleic acid sequences that encode a signal peptide such as nucleic acid sequences that encode SEQ ID NO:17. Nucleic acid sequences that encode RSV M2-1 immunogens may encode

proteins that comprises an immunogenic fragment of SEQ ID NO:4 and signal peptide SEQ ID NO:17. Nucleic acid sequences that encode RSV F immunogens may encode proteins that may be an immunogenic fragment of SEQ ID NO:12 that comprises SEQ ID NO:17. Nucleic acid sequences that encode RSV M2-1 immunogens may encode proteins that comprise a protein that is at least 98% homologous to SEQ ID NO:4, and in some embodiments may encodes a protein that is at least 99% homologous to SEQ ID NO:4. Nucleic acid sequences that encode RSV M2-1 immunogens may encode proteins that comprise SEQ ID NO:4. In each instance nucleic acid sequences that encode RSV M2-1 immunogens may optionally further comprise nucleic acid sequences that encode a signal peptide such as SEQ ID NO:17. Nucleic acid sequences that encode RSV M2-1 immunogens may encode a protein that comprises SEQ ID NO:4 and signal peptide SEQ ID NO:17. Nucleic acid sequences that encode RSV M2-1 immunogens may encode a protein that comprises SEQ ID NO:12.

Nucleic acid sequences that encode RSV M2-1 immunogens may comprise fragments of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:3. The nucleic acid sequence that is at least 98% homologous to SEQ ID NO:3 encodes a protein that is at least 98% homologous to SEQ ID NO:4. The fragment encodes an immunogenic fragment of the protein that is at least 98% homologous to SEQ ID NO:4. In some embodiments, the nucleic acid sequences that encodes the RSV M2-1 immunogen may comprise fragments of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:3 wherein the nucleic acid sequence encodes a protein that is at least 99% homologous to SEQ ID NO:4, in some embodiments nucleic acid sequence encodes a protein that comprises SEQ ID NO:4, and the fragment of the nucleic acid sequence encodes an immunogenic fragment of the protein that is at least 99% homologous to SEQ ID NO:4 such as an immunogenic fragment SEQ ID NO:4. In some such embodiments, the nucleic acid sequences that encodes the RSV M2-1 immunogen may comprise fragments of a nucleic acid sequence that is at least 99% homologous to SEQ ID NO:3 and in some embodiments, fragments of SEQ ID NO:3. In some embodiments, the nucleic acid sequences that encode RSV M2-1 immunogens may optionally further comprise nucleic acid sequences that encode a signal peptide such as SEQ ID NO:17. Nucleic acid sequences that encode RSV M2-1 immunogens may comprise fragments of SEQ ID NO:4 and coding sequence

encoding signal peptide SEQ ID NO:17. Nucleic acid sequences that encode RSV M2-1 immunogens may comprise fragments of SEQ ID NO:11 that include coding sequence encoding signal peptide SEQ ID NO:17.

Coding sequences may encode fusion proteins which comprise a combination of two or more of the RSV proteins set forth herein. Coding sequences may encode fusion proteins that include combinations of consensus proteins and/or fragments of consensus protein and/or variants of consensus protein and/or fragments of variants consensus proteins. The consensus proteins and/or fragments of consensus protein and/or variants of consensus protein and/or fragments of variants consensus proteins may be linked directly adjacent to each other or linked with a spacer or one more amino acids in between. In some embodiments, the space may be a proteolyic cleavage site. In some embodiments, the space may be a proteolyic cleavage site recognized by a protease found in cells to which the vaccine is intended to be administered and/or taken up.

In some embodiments, coding sequences encode fusion proteins which comprise a combination of two or more of the RSV proteins set forth herein. For example, coding sequences may encode fusion proteins that comprise: SEQ ID NO:2 or an immunogenic fragment thereof and SEQ ID NO:4 or an immunogenic fragment thereof, such as SEQ ID NO:1 or an immunogenic fragment-encoding fragment thereof and SEQ ID NO:3 or an immunogenic fragment thereof-encoding fragment thereof; SEQ ID NO:2 or an immunogenic fragment thereof and SEQ ID NO:6 or an immunogenic fragment thereof, such as SEQ ID NO:1 or an immunogenic fragment-encoding fragment thereof and SEQ ID NO:5 or an immunogenic fragment thereof-encoding fragment thereof; SEQ ID NO:2 or an immunogenic fragment thereof and SEQ ID NO:8 or an immunogenic fragment thereof, such as SEQ ID NO:1 or an immunogenic fragment-encoding fragment thereof and SEQ ID NO:7 or an immunogenic fragment thereof-encoding fragment thereof; SEQ ID NO:4 or an immunogenic fragment thereof and SEQ ID NO:6 or an immunogenic fragment thereof, such as SEQ ID NO:3 or an immunogenic fragment-encoding fragment thereof and SEQ ID NO:5 or an immunogenic fragment thereof-encoding fragment thereof; SEQ ID NO:4 or an immunogenic fragment thereof and SEQ ID NO:8 or an immunogenic fragment thereof, such as SEQ ID NO:3 or an

immunogenic fragment-encoding fragment thereof and SEQ ID NO:5 or an immunogenic fragment thereof-encoding fragment thereof; and SEQ ID NO:6 or an immunogenic fragment thereof and SEQ ID NO:8 or an immunogenic fragment thereof, such as SEQ ID NO:5 or an immunogenic fragment-encoding fragment thereof and SEQ ID NO:7 or an immunogenic fragment thereof-encoding fragment thereof. Coding sequences may encode fusion proteins which comprise a combination of three RSV proteins set forth herein. For example, coding sequences may encode fusion proteins that comprise: SEQ ID NO:2 or an immunogenic fragment thereof , SEQ ID NO:4 or an immunogenic fragment thereof and SEQ ID NO:6 or an immunogenic fragment thereof, such as SEQ ID NO:1 or an immunogenic fragment-encoding fragment thereof, SEQ ID NO:3 or an immunogenic fragment thereof-encoding fragment thereof and SEQ ID NO:5 or an immunogenic fragment-encoding fragment thereof; SEQ ID NO:2 or an immunogenic fragment thereof , SEQ ID NO:4 or an immunogenic fragment thereof and SEQ ID NO:8 or an immunogenic fragment thereof, such as SEQ ID NO:1 or an immunogenic fragment-encoding fragment thereof, SEQ ID NO:3 or an immunogenic fragment thereof-encoding fragment thereof and SEQ ID NO:7 or an immunogenic fragment-encoding fragment thereof; and SEQ ID NO:4 or an immunogenic fragment thereof , SEQ ID NO:6 or an immunogenic fragment thereof and SEQ ID NO:8 or an immunogenic fragment thereof, such as SEQ ID NO:3 or an immunogenic fragment-encoding fragment thereof, SEQ ID NO:5 or an immunogenic fragment thereof-encoding fragment thereof and SEQ ID NO:7 or an immunogenic fragment-encoding fragment thereof. Coding sequences may encode fusion proteins which comprise a combination of the four RSV proteins set forth herein such as coding sequences that encode SEQ ID NO:2 or an immunogenic fragment thereof , SEQ ID NO:4 or an immunogenic fragment thereof, SEQ ID NO:6 or an immunogenic fragment thereof, and SEQ ID NO:8 or an immunogenic fragment thereof , such as SEQ ID NO:1 or an immunogenic fragment-encoding fragment thereof, SEQ ID NO:3 or an immunogenic fragment thereof-encoding fragment thereof, SEQ ID NO:5 or an immunogenic fragment-encoding fragment thereof and SEQ ID NO:7 or an immunogenic fragment-encoding fragment thereof. Alternatively coding sequence encode one or more of the fusion proteins above may comprise a sequence that encodes a protein that is at least 98% or at least 99% homologous to SEQ ID NO:2 or an immunogenic fragment

thereof in place of the portion of the fusion protein described above corresponding to SEQ ID NO:2 or an immunogenic fragment. Likewise, coding sequences for one or more of the fusion proteins above comprise coding sequence at least 98% or at least 99% homologous to SEQ ID NO:4 or an immunogenic fragment thereof in place of the portion of the fusion protein described above corresponding to SEQ ID NO:4 or an immunogenic fragment; one or more of the fusion proteins above may comprise a sequence at least 98% or at least 99% homologous to SEQ ID NO:6 or an immunogenic fragment thereof in place of the portion of the fusion protein described above corresponding to SEQ ID NO:6 or an immunogenic fragment; and/or one or more of the fusion proteins above may comprise a sequence at least 98% or at least 99% homologous to SEQ ID NO:8 or an immunogenic fragment thereof in place of the portion of the fusion protein described above corresponding to SEQ ID NO:8 or an immunogenic fragment. In some embodiments, coding sequence encode one or more of the fusion proteins above may comprise a sequence that e is at least 98% or at least 99% homologous to SEQ ID NO:1 or fragment thereof that is at least 98% or at least 99% homologous to an immunogenic fragment of SEQ ID NO:2 in place of the portion of the coding sequence of the fusion protein described above corresponding to portion that encodes SEQ ID NO:2 or an immunogenic fragment. Coding sequence encode one or more of the fusion proteins above may comprise a sequence that is at least 98% or at least 99% homologous to SEQ ID NO:3 or fragment thereof that is at least 98% or at least 99% homologous to an immunogenic fragment of SEQ ID NO:4 in place of the portion of the coding sequence of the fusion protein described above corresponding to portion that encodes SEQ ID NO:4 or an immunogenic fragment. Coding sequence encode one or more of the fusion proteins above may comprise a sequence that is at least 98% or at least 99% homologous to SEQ ID NO:5 or fragment thereof that is at least 98% or at least 99% homologous to an immunogenic fragment of SEQ ID NO:6 in place of the portion of the coding sequence of the fusion protein described above corresponding to portion that encodes SEQ ID NO:6 or an immunogenic fragment. Coding sequence encode one or more of the fusion proteins above may comprise a sequence that is at least 98% or at least 99% homologous to SEQ ID NO:7 or fragment thereof that is at least 98% or at least 99% homologous to an immunogenic fragment of SEQ ID NO:8 in place of the portion of the coding sequence of the fusion protein described above corresponding

to portion that encodes SEQ ID NO:8 or an immunogenic fragment. Fusion proteins may include combinations of consensus proteins and/or fragments of consensus protein and/or variants of consensus protein and/or fragments of variants consensus proteins. The consensus proteins and/or fragments of consensus protein and/or variants of consensus protein and/or fragments of variants consensus proteins may be linked directly adjacent to each other or linked with a spacer or one more amino acids in between. In some embodiments, the space may be a proteolytic cleavage site. In some embodiments, the space may be a proteolytic cleavage site recognized by a protease found in cells to which the vaccine is intended to be administered and/or taken up.

4. Plasmid

Provided herein is a vector that is capable of expressing one or more RSV proteins in the cell of a mammal in a quantity effective to elicit an immune response in the mammal. The vector may comprise heterologous nucleic acid encoding the one or more RSV antigens. The vector may be a plasmid. The plasmid may be useful for transfecting cells with nucleic acid encoding an RSV antigen, which the transformed host cell is cultured and maintained under conditions wherein expression of the RSV antigen takes place.

The plasmid may comprise a nucleic acid sequence that encodes one or more of the various RSV proteins disclosed above including coding sequences that encode synthetic, consensus antigen capable of eliciting an immune response against RSV are provided for each of the following three proteins: F, Ga and Gb, fragments of such proteins, variants of such proteins, fragments of variants or fusion proteins which are made up of combinations of consensus proteins and/or fragments of consensus protein and/or variants of consensus protein and/or fragments of variants consensus proteins. The plasmid may comprise a nucleic acid sequence that encodes RSV M2-1 proteins disclosed above including coding sequences that encode synthetic, consensus antigen capable of eliciting an immune response against RSV M2-1, fragments of such proteins, variants of such proteins, fragments of variants or fusion proteins which are made up of combinations of consensus proteins and/or fragments of consensus protein and/or variants of consensus protein and/or fragments of variants consensus proteins.

A single plasmid may contain coding sequence for a single RSV Immunogen, coding sequence for two RSV Immunogens, or coding sequence for three RSV Immunogens. In some embodiments, a coding sequence for a fourth RSV Immunogen ay be provided. A single plasmid may contain coding sequence for RSV F Immunogen. A single plasmid may contain coding sequence for RSV Ga Immunogen. A single plasmid may contain coding sequence for RSV Gb Immunogen. A single plasmid may contain coding sequence for RSV F Immunogen and coding sequence for RSV Ga Immunogen. A single plasmid may contain coding sequence for RSV F Immunogen and coding sequence for RSV Gb Immunogen. A single plasmid may contain coding sequence for RSV Ga Immunogen and coding sequence for RSV Gb Immunogen. A single plasmid may contain coding sequence for RSV F Immunogen, coding sequence for RSV Ga Immunogen and coding sequence for RSV Gb Immunogen.

If RSV M2-1 coding sequence is provided, a single plasmid may contain coding sequence for RSV M2-1 Immunogen. A single plasmid may contain coding sequence for RSV F Immunogen and coding sequence for RSV M2-1 Immunogen. A single plasmid may contain coding sequence for RSV M2-1 Immunogen and coding sequence for RSV Ga Immunogen. A single plasmid may contain coding sequence for RSV M2-1 Immunogen and coding sequence for RSV Gb Immunogen. A single plasmid may contain coding sequence for RSV F Immunogen, coding sequence for RSV M2-1 Immunogen and coding sequence for RSV Ga Immunogen. A single plasmid may contain coding sequence for RSV F Immunogen, coding sequence for RSV M2-1 Immunogen and coding sequence for RSV Gb Immunogen. A single plasmid may contain coding sequence for RSV M2-1 Immunogen, coding sequence for RSV Ga Immunogen and coding sequence for RSV Gb Immunogen. A single plasmid may contain coding sequence for RSV F Immunogen, coding sequence for RSV M2-1 Immunogen, coding sequence for RSV Ga Immunogen and coding sequence for RSV Gb Immunogen.

In some embodiments, a plasmid may comprise coding sequence that encodes CCR20 alone or as part of one these plasmids. Similarly, plasmids may further comprise coding sequences for IL-12, IL-15 and/or IL-28.

The plasmid may further comprise an initiation codon, which may be upstream of the coding sequence, and a stop codon, which may be downstream of the coding sequence. The initiation and termination codon may be in frame with the coding sequence.

The plasmid may also comprise a promoter that is operably linked to the coding sequence The promoter operably linked to the coding sequence may be a promoter from simian virus 40 (SV40), a mouse mammary tumor virus (MMTV) promoter, a human immunodeficiency virus (HIV) promoter such as the bovine immunodeficiency virus (BIV) long terminal repeat (LTR) promoter, a Moloney virus promoter, an avian leukosis virus (ALV) promoter, a cytomegalovirus (CMV) promoter such as the CMV immediate early promoter, Epstein Barr virus (EBV) promoter, or a Rous sarcoma virus (RSV) promoter. The promoter may also be a promoter from a human gene such as human actin, human myosin, human hemoglobin, human muscle creatine, or human metalothionein. The promoter may also be a tissue specific promoter, such as a muscle or skin specific promoter, natural or synthetic. Examples of such promoters are described in US patent application publication no. US20040175727, the contents of which are incorporated herein in its entirety.

The plasmid may also comprise a polyadenylation signal, which may be downstream of the coding sequence. The polyadenylation signal may be a SV40 polyadenylation signal, LTR polyadenylation signal, bovine growth hormone (bGH) polyadenylation signal, human growth hormone (hGH) polyadenylation signal, or human β-globin polyadenylation signal. The SV40 polyadenylation signal may be a polyadenylation signal from a pCEP4 plasmid (Invitrogen, San Diego, CA).

The plasmid may also comprise an enhancer upstream of the coding sequence. The enhancer may be human actin, human myosin, human hemoglobin, human muscle creatine or a viral enhancer such as one from CMV, FMDV, RSV or EBV. Polynucleotide function enhances are described in U.S. Patent Nos. 5,593,972, 5,962,428, and WO94/016737, the contents of each are fully incorporated by reference.

The plasmid may also comprise a mammalian origin of replication in order to maintain the plasmid extrachromosomally and produce multiple copies of the plasmid in a cell. The plasmid may be pVAX1, pCEP4 or pREP4 from Invitrogen (San Diego, CA), which may

comprise the Epstein Barr virus origin of replication and nuclear antigen EBNA-1 coding region, which may produce high copy episomal replication without integration. The backbone of the plasmid may be pAV0242. The plasmid may be a replication defective adenovirus type 5 (Ad5) plasmid.

The plasmid may also comprise a regulatory sequence, which may be well suited for gene expression in a cell into which the plasmid is administered. The coding sequence may comprise a codon that may allow more efficient transcription of the coding sequence in the host cell.

The coding sequence may also comprise an Ig leader sequence. The leader sequence may be 5' of the coding sequence. The consensus antigens encoded by this sequence may comprise an N-terminal Ig leader followed by a consensus antigen protein. The N-terminal Ig leader may be IgE or IgG.

The plasmid may be pSE420 (Invitrogen, San Diego, Calif.), which may be used for protein production in *Escherichia coli* (E.coli). The plasmid may also be pYES2 (Invitrogen, San Diego, Calif.), which may be used for protein production in Saccharomyces cerevisiae strains of yeast. The plasmid may also be of the MAXBAC™ complete baculovirus expression system (Invitrogen, San Diego, Calif.), which may be used for protein production in insect cells. The plasmid may also be pcDNA I or pcDNA3 (Invitrogen, San Diego, Calif.), which may be used for protein production in mammalian cells such as Chinese hamster ovary (CHO) cells.

5. Compositions

Compositions are provided which comprise nucleic acid molecules. The compositions may comprise a plurality of copies of a single nucleic acid molecule such a single plasmid, a plurality of copies of a two or more different nucleic acid molecules such as two or more different plasmids. For example a compositions may comprise plurality of two, three, four, five, six, seven, eight, nine or ten or more different nucleic acid molecules. Such a compositions may comprise plurality of two, three, four, five, six, seven, eight, nine or ten or more different plasmids.

Compositions may comprise nucleic acid molecules, such as plasmids, that collectively contain coding sequence for a single RSV Immunogen, coding sequence for two RSV

Immunogens, or coding sequence for three RSV Immunogens. In some instances, coding sequence for a fourth RSV Immunogen may be provided.

Compositions comprising coding sequence of two RSV Immunogens may be on a single nucleic acid molecule such as a single plasmid or the compositions may comprise two different nucleic acid molecule such as two different plasmids wherein one nucleic acid molecule comprises the coding sequence one RSV Immunogen and the other nucleic acid molecule comprises the coding sequence different RSV Immunogen. Similarly, compositions comprising coding sequence three RSV Immunogens may comprise a single nucleic acid molecule such as a single plasmid, two different nucleic acid molecules or three different nucleic acid molecules. Likewise, compositions comprising coding sequence four RSV Immunogens may comprise a single nucleic acid molecule such as a single plasmid, two different nucleic acid molecules, three different nucleic acid molecules or four different nucleic acid molecule.

In some embodiments, a composition comprises a plurality single nucleic acid molecule encoding one RSV Immunogen such as RSV F Immunogen, RSV Ga Immunogen or RSV Gb Immunogen. In some embodiments, a composition comprises a plurality single nucleic acid molecule, such a single plasmid encoding two RSV Immunogen such as RSV F Immunogen and RSV Ga Immunogen, RSV F Immunogen and RSV Gb Immunogen, and RSV Ga Immunogen and RSV Gb Immunogen. In some embodiments, a composition comprises a plurality single nucleic acid molecule, such a single plasmid encoding three RSV Immunogen such as RSV Ga Immunogen, RSV F Immunogen and RSV Gb Immunogen.

In some embodiments, a composition comprises a plurality two different nucleic acid molecules, such as two plasmids, each different nucleic acid molecule comprising a single different coding sequence for a different RSV Immunogen wherein pairs of different nucleic acid molecule comprise RSV F Immunogen and RSV Ga Immunogen, RSV F Immunogen and RSV Gb Immunogen, and RSV Ga Immunogen and RSV Gb Immunogen.

In some embodiments, a composition comprises a plurality two different nucleic acid molecules, such as two plasmids, which collectively comprising coding sequence for three different RSV Immunogen. In some embodiments, one encodes RSV F Immunogen and RSV Ga Immunogen, and the second encodes RSV Gb Immunogen. In some embodiments, one

encodes RSV F Immunogen and RSV Gb Immunogen, and the second encodes RSV Ga Immunogen. In some embodiments, one encodes Ga Immunogen and RSV Gb Immunogen, and the second encodes RSV F Immunogen.

In some embodiments, a composition comprises a plurality single nucleic acid molecule encoding RSV M2-1 Immunogen.

In some embodiments in which coding sequences for RSV M2-1 are provided, a composition comprises a plurality single nucleic acid molecules, such a single plasmid encoding two RSV Immunogen such as RSV F Immunogen and RSV M2-1 Immunogen, RSV M2-1 Immunogen and RSV Ga Immunogen, and RSV M2-1 Immunogen and RSV Gb Immunogen.

In some embodiments in which coding sequences for RSV M2-1 are provided, a plurality single nucleic acid molecules, such a single plasmid encoding three RSV Immunogen such as RSV F Immunogen, RSV M2-1 Immunogen and RSV Ga Immunogen, RSV F Immunogen, RSV M2-1 Immunogen and RSV Gb Immunogen, RSV M2-1 Immunogen, RSV Ga Immunogen and RSV Gb Immunogen. In some embodiments, a composition comprises a plurality single nucleic acid molecule, such a single plasmid encoding three RSV Immunogen such as RSV F Immunogen, RSV M2-1 Immunogen, RSV Ga Immunogen and RSV Gb Immunogen.

In some embodiments in which coding sequences for RSV M2-1 are provided, a composition comprises a plurality two different nucleic acid molecules, such as two plasmids, each different nucleic acid molecule comprising a single different coding sequence for a different RSV Immunogen wherein pairs of different nucleic acid molecule comprise RSV F Immunogen and RSV M2-1 Immunogen, RSV M2-1 Immunogen and RSV Ga Immunogen, and RSV M2-1 Immunogen and RSV Gb Immunogen.

In some embodiments in which coding sequences for RSV M2-1 are provided, a composition comprises a plurality two different nucleic acid molecules, such as two plasmids, which collectively comprising coding sequence for three different RSV Immunogen or which one is RSV M2-1. Embodiments may be selected from the group consisting of: one nucleic acid molecule encodes RSV F Immunogen and RSV M2-1 Immunogen and the second encodes RSV Ga Immunogen; one nucleic acid molecule encodes RSV F Immunogen and RSV M2-1 Immunogen and the second encodes RSV Gb Immunogen; one encodes RSV F Immunogen and

RSV Ga Immunogen, and the second encodes RSV M2-1 Immunogen; one encodes RSV F Immunogen and RSV Gb Immunogen, and the second encodes RSV M2-1 Immunogen; one encodes RSV M2-1 Immunogen and RSV Ga Immunogen, and the second encodes RSV F Immunogen; one encodes RSV M2-1 Immunogen and RSV Ga Immunogen, and the second encodes RSV Gb Immunogen; one encodes RSV M2-1 Immunogen and RSV Gb Immunogen, and the second encodes RSV F Immunogen; one encodes RSV M2-1 Immunogen and RSV Gb Immunogen, and the second encodes RSV Ga Immunogen; and one encodes Ga Immunogen and RSV Gb Immunogen, and the second encodes RSV M2-1 Immunogen.

In some embodiments in which coding sequences for RSV M2-1 are provided, a composition comprises a plurality two different nucleic acid molecules, such as two plasmids, which collectively comprising coding sequence for four different RSV Immunogen. In some embodiments: one nucleic acid molecule encodes RSV F Immunogen and the second encodes RSV M2-1 Immunogen, RSV Ga Immunogen and RSV Gb Immunogen; one nucleic acid molecule encodes RSV M2-1 Immunogen and the second encodes RSV F Immunogen, RSV Ga Immunogen and RSV Gb Immunogen; one encodes RSV Ga Immunogen and the second encodes RSV F Immunogen, RSV M2-1 Immunogen and RSV Gb Immunogen, one encodes RSV Gb Immunogen and the second encodes RSV F Immunogen, RSV M2-1 Immunogen and RSV Ga Immunogen, one encodes RSV F and RSV M2-1 Immunogen the second encodes RSV Ga Immunogen and RSV Gb Immunogen, one encodes RSV F and RSV Ga Immunogen the second encodes RSV M2-1 Immunogen and RSV Gb Immunogen, and one encodes RSV F and RSV Gb Immunogen the second encodes RSV M2-1 Immunogen and RSV Ga Immunogen. In some embodiments, a composition comprises a plurality three different nucleic acid molecules, such as three plasmids, which collectively comprising coding sequence for three different RSV Immunogen. In some embodiments: one nucleic acid molecule encodes RSV F Immunogen, one encodes RSV M2-1 Immunogen and the third second encodes RSV Ga Immunogen; one nucleic acid molecule encodes RSV F Immunogen, one encodes RSV M2-1 Immunogen and the third second encodes RSV Gb Immunogen; one nucleic acid molecule encodes RSV F Immunogen, one encodes RSV Ga Immunogen and the third second encodes RSV Gb Immunogen; and one nucleic acid molecule encodes RSV M2-1 Immunogen, one encodes RSV Ga Immunogen and

the third second encodes RSV Gb Immunogen. In some embodiments, a composition comprises a plurality three different nucleic acid molecules, such as three plasmids, which collectively comprising coding sequence for four different RSV Immunogens. In some embodiments: one nucleic acid molecule encodes RSV F Immunogen, the second encodes RSV M2-1 Immunogen and the third encodes RSV Ga Immunogen and RSV Gb Immunogen; one nucleic acid molecule encodes RSV F Immunogen, the second encodes RSV Ga Immunogen and the third encodes RSV M2-1 Immunogen and RSV Gb Immunogen; one nucleic acid molecule encodes RSV F Immunogen, the second encodes RSV Gb Immunogen and the third encodes RSV M2-1 Immunogen and RSV Ga Immunogen; one nucleic acid molecule encodes RSV M2-1 Immunogen, the second encodes RSV Ga Immunogen and the third encodes RSV F Immunogen and RSV Gb Immunogen; one nucleic acid molecule encodes RSV M2-1 Immunogen, the second encodes RSV Gb Immunogen, and the third encodes RSV F Immunogen and RSV Ga Immunogen; and one encodes RSV Ga Immunogen, the second encodes RSV Gb Immunogen, and the third encodes RSV F Immunogen and RSV M2-1. In some embodiments, a composition comprises a plurality four different nucleic acid molecules, such as four plasmids, which collectively comprising coding sequence for four different RSV Immunogens, i.e. one nucleic acid molecule encodes RSV F Immunogen, the second encodes RSV M2-1 Immunogen, the third encodes RSV Ga Immunogen and the fourth encodes RSV Gb Immunogen.

In some embodiments, a composition further comprises coding sequence for chemokine CCL20, IL-12, IL-15 and/or IL-28. Coding sequence for chemokine CCL20, IL-12, IL-15 and/or IL-28 may be included on one or more nucleic acid molecules that comprise .coding sequence for one or more RSV Immunogens. Coding sequence for chemokine CCL20, IL-12, IL-15 and/or IL-28 may be included on a separate nucleic acid molecules such as a separate plasmid.

6. Vaccine

Provided herein is a vaccine capable of generating in a mammal an immune response against RSV. The vaccine may comprise each plasmid as discussed above. The vaccine may comprise a plurality of the plasmids, or combinations thereof. The vaccine may be provided to induce a therapeutic or prophylactic immune response.

In some embodiments, the vaccine is preferably Thl biased so as not to play into prior Th2 related pathogenesis and it should be able to function in a neutralization sensitive environment. Preferably, it should be non-live.

Vaccines may be used to deliver one or more immunogenic targets selected from the group consisting of: F, Ga and Gb, fragments of such proteins, variants of such proteins, fragments of variants. In the case of delivery of multiple targets, vaccines may include multiple compositions or a single compositions. Plasmids may be used which encode multiple proteins on a single plasmid or compositions which comprise different plasmids that encode different proteins. In some embodiments, vaccines may be used to deliver M2, fragments thereof , variants of M2-1, fragments of variants, alone or in combination with one or more immunogenic targets selected from the group consisting of: F, Ga and Gb, fragments of such proteins, variants of such proteins, fragments of variants.. In the case of delivery of multiple targets, vaccines may include multiple compositions or a single compositions. Plasmids may be used which encode multiple proteins on a single plasmid or compositions which comprise different plasmids that encode different proteins.

The vaccine may further comprise a pharmaceutically acceptable excipient. The pharmaceutically acceptable excipient may be functional molecules as vehicles, adjuvants, carriers, or diluents. The pharmaceutically acceptable excipient may be a transfection facilitating agent, which may include surface active agents, such as immune-stimulating complexes (ISCOMS), Freunds incomplete adjuvant, LPS analog including monophosphoryl lipid A, muramyl peptides, quinone analogs, vesicles such as squalene and squalene, hyaluronic acid, lipids, liposomes, calcium ions, viral proteins, polyanions, polycations, or nanoparticles, or other known transfection facilitating agents.

The transfection facilitating agent is a polyanion, polycation, including poly-L-glutamate (LGS), or lipid. The transfection facilitating agent is poly-L-glutamate, and more preferably, the poly-L-glutamate is present in the vaccine at a concentration less than 6 mg/ml. The transfection facilitating agent may also include surface active agents such as immune-stimulating complexes (ISCOMS), Freunds incomplete adjuvant, LPS analog including monophosphoryl lipid A, muramyl peptides, quinone analogs and vesicles such as squalene and squalene, and hyaluronic

acid may also be used administered in conjunction with the genetic construct. In some embodiments, the DNA plasmid vaccines may also include a transfection facilitating agent such as lipids, liposomes, including lecithin liposomes or other liposomes known in the art, as a DNA-liposome mixture (see for example W09324640), calcium ions, viral proteins, polyanions, polycations, or nanoparticles, or other known transfection facilitating agents. Preferably, the transfection facilitating agent is a polyanion, polycation, including poly-L-glutamate (LGS), or lipid. Concentration of the transfection agent in the vaccine is less than 4 mg/ml, less than 2 mg/ml, less than 1 mg/ml, less than 0.750 mg/ml, less than 0.500 mg/ml, less than 0.250 mg/ml, less than 0.100 mg/ml, less than 0.050 mg/ml, or less than 0.010 mg/ml.

The pharmaceutically acceptable excipient may be one or more adjuvants. An adjuvant may be other genes that are expressed from the same or from an alternative plasmid or are delivered as proteins in combination with the plasmid above in the vaccine. The one or more adjuvants may be proteins and/or nucleic acid molecules that encode proteins selected from the group consisting of: CCL20, α-interferon (IFN- α), β-interferon (IFN-β), γ-interferon, platelet derived growth factor (PDGF), TNFα, TNFβ, GM-CSF, epidermal growth factor (EGF), cutaneous T cell-attracting chemokine (CTACK), epithelial thymus-expressed chemokine (TECK), mucosae-associated epithelial chemokine (MEC), IL-12, IL-15 including IL-15 having the signal sequence or coding sequence that encodes the signal sequence deleted and optionally including a different signal peptide such as that from IgE or coding sequence that encodes a difference signal peptide such as that from IgE, IL-28, MHC, CD80, CD86, IL-1, IL-2, IL-4, IL-5, IL-6, IL-10, IL-18, MCP-1, MIP-lα, MIP-1β, IL-8, L-selectin, P-selectin, E-selectin, CD34, GlyCAM-1, MadCAM-1, LFA-1, VLA-1, Mac-1, pl50.95, PECAM, ICAM-1, ICAM-2, ICAM-3, CD2, LFA-3, M-CSF, G-CSF, mutant forms of IL-18, CD40, CD40L, vascular growth factor, fibroblast growth factor, IL-7, nerve growth factor, vascular endothelial growth factor, Fas, TNF receptor, Flt, Apo-1, p55, WSL-1, DR3, TRAMP, Apo-3, AIR, LARD, NGRF, DR4, DR5, KILLER, TRAIL-R2, TRICK2, DR6, Caspase ICE, Fos, c-jun, Sp-1, Ap-1, Ap-2, p38, p65Rel, MyD88, IRAK, TRAF6, IkB, Inactive NIK, SAP K, SAP-1, JNK, interferon response genes, NFkB, Bax, TRAIL, TRAILrec, TRAILrecDRC5, TRAIL-R3, TRAIL-R4, RANK, RANK LIGAND, Ox40, Ox40 LIGAND, NKG2D, MICA, MICB, NKG2A, NKG2B, NKG2C, NKG2E,

NKG2F, TAP1, TAP2 and functional fragments thereof. or a combination thereof. In some embodiments adjuvant may be one or more proteins and/or nucleic acid molecules that encode proteins selected from the group consisting of: CCL-20, IL-12, IL-15, IL-28, CTACK, TECK, MEC or RANTES. Examples of IL-12 constructs and sequences are disclosed in PCT application no. PCT/US1997/019502 and corresponding US Application Serial No. 08/956,865, and U.S. Provisional Application Serial No 61/569600 filed December 12, 2011, which are each incorporated herein by reference. Examples of IL-15 constructs and sequences are disclosed in PCT application no. PCT/US04/18962 and corresponding US Application Serial No. 10/560,650, and in PCT application no. PCT/US07/00886 and corresponding U.S. Application Serial No. 12/160,766, and in PCT application no. PCT/US10/048827, which are each incorporated herein by reference. Examples of IL-28 constructs and sequences are disclosed in PCT application no. PCT/US09/039648 and corresponding U.S. Application Serial No. 12/936,192, which are each incorporated herein by reference. Examples of RANTES and other constructs and sequences are disclosed in PCT application no. PCT/US1999/004332 and corresponding U.S. Application Serial No. and 09/622452, which are each incorporated herein by reference. Other examples of RANTES constructs and sequences are disclosed in PCT application no. PCT/US11/024098, which is incorporated herein by reference. Examples of RANTES and other constructs and sequences are disclosed in PCT application no. PCT/US1999/004332 and corresponding U.S. Application Serial No. 09/622452, which are each incorporated herein by reference. Other examples of RANTES constructs and sequences are disclosed in PCT application no. PCT/US11/024098, which is incorporated herein by reference. Examples of chemokines CTACK, TECK and MEC constructs and sequences are disclosed in PCT application no. PCT/US2005/042231 and corresponding U.S. Application Serial No. 11/719,646, which are each incorporated herein by reference. Examples of OX40 and other immunomodulators are disclosed in U.S. Application Serial No. 10/560,653, which is incorporated herein by reference. Examples of DR5 and other immunomodulators are disclosed in U.S. Application Serial No. 09/622452, which is incorporated herein by reference.

The vaccine may further comprise a genetic vaccine facilitator agent as described in U.S. Serial No. 021,579 filed April 1, 1994, which is fully incorporated by reference.

The vaccine may comprise the consensus antigens and plasmids at quantities of from about 1 nanogram to 100 milligrams; about 1 microgram to about 10 milligrams; or preferably about 0.1 microgram to about 10 milligrams; or more preferably about 1 milligram to about 2 milligram. In some preferred embodiments, pharmaceutical compositions according to the present invention comprise about 5 nanogram to about 1000 micrograms of DNA. In some preferred embodiments, the pharmaceutical compositions contain about 10 nanograms to about 800 micrograms of DNA. In some preferred embodiments, the pharmaceutical compositions contain about 0.1 to about 500 micrograms of DNA. In some preferred embodiments, the pharmaceutical compositions contain about 1 to about 350 micrograms of DNA. In some preferred embodiments, the pharmaceutical compositions contain about 25 to about 250 micrograms, from about 100 to about 200 microgram, from about 1 nanogram to 100 milligrams; from about 1 microgram to about 10 milligrams; from about 0.1 microgram to about 10 milligrams; from about 1 milligram to about 2 milligram, from about 5 nanogram to about 1000 micrograms, from about 10 nanograms to about 800 micrograms, from about 0.1 to about 500 micrograms, from about 1 to about 350 micrograms, from about 25 to about 250 micrograms, from about 100 to about 200 microgram of the consensus antigen or plasmid thereof.

The vaccine may be formulated according to the mode of administration to be used. An injectable vaccine pharmaceutical composition may be sterile, pyrogen free and particulate free. An isotonic formulation or solution may be used. Additives for isotonicity may include sodium chloride, dextrose, mannitol, sorbitol, and lactose. The vaccine may comprise a vasoconstriction agent. The isotonic solutions may include phosphate buffered saline. Vaccine may further comprise stabilizers including gelatin and albumin. The stabilizing may allow the formulation to be stable at room or ambient temperature for extended periods of time such as LGS or polycations or polyanions to the vaccine formulation.

7. Methods of Delivery the Vaccine

Provided herein is a method for delivering the vaccine for providing genetic constructs and proteins of the consensus antigen which comprise epitopes that make them particular effective against inmmunogens of RSV against which an immune response can be induced. The method of delivering the vaccine or vaccination may be provided to induce a therapeutic and

prophylactic immune response. The vaccination process may generate in the mammal an immune response against RSV. The vaccine may be delivered to an individual to modulate modulate the activity of the mammal's immune system and enhance the immune response. The delivery of the vaccine may be the transfection of the consensus antigen as a nucleic acid molecule that is expressed in the cell and delivered to the surface of the cell upon which the immune system recognized and induces a cellular, humoral, or cellular and humoral response. The delivery of the vaccine may be used to induce or elicit and immune response in mammals against RSV by administering to the mammals the vaccine as discussed above.

Upon delivery of the vaccine and plasmid into the cells of the mammal, the transfected cells will express and secrete consensus antigens for each of the plasmids injected from the vaccine. These proteins will be recognized as foreign by the immune system and antibodies will be made against them. These antibodies will be maintained by the immune system and allow for an effective response to subsequent RSV infections.

The vaccine may be administered to a mammal to elicit an immune response in a mammal. The mammal may be human, primate, non-human primate, cow, cattle, sheep, goat, antelope, bison, water buffalo, bison, bovids, deer, hedgehogs, elephants, llama, alpaca, mice, rats, and chicken.

a. Combination Treatments

The vaccine may be administered in combination with other proteins and/or genes encoding CCL20, α-interferon, γ-interferon, platelet derived growth factor (PDGF), TNFα, TNFβ, GM-CSF, epidermal growth factor (EGF), cutaneous T cell-attracting chemokine (CTACK), epithelial thymus-expressed chemokine (TECK), mucosae-associated epithelial chemokine (MEC), IL-12, IL-15 including IL-15 having the signal sequence deleted and optionally including the different signal peptide such as the IgE signal peptide, MHC, CD80, CD86, IL-28, IL-1, IL-2, IL-4, IL-5, IL-6, IL-10, IL-18, MCP-1, MIP-lα, MIP-1β, IL-8, RANTES, L-selectin, P-selectin, E-selectin, CD34, GlyCAM-1, MadCAM-1, LFA-1, VLA-1, Mac-1, pl50.95, PECAM, ICAM-1, ICAM-2, ICAM-3, CD2, LFA-3, M-CSF, G-CSF, mutant forms of IL-18, CD40, CD40L, vascular growth factor, fibroblast growth factor, IL-7, nerve growth factor, vascular endothelial growth factor, Fas, TNF receptor, Flt, Apo-1, p55, WSL-1,

DR3, TRAMP, Apo-3, AIR, LARD, NGRF, DR4, DR5, KILLER, TRAIL-R2, TRICK2, DR6, Caspase ICE, Fos, c-jun, Sp-1, Ap-1, Ap-2, p38, p65Rel, MyD88, IRAK, TRAF6, IkB, Inactive NIK, SAP K, SAP-1, JNK, interferon response genes, NFkB, Bax, TRAIL, TRAILrec, TRAILrecDRC5, TRAIL-R3, TRAIL-R4, RANK, RANK LIGAND, Ox40, Ox40 LIGAND, NKG2D, MICA, MICB, NKG2A, NKG2B, NKG2C, NKG2E, NKG2F, TAP1, TAP2 and functional fragments thereof or combinations thereof. In some embodiments, the vaccine is administered in combination with one or more of the following nucleic acid molecules and/or proteins: nucleic acid molecules selected from the group consisting of nucleic acid molecules comprising coding sequence that encode one or more of CCL20, IL-12, IL-15, IL-28, CTACK, TECK, MEC and RANTES or functional fragments thereof, and proteins selected from the group consisting of: CCL02, IL-12 protein, IL-15 protein, IL-28 protein, CTACK protein, TECK protein, MEC protein or RANTES protein or functional fragments thereof.

The vaccine may be administered by different routes including orally, parenterally, sublingually, transdermally, rectally, transmucosally, topically, via inhalation, via buccal administration, intrapleurally, intravenous, intraarterial, intraperitoneal, subcutaneous, intramuscular, intranasal intrathecal, and intraarticular or combinations thereof. For veterinary use, the composition may be administered as a suitably acceptable formulation in accordance with normal veterinary practice. The veterinarian can readily determine the dosing regimen and route of administration that is most appropriate for a particular animal.. The vaccine may be administered by traditional syringes, needleless injection devices, "microprojectile bombardment gone guns", or other physical methods such as electroporation (“EP”), “hydrodynamic method”, or ultrasound.

The plasmid of the vaccine may be delivered to the mammal by several well known technologies including DNA injection (also referred to as DNA vaccination) with and without in vivo electroporation, liposome mediated, nanoparticle facilitated, recombinant vectors such as recombinant adenovirus, recombinant adenovirus associated virus and recombinant vaccinia. The consensus antigen may be delivered via DNA injection and along with in vivo electroporation.

b. Electroporation

Administration of the vaccine via electroporation of the plasmids of the vaccine may be accomplished using electroporation devices that can be configured to deliver to a desired tissue of a mammal a pulse of energy effective to cause reversible pores to form in cell membranes, and preferable the pulse of energy is a constant current similar to a preset current input by a user. The electroporation device may comprise an electroporation component and an electrode assembly or handle assembly. The electroporation component may include and incorporate one or more of the various elements of the electroporation devices, including: controller, current waveform generator, impedance tester, waveform logger, input element, status reporting element, communication port, memory component, power source, and power switch. The electroporation may be accomplished using an in vivo electroporation device, for example CELLECTRA EP system (VGX Pharmaceuticals, Blue Bell, PA) or Elgen electroporator (Genetronics, San Diego, CA) to facilitate transfection of cells by the plasmid.

The electroporation component may function as one element of the electroporation devices, and the other elements are separate elements (or components) in communication with the electroporation component. The electroporation component may function as more than one element of the electroporation devices, which may be in communication with still other elements of the electroporation devices separate from the electroporation component. The elements of the electroporation devices existing as parts of one electromechanical or mechanical device may not limited as the elements can function as one device or as separate elements in communication with one another. The electroporation component may be capable of delivering the pulse of energy that produces the constant current in the desired tissue, and includes a feedback mechanism. The electrode assembly may include an electrode array having a plurality of electrodes in a spatial arrangement, wherein the electrode assembly receives the pulse of energy from the electroporation component and delivers same to the desired tissue through the electrodes. At least one of the plurality of electrodes is neutral during delivery of the pulse of energy and measures impedance in the desired tissue and communicates the impedance to the electroporation component. The feedback mechanism may receive the measured impedance and

can adjust the pulse of energy delivered by the electroporation component to maintain the constant current.

A plurality of electrodes may deliver the pulse of energy in a decentralized pattern. The plurality of electrodes may deliver the pulse of energy in the decentralized pattern through the control of the electrodes under a programmed sequence, and the programmed sequence is input by a user to the electroporation component. The programmed sequence may comprise a plurality of pulses delivered in sequence, wherein each pulse of the plurality of pulses is delivered by at least two active electrodes with one neutral electrode that measures impedance, and wherein a subsequent pulse of the plurality of pulses is delivered by a different one of at least two active electrodes with one neutral electrode that measures impedance.

The feedback mechanism may be performed by either hardware or software. The feedback mechanism may be performed by an analog closed-loop circuit. The feedback occurs every 50 μs, 20 μs, 10 μs or 1 μs, but is preferably a real-time feedback or instantaneous (i.e., substantially instantaneous as determined by available techniques for determining response time). The neutral electrode may measure the impedance in the desired tissue and communicates the impedance to the feedback mechanism, and the feedback mechanism responds to the impedance and adjusts the pulse of energy to maintain the constant current at a value similar to the preset current. The feedback mechanism may maintain the constant current continuously and instantaneously during the delivery of the pulse of energy.

Examples of electroporation devices and electroporation methods that may facilitate delivery of the DNA vaccines of the present invention, include those described in U.S. Patent No. 7,245,963 by Draghia-Akli, et al., U.S. Patent Pub. 2005/0052630 submitted by Smith, et al., the contents of which are hereby incorporated by reference in their entirety. Other electroporation devices and electroporation methods that may be used for facilitating delivery of the DNA vaccines include those provided in co-pending and co-owned U.S. Patent Application, Serial No. 11/874072, filed October 17, 2007, which claims the benefit under 35 USC 119(e) to U.S. Provisional Applications Ser. Nos. 60/852,149, filed October 17, 2006, and 60/978,982, filed October 10, 2007, all of which are hereby incorporated in their entirety.

U.S. Patent No. 7,245,963 by Draghia-Akli, et al. describes modular electrode systems and their use for facilitating the introduction of a biomolecule into cells of a selected tissue in a body or plant. The modular electrode systems may comprise a plurality of needle electrodes; a hypodermic needle; an electrical connector that provides a conductive link from a programmable constant-current pulse controller to the plurality of needle electrodes; and a power source. An operator can grasp the plurality of needle electrodes that are mounted on a support structure and firmly insert them into the selected tissue in a body or plant. The biomolecules are then delivered via the hypodermic needle into the selected tissue. The programmable constant-current pulse controller is activated and constant-current electrical pulse is applied to the plurality of needle electrodes. The applied constant-current electrical pulse facilitates the introduction of the biomolecule into the cell between the plurality of electrodes. The entire content of U.S. Patent No. 7,245,963 is hereby incorporated by reference.

U.S. Patent Pub. 2005/0052630 submitted by Smith, et al. describes an electroporation device which may be used to effectively facilitate the introduction of a biomolecule into cells of a selected tissue in a body or plant. The electroporation device comprises an electro-kinetic device ("EKD device") whose operation is specified by software or firmware. The EKD device produces a series of programmable constant-current pulse patterns between electrodes in an array based on user control and input of the pulse parameters, and allows the storage and acquisition of current waveform data. The electroporation device also comprises a replaceable electrode disk having an array of needle electrodes, a central injection channel for an injection needle, and a removable guide disk. The entire content of U.S. Patent Pub. 2005/0052630 is hereby incorporated by reference.

The electrode arrays and methods described in U.S. Patent No. 7,245,963 and U.S. Patent Pub. 2005/0052630 may be adapted for deep penetration into not only tissues such as muscle, but also other tissues or organs. Because of the configuration of the electrode array, the injection needle (to deliver the biomolecule of choice) is also inserted completely into the target organ, and the injection is administered perpendicular to the target issue, in the area that is pre-delineated by the electrodes The electrodes described in U.S. Patent No. 7,245,963 and U.S. Patent Pub. 2005/005263 are preferably 20 mm long and 21 gauge.

Additionally, contemplated in some embodiments that incorporate electroporation devices and uses thereof, there are electroporation devices that are those described in the following patents: US Patent 5,273,525 issued December 28, 1993, US Patents 6,110,161 issued August 29, 2000, 6,261,281 issued July 17, 2001, and 6,958,060 issued October 25, 2005, and US patent 6,939,862 issued September 6, 2005. Furthermore, patents covering subject matter provided in US patent 6,697,669 issued February 24, 2004, which concerns delivery of DNA using any of a variety of devices, and US patent 7,328,064 issued February 5, 2008, drawn to method of injecting DNA are contemplated herein. The above-patents are incorporated by reference in their entirety.

c. Method of Preparing DNA Plasmids

Provided herein is methods for preparing the DNA plasmids that comprise the DNA vaccines discussed herein. The DNA plasmids, after the final subcloning step into the mammalian expression plasmid, can be used to inoculate a cell culture in a large scale fermentation tank, using known methods in the art.

The DNA plasmids for use with the EP devices of the present invention can be formulated or manufactured using a combination of known devices and techniques, but preferably they are manufactured using an optimized plasmid manufacturing technique that is described in a licensed, co-pending U.S. provisional application U.S. Serial No. 60/939,792, which was filed on May 23, 2007. In some examples, the DNA plasmids used in these studies can be formulated at concentrations greater than or equal to 10 mg/mL. The manufacturing techniques also include or incorporate various devices and protocols that are commonly known to those of ordinary skill in the art, in addition to those described in U.S. Serial No. 60/939792, including those described in a licensed patent, US Patent No. 7,238,522, which issued on July 3, 2007. The above-referenced application and patent, US Serial No. 60/939,792 and US Patent No. 7,238,522, respectively, are hereby incorporated in their entirety.

EXAMPLES

The present invention is further illustrated in the following Examples. It should be understood that these Examples, while indicating preferred embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, various modifications of the invention in addition to those shown and described herein will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims

Example 1

With expected continued increases in the number of individuals at high risk for RSV infection in the coming years (*e.g.*, young infants and the elderly), there is a dire need for the development of an efficacious and financially manageable treatment for RSV infection. DNA vaccines, small bacterial plasmids genetically engineered to express an encoded protein of interest upon immunization, offer several significant advantages that may help in circumventing many of the hurdles involved in designing an efficacious RSV vaccine. For instance, to prevent clinically significant illness and re-infection, the vaccine would have to confer greater and longer lasting immunity than that which occurs from natural RSV infection. DNA vaccines not only elicit both humoral (*e.g.*, neutralization antibody) and cellular (*e.g.*, cytotoxic T lymphocytes) immune response, but can also elicit lifelong immunity against many viruses. In addition, the primary targets for RSV vaccination are neonates and young infants. Neonatal immune system is functionally immature and thus, develops only a weak immune response to conventional vaccines (*e.g.*, live attenuated or inactivated virus). DNA vaccines, on the other hand, have been shown to be safe and immunogenic in neonatal animal models. Furthermore, the presence of maternal antibody at the time of immunization has been known to suppress or inhibit immune responses to a variety of conventional vaccines. With DNA vaccine, however, the immunizing protein is not present in the vaccine preparation and therefore, should not be susceptible to direct inactivation by maternal antibodies.

Currently, there are three main RSV vaccines in or near early stages of clinical trials: 1) live attenuated RSV (MedImmune, Philadelphia, PA); 2) Sendai virus expressing the RSV F and/or G proteins (AmVac, Switzerland); and 3) virus-like particles containing the G protein of RSV (Novavax, Rockville, MD) (http://www.clinicaltrials.gov/ct2/results?term=RSV&pg=l). Although these approaches have shown some degree of efficacy in the prevention of RSV infection, there are other inherent disadvantages associated with such vaccine designs. For instance, the biggest issues with live attenuated virus are under-attenuation or mutations leading to reversion to virulence and therefore, should not be administered to immunocompromised individuals. A common drawback to all viral vectors is the host immune response as recognition of viral proteins leads to the generation of neutralizing antibodies that may significantly reduce the efficacy of the vaccine upon subsequent immunization. Lastly, the G protein of RSV is highly variable between the two subtypes of RSV and therefore, any vaccine targeting the G protein is not likely to provide cross protection.

In contrast, DNA vaccines have been shown to be highly immunogenic and lack many of the disadvantages associated with the above described vaccine approach . When delivered via electroporation, DNA vaccine immunogenicity further improves. DNA vaccines contain less than a complete set of viral components and like protein subunit vaccines have no risk for virulence reversion or infection of the host making them safe to use in both infants and in immunocompromised individuals.

Using consensus sequences of both subtype A and B of RSV provides the basis to obtain broader protection against both strains of the virus. Such advantages make consensus RSV DNA vaccine much more beneficial and potentially more efficacious than any other approaches suggested in the past

Major RSV proteins - fusion (F), glycoprotein (Ga), and glycoprotein (Ga) were chosen as targets for new RSV vaccines. Alsochosen for evaluation was the second matrix protein (M2). As mentioned above, F and G proteins are expressed on the surface and are ideal targets for neutralizing antibodies. M2 protein contains the major immunodominant CD8 T cell epitope. For each of these RSV proteins, a single consensus sequence was generated based on the amino acid sequences provided by PUBMED for both subtype A and B of RSV (Figure 1 shows the

phylogenetic tree of RSV-F Protein, the consensus RSV F protein amino acid sequence is set forth in SEQ ID NO:2; Figure 2 shows the phylogenetic tree of RSV M2-1 Protein, the consensus RSV M2-1 protein amino acid sequence is set forth in SEQ ID NO:4. In a preferred embodiments, codon and RNA optimized DNA sequences that encode the consensus protein sequences are produced and used to make DNA vaccines which may be delivered using electroporation.

Example 2

Successful expression of RSV consensus F protein was confirmed through immunofluorescence microscopy and/or western blot analysis. Constructs were used in immunization studies.

Mice were immunized with varying dosages of the different RSV DNA vaccine constructs. One group received the DNA vaccine backbone plasmid pVAX as a control. One group received 5 μg dose of plasmid RSV-F, one group received 15 μg dose of plasmid RSV-F; one group received 30 μg dose of plasmid RSV-F; and one group received 60 μg dose of plasmid RSV-F.

Mice were immunized 2 weeks apart for a total of 3 immunizations (weeks 0, 2 and 4) intramuscularly with electroporation. Mice were bled at weeks 2, 4, 6 and 12 after each immunization and the total RSV F protein specific IgG and IgA antibody was measured in the sera. Figure 3 shows anti-RSV F protein IgG after immunization 1 (i.e. at week 0), immunization 2 (i.e. week 2) and after immunizations 3 (i.e. week 4). Figure 4 shows anti-RSV F protein IgA after immunization 1 (i.e. at week 0), immunization 2 (i.e. week 2) and after immunizations 3 (i.e. week 4). Mice were sacrificed at week 12. In addition to comparisons of total IgG in sera and of total IgA in sera, a comparison of IFN-γ production at different dosage of RSV-F vaccine approximately 8 weeks after last immunization was done.

RSV-F DNA vaccine elicited high levels of both IgG and IgA in sera. Responses were detectable as early as 2 weeks after 2nd immunization for all dosage studied. The antibody responses induced appear to be strongly Thl biased, thus avoiding prior issues of the side effects of Th2 immune responses to RSV. RSV-F specific CD8 T cells were observed even after 8

weeks after the last immunization. Figure 5 shows results from a comparison of IFN-γ production at different dosage of RSV-F vaccine approximately 8 weeks after last immunization.

Example 3

Chemokines are a family of small proteins secreted by cells with ability to induce directed chemotaxis toward a higher concentration gradient in nearby responsive cells. Various groups have investigated the feasibility of using chemokines as immune adjuvants.

CCL28/MEC (mucosa-associated epithelial chemokine) which binds CCR10 is expressed on epithelial cells in the gut, lung, breast, and the salivary glands. Use of mucosal chemokines in previous studies showed their utility as immune adjuvants, inducing better immune response in the mucosal tissues after systemic immunization.

Experiments were performed to test the use of a CCL28 genetic construct as an adjuvant for the consensus RSV F protein DNA vaccine construct. A genetic construct that comprised nucleotide sequence encoding CCL20, also referred to a liver activation regulated chemokine (LARC) or Macrophage Inflammatory Protein-3 (MIP3A), was also tested as a possible adjuvant for the consensus RSV F protein DNA vaccine construct.

The immunization schedule for RSV-F plus chemokine experiments utilized three groups of mice: one receiving RSV-F construct only; one receiving RSV-F construct plus CCL20 construct; and one receiving RSV-F construct plus CCL28 construct. Mice were immunized a total of three times: at week 0, at week 2 and at week 4. Mice were bled at week 2, week 4 and week 5. At week 5, mice underwent vaginal wash to collect vaginal samples and were sacrificed after which lungs and spleens were collected.

Comparisons were made of IFN-γ+ CD8 and CD4 T cells in spleen after RSV-F +/- chemokine immunization in the spleen (Figure 6) and in lung (Figure 7) after RSV-F +/- chemokine immunization.

IgG Endpoint Titer in Sera was measured and IgG subtypes (IgG1 vs IgG2a) in Sera were compared (Figure 8). A comparison of IgG2a/IgG1 ratio was also done (Figure 9).

CCL20 and CCL28 chemokines did not appear to enhance CD8 and CD4 T cell immune responses after RSV-F DNA vaccination in either the spleen or the lung (based on IFN- γ ICS) although it is unclear if the dosage of chemokine construct was insufficient.

Animals receiving the CCL20 immune adjuvant exhibited a heavy bias towards a Th1 immune response (primarily IgG2a) after RSV-F DNA vaccination. Driving immune response away from Th2 and towards Th1 may be useful in order to avoid vaccine-associated enhanced disease after RSV infection.

Example 4

As noted above, administration of coding sequences that encode CCL20 led to a preferential Th1 immune response and away from Th2 immune responses. This property of CCL20 administration may be useful in the context of both an immunotherapeutic as well as a vaccine adjuvant for other targets in which a shift increasing Th1 immune responses and decreasing Th2 immune responses is desirable.

For example, CCL20 or administration of nucleic acid molecules that encode CCL20 may be useful to treat individuals who have autoimmune diseases or inflammatory conditions characterized by Th2 immune responses. The ability to decrease Th2 immune responses as shown herein can be used to reduce Th2 immune responses associated with certain autoimmune diseases or inflammatory conditions including for example.

In addition, CCL20 may be used as a vaccine adjuvant as described herein with vaccines targeting other infection diseases as well as cancer. The Th1 increasing/Th2 decreasing properties associated with the use of CCL20 as a vaccine adjuvant may be useful in vaccines. SEQ ID NO:18 and SEQ ID NO:20 contains high expression coding sequences for CCL20 with and without coding sequences encoding the IgE signal peptide. In some embodiments, SEQ ID NO:18 further comprises a start codon. SEQ ID NO:19 and SEQ ID NO:21 contains consensus sequences for CCL20 with and without coding sequences encoding the IgE signal peptide. In some embodiments, SEQ ID NO:19 further comprises an N terminal; methionine

Example 5

The Luciferase Reporter Gene Assay in TZM-bl Cells is based on Single-Round Infection with Molecularly Cloned Env-Pseudotyped Viruses. TZM-bl (JC53-bl) is a genetically

engineered HeLa cell line that expresses CD4, CXCR4 and CCR5 and contains Tat-inducible Luc and β-Gal reporter genes. The assay provides high success rate in single-round infections, increased assay capacity (2-day assay), increased precision (accurately measure 50% neutralization), improved level of standardization (stable cell line), and an optimized and validated assay platform. Montefiori, D.C. (2004) Current Protocols in Immunology, John Wiley & Sons, 12.11.1-12.11.15, which is incorporated herein by reference, described the assay.

Figure 10 depicts Env-Pseudotyped viruses in TZM-BL Cells/ "ON".

Figure 11 depicts Env-Pseudotyped viruses in TZM-BL Cells/ "OFF".

Figure 12 depicts RSV-Fusion Pseudo viral production in 293T cells.

Figure 13 shows the immunization protocol used for a RSV-F Neutralization Study using the assay.

Figure 14 shows data from quantification of RSV-F mediated infection in different target cells. Infectivity of pseudotypes bearing RSV-F proteins. Pseudotype viruses were pelleted, and the particle amounts were estimated by quantifying HIV p24 antigen. Cells were infected with pseudotype viruses or no envelope bearing a panel of diverse cell lines containing 10ng of particulate HIV p24 antigen. At 72 h postinfection, cells supernatant and assayed for p24 antigen. Values are the means of triplicate wells with the standard deviations.

Figure 15 shows data from quantification of RSV-F neutralization activity in TZM-bl HeLa cells. The data show results of a comparison of neutralizing ability to RSV-F using luciferase assay from RSV-F immunized serum samples from DNA vaccinated animals that can block the HIV-Luc/FMDV pseudovirus from entering and infecting target cells. The titer is defined as the reciprocal of the dilution of plasma that produces 50% inhibition of virus. The ability of the serum sample from the vaccinated mice to neutralize and block the infectivity of HIV-1 Luc/RSV-F the absence of such blocking activity in normal mouse serum (NMS).

Figure 16 shows data from quantification of cell death activity by RSV-F pseudo virus in HeLa cells. The results show that consensus RSV-F DNA plasmid elicits potent humoral and cellular immune responses in mice after 3 vaccinations with electroporation. A high level of RSV-F specific IgG in sera was observed as early as 1 week post 2nd immunization with just 5 ug DNA. Cell-mediated immune response were detectable as far out as 8 weeks post 3rd

immunization suggesting the long-term persistence of immune response after RSV-F DNA vaccination. Increase in RSV-F DNA concentration did not result in increased immune response. Antibodies generated after 3 vaccinations with RSV-F DNA plus electroporation were capable of neutralizing infection as measured by pseudo-virus neutralization assay. Results also showed mitigated RSV-F mediated apoptosis of target cells.

Example 6

Figure 17 shows the immunization schedule for RSV-M2 dosage study that was undertaken. Data collected from that study includes a comparison of IFN-g production at different dosages of RSV-M2 vaccine 1 week after last immunization (Figure 18). The results showed that a consensus-based RSV-M2 DNA plasmid elicited significant cellular immune responses in mice after 3 vaccinations with electroporation. High level of IFN-g+ T cells specific for either the immunodominant or subdominant epitopes of RSV-M2 in both spleen and in lung were observed. Maximum immune response was observed with 60 ug DNA (however, response not much greater than that observed with 10 ug DNA).

Example 7

Further study was undertaken to evaluate CCL20 as potential immune adjuvant to enhance mucosal immune response after DNA immunization. CCL20 (liver activation regulated chemokine – LARC; macrophage inflammatory protein 3 – MIP3a) binds to CCR6 and is expressed in lymph nodes, liver, appendix, fetal lung (lower levels in thymus, testis, prostate, and gut).

Figure 19 depicts an immunization schedule for evaluation of the combination of RSV-F vaccine with CCL20.

Figure 20 shows data from the study including data from a comparison of IFN-g production in each of spleen, liver and lung with different dosages of CCL20 at 1 week after last immunization.

Figure 21 shows data of a comparison of IgG subtypes (IgG1 vs IgG2a) in Sera.

Results show that co-immunization with CCL20 does not enhance cell-mediated immune response in both the lung and the liver after RSV-F DNA immunization. CCL20 appeared to bias RSV-F specific IgG antibodies generated after DNA immunization towards a Th1-isotype.

Less IgG1 and more IgG2a were observed in sera after CCL20 + RSV-F co-immunization compared to RSV-F immunization alone.

Thus, provided are improved vaccines, improved methods for inducing immune such responses, including mucosal immune responses, and for prophylactically and/or therapeutically immunizing individuals against immunogens, particularly those associated with pathogens and other agents which enter the host via mucosal tissue. For examples, infectious agents commonly enter the host across a mucosal tissue such as the oral mucosa and other mucosa of the alimentary canal, the respiratory tract including olfactory and conjunctival mucosa, the mammary glands, and the genitourinary tract.

Composition comprising nucleic acid sequences that encodes an immunogen in combination with nucleic acid sequences that encodes CCL20 and functional fragments thereof are provided. The composition may comprise an isolated nucleic acid molecule that encodes both an immunogen and CCL20 and functional fragments thereof and/or compositions comprising an isolated nucleic acid molecule that encodes an immunogen in combination with an isolated nucleic acid molecule that encodes CCL20 and functional fragments thereof. Such compositions may be provided as injectable pharmaceutical.

The compositions may be used in methods of inducing an immune response in an individual against an immunogen, comprising administering to the individual such a composition.

Recombinant vaccines comprising a nucleotide sequence that encodes an immunogen operably linked to regulatory elements and a nucleotide sequences that CCL20 or functional fragments thereof are provided as are methods of inducing an immune response, including methods of inducing a mucosal immune response, in an individual against an immunogen comprising administering such a recombinant vaccine to an individual.

Live attenuated pathogenscomprising a nucleotide sequence that CCL20 or functional fragments thereof, and to methods of inducing an immune response, including methods of inducing a mucosal immune response, in an individual against a pathogen comprising administering the live attenuated pathogen to an individual are also provided.

Methods of inducing an immune response in an individual against an immunogen comprising administering to said individual CCL20 protein or a functional fragment thereof in combination with an isolated nucleic acid molecule that encodes an immunogen; and/or a recombinant vaccine that encodes an immunogen and/or a subunit vaccine that comprises an immunogen and/or a live attenuated vaccine and/or a killed vaccine. Compositions comprising CCL20 protein or a functional fragments thereof in combination with one or more of an isolated nucleic acid molecule that encodes an immunogen; a recombinant vaccine comprising a nucleotide sequence that encodes an immunogen operably linked to regulatory elements; a live attenuated pathogen; and a killed pathogen are provided. As used herein, "functional fragment of CCL20" is meant to refer to a fragment of an iCCl20protein that, when delivered in conjunction with an immunogen, provides an modified immune response compared to the immune response that is induced when the immunogen is delivered without the fragment. Fragments are generally 10 or more amino acids in length. In some embodiments, functional fragments of CCL20 may comprise 20% or more, 25% or more, 30% or more, 35% or more, 40% or more, 45% or more, 50% or more, 55% or more, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 91% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, 99% or more percent of the length of the particular full length CCL20 protein.

When delivered in combination with an immunogen, CCL20 and functional fragments thereof, and combinations thereof modulates immune responses. Accordingly, a combination of these proteins may be delivered as components of a DNA or protein based vaccine in order to induce a therapeutic or prophylactic immune response or in compositions useful to induce an immune response. In some embodiments, the means to deliver the immunogen is a DNA vaccine, a recombinant vaccine, a protein subunit vaccine, an attenuated vaccine or a killed vaccine. In some embodiments, the means to deliver one or more of CCL20 and functional fragments thereof is by expression of coding sequences included in a DNA vaccine, a recombinant vaccine or an attenuated vaccine.

Immune responses result in the production of antigen specific antibodies and/or antigen specific T- and B-cells. Antigen specific antibodies and/or cells provide the means to protect

against infection, to reduce or to clear existing infection. They can also be isolated from the individual and used in other applications such as passive immunity protocols, immunocolumns or as reagents.

In some embodiments, CCL20 is useful to induce mucosal immune responses, particularly increased Th1 responses characterized by increased IgG2a responses and descreased IgG1 responses, even in protocols where the composition is delivered systemically. Co-immunization with an immunogen such as by DNA vaccine or other means plasmid plus one or more of these chemokines, such as in a DNA vaccine or part of the coding sequence of another type of vaccine, will provide a unique adjuvanting property.

CCL20 protein and nucleotides which encode it can be obtained from many sources, natural and sythentic. In some embodiments for which protein is used, for example, one having ordinary skill in the art can, using well known techniques, isolates CCL20 protein from natural sources using, for example, immuno columns which contain antibodies that specifically bind to the protein. Alternatively, the protein may be separated using electrophoresis, isolated from the electrophoresis matrix and purified by for example dialysis to yield essentially pure protein. Other well known protein purification technologies can be employed to produce isolated, essentially pure protein. In some embodiments for which protein is used, for example, one having ordinary skill in the art can, using well known techniques, inserts DNA molecules that encode any of CCL20 and functional fragments thereof into a commercially available expression vector for use in well known expression systems. In addition to isolating proteins from natural sources or producing proteins by recombinant techniques, automated peptide synthesizers may also be employed to produce isolated, essentially pure protein.

According to some aspects of the present invention, compositions and methods are provided which prophylactically and/or therapeutically immunize an individual against an immunogen such as an allergen, a pathogen or abnormal, disease-related cells. The vaccine may be any type of vaccine such as, a live attenuated vaccine, a cell vaccine, a recombinant vaccine or a nucleic, acid or DNA vaccine. By delivering nucleic acid molecules that encode an immunogen and one or more of CL20 and functional fragments thereof the immune response induced by the vaccine may be modulated.

Compositions for delivering immunogens and CCL20 are provided, particularly those comprising one or more nucleic acid molecules that comprise a nucleotide sequence that encodes one or more of CCL20 and functional fragments thereof operably linked to regulatory elements in combination with a nucleotide sequence that encodes an immunogen operably linked to regulatory elements. The nucleic acid sequences encoding CCL20 and the immunogen may be one the same molecule or on separate molecules.

The nucleic acid molecules may be delivered using any of several well known technologies including DNA injection (also referred to as DNA vaccination), recombinant vectors such as recombinant adenovirus, recombinant adenovirus associated virus and recombinant vaccinia. In some embodiments, DNA vaccines are constructed as described herein and delivered using electroporation as described herein. The descriptions for RSV vacicnes set forth herein, for example, may be applied to the use of CCL20 with other immunogens. DNA vaccines are described in U.S. Pat. Nos. 5,593,972, 5,739,118, 5,817,637, 5,830,876, 5,962,428, 5,981,505, 5,580,859, 5,703,055, 5,676,594, and the priority applications cited therein, which are each incorporated herein by reference. In addition to the delivery protocols described in those applications, alternative methods of delivering DNA are described in U.S. Pat. Nos. 4,945,050 and 5,036,006, which are both incorporated herein by reference. Routes of administration include, but are not limited to, intramuscular, intranasally, intraperitoneal, intradermal, subcutaneous, intravenous, intraarterially, intraocularly and oral as well as topically, transdermally, by inhalation or suppository or to mucosal tissue such as by lavage to vaginal, rectal, urethral, buccal and sublingual tissue. Preferred routes of administration include intramuscular, intraperitoneal, intradermal and subcutaneous injection. In some embodiments, nucleotide sequences that encode CCL20 or functional fragments thereof may be linked to IgE signal peptide. In some embodiments, the immunogen is a pathogen antigen, a cancer-associated antigen or an antigen linked to cells associated with autoimmune diseases. The present invention may be used to immunize an individual against all pathogens such as viruses, prokaryote and pathogenic eukaryotic organisms such as unicellular pathogenic organisms and multicellular parasites. The present invention is particularly useful to immunize an individual against those pathogens which infect cells and which are not encapsulated such as viruses, and prokaryote

such as gonorrhea, listeria and shigella. In addition, the present invention is also useful to immunize an individual against protozoan pathogens that include a stage in the life cycle where they are intracellular pathogens. Examples of pathogens include the following. Viruses in the Picornavirus Family Genera such as rhinoviruses (responsible for - 50% cases of the common cold), Etheroviruses, (include polioviruses, coxsackieviruses, echoviruses, and human enteroviruses such as hepatitis A virus), and Apthoviruses (Veterinary; target antigens include: VP1, VP2, VP3, VP4, VPG). Viruses in the Calcivirus Family Genera include Norwalk Group of Viruses (causative agent of epidemic gastroenteritis). Viruses of the Togavirus Family Genera include Alphaviruses: (examples include Senilis viruses, RossRiver virus and Eastern & Western, Equine encephalitis). Reovirus include Rubella virus. Flariviridue Family Examples include dengue, yellow fever, Japanese encephalitis, St. Louis encephalitis and tick borne encephalitis viruses. West Nile virus (Genbank NC001563, AF533540, AF404757, AF404756, AF404755, AF404754, AF404753, AF481864, M12294, AF317203, AF196835, AF260969, AF260968, AF260967, AF206518 and AF202541) Representative Target antigens: E NS5 C. Hepatitis C Virus. Coronavirus Family include infectious bronchitis virus. porcine transmissible gastroenteric virus, Porcine hemaglutinating encephalomyelitis virus, Feline infectious peritonitis virus, Feline enteric coronavirus, Canine coronavirus, SARS associated coronavirus, human respiratory coronaviruses cause ~40 cases of common cold. EX. 224E, OC43. Target antigens: E1 - also called M or matrix protein E2 - also called S or Spike protein E3 - also called BE or hemagglutin- elterose glycoprotein N – nucleocapsid. Rhabdovirus Family Genera include Vesiliovirus Lyssavirus such as rabies. Target antigen: G protein N protein. Filoviridue Family includes Hemorrhagic fever viruses such as (Medical) Marburg and Ebola virus. Paramyxovirus Genera includes paramyxovirus: Family such as Mumps virus, New Castle disease virus, Morbillivirus such as Measles, canine distemper, Pneuminvirus such as Respiratory syncytial virus, Orthomyxovirus. The Influenza virus Family includes Bungavirus Family Genera such as bungavirus, California encephalitis, LA Crosse, Phlebovirus such as Rift Valley Fever, Hantavirus such as puremala, a hemahagin fever virus, Nairvirus, unassigned bungaviruses Arenavirus Family LCM, and Lassi fever virus. Reovirus Family Genera includes Reovirus, Rotavirus, Orbiviruses such as Colorado Tick fever, Lebombo, equine encephalosis, blue tongue.

Retrovirus Family Sub-Family includes oncorivirinal such as leukemia virus, HTLVI and HTLVII, Lentivirinal such as HIV, feline immunodeficiency virus, equine infections, and anemia virus. Spumavirinal Papovavirus Family Sub-Family includes Polyomaviruses such as BKU and JCU viruses, Sub-Family Papillomavirus. Adenovirus such as EX AD7, ARD., O.B., 275. Parvovirus Family includes Feline parvovirus, Feline panleucopeniavirus, Canine parvovirus, Porcine parvovirus. Herpesvirus Family Sub-Family includes alpha-Genera Simplexvirus herpesviridue HSVI (Genbank X14112, NC001806), HSVII (NC001798) Varicellovinis, pseudorabies, varicella zoster. Sub-Family - beta- incoudes Genera Cytomegalovirus such as herpesviridue HCMV. Muromegalovirus Sub-Family includes Genera: Lymphocryptovirus, Gamma- EBV - (Burkitts lympho), herpesviridue. Rhadinovirus Poxvirus Family Sub-Family Genera Variola includes (Smallpox) Chordopoxviridue Vaccinia (Cowpox) (Parapoxivirus) Auipoxvirus, Capripoxvirus Leporipoxvirus. Suipoxviru's Sub-Family includes Entemopoxviridue Hepadnavirus. Hepatitis B virus Family Unclassified Hepatitis delta virus. Bacterial pathogens include pathogenic gram-positive cocci such as pneurnococcal; staphylococcal; and streptococcal. Pathogenic gram-negative cocci include: meningococcal; and gonococcal. Pathogenic enteric gram-negative bacilli include: enterobacteriaceae; pseudomonas, acinetobacteria and eikenella, melioidosis;, sahnonella; shigellosis; hemophilus; chancroid; brucellosis; tularemia; yersinia (pasteurella); streptobacillus mortiliformis and spirillum; listeria monocytogenes; erysipelothrix rhusiopathiae; diphtheria, cholera, anthrax; donovanosis (granuloma inguinale); and bartonellosis. Pathogenic anaerobic bacteria include: tetanus; botulism; other clostridia; tuberculosis; leprosy; and other mycobacteria. Pathogenic spirochetal diseases include: syphilis; - treponematoses: yaws, pinta and endemic syphilis; and leptospirosis. Other infections caused by higher pathogen bacteria and pathogenic fungi include: actinomycosis; .nocardiosis; cryptococcosis, blastomycosis, histoplasmosis and coccidioidomycosis; candidiasis, aspergillosis, and mucormycosis; sporotrichosis; paracoccidiodomycosis, petriellidiosis, torulopsosis, mycetoma, and chromomycosis; and dermatophytosis. Rickettsial infections include rickettsial and rickettsioses. Examples of mycoplasma and chlarnydial infections include: mycoplasma pneurnoniae; lymphogranuloma venereum; psittacosis; and perinatal chlamydial infections. Pathogenic eukaryotes Pathogenic

protozoans and helminths and infections thereby include: amebiasis; malaria; leishmaniasis; trypanosomiasis; toxoplasmosis; pneurnocystis carinii; babesiosis; giardiasis; trichinosis; filariasis; schistosomiasis; nematodes; trematodes or flukes; and cestode (tapeworm) infections. Vaccines can be made such as DNA constructs that comprise DNA sequences that encode the peptides that comprise at least an epitope identical or substantially similar to an epitope displayed on a pathogen antigen. In some embodiments, the methods of immunizing an individual against a pathogen are directed against RSV, HIV, HSV, HCV, WNV or HBV.

Methods of conferring a protective immune response against hyperproliferating cells utilize as immunogens proteins that are characteristic in hyperproliferative diseases. These are useful in vaccines and methods of treating individuals suffering from hyperproliferative diseases. Examples of hyperproliferative diseases include all forms of cancer and psoriasis. Examples of target proteins against hyperproliferative diseases include those which are proteins encoded by oncogenes such as myb, myc, fyn, and the translocation gene bcr/abl, ras, src, P53, neu, trk and EGRF. In addition to oncogene products as target antigens, target proteins for anti-cancer treatments and protective regimens include variable regions of antibodies made by B cell lymphomas and variable regions of T cell receptors of T cell lymphomas which, in some embodiments, are also used target antigens for autoimmune disease. Other tumor-associated proteins can be used as target proteins such as proteins that are found at higher levels in tumor cells including the protein recognized by monoclonal antibody 17-IA and folate binding proteins or PSA. Methods of treating individuals suffering from autoimmune diseases and disorders by conferring a broad based protective immune response against targets that are associated with autoimmunity including cell receptors and cells which produce "self"-directed antibodies are also provided. T cell mediated autoimmune diseases include Rheumatoid arthritis (RA), multiple sclerosis (MS), Sjogren's syndrome, sarcoidosis, insulin dependent diabetes mellitus (1DDM), autoimmune thyroiditis, reactive arthritis, ankylosing spondylitis, scleroderma, polymyositis, dermatomyositis, psoriasis, vasculitis, Wegener's granulomatosis, Crohn's disease and ulcerative colitis. Each of these diseases is characterized by T cell receptors that bind to endogenous antigens and initiate the inflammatory cascade associated with autoimmune diseases. Vaccination against the variable region of the T cells would elicit an immune response including

CTLs to eliminate those T cells. In RA, several specific variable regions of T cell receptors (TCRs) that are involved in the disease have been characterized. These TCRs include V.beta.-3, V.beta.-14, 20 V.beta.-17 and V.alpha.-17. Thus, vaccination with a DNA construct that encodes at least one of these proteins will elicit an immune response that will target T cells involved in RA. See: Howell, M. D., et al., 1991 Proc. Nat. Acad. Sci. USA 88:10921-10925; Piliard, X., et al, 1991 Science 253:325-329; Williams, W. V., et al., 1992 J Clin. Invest. 90:326-333; each of which is incorporated herein by reference. In MS, several specific variable regions of TCRs that are involved in the disease have been characterized. These TCRs include VfP and V.alpha.-10. Thus, vaccination with a DNA construct that encodes at least one of these proteins will elicit an immune response that will target T cells involved in MS. See: Wucherpfennig, K. W., et al., 1990 Science 248:1016-1019; Oksenberg, J. R., et al, 1990 Nature 345:344-346; each of which is incorporated herein by reference. In scleroderma, several specific variable regions of TCRs that are involved in the disease have been characterized. These TCRs include V.beta.-6, V.beta.-g, V.beta.-14 and V.alpha.-16, V.alpha.-3C, V.alpha.-7, V.alpha.-14, V.alpha.-15, V.alpha.-16, V.alpha.-28 and V.alpha.-12. Thus, vaccination with a DNA construct that encodes at least one of these proteins will elicit an immune response that will target T cells involved in scleroderma. In order to treat patients suffering from a T cell mediated autoimmune disease, particularly those for which the variable region of the TCR has yet to be characterized, a synovial biopsy can be performed. Samples of the T cells present can be taken and the variable region of those TCRs identified using standard techniques. Genetic vaccines can be prepared using this information. B cell mediated autoimmune diseases include Lupus (SLE), Grave's disease, myasthenia gravis, autoimmune hemolytic anemia, autoimmune thrombocytopenia, asthma, cryoglobulineinia, primary biliary sclerosis and pernicious anemia. Each of these diseases is characterized by antibodies that bind to endogenous antigens and initiate the inflammatory cascade associated with autoimmune diseases. Vaccination against the variable region of antibodies would elicit an immune response including CTLs to eliminate those B cells that produce the antibody.

In order to treat patients suffering from a B cell mediated autoimmune disease, the variable region of the antibodies involved in the autoimmune activity must be identified. A biopsy can be performed and samples of the antibodies present at a site of inflammation can be

taken. The variable region of those antibodies can be identified using standard techniques. Genetic vaccines can be prepared using this information. In the case of SLE, one antigen is believed to be DNA. Thus, in patients to be immunized against SLE, their sera can be screened for anti-DNA antibodies and a vaccine can be prepared which includes DNA constructs that encode the variable region of such anti-DNA antibodies found in the sera. Common structural features among the variable regions of both TCRs and antibodies are well known. The DNA sequence encoding a particular TCR or antibody can generally be found following well known methods such as those described in Kabat, et al 1987 Sequence of Proteins of Immunological Interest U.S. Department of Health and Human Services, Bethesda Md., which is incorporated herein by reference. In addition, a general method for cloning functional variable regions from antibodies can be found in Chaudhary, V. K., et al, 1990 Proc. Natl. Acad Sci. USA 87:1066, which is incorporated herein by reference.

In addition to using expressible forms of CCL20 protein coding sequence to improve DNA vaccines, improved attenuated live vaccines, improved killed vaccines and improved vaccines that use recombinant vectors to deliver foreign genes that encode antigens and well as subunit and glycoprotein vaccines are provided which comprise CCL20 protein or a functional fragment thereof and/or coding sequences that encode CCL20 or a functional fragment thereof. Examples of attenuated live vaccines, those using recombinant vectors to deliver foreign antigens, subunit vaccines and glycoprotein vaccines are described in U.S. Pat. Nos. 4,510,245; 4,797,368; 4,722,848; 4,790,987; 4,920,209; 5,017,487; 5,077,044; 5,110,587; 5,112,749; 5,174,993; 5,223,424; 5,225,336; 5,240,703; 5,242,829; 5,294,441; 5,294,548; 5,310,668; 5,387,744; 5,389,368; 5,424,065; 5,451,499; 5,453,364; 5,462,734; 5,470,734; 5,474,935; 5,482,713; 5,591,439; 5,643,579; 5,650,309; 5,698,202; 5,955,088; 6,034,298; 6,042,836; 6,156,319 and 6,589,529, which are each incorporated herein by reference.

Example 8

Using consensus sequences of RSV immunogens F, G(A) and G(B) provides the basis to obtain neutiralizing immune responses. Such advantages make consensus RSV DNA vaccine much more beneficial and potentially more efficacious than any other approaches suggested in the past

Major RSV proteins - fusion (F), glycoprotein (Ga), and glycoprotein (Ga) were chosen as targets for new RSV vaccines. As mentioned above, F and G proteins are expressed on the surface and are ideal targets for neutralizing antibodies. A cocktail DNA vaccine was prepared comprising plasmids that have sequence which encode and express consensus RSV-F, plasmids that have sequence which encode and express consensus RSV-G(A) and plasmids that have sequence which encode and express consensus RSV-G(B). The individual plasmids as well as the cocktail were used in studies. The cocktail vaccine was used in studies with rabbits and non-human primates and the results of those studies show that high levels of neutralizing immune response are induced, indicating that the cocktail vaccine can be used in both prophylactic and therapeutic applications with particular value in protecting against RSV infection.

As shown in Figures 22A, Figures 22B and Figures 22C, single insert plasmids for RSV-F, RSV-G(A), and RSV-G(B), respectively, were constructed using the pVAX vector. Consensus sequences for each respective plasmid were cloned into the pVAX vector between the BamHI and XhoI sites. The resulting plasmids were: pRSV-F, which comprises an 1813 base pair insert that encodes consensus RSV-F cloned into pVAX to produce a plasmid having 4812 base pairs; pRSV-G(A), which comprises a 945 base pair insert that encodes consensus RSV-G(A) cloned into pVAX to produce a plasmid having 3944 base pairs; and pRSV-G(B), which comprises a 930 base pair insert that encodes consensus RSV-G(B) cloned into pVAX to produce a plasmid having 3929 base pairs.

To confirm expression of RSV-F, RSV-G(A), and RSV-G(B) constructs, RD cells were transfected with pRSV-F, pRSV-G(A) and pRSV-G(B), respectively. Immunofluorescent evaluation was performed using immunized, pooled rabbit sera. RSV Immunogens were visualized by immunofluorescence by binding anti-RSV immunogen antibodies chemically conjugated with fluorescein isothiocyanate (FITC). DAPI was used as a nuclear counterstain. Results in Figure 23 show RD cells transfected with pVAX showed no immunostaining by FITC labeled anti-RSV immunogen antibodies.

An RSV-F Dosage Study was done using mice. The immunization schedule is shown in Figure 24. Five groups of mice were used. Group 1 were control injected with pVAX. Group 2 were injected with 5μg pRSV-F construct. Group 3 were injected with 15μg pRSV-F construct.

Group 4 were injected with 30μg pRSV-F construct. Group 5 were injected with 60μg pRSV-F construct. Animals were immunized a total of three times using electroporation: first immunization at Week 0, second immunization at Week 2 and third immunization at Week 4. Sera was collected and IgG measured a total of four times: first collection at Week 2, second collection at Week 4, third collection at Week 6 and fourth collection at Week 12. Animals were sacrificed at Week 12, spleens were harvested and IFN-gamma was measured via ELISPOT.

Figure 25 shows data from the RSV-F Dosage comparing total RSV-F IgG in Sera. Data is shown from sera collected after immunization #1, after immunization #2, and after immunization #3. Anti-RSV-F IgG was measured at reciprocal serum dilutions of 10, 100, 1,000 and 10,000. Data show pVAX did not induce antibodies after any immunization. Data show pRSV-F construct did not induce antibodies after first immunization. Data show pRSV-F construct induced antibodies after second and third immunization.

Figure 26 shows data from the RSV-F Dosage comparing IFN-γ production induced by RSV-F vaccination approximately eight weeks after last immunization. Data is shown comparing IFN-γ production (SFU/106 splenocytes) from animals injected with pVAX and 15 μg average dose pRSV-F construct.

An RSV-F PSEUDO Neutralization Study was done using mice. The immunization schedule is shown in Figure 27. Mice were immunized with 15μg pRSV-F construct. Animals were immunized a total of three times using electroporation: first immunization at Week 0, second immunization at Week 2 and third immunization at Week 4. Sera was collected and neutralization assay performed at Week 5.

Data from the RSV-F PSEUDO Neutralization Study is shown in Figure 28. RSV-F pseudo-type neutralization activity in TZM-bl HeLa cells was quantified. A comparison of neutralizing ability of RSV-F using luciferase assay from RSV-F immunized serum samples from DNA vaccinated animals that can block the HIV-Luc/RSV-F pseudovirus from entering and infecting target cells. The titer is defined as the reciprocal of the dilution of plasma that produces 50% inhibition of virus. Note the ability of the serum sample from the vaccinated mice to neutralize and block infectivity of HIV-Luc/RSV-F in the absence of such blocking activity in normal mouse serum (NMS).

As shown in Figure 29, two comparison were done using sera from two animals vaccinated with pRSV-F (RSV-F2 and RSV-F5) versus sera from pVAX vaccinated animals to determine inhibition of apoptotic cell death activity by RSV-F pseudovirus in HeLa cells. For each comparison, sera was diluted 1:1, 1:10, 1:100 and 1:1000. The level of cell death expressed as a percentage was determined showing that sera from animals vaccinated with pRSV-F inhibited apoptotic cell death of HeLa cell exposed to RSV-F pseudovirus in the presence of sera.

An RSV-G(A) and RSV-(B) Dosage Study was done using mice. The immunization schedule is shown in Figure 30. Six groups of mice were used. Group 1 were control injected with pVAX. Group 2 were injected with 1μg pRSV-G(A) or pRSV-G(B) construct. Group 3 were injected with 5μg pRSV-G(A) or pRSV-G(B) construct. Group 4 were injected with 10μg pRSV-G(A) or pRSV-G(B) construct. Group 5 were injected with 20μg pRSV-G(A) or pRSV-G(B) construct. Group 6 were injected with 40μg pRSV-G(A) or pRSV-G(B) construct. Animals were immunized a total of three times using electroporation: first immunization at Week 0, second immunization at Week 2 and third immunization at Week 4. Sera was collected and IgG measured a total of two times: first collection at Week 4, and the second collection at Week 6.

Figure 31 shows data for IgG in sera after RSV-G(A) DNA immunization in the RSV(A) or RSV-G(B) Dosage Study. Total anti-RSV-G(A) IgG in Sera was measured from the Dosage Study. Data is shown from sera collected at various doses as set out in the x-axis of the graph in Figure 31. Anti-RSV-G(A) IgG was measured at serum dilution of 1:100.

Figure 32 shows data for IgG in sera after RSV-G(B) DNA immunization in the RSV(A) or RSV-G(B) Dosage Study. Total anti-RSV-G(B) IgG in Sera was measured from the Dosage Study. Data is shown from sera collected at various doses as set out in the x-axis of the graph in Figure 31. Anti-RSV-G(B) IgG was measured at serum dilution of 1:100.

Figure 33 is a chart showing s summary of RSV Preliminary Neutralization results using Mono Vaccines in Mouse Studies. Neutralization titers from sera collected 3 days post immunization were measured. Negative serum was used as a negative control. Positive controls included an anti-RSV monoclonal antibody and anti-RSV polyclonal serum. Neutralization

titers were measured for controls and for sera from two mice vaccinated with pRSV-F, two mice vaccinated with pRSV-G(A) and one mouse vaccinated with pRSV-G(B).

An RSV Rabbit Study was designed and undertaken. The RSV Rabbit Study Design is shown in Figure 34. Four female New Zealand white (NZW) rabbits were immunized with a cocktail containing pRSV-F, pRSV-G(A), and pRSV-G(B). Each animal received 200 μg/plasmid in a final 100 μl volume. Animals were immunized a total of four times using intramuscular/electroporation immunization. The first immunization was done at Week 0, the second immunization at Week 3, the third immunization at Week 6 and the fourth at Week 9. Blood was collected from animals six times: Week -2 (Pre-bleed - 2 weeks prior to first immunization), Week 0 (Pre-bleed prior to first immunization), Week 2, Week 5, Week 8 and Week 13. Blood was analyzed using ELISA and virus neutralization assays.

Data from the Rabbit Study is shown in Figure 35. Humoral immunity against RSV-F was measured. Anti-RSV-F IgG from pre-bleed samples taken at Weeks -2 and 0 and from samples taken at Week 2, at Week 5, at Week 8 and at Week 13. At Week 2, animals from which samples were collected had been immunized one time and the sample was collected 2 weeks post immunization. At Week 5, animals from which samples were collected had been immunized two times and the sample was collected 2 weeks after the second immunization. At Week 8, animals from which samples were collected had been immunized three times and the sample was collected 2 weeks after the third immunization. At Week 13, animals from which samples were collected had been immunized four times and the sample was collected 4 weeks after the fourth immunization.

Data from the Rabbit Study is shown in Figure 36. Humoral immunity against RSV-G was measured. Anti-RSV-G IgG from pre-bleed samples taken at Weeks -2 and 0 and from samples taken at Week 2, at Week 5, at Week 8 and at Week 13. At Week 2, animals from which samples were collected had been immunized one time and the sample was collected 2 weeks post immunization. At Week 5, animals from which samples were collected had been immunized two times and the sample was collected 2 weeks after the second immunization. At Week 8, animals from which samples were collected had been immunized three times and the sample was collected 2 weeks after the third immunization. At Week 13, animals from which

samples were collected had been immunized four times and the sample was collected 4 weeks after the fourth immunization.

An RSV Non-human Primate Study was undertaken. The purpose of the study was to evaluate the humoral immunogenicity of the single-insert pDNA RSV vaccines administered as a cocktail in the non-human primate (NHP). Also, the study was used to evaluate and determine whether factors such as multiple site delivery of the vaccine, electroporation method, and delivery with an adjuvant would increase the breadth of the humoral responses. The experiments were performed to determine if the synthetic DNA vaccines against RSV-F, RSV-G(A), and RSV-G(B) stimulate high titer of anti-RSV antibodies, to analyze the specific immune responses in animals vaccinated with the RSV cocktail and to evaluate in the effect of adjuvant and/or multiple site delivery on the immune responses generated. The RSV Non-Human Primate Study Design is shown in Figure 37. The non-human primate used were Rhesus macaques. In the study, 15 Rhesus macaques were divided into 4 groups: three groups contained four animals each and one group contained three animals. The groups in which n = 4 were treated groups, i.e. they were vaccinated. The group in which n = 3 was the untreated group which served as a control. The groups were treated as follows.

Group 1 (n=4): each animal was administered a cocktail of 1 mg RSV-F, 1 mg RSV-G(A), 1 mg RSV-G(B), mixed and delivered IM in 1 site.

Group 2 (n=4): each animal was administered a cocktail of 1 mg RSV-F, 1 mg RSV-G(A), 1 mg RSV-G(B), 1 mg rhMEC, mixed and delivered IM in 1 site.

Group 3 (n=4): each animal was administered a cocktail of 1 mg RSV-F, 1 mg RSV-G(A), 1 mg RSV-G(B), 1 mixed and delivered ID in 3 sites.

Group 4 (n=3): each animal was untreated.

Animals were immunized a total of three times using electroporation. The first immunization was done at Week 1, the second immunization at Week 5, and the third immunization at Week 13. Blood was collected from animals six times: Week 0 (Pre-bleed prior to first immunization),

Week 3, Week 7, Week 15, Week 17 and Week 18. Blood was analyzed using ELISA and virus neutralization assays.

Figure 38A-38D show data from the RSV Non-Human Primate Study of measured humoral immunity against RSV-F. Figure 38A shows data from measured humoral immunity against RSV-F in naïve animals. Figure 38B shows data from measured humoral immunity against RSV-F in animals who received the cocktail vaccine IM at one site. Figure 38C shows data from measured humoral immunity against RSV-F in animals who received the cocktail vaccine plus rhMEC construct IM at one site. Figure 38D shows data from measured humoral immunity against RSV-F in animals who received the cocktail vaccine ID at three sites. Anti-RSV-F IgG from pre-bleed samples taken at Week 0 and from samples taken at Week 3, at Week 15 and at Week 18. At Week 3, animals from which samples were collected had been immunized one time and the sample was collected 2 weeks post immunization. At Week 15, animals from which samples were collected had been immunized three times and the sample was collected 2 weeks after the third immunization. At Week 18, animals from which samples were collected had been immunized three times and the sample was collected 5 weeks after the third immunization.

Figure 39A-39D show data from the RSV Non-Human Primate Study of measured humoral immunity against RSV-G. Figure 39A shows data from measured humoral immunity against RSV-G in naïve animals. Figure 39B shows data from measured humoral immunity against RSV-G in animals who received the cocktail vaccine IM at one site. Figure 39C shows data from measured humoral immunity against RSV-G in animals who received the cocktail vaccine plus rhMEC construct IM at one site. Figure 39D shows data from measured humoral immunity against RSV-G in animals who received the cocktail vaccine ID at three sites. Anti-RSV-G IgG from pre-bleed samples taken at Week 0 and from samples taken at Week 3, at Week 15 and at Week 18. At Week 3, animals from which samples were collected had been immunized one time and the sample was collected 2 weeks post immunization. At Week 15, animals from which samples were collected had been immunized three times and the sample was collected 2 weeks after the third immunization. At Week 18, animals from which samples were

collected had been immunized three times and the sample was collected 5 weeks after the third immunization.

Figure 40 is a chart showing a summary of RSV Preliminary Neutralization results using a cocktail vaccines in Rabbit Non-Human Primate Studies. Neutralization titers from sera collected 3 days post immunization were measured. Negative serum was used as a negative control. Positive controls included an anti-RSV monoclonal antibody and anti-RSV polyclonal serum. Neutralization titers were measured for controls and for sera from two rabbits vaccinated with the cocktail vaccine and two non-human primates vaccinated with the cocktail vaccine. Data from pre-bleeds shows that neutralization titers in the rabbits and the non-human primates were generated post vaccination.

CLAIMS

1. A composition comprising one or more nucleic acid sequences that encode an RSV immunogen selected from the group consisting of:

a) a nucleic acid coding sequence that encodes an RSV F immunogen, wherein the nucleic acid coding sequence that encodes the RSV F immunogen is selected from the group consisting of:

a nucleic acid sequence encoding SEQ ID NO:2 and optionally further comprising coding sequence that encodes a signal peptide operably linked to the sequence that encodes SEQ ID NO:2;

a nucleic acid sequence that encodes a fragment of SEQ ID NO:2 that is at least 115 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to the sequence that encodes the fragment of SEQ ID NO:2;

a nucleic acid sequence that encodes a protein that is at least 98% homologous to SEQ ID NO:2 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence that encodes that protein that is at least 98% homologous to SEQ ID NO:2; and

a nucleic acid sequence that encodes a fragment of a protein that is at least 98% homologous to SEQ ID NO:2 that is at least 115 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to the sequence that encodes the fragment of a protein that is at least 98% homologous to SEQ ID NO:2;

b) a nucleic acid coding sequence that encodes an RSV Ga immunogen, wherein the nucleic acid coding sequence that encodes the RSV Ga immunogen is selected from the group consisting of:

a nucleic acid sequence encoding SEQ ID NO:6 and optionally further comprising coding sequence that encodes a signal peptide operably linked to the sequence that encodes SEQ ID NO:6;

a nucleic acid sequence that encodes a fragment of SEQ ID NO:6 that is at least 125 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to the sequence that encodes the fragment of SEQ ID NO:6;

a nucleic acid sequence that encodes a protein that is at least 98% homologous to SEQ ID NO:6 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence that encodes that protein that is at least 98% homologous to SEQ ID NO:6; and

a nucleic acid sequence that encodes a fragment of a protein that is at least 98% homologous to SEQ ID NO:6 that is at least 125 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to the sequence that encodes the fragment of a protein that is at least 98% homologous to SEQ ID NO:6;

and

c) a nucleic acid coding sequence that encodes an RSV Gb immunogen, wherein the nucleic acid coding sequence that encodes the RSV Gb immunogen is selected from the group consisting of:

a nucleic acid sequence encoding SEQ ID NO:8 and optionally further comprising coding sequence that encodes a signal peptide operably linked to the sequence that encodes SEQ ID NO:8;

a nucleic acid sequence that encodes a fragment of SEQ ID NO:8 that is at least 125 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to the sequence that encodes the fragment of SEQ ID NO:8;

a nucleic acid sequence that encodes a protein that is at least 98% homologous to SEQ ID NO:8 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence that encodes that protein that is at least 98% homologous to SEQ ID NO:8; and

a nucleic acid sequence that encodes a fragment of a protein that is at least 98% homologous to SEQ ID NO:8 that is at least 125 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to the sequence that encodes the fragment of a protein that is at least 98% homologous to SEQ ID NO:8.

2. The composition of claim 1 comprising one or more nucleic acid sequences that encode an RSV immunogen selected from the group consisting of:

a) a nucleic acid coding sequence that encodes an RSV F immunogen, wherein the nucleic acid coding sequence that encodes the RSV F immunogen is selected from the group consisting of:

a nucleic acid sequence comprising SEQ ID NO:1 and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:1;

a nucleic acid sequence comprising a fragment of SEQ ID NO:1 encoding at least 115 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:1;

a nucleic acid sequence at least 98% homologous to SEQ ID NO:1 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence at least 98% homologous to SEQ ID NO:1; and

a fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:1 encoding at least 115 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:1;

b) a nucleic acid coding sequence that encodes an RSV Ga immunogen, wherein the nucleic acid coding sequence that encodes the RSV Ga immunogen is selected from the group consisting of:

a nucleic acid sequence comprising SEQ ID NO:5 and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:5;

a nucleic acid sequence comprising a fragment of SEQ ID NO:5 encoding at least 125 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:5;

a nucleic acid sequence at least 98% homologous to SEQ ID NO:5 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence at least 98% homologous to SEQ ID NO:5; and

a fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:5 encoding at least 125 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:5; and

c) a nucleic acid coding sequence that encodes an RSV Gb immunogen, wherein the nucleic acid coding sequence that encodes the RSV Gb immunogen is selected from the group consisting of:

a nucleic acid sequence comprising SEQ ID NO:7 and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:7;

a nucleic acid sequence comprising a fragment of SEQ ID NO:7 encoding at least 125 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:7;

a nucleic acid sequence at least 98% homologous to SEQ ID NO:7 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence at least 98% homologous to SEQ ID NO:7; and

a fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:7 encoding at least 125 or more amino acids and optionally further comprises coding

sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:7.

3. The composition of claim 1 comprising one or more nucleic acid sequences selected from the group consisting of:

a) a nucleic acid coding sequence that encodes an RSV F immunogen comprising SEQ ID NO:2 and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:2;

b) a nucleic acid coding sequence that encodes an RSV Ga immunogen comprising SEQ ID NO:6 and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:6;

and

c) a nucleic acid coding sequence that encodes an RSV Gb immunogen comprising SEQ ID NO:8 and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:8.

4. The composition of claim 3 comprising one or more nucleic acid sequences selected from the group consisting of:

a) a nucleic acid coding sequence that encodes an RSV F immunogen, the coding sequence comprising SEQ ID NO:1 and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:1;

b) a nucleic acid coding sequence that encodes an RSV Ga immunogen, the coding sequence comprising SEQ ID NO:5 and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:5;

and

c) a nucleic acid coding sequence that encodes an RSV Gb immunogen, the coding sequence comprising SEQ ID NO:7 and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:7.

5. The composition of claim 3 comprising one or more nucleic acid sequences selected from the group consisting of:

a) a nucleic acid sequence that encodes SEQ ID NO:10;

b) a nucleic acid sequence that encodes SEQ ID NO:14;

and

c) a nucleic acid sequence that encodes SEQ ID NO:16;

6. The composition of claim 3 comprising one or more nucleic acid sequences selected from the group consisting of:

a) a nucleic acid sequence comprising SEQ ID NO:9;

b) a nucleic acid sequence comprising SEQ ID NO:13;

and

c) a nucleic acid sequence comprising SEQ ID NO:15.

7. The composition of claim 1 comprising one or more nucleic acid sequences selected from the group consisting of:

a) a nucleic acid coding sequence that encodes an RSV F immunogen comprising a fragment of SEQ ID NO:2 having at least 345 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:2, or a fragment of a protein that is at least 98% homologous to SEQ ID NO:2 having at least 345 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that encodes a protein least 98% homologous to SEQ ID NO:2;

b) a nucleic acid coding sequence that encodes an RSV Ga immunogen comprising a fragment of SEQ ID NO:6 having at least 200 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:6, or a fragment of a protein that is at least 98% homologous to SEQ ID NO:6 having at least 200 or more amino acids and optionally further comprises coding sequence that encodes a signal

peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that encodes a protein least 98% homologous to SEQ ID NO:6;
and

c) a nucleic acid coding sequence that encodes an RSV Gb immunogen comprising a fragment of SEQ ID NO:8 having at least 200 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:8, or a fragment of a protein that is at least 98% homologous to SEQ ID NO:8 having at least 200 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that encodes a protein least 98% homologous to SEQ ID NO:8.

8. The composition of claim 7 comprising one or more nucleic acid sequences selected from the group consisting of:

a) a nucleic acid coding sequence that encodes an RSV F immunogen comprising a nucleic acid sequence comprising a fragment of SEQ ID NO:1 encoding at least 345 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:1, or a fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:1 encoding at least 345 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:1;

b) a nucleic acid coding sequence that encodes an RSV Ga immunogen, comprising a fragment of SEQ ID NO:5 encoding at least 200 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:5, or a fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:5 encoding at least 125 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:5;
and

c) a nucleic acid coding sequence that encodes an RSV Gb immunogen comprising a fragment of SEQ ID NO:7 encoding at least 200 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:7, or a fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:7 encoding at least 200 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:7.

9. The composition of claim 1 comprising one or more nucleic acid sequences selected from the group consisting of:

a) a nucleic acid coding sequence that encodes an RSV F immunogen comprising a fragment of SEQ ID NO:2 having at least 550 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:2, or a fragment of a protein that is at least 98% homologous to SEQ ID NO:2 having at least 550 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that encodes a protein least 98% homologous to SEQ ID NO:2;

b) a nucleic acid coding sequence that encodes an RSV Ga immunogen comprising a fragment of SEQ ID NO:6 having at least 270 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:6, or a fragment of a protein that is at least 98% homologous to SEQ ID NO:6 having at least 270 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that encodes a protein least 98% homologous to SEQ ID NO:6;

and

c) a nucleic acid coding sequence that encodes an RSV Gb immunogen comprising a fragment of SEQ ID NO:8 having at least 270 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:8, or a fragment of a protein that is at least 98% homologous to SEQ ID NO:8 having at least 270 or

more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that encodes a protein least 98% homologous to SEQ ID NO:8.

10. The composition of claim 9 comprising one or more nucleic acid sequences selected from the group consisting of:

a) a nucleic acid coding sequence that encodes an RSV F immunogen comprising a nucleic acid sequence comprising a fragment of SEQ ID NO:1 encoding at least 550 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:1, or a fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:1 encoding at least 550 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:1;

b) a nucleic acid coding sequence that encodes an RSV Ga immunogen, comprising a fragment of SEQ ID NO:5 encoding at least 270 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:5, or a fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:5 encoding at least 270 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:5;

and

c) a nucleic acid coding sequence that encodes an RSV Gb immunogen comprising a fragment of SEQ ID NO:7 encoding at least 270 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:7, or a fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:7 encoding at least 270 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:7.

11. The composition of claim 1 comprising one or more nucleic acid sequences selected from the group consisting of:

a) a nucleic acid coding sequence that encodes an RSV F immunogen at least 99% homologous to SEQ ID NO:2 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence at least 99% homologous to SEQ ID NO:2;

b) a nucleic acid coding sequence that encodes an RSV Ga immunogen at least 99% homologous to SEQ ID NO:6 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence at least 98% homologous to SEQ ID NO:6; and

c) a nucleic acid coding sequence that encodes an RSV Gb immunogen at least 99% homologous to SEQ ID NO:8 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence at least 98% homologous to SEQ ID NO:8.

12. The composition of claim 11 comprising one or more nucleic acid sequences selected from the group consisting of:

a) a nucleic acid coding sequence that encodes an RSV F immunogen, the coding sequence being at least 99% homologous to SEQ ID NO:1 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence at least 99% homologous to SEQ ID NO:1;

b) a nucleic acid coding sequence that encodes an RSV Ga immunogen, the coding sequence being at least 99% homologous to SEQ ID NO:5 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence at least 98% homologous to SEQ ID NO:5;

and

c) a nucleic acid coding sequence that encodes an RSV Gb immunogen, the coding sequence being at least 99% homologous to SEQ ID NO:7 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence at least 98% homologous to SEQ ID NO:7.

13. A composition of claim 1 comprising nucleic acid sequences selected from the group consisting of:

a nucleic acid coding sequence that encodes an RSV F immunogen,

a nucleic acid coding sequence that encodes an RSV Ga immunogen,

a nucleic acid coding sequence that encodes an RSV Gb immunogen,

a nucleic acid coding sequence that encodes an RSV F immunogen and a nucleic acid coding sequence that encodes an RSV Ga immunogen,

a nucleic acid coding sequence that encodes an RSV F immunogen and a nucleic acid coding sequence that encodes an RSV Gb immunogen,

a nucleic acid coding sequence that encodes an RSV Ga immunogen and a nucleic acid coding sequence that encodes an RSV Gb immunogen, and

a nucleic acid coding sequence that encodes an RSV F immunogen, a nucleic acid coding sequence that encodes an RSV Ga immunogen and a nucleic acid coding sequence that encodes an RSV Gb immunogen.

14. The composition of any of claims 1-13 further comprising a nucleic acid coding sequence that encodes CCL20.

15. The composition of any of claims 1-13 formulated for delivery to an individual using electroporation.

16. The composition of any of claims 1-13 further comprising nucleic acid sequences that encode one or more proteins selected from the group consisting of: IL-12, IL-15 and IL-28.

17. The composition of any of claims 1-13 wherein said one or more nucleic acid coding sequences are part of one or more plasmids.

18. The composition of any of claims 1-13 wherein each of the one or more nucleic acid coding sequences are part of incorporated into a separate plasmid.

19. A method of inducing an immune response against RSV comprising administering the composition of any of claims 1-13 to an individual in an amount effective to induce an immune response in said individual.

20. A method of treating an individual who has been diagnosed with RSV comprising administering a therapeutically effective amount of the composition of any of claims 1-13 to an individual.

21. A method of preventing RSV infection an individual comprising administering a prophylactically effective amount of the composition of any of claims 1-13 to an individual.

22. A protein selected from the group consisting of: SEQ ID NO:2; SEQ ID NO:6; SEQ ID NO:8; SEQ ID NO:10; SEQ ID NO:14; and SEQ ID NO:16.

23. A composition comprising a nucleic acid coding sequence that encodes an RSV M2-1 immunogen, wherein the nucleic acid coding sequence that encodes the RSV M2-1 immunogen is selected from the group consisting of:

a nucleic acid sequence encoding SEQ ID NO:4 and optionally further comprising coding sequence that encodes a signal peptide operably linked to the sequence that encodes SEQ ID NO:4;

a nucleic acid sequence that encodes a fragment of SEQ ID NO:4 that is at least 25 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to the sequence that encodes the fragment of SEQ ID NO:4;

a nucleic acid sequence that encodes a protein that is at least 98% homologous to SEQ ID NO:4 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence that encodes that protein that is at least 98% homologous to SEQ ID NO:4; and

a nucleic acid sequence that encodes a fragment of a protein that is at least 98% homologous to SEQ ID NO:4 that is at least 25 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to the sequence that encodes the fragment of a protein that is at least 98% homologous to SEQ ID NO:4.

24. The composition of claim 23 comprising a nucleic acid coding sequence that encodes an RSV M2-1 immunogen, wherein the nucleic acid coding sequence that encodes the RSV M2-1 immunogen is selected from the group consisting of:

a nucleic acid sequence comprising SEQ ID NO:3 and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:3;

a nucleic acid sequence comprising a fragment of SEQ ID NO:3 encoding at least 25 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:3;

a nucleic acid sequence at least 98% homologous to SEQ ID NO:3 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence at least 98% homologous to SEQ ID NO:3; and

a fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:3 encoding at least 25 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:3.

25. The composition of claim 23 comprising a nucleic acid coding sequence that encodes an RSV M2-1 immunogen comprising SEQ ID NO:4 and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:4.

26. The composition of claim 25 comprising a nucleic acid coding sequence that encodes an RSV M2-1 immunogen, the coding sequence comprising SEQ ID NO:3 and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:3.

27. The composition of claim 25 comprising a nucleic acid sequence that encodes SEQ ID NO:12.

28. The composition of claim 25 comprising a nucleic acid sequence comprising SEQ ID NO:11.

29. The composition of claim 23 comprising a nucleic acid coding sequence that encodes an RSV M2-1 immunogen comprising a fragment of SEQ ID NO:4 having at least 130 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:4, or a fragment of a protein that is at least 98% homologous to SEQ ID NO:4 having at least 130 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that encodes a protein least 98% homologous to SEQ ID NO:4.

30. The composition of claim 29 comprising a nucleic acid coding sequence that encodes an RSV M2-1 immunogen, comprising a fragment of SEQ ID NO:3 encoding at least 130 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:3, or a fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:3 encoding at least 130 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:3.

31. The composition of claim 23 comprising a nucleic acid coding sequence that encodes an RSV M2-1 immunogen comprising a fragment of SEQ ID NO:4 having at least 165 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide

operably linked to SEQ ID NO:4, or a fragment of a protein that is at least 98% homologous to SEQ ID NO:4 having at least 165 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that encodes a protein least 98% homologous to SEQ ID NO:4.

32. The composition of claim 31 comprising a nucleic acid coding sequence that encodes an RSV M2-1 immunogen, comprising a fragment of SEQ ID NO:3 encoding at least 165 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:3, or a fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:3 encoding at least 165 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:3.

33. The composition of claim 23 comprising a nucleic acid coding sequence that encodes the RSV M2-1 immunogen at least 99% homologous to SEQ ID NO:4 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence at least 99% homologous to SEQ ID NO:4.

34. The composition of claim 33 comprising nucleic acid coding sequence that encodes the RSV M2-1 immunogen, the coding sequence being at least 99% homologous to SEQ ID NO:3 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence at least 99% homologous to SEQ ID NO:3.

35. A composition of claim 23 comprising nucleic acid sequences selected from the group consisting of:

a nucleic acid coding sequence that encodes an RSV M2-1 immunogen,

a nucleic acid coding sequence that encodes an RSV F immunogen and a nucleic acid coding sequence that encodes an RSV M2-1 immunogen,

a nucleic acid coding sequence that encodes an RSV M2-1 immunogen and a nucleic acid coding sequence that encodes an RSV Ga immunogen,

a nucleic acid coding sequence that encodes an RSV M2-1 immunogen and a nucleic acid coding sequence that encodes an RSV Gb immunogen,

a nucleic acid coding sequence that encodes an RSV F immunogen, a nucleic acid coding sequence that encodes an RSV M2-1 immunogen and a nucleic acid coding sequence that encodes an RSV Ga immunogen,

a nucleic acid coding sequence that encodes an RSV F immunogen, a nucleic acid coding sequence that encodes an RSV M2-1 immunogen and a nucleic acid coding sequence that encodes an RSV Gb immunogen,

a nucleic acid coding sequence that encodes an RSV M2-1 immunogen, a nucleic acid coding sequence that encodes an RSV Ga immunogen and a nucleic acid coding sequence that encodes an RSV Gb immunogen, and

a nucleic acid coding sequence that encodes an RSV F immunogen, a nucleic acid coding sequence that encodes an RSV M2-1 immunogen, a nucleic acid coding sequence that encodes an RSV Ga immunogen and a nucleic acid coding sequence that encodes an RSV Gb immunogen.

36. The composition of any of claims 23-35 further comprising a nucleic acid coding sequence that encodes CCL20.

37. The composition of any of claims 23-35 formulated for delivery to an individual using electroporation.

38. The composition of any of claims 23-35 further comprising nucleic acid sequences that encode one or more proteins selected from the group consisting of: IL-12, IL-15 and IL-28.

39. The composition of any of claims 23-35 wherein said one or more nucleic acid coding sequences are part of one or more plasmids.

40. The composition of any of claims 23-35 wherein each of the one or more nucleic acid coding sequences are part of incorporated into a separate plasmid.

41. A method of inducing an immune response against RSV comprising administering the composition of any of claims 23-35 to an individual in an amount effective to induce an immune response in said individual.

42. A method of treating an individual who has been diagnosed with RSV comprising administering a therapeutically effective amount of the composition of any of claims 23-35 to an individual.

43. A method of preventing RSV infection an individual comprising administering a prophylactically effective amount of the composition of any of claims 23-35 to an individual.

44. A protein selected from the group consisting of: SEQ ID NO:4 and SEQ ID NO:12.

Figure 1

Phylogenetic Tree of RSV-F Protein

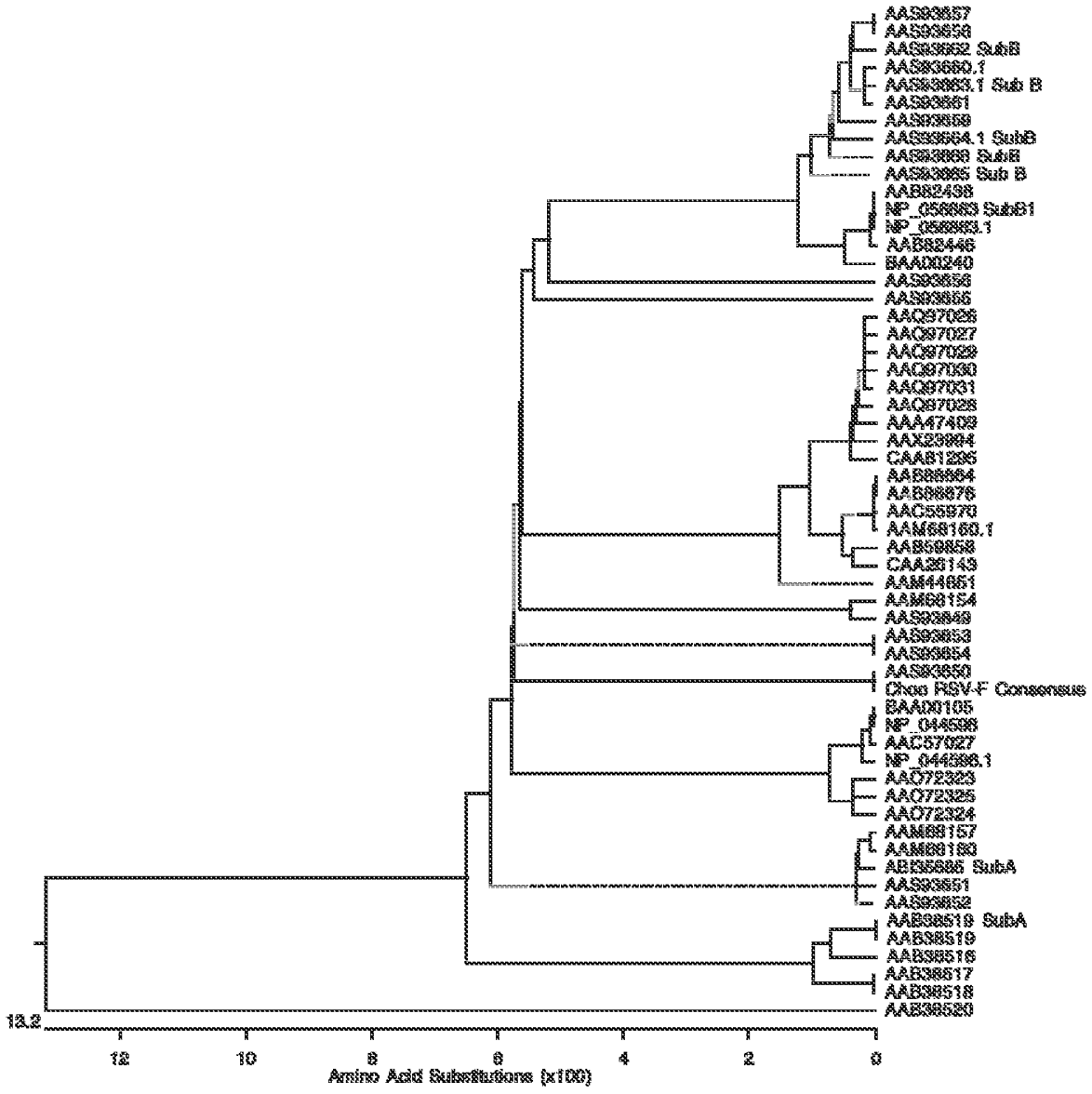


Figure 2

Phylogenetic Tree of RSV-M2 Protein

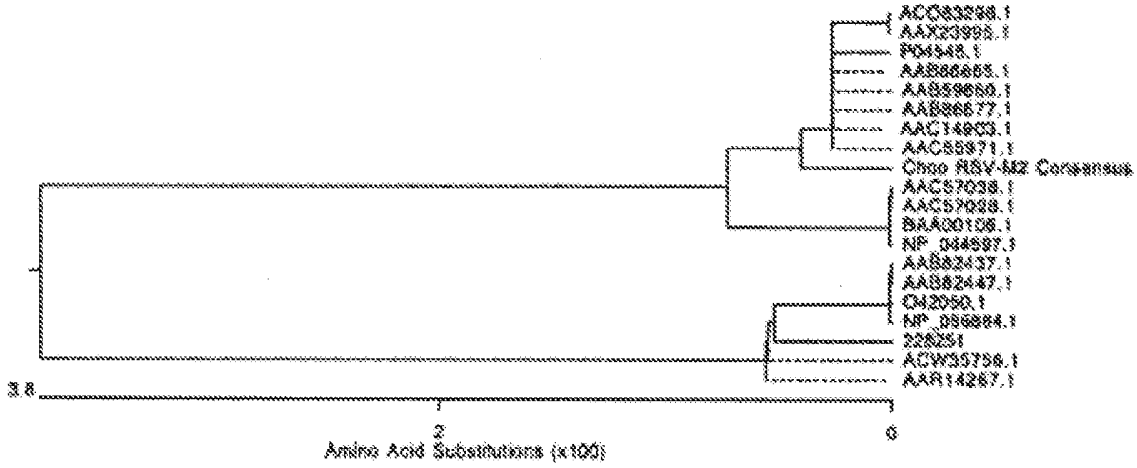


Figure 3

Comparison of total anti-RSV-F Protein IgG in Sera

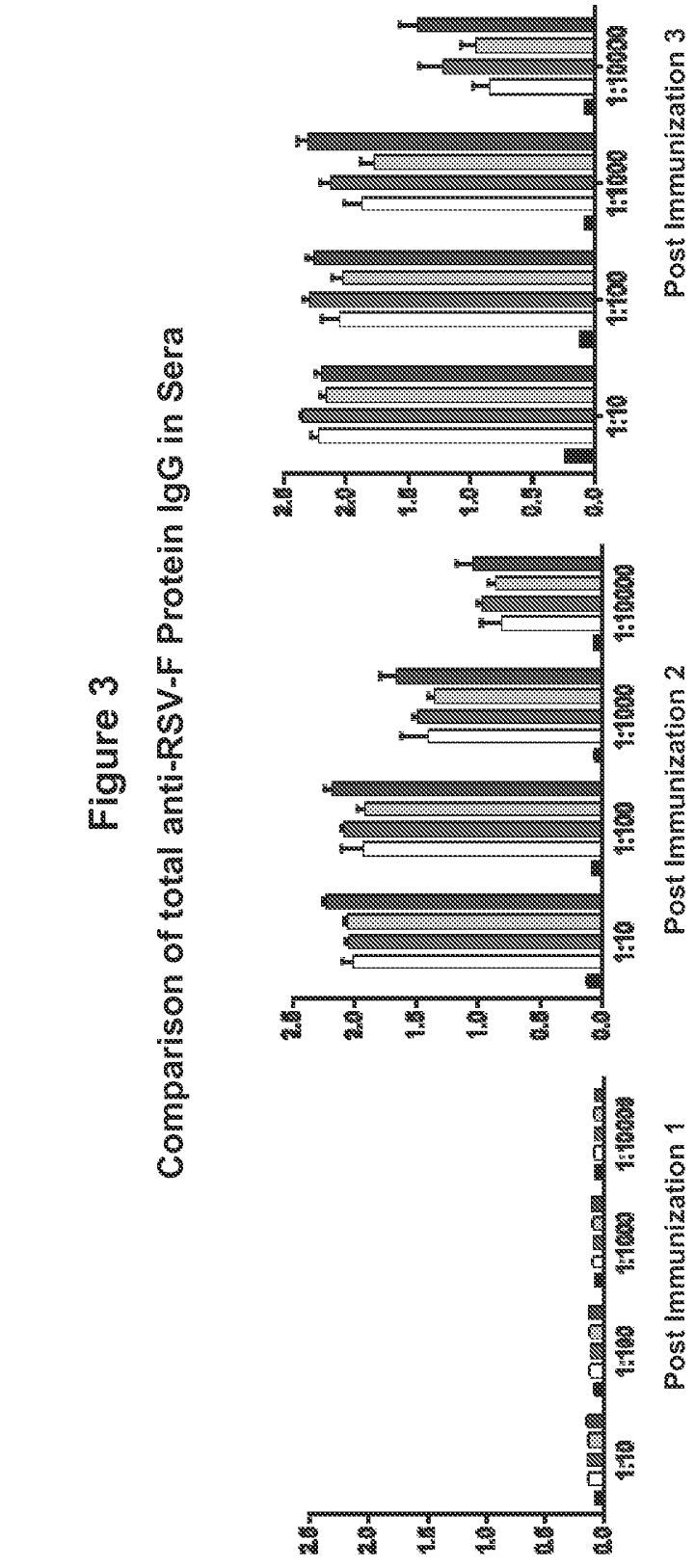


Figure 4

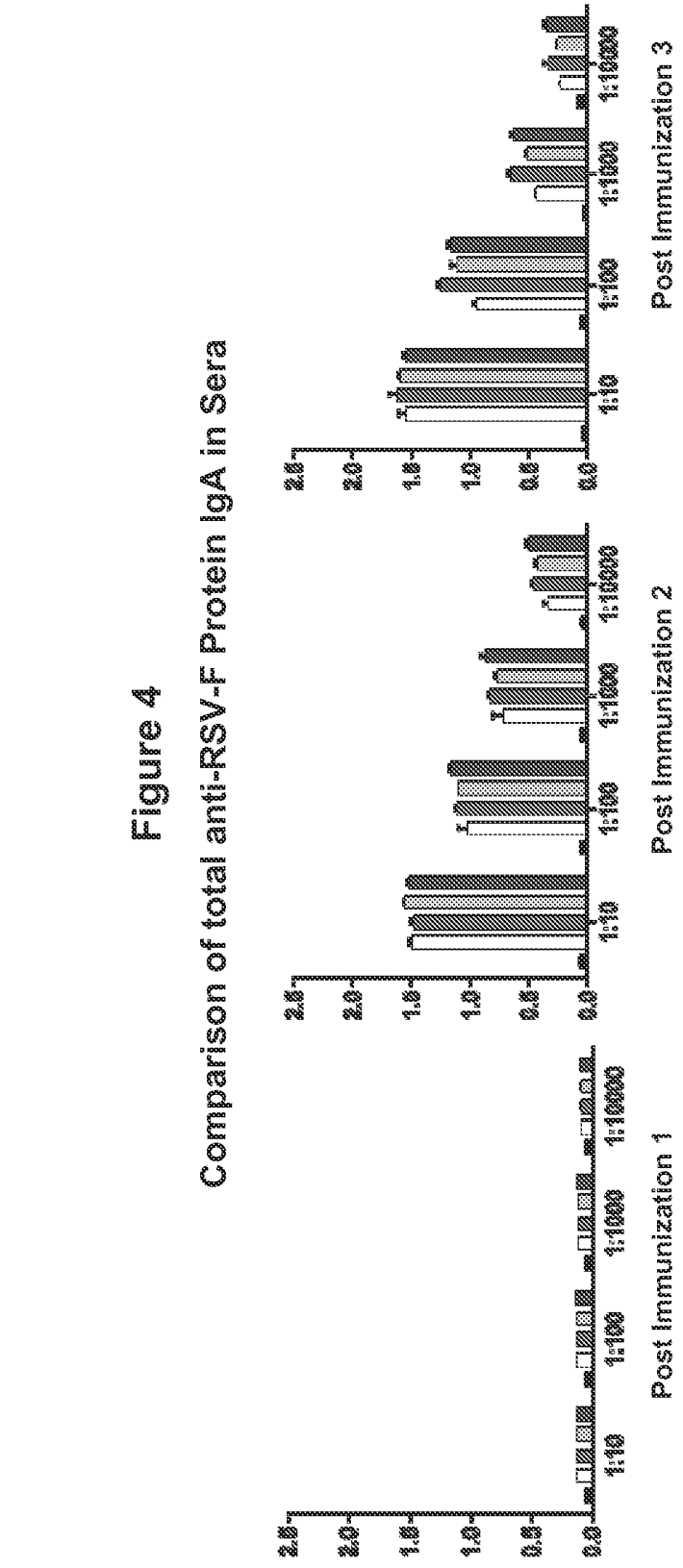

Comparison of total anti-RSV-F Protein IgA in Sera
Post Immunization 1
Post Immunization 2
Post Immunization 3
2.5
2.0
1.5
1.0
0.5
0.0
1:10
1:100
1:1000
1:10000

Figure 5

Comparison of IFN-g production at different dosage of RSV-F vaccine approximately 8 weeks after last immunization

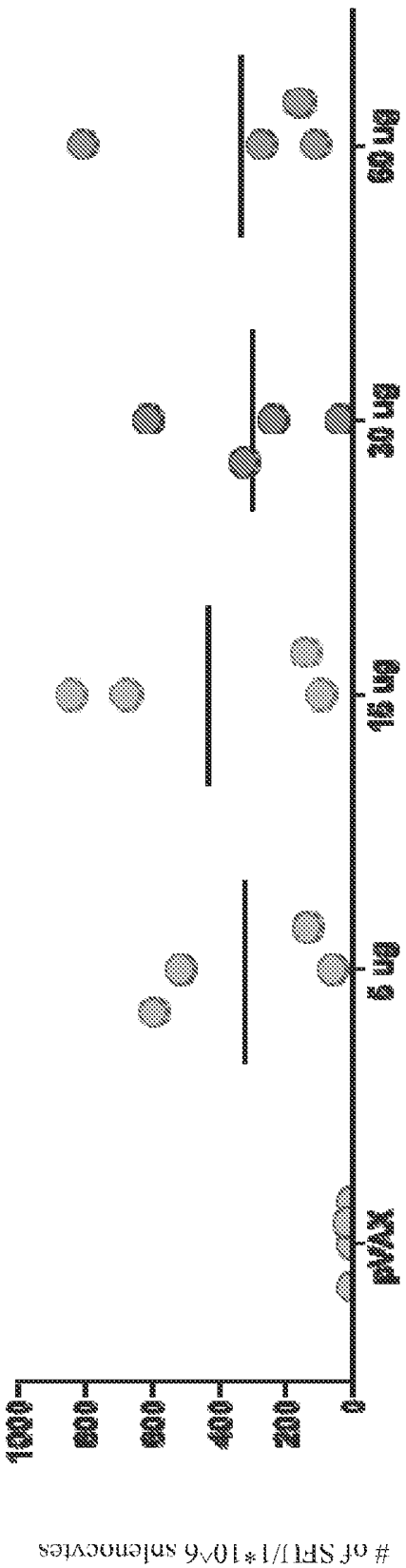


Figure 6

Comparison of IFN-γ+ CD8 or CD4 T cells in spleen after RSV-F +/- chemokine immunization

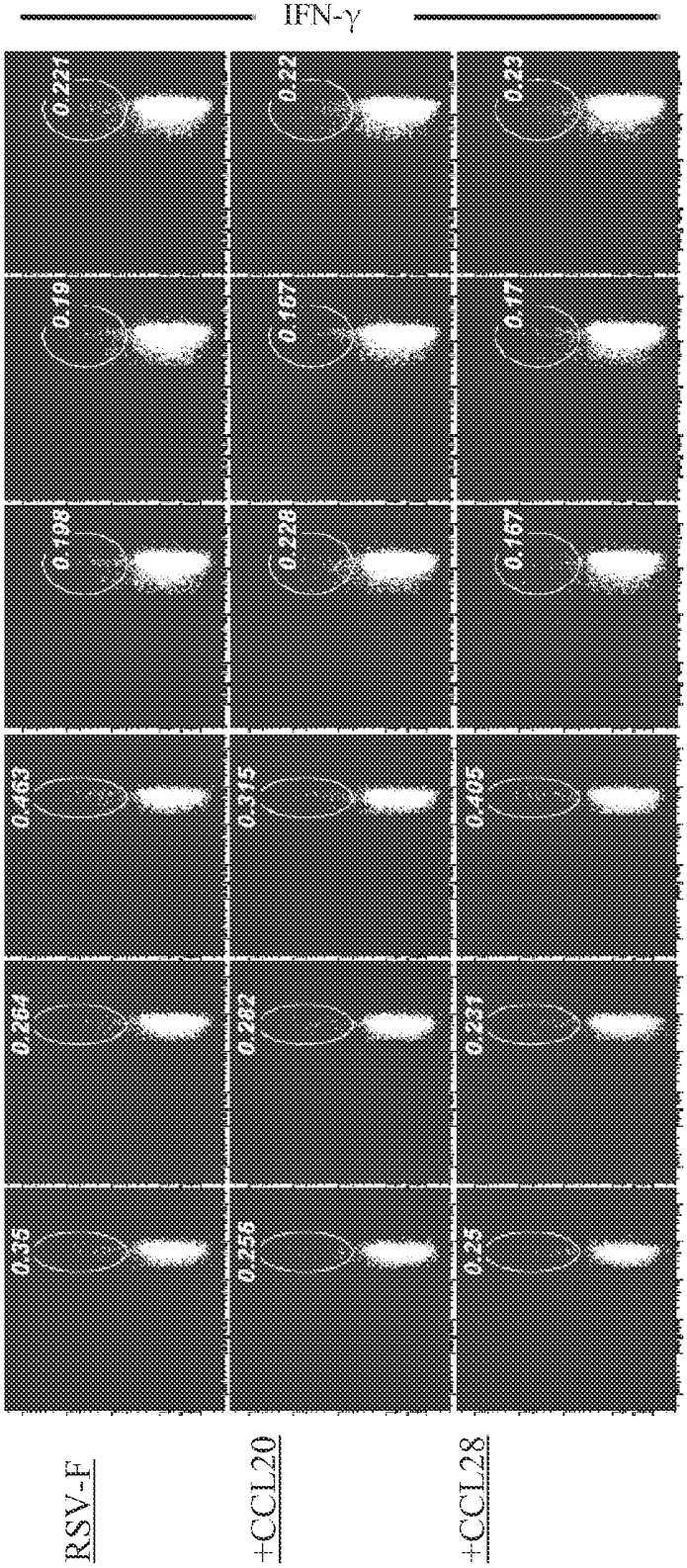


Figure 7

Comparison of IFN-γ+ CD8 or CD4 T cells in lung after RSV-F +/- chemokine immunization

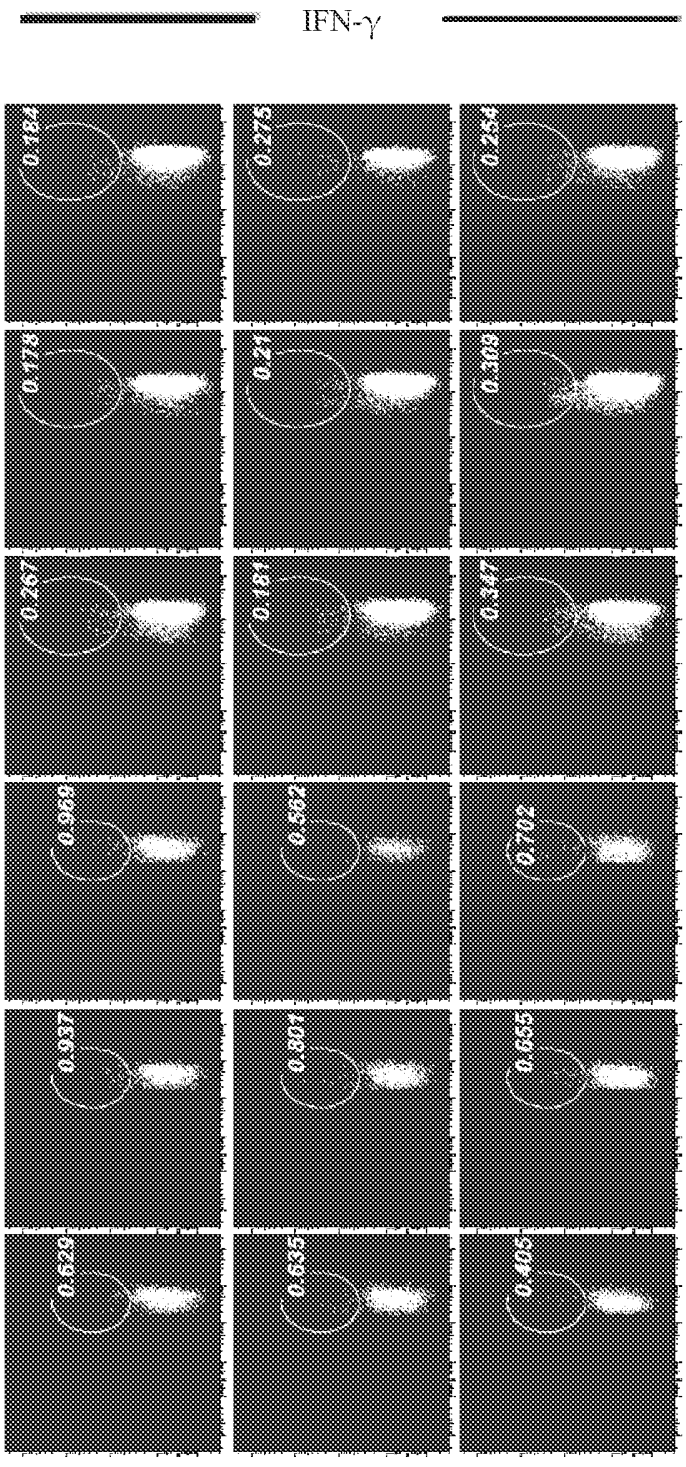


Figure 8
Comparison of IgG subtypes (IgG1 vs IgG2a) in Sera

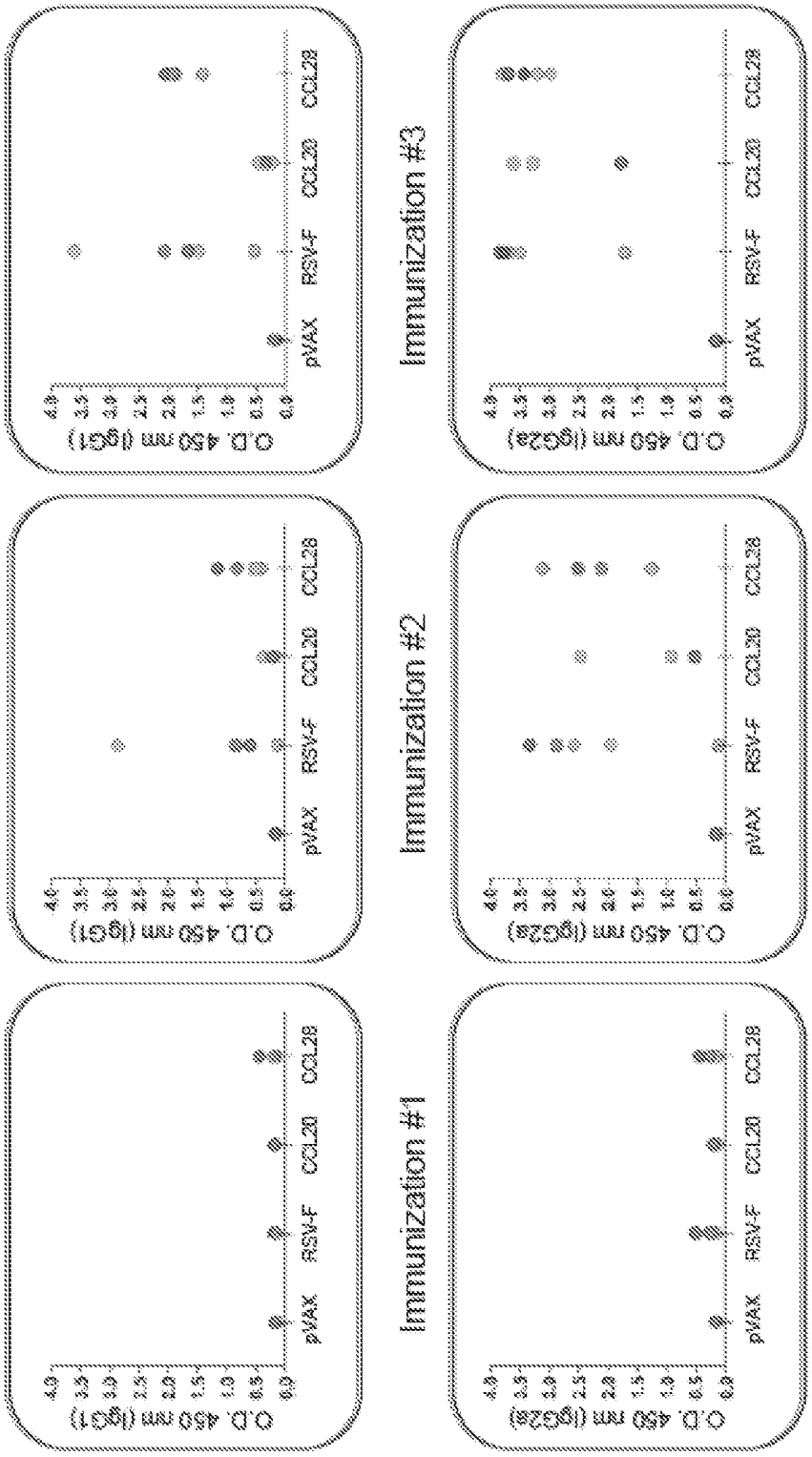


Figure 9

Comparison of IgG2a/IgG Ratio

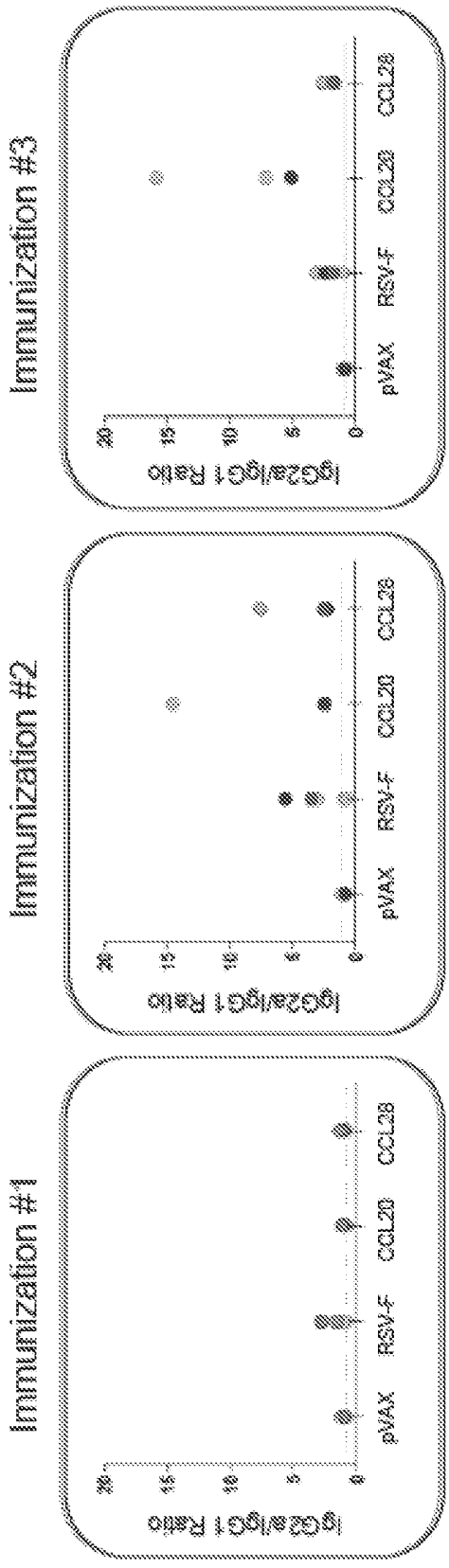


Figure 10

Env-Pseudotyped viruses in TZM-BL Cells/ "ON"

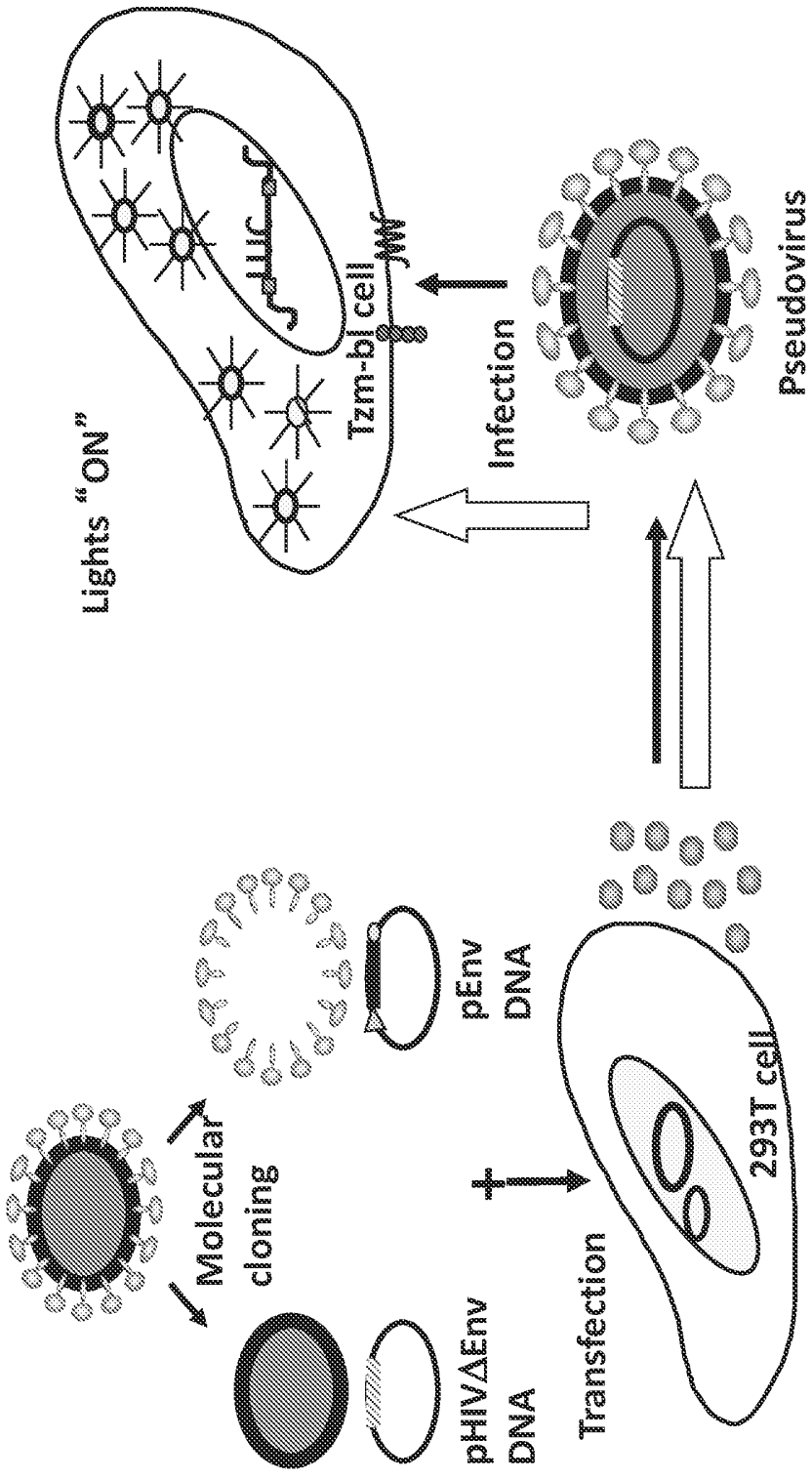


Figure 11

Env-Pseudotyped viruses in TZM-BL Cells/ "OFF"

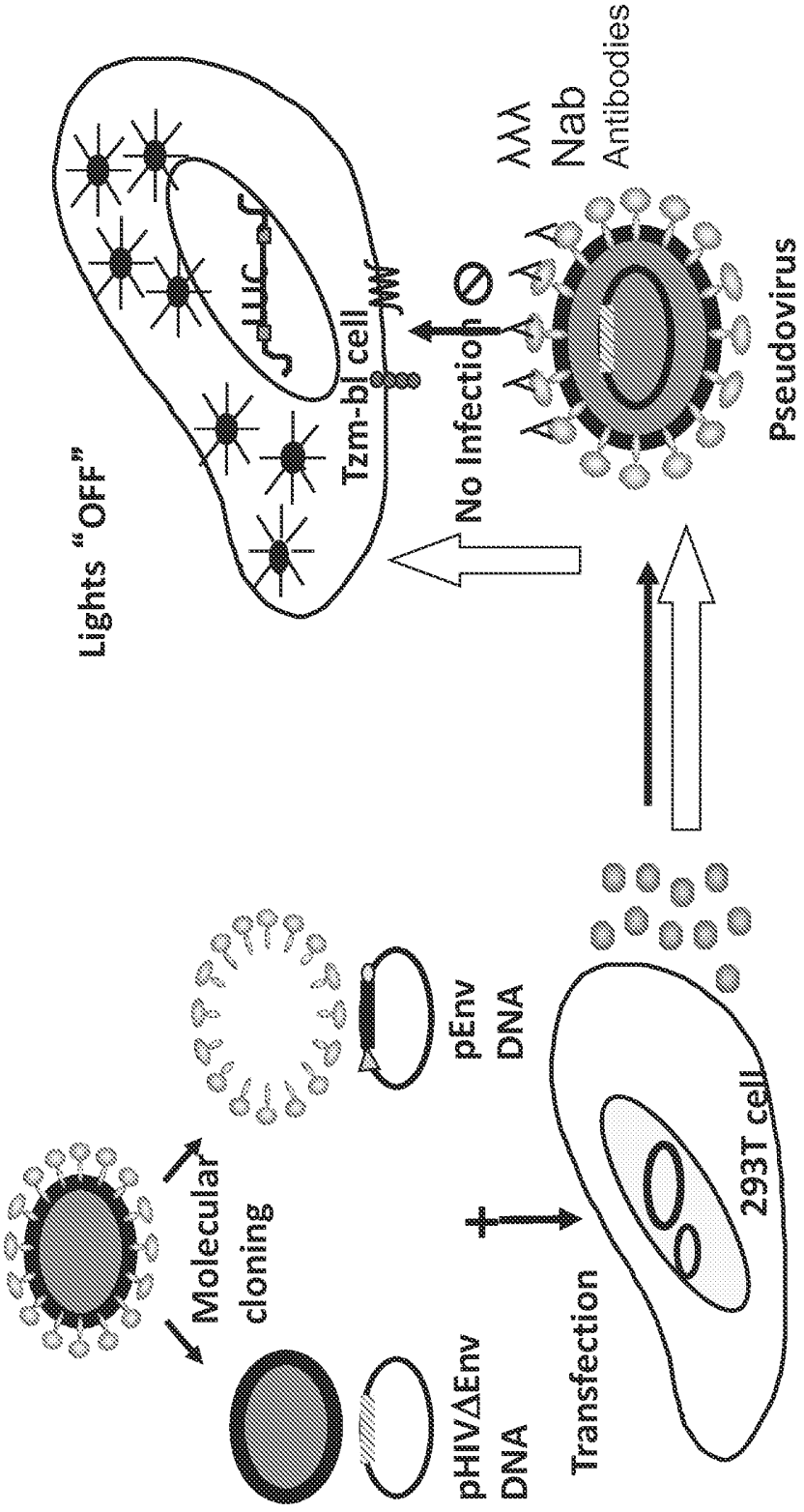


Figure 12

RSV-Fusion Pseudo viral production in 293T cells

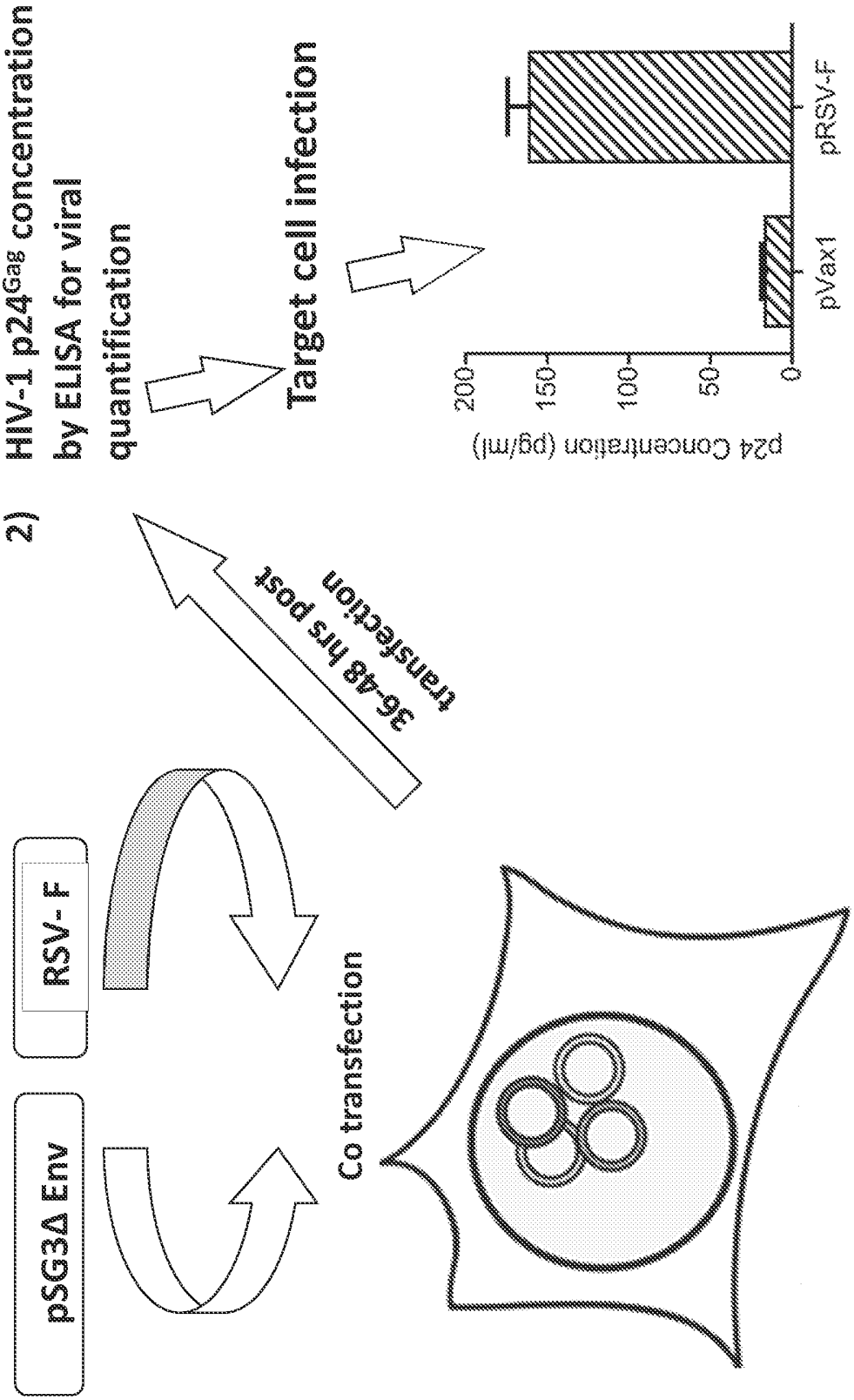


Figure 13
Immunization Schedule for RSV-F Neutralization Study

Figure 14

Quantification of RSV-F mediated infection in different target cells

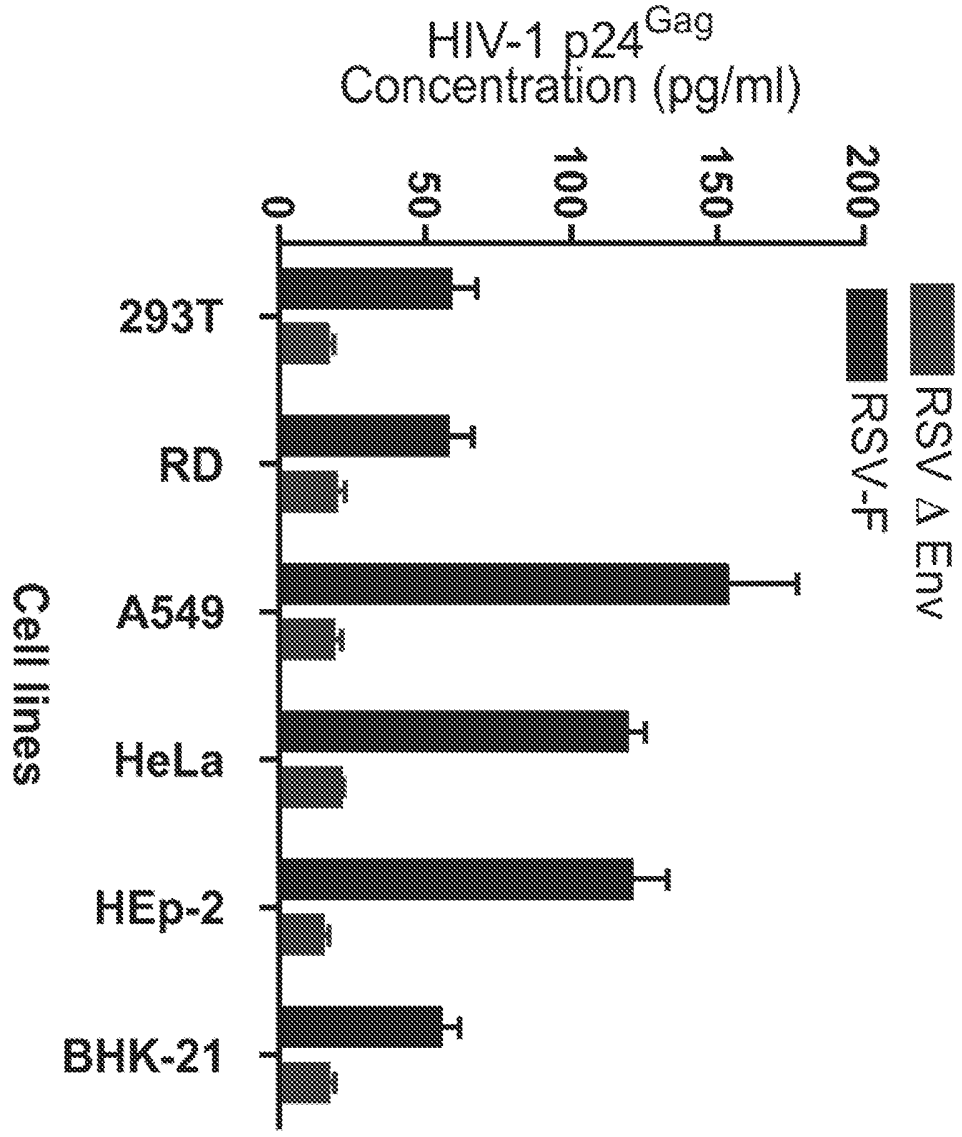


Figure 15

Quantification of RSV-F neutralization activity in TZM-bl HeLa cells

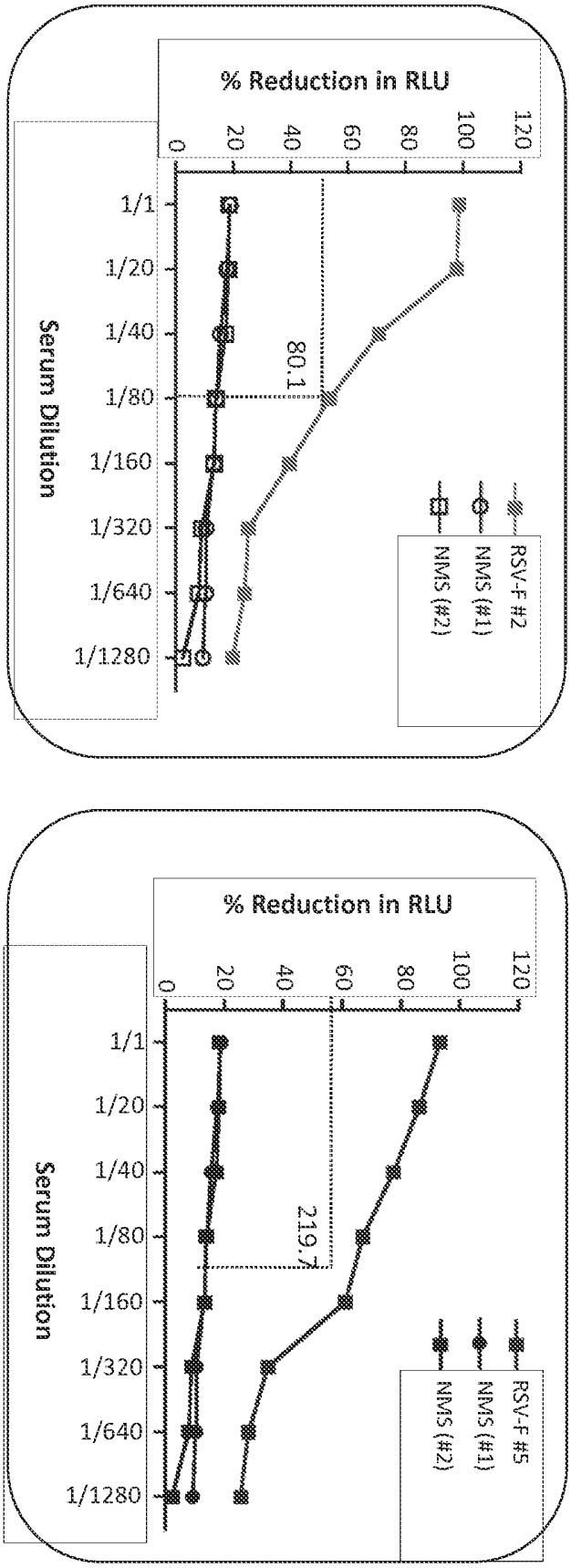


Figure 16
Quantification of cell death activity by RSV-F pseudo virus in HeLa cells

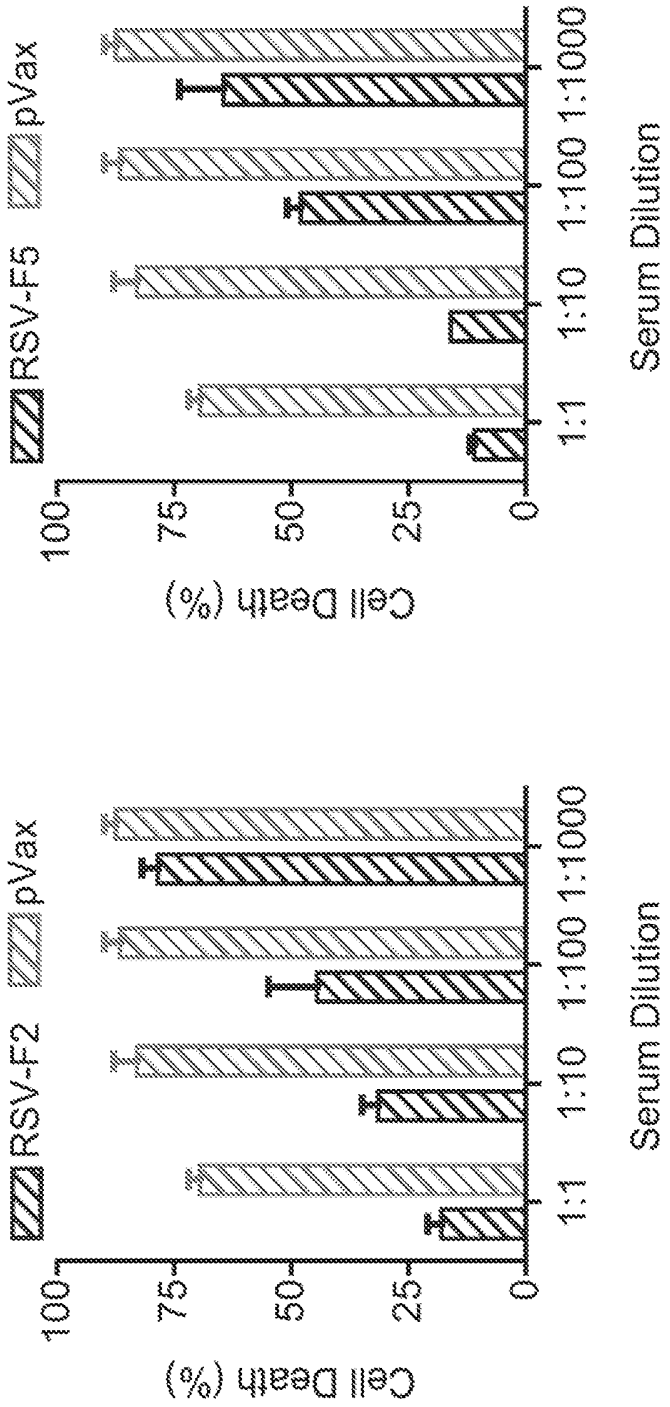


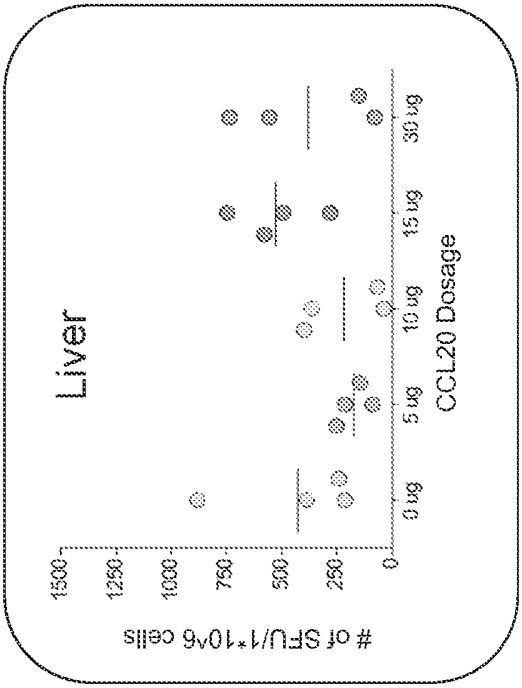
Figure 17
Immunization Schedule for RSV-M2 Dosage Study

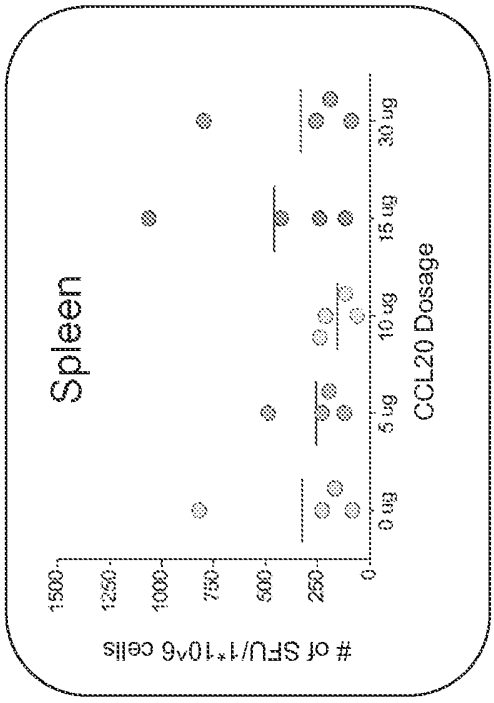
Figure 18
Comparison of IFN-g production at different dosages of RSV-M2 vaccine 1 week after last immunization

Figure 19
Immunization Schedule for RSV-F + CCL20 Study

Groups:
1.RSV-F (5 ug) alone
2.RSV-F (5 ug) + CCL20 (5 ug)
3.RSV-F (5 ug) + CCL20 (10 ug)
4.RSV-F (5 ug) + CCL20 (15 ug)
5.RSV-F (5 ug) + CCL20 (30 ug)

Figure 20
Comparison of IFN-g production with different dosages of CCL20 at 1 week after last immunization





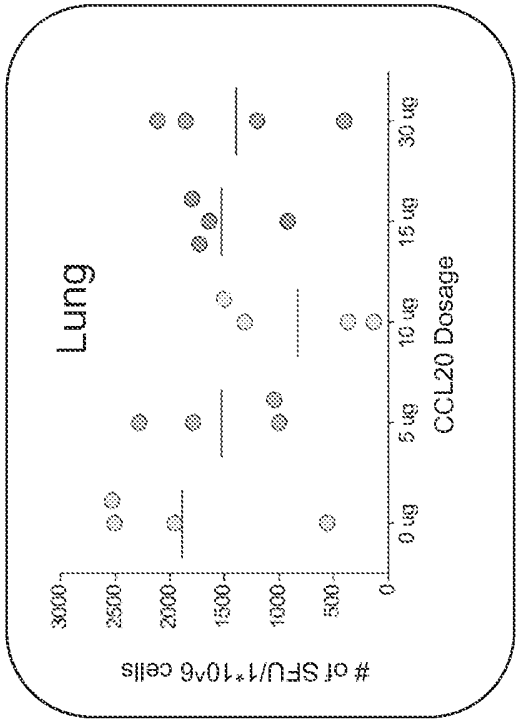


Figure 21
Comparison of IgG subtypes (IgG1 vs IgG2a) in Sera

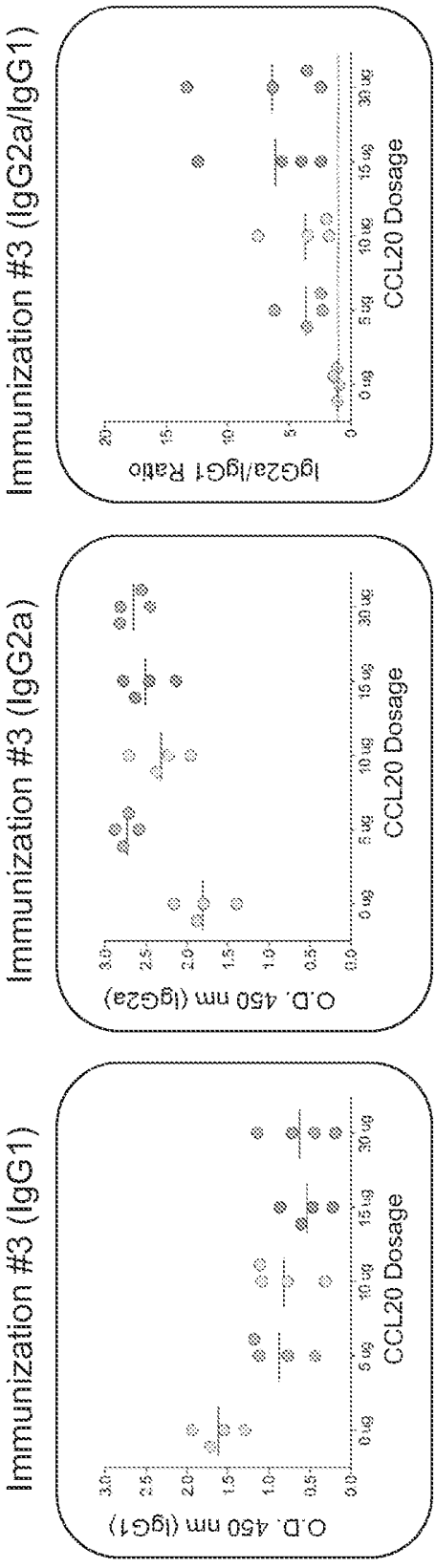


Figure 22A

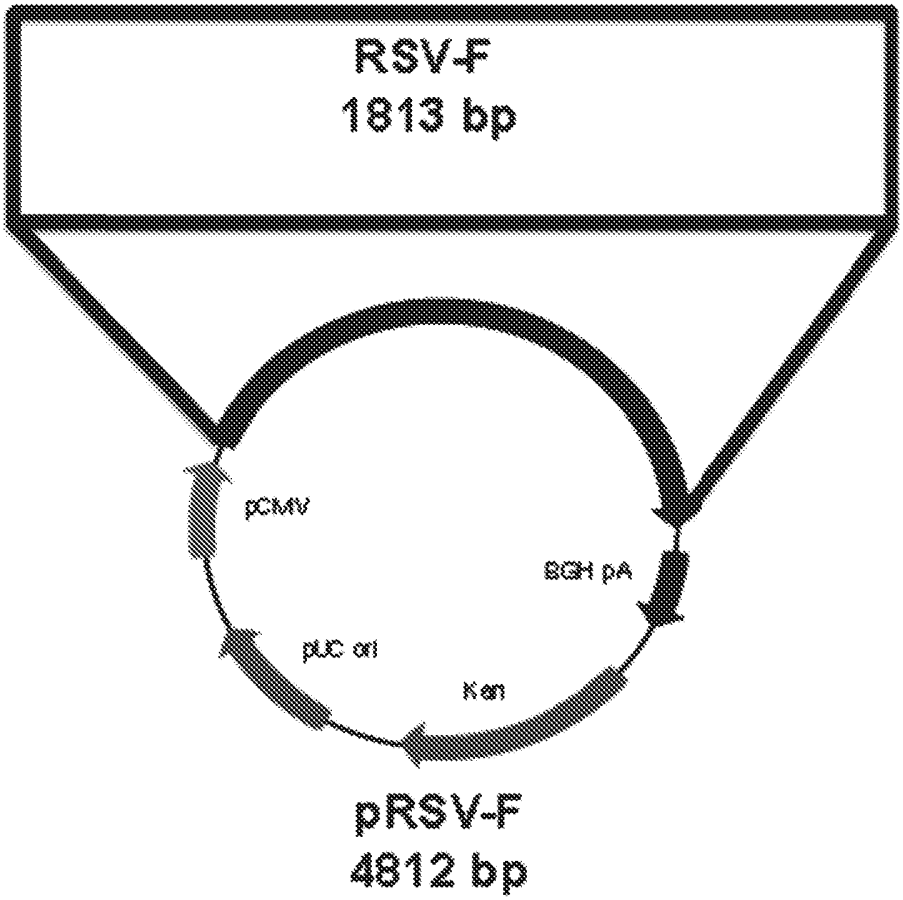
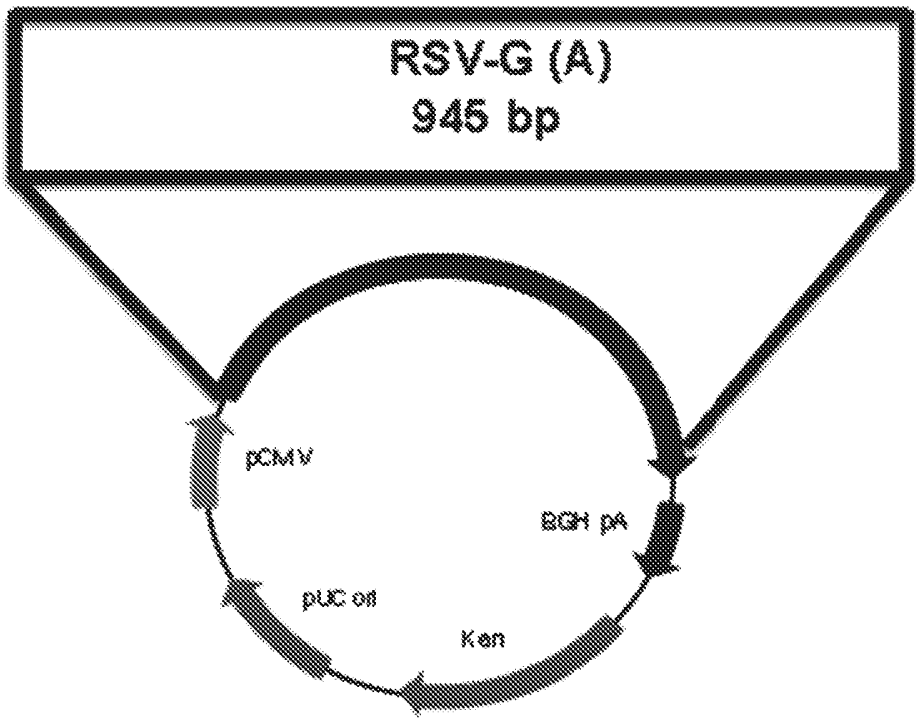


Figure 22B



pRSV-G (subtype A)
3944 bp

Figure 22C

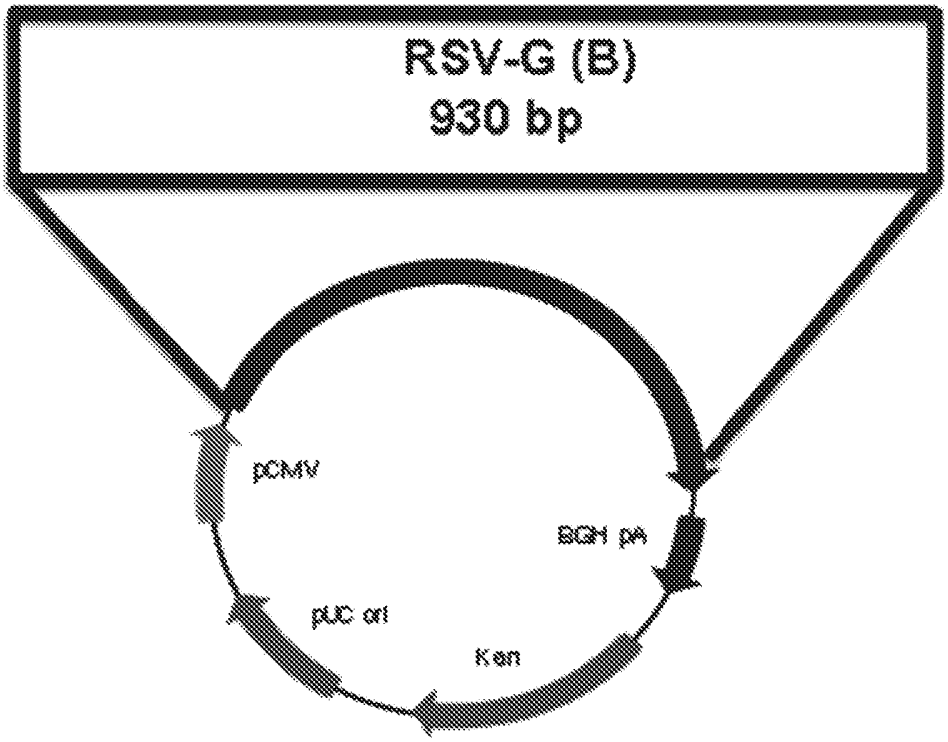


Figure 23

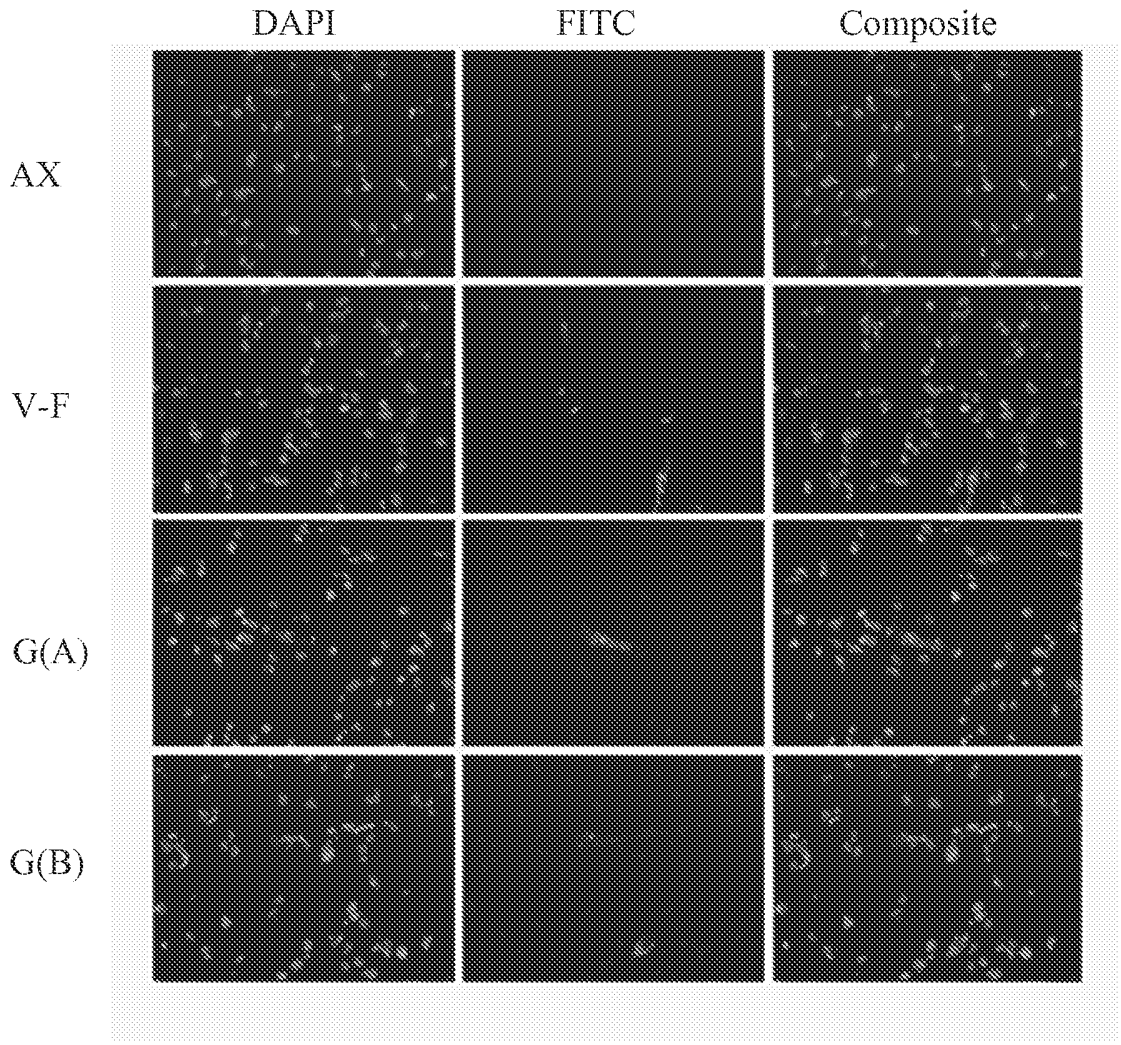


Figure 24

Immunization Schedule for RSV-F Dosage Study

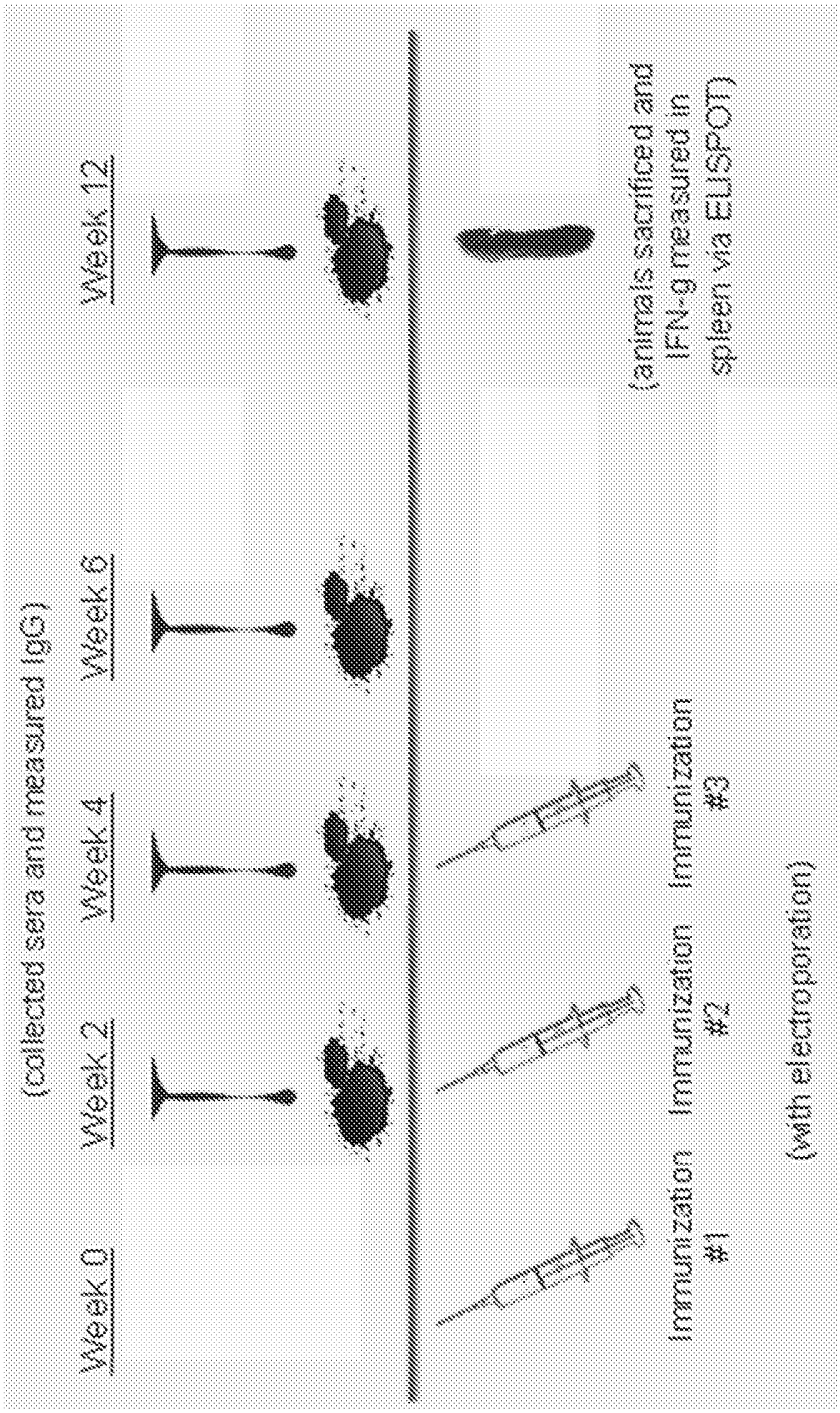


Figure 25

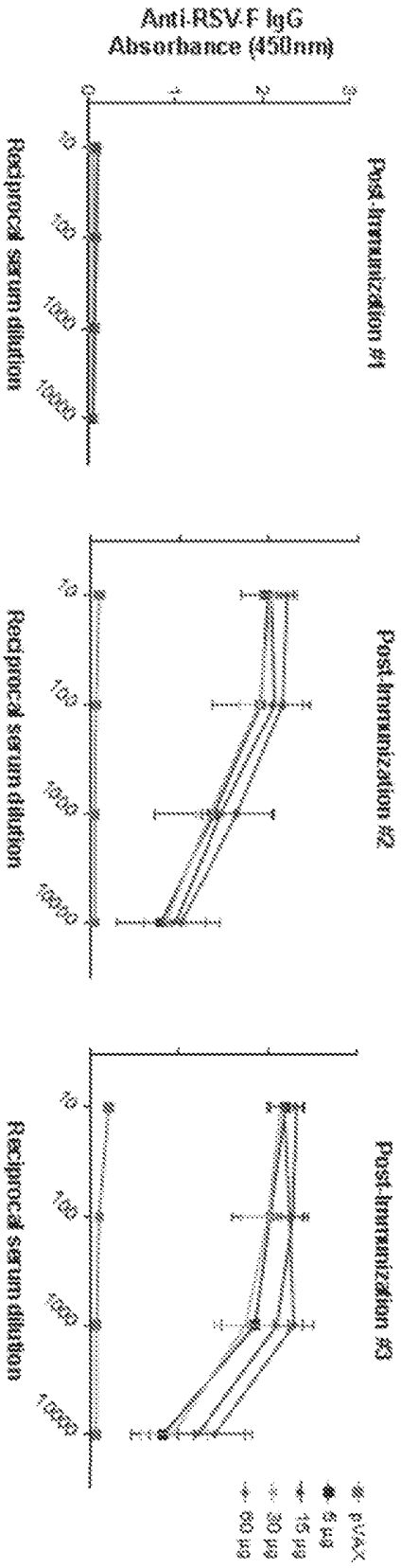

Anti-RSV-F IgG
Absorbance (450nm)
Post-Immunization #1
Post-Immunization #2
Post-Immunization #3
Reciprocal serum dilution
10
100
1000
10000
100000
pVAX
1 µg
5 µg
25 µg
50 µg

Figure 26

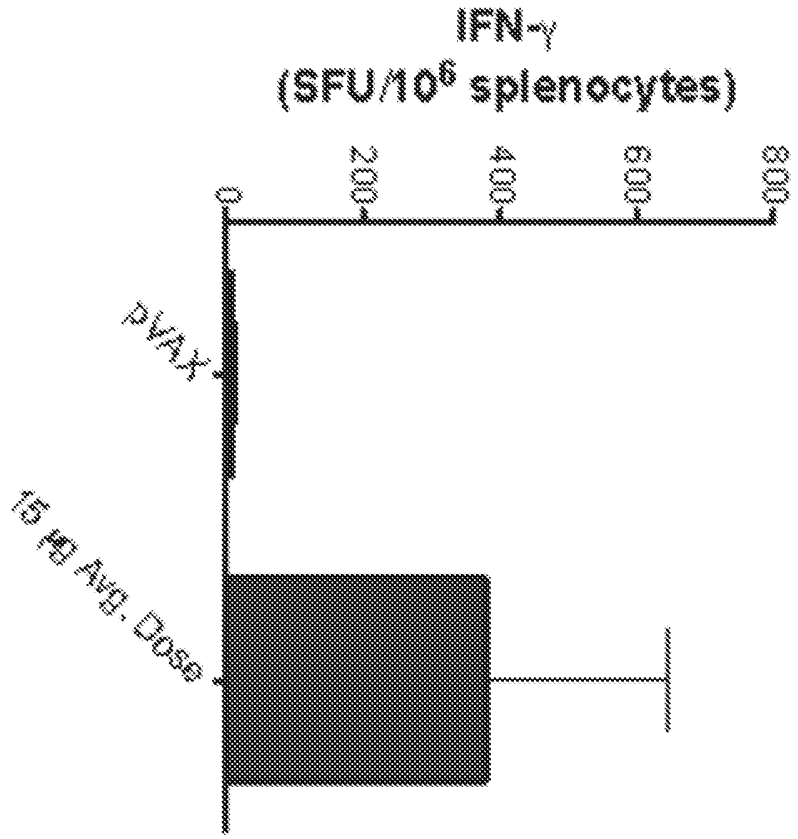


Figure 27

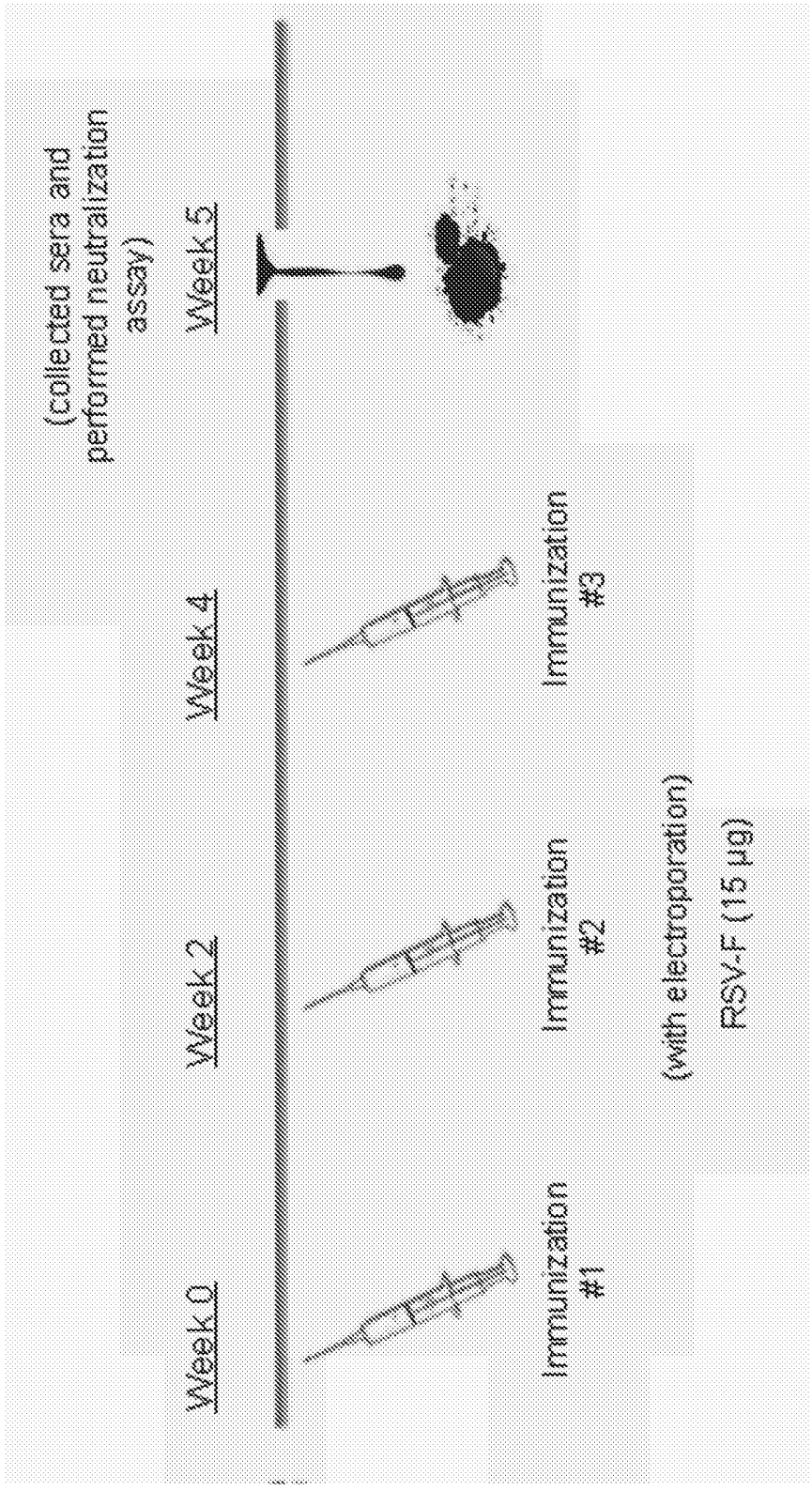

Week 0
Week 2
Week 4
Week 5
(collected sera and performed neutralization assay)
Immunization #1
Immunization #2
Immunization #3
(with electroporation)
RSV-F (15 µg)

Figure 28

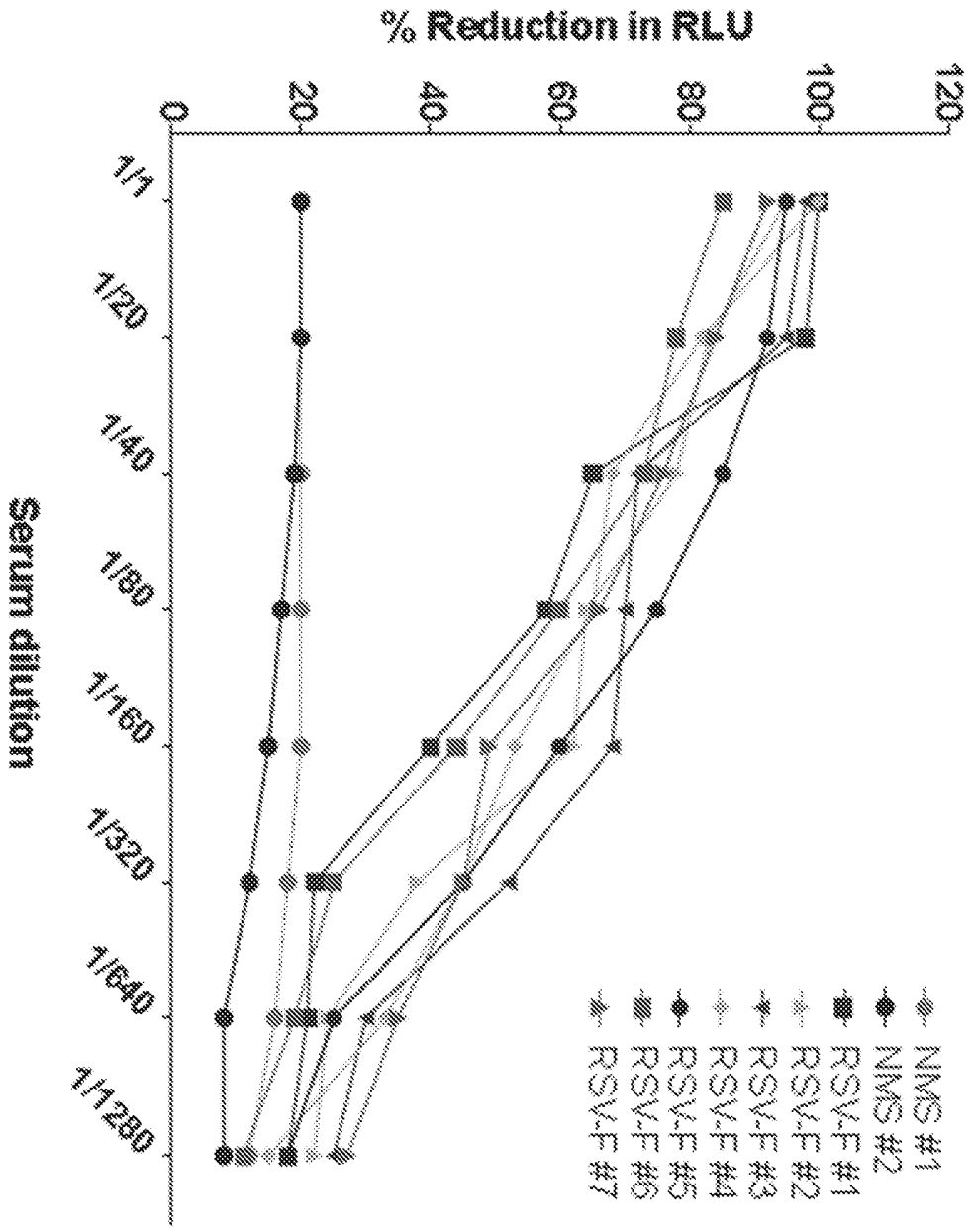

% Reduction in RLU
0
20
40
60
80
100
120
Serum dilution
1/1
1/20
1/40
1/80
1/160
1/320
1/640
1/1280
NMS #1
NMS #2
RSV-F #1
RSV-F #2
RSV-F #3
RSV-F #4
RSV-F #5
RSV-F #6
RSV-F #7

Figure 29

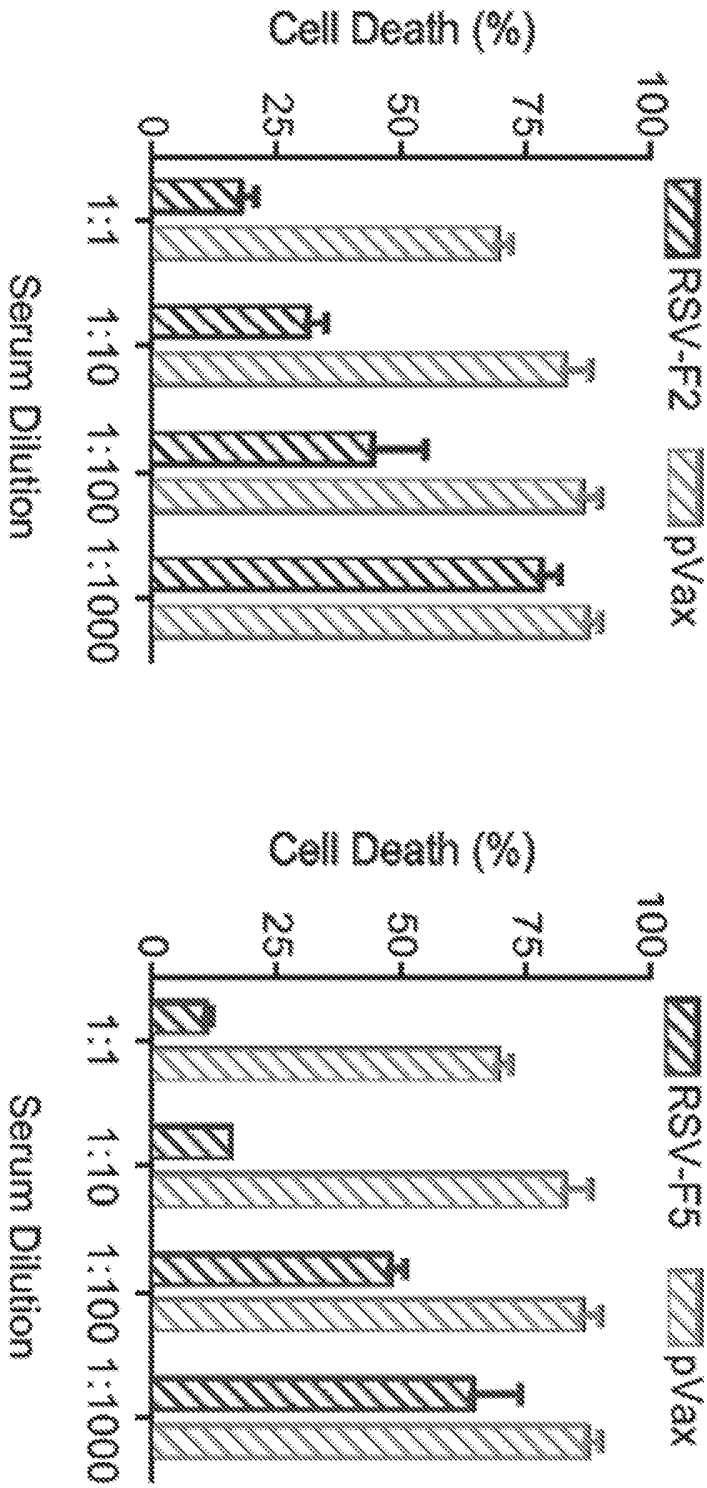

Cell Death (%)
0
25
50
75
100
1:1
1:10
1:100
1:1000
Serum Dilution
RSV-F2
pVax
Cell Death (%)
0
25
50
75
100
1:1
1:10
1:100
1:1000
Serum Dilution
RSV-F5
pVax

Figure 30

Immunization Schedule for RSV-G(A) and RSV-(B) Dosage Study

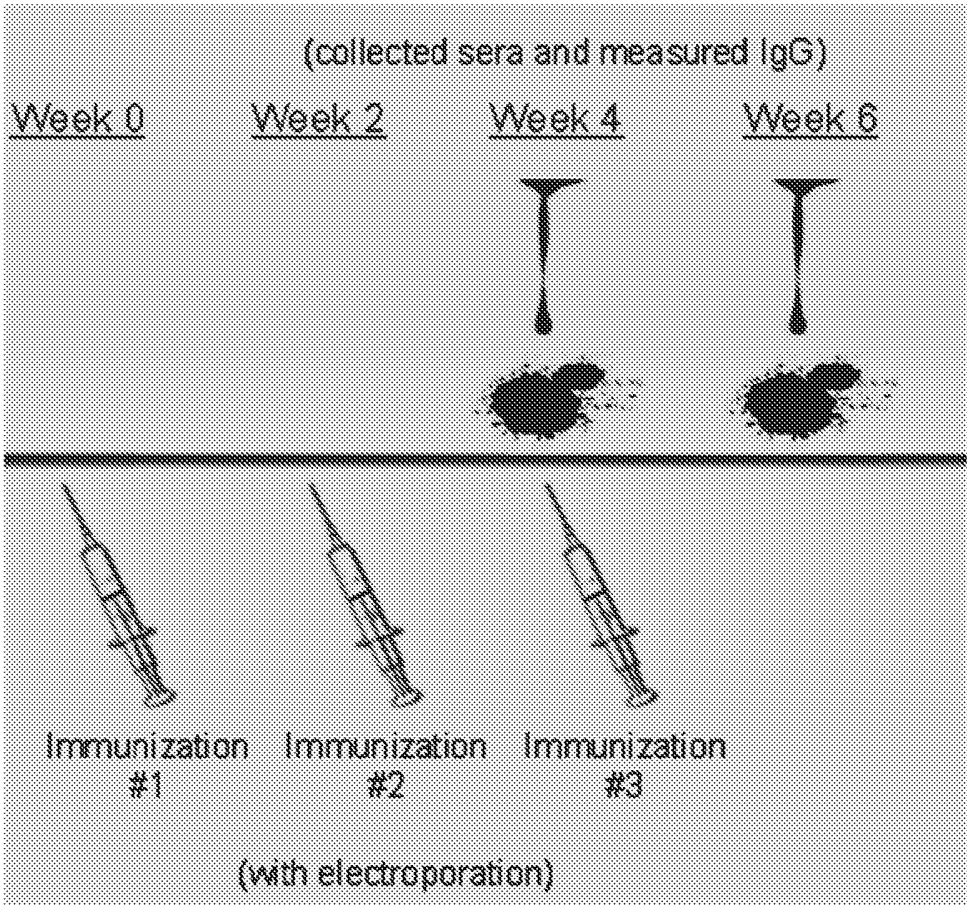


Figure 31

Total IgG in Sera after RSV-G(A) DNA immunization

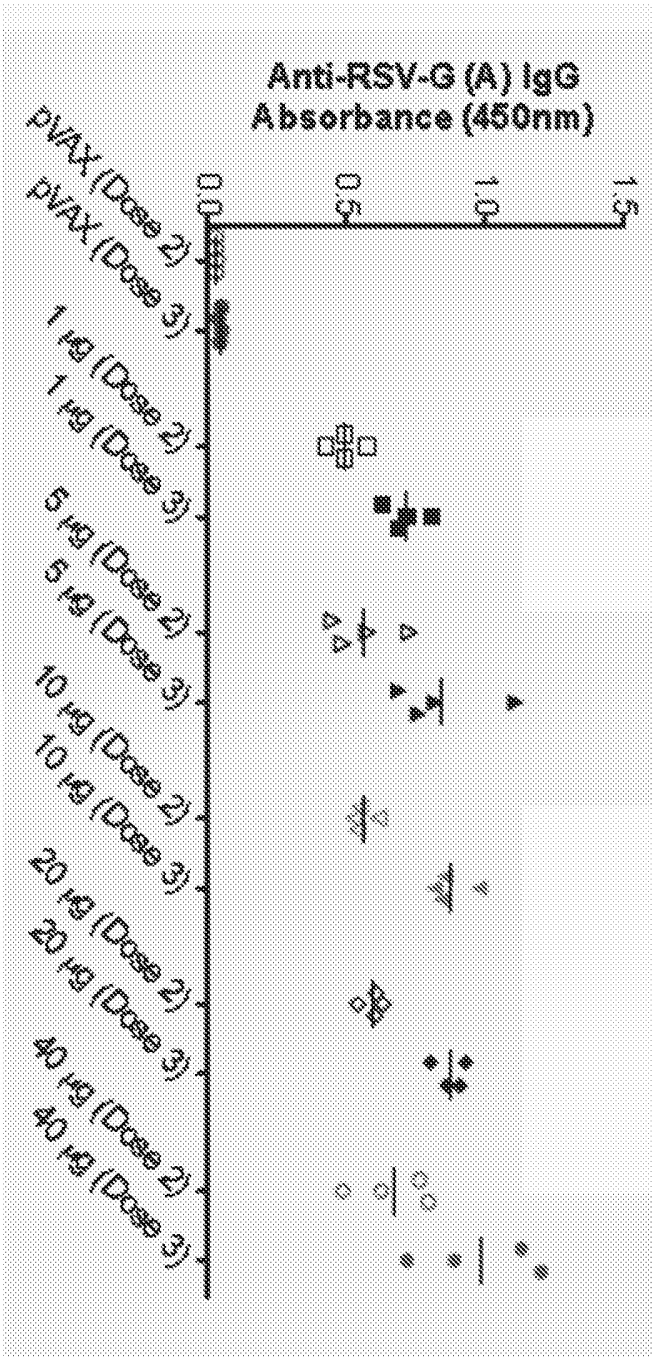


Figure 32

Total IgG in Sera after RSV-G(B) DNA immunization

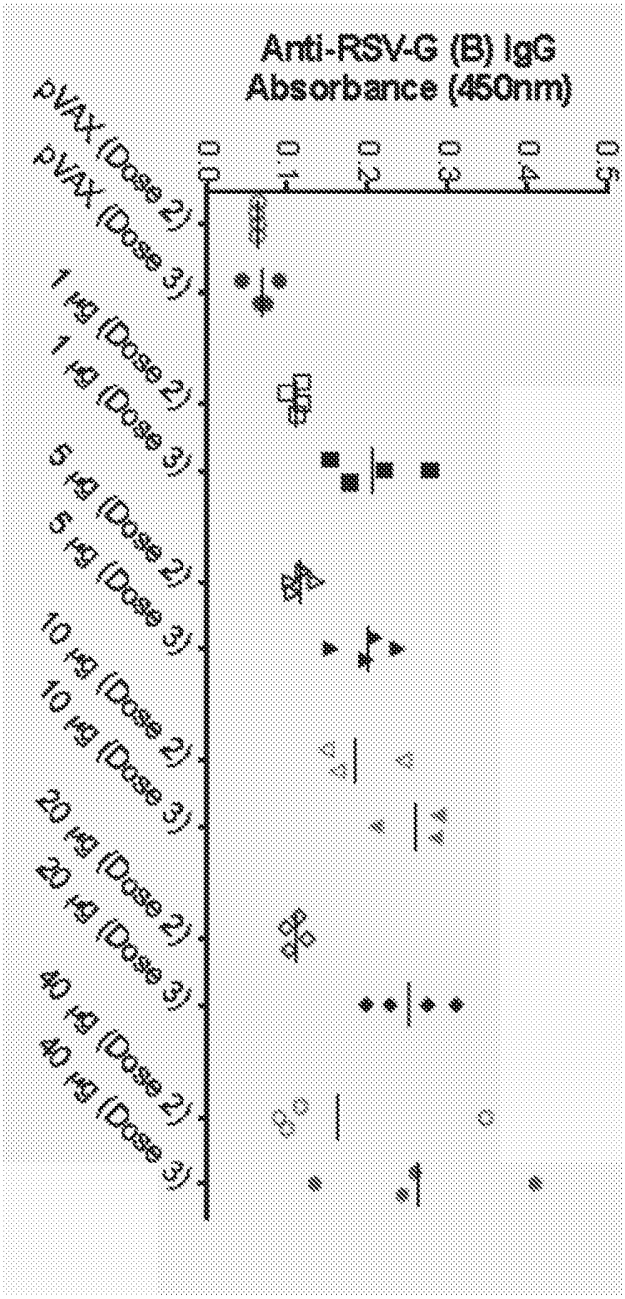


Figure 33

Preliminary Neutralization Results from Mono Vaccine Mouse Studies

Sample Tested	Neut Titer Post 3d Imm
Negative serum	0
Mab anti RSV	40
Polyclonal Serum	160
Mouse 1-F Ag	320
Mouse 2-F Ag	160
Mouse 3- GA	80
Mouse 4 - GA	320
Mouse 14 - GB	160

Figure 34

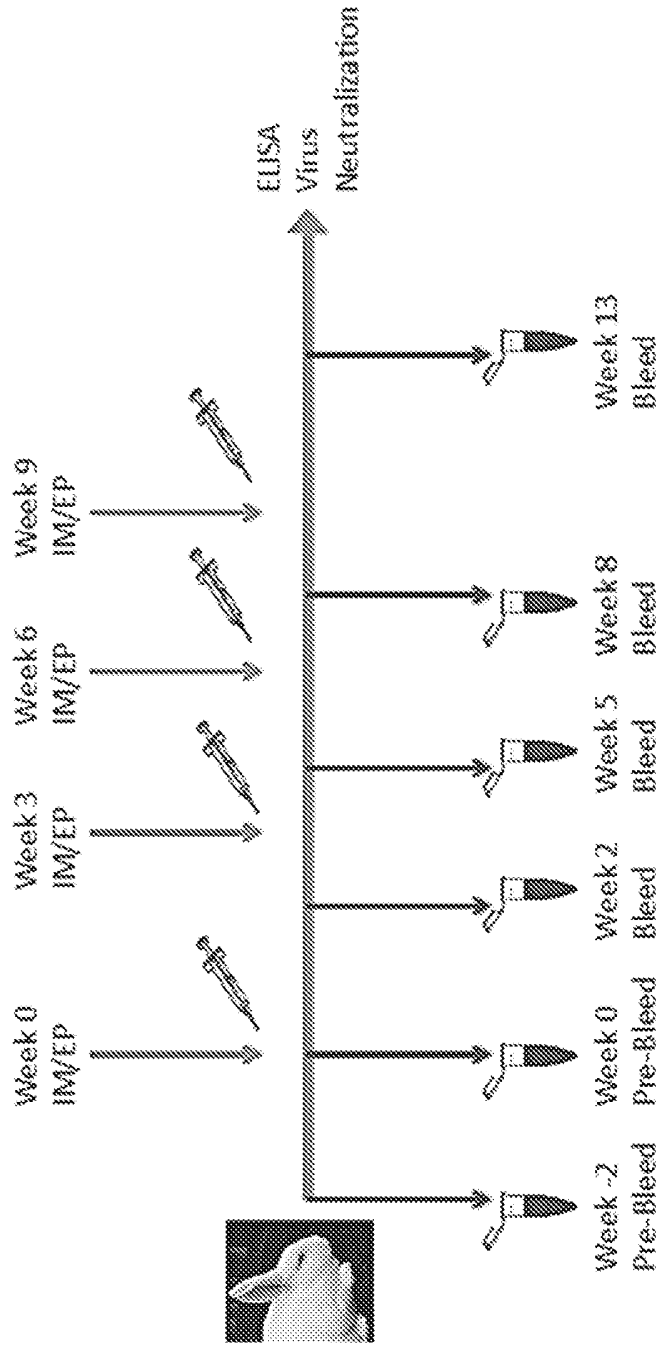

Week 0
IM/EP
Week 3
IM/EP
Week 6
IM/EP
Week 9
IM/EP
ELISA
Virus
Neutralization
Week -2
Pre-Bleed
Week 0
Pre-Bleed
Week 2
Bleed
Week 5
Bleed
Week 8
Bleed
Week 13
Bleed

Figure 35

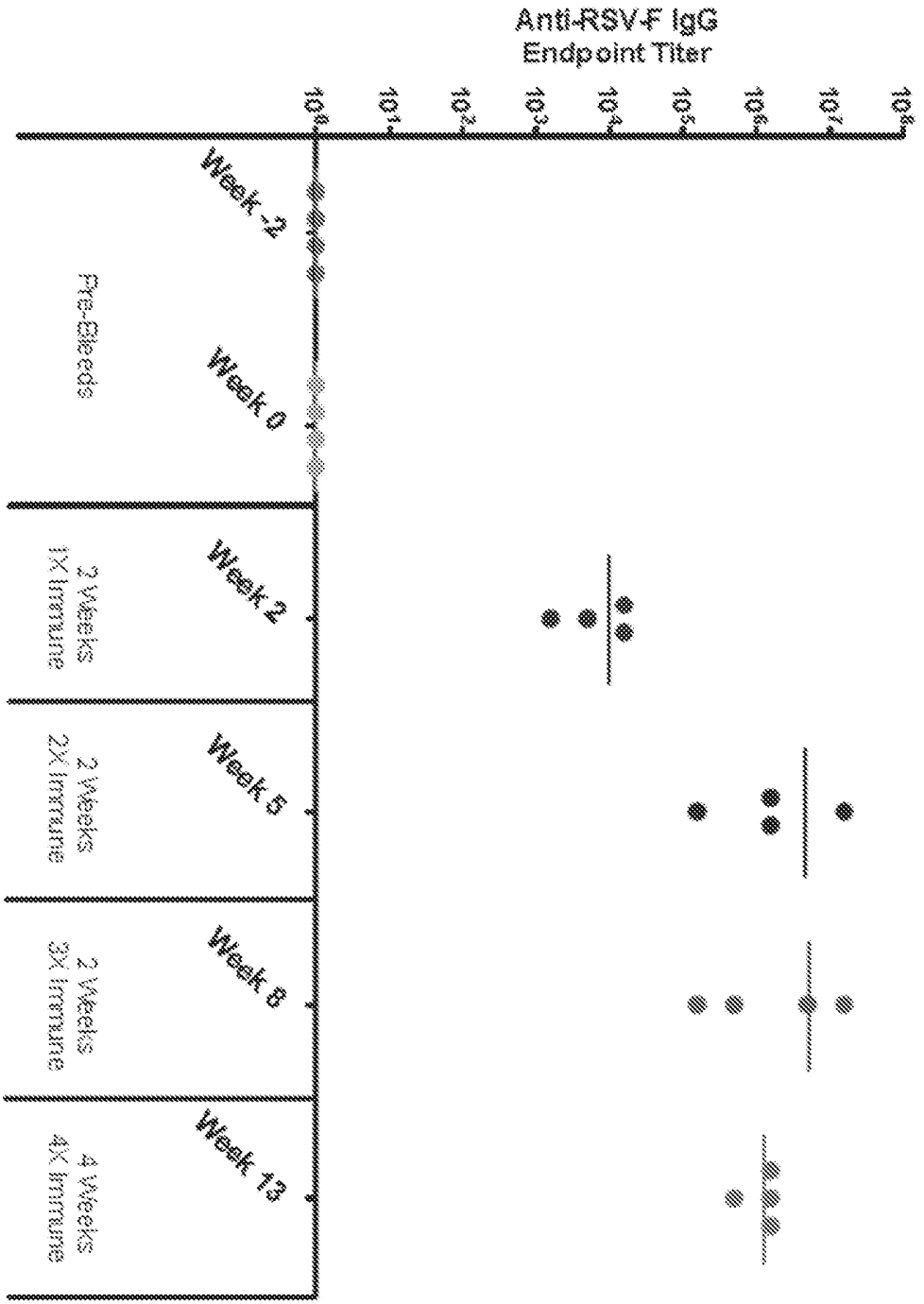

Anti-RSV-F IgG
Endpoint Titer
10^0
10^1
10^2
10^3
10^4
10^5
10^6
10^7
10^8
Week -2
Week 0
Week 2
Week 5
Week 6
Week 13
Pre-Bleeds
2 Weeks
1X Immune
2 Weeks
2X Immune
2 Weeks
3X Immune
4 Weeks
4X Immune

Figure 36

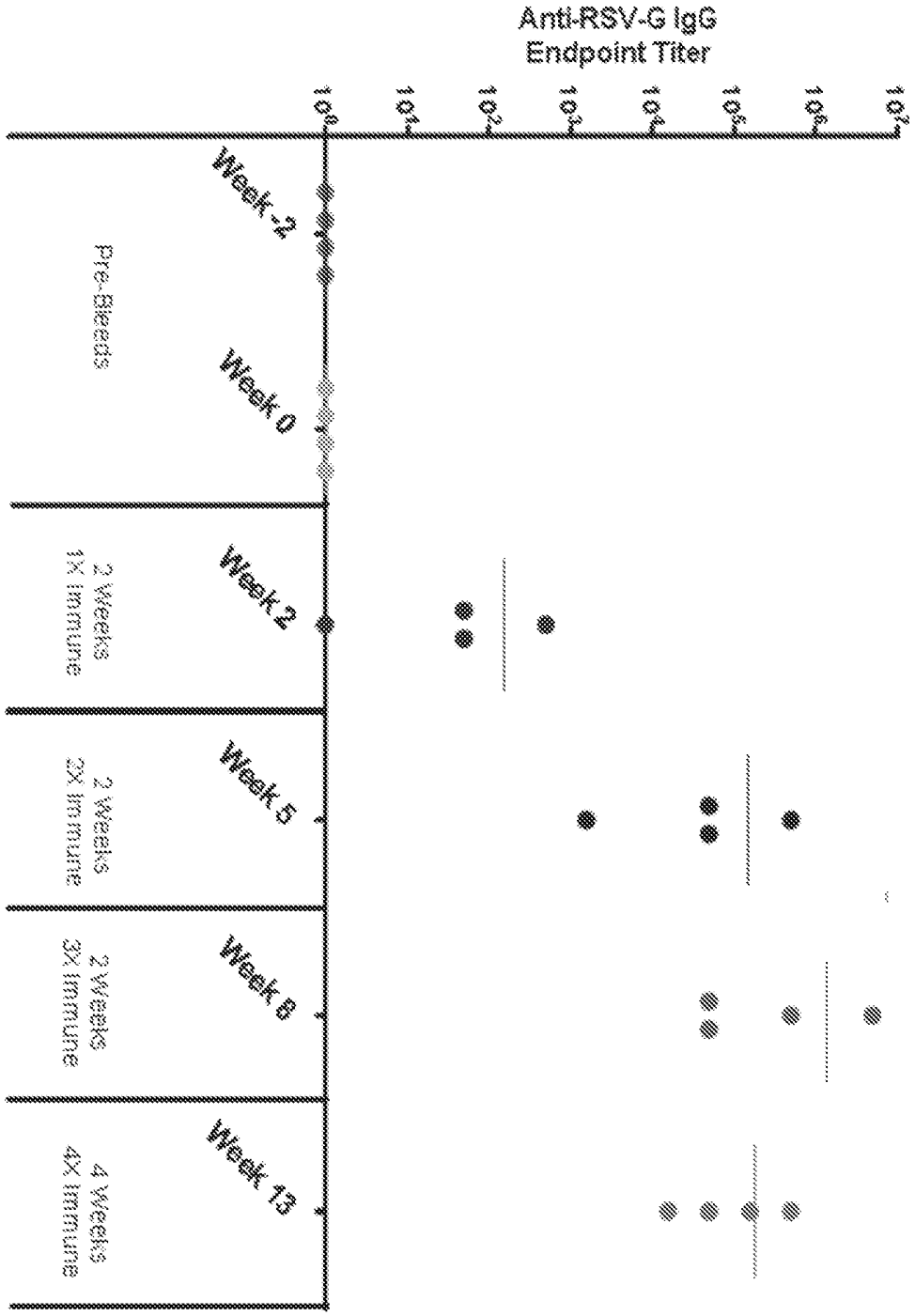

Anti-RSV-G IgG
Endpoint Titer
10^0
10^1
10^2
10^3
10^4
10^5
10^6
10^7
Week -2
Week 0
Week 2
Week 5
Week 8
Week 13
Pre-Bleeds
2 Weeks
1X Immune
2 Weeks
2X Immune
2 Weeks
3X Immune
4 Weeks
4X Immune

Figure 37

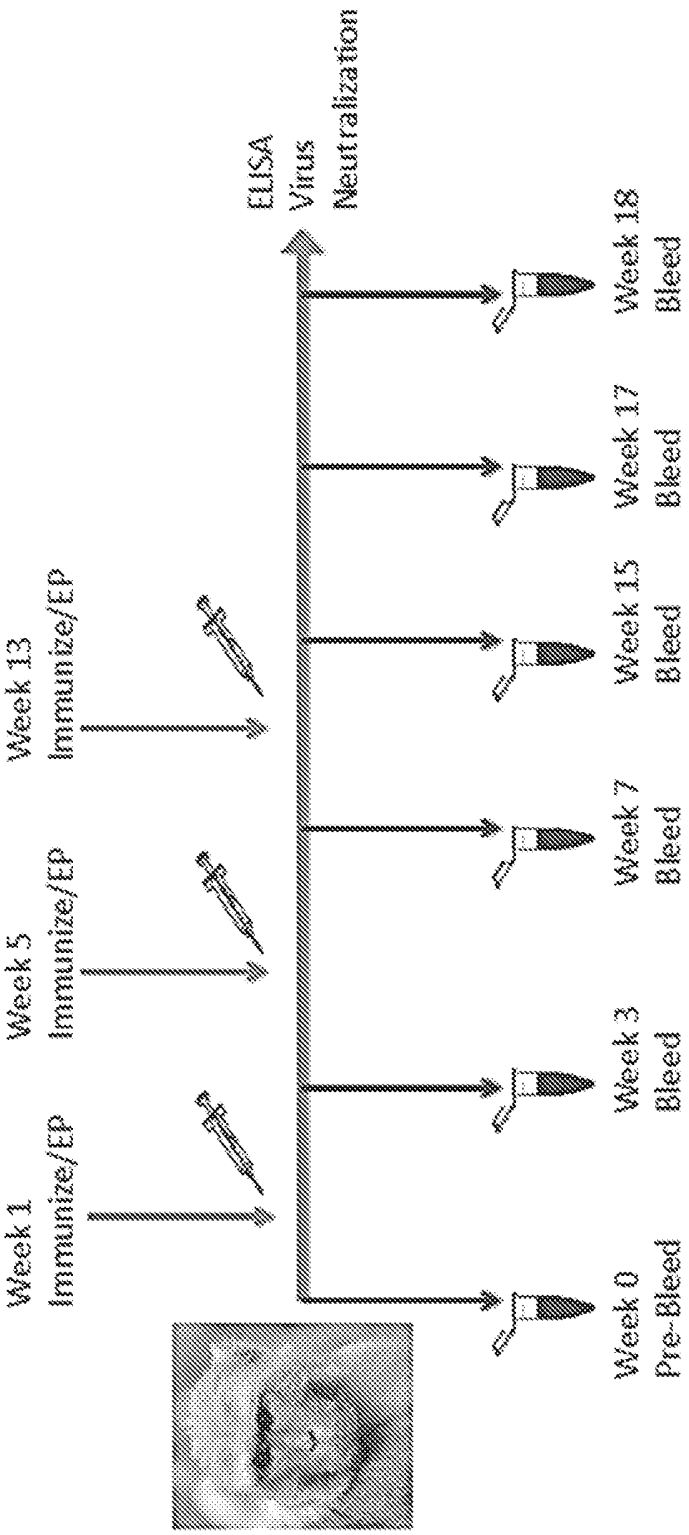

Week 1
Immunize/EP
Week 5
Immunize/EP
Week 13
Immunize/EP
ELISA
Virus
Neutralization
Week 0
Pre-Bleed
Week 3
Bleed
Week 7
Bleed
Week 15
Bleed
Week 17
Bleed
Week 18
Bleed

Figure 38A

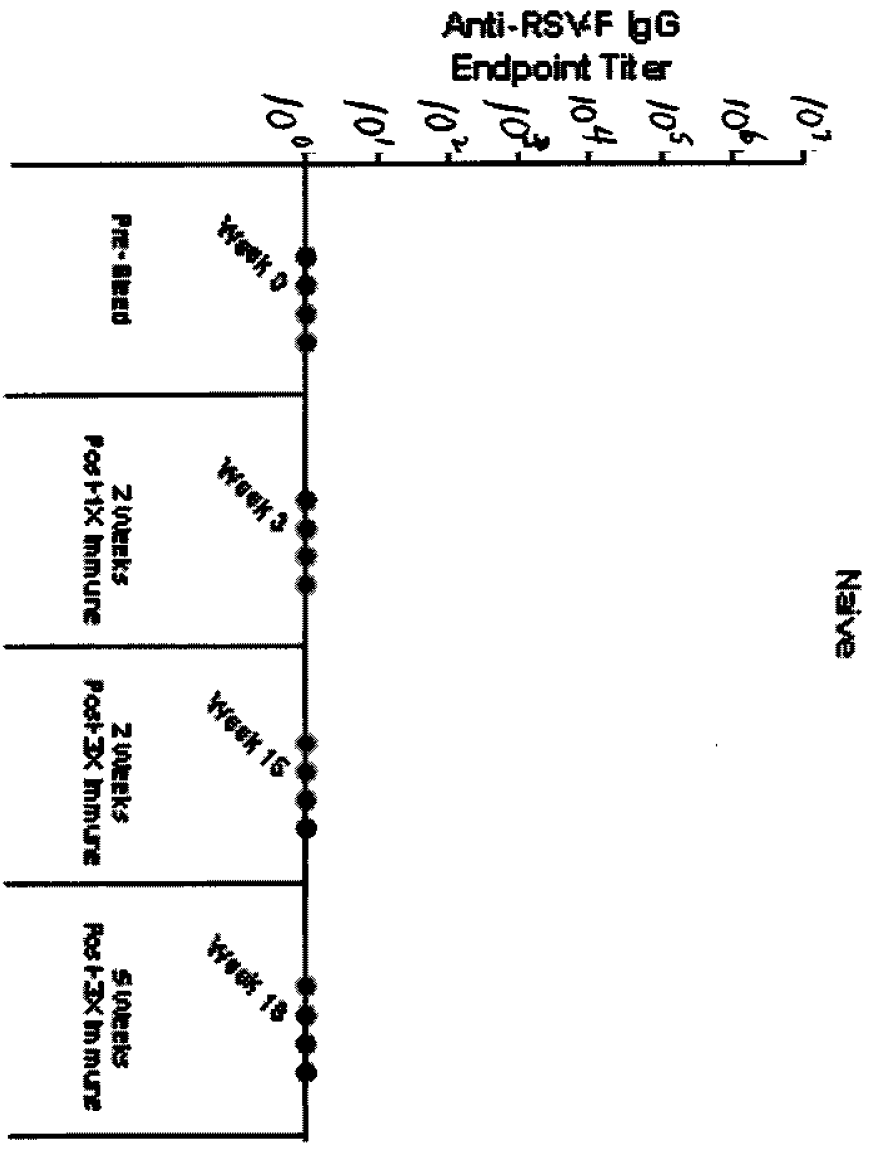

Anti-RSV-F IgG
Endpoint Titer
10^0
10^1
10^2
10^3
10^4
10^5
10^6
10^7
Pre-Bleed
Week 0
2 Weeks
Post-1X Immune
Week 3
2 Weeks
Post-2X Immune
Week 16
5 Weeks
Post-2X Immune
Week 18
NAIVE

Figure 38B

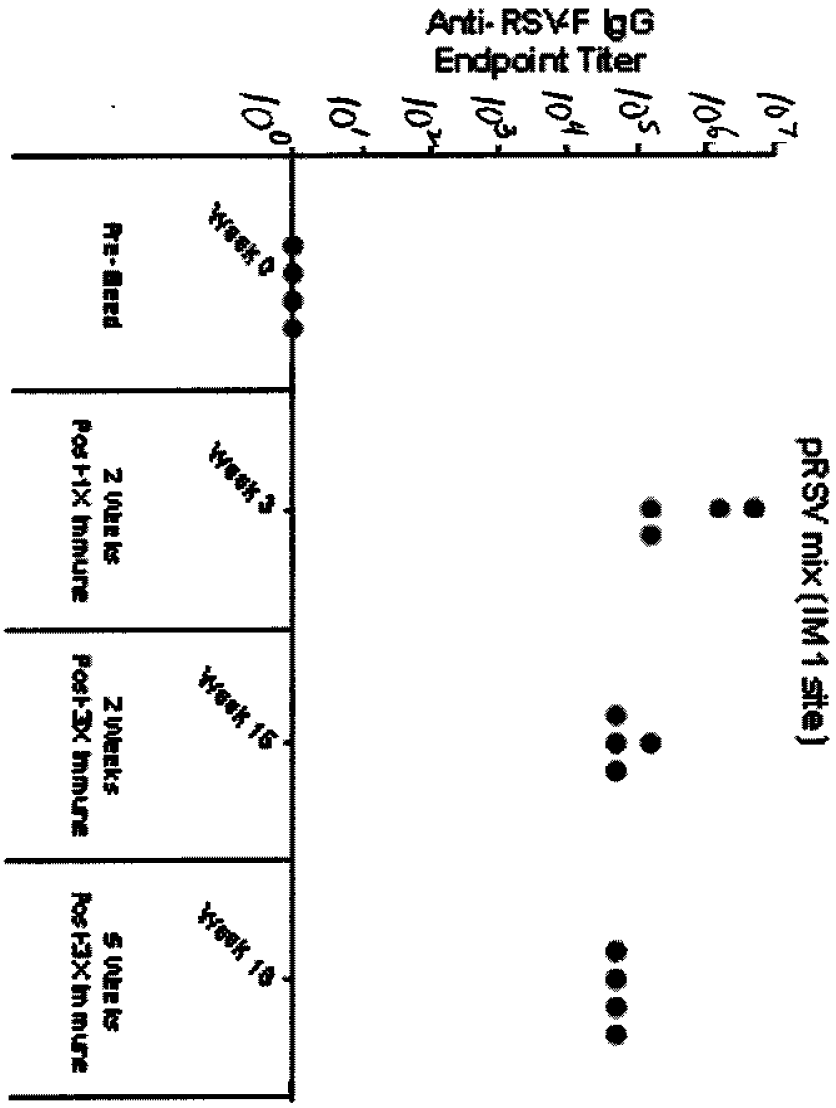

Anti-RSV-F IgG
Endpoint Titer
10^0
10^1
10^2
10^3
10^4
10^5
10^6
10^7
Week 0
Week 3
Week 15
Week 19
Pre-Bleed
2 Weeks
Post-1X Immune
2 Weeks
Post-2X Immune
5 Weeks
Post-3X Immune
pRSV mix (IM 1 site)

Figure 38C

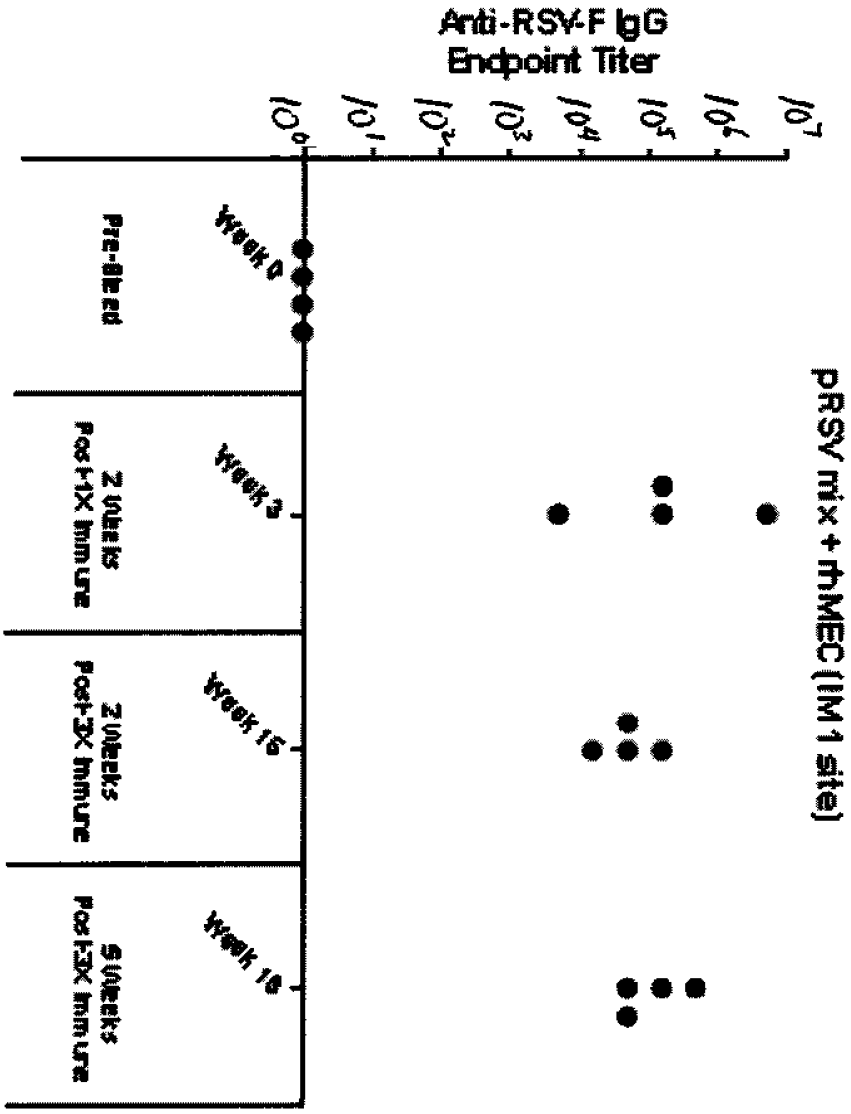

Anti-RSV-F IgG
Endpoint Titer
10^0
10^1
10^2
10^3
10^4
10^5
10^6
10^7
Week 0
Week 3
Week 15
Week 18
Pre-Bleed
2 Weeks Post-1X Immune
2 Weeks Post-2X Immune
5 Weeks Post-3X Immune
PRSV mix + rhMEC (IM 1 site)

Figure 38D

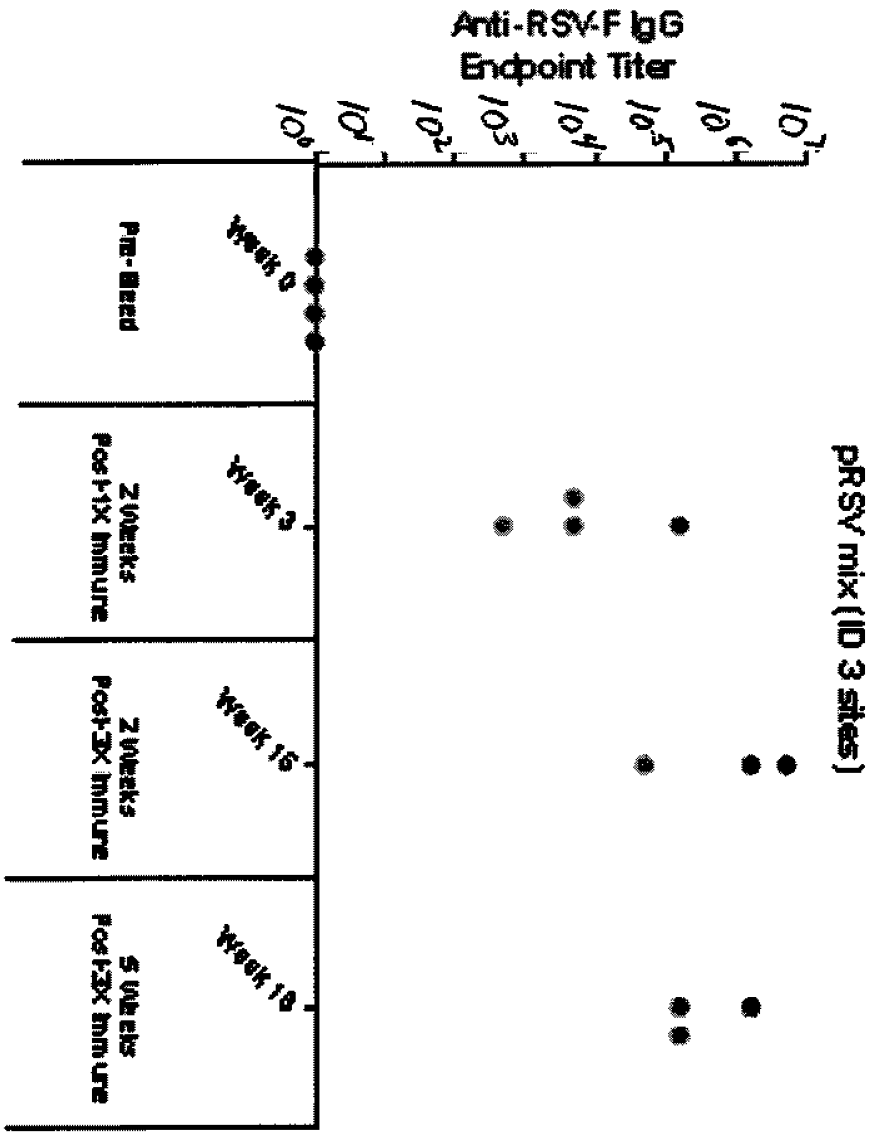

Anti-RSV-F IgG
Endpoint Titer
10^0
10^1
10^2
10^3
10^4
10^5
10^6
10^7
Week 0
Week 3
Week 16
Week 18
Pre-Bleed
2 Weeks Post 1X Immune
2 Weeks Post 2X Immune
5 Weeks Post 2X Immune
pRSV mix (ID 3 sites)

Figure 39A

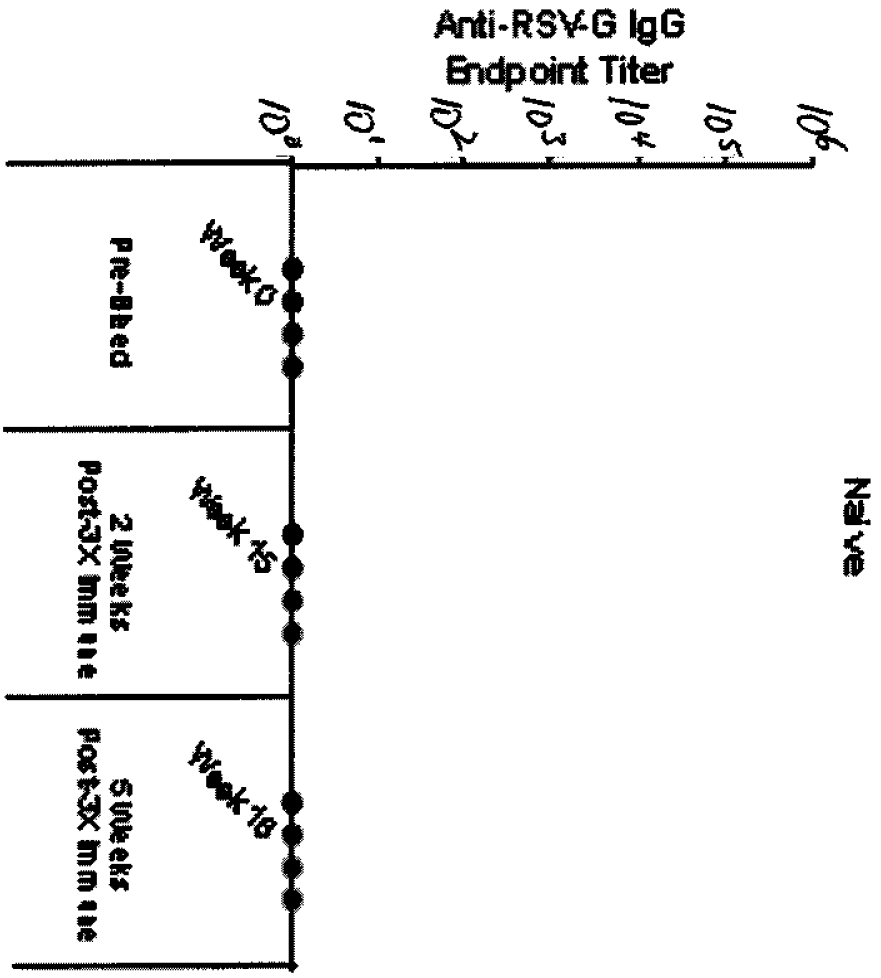

Anti-RSV-G IgG
Endpoint Titer
10^0
10^1
10^2
10^3
10^4
10^5
10^6
Week 0
Week 26
Week 39
Pre-Bleed
2 Weeks
post-3X Immune
5 Weeks
post-3X Immune
Naïve

Figure 39B

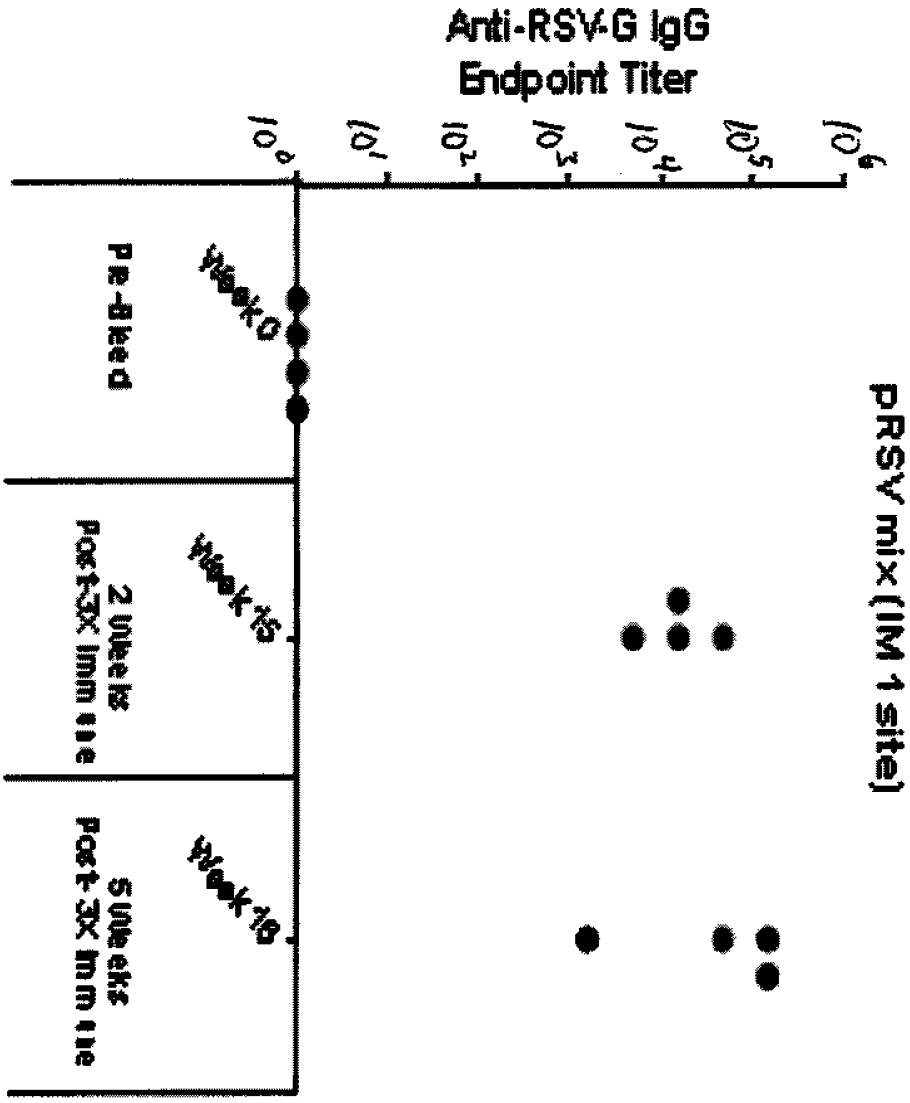

Anti-RSV-G IgG
Endpoint Titer
10^0
10^1
10^2
10^3
10^4
10^5
10^6
Pre-Bleed
Week 0
2 Weeks Post-3X Immune
Week 4
5 Weeks Post-3X Immune
Week 7
PRSV mix (IM 1 site)

Figure 39C

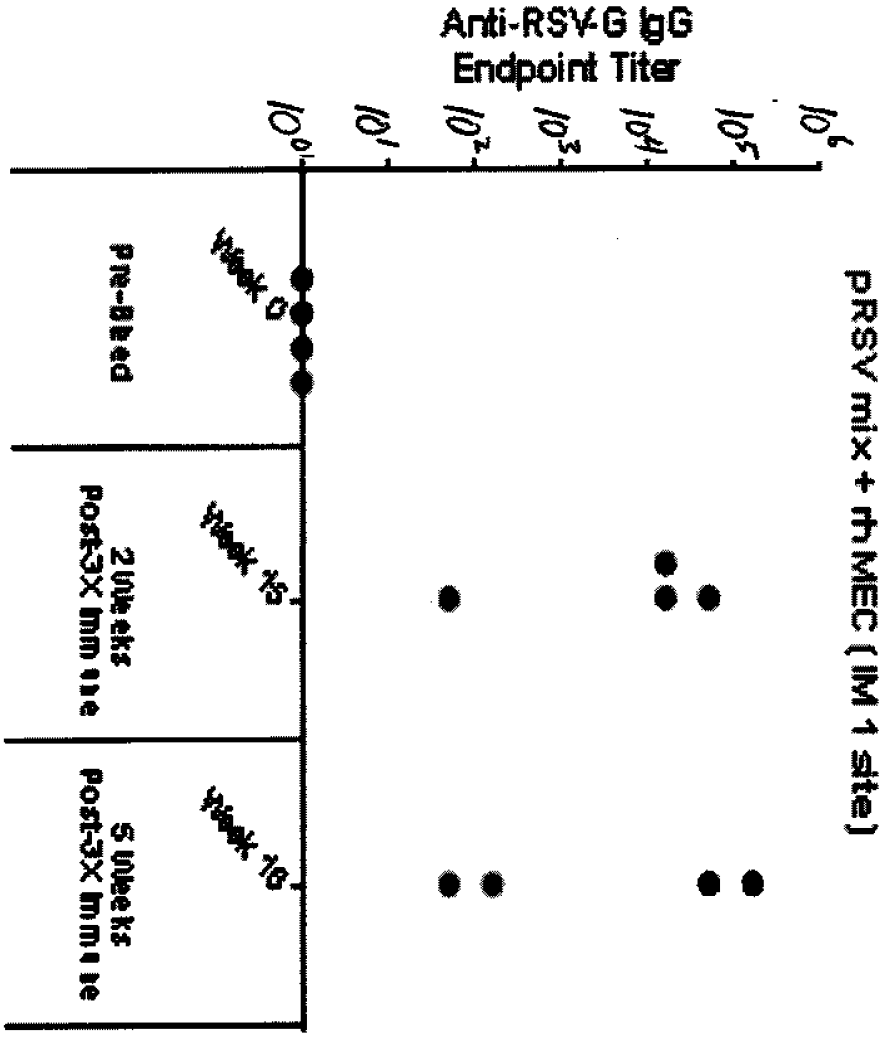

Anti-RSV-G IgG
Endpoint Titer
10^0
10^1
10^2
10^3
10^4
10^5
10^6
Week 0
Week 26
Week 78
Pre-Bleed
2 Weeks
Post-3X Immune
5 Weeks
Post-3X Immune
PRSV mix + rhMEC (IM 1 site)

Figure 39D

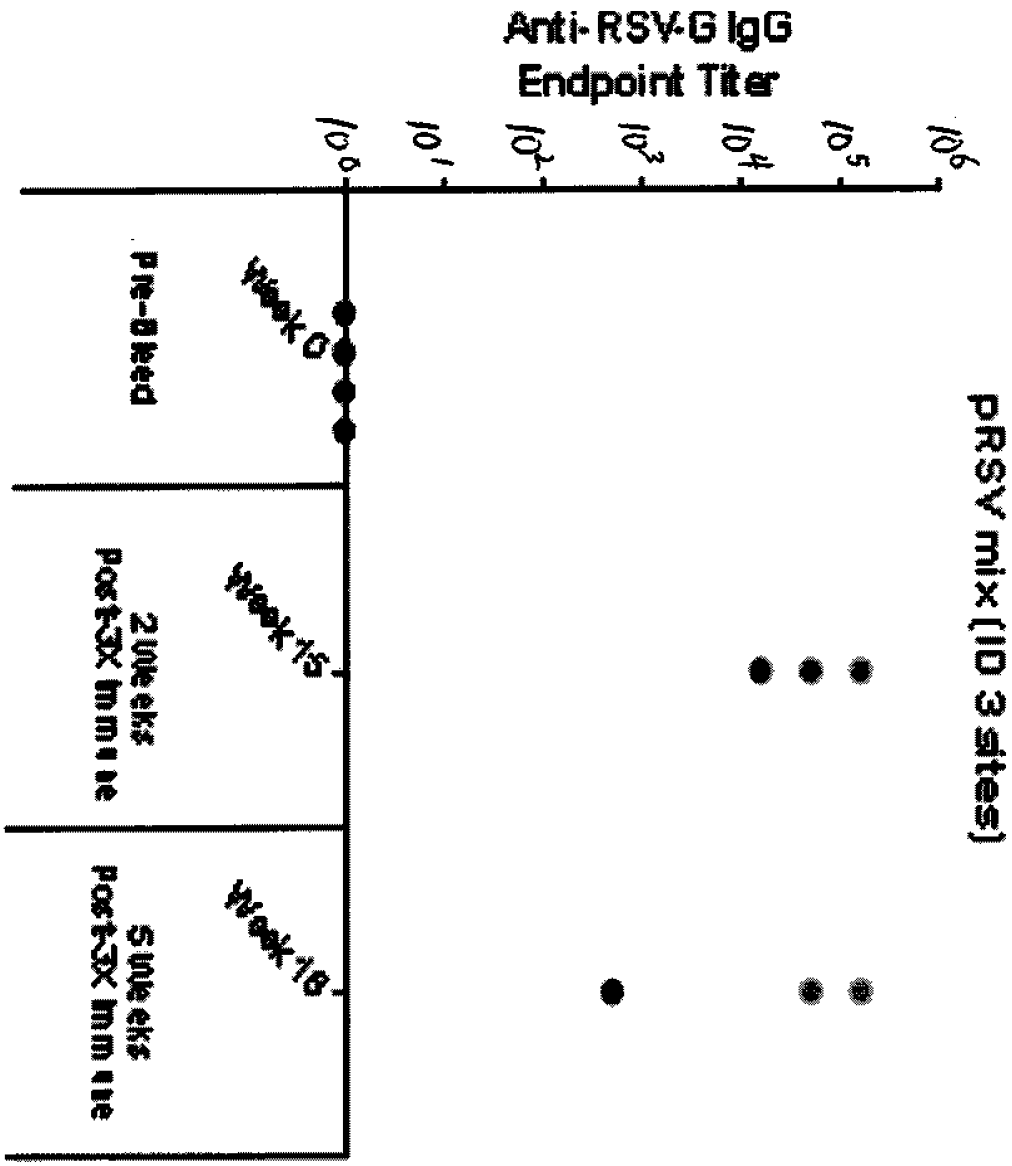

Anti-RSV-G IgG
Endpoint Titer
10^0
10^1
10^2
10^3
10^4
10^5
10^6
Week 0
Week 16
Week 19
Pre-Bleed
2 Weeks Post-3X Immune
5 Weeks Post-3X Immune
PRSV mix (10^3 sites)

Figure 40

Preliminary Neutralization Results from Cocktail Vaccine in Rabbit and Non-Human Primate Studies

Samples Tested	Predilutions	Neutralizing Titer Post-3d Imm
Negative serum		0
Mab anti RSV		40
Polyclonal Serum		160
Rabbit 71	0	640
Rabbit 72	0	320
NHP IM-1	0	640
NHP IM-2	0	320

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/036008

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C07K 14/135 (2013.01)
USPC - 424/211.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - A61K 39/12, 39/155; A61P 31/14, 37/04; C07K 14/115, 14/135 (2013.01)
USPC - 424/186.1, 199.1, 211.1; 435/69.1; 530/350

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

CPC: A61K 39/00, 39/155, 2039/5256; C07K 14/005, 14/135, 16/1027; C12N 2760/18522, 2760/18534

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Patbase, Google Patents, Google, Pubmed

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2008/114149 A2 (BLAIS et al) 25 September 2008 (25.09.2008) entire document	1-22
A	US 2011/0014220 A1 (CHOW et al) 20 January 2011 (20.01.2011) entire document	1-22
A	WO 2009/039178 A1 (KALERGIS et al) 26 March 2009 (26.03.2009) entire document	1-22
A	WO 2004/010935 A2 (YOUNG et al) 05 February 2004 (05.02.2004) entire document This document can be viewed by entering the doc number at the following url: http://worldwide.espacenet.com/numberSearch?locale=en_EP	1-22
P, Y	US 2012/0276142 A1 (WEINER et al) 01 November 2012 (01.11.2012) entire document	1-22

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 September 2013	20 SEP 2013
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/036008

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

   a. (means)

   ☐ on paper

   ☒ in electronic form

   b. (time)

   ☒ in the international application as filed

   ☐ together with the international application in electronic form

   ☐ subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

Specifically, SEQ ID NOs:1, 2, 9, and 10 were searched.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/036008

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See Extra Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-22, limited to a composition comprising an RSV F immunogen, wherein the RSV F immunogen is selected to be SEQ ID NO:2, further comprising nucleic acid sequence SEQ ID NO:1 that encodes for SEQ ID NO:2; the composition further comprising the RSV F immunogen of SEQ ID NO:2 operably linked to a signal peptide, wherein the RSV F immunogen and signal peptide fusion protein is selected to be SEQ ID NO:10, encoded by nucleic acid sequence SEQ ID NO:9.

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

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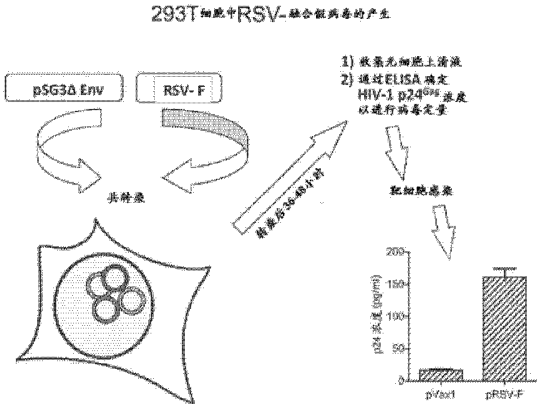
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(54)发明名称

人呼吸道合胞病毒共有抗原、由其制备的核酸构建体和疫苗及其使用方法

(57)摘要

本发明公开了包含一个或多个编码RSV免疫原的核酸序列的核酸分子和组合物。所公开的核酸分子包含了编码共有RSV F蛋白或其免疫原性片段的序列、编码RSV G(A)蛋白或其免疫原性片段的序列及编码RSV G(B)蛋白或其免疫原性片段的序列。本发明公开了包含三个序列中的一个序列、两个或全部三个序列的组合的组合物。这些编码序列任选地包括可操作地连接的编码信号肽的编码序列。还公开了包含趋化因子CC20和/或共有RSV M2-1蛋白或其免疫原性片段的核酸分子和组合物。本发明公开了免疫调节方法和诱导针对RSV的免疫应答的方法。本发明公开了防止RSV感染的方法及治疗感染RSV的个体的方法。



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1. 一种包含一个或多个编码 RSV 免疫原的核酸序列的组合物，所述核酸序列选自由以下组成的组：

a) 编码 RSV F 免疫原的核酸编码序列，其中所述编码 RSV F 免疫原的核酸编码序列选自由以下组成的组：

编码 SEQ ID NO:2 的核酸序列并且其任选地另外包含编码信号肽的编码序列，所述编码序列可操作地连接到编码 SEQ ID NO:2 的序列；

编码 SEQ ID NO:2 的至少 115 个或更多氨基酸的片段的核酸序列并且其任选地另外包含编码信号肽的编码序列，所述编码序列可操作地连接到编码所述 SEQ ID NO:2 的片段的序列；

编码与 SEQ ID NO:2 具有至少 98％同源性的蛋白质的核酸序列并且其任选地另外包含编码信号肽的编码序列，所述编码序列可操作地连接到编码所述与 SEQ ID NO:2 具有至少 98％同源性的蛋白质的编码序列；及

编码与 SEQ ID NO:2 具有至少 98％同源性的蛋白质的至少 115 个或更多氨基酸的片段的核酸序列并且其任选地另外包含编码信号肽的编码序列，所述编码序列可操作地连接到编码与 SEQ ID NO:2 具有至少 98％同源性的蛋白质的片段的序列；

b) 编码 RSV Ga 免疫原的核酸编码序列，其中所述编码 RSV Ga 免疫原的核酸编码序列选自由以下组成的组：

编码 SEQ ID NO:6 的核酸序列并且其任选地另外包含编码信号肽的编码序列，所述编码序列可操作地连接到编码 SEQ ID NO:6 的序列；

编码 SEQ ID NO:6 的至少 125 个或更多氨基酸的片段的核酸序列并且其任选地另外包含编码信号肽的编码序列，所述编码序列可操作地连接到编码所述 SEQ ID NO:6 的片段的序列；

编码与 SEQ ID NO:6 具有至少 98％同源性的蛋白质的核酸序列并且其任选地另外包含编码信号肽的编码序列，所述编码序列可操作地连接到编码所述与 SEQ ID NO:6 具有至少 98％同源性的蛋白质的编码序列；及

编码与 SEQ ID NO:6 具有至少 98％同源性的蛋白质的至少 125 个或更多氨基酸的片段的核酸序列并且其任选地另外包含编码信号肽的编码序列，所述编码序列可操作地连接到编码与 SEQ ID NO:6 具有至少 98％同源性的蛋白质的片段的序列；

和

c) 编码 RSV Gb 免疫原的核酸编码序列，其中所述编码 RSV Gb 免疫原的核酸编码序列选自由以下组成的组：

编码 SEQ ID NO:8 的核酸序列并且其任选地另外包含编码信号肽的编码序列，所述编码序列可操作地连接到编码 SEQ ID NO:8 的序列；

编码 SEQ ID NO:8 的至少 125 个或更多氨基酸的片段的核酸序列并且其任选地另外包含编码信号肽的编码序列，所述编码序列可操作地连接到编码所述 SEQ ID NO:8 的片段的序列；

编码与 SEQ ID NO:8 具有至少 98％同源性的蛋白质的核酸序列并且其任选地另外包含编码信号肽的编码序列，所述编码序列可操作地连接到编码所述与 SEQ ID NO:8 具有至少 98％同源性的蛋白质的编码序列；及

编码与SEQ ID NO:8具有至少98％同源性的蛋白质的至少125个或更多氨基酸的片段的核酸序列并且其任选地另外包含编码信号肽的编码序列，所述编码序列可操作地连接到编码与SEQ ID NO:8具有至少98％同源性的蛋白质的片段的序列。

2.如权利要求1所述的组合物，包含一个或多个编码RSV免疫原的核酸序列，所述核酸序列选自由以下组成的组：

a)编码RSV F免疫原的核酸编码序列，其中所述编码RSV F免疫原的核酸编码序列选自由以下组成的组：

包含SEQ ID NO:1的核酸序列并且其任选地另外包含编码信号肽的编码序列，所述编码序列可操作地连接到SEQ ID NO:1；

包含SEQ ID NO:1的编码至少115个或更多氨基酸的片段的核酸序列并且其任选地另外包含编码信号肽的编码序列，所述编码序列可操作地连接到SEQ ID NO:1；

与SEQ ID NO:1具有至少98％同源性的核酸序列并且其任选地另外包含编码信号肽的编码序列，所述编码序列可操作地连接到与SEQ ID NO:1具有至少98％同源性的编码序列；及

与SEQ ID NO:1具有至少98％同源性的核酸序列的编码至少115个或更多氨基酸的片段并且所述核酸序列任选地另外包含编码信号肽的编码序列，所述编码序列可操作地连接到与SEQ ID NO:1具有至少98％同源性的核酸序列的片段的编码序列；

b)编码RSV Ga免疫原的核酸编码序列，其中所述编码RSV Ga免疫原的核酸编码序列选自由以下组成的组：

包含SEQ ID NO:5的核酸序列并且其任选地另外包含编码信号肽的编码序列，所述编码序列可操作地连接到SEQ ID NO:5；

包含SEQ ID NO:5的编码至少125个或更多氨基酸的片段的核酸序列并且其任选地另外包含编码信号肽的编码序列，所述编码序列可操作地连接到SEQ ID NO:5；

与SEQ ID NO:5具有至少98％同源性的核酸序列并且其任选地另外包含编码信号肽的编码序列，所述编码序列可操作地连接到与SEQ ID NO:5具有至少98％同源性的编码序列；及

与SEQ ID NO:5具有至少98％同源性的核酸序列的编码至少125个或更多氨基酸的片段并且所述核酸序列任选地另外包含编码信号肽的编码序列，所述编码序列可操作地连接到与SEQ ID NO:5具有至少98％同源性的核酸序列的片段的编码序列；

和

c)编码RSV Gb免疫原的核酸编码序列，其中所述编码RSV Gb免疫原的核酸编码序列选自由以下组成的组：

包含SEQ ID NO:7的核酸序列并且其任选地另外包含编码信号肽的编码序列，所述编码序列可操作地连接到SEQ ID NO:7；

包含SEQ ID NO:7的编码至少125个或更多氨基酸的片段的核酸序列并且其任选地另外包含编码信号肽的编码序列，所述编码序列可操作地连接到SEQ ID NO:7；

与SEQ ID NO:7具有至少98％同源性的核酸序列并且其任选地另外包含编码信号肽的编码序列，所述编码序列可操作地连接到与SEQ ID NO:7具有至少98％同源性的编码序列；及

与SEQ ID NO:7具有至少98％同源性的核酸序列的编码至少125个或更多氨基酸的片段并且所述核酸序列任选地另外包含编码信号肽的编码序列，所述编码序列可操作地连接到与SEQ ID NO:7具有至少98％同源性的核酸序列的片段的编码序列。

3.如权利要求1所述的组合物，包含一个或多个核酸序列，所述核酸序列选自由以下组成的组：

a)编码包含SEQ ID NO:2的RSV F免疫原的核酸编码序列并且其任选地另外包含编码信号肽的编码序列，所述信号肽可操作地连接到SEQ ID NO:2；

b)编码包含SEQ ID NO:6的RSV Ga免疫原的核酸编码序列并且其任选地另外包含编码信号肽的编码序列，所述信号肽可操作地连接到SEQ ID NO:6；

和

c)编码包含SEQ ID NO:8的RSV Gb免疫原的核酸编码序列并且其任选地另外包含编码信号肽的编码序列，所述信号肽可操作地连接到SEQ ID NO:8。

4.如权利要求3所述的组合物，包含一个或多个核酸序列，所述核酸序列选自由以下组成的组：

a)编码RSV F免疫原的核酸编码序列，所述编码序列包含SEQ ID NO:1并且任选地另外包含可操作地连接到SEQ ID NO:1的编码信号肽的编码序列；

b)编码RSV Ga免疫原的核酸编码序列，所述编码序列包含SEQ ID NO:5并且任选地另外包含可操作地连接到SEQ ID NO:5的编码信号肽的编码序列；

和

c)编码RSV Gb免疫原的核酸编码序列，所述编码序列包含SEQ ID NO:7并且任选地另外包含可操作地连接到SEQ ID NO:7的编码信号肽的编码序列。

5.如权利要求3所述的组合物，包含一个或多个核酸序列，所述核酸序列选自由以下组成的组：

a)编码SEQ ID NO:10的核酸序列；

b)编码SEQ ID NO:14的核酸序列；

和

c)编码SEQ ID NO:16的核酸序列；

6.如权利要求3所述的组合物，包含一个或多个核酸序列，所述核酸序列选自由以下组成的组：

a)包含SEQ ID NO:9的核酸序列；

b)包含SEQ ID NO:13的核酸序列；

和

c)包含SEQ ID NO:15的核酸序列。

7.如权利要求1所述的组合物，包含一个或多个核酸序列，所述核酸序列选自由以下组成的组：

a)编码RSV F免疫原的核酸编码序列，所述RSV F免疫原包含SEQ ID NO:2的具有至少345个或更多氨基酸的片段并且所述核酸编码序列任选地另外包含编码可操作地连接到SEQ ID NO:2的信号肽的编码序列，或所述RSV F免疫原包含与SEQ ID NO:2具有至少98％同源性的蛋白质的具有至少345个或更多氨基酸的片段并且所述核酸编码序列任选

地另外包含编码信号肽的编码序列，所述编码序列可操作地连接到编码与 SEQ ID NO:2 具有至少 98％同源性的蛋白质的核酸序列的片段的编码序列；

b) 编码 RSV Ga 免疫原的核酸编码序列，所述 RSV Ga 免疫原包含 SEQ ID NO:6 的具有至少 200 个或更多氨基酸的片段并且所述核酸编码序列任选地另外包含编码可操作地连接到 SEQ ID NO:6 的信号肽的编码序列，或所述 RSV Ga 免疫原包含与 SEQ ID NO:6 具有至少 98％同源性的蛋白质的具有至少 200 个或更多氨基酸的片段并且所述核酸编码序列任选地另外包含编码信号肽的编码序列，所述编码序列可操作地连接到编码与 SEQ ID NO:6 具有至少 98％同源性的蛋白质的核酸序列的片段的编码序列；

和

c) 编码 RSV Gb 免疫原的核酸编码序列，所述 RSV Gb 免疫原包含 SEQ ID NO:8 的具有至少 200 个或更多氨基酸的片段并且所述核酸编码序列任选地另外包含编码可操作地连接到 SEQ ID NO:8 的信号肽的编码序列，或所述 RSV Gb 免疫原包含与 SEQ ID NO:8 具有至少 98％同源性的蛋白质的具有至少 200 个或更多氨基酸的片段并且所述核酸编码序列任选地另外包含编码信号肽的编码序列，所述编码序列可操作地连接到编码与 SEQ ID NO:8 具有至少 98％同源性的蛋白质的核酸序列的片段的编码序列。

8. 如权利要求 7 所述的组合物，包含一个或多个核酸序列，所述核酸序列选自由以下组成的组：

a) 编码 RSV F 免疫原的核酸编码序列，所述核酸编码序列包含了包括 SEQ ID NO:1 的编码至少 345 个或更多氨基酸的片段的核酸序列并且任选地另外包含可操作地连接到 SEQ ID NO:1 的编码信号肽的编码序列，或所述核酸编码序列包含与 SEQ ID NO:1 具有至少 98％同源性的核酸序列的编码至少 345 个或更多氨基酸的片段并且任选地另外包含编码信号肽的编码序列，所述编码序列可操作地连接到与 SEQ ID NO:1 具有至少 98％同源性的核酸序列的片段的编码序列；

b) 编码 RSV Ga 免疫原的核酸编码序列，所述核酸编码序列包含 SEQ ID NO:5 的编码至少 200 个或更多氨基酸的片段并且任选地另外包含可操作地连接到 SEQ ID NO:5 的编码信号肽的编码序列，或所述核酸编码序列包含与 SEQ ID NO:5 具有至少 98％同源性的核酸序列的编码至少 125 个或更多氨基酸的片段并且任选地另外包含编码信号肽的编码序列，所述编码序列可操作地连接到与 SEQ ID NO:5 具有至少 98％同源性的核酸序列的片段的编码序列；

和

c) 编码 RSV Gb 免疫原的核酸编码序列，所述核酸编码序列包含 SEQ ID NO:7 的编码至少 200 个或更多氨基酸的片段并且任选地另外包含可操作地连接到 SEQ ID NO:7 的编码信号肽的编码序列，或所述核酸编码序列包含与 SEQ ID NO:7 具有至少 98％同源性的核酸序列的编码至少 200 个或更多氨基酸的片段并且任选地另外包含编码信号肽的编码序列，所述编码序列可操作地连接到与 SEQ ID NO:7 具有至少 98％同源性的核酸序列的片段的编码序列。

9. 如权利要求 1 所述的组合物，包含一个或多个核酸序列，所述核酸序列选自由以下组成的组：

a) 编码 RSV F 免疫原的核酸编码序列，所述 RSV F 免疫原包含 SEQ ID NO:2 的具有至

少 550 个或更多氨基酸的片段并且任选地另外包含编码可操作地连接到 SEQ ID NO:2 的信号肽的编码序列，或所述 RSV F 免疫原包含与 SEQ ID NO:2 具有至少 98％同源性的蛋白质的具有至少 550 个或更多氨基酸的片段并且任选地另外包含编码信号肽的编码序列，所述编码序列可操作地连接到编码与 SEQ ID NO:2 具有至少 98％同源性的蛋白质的核酸序列的片段的编码序列；

b) 编码 RSV Ga 免疫原的核酸编码序列，所述 RSV Ga 免疫原包含 SEQ ID NO:6 的具有至少 270 个或更多氨基酸的片段并且任选地另外包含编码可操作地连接到 SEQ ID NO:6 的信号肽的编码序列，或所述 RSV Ga 免疫原包含与 SEQ ID NO:6 具有至少 98％同源性的蛋白质的具有至少 270 个或更多氨基酸的片段并且任选地另外包含编码信号肽的编码序列，所述编码序列可操作地连接到编码与 SEQ ID NO:6 具有至少 98％同源性的蛋白质的核酸序列的片段的编码序列；

和

c) 编码 RSV Gb 免疫原的核酸编码序列，所述 RSV Gb 免疫原包含 SEQ ID NO:8 的具有至少 270 个或更多氨基酸的片段并且任选地另外包含编码可操作地连接到 SEQ ID NO:8 的信号肽的编码序列，或所述 RSV Gb 免疫原包含与 SEQ ID NO:8 具有至少 98％同源性的蛋白质的具有至少 270 个或更多氨基酸的片段并且任选地另外包含编码信号肽的编码序列，所述编码序列可操作地连接到编码与 SEQ ID NO:8 具有至少 98％同源性的蛋白质的核酸序列的片段的编码序列。

10. 如权利要求 9 所述的组合物，包含一个或多个核酸序列，所述核酸序列选自由以下组成的组：

a) 编码 RSV F 免疫原的核酸编码序列，所述 RSV F 免疫原包含了包括 SEQ ID NO:1 的编码至少 550 个或更多氨基酸的片段的核酸序列并且任选地另外包含了可操作地连接到 SEQ ID NO:1 的编码信号肽的编码序列，或所述 RSV F 免疫原包含与 SEQ ID NO:1 具有至少 98％同源性的核酸序列的编码至少 550 个或更多氨基酸的片段并且任选地另外包含了编码信号肽的编码序列，所述编码序列可操作地连接到与 SEQ ID NO:1 具有至少 98％同源性的核酸序列的片段的编码序列；

b) 编码 RSV Ga 免疫原的核酸编码序列，所述 RSV Ga 免疫原包含了 SEQ ID NO:5 的编码至少 270 个或更多氨基酸的片段并且任选地另外包含了可操作地连接到 SEQ ID NO:5 的编码信号肽的编码序列，或所述 RSV Ga 免疫原包含与 SEQ ID NO:5 具有至少 98％同源性的核酸序列的编码至少 270 个或更多氨基酸的片段并且任选地另外包含了编码信号肽的编码序列，所述编码序列可操作地连接到与 SEQ ID NO:5 具有至少 98％同源性的核酸序列的片段的编码序列；

和

c) 编码 RSV Gb 免疫原的核酸编码序列，所述 RSV Gb 免疫原包含 SEQ ID NO:7 的编码至少 270 个或更多氨基酸的片段并且任选地另外包含了可操作地连接到 SEQ ID NO:7 的编码信号肽的编码序列，或所述 RSV Gb 免疫原包含与 SEQ ID NO:7 具有至少 98％同源性的核酸序列的编码至少 270 个或更多氨基酸的片段并且任选地另外包含了编码信号肽的编码序列，所述编码序列可操作地连接到与 SEQ ID NO:7 具有至少 98％同源性的核酸序列的片段的编码序列。

11. 如权利要求 1 所述的组合物，包含一个或多个核酸序列，所述核酸序列选自由以下组成的组：

a) 编码与 SEQ ID NO:2 具有至少 99％同源性的 RSV F 免疫原的核酸编码序列并且其任选地另外包含编码信号肽的编码序列，所述信号肽可操作地连接到与 SEQ ID NO:2 具有至少 99％同源性的编码序列；

b) 编码与 SEQ ID NO:6 具有至少 99％同源性的 RSV Ga 免疫原的核酸编码序列并且其任选地另外包含编码信号肽的编码序列，所述信号肽可操作地连接到与 SEQ ID NO:6 具有至少 98％同源性的编码序列；

和

c) 编码与 SEQ ID NO:8 具有至少 99％同源性的 RSV Gb 免疫原的核酸编码序列并且其任选地另外包含编码信号肽的编码序列，所述信号肽可操作地连接到与 SEQ ID NO:8 具有至少 98％同源性的编码序列。

12. 如权利要求 11 所述的组合物，包含一个或多个核酸序列，所述核酸序列选自由以下组成的组：

a) 编码 RSV F 免疫原的核酸编码序列，所述编码序列与 SEQ ID NO:1 具有至少 99％同源性并且任选地另外包含可操作地连接到与 SEQ ID NO:1 具有至少 99％同源性的编码序列的编码信号肽的编码序列；

b) 编码 RSV Ga 免疫原的核酸编码序列，所述编码序列与 SEQ ID NO:5 具有至少 99％同源性并且任选地另外包含可操作地连接到与 SEQ ID NO:5 具有至少 98％同源性的编码序列的编码信号肽的编码序列；

和

c) 编码 RSV Gb 免疫原的核酸编码序列，所述编码序列与 SEQ ID NO:7 具有至少 99％同源性并且任选地另外包含可操作地连接到与 SEQ ID NO:7 具有至少 98％同源性的编码序列的编码信号肽的编码序列。

13. 如权利要求 1 所述的组合物，包含了选自由以下组成的组的核酸序列：

编码 RSV F 免疫原的核酸编码序列，

编码 RSV Ga 免疫原的核酸编码序列，

编码 RSV Gb 免疫原的核酸编码序列，

编码 RSV F 免疫原的核酸编码序列和编码 RSV Ga 免疫原的核酸编码序列，

编码 RSV F 免疫原的核酸编码序列和编码 RSV Gb 免疫原的核酸编码序列，

编码 RSV Ga 免疫原的核酸编码序列和编码 RSV Gb 免疫原的核酸编码序列，及

编码 RSV F 免疫原的核酸编码序列、编码 RSV Ga 免疫原的核酸编码序列及编码 RSV Gb 免疫原的核酸编码序列。

14. 如权利要求 1 到 13 中任一项所述的组合物，另外包含编码 CCL20 的核酸编码序列。

15. 如权利要求 1 到 13 中任一项所述的组合物，所述组合物被配制成使用电穿孔法传递给个体。

16. 如权利要求 1 到 13 中任一项所述的组合物，另外包含编码一种或多种选自由 IL-12、IL-15 和 IL-28 组成的组的蛋白质的核酸序列。

17. 如权利要求 1 到 13 中任一项所述的组合物，其中所述一个或多个核酸编码序列是

一个或多个质粒的一部分。

18. 如权利要求 1 到 13 中任一项所述的组合物，其中所述一个或多个核酸编码序列中的每一个为并入单独质粒中的一部分。

19. 一种诱导针对 RSV 的免疫应答的方法，包括向个体施用有效诱导所述个体中的免疫应答的量的如权利要求 1 到 13 中任一项所述的组合物。

20. 一种治疗被诊断带有 RSV 的个体的方法，包括向个体施用治疗有效量的如权利要求 1 到 13 中任一项所述的组合物。

21. 一种防止个体感染 RSV 的方法，包括向个体施用预防有效量的如权利要求 1 到 13 中任一项所述的组合物。

22. 一种蛋白质，选自由以下组成的组 ：SEQ ID NO:2 ；SEQ ID NO:6 ；SEQ ID NO:8 ；SEQ ID NO:10 ；SEQ ID NO:14 ；及 SEQ ID NO:16。

23. 一种包含编码 RSV M2-1 免疫原的核酸编码序列的组合物，其中所述编码 RSV M2-1 免疫原的核酸编码序列选自由以下组成的组 ：

编码 SEQ ID NO:4 的核酸序列并且其任选地另外包含编码信号肽的编码序列，所述编码序列可操作地连接到编码 SEQ ID NO:4 的序列 ；

编码 SEQ ID NO:4 的至少 25 个或更多氨基酸的片段的核酸序列并且其任选地另外包含编码信号肽的编码序列，所述编码序列可操作地连接到编码所述 SEQ ID NO:4 的片段的序列 ；

编码与 SEQ ID NO:4 具有至少 98％同源性的蛋白质的核酸序列并且其任选地另外包含编码信号肽的编码序列，所述编码序列可操作地连接到编码所述与 SEQ ID NO:4 具有至少 98％同源性的蛋白质的编码序列 ；及

编码与 SEQ ID NO:4 具有至少 98％同源性的蛋白质的至少 25 个或更多氨基酸的片段的核酸序列并且其任选地另外包含编码信号肽的编码序列，所述编码序列可操作地连接到编码与 SEQ ID NO:4 具有至少 98％同源性的蛋白质的片段的序列。

24. 如权利要求 23 所述的包含编码 RSV M2-1 免疫原的核酸编码序列的组合物，其中所述编码 RSV M2-1 免疫原的核酸编码序列选自由以下组成的组 ：

包含 SEQ ID NO:3 的核酸序列并且其任选地另外包含编码信号肽的编码序列，所述编码序列可操作地连接到 SEQ ID NO:3 ；

包含 SEQ ID NO:3 的编码至少 25 个或更多氨基酸的片段的核酸序列并且其任选地另外包含编码信号肽的编码序列，所述编码序列可操作地连接到 SEQ ID NO:3 ；

与 SEQ ID NO:3 具有至少 98％同源性的核酸序列并且其任选地另外包含编码信号肽的编码序列，所述编码序列可操作地连接到与 SEQ ID NO:3 具有至少 98％同源性的编码序列 ；及

与 SEQ ID NO:3 具有至少 98％同源性的核酸序列的编码至少 25 个或更多氨基酸的片段并且所述核酸序列任选地另外包含编码信号肽的编码序列，所述编码序列可操作地连接到与 SEQ ID NO:3 具有至少 98％同源性的核酸序列的片段的编码序列。

25. 如权利要求 23 所述的包含编码 RSV M2-1 免疫原的核酸编码序列的组合物，包含 SEQ ID NO:4 并且任选地另外包含编码信号肽的编码序列，所述信号肽可操作地连接到 SEQ ID NO:4。

26. 如权利要求 25 所述的包含编码 RSV M2-1 免疫原的核酸编码序列的组合物，所述编码序列包含 SEQ ID NO:3 并且任选地另外包含可操作地连接到 SEQ ID NO:3 的编码信号肽的编码序列。

27. 如权利要求 25 所述的组合物，包含编码 SEQ ID NO:12 的核酸序列。

28. 如权利要求 25 所述的组合物，包含了含 SEQ ID NO:11 的核酸序列。

29. 如权利要求 23 所述的包含编码 RSV M2-1 免疫原的核酸编码序列的组合物，所述 RSV M2-1 免疫原包含 SEQ ID NO:4 的具有至少 130 个或更多氨基酸的片段并且所述核酸编码序列任选地另外包含编码可操作地连接到 SEQ ID NO:4 的信号肽的编码序列，或所述 RSV M2-1 免疫原包含与 SEQ ID NO:4 具有至少 98％同源性的蛋白质的具有至少 130 个或更多氨基酸的片段并且所述核酸编码序列任选地另外包含编码信号肽的编码序列，所述编码序列可操作地连接到编码与 SEQ ID NO:4 具有至少 98％同源性的蛋白质的核酸序列的片段的编码序列。

30. 如权利要求 29 所述的包含编码 RSV M2-1 免疫原的核酸编码序列的组合物，包含 SEQ ID NO:3 的编码至少 130 个或更多氨基酸的片段并且任选地另外包含可操作地连接到 SEQ ID NO:3 的编码信号肽的编码序列，或包含与 SEQ ID NO:3 具有至少 98％同源性的核酸序列的编码至少 130 个或更多氨基酸的片段并且任选地另外包含编码信号肽的编码序列，所述编码序列可操作地连接到与 SEQ ID NO:3 具有至少 98％同源性的核酸序列的片段的编码序列。

31. 如权利要求 23 所述的包含编码 RSV M2-1 免疫原的核酸编码序列的组合物，所述 RSV M2-1 免疫原包含 SEQ ID NO:4 的具有至少 165 个或更多氨基酸的片段并且所述核酸编码序列任选地另外包含编码可操作地连接到 SEQ ID NO:4 的信号肽的编码序列，或所述 RSV M2-1 免疫原包含与 SEQ ID NO:4 具有至少 98％同源性的蛋白质的具有至少 165 个或更多氨基酸的片段并且所述核酸编码序列任选地另外包含编码信号肽的编码序列，所述编码序列可操作地连接到编码与 SEQ ID NO:4 具有至少 98％同源性的蛋白质的核酸序列的片段的编码序列。

32. 如权利要求 31 所述的包含编码 RSV M2-1 免疫原的核酸编码序列的组合物，包含 SEQ ID NO:3 的编码至少 165 个或更多氨基酸的片段并且任选地另外包含可操作地连接到 SEQ ID NO:3 的编码信号肽的编码序列，或包含与 SEQ ID NO:3 具有至少 98％同源性的核酸序列的编码至少 165 个或更多氨基酸的片段并且任选地另外包含编码信号肽的编码序列，所述编码序列可操作地连接到与 SEQ ID NO:3 具有至少 98％同源性的核酸序列的片段的编码序列。

33. 如权利要求 23 所述的包含编码 RSV M2-1 免疫原的核酸编码序列的组合物，所述 RSV M2-1 免疫原与 SEQ ID NO:4 具有至少 99％同源性并且所述核酸编码序列任选地另外包含编码信号肽的编码序列，所述信号肽可操作地连接到与 SEQ ID NO:4 具有至少 99％同源性的编码序列。

34. 如权利要求 33 所述的包含编码 RSV M2-1 免疫原的核酸编码序列的组合物，所述编码序列与 SEQ ID NO:3 具有至少 99％同源性并且任选地另外包含可操作地连接到与 SEQ ID NO:3 具有至少 99％同源性的编码序列的编码信号肽的编码序列。

35. 如权利要求 23 所述的组合物，包含选自由以下组成的组的核酸序列：

编码 RSV M2-1 免疫原的核酸编码序列，

编码 RSV F 免疫原的核酸编码序列和编码 RSV M2-1 免疫原的核酸编码序列，

编码 RSV M2-1 免疫原的核酸编码序列和编码 RSV Ga 免疫原的核酸编码序列，

编码 RSV M2-1 免疫原的核酸编码序列和编码 RSV Gb 免疫原的核酸编码序列，

编码 RSV F 免疫原的核酸编码序列、编码 RSV M2-1 免疫原的核酸编码序列及编码 RSV Ga 免疫原的核酸编码序列，

编码 RSV F 免疫原的核酸编码序列、编码 RSV M2-1 免疫原的核酸编码序列及编码 RSV Gb 免疫原的核酸编码序列，

编码 RSV M2-1 免疫原的核酸编码序列、编码 RSV Ga 免疫原的核酸编码序列及编码 RSV Gb 免疫原的核酸编码序列，及

编码 RSV F 免疫原的核酸编码序列、编码 RSV M2-1 免疫原的核酸编码序列、编码 RSV Ga 免疫原的核酸编码序列及编码 RSV Gb 免疫原的核酸编码序列。

36. 如权利要求 23 到 35 中任一项所述的组合物，另外包含编码 CCL20 的核酸编码序列。

37. 如权利要求 23 到 35 中任一项所述的组合物，所述组合物被配制成使用电穿孔法传递给个体。

38. 如权利要求 23 到 35 中任一项所述的组合物，另外包含编码一种或多种选自由 IL-12、IL-15 和 IL-28 组成的组的蛋白质的核酸序列。

39. 如权利要求 23 到 35 中任一项所述的组合物，其中所述一个或多个核酸编码序列是一个或多个质粒的一部分。

40. 如权利要求 23 到 35 中任一项所述的组合物，其中所述一个或多个核酸编码序列中的每一个为并入单独质粒中的一部分。

41. 一种诱导针对 RSV 的免疫应答的方法，包括向个体施用有效诱导所述个体中的免疫应答的量的如权利要求 23 到 35 中任一项所述的组合物。

42. 一种治疗被诊断带有 RSV 的个体的方法，包括向个体施用治疗有效量的如权利要求 23 到 35 中任一项所述的组合物。

43. 一种防止个体感染 RSV 的方法，包括向个体施用预防有效量的如权利要求 23 到 35 中任一项所述的组合物。

44. 一种蛋白质，选自由以下组成的组 :SEQ ID NO:4 及 SEQ ID NO:12。

人呼吸道合胞病毒共有抗原、由其制备的核酸构建体和疫苗及其使用方法

技术领域

[0001] 本发明涉及共有抗原性呼吸道合胞病毒蛋白质和编码这些蛋白质的核酸分子；包括这些蛋白质和/或核酸分子的改良的呼吸道合胞病毒疫苗；及使用这些疫苗诱导免疫应答和防止呼吸道合胞病毒感染和/或治疗感染呼吸道合胞病毒的个体的方法。

背景技术

[0002] 本申请要求美国临时申请号61/622,279的优先权，该案以引用的方式整体并入本文中。

[0003] 人呼吸道合胞病毒(Human Respiratory Syncytial Virus，RSV)是导致婴儿和幼儿(早产婴儿尤其易于患病)上呼吸道和下呼吸道感染的最常见原因。在世界范围内，RSV是引起全世界婴儿(尤其是在早产并且患有慢性肺病或先天性心脏病的那些婴儿中)和幼儿的严重下呼吸道感染的主导原因，并且引起多种疾病，包括住院儿童中的20-25％的肺炎病例和45-50％的细支气管炎病例。在美国，RSV的发生每年导致约120,000例住院治疗和500+例死亡。在不到1岁的人群中，RSV是婴儿病毒死亡的主导原因并且在这一人群中的死亡率是因流感感染引起的死亡率的10倍。估计全球每年的发病率和死亡率分别为64,000,000例和160,000例。RSV相关性医疗成本估计每年超过$65,000万。

[0004] 尽管基本上所有的儿童在两岁时都经历感染，但严重RSV感染的峰值年龄是在2-6个月。大多数感染都平安无事地度过。然而，对于一些儿童来说，感染可能使儿童在以后的生活中易患上哮喘和气道高反应性。自然的RSV感染不会赋予终生免疫性，并因此，个体可能会反复地感染。

[0005] RSV感染对于老年人和其它易患病人群来说是个重要的问题。在老年人中，RSV是病毒死亡的第二大主导原因。移植物接受者和其它免疫力低下的人群以及罹患囊肿性纤维化的个体会因为感染而易于发生严重健康问题。

[0006] 呼吸道合胞病毒(RSV)是副粘病毒科(Paramyxoviridae；一种包括如流感病毒等常见呼吸道病毒及引起腮腺炎和麻疹的那些病毒的病毒科)的一种有包膜、负链单股RNA病毒。总体来说，RSV具有十个基因，编码十一种不同的蛋白质。这十一种RSV蛋白包括：蛋白质1)蛋白质“NS1”和2)蛋白质“NS2”，这些蛋白质抑制I型干扰素活性；3)蛋白质“N”，与RNA缔合形成核壳体的核壳体蛋白；4)蛋白质“P”，该蛋白质是蛋白质L的辅因子；5)蛋白质“M”，病毒组装所需的基质蛋白；6)蛋白质“SH”，该蛋白质在病毒表面上表达以与蛋白质G和蛋白质F形成病毒衣壳；7)蛋白质“G”，该蛋白质是高度糖基化的，在表面上表达，涉及病毒附着并且结合糖胺聚糖(glycosaminoglycan，GAG)；8)蛋白质“F”，该蛋白质在表面上表达，涉及病毒-细胞膜融合并且介导融合以允许病毒进入到细胞质中；9)蛋白质“M2-1”，该蛋白质是一种基质蛋白和延伸因子；10)蛋白质“M2-2”，该蛋白质是一种基质蛋白和转录因子；以及11)蛋白质“L”，该蛋白质是RNA聚合酶。M2基因在重叠的开放阅读框中编码蛋白质M2-1和蛋白质M2-2。原代CD8 T细胞表位是由M2基因编码。人RSV存在

两个主要亚型—A 和 B。这两个亚型之间的主要差异存在于 G 蛋白内。

[0007] RSV 感染通常同时引起免疫介导的病理学和病毒介导的病理学。原发性 RSV 感染通常导致急性细支气管炎，由此导致炎症诱发的气道阻塞。经显示，RSV F 结合可诱导细胞凋亡，从而引起纤毛上皮细胞脱落、肺清除受损及随之发生的继发性感染。

[0008] 不幸的是，尽管进行了大量尝试，仍没有可用于 RSV 的有效疫苗。在 1966-1967 间，首个 RSV 疫苗候选物，一种福尔马林灭活、明矾沉淀的 RSV 制剂 (FI-RSV 疫苗) 使接种疫苗的儿童在随后自然感染之后疾病增强。对死于由接种疫苗之后感染所引起的疾病增强的儿童的肺部的组织学分析揭露了广泛的单核细胞浸润，包括肺嗜酸性粒细胞增多。由于变性的 F 蛋白的存在，FI-RSV 疫苗只是产生结合抗体，没有中和活性，并且不能诱导 CTL 活性。

[0009] 随后的实验已经提出，这一肺部疾病增强与 CD4 T 细胞对 Th2 型细胞因子的反应增加、CD8 T 细胞的细胞溶解反应不良及中和抗体反应较弱有关。FI-RSV 疫苗接种的 BALB/c 小鼠和 C57BL/6 小鼠感染 RSV 导致在 FI-RSV 疫苗接种的儿童中所观察到的疾病增强。Th2 介导的免疫应答的特征表明，疫苗使得被免疫的儿童对 Th2 免疫应答产生易感性。展现出 Th2 相关性细胞因子 IL-5、IL-4 和 IL-13 以及嗜酸性粒细胞活化趋化因子 (chemokineeotaxin) 的水平增加，以及 Th1 相关性细胞因子 IL-12 减少。IL-4、IL-10 或 IL-13 耗尽导致在 RSV 攻击之后疾病增强的显著减轻。

[0010] 减毒活病毒疫苗和灭活全病毒疫苗也无法提供保护作用。这些候选疫苗是减毒不充分的或展示出疾病增强的可能性。在 1982 年，发现了一种减毒的活 RSV 疫苗是安全的，但无法有效预防 RSV 疾病。

[0011] 在 1983 年，一名被认为患上 B 族链球菌疾病但实际上是感染 RSV 的美国原住民婴儿“Moose 宝宝”当接受 IGIV 时，偶然发现疾病好转。这一结果推动了 IGIV 用于 RSV 疾病的研究。在 1980 年代中期到 1990 年，有关标准 IGIV 用于治疗和预防 RSV 疾病的研究验证了抗体在预防 RSV 疾病方面的作用。

[0012] 在 1990 年代早期，使用了各种亚单位变化来重新起始 RSV 疫苗研究。这些试验未能显示对疾病的显著防护作用。

[0013] 从 1990 年代中期到 2002 年早期，有关帕利珠单抗 (palivizumab ；一种对 RSV-F 具有特异性的单克隆抗体) 用于预防由 RSV 引起的严重呼吸道疾病的临床试验取得了积极的成果。在 2003 年 9 月，帕利珠单抗被批准用于高风险儿童以预防 RSV 相关性疾病。用帕利珠单抗进行的预防性治疗可有效降低疾病的严重程度，但由于治疗所涉及的高成本而只被推荐用于高风险患者。

[0014] 尽管过去的研究未能得到有效的 RSV 疫苗，但这些研究已经令人信服地展示了免疫应答在提供针对 RSV 感染的全面防护方面的重要性。研究已经发现了支持体液反应和抗体在防止 RSV 介导的疾病方面的重要性的证据。肺中 IgG 抗体的存在与病毒负荷减小直接相关，并且患有不太严重的 RSV 疾病的儿童在感染之前通常具有明显较高的抗 RSV 抗体滴度。针对融合蛋白的抗体与免疫性具有重要关联。未感染 RSV 的婴儿的 IgG 平均滴度高于受感染的婴儿，并且其母亲的母体 RSV 特异性 IgG 抗体水平明显高于受感染婴儿的母亲。抗体在介导防护作用方面的重要性有助于解释早产儿在 RSV 感染之后发生严重疾病的风险如此高的原因。在妊娠第三期之前，母体 IgG 并未有效地转移到胎儿。

[0015] 类似地，细胞免疫应答在针对 RSV 感染提供全面防护方面的重要性也已经得到证

实。缺乏T细胞的儿童很难清除病毒并且更易于随后发生RSV感染。在动物研究中，在小鼠中仅耗尽CD8 T细胞不会引起慢性感染，但会导致病毒清除延迟。清除需要IFN-γ、FasL、TNF-α。这些因子中任一个的缺乏都会引起病毒清除延迟。此外，用VV-G免疫的C57BL/6小鼠未发生RSV相关性疾病增强，但在免疫之前耗尽CD8 T细胞会导致疾病。

[0016] 仍然需要一种诱导针对RSV的长期防护作用的RSV疫苗，包括免疫原性抗原。还需要一种能够针对RSV进行大规模预防性疫苗接种的具有成本效益的传递系统。还需要用以治疗感染RSV的个体的其它治疗剂。

发明内容

[0017] 发明概要

[0018] 本发明提供了核酸分子，这些核酸分子包含编码一种或多种共有RSV免疫原的一个或多个核酸序列。这些RSV免疫原包括共有RSV F免疫原、共有RSV-G(A)免疫原和共有RSV G(B)免疫原。

[0019] 本发明提供了包含一个或多个编码RSV免疫原的核酸序列的组合物。这些核酸选自由以下组成的组：a)编码RSV F免疫原的核酸编码序列；b)编码RSV Ga免疫原的核酸编码序列；及c)编码RSV Gb免疫原的核酸编码序列。

[0020] 在一些实施方案中，编码RSV F免疫原的核酸编码序列编码一种RSV F免疫原，该免疫原选自由以下组成的组：SEQ ID NO:2、SEQ ID NO:2的片段、与SEQ ID NO:2具有至少98％同源性的蛋白质及与SEQ ID NO:2具有至少98％同源性的蛋白质的片段。在每一情形中，编码RSV F免疫原的核酸编码序列都可以任选地另外包含可操作地连接到该序列的编码信号肽的编码序列。

[0021] 在一些实施方案中，编码RSV Ga免疫原的核酸编码序列编码一种RSV Ga免疫原，该免疫原选自由以下组成的组：SEQ ID NO:6、SEQ ID NO:6的片段、与SEQ ID NO:6具有至少98％同源性的蛋白质及与SEQ ID NO:6具有至少98％同源性的蛋白质的片段。在每一情形中，编码RSV Ga免疫原的核酸编码序列可以任选地另外包含可操作地连接到该序列的编码信号肽的编码序列。

[0022] 在一些实施方案中，编码RSV Gb免疫原的核酸编码序列编码一种RSV Gb免疫原，该免疫原选自由以下组成的组：SEQ ID NO:8、SEQ ID NO:8的片段、与SEQ ID NO:8具有至少98％同源性的蛋白质及与SEQ ID NO:8具有至少98％同源性的蛋白质的片段。在每一情形中，编码RSV Gb免疫原的核酸编码序列可以任选地另外包含可操作地连接到该序列的编码信号肽的编码序列。

[0023] 在一些实施方案中，编码RSV F免疫原的核酸编码序列是选自由以下组成的组：包含SEQ ID NO:1的核酸序列、包含SEQ ID NO:1的片段的核酸序列、包含与SEQ ID NO:1具有至少98％同源性的序列的核酸序列，及包含与SEQ ID NO:1具有至少98％同源性的序列的片段的核酸序列。在每一情形中，编码RSV F免疫原的核酸编码序列可以任选地另外包含可操作地连接到该序列的编码信号肽的编码序列。包含与SEQ ID NO:1具有至少98％同源性的序列的核酸序列优选编码与SEQ ID NO:2具有至少98％同源性的蛋白质。包含与SEQ ID NO:1具有至少98％同源性的序列的片段的核酸序列优选编码与SEQ ID NO:2具有至少98％同源性的蛋白质的片段。

[0024] 在一些实施方案中，编码 RSV Ga 免疫原的核酸编码序列是选自由以下组成的组：包含 SEQ ID NO:5 的核酸序列、包含 SEQ ID NO:5 的片段的核酸序列、包含与 SEQ ID NO:5 具有至少 98％同源性的序列的核酸序列，及包含与 SEQ ID NO:5 具有至少 98％同源性的序列的片段的核酸序列。在每一情形中，编码 RSV Ga 免疫原的核酸编码序列可以任选地另外包含可操作地连接到该序列的编码信号肽的编码序列。包含与 SEQ ID NO:5 具有至少 98％同源性的序列的核酸序列优选编码一种与 SEQ ID NO:6 具有至少 98％同源性的蛋白质。包含与 SEQ ID NO:5 具有至少 98％同源性的序列的片段的核酸序列优选编码与 SEQ ID NO:6 具有至少 98％同源性的蛋白质的片段。

[0025] 在一些实施方案中，编码 RSV Gb 免疫原的核酸编码序列是选自由以下组成的组：包含 SEQ ID NO:7 的核酸序列、包含 SEQ ID NO:7 的片段的核酸序列、包含与 SEQ ID NO:7 具有至少 98％同源性的序列的核酸序列，及包含与 SEQ ID NO:7 具有至少 98％同源性的序列的片段的核酸序列。在每一情形中，编码 RSV Gb 免疫原的核酸编码序列可以任选地另外包含可操作地连接到该序列的编码信号肽的编码序列。包含与 SEQ ID NO:7 具有至少 98％同源性的序列的核酸序列优选编码一种与 SEQ ID NO:8 具有至少 98％同源性的蛋白质。包含与 SEQ ID NO:7 具有至少 98％同源性的序列的片段的核酸序列优选编码与 SEQ ID NO:8 具有至少 98％同源性的蛋白质的片段。

[0026] 在一些实施方案中，所述组合物包含一个或多个选自由以下组成的组的核酸序列：编码 SEQ ID NO:10 的核酸序列；编码 SEQ ID NO:14 的核酸序列；及编码 SEQ ID NO:16 的核酸序列。

[0027] 在一些实施方案中，所述组合物包含一个或多个选自由以下组成的组的核酸序列：包含 SEQ ID NO:9 的核酸序列；包含 SEQ ID NO:13 的核酸序列；及包含 SEQ ID NO:15 的核酸序列。

[0028] 在一些实施方案中，所述组合物包含了选自由以下组成的组的核酸序列：

[0029] 编码 RSV F 免疫原的核酸编码序列，

[0030] 编码 RSV Ga 免疫原的核酸编码序列，

[0031] 编码 RSV Gb 免疫原的核酸编码序列，

[0032] 编码 RSV F 免疫原的核酸编码序列和编码 RSV Ga 免疫原的核酸编码序列，

[0033] 编码 RSV F 免疫原的核酸编码序列和编码 RSV Gb 免疫原的核酸编码序列，

[0034] 编码 RSV Ga 免疫原的核酸编码序列和编码 RSV Gb 免疫原的核酸编码序列，

[0035] 编码 RSV F 免疫原的核酸编码序列、编码 RSV Ga 免疫原的核酸编码序列及编码 RSV Gb 免疫原的核酸编码序列。

[0036] 所述组合物可以另外包含一种编码 CCL20 的核酸编码序列，和 / 或被配制成使用电穿孔法传递到个体，和 / 或另外包含编码一种或多种选自由 IL-12、IL-15 和 IL-28 组成的组的蛋白质的核酸序列。

[0037] 在一些实施方案中，一个或多个核酸编码序列是一个或多个质粒的一部分。在一些实施方案中，一个或多个核酸编码序列各自被并入一个独立的质粒中。

[0038] 本发明提供了用于诱导针对 RSV 的免疫应答的方法，该方法包括向个体施用有效诱导所述个体的免疫应答的量的组合物。

[0039] 本发明提供了用于治疗被诊断带有 RSV 的个体的方法，该方法包括向个体施用治

疗有效量的组合物。

[0040] 本发明提供了用于防止个体感染 RSV 的方法，该方法包括向个体施用预防有效量的组合物。

[0041] 提供了新颖的“RSV F 免疫原”、“RSV Ga 免疫原”和“RSV Gb 免疫原”。在一些实施方案中，提供了选自由以下组成的组的蛋白质 ：SEQ ID NO:2 ；SEQ ID NO:6 ；SEQ ID NO:8 ；SEQ ID NO:10 ；SEQ ID NO:14 ；及 SEQ ID NO:16。

[0042] 还提供了使用编码趋化因子 CCL20 的核酸编码序列调节免疫应答的方法。

[0043] 此外，本文提供了一种编码共有 RSV M2-1 免疫原的核酸编码序列。在一些实施方案中，编码 RSV M2-1 免疫原的核酸编码序列编码一种 RSV M2-1 免疫原，该免疫原选自由以下组成的组 ：SEQ ID NO:4、SEQ ID NO:4 的片段、与 SEQ ID NO:4 具有至少 98％同源性的蛋白质及与 SEQ ID NO:4 具有至少 98％同源性的蛋白质的片段。在每一情形中，编码 RSV M2-1 免疫原的核酸编码序列可以任选地另外包含可操作地连接到该序列的编码信号肽的编码序列。

[0044] 在一些实施方案中，编码 RSV M2-1 免疫原的核酸编码序列是选自由以下组成的组 ：包含 SEQ ID NO:3 的核酸序列、包含 SEQ ID NO:3 的片段的核酸序列、包含与 SEQ ID NO:3 具有至少 98％同源性的序列的核酸序列，及包含与 SEQ ID NO:3 具有至少 98％同源性的序列的片段的核酸序列。在每一情形中，编码 RSV M2-1 免疫原的核酸编码序列可以任选地另外包含可操作地连接到该序列的编码信号肽的编码序列。包含与 SEQ ID NO:3 具有至少 98％同源性的序列的核酸序列优选编码一种与 SEQ ID NO:4 具有至少 98％同源性的蛋白质。包含与 SEQ ID NO:3 具有至少 98％同源性的序列的片段的核酸序列优选编码与 SEQ ID NO:4 具有至少 98％同源性的蛋白质的片段。

[0045] 在一些实施方案中，提供了包含一个或多个核酸序列（包括编码 SEQ ID NO:12 的核酸序列在内）的组合物。

[0046] 在一些实施方案中，提供了包含一个或多个核酸序列（包括了含 SEQ ID NO:11 的核酸序列在内）的组合物。

[0047] 在一些实施方案中，所述组合物包含了选自由以下组成的组的核酸序列 ：编码 RSV M2-1 免疫原的核酸编码序列 ；编码 RSV F 免疫原的核酸编码序列和编码 RSV M2-1 免疫原的核酸编码序列 ；编码 RSV M2-1 免疫原的核酸编码序列和编码 RSV Ga 免疫原的核酸编码序列 ；编码 RSV M2-1 免疫原的核酸编码序列和编码 RSV Gb 免疫原的核酸编码序列 ；编码 RSV F 免疫原的核酸编码序列、编码 RSV M2-1 免疫原的核酸编码序列和编码 RSV Ga 免疫原的核酸编码序列 ；编码 RSV F 免疫原的核酸编码序列、编码 RSV M2-1 免疫原的核酸编码序列和编码 RSV Gb 免疫原的核酸编码序列 ；编码 RSV M2-1 免疫原的核酸编码序列、编码 RSV Ga 免疫原的核酸编码序列和编码 RSV Gb 免疫原的核酸编码序列 ；及编码 RSV F 免疫原的核酸编码序列、编码 RSV M2-1 免疫原的核酸编码序列、编码 RSV Ga 免疫原的核酸编码序列和编码 RSV Gb 免疫原的核酸编码序列。

[0048] 还提供了新颖的“RSV M2-1 免疫原”。在一些实施方案中，提供了选自由以下组成的组 SEQ ID NO:4 和 SEQ ID NO:12 的蛋白质。

附图说明

[0049] 图 1 示出了 RSV-F 蛋白的系统发生树，共有 RSV F 蛋白的氨基酸序列以 SEQ ID NO:2 陈述。

[0050] 图 2 示出了 RSV M2-1 蛋白的系统发生树，共有 RSV M2-1 蛋白的氨基酸序列以 SEQ ID NO:4 陈述。

[0051] 图 3 示出了在用 RSV F 疫苗进行免疫之后在不同时间点时抗 RSV F 蛋白 IgG 的结果。

[0052] 图 4 示出了在用 RSV F 疫苗进行免疫之后在不同时间点时抗 RSV F 蛋白 IgA 的结果。

[0053] 图 5 示出了在最后一次免疫之后约 8 周，通过比较在不同 RSV-F 疫苗剂量下的 IFN-γ 产量得到的结果。

[0054] 图 6 示出了在脾中用 RSV-F+/- 趋化因子进行免疫之后，通过比较脾中 IFN-γ +CD8 和 CD4 T 细胞得到的结果。

[0055] 图 7 示出了在肺中用 RSV-F+/- 趋化因子进行免疫之后，通过比较脾中 IFN-γ +CD8 和 CD4 T 细胞得到的结果。

[0056] 图 8 示出了由血清中的 IgG 终点滴度及血清中 IgG 亚型 (IgG1 对比 IgG2a) 的比较得到的结果。

[0057] 图 9 示出了 IgG2a/IgG1 比率的比较。

[0058] 图 10 描绘了 TZM-BL 细胞 /“打开”中的包膜假型病毒 (Env-Pseudotyped virus)。

[0059] 图 11 描绘了 TZM-BL 细胞 / “关闭”中的包膜假型病毒。

[0060] 图 12 描绘了 293T 细胞中 RSV- 融合假病毒的产生。

[0061] 图 13 示出了用于一项使用该检验的 RSV-F 中和研究中的免疫方案。

[0062] 图 14 示出了通过对不同靶细胞中 RSV-F 介导的感染进行定量得到的数据。

[0063] 图 15 示出了通过对 TZM-bl HeLa 细胞中的 RSV-F 中和活性进行定量得到的数据。

[0064] 图 16 示出了通过对 HeLa 细胞中由 RSV-F 假病毒引起的细胞死亡活性进行定量得到的数据。

[0065] 图 17 示出了所采取的 RSV-M2 剂量研究的免疫时程安排。

[0066] 图 18 示出了从该研究收集的数据，包括在最后一次免疫之后 1 周在不同 RSV-M2 疫苗剂量下 IFN-g 产量的比较。

[0067] 图 19 描绘了用于评价 RSV-F 疫苗与 CCL20 的组合的免疫时程安排。

[0068] 图 20 示出了从该研究得到的数据，包括通过比较在最后一次免疫之后 1 周在利用不同 CCL20 剂量下脾、肝和肺中的每一个的 IFN-g 产量得到的数据。

[0069] 图 21 示出了血清中 IgG 亚型 (IgG1 对比 IgG2a) 的比较数据。

[0070] 图 22A-22C 示出了 RSV-F、RSV-G(A) 和 RSV-G(B) 构建体的质粒设计和构建。图 22A 示出了 RSV-F 构建体的质粒设计和构建。图 22B 示出了 RSV-G(A) 构建体的质粒设计和构建。图 22C 示出了 RSV-G(B) 构建体的质粒设计和构建。

[0071] 图 23 示出了证实 RSV-F、RSV-G(A) 和 RSV-G(B) 构建体的体外表达的结果。

[0072] 图 24 示出了 RSV-F 剂量研究的免疫时程安排。

[0073] 图 25 示出了由 RSV-F 剂量研究得到的 IgG 数据。

[0074] 图 26 示出了由 RSV-F 剂量研究得到的 IFN-γ 数据。

[0075] 图 27 示出了 RSV-F PSEUDO 中和研究的免疫时程安排。

[0076] 图 28 示出了由 RSV-F PSEUDO 中和研究得到的数据。

[0077] 图 29 示出了由确定对 HeLa 细胞中由 RSV-F 假病毒引起的凋亡细胞死亡活性的抑制作用的两项比较得到的数据。

[0078] 图 30 示出了 RSV-G(A) 和 RSV-(B) 剂量研究的免疫时程安排。

[0079] 图 31 示出了 RSV-G(A) 和 RSV-(B) 剂量研究中由 RSV-G(A) 得到的 IgG 数据。

[0080] 图 32 示出了 RSV-G(A) 和 RSV-(B) 剂量研究中由 RSV-G(A) 得到的 IgG 数据。

[0081] 图 33 是示出了在小鼠研究中使用 Mono 疫苗的 RSV 初步中和结果的概述的图表。

[0082] 图 34 示出了以下 RSV 兔研究设计。

[0083] 图 35 示出了由测量针对 RSV-F 的体液免疫的 RSV 兔研究得到的数据。

[0084] 图 36 示出了由测量针对 RSV-G 的体液免疫的 RSV 兔研究得到的数据。

[0085] 图 37 示出了以下 RSV 非人类灵长类动物研究设计。

[0086] 图 38A-38D 示出了由测量针对 RSV-F 的体液免疫的 RSV 非人类灵长类动物研究得到的数据。图 38A 示出了在原生动物中由测量针对 RSV-F 的体液免疫得到的数据。图 38B 示出了在一个部位接受混合疫苗 (IM) 的动物中由测量针对 RSV-F 的体液免疫得到的数据。图 38C 示出了在一个部位接受混合疫苗加 rhMEC 构建体 (IM) 的动物中由测量针对 RSV-F 的体液免疫得到的数据。图 38D 示出了在三个部位接受混合疫苗 (ID) 的动物中由测量针对 RSV-F 的体液免疫得到的数据。

[0087] 图 39A-39D 示出了由测量针对 RSV-G 的体液免疫的 RSV 非人类灵长类动物研究得到的数据。图 39A 示出了在原生动物中由测量针对 RSV-G 的体液免疫得到的数据。图 39B 示出了在一个部位接受混合疫苗 (IM) 的动物中由测量针对 RSV-G 的体液免疫得到的数据。图 39C 示出了在一个部位接受混合疫苗加 rhMEC 构建体 (IM) 的动物中由测量针对 RSV-G 的体液免疫得到的数据。图 39D 示出了在三个部位接受混合疫苗 (ID) 的动物中由测量针对 RSV-G 的体液免疫得到的数据。

[0088] 图 40 是示出了在兔非人类灵长类动物研究中使用混合疫苗的 RSV 初步中和结果的概述的图表。

具体实施方式

[0089] 在本发明的一个方面，希望的是，共有抗原提供改良的转录和翻译，包括具有以下一种或多种特性：用以增加转录的低 GC 含量的前导序列；mRNA 稳定性和密码子优化；在一定程度上消除可能的顺式作用序列基元（即，内部 TATA 盒）。

[0090] 在本发明的一些方面，希望产生一种共有抗原，该抗原在多种品系中产生广泛免疫应答，包括具有以下一种或多种特性：并入所有可用的全长序列；计算机产生在每个位置处利用最常见氨基酸的序列；及增加各品系之间的交叉反应性。

[0091] 所附序列表包括 SEQ ID NO:1-16，这些序列是各种 RSV 序列和实施方案。

[0092] SEQ ID NO:1 示出了编码 RSV F 并且不含起始密码子 (ATG) 或信号肽的核苷酸序列。

[0093] SEQ ID NO:2 示出了编码 RSV F 并且不含由起始密码子 (ATG) 编码的 Met 或信号肽的氨基酸序列。

[0094] SEQ ID NO:3示出了编码RSV M2-1并且不含起始密码子(ATG)或信号肽的核苷酸序列。

[0095] SEQ ID NO:4示出了编码RSV M2-1并且不含由起始密码子(ATG)编码的Met或信号肽的氨基酸序列。

[0096] SEQ ID NO:5示出了编码RSV Ga并且不含起始密码子(ATG)或信号肽的核苷酸序列。

[0097] SEQ ID NO:6示出了编码RSV Ga并且不含由起始密码子(ATG)编码的Met或信号肽的氨基酸序列。

[0098] SEQ ID NO:7示出了编码RSV Gb并且不含起始密码子(ATG)或信号肽的核苷酸序列。

[0099] SEQ ID NO:8示出了编码RSV Gb并且不含由起始密码子(ATG)编码的Met或信号肽的氨基酸序列。

[0100] SEQ ID NO:9示出了编码RSV F并且包括起始密码子(ATG)/编码IgE信号肽的序列的核苷酸序列。

[0101] SEQ ID NO:10示出了编码RSV F并且具有由起始密码子编码的Met/IgE信号肽的氨基酸序列。

[0102] SEQ ID NO:11示出了编码RSV M2-1并且包括起始密码子(ATG)/编码IgE信号肽的序列的核苷酸序列。

[0103] SEQ ID NO:12示出了编码RSV M2-1并且具有由起始密码子编码的Met/IgE信号肽的氨基酸序列。

[0104] SEQ ID NO:13示出了编码RSV Ga并且包括起始密码子(ATG)/编码IgE信号肽的序列的核苷酸序列。

[0105] SEQ ID NO:14示出了编码RSV Ga并且具有由起始密码子编码的Met/IgE信号肽的氨基酸序列。

[0106] SEQ ID NO:15示出了编码RSV Gb并且包括起始密码子(ATG)/编码IgE信号肽的序列的核苷酸序列。

[0107] SEQ ID NO:16示出了编码RSV Gb并且具有由起始密码子编码的Met/IgE信号肽的氨基酸序列。

[0108] 所附序列表包括SEQ ID NO:17,该序列为IgE信号肽的氨基酸序列。

[0109] SEQ ID NO:17示出了IgE信号肽的氨基酸序列。

[0110] 所附序列表包括SEQ ID NO:18-21,这些序列是各种CCL20序列和实施方案。

[0111] SEQ ID NO:18示出了编码CCL20并且不含起始密码子(ATG)或信号肽的核苷酸序列。

[0112] SEQ ID NO:19不含由起始密码子(ATG)编码的Met或信号肽的CCL20

[0113] SEQ ID NO:20示出了编码CCL20并且包括起始密码(ATG)/编码IgE信号肽的序列的核苷酸序列。

[0114] SEQ ID NO:21示出了编码CCL20并且含有由起始密码子编码的Met/IgE信号肽的氨基酸序列。

[0115] 所附序列表包括SEQ ID NO:22-31,这些序列是来源于RSV免疫原的氨基酸序列。

[0116] SEQ ID NO:22 是 RSV F 免疫原的 T 细胞表位。

[0117] SEQ ID NO:23 是 RSV F 免疫原的 T 细胞表位。

[0118] SEQ ID NO:24 是 RSV F 免疫原的 T 细胞表位。

[0119] SEQ ID NO:25 是 RSV F 免疫原的 T 细胞表位。

[0120] SEQ ID NO:26 是 RSV F 免疫原蛋白酶裂解位点。

[0121] SEQ ID NO:27 是 RSV F 免疫原蛋白酶裂解位点。

[0122] SEQ ID NO:28 是 RSV F 免疫原融合肽。

[0123] SEQ ID NO:29 是 RSV F 免疫原的帕利珠单抗结合位点。

[0124] SEQ ID NO:30 是 RSV M2-1 免疫优势 T 细胞表位。

[0125] SEQ ID NO:31 是 RSV M2-1 次优势 T 细胞表位。

[0126] 在本发明的一些方面中，提供了 RSV F 免疫原和编码 RSV F 免疫原的序列。在本发明的一些方面中，提供了 RSV Ga 免疫原和编码 RSV Ga 免疫原的序列。在本发明的一些方面中，提供了 RSV Gb 免疫原和编码 RSV Gb 免疫原的序列。本发明提供了这样一些组合物，所述组合物包含了编码 RSV F 免疫原的序列、编码 RSV Ga 免疫原的序列与编码 RSV Gb 免疫原的序列的组合，包括了编码 RSV F 免疫原的序列、编码 RSV Ga 免疫原的序列和编码 RSV Gb 免疫原的序列中的每一个。

[0127] 还提供了 RSV M2-1 免疫原和编码 RSV M2-1 免疫原的序列，并且其可以单独使用或与其它蛋白质和 / 或核酸序列组合使用。

[0128] 1. 定义 .

[0129] 本文所使用的术语只是用于描述特定实施方案的目的，并且不打算作为限制。除非上下文另外清楚地规定，否则如本说明书和所附权利要求中所使用，单数形式“一个（种）”(a/an) 和“该”包括复数个参照物。

[0130] 对于本文所陈述的数字范围，清楚地涵盖其间具有相同精确度的每个中间数字。举例来说，对于范围 6-9，除 6 和 9 外，还涵盖数字 7 和 8，并且对于范围 6.0-7.0，明确地涵盖数字 6.0、6.1、6.2、6.3、6.4、6.5、6.6、6.7、6.8、6.9 及 7.0。

[0131] a. 佐剂

[0132] 如本文所使用，“佐剂”可以指添加到本文所描述的 DNA 质粒疫苗中以增强由这些 DNA 质粒所编码的一种或多种呼吸道合胞病毒 (RSV.) 抗原的免疫原性并且编码下文所描述的核酸序列的任何分子。

[0133] b. 抗体

[0134] “抗体”可以指属于类别 IgG、IgM、IgA、IgD 或 IgE 的抗体，或片段、片段或其衍生物，包括 Fab、F(ab')2、Fd 及单链抗体、微型双功能抗体、双特异性抗体、双功能抗体及其衍生物。抗体可以是从哺乳动物的血清样品分离的抗体、多克隆抗体、亲和纯化的抗体，或其混合物，其对所希望的表位或衍生自其的序列展现足够的结合特异性。

[0135] c. 编码序列

[0136] 如本文所使用，“编码序列”或“编码核酸”可以指包含了编码蛋白质的核苷酸序列的核酸 (RNA 或 DNA 分子）。编码序列可以另外包括可操作地连接到调控元件的起始和终止信号，包括启动子和聚腺苷酸化信号，它能够引导施用该核酸的个体或哺乳动物的细胞中的表达。

[0137] 在一些实施方案中，以 SEQ ID NO:2 陈述的共有 RSV F 免疫原的氨基酸序列的编码序列为 SEQ ID NO:1。该编码序列可以任选地另外包含一个编码 N 末端甲硫氨酸的起始密码子或一个信号肽，如 IgE 或 IgG 信号肽。SEQ ID NO:10 对应于具有 IgE 信号肽 (SEQ ID NO:17) 的 SEQ ID NO:2。SEQ ID NO:9 编码 SEQ ID NO:10。

[0138] 在一些实施方案中，以 SEQ ID NO:6 陈述的共有 RSV Ga 免疫原的氨基酸序列的编码序列为 SEQ ID NO:5。该编码序列可以任选地另外包含一个编码 N 末端甲硫氨酸的起始密码子或一个信号肽，如 IgE 或 IgG 信号肽。SEQ ID NO:14 对应于具有 IgE 信号肽 (SEQ ID NO:17) 的 SEQ ID NO:6。SEQ ID NO:13 编码 SEQ ID NO:14。

[0139] 在一些实施方案中，以 SEQ ID NO:8 陈述的共有 RSV Gb 免疫原的氨基酸序列的编码序列为 SEQ ID NO:7。该编码序列可以任选地另外包含一个编码 N 末端甲硫氨酸的起始密码子或一个信号肽，如 IgE 或 IgG 信号肽。SEQ ID NO:16 对应于具有 IgE 信号肽 (SEQ ID NO:17) 的 SEQ ID NO:8。SEQ ID NO:15 编码 SEQ ID NO:16。

[0140] 还提供了一个编码序列 SEQ ID NO:3，该序列编码共有 RSV M2-1 免疫原的氨基酸序列，该氨基酸序列以 SEQ ID NO:4 陈述。该编码序列可以任选地另外包含一个编码 N 末端甲硫氨酸的起始密码子或一个信号肽，如 IgE 或 IgG 信号肽。SEQ ID NO:12 对应于具有 IgE 信号肽 (SEQ ID NO:17) 的 SEQ ID NO:4。SEQ ID NO:11 编码 SEQ ID NO:12。

[0141] d. 补体

[0142] 如本文所使用，“补体”或“互补”可以指核苷酸或核酸分子的核苷酸类似物之间的 Watson-Crick (例如 A-T/U 和 C-G) 或 Hoogsteen 碱基配对。

[0143] e. 共有或共有序列

[0144] 如本文所使用，“共有”或“共有序列”可以指基于特定 RSV 抗原的多种亚型的比对分析构建的合成核酸序列，或相应的多肽序列，其可以被用于诱导针对特定 RSV 抗原的多种亚型或血清型的广泛免疫性。共有 RSV 抗原可以包括蛋白质 F、蛋白质 G 和蛋白质 M2 的共有氨基酸序列。还提供了编码这些共有氨基酸序列的核苷酸序列。另外，可以对合成抗原 (如融合蛋白) 进行操作以使其包括共有序列 (或共有抗原)。

[0145] 共有 RSV F 免疫原的氨基酸序列以 SEQ ID NO:2 陈述。该序列可以任选地包含 N 末端甲硫氨酸或信号肽，如 IgE 或 IgG 信号肽。SEQ ID NO:10 对应于具有 IgE 信号肽 (SEQ ID NO:17) 的 SEQ ID NO:2。

[0146] 共有 RSV Ga 免疫原的氨基酸序列以 SEQ ID NO:6 陈述。该序列可以任选地包含 N 末端甲硫氨酸或信号肽，如 IgE 或 IgG 信号肽。SEQ ID NO:14 对应于具有 IgE 信号肽 (SEQ ID NO:17) 的 SEQ ID NO:6。

[0147] 共有 RSV Gb 免疫原的氨基酸序列以 SEQ ID NO:8 陈述。该序列可以任选地包含 N 末端甲硫氨酸或信号肽，如 IgE 或 IgG 信号肽。SEQ ID NO:16 对应于具有 IgE 信号肽 (SEQ ID NO:17) 的 SEQ ID NO:8。

[0148] 还提供了共有 RSV M2-1 免疫原的氨基酸序列，该序列以 SEQ ID NO:4 陈述。该序列可以任选地包含 N 末端甲硫氨酸或信号肽，如 IgE 或 IgG 信号肽。SEQ ID NO:12 对应于具有 IgE 信号肽 (SEQ ID NO:17) 的 SEQ ID NO:4。

[0149] f. 恒定电流

[0150] 如本文所使用，“恒定电流”定义为由组织或界定所述组织的细胞在向该组织传递

电脉冲期间所接收或经历的电流。该电脉冲是由本文所描述的电穿孔装置传递。这一电流在电脉冲施加期间于所述组织中保持在恒定电流强度，因为本文提供的电穿孔装置具有一个反馈元件，优选地具有即时反馈。该反馈元件可以测量在整个脉冲持续期间组织（或细胞）的电阻，并且引起该电脉冲装置改变其电能输出（例如，增加电压），因此在该组织中的电流在整个电脉冲（约数微妙）中并且随脉冲变化而保持恒定。在一些实施方案中，该反馈元件包含控制器。

[0151] g. 电流反馈或反馈

[0152] 如本文所使用，“电流反馈”或“反馈”可以互换使用，并且可以指所提供的电穿孔装置的主动响应，其包括测量组织中各电极之间的电流，及相应地改变 EP 装置所传递的能量输出，以便将电流维持在恒定水平。这一恒定水平是由用户在起始脉冲序列或电处理之前预先设定的。该反馈可以通过电穿孔装置的电穿孔组件（例如控制器）实现，因为其中的电路能够持续地监测组织中各电极之间的电流并且将该电流监测值（或组织内的电流）与预先设定的电流相比较，并且不断地作出能量输出调整以将所监测的电流维持在预先设定的水平。反馈循环可以是即时的，因为它是一个模拟闭环反馈。

[0153] h. 分散电流

[0154] 如本文所使用，“分散电流”可以指由本文所描述的电穿孔装置的各种针式电极阵列所传递的电流模式，其中这些模式使得在进行电穿孔的组织的任何区域上出现的电穿孔相关热应力减到最小或优选地消除。

[0155] i. 电穿孔

[0156] “电穿孔”、“电渗透”或“电动力学强化”（“EP”）在本文中可互换使用，可以指使用跨膜电场脉冲在生物膜中诱导微观路径（孔）；其存在允许如质粒、寡核苷酸、siRNA、药物、离子和水等生物分子从细胞膜的一侧到达另一侧。

[0157] j. 反馈机制

[0158] 如本文所使用，“反馈机制”可以指由软件或硬件（或固件）执行的一种工艺，该工艺接收所希望的组织的阻抗（在传递能量脉冲之前、期间和 / 或之后）并将其与预先设定的值（优选为电流）相比较，并且调整所传递的能量脉冲以达到预先设定的值。反馈机制可以由模拟闭环电路执行。

[0159] k. 片段

[0160] “片段”可以指能够在哺乳动物中通过识别特定 RSV 抗原而引起针对 RSV 的免疫应答的 RSV 免疫原的多肽片段。RSV 免疫原是指本文所陈述的 RSV F 免疫原、RSV M2-1 免疫原、RSV Ga 免疫原、RSV Gb 免疫原的共有序列，在每一情形中，这些共有序列含有或不含信号肽和 / 或在 1 位的甲硫氨酸；与本文陈述的这些共有序列具有 98％或更高同源性的蛋白质、与本文陈述的这些共有序列具有 99％或更高同源性的蛋白质及与本文所陈述的这些共有序列 100％同一的蛋白质，在每一情形中，这些蛋白质含有或不含信号肽和 / 或在 1 位的甲硫氨酸。片段可以包含或可以不包含例如连接到信号肽，如免疫球蛋白信号肽，例如 IgE 信号肽 (SEQ ID NO:17) 或 IgG 信号肽的 RSV 免疫原的片段。

[0161] RSV F共有免疫原的片段可以是以下各序列的片段：SEQ ID NO:2、SEQ ID NO:10、与本文所陈述的 RSV F 共有免疫原序列具有 98％或更高同源性的蛋白质、与本文所陈述的 RSV F 共有免疫原序列具有 99％或更高同源性的蛋白质或与本文所陈述的共有序列具有

100％同一性的 RSV F 共有免疫原，在每一情形中均含有或不含信号肽和 / 或在 1 位的甲硫氨酸。这些片段可以占本文所陈述的特定全长 RSV F 共有免疫原序列的长度的 20％或更高，25％或更高，30％或更高，35％或更高，40％或更高，45％或更高，50％或更高，55％或更高，60％或更高，65％或更高，70％或更高，75％或更高，80％或更高，85％或更高，90％或更高，91％或更高，92％或更高，93％或更高，94％或更高，95％或更高，96％或更高，97％或更高，98％或更高，99％或更高百分比，不包括添加的任何非 RSV 信号肽。片段是指与本文所陈述的 RSV F 共有免疫原的序列具有 98％或更高，或 99％或更高同源性以及与该序列具有 100％同一性的多肽的片段，在每一情形中均含有或不含信号肽和 / 或在 1 位的甲硫氨酸。片段可以包含与 RSV F 共有免疫原具有 98％或更高同源性，99％或更高同源性，或具有 100％同一性并且另外包含当计算同源性百分比时未包括在内的信号肽（如免疫球蛋白信号肽）的多肽的片段。在一些实施方案中，RSV F 免疫原的片段可以包含 SEQ ID NO:2 的片段。该片段可以包含连接到信号肽，如免疫球蛋白信号肽，例如 IgE 信号肽 (SEQ ID NO:17) 或 IgG 信号肽的 SEQ ID NO:2 的片段。因此，在一些实施方案中，该片段是包含 SEQ ID NO:17 的 SEQ ID NO:10 的片段。该片段可以包括连接到 N 末端甲硫氨酸的 SEQ ID NO:2 的片段。片段还指与 SEQ ID NO:2 具有 98％或更高，或 99％或更高同源性的多肽的片段。该片段可以包含与 SEQ ID NO:2 具有 98％或更高同源性并且另外包含当计算同源性百分比时未包括在内的信号肽（如免疫球蛋白信号肽）的多肽的片段。该片段可以包含与 SEQ ID NO:2 具有 99％或更高同源性并且另外包含当计算同源性百分比时未包括在内的信号肽（如免疫球蛋白信号肽）的多肽的片段。该片段可以包含与 SEQ ID NO:2 具有 98％或更高同源性并且另外包含当计算同源性百分比时未包括在内的 N 末端甲硫氨酸的多肽的片段。该片段可以包含与 SEQ ID NO:2 具有 99％或更高同源性并且另外包含当计算同源性百分比时未包括在内的 N 末端甲硫氨酸的多肽的片段。RSV F 免疫原具有 575 个氨基酸。在一些实施方案中，其片段可以为 115 个或更多氨基酸的长度，163 个或更多、230 个或更多、288 个或更多、345 个或更多、403 个或更多、460 个或更多、515 个或更多、520 个或更多、525 个或更多、530 个或更多、535 个或更多、540 个或更多、545 个或更多、550 个或更多、555 个或更多、560 个或更多、565 个或更多、570 个或更多、574 或更多氨基酸的长度。多肽片段可以为不到 140 个、不到 190 个、不到 250 个、不到 283 个、不到 300 个、不到 365 个、不到 425 个、不到 495 个、不到 518 个、不到 523 个、不到 528 个、不到 533 个、不到 547 个、不到 552 个、不到 563 个或不到 572 个氨基酸的长度。片段可以另外包含 N 末端甲硫氨酸和 / 或信号肽，如免疫球蛋白信号肽，例如 IgE 或 IgG 信号肽。N 末端甲硫氨酸和 / 或信号肽可以连接到 574 个氨基酸或其更小的片段。N 末端甲硫氨酸和 / 或信号肽可以连接到与该 574 个氨基酸的序列具有 98％同源性的多肽，或连接到与该 574 个氨基酸的序列具有 98％同源性的多肽的更小的片段。N 末端甲硫氨酸和 / 或信号肽可以连接到与该 574 个氨基酸的序列具有 99％同源性的多肽，或连接到与该 574 个氨基酸的序列具有 99％同源性的多肽的更小的片段。在计算同源性程度时，多肽具有 SEQ ID NO:2 或其片段，在该计算中未包括 N 末端甲硫氨酸和 / 或任何信号肽。在测定同源性时，未使用信号肽序列。因此，例如，尽管 SEQ ID NO:10 包含了可操作地连接到信号肽 SEQ ID NO:17 的 SEQ ID NO:2，但 SEQ ID NO:10 包含 SEQ ID NO:2 的片段，其与 SEQ ID NO:2 的片段具有 100％同源性，虽然在 SEQ ID NO:2 中不存在信号肽。因此，包含了与 SEQ ID NO:2 的片段具有至少 98％同源性的多肽的片段的

蛋白质拟指这样一些蛋白质，其中与 SEQ ID NO:2 的片段具有至少 98％同源性的多肽的片段为至少 115 个氨基酸并且可以任选地连接到例如信号肽。SEQ ID NO:22、SEQ ID NO:23、SEQ ID NO:24 及 SEQ ID NO:25 对应于 SEQ ID NO:2 中所陈述的 RSV F 免疫原的 T 细胞表位。在一些实施方案中，片段包含这些序列或与其具有 98％或更高，或 99％或更高同源性的序列中一个或多个序列的全部或一部分。SEQ ID NO:26 和 SEQ ID NO:27 是 RSV F 免疫原蛋白酶裂解位点。SEQ ID NO:28 是 RSV F 免疫原融合肽。SEQ ID NO:29 是 RSV F 免疫原的帕利珠单抗结合位点。

[0162] RSV Ga 共有免疫原的片段可以是以下各序列的片段：SEQ ID NO:6、SEQ ID NO:14、与本文所陈述的 RSV Ga 共有免疫原序列具有 98％或更高同源性的蛋白质、与本文所陈述的 RSV Ga 共有免疫原序列具有 99％或更高同源性的蛋白质或与本文所陈述的共有序列具有 100％同一性的 RSV Ga 共有免疫原，在每一情形中均含有或不含信号肽和 / 或在 1 位的甲硫氨酸。这些片段可以占本文所陈述的特定全长 RSV Ga 共有免疫原序列的长度的 20％或更高，25％或更高，30％或更高，35％或更高，40％或更高，45％或更高，50％或更高，55％或更高，60％或更高，65％或更高，70％或更高，75％或更高，80％或更高，85％或更高，90％或更高，91％或更高，92％或更高，93％或更高，94％或更高，95％或更高，96％或更高，97％或更高，98％或更高，99％或更高百分比，不包括添加的任何非 RSV 信号肽。片段是指与本文所陈述的 RSV Ga 共有免疫原的序列具有 98％或更高，或 99％或更高同源性以及与该序列具有 100％同一性的多肽的片段，在每一情形中均含有或不含信号肽和 / 或在 1 位的甲硫氨酸。片段可以包含与 RSV Ga 共有免疫原具有 98％或更高同源性，99％或更高同源性，或具有 100％同一性并且另外包含当计算同源性百分比时未包括在内的信号肽（如免疫球蛋白信号肽）的多肽的片段。在一些实施方案中，RSV Ga 免疫原的片段可以包含 SEQ ID NO:6 的片段。该片段可以包含连接到信号肽，如免疫球蛋白信号肽，例如 IgE 信号肽 (SEQ ID NO:17) 或 IgG 信号肽的 SEQ ID NO:6 的片段。因此，在一些实施方案中，该片段是包含 SEQ ID NO:17 的 SEQ ID NO:14 的片段。该片段可以包括连接到 N 末端甲硫氨酸的 SEQ ID NO:6 的片段。片段还指与 SEQ ID NO:6 具有 98％或更高，或 99％或更高同源性的多肽的片段。该片段可以包含与 SEQ ID NO:6 具有 98％或更高同源性并且另外包含当计算同源性百分比时未包括在内的信号肽（如免疫球蛋白信号肽）的多肽的片段。该片段可以包含与 SEQ ID NO:6 具有 99％或更高同源性并且另外包含当计算同源性百分比时未包括在内的信号肽（如免疫球蛋白信号肽）的多肽的片段。该片段可以包含与 SEQ ID NO:6 具有 98％或更高同源性并且另外包含当计算同源性百分比时未包括在内的 N 末端甲硫氨酸的多肽的片段。该片段可以包含与 SEQ ID NO:6 具有 99％或更高同源性并且另外包含当计算同源性百分比时未包括在内的 N 末端甲硫氨酸的多肽的片段。RSV Ga 免疫原具有 297 个氨基酸，不包括 N 末端甲硫氨酸和 / 或信号肽。在一些实施方案中，其片段可以为 125 个或更多氨基酸的长度，130 个或更多、140 个或更多、150 个或更多、160 个或更多、170 个或更多、180 个或更多、190 个或更多、200 个或更多、210 个或更多、220 个或更多、230 个或更多、240 个或更多、250 个或更多、260 个或更多、270 个或更多、280 个或更多、285 个或更多、290 个或更多、295 个或更多氨基酸的长度。多肽片段可以为不到 135 个、不到 145 个、不到 155 个、不到 165 个、不到 175 个、不到 185 个、不到 195 个、不到 205 个、不到 215 个、不到 225 个、不到 235 个、不到 245 个、不到 255 个、不到 265 个、不到 275 个、不到 285 个、不到 288

个、不到 292 个或不到 296 个氨基酸的长度。片段可以另外包含 N 末端甲硫氨酸和 / 或信号肽，如免疫球蛋白信号肽，例如 IgE 或 IgG 信号肽。N 末端甲硫氨酸和 / 或信号肽可以连接到 296 个氨基酸或其更小的片段。N 末端甲硫氨酸和 / 或信号肽可以连接到与该 296 个氨基酸的序列具有 98％同源性的多肽，或连接到与该 296 个氨基酸的序列具有 98％同源性的多肽的更小的片段。N 末端甲硫氨酸和 / 或信号肽可以连接到与该 296 个氨基酸的序列具有 99％同源性的多肽，或连接到与该 296 个氨基酸的序列具有 99％同源性的多肽的更小的片段。在计算同源性程度时，多肽具有 SEQ ID NO:6 或其片段，在该计算中未包括 N 末端甲硫氨酸和 / 或任何信号肽。在测定同源性时，未使用信号肽序列。因此，例如，尽管 SEQ ID NO:14 包含了可操作地连接到信号肽 SEQ ID NO:17 的 SEQ ID NO:6，但 SEQ ID NO:14 包含 SEQ ID NO:6 的片段，其与 SEQ ID NO:6 的片段具有 100％同源性，虽然在 SEQ ID NO:6 中不存在信号肽。因此，包含了与 SEQ ID NO:6 的片段具有至少 98％同源性的多肽的片段的蛋白质拟指这样一些蛋白质，其中与 SEQ ID NO:6 的片段具有至少 98％同源性的多肽的片段为至少 125 个氨基酸并且可以任选地连接到例如信号肽。

[0163] RSV Gb 共有免疫原的片段，如 SEQ ID NO:8、SEQ ID NO:16、与本文陈述的 RSV Gb 共有免疫原序列具有 98％或更高同源性的蛋白质、与本文陈述的 RSV Gb 共有免疫原序列具有 99％或更高同源性的蛋白质及与本文陈述的共有序列具有 100％同一性的 RSV Gb 共有免疫原中所公开的片段（在每一情形中均含有或不含信号肽和 / 或在 1 位的甲硫氨酸），可以占本文陈述的特定全长 RSV Gb 共有免疫原序列的长度的 20％或更高，25％或更高，30％或更高，35％或更高，40％或更高，45％或更高，50％或更高，55％或更高，60％或更高，65％或更高，70％或更高，75％或更高，80％或更高，85％或更高，90％或更高，91％或更高，92％或更高，93％或更高，94％或更高，95％或更高，96％或更高，97％或更高，98％或更高，99％或更高百分比，不包括添加的任何非 RSV 信号肽。片段是指与本文所陈述的 RSV Gb 共有免疫原的序列具有 98％或更高，或 99％或更高同源性以及与该序列具有 100％同一性的多肽的片段，在每一情形中均含有或不含信号肽和 / 或在 1 位的甲硫氨酸。片段可以包含与 RSV Gb 共有免疫原具有 98％或更高同源性，99％或更高同源性，或具有 100％同一性并且另外包含当计算同源性百分比时未包括在内的信号肽（如免疫球蛋白信号肽）的多肽的片段。在一些实施方案中，RSV Gb 免疫原的片段可以包含 SEQ ID NO:8 的片段。该片段可以包含连接到信号肽，如免疫球蛋白信号肽，例如 IgE 信号肽 (SEQ ID NO:17) 或 IgG 信号肽的 SEQ ID NO:8 的片段。因此，在一些实施方案中，该片段是包含了 SEQ ID NO:17 的 SEQ ID NO:16 的片段。该片段可以包括连接到 N 末端甲硫氨酸的 SEQ ID NO:8 的片段。片段还指与 SEQ ID NO:8 具有 98％或更高，或 99％或更高同源性的多肽的片段。该片段可以包含与 SEQ ID NO:8 具有 98％或更高同源性并且另外包含当计算同源性百分比时未包括在内的信号肽（如免疫球蛋白信号肽）的多肽的片段。该片段可以包含与 SEQ ID NO:8 具有 99％或更高同源性并且另外包含当计算同源性百分比时未包括在内的信号肽（如免疫球蛋白信号肽）的多肽的片段。该片段可以包含与 SEQ ID NO:8 具有 98％或更高同源性并且另外包含当计算同源性百分比时未包括在内的 N 末端甲硫氨酸的多肽的片段。该片段可以包含与 SEQ ID NO:8 具有 99％或更高同源性并且另外包含当计算同源性百分比时未包括在内的 N 末端甲硫氨酸的多肽的片段。RSV Gb 免疫原具有 291 个氨基酸，不包括 N 末端甲硫氨酸和 / 或信号肽。在一些实施方案中，其片段可以为 125 个或更多氨基酸的长度，130 个或更

多、140 个或更多、150 个或更多、160 个或更多、170 个或更多、180 个或更多、190 个或更多、200 个或更多、210 个或更多、220 个或更多、230 个或更多、240 个或更多、250 个或更多、260 个或更多、270 个或更多、280 个或更多、285 个或更多、288 个或更多氨基酸的长度。多肽片段可以为不到 135 个、不到 145 个、不到 155 个、不到 165 个、不到 175 个、不到 185 个、不到 195 个、不到 205 个、不到 215 个、不到 225 个、不到 235 个、不到 245 个、不到 255 个、不到 265 个、不到 275 个、不到 285 个、不到 288 个、不到 290 个氨基酸的长度。片段可以另外包含 N 末端甲硫氨酸和 / 或信号肽，如免疫球蛋白信号肽，例如 IgE 或 IgG 信号肽。N 末端甲硫氨酸和 / 或信号肽可以连接到 290 个氨基酸或其更小的片段。N 末端甲硫氨酸和 / 或信号肽可以连接到与该 290 个氨基酸的序列具有 98％同源性的多肽，或连接到与该 290 个氨基酸的序列具有 98％同源性的多肽的更小的片段。N 末端甲硫氨酸和 / 或信号肽可以连接到与该 290 个氨基酸的序列具有 99％同源性的多肽，或连接到与该 290 个氨基酸的序列具有 99％同源性的多肽的更小的片段。在计算同源性程度时，多肽具有 SEQ ID NO:6 或其片段，在该计算中未包括 N 末端甲硫氨酸和 / 或任何信号肽。在测定同源性时，未使用信号肽序列。因此，例如，尽管 SEQ ID NO:16 包含了可操作地连接到信号肽 SEQ ID NO:17 的 SEQ ID NO:8，但 SEQ ID NO:16 包含 SEQ ID NO:8 的片段，其与 SEQ ID NO:8 的片段具有 100％同源性，虽然在 SEQ ID NO:8 中不存在信号肽。因此，包含了与 SEQ ID NO:8 的片段具有至少 98％同源性的多肽的片段的蛋白质拟指这样一些蛋白质，其中与 SEQ ID NO:8 的片段具有至少 98％同源性的多肽的片段为至少 125 个氨基酸并且可以任选地连接到例如信号肽。

[0164] 此外，RSV M2-1 共有免疫原的片段可以是以下各序列的片段 :SEQ ID NO:4、SEQ ID NO:12、与本文所陈述的 RSV M2-1 共有免疫原序列具有 98％或更高同源性的蛋白质、与本文所陈述的 RSV M2-1 共有免疫原序列具有 99％或更高同源性的蛋白质或与本文所陈述的共有序列具有 100％同一性的 RSV M2-1 共有免疫原，在每一情形中均含有或不含信号肽和 / 或在 1 位的甲硫氨酸。这些片段可以占本文所陈述的特定全长 RSV M2-1 共有免疫原序列的长度的 20％或更高，25％或更高，30％或更高，35％或更高，40％或更高，45％或更高，50％或更高，55％或更高，60％或更高，65％或更高，70％或更高，75％或更高，80％或更高，85％或更高，90％或更高，91％或更高，92％或更高，93％或更高，94％或更高，95％或更高，96％或更高，97％或更高，98％或更高，99％或更高百分比，不包括添加的任何非 RSV 信号肽。片段是指与本文所陈述的 RSV M2-1 共有免疫原的序列具有 98％或更高，或 99％或更高同源性以及与该序列具有 100％同一性的多肽的片段，在每一情形中均含有或不含信号肽和 / 或在 1 位的甲硫氨酸。片段可以包含与 RSV M2-1 共有免疫原具有 98％或更高同源性，99％或更高同源性，或具有 100％同一性并且另外包含当计算同源性百分比时未包括在内的信号肽（如免疫球蛋白信号肽）的多肽的片段。在一些实施方案中，RSV M2-1 免疫原的片段可以包含 SEQ ID NO:4 的片段。该片段可以包含连接到信号肽，如免疫球蛋白信号肽，例如 IgE 信号肽 (SEQ ID NO:17) 或 IgG 信号肽的 SEQ ID NO:4 的片段。因此，在一些实施方案中，该片段是包含 SEQ ID NO:17 的 SEQ ID NO:12 的片段。该片段可以包括连接到 N 末端甲硫氨酸的 SEQ ID NO:4 的片段。片段还指与 SEQ ID NO:4 具有 98％或更高，或 99％或更高同源性的多肽的片段。该片段可以包含与 SEQ ID NO:4 具有 98％或更高同源性并且另外包含当计算同源性百分比时未包括在内的信号肽（如免疫球蛋白信号肽）的多肽的片段。该片段可以包含与 SEQ ID NO:4 具有 99％或更高同源性并且另外包含当计算

同源性百分比时未包括在内的信号肽（如免疫球蛋白信号肽）的多肽的片段。该片段可以包含与SEQ ID NO:4具有98％或更高同源性并且另外包含当计算同源性百分比时未包括在内的N末端甲硫氨酸的多肽的片段。该片段可以包含与SEQ ID NO:4具有99％或更高同源性并且另外包含当计算同源性百分比时未包括在内的N末端甲硫氨酸的多肽的片段。RSV M2-1免疫原具有195个氨基酸。在一些实施方案中，其片段可以为25个或更多氨基酸的长度，30个或更多、40个或更多、50个或更多、60个或更多、70个或更多、80个或更多、90个或更多、100个或更多、110个或更多、120个或更多、130个或更多、140个或更多、150个或更多、160个或更多、170个或更多、180个或更多、184个或更多、190个或更多、193或更多氨基酸的长度。多肽片段可以为不到35个、不到45个、不到55个、不到65个、不到75个、不到85个、不到95个、不到105个、不到115个、不到125个、不到135个、不到145个、不到155个、不到165个、不到175个、不到185个、不到188个、不到192个或不到194个氨基酸的长度。片段可以另外包含N末端甲硫氨酸和/或信号肽，如免疫球蛋白信号肽，例如IgE或IgG信号肽。N末端甲硫氨酸和/或信号肽可以连接到194个氨基酸或其更小的片段。N末端甲硫氨酸和/或信号肽可以连接到与该194个氨基酸的序列具有98％同源性的多肽，或连接到与该194个氨基酸的序列具有98％同源性的多肽的更小的片段。N末端甲硫氨酸和/或信号肽可以连接到与该194个氨基酸的序列具有99％同源性的多肽，或连接到与该194个氨基酸的序列具有99％同源性的多肽的更小的片段。在计算同源性程度时，多肽具有SEQ ID NO:4或其片段，在该计算中未包括N末端甲硫氨酸和/或任何信号肽。在测定同源性时，未使用信号肽序列。因此，例如，尽管SEQ ID NO:12包含了可操作地连接到信号肽SEQ ID NO:17的SEQ ID NO:4，但SEQ ID NO:12包含SEQ ID NO:4的片段，其与SEQ ID NO:4的片段具有100％同源性，虽然在SEQ ID NO:4中不存在信号肽。因此，包含了与SEQ ID NO:4的片段具有至少98％同源性的多肽的片段的蛋白质拟指这样一些蛋白质，其中与SEQ ID NO:4的片段具有至少98％同源性的多肽的片段为至少25个氨基酸并且可以任选地连接到例如信号肽。SEQ ID NO:30对应于SEQ ID NO:4中陈述的RSV M2-1免疫原的免疫优势T细胞表位。SEQ ID NO:31是指对应于SEQ ID NO:4中陈述的RSV M2-1免疫原的免疫优势T细胞表位的次优势T细胞表位。在一些实施方案中，片段包含这些序列或与其具有98％或更高，或99％或更高同源性的序列中一个或多个序列的全部或一部分。

[0165] “片段”还可以指编码以上陈述的RSV免疫原（即，RSV F免疫原、RSV M2-1免疫原、RSV Ga免疫原、RSV Gb免疫原，在每一情形中均含有或不含信号肽和/或在1位的甲硫氨酸）的片段、与本文陈述的这些共有序列具有98％或更高同源性的蛋白质、与本文陈述的这些共有序列具有99％或更高同源性的蛋白质及与本文所陈述的这些共有序列具有100％同一性的蛋白质的核酸片段，在每一情形中，这些蛋白质含有或不含信号肽和/或在1位的甲硫氨酸。片段可以包含或可以不包含例如连接到N末端甲硫氨酸的编码序列（即，起始密码子）或编码信号肽（如免疫球蛋白信号肽，例如IgE信号肽(SEQ ID NO:17)或IgG信号肽）的编码序列的编码RSV免疫原的核酸序列的片段。编码RSV免疫原的编码序列的片段可以占本文所陈述的特定全长RSV免疫原序列的编码序列的长度的20％或更高，25％或更高，30％或更高，35％或更高，40％或更高，45％或更高，50％或更高，55％或更高，60％或更高，65％或更高，70％或更高，75％或更高，80％或更高，85％或更高，90％或更高，91％或更高，92％或更高，93％或更高，94％或更高，95％或更高，96％或更高，97％或更高，

98％或更高，99％或更高百分比，不包括添加的编码非RSV信号肽的任何编码序列。片段还指编码与本文陈述的RSV免疫原序列具有98％或更高，或99％或更高同源性的多肽的编码序列的片段。该片段可以包含与具有98％或更高同源性并且另外包含当计算同源性百分比时未包括在内的信号肽（如免疫球蛋白信号肽）的多肽的片段。

[0166] 在一些实施方案中，这些片段是与SEQ ID NO:1具有98％或更高同源性的核酸序列的片段。在一些实施方案中，这些片段是与SEQ ID NO:1具有99％或更高同源性的核酸序列的片段。在一些实施方案中，这些片段是SEQ ID NO:1的片段。预期明确地公开了编码本文所描述的多肽片段的核酸序列，即，编码了具有这些肽片段的大小和同源性的蛋白质的核酸序列的片段。在一些实施方案中，编码了具有这些肽片段的大小和同源性的蛋白质的核酸序列的片段是SEQ ID NO:1的片段。编码RSV F共有免疫原，如SEQ ID NO:2、SEQ ID NO:10、与本文陈述的RSV F共有免疫原序列具有98％或更高同源性的蛋白质、与本文陈述的RSV F共有免疫原序列具有99％或更高同源性的蛋白质及与本文陈述的共有序列具有100％同一性的RSV F共有免疫原中所公开的序列（在每一情形中均含有或不含信号肽和/或在1位的甲硫氨酸）的编码序列（如例如SEQ ID NO:1或EQ ID NO:9）的片段，可以占本文陈述的特定全长RSV F共有免疫原序列的长度的20％或更高，25％或更高，30％或更高，35％或更高，40％或更高，45％或更高，50％或更高，55％或更高，60％或更高，65％或更高，70％或更高，75％或更高，80％或更高，85％或更高，90％或更高，91％或更高，92％或更高，93％或更高，94％或更高，95％或更高，96％或更高，97％或更高，98％或更高，99％或更高百分比，不包括添加的任何非RSV信号肽。片段可以编码与本文所陈述的RSV F共有免疫原的序列具有98％或更高，或99％或更高同源性以及与该序列具有100％同一性的多肽的片段，在每一情形中均含有或不含信号肽和/或在1位的甲硫氨酸。片段可以编码与RSV F共有免疫原具有98％或更高同源性，99％或更高同源性，或具有100％同一性并且另外包含当计算同源性百分比时未包括在内的信号肽（如免疫球蛋白信号肽）的多肽的片段。在一些实施方案中，编码RSV F免疫原的片段可以编码SEQ ID NO:2的片段并且包含SEQ ID NO:1的片段。该片段可以编码连接到信号肽，如免疫球蛋白信号肽，例如IgE信号肽（SEQ ID NO:17）或IgG信号肽的SEQ ID NO:2的片段。因此，在一些实施方案中，该片段是包含SEQ ID NO:17的SEQ ID NO:10的片段并且可以由SEQ ID NO:9编码。该片段可以包含连接到起始密码子的SEQ ID NO:1的片段。片段还编码与SEQ ID NO:2具有98％或更高，或99％或更高同源性的多肽的片段。该片段可以编码与SEQ ID NO:2具有98％或更高同源性并且另外包含当计算同源性百分比时未包括在内的信号肽（如免疫球蛋白信号肽）的多肽的片段。该片段可以编码与SEQ ID NO:2具有99％或更高同源性并且另外包含当计算同源性百分比时未包括在内的信号肽（如免疫球蛋白信号肽）的多肽的片段。该片段可以编码与SEQ ID NO:2具有98％或更高同源性并且另外包含当计算同源性百分比时未包括在内的N末端甲硫氨酸的多肽的片段。该片段可以编码与SEQ ID NO:2具有99％或更高同源性并且另外包含当计算同源性百分比时未包括在内的N末端甲硫氨酸的多肽的片段。片段可以与SEQ ID NO:1具有98％或更高，或99％或更高同源性。该片段可以与SEQ ID NO:1具有98％或更高同源性并且另外包含当计算同源性百分比时未包括在内的信号肽，如免疫球蛋白信号肽。该片段可以与SEQ ID NO:1具有99％或更高同源性并且另外包含当计算同源性百分比时未包括在内的信号肽，如免疫球蛋白信号肽。该片段可以与SEQ ID NO:1具

有98％或更高同源性并且另外包含当计算同源性百分比时未包括在内的N末端甲硫氨酸。该片段可以与SEQ ID NO:1具有99％或更高同源性并且另外包含当计算同源性百分比时未包括在内的N末端甲硫氨酸。SEQ ID NO:1的片段、与SEQ ID NO:1具有98％或更高同源性的核酸序列的片段及与SEQ ID NO:1具有99％或更高同源性的核酸序列的片段（在每一情形中均含有或不含起始密码子或编码信号肽的编码序列）可以编码RSV F免疫原的片段，这些片段可以为115个或更多氨基酸的长度、163个或更多、230个或更多、288个或更多、345个或更多、403个或更多、460个或更多、515个或更多、520个或更多、525个或更多、530个或更多、535个或更多、540个或更多、545个或更多、550个或更多、555个或更多、560个或更多、565个或更多、570个或更多、574个或更多氨基酸的长度。多肽片段可以为不到140个、不到190个、不到250个、不到283个、不到300个、不到365个、不到425个、不到495个、不到518个、不到523个、不到528个、不到533个、不到547个、不到552个、不到563个或不到572个氨基酸的长度。片段可以另外编码N末端甲硫氨酸和/或信号肽，如免疫球蛋白信号肽，例如IgE或IgG信号肽。N末端甲硫氨酸和/或信号肽可以连接到编码574个氨基酸或其更小的片段的序列。该片段可以编码连接到与该574个氨基酸的序列具有98％同源性的多肽，或连接到与该574个氨基酸的序列具有98％同源性的多肽的更小的片段的N末端甲硫氨酸和/或信号肽。该片段可以编码连接到与该574个氨基酸的序列具有99％同源性的多肽，或连接到与该574个氨基酸的序列具有99％同源性的多肽的更小的片段的N末端甲硫氨酸和/或信号肽。在计算同源性程度时，多肽具有SEQ ID NO:2或其片段，在该计算中未包括N末端甲硫氨酸和/或任何信号肽。在测定同源性时，未使用信号肽序列。因此，例如，尽管SEQ ID NO:7包含了可操作地连接到编码信号肽SEQ ID NO:17的编码序列的SEQ ID NO:1，但SEQ ID NO:7包含SEQ ID NO:1的片段，其与SEQ ID NO:1的片段具有100％同源性，虽然在SEQ ID NO:1中不存在信号肽编码序列。SEQ ID NO:22、SEQ ID NO:23、SEQ ID NO:24及SEQ ID NO:25对应于SEQ ID NO:2中所陈述的RSV F免疫原的T细胞表位。在一些实施方案中，片段包含了编码这些序列或与其具有98％或更高，或99％或更高同源性的序列中的一个或多个序列的全部或一部分的编码序列，包括SEQ ID NO:1或与SEQ ID NO:1具有98％或更高，或99％或更高同源性的序列的片段。

[0167] 在一些实施方案中，这些片段是与SEQ ID NO:5具有98％或更高同源性的核酸序列的片段。在一些实施方案中，这些片段是与SEQ ID NO:5具有99％或更高同源性的核酸序列的片段。在一些实施方案中，这些片段是SEQ ID NO:5的片段。预计本发明明确地公开了编码本文所描述的多肽片段的核酸序列，即，编码了具有这些肽片段的大小和同源性的蛋白质的核酸序列的片段。在一些实施方案中，编码了具有这些肽片段的大小和同源性的蛋白质的核酸序列的片段是SEQ ID NO:5的片段。编码RSV Ga共有免疫原，如SEQ ID NO:6、SEQ ID NO:14、与本文陈述的RSV Ga共有免疫原序列具有98％或更高同源性的蛋白质、与本文陈述的RSV Ga共有免疫原序列具有99％或更高同源性的蛋白质及与本文陈述的共有序列具有100％同一性的RSV Ga共有免疫原中所公开的序列（在每一情形中均含有或不含信号肽和/或在1位的甲硫氨酸）的编码序列（如例如SEQ ID NO:5或EQ ID NO:13）的片段，可以占本文陈述的特定全长RSV Ga共有免疫原序列的长度的20％或更高，25％或更高，30％或更高，35％或更高，40％或更高，45％或更高，50％或更高，55％或更高，60％或更高，65％或更高，70％或更高，75％或更高，80％或更高，85％或更高，90％或更高，91％或

更高，92％或更高，93％或更高，94％或更高，95％或更高，96％或更高，97％或更高，98％或更高，99％或更高百分比，不包括添加的任何非 RSV 信号肽。片段可以编码与本文所陈述的 RSV Ga 共有免疫原的序列具有 98％或更高，或 99％或更高同源性以及与该序列具有 100％同一性的多肽的片段，在每一情形中均含有或不含信号肽和 / 或在 1 位的甲硫氨酸。片段可以编码与 RSV Ga 共有免疫原具有 98％或更高同源性，99％或更高同源性，或具有 100％同一性并且另外包含当计算同源性百分比时未包括在内的信号肽（如免疫球蛋白信号肽）的多肽的片段。在一些实施方案中，编码 RSV Ga 免疫原的片段可以编码 SEQ ID NO:6 的片段并且包含 SEQ ID NO:5 的片段。该片段可以编码连接到信号肽，如免疫球蛋白信号肽，例如 IgE 信号肽 (SEQ ID NO:17) 或 IgG 信号肽的 SEQ ID NO:6 的片段。因此，在一些实施方案中，该片段是包含了 SEQ ID NO:17 的 SEQ ID NO:14 的片段并且可以由 SEQ ID NO:13 编码。该片段可以包含连接到起始密码子的 SEQ ID NO:5 的片段。片段还编码与 SEQ ID NO:6 具有 98％或更高，或 99％或更高同源性的多肽的片段。该片段可以编码与 SEQ ID NO:6 具有 98％或更高同源性并且另外包含当计算同源性百分比时未包括在内的信号肽（如免疫球蛋白信号肽）的多肽的片段。该片段可以编码与 SEQ ID NO:6 具有 99％或更高同源性并且另外包含当计算同源性百分比时未包括在内的信号肽（如免疫球蛋白信号肽）的多肽的片段。该片段可以编码与 SEQ ID NO:6 具有 98％或更高同源性并且另外包含当计算同源性百分比时未包括在内的 N 末端甲硫氨酸的多肽的片段。该片段可以编码与 SEQ ID NO:6 具有 99％或更高同源性并且另外包含当计算同源性百分比时未包括在内的 N 末端甲硫氨酸的多肽的片段。片段可以与 SEQ ID NO:5 具有 98％或更高，或 99％或更高同源性。该片段可以与 SEQ ID NO:5 具有 98％或更高同源性并且另外包含当计算同源性百分比时未包括在内的信号肽，如免疫球蛋白信号肽。该片段可以与 SEQ ID NO:5 具有 99％或更高同源性并且另外包含当计算同源性百分比时未包括在内的信号肽，如免疫球蛋白信号肽。该片段可以与 SEQ ID NO:5 具有 98％或更高同源性并且另外包含当计算同源性百分比时未包括在内的 N 末端甲硫氨酸。该片段可以与 SEQ ID NO:5 具有 99％或更高同源性并且另外包含当计算同源性百分比时未包括在内的 N 末端甲硫氨酸。SEQ ID NO:5 的片段、与 SEQ ID NO:5 具有 98％或更高同源性的核酸序列的片段及与 SEQ ID NO:5 具有 99％或更高同源性的核酸序列的片段（在每一情形中均含有或不含起始密码子或编码信号肽的编码序列）可以编码 RSV Ga 免疫原的片段，在一些实施方案中，这些片段可以为 125 个或更多氨基酸的长度、130 个或更多、140 个或更多、150 个或更多、160 个或更多、170 个或更多、180 个或更多、190 个或更多、200 个或更多、210 个或更多、220 个或更多、230 个或更多、240 个或更多、250 个或更多、260 个或更多、270 个或更多、280 个或更多、285 个或更多、290 个或更多、295 个或更多氨基酸的长度。多肽片段可以为不到 135 个、不到 145 个、不到 155 个、不到 165 个、不到 175 个、不到 185 个、不到 195 个、不到 205 个、不到 215 个、不到 225 个、不到 235 个、不到 245 个、不到 255 个、不到 265 个、不到 275 个、不到 285 个、不到 288 个、不到 292 个或不到 296 个氨基酸的长度。片段可以另外编码 N 末端甲硫氨酸和 / 或信号肽，如免疫球蛋白信号肽，例如 IgE 或 IgG 信号肽。N 末端甲硫氨酸和 / 或信号肽可以连接到编码 296 个氨基酸或其更小的片段的序列。该片段可以编码连接到与该 296 个氨基酸的序列具有 98％同源性的多肽，或连接到与该 296 个氨基酸的序列具有 98％同源性的多肽的更小的片段的 N 末端甲硫氨酸和 / 或信号肽。该片段可以编码连接到与该 296 个氨基酸的序列具有 99％

同源性的多肽，或连接到与该 296 个氨基酸的序列具有 99％同源性的多肽的更小的片段的 N 末端甲硫氨酸和 / 或信号肽。在计算同源性程度时，多肽具有 SEQ ID NO:6 或其片段，在该计算中未包括 N 末端甲硫氨酸和 / 或任何信号肽。在测定同源性时，未使用信号肽序列。因此，例如，尽管 SEQ ID NO:13 包含了可操作地连接到编码信号肽 SEQ ID NO:17 的编码序列的 SEQ ID NO:5，但 SEQ ID NO:13 包含 SEQ ID NO:5 的片段，其与 SEQ ID NO:5 的片段具有 100％同源性，虽然在 SEQ ID NO:5 中不存在信号肽编码序列。

[0168] 在一些实施方案中，这些片段是与 SEQ ID NO:7 具有 98％或更高同源性的核酸序列的片段。在一些实施方案中，这些片段是与 SEQ ID NO:7 具有 99％或更高同源性的核酸序列的片段。在一些实施方案中，这些片段是 SEQ ID NO:7 的片段。预计本发明明确地公开了编码本文所描述的多肽片段的核酸序列，即，编码了具有这些肽片段的大小和同源性的蛋白质的核酸序列的片段。在一些实施方案中，编码了具有这些肽片段的大小和同源性的蛋白质的核酸序列的片段是 SEQ ID NO:7 的片段。编码 RSV Gb 共有免疫原，如 SEQ ID NO:8、SEQ ID NO:16、与本文陈述的 RSV Gb 共有免疫原序列具有 98％或更高同源性的蛋白质、与本文陈述的 RSV Gb 共有免疫原序列具有 99％或更高同源性的蛋白质及与本文陈述的共有序列具有 100％同一性的 RSV Gb 共有免疫原中所公开的序列（在每一情形中均含有或不含信号肽和 / 或在 1 位的甲硫氨酸）的编码序列（如例如 SEQ ID NO:7 或 SEQ ID NO:15）的片段，可以占本文陈述的特定全长 RSV Gb 共有免疫原序列的长度的 20％或更高，25％或更高，30％或更高，35％或更高，40％或更高，45％或更高，50％或更高，55％或更高，60％或更高，65％或更高，70％或更高，75％或更高，80％或更高，85％或更高，90％或更高，91％或更高，92％或更高，93％或更高，94％或更高，95％或更高，96％或更高，97％或更高，98％或更高，99％或更高百分比，不包括添加的任何非 RSV 信号肽。片段可以编码与本文所陈述的 RSV Gb 共有免疫原的序列具有 98％或更高，或 99％或更高同源性以及与该序列具有 100％同一性的多肽的片段，在每一情形中均含有或不含信号肽和 / 或在 1 位的甲硫氨酸。片段可以编码与 RSV Gb 共有免疫原具有 98％或更高同源性，99％或更高同源性，或具有 100％同一性，并且另外包含当计算同源性百分比时未包括在内的信号肽（如免疫球蛋白信号肽）的多肽的片段。在一些实施方案中，编码 RSV Gb 免疫原的片段可以编码 SEQ ID NO:8 的片段并且包含 SEQ ID NO:7 的片段。该片段可以编码连接到信号肽，如免疫球蛋白信号肽，例如 IgE 信号肽 (SEQ ID NO:17) 或 IgG 信号肽的 SEQ ID NO:8 的片段。因此，在一些实施方案中，该片段是包含了 SEQ ID NO:17 的 SEQ ID NO:16 的片段并且可以由 SEQ ID NO:15 编码。该片段可以包含连接到起始密码子的 SEQ ID NO:7 的片段。片段还编码与 SEQ ID NO:8 具有 98％或更高，或 99％或更高同源性的多肽的片段。该片段可以编码与 SEQ ID NO:8 具有 98％或更高同源性并且另外包含当计算同源性百分比时未包括在内的信号肽（如免疫球蛋白信号肽）的多肽的片段。该片段可以编码与 SEQ ID NO:8 具有 99％或更高同源性并且另外包含当计算同源性百分比时未包括在内的信号肽（如免疫球蛋白信号肽）的多肽的片段。该片段可以编码与 SEQ ID NO:8 具有 98％或更高同源性并且另外包含当计算同源性百分比时未包括在内的 N 末端甲硫氨酸的多肽的片段。该片段可以编码与 SEQ ID NO:8 具有 99％或更高同源性并且另外包含当计算同源性百分比时未包括在内的 N 末端甲硫氨酸的多肽的片段。片段可以与 SEQ ID NO:7 具有 98％或更高，或 99％或更高同源性。该片段可以与 SEQ ID NO:7 具有 98％或更高同源性并且另外包含当计算同源性百分比时未

包括在内的信号肽，如免疫球蛋白信号肽。该片段可以与 SEQ ID NO:7 具有 99％或更高同源性并且另外包含当计算同源性百分比时未包括在内的信号肽，如免疫球蛋白信号肽。该片段可以与 SEQ ID NO:7 具有 98％或更高同源性并且另外包含当计算同源性百分比时未包括在内的 N 末端甲硫氨酸。该片段可以与 SEQ ID NO:7 具有 99％或更高同源性并且另外包含当计算同源性百分比时未包括在内的 N 末端甲硫氨酸。SEQ ID NO:7 的片段、与 SEQ ID NO:7 具有 98％或更高同源性的核酸序列的片段及与 SEQ ID NO:7 具有 99％或更高同源性的核酸序列的片段（在每一情形中均含有或不含起始密码子或编码信号肽的编码序列）可以编码 RSV Gb 免疫原的片段，这些片段可以为 125 个或更多氨基酸的长度、130 个或更多、140 个或更多、150 个或更多、160 个或更多、170 个或更多、180 个或更多、190 个或更多、200 个或更多、210 个或更多、220 个或更多、230 个或更多、240 个或更多、250 个或更多、260 个或更多、270 个或更多、280 个或更多、285 个或更多、288 个或更多氨基酸的长度。多肽片段可以为不到 135 个、不到 145 个、不到 155 个、不到 165 个、不到 175 个、不到 185 个、不到 195 个、不到 205 个、不到 215 个、不到 225 个、不到 235 个、不到 245 个、不到 255 个、不到 265 个、不到 275 个、不到 285 个、不到 288 个、不到 290 个氨基酸的长度。片段可以另外编码 N 末端甲硫氨酸和 / 或信号肽，如免疫球蛋白信号肽，例如 IgE 或 IgG 信号肽。N 末端甲硫氨酸和 / 或信号肽可以连接到编码 290 个氨基酸或其更小的片段的序列。该片段可以编码连接到与该 290 个氨基酸的序列具有 98％同源性的多肽，或连接到与该 290 个氨基酸的序列具有 98％同源性的多肽的更小的片段的 N 末端甲硫氨酸和 / 或信号肽。该片段可以编码连接到与该 290 个氨基酸的序列具有 99％同源性的多肽，或连接到与该 290 个氨基酸的序列具有 99％同源性的多肽的更小的片段的 N 末端甲硫氨酸和 / 或信号肽。在计算同源性程度时，多肽具有 SEQ ID NO:8 或其片段，在该计算中未包括 N 末端甲硫氨酸和 / 或任何信号肽。在测定同源性时，未使用信号肽序列。因此，例如，尽管 SEQ ID NO:15 包含了可操作地连接到编码信号肽 SEQ ID NO:17 的编码序列的 SEQ ID NO:7，但 SEQ ID NO:15 包含 SEQ ID NO:7 的片段，其与 SEQ ID NO:7 的片段具有 100％同源性，虽然在 SEQ ID NO:7 中不存在信号肽编码序列。

[0169] 还提供了与 RSV M2-1 相关的序列，包括与 SEQ ID NO:3 具有 98％或更高同源性的核酸序列的片段。在一些实施方案中，这些片段是与 SEQ ID NO:3 具有 99％或更高同源性的核酸序列的片段。在一些实施方案中，这些片段是 SEQ ID NO:3 的片段。预计本发明明确地公开了编码本文所描述的多肽片段的核酸序列，即，编码了具有这些肽片段的大小和同源性的蛋白质的核酸序列的片段。在一些实施方案中，编码了具有这些肽片段的大小和同源性的蛋白质的核酸序列的片段是 SEQ ID NO:3 的片段。编码 RSV M2-1 共有免疫原，如 SEQ ID NO:4、SEQ ID NO:12、与本文陈述的 RSV M2-1 共有免疫原序列具有 98％或更高同源性的蛋白质、与本文陈述的 RSV M2-1 共有免疫原序列具有 99％或更高同源性的蛋白质及与本文陈述的共有序列具有 100％同一性的 RSV M2-1 共有免疫原中所公开的序列（在每一情形中均含有或不含信号肽和 / 或在 1 位的甲硫氨酸）的编码序列（如例如 SEQ ID NO:3 或 SEQ ID NO:11）的片段，可以占本文陈述的特定全长 RSV M2-1 共有免疫原序列的长度的 20％或更高，25％或更高，30％或更高，35％或更高，40％或更高，45％或更高，50％或更高，55％或更高，60％或更高，65％或更高，70％或更高，75％或更高，80％或更高，85％或更高，90％或更高，91％或更高，92％或更高，93％或更高，94％或更高，95％或更高，96％或更高，

97％或更高，98％或更高，99％或更高百分比，不包括添加的任何非 RSV 信号肽。片段可以编码与本文所陈述的 RSV M2-1 共有免疫原的序列具有 98％或更高，或 99％或更高同源性以及与该序列具有 100％同一性的多肽的片段，在每一情形中均含有或不含信号肽和 / 或在 1 位的甲硫氨酸。片段可以编码与 RSV M2-1 共有免疫原具有 98％或更高同源性，99％或更高同源性，或具有 100％同一性，并且另外包含当计算同源性百分比时未包括在内的信号肽（如免疫球蛋白信号肽）的多肽的片段。在一些实施方案中，编码 RSV M2-1 免疫原的片段可以编码 SEQ ID NO:4 的片段并且包含 SEQ ID NO:3 的片段。该片段可以编码连接到信号肽，如免疫球蛋白信号肽，例如 IgE 信号肽 (SEQ ID NO:17) 或 IgG 信号肽的 SEQ ID NO:4 的片段。因此，在一些实施方案中，该片段是包含了 SEQ ID NO:17 的 SEQ ID NO:12 的片段并且可以由 SEQ ID NO:11 编码。该片段可以包含连接到起始密码子的 SEQ ID NO:3 的片段。片段还编码与 SEQ ID NO:4 具有 98％或更高，或 99％或更高同源性的多肽的片段。该片段可以编码与 SEQ ID NO:4 具有 98％或更高同源性并且另外包含当计算同源性百分比时未包括在内的信号肽（如免疫球蛋白信号肽）的多肽的片段。该片段可以编码与 SEQ ID NO:4 具有 99％或更高同源性并且另外包含当计算同源性百分比时未包括在内的信号肽（如免疫球蛋白信号肽）的多肽的片段。该片段可以编码与 SEQ ID NO:4 具有 98％或更高同源性并且另外包含当计算同源性百分比时未包括在内的 N 末端甲硫氨酸的多肽的片段。该片段可以编码与 SEQ ID NO:4 具有 99％或更高同源性并且另外包含当计算同源性百分比时未包括在内的 N 末端甲硫氨酸的多肽的片段。片段可以与 SEQ ID NO:3 具有 98％或更高，或 99％或更高同源性。该片段可以与 SEQ ID NO:3 具有 98％或更高同源性并且另外包含当计算同源性百分比时未包括在内的信号肽，如免疫球蛋白信号肽。该片段可以与 SEQ ID NO:3 具有 99％或更高同源性并且另外包含当计算同源性百分比时未包括在内的信号肽，如免疫球蛋白信号肽。该片段可以与 SEQ ID NO:3 具有 98％或更高同源性并且另外包含当计算同源性百分比时未包括在内的 N 末端甲硫氨酸。该片段可以与 SEQ ID NO:3 具有 99％或更高同源性并且另外包含当计算同源性百分比时未包括在内的 N 末端甲硫氨酸。SEQ ID NO:3 的片段、与 SEQ ID NO:3 具有 98％或更高同源性的核酸序列的片段及与 SEQ ID NO:3 具有 99％或更高同源性的核酸序列的片段（在每一情形中均含有或不含起始密码子或编码信号肽的编码序列）可以编码 RSV M2-1 免疫原的片段，这些片段可以为 25 个或更多氨基酸的长度、30 个或更多、40 个或更多、50 个或更多、60 个或更多、70 个或更多、80 个或更多、90 个或更多、100 个或更多、110 个或更多、120 个或更多、130 个或更多、140 个或更多、150 个或更多、160 个或更多、170 个或更多、180 个或更多、184 个或更多、190 个或更多、193 个或更多氨基酸的长度。多肽片段可以为不到 35 个、不到 45 个、不到 55 个、不到 65 个、不到 75 个、不到 85 个、不到 95 个、不到 105 个、不到 115 个、不到 125 个、不到 135 个、不到 145 个、不到 155 个、不到 165 个、不到 175 个、不到 185 个、不到 188 个、不到 192 个或不到 194 个氨基酸的长度。片段可以另外编码 N 末端甲硫氨酸和 / 或信号肽，如免疫球蛋白信号肽，例如 IgE 或 IgG 信号肽。N 末端甲硫氨酸和 / 或信号肽可以连接到编码 194 个氨基酸或其更小的片段的序列。该片段可以编码连接到与该 194 个氨基酸的序列具有 98％同源性的多肽，或连接到与该 194 个氨基酸的序列具有 98％同源性的多肽的更小的片段的 N 末端甲硫氨酸和 / 或信号肽。该片段可以编码连接到与该 194 个氨基酸的序列具有 99％同源性的多肽，或连接到与该 194 个氨基酸的序列具有 99％同源性的多肽的更小的片段的

N 末端甲硫氨酸和 / 或信号肽。在计算同源性程度时，多肽具有 SEQ ID NO:4 或其片段，在该计算中未包括 N 末端甲硫氨酸和 / 或任何信号肽。在测定同源性时，未使用信号肽序列。因此，例如，尽管 SEQ ID NO:9 包含了可操作地连接到编码信号肽 SEQ ID NO:17 的编码序列的 SEQ ID NO:3，但 SEQ ID NO:9 包含 SEQ ID NO:3 的片段，其与 SEQ ID NO:3 的片段具有 100％同源性，虽然在 SEQ ID NO:3 中不存在信号肽编码序列。SEQ ID NO:30 对应于 SEQ ID NO:4 中陈述的 RSV M2-1 免疫原的免疫优势 T 细胞表位。SEQ ID NO:31 是指对应于 SEQ ID NO:4 中陈述的 RSV M2-1 免疫原的免疫优势 T 细胞表位的次优势 T 细胞表位。在一些实施方案中，片段包含了编码这些序列或与其具有 98％或更高，或 99％或更高同源性的序列中的一个或多个序列的全部或一部分的编码序列，包括 SEQ ID NO:3 或与 SEQ ID NO:3 具有 98％或更高，或 99％或更高同源性的序列的片段。

[0170] 1. 同一

[0171] 在两个或更多个核酸或多肽序列的情况下，如本文所使用，“同一”或“同一性”可以指这些序列在指定区域内具有指定百分比的残基相同。该百分比可以通过以下方式计算 :最佳地对准两个序列，在指定区域内比较两个序列，测定在两个序列中出现相同残基的位置的数量以得到匹配位置的数量，用匹配位置的数量除以指定区域中的总位置数并且将结果乘以 100，得到序列同一性百分比。在两个序列具有不同长度或该对准产生一个或多个交错的末端并且比较的指定区域仅包括单一序列的情况下，在计算的分母而非分子中包括单一序列的残基。当比较 DNA 和 RNA 时，胸腺嘧啶 (T) 和尿嘧啶 (U) 可以视为相同的。同一性可以手动地得到或通过使用如 BLAST 或 BLAST 2.0 等计算机序列算法得到。

[0172] m. 阻抗

[0173] 如本文所使用，“阻抗”可以在论述反馈机制时使用，并且可以根据欧姆定律 (Ohm’s law) 换算成电流值，由此能够与预先设定的电流相比较。

[0174] n. 免疫应答

[0175] 如本文所使用，“免疫应答”可以指宿主免疫系统（例如哺乳动物免疫系统）经由所提供的 DNA 质粒疫苗而响应一种一种或多种 RSV 共有抗原的引入而发生活化。免疫应答可以是细胞应答或体液应答，或两者的形式。

[0176] o. 核酸

[0177] 如本文所使用，“核酸”或“寡核苷酸”或“多核苷酸”可以指共价连接在一起的至少两个核苷酸。单链的描绘也定义互补链的序列。因此，核酸还涵盖所描绘的单链的互补链。许多核酸变体可以被用于与指定核酸相同的目的。因此，核酸还涵盖基本上同一的核酸和其补体。单链提供了可以在严格杂交条件下与靶序列杂交的探针。因此，核酸还涵盖在严格杂交条件下杂交的探针。

[0178] 核酸可以是单链或双链的，或可以含有双链和单链序列的部分。核酸可以是 DNA（基因组 DNA 和 cDNA）、RNA 或杂交体，其中核酸可以含有脱氧核糖核苷酸和核糖核苷酸的组合，以及包括尿嘧啶、腺嘌呤、胸腺嘧啶、胞嘧啶、鸟嘌呤、肌苷、黄嘌呤、次黄嘌呤、异胞嘧啶和异鸟嘌呤在内的碱基的组合。核酸可以通过化学合成法或通过重组方法获得。

[0179] p. 可操作地连接

[0180] 如本文所使用，“可操作地连接”可以指基因的表达处于在空间上与其连接的启动子的控制下。启动子可以使定位于 5’（上游）或 3’（下游）基因处于其控制下。启动子与

基因之间的距离可以同该启动子与衍生得到该启动子的基因中其所控制的基因之间的距离大致相同。如本领域中所知，在不损失启动子功能的情况下，可以实现这一距离的变化。

[0181] q. 启动子

[0182] 如本文所使用，“启动子”可以指能够赋予、活化或增进细胞中核酸的表达的合成或天然来源的分子。启动子可以包含一个或多个特定转录调控序列，用以进一步增进表达和/或改变其空间表达和/或时程安排达。启动子还可以包含远端增强子或阻遏子元件，这些元件可以位于距转录起始位点数千碱基对处。启动子可以来源于包括病毒、细菌、真菌、植物、昆虫和动物在内的来源。启动子可以组成性地，或相对于发生表达的细胞、组织或器官，或相对于发生表达的发育阶段，或响应于如生理应激、病原体、金属离子或诱导剂等外部刺激物而有差别地调控基因组分的表达。启动子的代表性实例包括噬菌体 T7 启动子、噬菌体 T3 启动子、SP6 启动子、lac 操作子 - 启动子、tac 启动子、SV40 晚期启动子、SV40 早期启动子、RSV-LTR 启动子、CMV IE 启动子、SV40 早期启动子或 SV40 晚期启动子及 CMV IE 启动子。

[0183] r. 严格杂交条件

[0184] 如本文所使用，“严格杂交条件”可以指第一核酸序列（例如探针）将与第二核酸序列（例如靶），如以复杂核酸混合物形式杂交的条件。严格条件是序列依赖性的并且在不同情况下将不同。所选严格杂交条件可以比在指定离子强度 pH 下特定序列的热熔融温度（T_m）低约 5℃ -10℃。T_m可以是在平衡时，与靶互补的探针中有 50％与靶序列杂交（当靶序列过量存在时，在 T_m下，在平衡时 50％的探针被占据）的温度（在指定离子强度、pH 和核酸浓度下）。严格条件可以是在 pH 7.0 到 8.3 下盐浓度低于约 1.0M 钠离子，如为约 0.01-1.0M 钠离子浓度（或其它盐）并且温度对于短探针（例如约 10-50 个核苷酸）为至少约 30℃并且对于长探针（例如超过约 50 个核苷酸）为至少约 60℃的那些条件。严格条件也可以通过添加去稳定剂（如甲酰胺）实现。对于选择性或特异性杂交，阳性信号可以为背景杂交的至少 2 到 10 倍。示范性严格杂交条件包括以下条件 :50％甲酰胺、5x SSC 及 1％ SDS，在 42℃下温育 ;或 5x SSC、1％ SDS，在 65℃下温育，并且在 65℃下在 0.2x SSC 和 0.1％ SDS 中洗涤。

[0185] s. 基本上互补

[0186] 如本文所使用，“基本上互补”可以指第一序列与第二序列的补体在 8、9、10、11、12、13、14、15、16、17、18、19、20、21、22、23、24、25、30、35、40、45、50、55、60、65、70、75、80、85、90、95、100 个或更多核苷酸或氨基酸的区域内至少 60％、65％、70％、75％、80％、85％、90％、95％、97％、98％或 99％同一，或这两个序列在严格杂交条件下杂交。

[0187] t. 基本上同一

[0188] 如本文所使用，“基本上同一”可以指第一序列与第二序列在 8、9、10、11、12、13、14、15、16、17、18、19、20、21、22、23、24、25、30、35、40、45、50、55、60、65、70、75、80、85、90、95、100 个或更多核苷酸或氨基酸的区域内至少 60％、65％、70％、75％、80％、85％、90％、95％、97％、98％或 99％同一，或就核酸来说，第一序列与第二序列的补体基本上互补。

[0189] u. 变体

[0190] 本文相对于核酸所使用的“变体”可以指 (i) 所提到的核苷酸序列的一部分或片段 ;(ii) 所提到的核苷酸序列的补体或其一部分 ;(iii) 与所提到的核酸或其补体基本上

同一的核酸；或 (iv) 在严格条件下于所提到的核酸、其补体或与其基本上同一的序列杂交的核酸。

[0191] 关于肽或多肽的“变体”因氨基酸的插入、缺失或保守取代而在氨基酸序列中存在差异，但保留了至少一种生物活性。变体还可以指氨基酸序列与具有保留了至少一种生物活性的氨基酸序列的参照蛋白质基本上同一的蛋白质。氨基酸的保守取代，即，一个氨基酸被具有类似特性（例如，亲水性，以及带电区域的程度和分布）的不同氨基酸置换，在本领域中被公认为典型地涉及极小变化。如本领域中所了解，这些极小变化可以部分通过考虑氨基酸的亲水指数来鉴别。Kyte 等人，J.Mol.Biol.157:105-132(1982)。氨基酸的亲水指数是基于考虑其疏水性和电荷。本领域中已知，具有类似亲水指数的氨基酸可以被取代并且仍保持蛋白质功能。在一个方面，亲水指数为 ±2 的氨基酸被取代。氨基酸的亲水性也可以用于揭露将产生保留生物功能的蛋白质的取代。在肽的情形下，考虑氨基酸的亲水性容许计算出该肽的最大局部平均亲水性，这是已报导的与抗原性和免疫原性充分相关的一种有用措施。美国专利号 4,554,101，通过引用完全并入本文中。如本领域中所了解，具有类似亲水性值的氨基酸的取代可以产生保留生物活性（例如免疫原性）的肽。取代可以用亲水性值彼此差异在 ±2 范围内的氨基酸进行。氨基酸的疏水指数和亲水性值都受该氨基酸的特定侧链的影响。与该观察结果一致，如通过疏水性、亲水性、电荷、大小和其它特性所揭露，与生物功能相容的氨基酸取代应当理解为取决于氨基酸的相对相似性及这些氨基酸的特定侧链。

[0192] v. 载体

[0193] 本文所使用的“载体”可以指含有复制起点的核酸序列。载体可以是质粒、噬菌体、细菌人工染色体或酵母人工染色体。载体可以是 DNA 或 RNA 载体。载体可以为自我复制的染色体外载体或整合到宿主基因组中的载体。

[0194] 2.RSV 蛋白

[0195] 本文提供了 RSV 蛋白，在本文又称为“RSV 免疫原”，是能够引起针对 RSV 的免疫应答的共有抗原。这些共有 RSV 免疫原中的三种为：RSV F 免疫原，以及两种形式的 RSV G 免疫原，在本文中称为 RSV Ga 免疫原和 RSV Gb 免疫原。两种 RSV G 免疫原形式是由于 RSV G 蛋白的多样性而提供。在一些实施方案中，这些 RSV 免疫原可以包含来自不同蛋白质（如免疫球蛋白）的信号肽，例如 IgE 信号肽 (SEQ ID NO:17) 或 IgG 信号肽。RSV 免疫原包括 RSV F 免疫原、RSV Ga 免疫原和 RSV Gb 免疫原。本文还提供了作为能够引起针对 RSV M2-1 的免疫应答的共有抗原的“RSV 免疫原”，即，RSV M2-1 免疫原，该免疫原可以任选地包含来自不同蛋白质（如免疫球蛋白）的信号肽，例如 IgE 信号肽 (SEQ ID NO:17) 或 IgG 信号肽。

[0196] “RSV F 免疫原”是指包含与 SEQ ID NO:2 具有至少 98％同源性的蛋白质的免疫原性片段的蛋白质。RSV F 免疫原可以包含与 SEQ ID NO:2 具有至少 99％同源性的蛋白质的免疫原性片段。RSV F 免疫原可以包含 SEQ ID NO:2 的免疫原性片段。在每一情形中，这些免疫原性片段可以任选地另外包含信号肽，如 SEQ ID NO:17。包含 SEQ ID NO:2 的免疫原性片段和信号肽 SEQ ID NO:17 的 RSV F 免疫原可以是包含 SEQ ID NO:17 的 SEQ ID NO:10 的免疫原性片段。RSV F 免疫原可以包含与 SEQ ID NO:2 具有至少 98％同源性的蛋白质，并且在一些实施方案中可以包含与 SEQ ID NO:2 具有至少 99％同源性的蛋白质。RSV F 免疫原可以包含 SEQ ID NO:2。在每一情形中，RSV F 免疫原可以任选地另外包含信号肽，如

SEQ ID NO:17。包含 SEQ ID NO:2 和信号肽 SEQ ID NO:17 的 RSV F 免疫原可以包含 SEQ ID NO:10。

[0197] “RSV Ga 免疫原”是指包含与 SEQ ID NO:6 具有至少 98％同源性的蛋白质的免疫原性片段的蛋白质。RSV Ga 免疫原可以包含与 SEQ ID NO:6 具有至少 99％同源性的蛋白质的免疫原性片段。RSV Ga 免疫原可以包含 SEQ ID NO:6 的免疫原性片段。在每一情形中，这些免疫原性片段可以任选地另外包含信号肽，如 SEQ ID NO:17。包含 SEQ ID NO:6 的免疫原性片段和信号肽 SEQ ID NO:17 的 RSV Ga 免疫原可以是包含 SEQ ID NO:17 的 SEQ ID NO:14 的免疫原性片段。RSV Ga 免疫原可以包含与 SEQ ID NO:6 具有至少 98％同源性的蛋白质，并且在一些实施方案中可以包含与 SEQ ID NO:6 具有至少 99％同源性的蛋白质。RSV Ga 免疫原可以包含 SEQ ID NO:6。在每一情形中，RSV Ga 免疫原可以任选地另外包含信号肽，如 SEQ ID NO:17。包含 SEQ ID NO:6 和信号肽 SEQ ID NO:17 的 RSV Ga 免疫原可以包含 SEQ ID NO:14。

[0198] “RSV Gb 免疫原”是指包含与 SEQ ID NO:8 具有至少 98％同源性的蛋白质的免疫原性片段的蛋白质。RSV Gb 免疫原可以包含与 SEQ ID NO:8 具有至少 99％同源性的蛋白质的免疫原性片段。RSV Gb 免疫原可以包含 SEQ ID NO:8 的免疫原性片段。在每一情形中，这些免疫原性片段可以任选地另外包含信号肽，如 SEQ ID NO:17。包含 SEQ ID NO:8 的免疫原性片段和信号肽 SEQ ID NO:17 的 RSV Gb 免疫原可以是包含 SEQ ID NO:17 的 SEQ ID NO:16 的免疫原性片段。RSV Gb 免疫原可以包含与 SEQ ID NO:8 具有至少 98％同源性的蛋白质，并且在一些实施方案中可以包含与 SEQ ID NO:8 具有至少 99％同源性的蛋白质。RSV Gb 免疫原可以包含 SEQ ID NO:8。在每一情形中，RSV Gb 免疫原可以任选地另外包含信号肽，如 SEQ ID NO:17。包含 SEQ ID NO:8 和信号肽 SEQ ID NO:17 的 RSV Gb 免疫原可以包含 SEQ ID NO:16。

[0199] 在一些实施方案中，提供了包含本文所陈述的两种或更多种 RSV 蛋白的组合的融合蛋白。举例来说，融合蛋白可以包含 ：SEQ ID NO:2 或其免疫原性片段和 SEQ ID NO:6 或其免疫原性片段 ；SEQ ID NO:2 或其免疫原性片段和 SEQ ID NO:8 或其免疫原性片段 ；或 SEQ ID NO:6 或其免疫原性片段和 SEQ ID NO:8 或其免疫原性片段。融合蛋白可以包含 ：SEQ ID NO:2 或其免疫原性片段、SEQ ID NO:6 或其免疫原性片段及 SEQ ID NO:8 或其免疫原性片段。作为替代方案，以上一种或多种融合蛋白可以包含与 SEQ ID NO:2 具有至少 98％或至少 99％同源性的序列或其免疫原性片段代替以上所描述的融合蛋白中对应于 SEQ ID NO:2 或其免疫原性片段的部分。同样，以上一种或多种融合蛋白可以包含与 SEQ ID NO:6 或其免疫原性片段具有至少 98％或至少 99％同源性的序列代替以上所描述的融合蛋白中对应于 SEQ ID NO:6 或其免疫原性片段的部分 ；和 / 或以上一种或多种融合蛋白可以包含与 SEQ ID NO:8 或其免疫原性片段具有至少 98％或至少 99％同源性的序列代替以上所描述的融合蛋白中对应于 SEQ ID NO:8 或其免疫原性片段的部分。融合蛋白可以包括共有蛋白和 / 或共有蛋白的片段和 / 或共有蛋白的变体和 / 或共有蛋白变体的片段的组合。所述共有蛋白和 / 或共有蛋白的片段和 / 或共有蛋白的变体和 / 或共有蛋白变体的片段可以直接彼此邻接或以其间的间隔子或者一个多个氨基酸连接。在一些实施方案中，该间隔子可以为蛋白水解裂解位点。在一些实施方案中，该间隔子可以是被打算施用和 / 或吸收疫苗的细胞中所见的蛋白酶识别的蛋白水解裂解位点。

[0200] “RSV M2-1 免疫原”是指包含与 SEQ ID NO:4 具有至少 98％同源性的蛋白质的免疫原性片段的蛋白质。RSV M2-1 免疫原可以包含与 SEQ ID NO:4 具有至少 99％同源性的蛋白质的免疫原性片段。RSV M2-1 免疫原可以包含 SEQ ID NO:4 的免疫原性片段。在每一情形中，这些免疫原性片段可以任选地另外包含信号肽，如 SEQ ID NO:17。包含 SEQ ID NO:4 的免疫原性片段和信号肽 SEQ ID NO:17 的 RSV M2-1 免疫原可以是包含 SEQ ID NO:17 的 SEQ ID NO:12 的免疫原性片段。RSV M2-1 免疫原可以包含与 SEQ ID NO:4 具有至少 98％同源性的蛋白质，并且在一些实施方案中可以包含与 SEQ ID NO:4 具有至少 99％同源性的蛋白质。RSV M2-1 免疫原可以包含 SEQ ID NO:4。在每一情形中，RSV M2-1 免疫原可以任选地另外包含信号肽，如 SEQ ID NO:17。包含 SEQ ID NO:4 和信号肽 SEQ ID NO:17 的 RSV M2-1 免疫原可以包含 SEQ ID NO:12。在一些实施方案中，提供了包含 RSV M2-1 与本文所陈述的另一 RSV 免疫原蛋白的组合的融合蛋白。举例来说，融合蛋白可以包含 ：SEQ ID NO:2 或其免疫原性片段和 SEQ ID NO:4 或其免疫原性片段 ；SEQ ID NO:4 或其免疫原性片段和 SEQ ID NO:6 或其免疫原性片段 ；或 SEQ ID NO:4 或其免疫原性片段和 SEQ ID NO:8 或其免疫原性片段 ；或 SEQ ID NO:6 或其免疫原性片段和 SEQ ID NO:8 或其免疫原性片段。融合蛋白可以包含 ：SEQ ID NO:4 或其免疫原性片段。作为替代方案，以上一种或多种融合蛋白可以包含与 SEQ ID NO:4 具有至少 98％或至少 99％同源性的序列或其免疫原性片段代替以上所描述的融合蛋白中对应于 SEQ ID NO:4 或其免疫原性片段的部分。

[0201] 3. 编码 RSV 蛋白的编码序列

[0202] 本文提供了编码本文所陈述的“RSV 免疫原”的核酸序列。施用包含这些核酸序列的核酸分子当被细胞吸收并表达时，会引起针对 RSV 的广泛免疫应答。提供了 RSV 免疫原的编码序列，即，编码 RSV F 免疫原的核酸序列、编码 RSV Ga 免疫原的核酸序列和编码 RSV Gb 免疫原的核酸序列，以及编码 RSV M2-1 免疫原的核酸序列。在一些实施方案中，编码 RSV 免疫原的核酸序列可以包含编码来自不同蛋白质（如免疫球蛋白）的信号肽（例如 IgE 信号肽 (SEQ ID NO:17) 或 IgG 信号肽）的核酸序列。

[0203] 编码 RSV F 免疫原的核酸序列可以编码包含与 SEQ ID NO:2 具有至少 98％同源性的蛋白质的免疫原性片段的蛋白质。编码 RSV F 免疫原的核酸序列可以编码包含与 SEQ ID NO:2 具有至少 99％同源性的蛋白质的免疫原性片段的蛋白质。编码 RSV F 免疫原的核酸序列可以编码包含 SEQ ID NO:2 的免疫原性片段的蛋白质。在每一情形中，编码包含这些免疫原性片的蛋白质的核酸序列可以任选地另外包含编码信号肽的核酸序列，如编码 SEQ ID NO:17 的核酸序列。编码 RSV F 免疫原的核酸序列可以编码包含 SEQ ID NO:2 的免疫原性片段和信号肽 SEQ ID NO:17 的蛋白质。编码 RSV F 免疫原的核酸序列可以编码的蛋白质可以为包含 SEQ ID NO:17 的 SEQ ID NO:10 的免疫原性片段。编码 RSV F 免疫原的核酸序列可以编码包含与 SEQ ID NO:2 具有至少 98％同源性的蛋白质的蛋白质，并且在一些实施方案中可以编码与 SEQ ID NO:2 具有至少 99％同源性的蛋白质。编码 RSV F 免疫原的核酸序列可以编码包含 SEQ ID NO:2 的蛋白质。在每一情形中，编码 RSV F 免疫原的核酸序列可以任选地另外包含编码信号肽（如 SEQ ID NO:17) 的核酸序列。编码 RSV F 免疫原的核酸序列可以编码一种包含 SEQ ID NO:2 和信号肽 SEQ ID NO:17 的蛋白质。编码 RSV F 免疫原的核酸序列可以编码一种包含 SEQ ID NO:10 的蛋白质。

[0204] 编码 RSV F 免疫原的核酸序列可以包含与 SEQ ID NO:1 具有至少 98％同源性的核

酸序列的片段。与 SEQ ID NO:1 具有至少 98％同源性的核酸序列编码一种与 SEQ ID NO:2 具有至少 98％同源性的蛋白质。该片段编码与 SEQ ID NO:2 具有至少 98％同源性的蛋白质的免疫原性片段。在一些实施方案中，编码 RSV F 免疫原的核酸序列可以包含与 SEQ ID NO:1 具有至少 98％同源性的核酸序列的片段，其中该核酸序列编码一种与 SEQ ID NO:2 具有至少 99％同源性的蛋白质，在一些实施方案中，核酸序列编码一种包含 SEQ ID NO:2 的蛋白质，并且该核酸序列的片段编码与 SEQ ID NO:2 具有至少 99％同源性的蛋白质的免疫原性片段，如 SEQ ID NO:2 的免疫原性片段。在一些此类实施方案中，编码 RSV F 免疫原的核酸序列可以包含与 SEQ ID NO:1 具有至少 99％同源性的核酸序列的片段，并且在一些实施例中包含 SEQ ID NO:1 的片段。在一些实施方案中，编码 RSV F 免疫原的核酸序列可以任选地另外包含编码信号肽（如 SEQ ID NO:17）的核酸序列。编码 RSV F 免疫原的核酸序列可以包含 SEQ ID NO:2 的片段和编码信号肽 SEQ ID NO:17 的编码序列。编码 RSV F 免疫原的核酸序列可以包含 SEQ ID NO:9 的片段，其包括编码信号肽 SEQ ID NO:17 的编码序列。

[0205] 编码 RSV Ga 免疫原的核酸序列可以编码包含与 SEQ ID NO:6 具有至少 98％同源性的蛋白质的免疫原性片段的蛋白质。编码 RSV Ga 免疫原的核酸序列可以编码包含与 SEQ ID NO:6 具有至少 99％同源性的蛋白质的免疫原性片段的蛋白质。编码 RSV Ga 免疫原的核酸序列可以编码包含 SEQ ID NO:6 的免疫原性片段的蛋白质。在每一情形中，编码包含这些免疫原性片的蛋白质的核酸序列可以任选地另外包含编码信号肽的核酸序列，如编码 SEQ ID NO:17 的核酸序列。编码 RSV Ga 免疫原的核酸序列可以编码包含 SEQ ID NO:6 的免疫原性片段和信号肽 SEQ ID NO:17 的蛋白质。编码 RSV Ga 免疫原的核酸序列可以编码的蛋白质可以为包含 SEQ ID NO:17 的 SEQ ID NO:14 的免疫原性片段。编码 RSV Ga 免疫原的核酸序列可以编码包含与 SEQ ID NO:6 具有至少 98％同源性的蛋白质的蛋白质，并且在一些实施方案中可以编码与 SEQ ID NO:6 具有至少 99％同源性的蛋白质。编码 RSV Ga 免疫原的核酸序列可以编码包含 SEQ ID NO:6 的蛋白质。在每一情形中，编码 RSV Ga 免疫原的核酸序列可以任选地另外包含编码信号肽（如 SEQ ID NO:17）的核酸序列。编码 RSV Ga 免疫原的核酸序列可以编码一种包含 SEQ ID NO:6 和信号肽 SEQ ID NO:17 的蛋白质。编码 RSV Ga 免疫原的核酸序列可以编码一种包含 SEQ ID NO:14 的蛋白质。

[0206] 编码 RSV Ga 免疫原的核酸序列可以包含与 SEQ ID NO:5 具有至少 98％同源性的核酸序列的片段。与 SEQ ID NO:5 具有至少 98％同源性的核酸序列编码一种与 SEQ ID NO:6 具有至少 98％同源性的蛋白质。该片段编码与 SEQ ID NO:6 具有至少 98％同源性的蛋白质的免疫原性片段。在一些实施方案中，编码 RSV Ga 免疫原的核酸序列可以包含与 SEQ ID NO:5 具有至少 98％同源性的核酸序列的片段，其中该核酸序列编码一种与 SEQ ID NO:6 具有至少 99％同源性的蛋白质，在一些实施方案中，核酸序列编码一种包含 SEQ ID NO:6 的蛋白质，并且该核酸序列的片段编码与 SEQ ID NO:6 具有至少 99％同源性的蛋白质的免疫原性片段，如 SEQ ID NO:6 的免疫原性片段。在一些此类实施方案中，编码 RSV Ga 免疫原的核酸序列可以包含与 SEQ ID NO:5 具有至少 99％同源性的核酸序列的片段，并且在一些实施例中包含 SEQ ID NO:5 的片段。在一些实施方案中，编码 RSV Ga 免疫原的核酸序列可以任选地另外包含编码信号肽（如 SEQ ID NO:17）的核酸序列。编码 RSV Ga 免疫原的核酸序列可以包含 SEQ ID NO:6 的片段和编码信号肽 SEQ ID NO:17 的编码序列。编码 RSV Ga

免疫原的核酸序列可以包含 SEQ ID NO:13 的片段，其包括编码信号肽 SEQ ID NO:17 的编码序列。

[0207] 编码 RSV Gb 免疫原的核酸序列可以编码包含与 SEQ ID NO:8 具有至少 98％同源性的蛋白质的免疫原性片段的蛋白质。编码 RSV Gb 免疫原的核酸序列可以编码包含与 SEQ ID NO:8 具有至少 99％同源性的蛋白质的免疫原性片段的蛋白质。编码 RSV Gb 免疫原的核酸序列可以编码包含 SEQ ID NO:8 的免疫原性片段的蛋白质。在每一情形中，编码包含这些免疫原性片的蛋白质的核酸序列可以任选地另外包含编码信号肽的核酸序列，如编码 SEQ ID NO:17 的核酸序列。编码 RSV Gb 免疫原的核酸序列可以编码包含 SEQ ID NO:8 的免疫原性片段和信号肽 SEQ ID NO:17 的蛋白质。编码 RSV Gb 免疫原的核酸序列可以编码的蛋白质可以为包含 SEQ ID NO:17 的 SEQ ID NO:16 的免疫原性片段。编码 RSV Gb 免疫原的核酸序列可以编码包含与 SEQ ID NO:8 具有至少 98％同源性的蛋白质的蛋白质，并且在一些实施方案中可以编码与 SEQ ID NO:8 具有至少 99％同源性的蛋白质。编码 RSV Gb 免疫原的核酸序列可以编码包含 SEQ ID NO:8 的蛋白质。在每一情形中，编码 RSV Gb 免疫原的核酸序列可以任选地另外包含编码信号肽（如 SEQ ID NO:17）的核酸序列。编码 RSV Gb 免疫原的核酸序列可以编码包含 SEQ ID NO:8 和信号肽 SEQ ID NO:17 的蛋白质。编码 RSV Gb 免疫原的核酸序列可以编码一种包含 SEQ ID NO:16 的蛋白质。

[0208] 编码 RSV Gb 免疫原的核酸序列可以包含与 SEQ ID NO:7 具有至少 98％同源性的核酸序列的片段。与 SEQ ID NO:7 具有至少 98％同源性的核酸序列编码一种与 SEQ ID NO:8 具有至少 98％同源性的蛋白质。该片段编码与 SEQ ID NO:8 具有至少 98％同源性的蛋白质的免疫原性片段。在一些实施方案中，编码 RSV Gb 免疫原的核酸序列可以包含与 SEQ ID NO:7 具有至少 98％同源性的核酸序列的片段，其中该核酸序列编码一种与 SEQ ID NO:8 具有至少 99％同源性的蛋白质，在一些实施方案中，核酸序列编码一种包含 SEQ ID NO:8 的蛋白质，并且该核酸序列的片段编码与 SEQ ID NO:8 具有至少 99％同源性的蛋白质的免疫原性片段，如 SEQ ID NO:8 的免疫原性片段。在一些实施方案中，编码 RSV Gb 免疫原的核酸序列可以包含与 SEQ ID NO:7 具有至少 99％同源性的核酸序列的片段，并且在一些实施例中包含 SEQ ID NO:7 的片段。在一些实施方案中，编码 RSV Gb 免疫原的核酸序列可以任选地另外包含编码信号肽（如 SEQ ID NO:17）的核酸序列。编码 RSV Gb 免疫原的核酸序列可以包含 SEQ ID NO:8 的片段和编码信号肽 SEQ ID NO:17 的编码序列。编码 RSV Gb 免疫原的核酸序列可以包含 SEQ ID NO:15 的片段，其包括编码信号肽 SEQ ID NO:17 的编码序列。

[0209] 编码 RSV M2-1 免疫原的核酸序列可以编码包含与 SEQ ID NO:4 具有至少 98％同源性的蛋白质的免疫原性片段的蛋白质。编码 RSV M2-1 免疫原的核酸序列可以编码包含与 SEQ ID NO:4 具有至少 99％同源性的蛋白质的免疫原性片段的蛋白质。编码 RSV M2-1 免疫原的核酸序列可以编码包含 SEQ ID NO:4 的免疫原性片段的蛋白质。在每一情形中，编码包含这些免疫原性片的蛋白质的核酸序列可以任选地另外包含编码信号肽的核酸序列，如编码 SEQ ID NO:17 的核酸序列。编码 RSV M2-1 免疫原的核酸序列可以编码包含 SEQ ID NO:4 的免疫原性片段和信号肽 SEQ ID NO:17 的蛋白质。编码 RSV F 免疫原的核酸序列可以编码的蛋白质可以为包含 SEQ ID NO:17 的 SEQ ID NO:12 的免疫原性片段。编码 RSV M2-1 免疫原的核酸序列可以编码包含与 SEQ ID NO:4 具有至少 98％同源性的蛋白质的蛋

白质，并且在一些实施方案中可以编码与 SEQ ID NO:4 具有至少 99％同源性的蛋白质。编码 RSV M2-1 免疫原的核酸序列可以编码包含 SEQ ID NO:4 的蛋白质。在每一情形中，编码 RSV M2-1 免疫原的核酸序列可以任选地另外包含编码信号肽（如 SEQ ID NO:17）的核酸序列。编码 RSV M2-1 免疫原的核酸序列可以编码包含 SEQ ID NO:4 和信号肽 SEQ ID NO:17 的蛋白质。编码 RSV M2-1 免疫原的核酸序列可以编码一种包含 SEQ ID NO:12 的蛋白质。

[0210] 编码 RSV M2-1 免疫原的核酸序列可以包含与 SEQ ID NO:3 具有至少 98％同源性的核酸序列的片段。与 SEQ ID NO:3 具有至少 98％同源性的核酸序列编码一种与 SEQ ID NO:4 具有至少 98％同源性的蛋白质。该片段编码与 SEQ ID NO:4 具有至少 98％同源性的蛋白质的免疫原性片段。在一些实施方案中，编码 RSV M2-1 免疫原的核酸序列可以包含与 SEQ ID NO:3 具有至少 98％同源性的核酸序列的片段，其中该核酸序列编码一种与 SEQ ID NO:4 具有至少 99％同源性的蛋白质，在一些实施方案中，核酸序列编码一种包含 SEQ ID NO:4 的蛋白质，并且该核酸序列的片段编码与 SEQ ID NO:4 具有至少 99％同源性的蛋白质的免疫原性片段，如 SEQ ID NO:4 的免疫原性片段。在一些此类实施方案中，编码 RSV M2-1 免疫原的核酸序列可以包含与 SEQ ID NO:3 具有至少 99％同源性的核酸序列的片段，并且在一些实施例中包含 SEQ ID NO:3 的片段。在一些实施方案中，编码 RSV M2-1 免疫原的核酸序列可以任选地另外包含编码信号肽（如 SEQ ID NO:17）的核酸序列。编码 RSV M2-1 免疫原的核酸序列可以包含 SEQ ID NO:4 的片段和编码信号肽 SEQ ID NO:17 的编码序列。编码 RSV M2-1 免疫原的核酸序列可以包含 SEQ ID NO:11 的片段和编码信号肽 SEQ ID NO:17 的编码序列。

[0211] 编码序列可以编码包含本文所陈述的两种或更多种 RSV 蛋白的组合的融合蛋白。编码序列可以编码包括共有蛋白和 / 或共有蛋白的片段和 / 或共有蛋白的变体和 / 或共有蛋白变体的片段的组合的融合蛋白。所述共有蛋白和 / 或共有蛋白的片段和 / 或共有蛋白的变体和 / 或共有蛋白变体的片段可以直接彼此邻接或以其间的间隔子或者一个或多个氨基酸连接。在一些实施方案中，该间隔子可以为蛋白水解裂解位点。在一些实施方案中，该间隔子可以是被打算施用和 / 或吸收疫苗的细胞中所见的蛋白酶识别的蛋白水解裂解位点。

[0212] 在一些实施方案中，编码序列编码了包含本文所陈述的两种或更多种 RSV 蛋白的组合的融合蛋白。举例来说，编码序列可以编码包含以下各序列的融合蛋白 ：SEQ ID NO:2 或其免疫原性片段和 SEQ ID NO:4 或其免疫原性片段，如 SEQ ID NO:1 或其免疫原性片段编码片段和 SEQ ID NO:3 或其免疫原性片段的编码片段 ；SEQ ID NO:2 或其免疫原性片段和 SEQ ID NO:6 或其免疫原性片段，如 SEQ ID NO:1 或其免疫原性片段编码片段和 SEQ ID NO:5 或其免疫原性片段编码片段 ；SEQ ID NO:2 或其免疫原性片段和 SEQ ID NO:8 或其免疫原性片段，如 SEQ ID NO:1 或其免疫原性片段编码片段和 SEQ ID NO:7 或其免疫原性片段编码片段 ；SEQ ID NO:4 或其免疫原性片段和 SEQ ID NO:6 或其免疫原性片段，如 SEQ ID NO:3 或其免疫原性片段编码片段和 SEQ ID NO:5 或其免疫原性片段编码片段 ；SEQ ID NO:4 或其免疫原性片段和 SEQ ID NO:8 或其免疫原性片段，如 SEQ ID NO:3 或其免疫原性片段编码片段和 SEQ ID NO:5 或其免疫原性片段编码片段 ；及 SEQ ID NO:6 或其免疫原性片段和 SEQ ID NO:8 或其免疫原性片段，如 SEQ ID NO:5 或其免疫原性片段编码片段和 SEQ ID NO:7 或其免疫原性片段编码片段。编码序列可以编码包含三种本文所陈述的 RSV 蛋白的

组合的融合蛋白。举例来说，编码序列可以编码包含以下各序列的融合蛋白：SEQ ID NO:2或其免疫原性片段、SEQ ID NO:4或其免疫原性片段和SEQ ID NO:6或其免疫原性片段，如SEQ ID NO:1或其免疫原性片段编码片段、SEQ ID NO:3或其免疫原性片段编码片段和SEQ ID NO:5或其免疫原性片段编码片段；SEQ ID NO:2或其免疫原性片段、SEQ ID NO:4或其免疫原性片段和SEQ ID NO:8或其免疫原性片段，如SEQ ID NO:1或其免疫原性片段编码片段、SEQ ID NO:3或其免疫原性片段编码片段和SEQ ID NO:7或其免疫原性片段编码片段；及SEQ ID NO:4或其免疫原性片段、SEQ ID NO:6或其免疫原性片段和SEQ ID NO:8或其免疫原性片段，如SEQ ID NO:3或其免疫原性片段编码片段、SEQ ID NO:5或其免疫原性片段编码片段和SEQ ID NO:7或其免疫原性片段编码片段。编码序列可以编码包含四种本文所陈述的RSV蛋白的组合的融合蛋白，如编码SEQ ID NO:2或其免疫原性片段、SEQ ID NO:4或其免疫原性片段、SEQ ID NO:6或其免疫原性片段及SEQ ID NO:8或其免疫原性片段的编码序列，如SEQ ID NO:1或其免疫原性片段编码片段、SEQ ID NO:3或其免疫原性片段编码片段、SEQ ID NO:5或其免疫原性片段编码片段及SEQ ID NO:7或其免疫原性片段编码片段。作为替代方案，编码序列编码以上一种或多种融合蛋白，这些融合蛋白可以包含与SEQ ID NO:2具有至少98％或至少99同源性的蛋白质或其免疫原性片段代替以上所描述的融合蛋白中对应于SEQ ID NO:2或其免疫原性片段的部分。同样，以上一种或多种融合蛋白的编码序列包含了与SEQ ID NO:4具有至少98％或至少99％同源性的编码序列或其免疫原性片段代替以上所描述的融合蛋白中对应于SEQ ID NO:4或其免疫原性片段的部分；以上一种或多种融合蛋白可以包含与SEQ ID NO:6具有至少98％或至少99％同源性的序列或其免疫原性片段代替以上所描述的融合蛋白中对应于SEQ ID NO:6或免疫原性片段的部分；和/或以上一种或多种融合蛋白可以包含与SEQ ID NO:8具有至少98％或至少99％同源性的序列或其免疫原性片段代替以上所描述的融合蛋白中对应于SEQ ID NO:8或免疫原性片段的部分。在一些实施方案中，编码以上一种或多种融合蛋白的编码序列可以包含与SEQ ID NO:1具有至少98％或至少99％同源性的序列或其片段（即，与SEQ ID NO:2的免疫原性片段具有至少98％或至少99％同源性）代替以上所描述的融合蛋白的编码序列中对应于编码SEQ ID NO:2或免疫原性片段的部分。编码以上一种或多种融合蛋白的编码序列可以包含与SEQ ID NO:3具有至少98％或至少99％同源性的序列或其片段（即，与SEQ ID NO:4的免疫原性片段具有至少98％或至少99％同源性）代替以上所描述的融合蛋白的编码序列中对应于编码SEQ ID NO:4或免疫原性片段的部分。编码以上一种或多种融合蛋白的编码序列可以包含与SEQ ID NO:5具有至少98％或至少99％同源性的序列或其片段（即，与SEQ ID NO:6的免疫原性片段具有至少98％或至少99％同源性）代替以上所描述的融合蛋白的编码序列中对应于编码SEQ ID NO:6或免疫原性片段的部分。编码以上一种或多种融合蛋白的编码序列可以包含与SEQ ID NO:7具有至少98％或至少99％同源性的序列或其片段（即，与SEQ ID NO:8的免疫原性片段具有至少98％或至少99％同源性）代替以上所描述的融合蛋白的编码序列中对应于编码SEQ ID NO:8或免疫原性片段的部分。融合蛋白可以包括共有蛋白和/或共有蛋白的片段和/或共有蛋白的变体和/或共有蛋白变体的片段的组合。所述共有蛋白和/或共有蛋白的片段和/或共有蛋白的变体和/或共有蛋白变体的片段可以直接彼此邻接或以其间的间隔子或者一个或多个氨基酸连接。在一些实施方案中，该间隔子可以为蛋白水解裂解位点。在一些实施方案中，该间隔子可以是被

打算施用和/或吸收疫苗的细胞中所见的蛋白酶识别的蛋白水解裂解位点。

[0213] 4. 质粒

[0214] 本文提供了一种能够在哺乳动物细胞中表达有效在该哺乳动物中引起免疫应答的量的一种或多种RSV蛋白的载体。该载体可以包含编码一种或多种RSV抗原的异源核酸。该载体可以为质粒。该质粒可以用于以编码RSV抗原的核酸转染细胞，在发生RSV抗原表达的条件下培养并维持该被转化的宿主细胞。

[0215] 质粒可以包含一种核酸序列，该核酸序列编码以上所公开的各种RSV蛋白中的一种或多种，包括了编码能够引起针对RSV的免疫应答的合成共有抗原的编码序列，提供的编码序列是针对以下三种蛋白质中的每一种：这些蛋白质的F、Ga和Gb片段、这些蛋白质的变体、变体或由共有蛋白和/或共有蛋白的片段和/或共有蛋白的变体和/或共有蛋白变体的片段的组合构成的融合蛋白的片段，。质粒可以包含一种核酸序列，该核酸序列编码以上所公开的RSV M2-1蛋白，包括了编码能够引起针对RSV M2-1、这些蛋白质的片段、这些蛋白质的变体、变体或由共有蛋白和/或共有蛋白的片段和/或共有蛋白的变体和/或共有蛋白变体的片段的组合构成的融合蛋白的片段的免疫应答的合成共有抗原的编码序列。

[0216] 单一质粒可以含有单一RSV免疫原的编码序列、两种RSV免疫原的编码序列或三种RSV免疫原的编码序列。在一些实施方案中，提供了四种RSV免疫原的编码序列。单一质粒可以含有RSV F免疫原的编码序列。单一质粒可以含有RSV Ga免疫原的编码序列。单一质粒可以含有RSV Gb免疫原的编码序列。单一质粒可以含有RSV F免疫原的编码序列和RSV Ga免疫原的编码序列。单一质粒可以含有RSV F免疫原的编码序列和RSV Gb免疫原的编码序列。单一质粒可以含有RSV Ga免疫原的编码序列和RSV Gb免疫原的编码序列。单一质粒可以含有RSV F免疫原的编码序列、RSV Ga免疫原的编码序列和RSV Gb免疫原的编码序列。

[0217] 如果提供RSV M2-1编码序列，那么单一质粒可以含有RSV M2-1免疫原的编码序列。单一质粒可以含有RSV F免疫原的编码序列和RSV M2-1免疫原的编码序列。单一质粒可以含有RSV M2-1免疫原的编码序列和RSV Ga免疫原的编码序列。单一质粒可以含有RSV M2-1免疫原的编码序列和RSV Gb免疫原的编码序列。单一质粒可以含有RSV F免疫原的编码序列、RSV M2-1免疫原的编码序列和RSV Ga免疫原的编码序列。单一质粒可以含有RSV F免疫原的编码序列、RSV M2-1免疫原的编码序列和RSV Gb免疫原的编码序列。单一质粒可以含有RSV M2-1免疫原的编码序列、RSV Ga免疫原的编码序列和RSV Gb免疫原的编码序列。单一质粒可以含有RSV F免疫原的编码序列、RSV M2-1免疫原的编码序列、RSV Ga免疫原的编码序列及RSV Gb免疫原的编码序列。

[0218] 在一些实施方案中，质粒可以包含仅编码CCR20或作为这些质粒之一的一部分的编码序列。类似地，质粒可以另外包含IL-12、IL-15和/或IL-28的编码序列。

[0219] 质粒可以另外包含起始密码子，该起始密码子可以在编码序列的上游；及终止密码子，该终止密码子可以在编码序列的下游。起始和终止密码子可以与编码序列同框。

[0220] 质粒还可以包含一个可操作地连接到编码序列的启动子。可操作地连接到编码序列的启动子可以是来自猿猴病毒40(simian virus 40,SV40)的启动子、小鼠乳房肿瘤病毒(mouse mammary tumor virus,MMTV)启动子、人免疫缺陷病毒(human immunodeficiency virus,HIV)启动子(如牛免疫缺陷病毒(bovine immunodeficiency virus,BIV)长末端重

复序列(long terminal repeat,LTR)启动子)、莫罗尼氏病毒(Moloney virus)启动子、禽白血病病毒(avian leukosis virus,ALV)启动子、巨细胞病毒(cytomegalovirus,CMV)启动子(如CMV立即早期启动子)、爱泼斯坦-巴尔病毒(Epstein Barr virus,EBV)启动子或劳氏肉瘤病毒(Rous sarcoma virus,RSV)启动子。启动子还可以是来自人类基因(如人肌动蛋白、人肌球蛋白、人血红蛋白、人肌肉肌酸或人金属硫蛋白)的启动子。启动子还可以是天然或合成的组织特异性启动子,如肌肉或皮肤特异性启动子。这些启动子的实例描述于美国专利申请公布号US20040175727中,其内容通过引用整体并入本文中。

[0221] 质粒也可以包含聚腺苷酸化信号,该信号可以在编码序列的下游。聚腺苷酸化信号可以为SV40聚腺苷酸化信号、LTR聚腺苷酸化信号、牛生长激素(bovine growth hormone,bGH)聚腺苷酸化信号、人生长激素(human growth hormone,hGH)聚腺苷酸化信号或人β-球蛋白聚腺苷酸化信号。SV40聚腺苷酸化信号可以是来自pCEP4质粒(Invitrogen,San Diego,CA)的聚腺苷酸化信号。

[0222] 质粒还可以包含在编码序列上游的增强子。增强子可以为人肌动蛋白、人肌球蛋白、人血红蛋白、人肌肉肌酸,或病毒增强子,如来自CMV、FMDV、RSV或EBV的增强子。多核苷酸功能增强描述于美国专利号5,593,972、5,962,428;以及WO94/016737中,其各自的内容通过引用完整地并入本文中。

[0223] 质粒还可以包含哺乳动物复制起点,以便在染色体外维持质粒并且在细胞中产生质粒的多个拷贝。质粒可以为来自Invitrogen公司(San Diego,CA)的pVAX1、pCEP4或pREP4,这些质粒可以包含爱泼斯坦-巴尔病毒复制起点和核抗原EBNA-1编码区,该编码区可以在不进行整合情况下产生高拷贝游离型复制。质粒的主链可以为pAV0242。质粒可以是复制缺陷性腺病毒型5(Ad5)质粒。

[0224] 质粒还可以包含调控序列,该调控序列可以特别适合于在施用该质粒的细胞中进行基因表达。编码序列可以包含一个能够允许编码序列在宿主细胞中更高效的转录的密码子。

[0225] 编码序列还可以包含Ig前导序列。前导序列可以在编码序列的5'端。由这一序列编码的共有抗原可以依序包含N末端Ig前导序列和共有抗原蛋白。N末端Ig前导序列可以为IgE或IgG。

[0226] 质粒可以为pSE420(Invitrogen,San Diego,Calif.),其可以用于在大肠杆菌(Escherichia coli,E.coli)中产生蛋白质。质粒还可以为pYES2(Invitrogen,San Diego,Calif.),其可以用于在酵母的酿酒酵母(Saccharomyces cerevisiae)菌株中产生蛋白质。质粒还可以属于MAXBAC™完全杆状病毒表达系统(Invitrogen,San Diego,Calif.),其可以用于在昆虫细胞中产生蛋白质。质粒还可以为pcDNA I或pcDNA3(Invitrogen,San Diego,Calif.),其可以用于在如中国仓鼠卵巢(Chinese hamster ovary,CHO)细胞等哺乳动物细胞中产生蛋白质。

[0227] 5.组合物

[0228] 提供了包含核酸分子的组合物。这些组合物可以包含单一核酸分子(如单一质粒)的多个拷贝、两个或更多个不同核酸分子(如两个或更多个不同质粒)的多个拷贝。举例来说,组合物可以包含许多两个、三个、四个、五个、六个、七个、八个、九个或十个或更多不同核酸分子。此类组合物可以包含许多两个、三个、四个、五个、六个、七个、八个、九个或

十个或更多不同核酸分子。

[0229] 组合物可以包含多个核酸分子，如质粒，这些核酸分子共同地含有单一 RSV 免疫原的编码序列、两种 RSV 免疫原的编码序列或三种 RSV 免疫原的编码序列。在一些情形中，可以提供四种 RSV 免疫原的编码序列。

[0230] 包含两种 RSV 免疫原的编码序列的组合物可以在单一核酸分子（如单一质粒）上，或这些组合物可以包含两个不同的核酸分子，如两个不同的质粒，其中一个核酸分子包含一种 RSV 免疫原的编码序列并且另一个核酸分子包含不同 RSV 免疫原的编码序列。类似地，包含三种 RSV 免疫原的编码序列的组合物可以包含单一核酸分子（如单一质粒）、两个不同的核酸分子或三个不同的核酸分子。同样，包含四种 RSV 免疫原的编码序列的组合物可以包含单一核酸分子（如单一质粒）、两个不同的核酸分子、三个不同的核酸分子或四个不同的核酸分子。

[0231] 在一些实施方案中，一种组合物包含多个编码一种 RSV 免疫原（如 RSV F 免疫原、RSV Ga 免疫原或 RSV Gb 免疫原）的单一核酸分子。在一些实施方案中，一种组合物包含多个单一核酸分子，如单一质粒，其编码两种 RSV 免疫原，如 RSV F 免疫原和 RSV Ga 免疫原、RSV F 免疫原和 RSV Gb 免疫原以及 RSV Ga 免疫原和 RSV Gb 免疫原。在一些实施方案中，一种组合物包含多个单一核酸分子，如单一质粒，其编码三种 RSV 免疫原，如 RSV Ga 免疫原、RSV F 免疫原和 RSV Gb 免疫原。

[0232] 在一些实施方案中，一种组合物包含多个两种不同的核酸分子，如两个质粒，每个不同的核酸分子包含不同 RSV 免疫原的单一不同核酸序列，其中不同核酸分子对包含 RSV F 免疫原和 RSV Ga 免疫原、RSV F 免疫原和 RSV Gb 免疫原，以及 RSV Ga 免疫原和 RSV Gb 免疫原。

[0233] 在一些实施方案中，一种组合物包含多个两种不同的核酸分子，如两个质粒，其共同地包含三种不同 RSV 免疫原的编码序列。在一些实施方案中，一个编码 RSV F 免疫原和 RSV Ga 免疫原，并且另一个编码 RSV Gb 免疫原。在一些实施方案中，一个编码 RSV F 免疫原和 RSV Gb 免疫原，并且另一个编码 RSV Ga 免疫原。在一些实施方案中，一个编码 Ga 免疫原和 RSV Gb 免疫原，并且另一个编码 RSV F 免疫原。

[0234] 在一些实施方案中，一种组合物包含多个编码 RSV M2-1 免疫原的单一核酸分子。

[0235] 在提供 RSV M2-1 的编码序列的一些实施方案中，一种组合物包含多个单一核酸分子，如单一质粒，其编码两种 RSV 免疫原，如 RSV F 免疫原和 RSV M2-1 免疫原、RSV M2-1 免疫原和 RSV Ga 免疫原，以及 RSV M2-1 免疫原和 RSV Gb 免疫原。

[0236] 在提供 RSV M2-1 的编码序列的一些实施方案中，多个单一核酸分子，如单一质粒，其编码三种 RSV 免疫原，如 RSV F 免疫原、RSV M2-1 免疫原和 RSV Ga 免疫原；RSV F 免疫原、RSV M2-1 免疫原和 RSV Gb 免疫原；RSV M2-1 免疫原、RSV Ga 免疫原和 RSV Gb 免疫原。在一些实施方案中，一种组合物包含多个单一核酸分子，如单一质粒，其编码三种 RSV 免疫原，如 RSV F 免疫原、RSV M2-1 免疫原、RSV Ga 免疫原和 RSV Gb 免疫原。

[0237] 在提供 RSV M2-1 的编码序列的一些实施方案中，一种组合物包含多个两种不同的核酸分子，如两个质粒，每个不同的核酸分子包含不同 RSV 免疫原的单一不同核酸序列，其中不同核酸分子对包含 RSV F 免疫原和 RSV M2-1 免疫原、RSV M2-1 免疫原和 RSV Ga 免疫原，以及 RSV M2-1 免疫原和 RSV Gb 免疫原。

[0238] 在提供RSV M2-1的编码序列的一些实施方案中，一种组合物包含多个两种不同的核酸分子，如两个质粒，其共同地包含三种不同RSV免疫原或其中之一为RSV M2-1的编码序列。实施方案可以选自由以下组成的组：一个核酸分子编码RSV F免疫原和RSV M2-1免疫原并且另一个编码RSV Ga免疫原；一个核酸分子编码RSV F免疫原和RSV M2-1免疫原并且另一个编码RSV Gb免疫原；一个编码RSV F免疫原和RSV Ga免疫原，并且另一个编码RSV M2-1免疫原；一个编码RSV F免疫原和RSV Gb免疫原，并且另一个编码RSV M2-1免疫原；一个编码RSV M2-1免疫原和RSV Ga免疫原，并且另一个编码RSV F免疫原；一个编码RSV M2-1免疫原和RSV Ga免疫原，并且另一个编码RSV Gb免疫原；一个编码RSV M2-1免疫原和RSV Gb免疫原，并且另一个编码RSV F免疫原；一个编码RSV M2-1免疫原和RSV Gb免疫原，并且另一个编码RSV Ga免疫原；以及一个编码Ga免疫原和RSV Gb免疫原，并且另一个编码RSV M2-1免疫原。

[0239] 在提供RSV M2-1的编码序列的一些实施方案中，一种组合物包含多个两种不同的核酸分子，如两个质粒，其共同地包含四种不同RSV免疫原的编码序列。在一些实施方案中：一个核酸分子编码RSV F免疫原并且另一个编码RSV M2-1免疫原、RSV Ga免疫原和RSV Gb免疫原；一个核酸分子编码RSV M2-1免疫原并且另一个编码RSV F免疫原、RSV Ga免疫原和RSV Gb免疫原；一个编码RSV Ga免疫原并且另一个编码RSV F免疫原、RSV M2-1免疫原和RSV Gb免疫原、一个编码RSV Gb免疫原并且另一个编码RSV F免疫原、RSV M2-1免疫原和RSV Ga免疫原、一个编码RSV F和RSV M2-1免疫原，另一个编码RSV Ga免疫原和RSV Gb免疫原；一个编码RSV F和RSV Ga免疫原，另一个编码RSV M2-1免疫原和RSV Gb免疫原；以及一个编码RSV F和RSV Gb免疫原，另一个编码RSV M2-1免疫原和RSV Ga免疫原。在一些实施方案中，一种组合物包含多个三种不同的核酸分子，如三个质粒，其共同地包含三种不同RSV免疫原的编码序列。在一些实施方案中：一个核酸分子编码RSV F免疫原，一个编码RSV M2-1免疫原并且第三个第二个编码RSV Ga免疫原；一个核酸分子编码RSV F免疫原，一个编码RSV M2-1免疫原并且第三个编码RSV Gb免疫原；一个核酸分子编码RSV F免疫原，一个编码RSV Ga免疫原并且第三个编码RSV Gb免疫原；以及一个核酸分子编码RSV M2-1免疫原，一个编码RSV Ga免疫原并且第三个编码RSV Gb免疫原。在一些实施方案中，一种组合物包含多个三种不同的核酸分子，如三个质粒，其共同地包含四种不同RSV免疫原的编码序列。在一些实施方案中：一个核酸分子编码RSV F免疫原，第二个编码RSV M2-1免疫原，并且第三个编码RSV Ga免疫原和RSV Gb免疫原；一个核酸分子编码RSV F免疫原，第二个编码RSV Ga免疫原，并且第三个编码RSV M2-1免疫原和RSV Gb免疫原；一个核酸分子编码RSV F免疫原，第二个编码RSV Gb免疫原，并且第三个编码RSV M2-1免疫原和RSV Ga免疫原；一个核酸分子编码RSV M2-1免疫原，第二个编码RSV Ga免疫原，并且第三个编码RSV F免疫原和RSV Gb免疫原；一个核酸分子编码RSV M2-1免疫原，第二个编码RSV Gb免疫原，，并且第三个编码RSV F免疫原和RSV Ga免疫原；以及一个编码RSV Ga免疫原，第二个编码RSV Gb免疫原，并且第三个编码RSV F免疫原和RSV M2-1。在一些实施方案中，一种组合物包含多个四种不同的核酸分子，如四个质粒，其共同地包含四种不同RSV免疫原的编码序列，即，一个核酸分子编码RSV F免疫原，第二个编码RSV M2-1免疫原，第三个编码RSV Ga免疫原并且第四个编码RSV Gb免疫原。

[0240] 在一些实施方案中，一种组合物另外包含趋化因子CCL20、IL-12、IL-15和/或

IL-28 的编码序列。趋化因子 CCL20、IL-12、IL-15 和 / 或 IL-28 的编码序列可以被包括在包含一种或多种 RSV 免疫原的编码序列的一个或多个核酸分子上。趋化因子 CCL20、IL-12、IL-15 和 / 或 IL-28 的编码序列可以被包括在单独核酸分子上，如单独质粒上。

[0241] 6. 疫苗

[0242] 本文提供了一种能够在哺乳动物中产生针对 RSV 的免疫应答的疫苗。该疫苗可以包含如以上所论述的每种质粒。该疫苗可以包含多个质粒，或其组合。提供的疫苗可以诱导治疗性或预防性免疫应答。

[0243] 在一些实施方案中，疫苗优选为 Th1 偏性的，由此不会在 Th2 相关性发病之前起作用，并且它应当能够在中和敏感性环境中起作用。优选地，它应当为失活疫苗。

[0244] 疫苗可以用于传递一种或多种免疫原性靶，这些靶选自由以下组成的组 ：这些蛋白质的 F、Ga 和 Gb 片段、这些蛋白质的变体、变体的片段。在传递多个靶的情况下，疫苗可以包括多种组合物或单一组合物。可以使用在单一质粒上编码多种蛋白质的质粒或包含编码不同蛋白质的不同质粒的组合物。在一些实施方案中，疫苗可以用于仅传递 M2、其片段、M2-1 的变体、变体的片段，或其与一种或多种免疫原靶的组合，这些靶选自由以下组成的组 ：这些蛋白质的 F、Ga 和 Gb 片段、这些蛋白质的变体、变体的片段。在传递多个靶的情况下，疫苗可以包括多种组合物或单一组合物。可以使用在单一质粒上编码多种蛋白质的质粒或包含编码不同蛋白质的不同质粒的组合物。

[0245] 疫苗可以另外包含药学上可接受的赋形剂。该药学上可接受的赋形剂可以是如媒剂、佐剂、载剂或稀释剂等功能分子。该药学上可接受的赋形剂可以是转染促进剂，其可以包括表面活性剂，如免疫刺激复合物 (immune-stimulating complex，ISCOMS)、弗氏不完全佐剂 (Freunds incomplete adjuvant)、包括单磷酰基脂质 A 的 LPS 类似物、胞壁酰肽、醌类似物、囊泡 (如角鲨烯和角鲨烯)、透明质酸、脂质、脂质体、钙离子、病毒蛋白、聚阴离子或纳米粒子，或其它已知的转染促进剂。

[0246] 转染促进剂为聚阴离子、聚阳离子 (包括聚 L- 谷氨酸 (L-glutamate，LGS)) 或脂质。转染促进剂为聚 L- 谷氨酸，并且更优选该聚 L- 谷氨酸是以低于 6mg/ml 的浓度存在于疫苗中。转染促进剂还可以包括表面活性剂，如免疫刺激复合物 (ISCOMS)、弗氏不完全佐剂、包括单磷酰基脂质 A 的 LPS 类似物、胞壁酰肽、醌类似物以及囊泡 (如角鲨烯和角鲨烯)，并且还可以将透明质酸结合遗传构建体一起施用。在一些实施方案中，DNA 质粒疫苗还可以包括一种转染促进剂，如脂质、脂质体 (包括卵磷脂脂质体或本领域中已知的其它脂质体，如 DNA- 脂质体混合物 (参看例如 WO9324640))、钙离子、病毒蛋白、聚阴离子、聚阳离子或纳米粒子，或其它已知的转染促进剂。优选地，转染促进剂为聚阴离子、聚阳离子 (包括聚 L- 谷氨酸 (LGS)) 或脂质。疫苗中转染剂的浓度低于 4mg/ml，低于 2mg/ml，低于 1mg/ml，低于 0.750mg/ml，低于 0.500mg/ml，低于 0.250mg/ml，低于 0.100mg/ml，低于 0.050mg/ml，或低于 0.010mg/ml。

[0247] 药学上可接受的赋形剂可以为一种或多种佐剂。佐剂可以是由相同质粒或由替代性质粒表达，或以蛋白质形式与以上质粒组合于疫苗中进行传递的其它基因。该一种或多种佐剂可以是选自由以下组成的组的蛋白质和 / 或编码这些蛋白质的核酸分子 ：CCL20、α - 干扰素 (IFN-α)、β - 干扰素 (IFN-β)、γ - 干扰素、血小板源性生长因子 (platelet derived growth factor，PDGF)、TNFα、TNFβ、GM-CSF、表皮生长因子 (EGF)、

皮肤 T 细胞吸引趋化因子 (cutaneous T cell-attracting chemokine，CTACK)、上皮胸腺表达趋化因子 (epithelial thymus-expressed chemokine，TECK)、粘膜相关上皮趋化因子 (mucosae-associated epithelial chemokine，MEC)、IL-12、IL-15(包括具有信号序列或编码缺失的信号序列并且任选包括如来自 IgE 的信号肽等不同信号肽的编码序列，或编码如来自 IgE 的信号肽等不同信号肽的编码序列的 IL-15)、IL-28、MHC、CD80、CD86、IL-1、IL-2、IL-4、IL-5、IL-6、IL-10、IL-18、MCP-1、MIP-1α、MIP-1β、IL-8、L- 选择素、P- 选择素、E- 选择素、CD34、GlyCAM-1、MadCAM-1、LFA-1、VLA-1、Mac-1、p150.95、PECAM、ICAM-1、ICAM-2、ICAM-3、CD2、LFA-3、M-CSF、G-CSF、IL-18 的突变形式、CD40、CD40L、血管生长因子、成纤维细胞生长因子、IL-7、神经生长因子、血管内皮生长因子、Fas、TNF 受体、Flt、Apo-1、p55、WSL-1、DR3、TRAMP、Apo-3、AIR、LARD、NGRF、DR4、DR5、KILLER、TRAIL-R2、TRICK2、DR6、Caspase ICE、Fos、c-jun、Sp-1、Ap-1、Ap-2、p38、p65Rel、MyD88、IRAK、TRAF6、IkB、无活性 NIK、SAP K、SAP-1、JNK、干扰素反应基因、NFkB、Bax、TRAIL、TRAILrec、TRAILrecDRC5、TRAIL-R3、TRAIL-R4、RANK、RANK LIGAND、Ox40、Ox40 LIGAND、NKG2D、MICA、MICB、NKG2A、NKG2B、NKG2C、NKG2E、NKG2F、TAP1、TAP2 及其功能片段，或其组合。在一些实施方案中，佐剂可以是一种或多种选自由以下组成的组的蛋白质和 / 或编码这些蛋白质的核酸分子：CCL-20、IL-12、IL-15、IL-28、CTACK、TECK、MEC 或 RANTES。IL-12 构建体和序列的实例公开于 PCT 申请号 PCT/US1997/019502 和相应美国申请序列号 08/956,865，以及 2011 年 12 月 12 日申请的美国临时申请序列号 61/569600 中，其各自通过引用并入本文中。IL-15 构建体和序列的实例公开于 PCT 申请号 PCT/US04/18962 和相应美国申请序列号 10/560,650，以及 PCT 申请号 PCT/US07/00886 和相应美国申请序列号 12/160,766，及 PCT 申请号 PCT/US10/048827 中，其各自通过引用并入本文中。IL-28 构建体和序列的实例公开于 PCT 申请号 PCT/US09/039648 和相应美国申请序列号 12/936,192 中，其各自通过引用并入本文中。RANTES 以及其它构建体和序列的实例公开于 PCT 申请号 PCT/US1999/004332 和相应美国申请序列号 09/622452 中，其各自通过引用并入本文中。RANTES 构建体和序列的其它实例公开于 PCT 申请号 PCT/US11/024098 中，该案通过引用并入本文中。RANTES 以及其它构建体和序列的实例公开于 PCT 申请号 PCT/US1999/004332 和相应美国申请序列号 09/622452 中，其各自通过引用并入本文中。RANTES 构建体和序列的其它实例公开于 PCT 申请号 PCT/US11/024098 中，该案通过引用并入本文中。趋化因子 CTACK、TECK 和 MEC 构建体和序列的实例公开于 PCT 申请号 PCT/US2005/042231 和相应美国申请序列号 11/719,646 中，其各自通过引用并入本文中。OX40 和其它免疫调节剂的实例公开于美国申请序列号 10/560,653 中，该案通过引用并入本文中。DR5 和其它免疫调节剂的实例公开于美国申请序列号 09/622452 中，该案通过引用并入本文中。

[0248] 疫苗可以另外包含如 1994 年 4 月 1 日申请的美国序列号 021,579(该案通过引用完整地并入本文中) 中所描述的基因疫苗促进剂。

[0249] 疫苗包含的共有抗原和质粒的量可以为约 1 纳克到 100 毫克；约 1 微克到约 10 毫克；或优选约 0.1 微克到约 10 毫克；或更优选约 1 毫克到约 2 毫克。在一些优选的实施方案中，根据本发明的药物组合物包含约 5 纳克到约 1000 毫克的 DNA。在一些优选的实施方案中，这些药物组合物含有约 10 纳克到约 800 毫克的 DNA。在一些优选的实施方案中，这些药物组合物含有约 0.1 到约 500 微克的 DNA。在一些优选的实施方案中，这些药物组合物

含有约 1 到约 350 微克的 DNA。在一些优选的实施方案中，这些药物组合物含有约 25 到约 250 微克，约 100 到约 200 微克，约 1 纳克到 100 毫克；约 1 微克到约 10 毫克；约 0.1 微克到约 10 毫克；约 1 毫克到约 2 毫克；约 5 纳克到约 1000 微克、约 10 纳克到约 800 微克、约 0.1 到约 500 微克、约 1 到约 350 微克、约 25 到约 250 微克、约 100 到约 200 微克的共有抗原或其质粒。

[0250] 疫苗可以根据打算使用的施药模式进行配制。可注射疫苗药物组合物可以是无菌、无热原质并且无颗粒状物的。可以使用等渗制剂或溶液。用于等渗性的添加剂可以包括氯化钠、右旋糖、甘露糖醇、山梨糖醇及乳糖。疫苗可以包含血管收缩剂。等渗溶液可以包括磷酸盐缓冲的生理盐水。疫苗可以另外包含稳定剂，包括明胶和白蛋白。稳定剂可以使该制剂在室温或环境温度下稳定保持较长时间段，如添加到疫苗制剂中的 LGS 或聚阴离子或聚阳离子。

[0251] 7. 疫苗传递方法

[0252] 本文提供了一种用于传递疫苗的方法，该疫苗将提供共有抗原的遗传构建体和蛋白质，其包含使其特别有效地针对 RSV 免疫原的表位，针对这些免疫原可以诱导免疫应答。提供的递送疫苗或疫苗接种的方法可以诱导治疗性和预防性免疫应答。疫苗接种过程可以在哺乳动物中产生针对 RSV 的免疫应答。该疫苗可以被传递给个体以调节哺乳动物免疫系统的活性并且增强免疫应答。疫苗的传递可以是呈核酸分子形式的共有抗原的转染，该核酸分子在细胞中表达并且被递送到细胞表面，在细胞表面上被免疫系统识别，并且诱导细胞、体液或细胞和体液反应。疫苗的传递可以用于在哺乳动物中诱导或引起针对 RSV 免疫应答，这是通过向哺乳动物施用如以上所论述的疫苗实现的。

[0253] 在将疫苗和质粒传递到哺乳动物细胞中之后，受转染的细胞将表达并分泌由该疫苗所注射的每个质粒的共有抗原。这些蛋白质将被免疫系统识别为外来物，并且将产生针对其的抗体。这些抗体将被免疫系统维持并允许对随后的 RSV 感染产生有效应答。

[0254] 该疫苗可以被施用给哺乳动物以在哺乳动物中引起免疫应答。该哺乳动物可以为人类、灵长类动物、非人类灵长类动物、母牛、牛、绵羊、山羊、羚羊、野牛、水牛、野牛、牛科动物、鹿、刺猬、大象、骆驼、羊驼、小鼠、大鼠和鸡。

[0255] a. 组合治疗

[0256] 该疫苗可以与其它蛋白质和 / 或编码以下各物的基因组合施用：CCL20、α - 干扰素、γ - 干扰素、血小板源性生长因子 (PDGF)、TNFα、TNFβ、GM-CSF、表皮生长因子 (EGF)、皮肤 T 细胞吸引趋化因子 (CTACK)、上皮胸腺表达趋化因子 (TECK)、粘膜相关上皮趋化因子 (MEC)、IL-12、IL-15(包括缺失信号序列并且任选包括如 IgE 信号肽等不同信号肽的 IL-15)、MHC、CD80、CD86、IL-28、IL-1、IL-2、IL-4、IL-5、IL-6、IL-10、IL-18、MCP-1、MIP-1α、MIP-1β、IL-8、RANTES、L- 选择素、P- 选择素、E- 选择素、CD34、GlyCAM-1、MadCAM-1、LFA-1、VLA-1、Mac-1、p150.95、PECAM、ICAM-1、ICAM-2、ICAM-3、CD2、LFA-3、M-CSF、G-CSF、IL-18 的突变形式、CD40、CD40L、血管生长因子、IL-7、神经生长因子、血管内皮生长因子、Fas、TNF 受体、Flt、Apo-1、p55、WSL-1、DR3、TRAMP、Apo-3、AIR、LARD、NGRF、DR4、DR5、KILLER、TRAIL-R2、TRICK2、DR6、Caspase ICE、Fos、c-jun、Sp-1、Ap-1、Ap-2、p38、p65Rel、MyD88、IRAK、TRAF6、IkB、无活性 NIK、SAP K、SAP-1、JNK、干扰素反应基因、NFkB、Bax、TRAIL、TRAILrec、TRAILrecDRC5、TRAIL-R3、TRAIL-R4、RANK、RANK LIGAND、Ox40、Ox40

LIGAND、NKG2D、MICA、MICB、NKG2A、NKG2B、NKG2C、NKG2E、NKG2F、TAP1、TAP2 及其功能片段，或其组合。在一些实施方案中，疫苗是与以下一种或多种核酸分子和 / 或蛋白质组合施用：选自由以下组成的组的核酸分子：包含了编码 CCL20、IL-12、IL-15、IL-28、CTACK、TECK、MEC 及 RANTES 中的一种或多种的编码序列或其功能片段的核酸分子；及选自由以下组成的组的蛋白质：CCL02、IL-12 蛋白、IL-15 蛋白、IL-28 蛋白、CTACK 蛋白、TECK 蛋白、MEC 蛋白或 RANTES 蛋白，或其功能片段。

[0257] 疫苗可以通过不同途径施用，这些途经包括口服、肠胃外、舌下、透皮、直肠、透粘膜、局部、经由吸入、经由口腔施用、经胸膜内、静脉内、动脉内、腹膜内、皮下、肌肉内、鼻内、鞘内及关节内，或其组合。对于兽医应用，该组合物可以根据标准兽医实践以适合地可接受的制剂施用。兽医可以容易地确定最适合特定动物施用的剂量方案和途径。疫苗可以通过传统注射器、无针注射装置、“微粒轰击基因枪”(microprojectile bombardment gone gun)，或如电穿孔法（“EP”）、流体动力学方法或超声波法等其它物理方法施用。

[0258] 疫苗的质粒可以通过若干众所周知的技术递送给哺乳动物，包括 DNA 注射（又称为 DNA 疫苗接种），并且利用和不利用了体内电穿孔法、脂质体介导、纳米粒子促进的重组载体，如重组腺病毒、重组腺病毒相关病毒及重组牛痘。该共有抗原可以经由 DNA 注射以及体内电穿孔法递送。

[0259] b. 电穿孔

[0260] 经由疫苗质粒的电穿孔来施用疫苗可以使用电穿孔装置实现，这些电穿孔装置可以被配置成向所希望的哺乳动物组织递送有效引起细胞膜中可逆孔形成的能量脉冲，并且优选该能量脉冲是类似于用户输入的预先设定的电流的恒定电流。电穿孔装置可以包含一个电穿孔组件和一个电极组合件或处理组合件。电穿孔组件可以包括并且并入电穿孔装置的各种元件中的一个或多个，包括：控制器、电流波形发生器、阻抗测试仪、波形记录器、输入元件、状态报告元件、通信端口、记忆组件、电源和电源开关。电穿孔可以使用体内电穿孔装置，例如 CELLECTRA EP 系统 (VGX Pharmaceuticals, Blue Bell, PA) 或 Elgen 电穿孔仪 (Genetronics, San Diego, CA) 实现，以促进质粒对细胞的转染。

[0261] 电穿孔组件可以起到电穿孔装置的一个元件的作用，并且其它元件是与电穿孔组件通信的单独元件（或组件）。电穿孔组件可以起到电穿孔装置的超过一个元件的作用，其可以同与电穿孔装置中与电穿孔组件分开的其它元件通信。作为一种电机械或机械装置的一部分存在的电穿孔装置的元件不受限制，因为这些元件可以起到一个装置或彼此通信的单独元件的作用。电穿孔组件能够传递能量脉冲以在所希望的组织中产生恒定电流，并且包括反馈机制。电极组合件可以包括一个电极阵列，该电极阵列具有呈空间布置的多个电极，其中该电极组合件从电穿孔组件接收能量脉冲并且通过电极将其传递给所希望的组织。这多个电极中的至少一个在能量脉冲的传递期间是呈中性的，并且测量所希望的组织中的阻抗，并将该阻抗传达给电穿孔组件。该反馈机制可以接收所测量的阻抗并且可以调整由电穿孔组件所传递的能量脉冲以维持恒定电流。

[0262] 多个电极可以按一种分散模式传递能量脉冲。该多个电极可以通过电极的控制以一种程序化的次序传递能量脉冲，并且该程序化的次序是由用户输入到电穿孔组件中。该程序化的次序可以包含依序传递的多个脉冲，其中该多个脉冲中的每个脉冲是通过至少两个有效电极和一个测量阻抗的中性电极传递，并且其中该多个脉冲中的随后脉冲是通过与

至少两个有效电极不同的一个电极和一个测量阻抗的中性电极传递。

[0263] 该反馈机制可以由硬件或软件进行。反馈机制可以由模拟闭环电路执行。反馈每50μs、20μs、10μs或1μs发生，但优选实时反馈或即时反馈（即，如由可用于测定反应时间的技术确定为基本上即时的）。该中性电极可以测量所希望的组织中的阻抗并且将该阻抗传达给反馈机制，并且该反馈机制对该阻抗作出响应并调整能量脉冲以将恒定电流维持在类似于预先设定的电流的值。反馈机制可以在能量脉冲传递期间持续地并且即时地维持恒定电流。

[0264] 可以有助于本发明的DNA疫苗的传递的电穿孔装置和电穿孔方法的实例包括Draghia-Akli等人的美国专利号7,245,963、Smith等人提交的美国专利公布2005/0052630中所描述的那些，这些专利的内容都通过引用整体并入本文中。可以用于促进DNA疫苗的传递的其它电穿孔装置和电穿孔方法包括2007年10月17日提交的同在申请并且共同拥有的美国专利申请序列号11/874072中所提供的那些，该案依据35 USC 119(e)要求2006年10月17日提交的美国临时申请序列号60/852,149和2007年10月10日提交的美国临时申请序列号60/978,982的权益，所有这些申请都整体并入本文中。

[0265] Draghia-Akli等人的美国专利号7,245,963描述了模块式电极系统及其用于促进将生物分子引入身体或植物中的所选组织的细胞中的用途。这些模块式电极系统可以包含多个针状电极；皮下注射针；提供从可程序化的恒定电流脉冲控制器到多个针状电极的导电链路的电连接器；及电源。操作人员可以抓住被安装在一个支撑结构上的多个针状电极，并且平稳地将其插入身体或植物中的所选组织中。然后，经由皮下注射针将生物分子传递到所选组织中。将可程序化的恒定电流脉冲控制器激活，并且将恒定电流电脉冲施加到该多个针状电极。所施加的恒定电流电脉冲促进将生物分子引入在多个电极之间的细胞中。美国专利号7,245,963的完整内容通过引用并入本文中。

[0266] 由Smith等人提交的美国专利公布2005/0052630描述了一种电穿孔装置，该装置可以用于有效地促进将生物分子引入身体或植物中的所选组织的细胞中。该电穿孔装置包含一个电动力学装置（“EKD装置”），其操作是由软件或固件指定的。EKD装置基于用户控制和脉冲参数的输入在呈阵列形式的电极之间产生一系列可程序化的恒定电流脉冲模式，并且允许储存和获取电流波形数据。电穿孔装置还包含一个可更换的电极盘，该电极盘具有一个由针状电极形成的阵列；一个用于注射针的中央注射通道；及一个可拆卸的导向盘。美国专利公布2005/0052630的完整内容通过引用并入本文中。

[0267] 美国专利号7,245,963和美国专利公布2005/0052630中所描述的电极阵列和方法都可以用于深穿透到不仅如肌肉等组织中，而且还深穿透到其它组织或器官中。归因于电极阵列的配置，注射针（用以传递所选生物分子）还能完全地插入靶器官中，并且垂直于靶组织，在由电极预先划定的区域中施用注射。美国专利号7,245,963和美国专利公布2005/005263中所描述的电极优选为20mm长度并且为21号。

[0268] 另外地，涵盖了并入电穿孔装置和其用途的一些实施方案，电穿孔装置是以下专利中所描述的那些：1993年12月28日颁布的美国专利5,273,525、2000年8月29日颁布的美国专利6,110,161、2001年7月17日颁布的6,261,281和2005年10月25日颁布的6,958,060，以及2005年9月6日颁布的美国专利6,939,862。此外，涵盖2004年2月24日颁布的美国专利6,697,669（涉及使用多种装置中的任一种传递DNA）和2008年2月5

日颁布的美国专利 7,328,064（涉及注射 DNA 的方法）中所提供的主题内容的专利也涵盖在本文中。以上专利都通过引用整体并入本文中。

[0269] c. 制备 DNA 质粒的方法

[0270] 本文提供了用于制备 DNA 质粒的方法，这些 DNA 质粒包含本文所论述的 DNA 疫苗。DNA 质粒在最后一个步骤亚克隆到哺乳动物表达质粒中之后，可以用于在大规模发酵罐中，使用本领域中已知的方法接种细胞培养基。

[0271] 用于本发明的 EP 装置的 DNA 质粒可以使用已知装置和技术的组合进行配制或制造，但优选使用优化的质粒制造技术进行制造，该技术描述于 2007 年 5 月 23 日提交的得到许可的同在申请的美国临时申请美国序列号 60/939,792 中。在一些实施例中，用于这些研究中的 DNA 质粒可以被配制成浓度大于或等于 10mg/mL。除美国序列号 60/939792 中所描述的那些，包括得到许可的专利美国专利号 7,238,522(2007 年 7 月 3 日颁布）中所描述的那些外，这些制造技术还包括或并入了本领域普通技术人员通常已知的各种装置和方案。以上参考的申请和专利（分别为美国序列号 60/939,792 和美国专利号 7,238,522) 都整体并入本文中。

[0272] 实施例

[0273] 以下实施例将进一步说明本发明。应了解，这些实施例尽管指示了本发明的优选实施方案，但仅出于说明的目的提供。从以上论述和这些实施例，本领域技术人员可以确定本发明的基本特征，并且在不偏离本发明的精神和范围的情况下，可以对本发明进行各种改变和修改以使其适于各种用法和条件。因此，本领域技术人员从前述说明将对除本文所显示并描述的那些外的本发明的各种修改显而易见。这些修改也意图在所附权利要求书的范围内。

[0274] 实施例 1

[0275] 预期在未来数年里，具有感染 RSV 的高风险的个体的数量将持续增加（例如年幼的婴儿和老年人），因此，迫切需要开发出针对 RSV 感染的有效并且在财政上易管理的治疗方法。DNA 疫苗，被基因工程改造成在免疫之后表达相关编码蛋白质的小细菌质粒，提供了若干重要益处，这些益处可以有助于解决在设计有效 RSV 疫苗中所涉及的许多障碍。举例来说，为了防止临床上显著疾病和再感染，该疫苗赋予的免疫性须比由自然 RSV 感染所引起的免疫性更强并且更持久。DNA 疫苗不仅引起体液（例如中和抗体）和细胞（例如细胞毒性 T 淋巴细胞）免疫应答，而且还可以引起针对许多病毒的终生免疫。此外，RSV 疫苗接种的主要目标是新生儿和年幼的婴儿。新生儿免疫系统在功能上是不成熟的，并因此对常规疫苗（例如减毒活病毒或失活病毒）仅产生较弱的免疫应答。另一方面，经显示，DNA 疫苗在新生动物模型中是安全并且具有免疫原性的。此外，已知在进行免疫时母体抗体的存在可压制或抑制针对多种常规疫苗的免疫应答。然而，利用 DNA 疫苗，免疫性蛋白质不存在于疫苗制剂中并因此，不应当受到母体抗体引导的失活影响。

[0276] 当前，有三种主要 RSV 疫苗进入或即将进入临床试验早期：1) 减毒活 RSV(MedImmune, Philadelphia, PA)；2) 表达 RSV F 和 / 或 G 蛋白的仙台病毒 (Sendai virus)(AmVac, Switzerland)；及 3) 含 RSV G 蛋白的病毒样颗粒 (Novavax, Rockville, MD)(http://www.clinicaltrials.gov/ct2/results？ term ＝ RSV&pg ＝ 1)。尽管这些方法在防止 RSV 感染方面已显示某种程度的功效，但这些疫苗设计仍存在其它固有的缺点。举

例来说，利用减毒活病毒的最大问题是减毒不良或突变，导致毒力返强，并因此不应当施用给免疫力低下的个体。所有病毒载体的一个共同的缺点是由于识别病毒蛋白引起的宿主免疫应答导致产生中和抗体，这些中和抗体可能显著地降低在后续免疫时疫苗的功效。最后，RSV G 蛋白在 RSV 两种亚型之间具有高度可变性，并因此，靶向该 G 蛋白的任何疫苗都不可能提供交叉防护。

[0277] 相比之下，经显示，DNA 疫苗具有高度免疫原性并且没有与以上描述的疫苗方法相关的许多缺点。当经由电穿孔法传递时，DNA 疫苗的免疫原性进一步提高。DNA 疫苗没有含有一整套病毒组分并且类蛋白质亚单位疫苗没有宿主毒力返强或感染的风险，使其可安全地用于婴儿和免疫力低下的个体。

[0278] 使用 RSV 的 A 和 B 亚型的共有序列，为获得针对该病毒的两个病毒株的更广泛防护作用提供了基础。这些益处使共有 RSV DNA 疫苗比过去曾提出的任何其它方法更有益并且可能更有效。

[0279] 选出大多数 RSV 蛋白作为新 RSV 疫苗的靶：融合蛋白 (F)、糖蛋白 (Ga) 和糖蛋白 (Ga)。另选出第二基质蛋白 (M2) 用于评价。如以上所提到的，F 蛋白和 G 蛋白在表面上表达并且是中和抗体的理想靶。M2 蛋白含有主要免疫优势 CD8 T 表位。对于这些 RSV 蛋白中的每一种，基于由 PUBMED 提供的有关 RSV 的 A 和 B 亚型的氨基酸序列，产生单一共有序列（图 1 示出了 RSV-F 蛋白的系统发生树，共有 RSV F 蛋白质氨基酸序列以 SEQ ID NO:2 陈述；图 2 示出了 RSV M2-1 蛋白的系统发生树，共有 RSV M2-1 蛋白质氨基酸序列以 SEQ ID NO:4 陈述）。在一个优选实施方案中，产生了编码这些共有蛋白质序列的密码子和 RNA 优化的 DNA 序列并将其用于可以使用电穿孔法传递的 DNA 疫苗中。

[0280] 实施例 2

[0281] 通过免疫荧光显微镜检术和 / 或蛋白质印迹分析 (western blot analysis) 确定 RSV 共有 F 蛋白的成功表达。构建体被用于免疫研究中。

[0282] 用不同剂量的不同 RSV DNA 疫苗构建体对小鼠进行免疫。一组接受 DNA 疫苗主链质粒 pVAX 作为对照。一组接受 5 μ g 剂量的质粒 RSV-F，一组接受 15 μ g 剂量的质粒 RSV-F；一组接受 30 μ g 剂量的质粒 RSV-F；并且一组接受 60 μ g 剂量的质粒 RSV-F。

[0283] 利用电穿孔法经肌肉内对小鼠进行免疫，总计免疫 3 次（第 0、2 和 4 周），每次间隔 2 周。在每次免疫之后第 2、4、6 和 12 周对小鼠抽血，并且测量血清中总 RSV F 蛋白特异性 IgG 和 IgA 抗体。图 3 示出了在第 1 次免疫（即，第 0 周）、第 2 次免疫（即，第 2 周）及第 3 次免疫（即，第 4 周）之后的抗 RSV F 蛋白 IgG。图 4 示出了在第 1 次免疫（即，第 0 周）、第 2 次免疫（即，第 2 周）及第 3 次免疫（即，第 4 周）之后的抗 RSV F 蛋白 IgA。在第 12 周处死小鼠。除了比较血清中的总 IgG 和血清中的总 IgA 之外，还对在最后一次免疫之后约 8 周在不同剂量 RSV-F 疫苗下的 IFN-γ 产量进行比较。

[0284] RSV-F DNA 疫苗在血清中引起高水平 IgG 和 IgA。对于所研究的所有剂量，早在第 2 次免疫之后 2 周即可检测到反应。所诱导的抗体反应看来具有较强的 Th1 偏性，由此避免了 Th2 预先对 RSV 产生免疫应答的副作用的问题。甚至在最后一次免疫之后 8 周后，仍观察到 RSV-F 特异性 CD8 T 细胞。图 5 示出了在最后一次免疫之后约 8 周，通过比较在不同 RSV-F 疫苗剂量下的 IFN-γ 产量得到的结果。

[0285] 实施例 3

[0286] 趋化因子是由细胞分泌的小蛋白质家族，能够在邻近应答细胞中诱导朝向较高浓度梯度的定向趋化作用。多个团体已对使用趋化因子作为免疫佐剂的可行性进行了研究。

[0287] CCL28/MEC（粘膜相关性上皮趋化因子）结合 CCR10，在肠、肺、乳房及唾液腺中的上皮细胞中表达。在先前的研究中使用粘膜趋化因子显示了其作为免疫佐剂的效用，在全身免疫之后在黏膜组织中更好地诱导免疫应答。

[0288] 已进行了实验来测试 CCL28 基因构建体作为佐剂用于共有 RSV F 蛋白 DNA 疫苗构建体的用途。还对包含了编码 CCL20 的核苷酸序列，又称为肝活化调控性趋化因子 (liver activation regulated chemokine，LARC) 或巨噬细胞炎性蛋白 -3(Macrophage Inflammatory Protein-3，MIP3A) 的基因构建体作为共有 RSV F 蛋白 DNA 疫苗构建体的可能佐剂进行了测试。

[0289] RSV-F 加趋化因子实验的免疫时程安排利用了三组小鼠：一种仅接受 RSV-F 构建体；一组接受 RSV-F 构建体加 CCL20 构建体；并且一组接受 RSV-F 构建体加 CCL28 构建体。对小鼠免疫总计三次：第 0 周、第 2 周和第 4 周。在第 2 周、第 4 周和第 5 周对小鼠抽血。在第 5 周，小鼠经历阴道清洗以收集阴道样品并将其处死，之后收集肺和脾。

[0290] 对于在 RSV-F+/- 趋化因子免疫之后脾中（图 6）和在 RSV-F+/- 趋化因子免疫之后肺中（图 7）的 IFN-γ+CD8 和 CD4 T 细胞进行比较。

[0291] 测量血清中 IgG 终点滴度并比较血清中的 IgG 亚型 (IgG1 对比 IgG2a)（图 8）。还对 IgG2a/IgG1 比率进行比较（图 9）。

[0292] CCL20 和 CCL28 趋化因子看来在脾或肺中进行 RSV-F DNA 疫苗接种之后不会增强 CD8 和 CD4 T 细胞免疫应答（基于 IFN-γ ICS)，不过趋化因子构建体的剂量是否不足尚不清楚。

[0293] 接受 CCL20 免疫佐剂的动物在 RSV-F DNA 疫苗接种之后展现出强烈的 Th1 免疫应答偏性（主要是 IgG2a)。驱使免疫应答远离 Th2 并且朝向 Th1 是有用的，由此避免了在 RSV 感染之后发生疫苗相关性疾病增强。

[0294] 实施例 4

[0295] 如以上所述，施用编码 CCL20 的编码序列优先引起 Th1 免疫应答并且远离 Th2 免疫应答。施用 CCL20 的这一特性在免疫治疗以及作为需要转换增加 Th1 免疫应答并且减少 Th2 免疫应答的其它靶的疫苗佐剂的情形中可为有用的。

[0296] 举例来说，CCL20 或编码 CCL20 的核酸分子的施用可以用于治疗患有以 Th2 免疫应答为特征的自体免疫疾病或炎性病症的个体。如本文所显示的减少 Th2 免疫应答的能力可以用于减少与包括例如某些自体免疫疾病或炎性病症相关的 Th2 免疫应答。

[0297] 此外，CCL20 可以用作疫苗佐剂（如本文所描述），其中疫苗靶向其它感染疾病以及癌症。与使用 CCL20 作为疫苗佐剂相关的 Th1 增加 /Th2 减少特性可以用于疫苗中。SEQ ID NO:18 和 SEQ ID NO:20 含有 CCL20 的高表达量编码序列，并且含有和不含编码 IgE 信号肽的编码序列。在一些实施方案中，SEQ ID NO:18 另外包含起始密码子。SEQ ID NO:19 和 SEQ ID NO:21 含有 CCL20 的共有序列，并且含有和不含编码 IgE 信号肽的编码序列。在一些实施方案中，SEQ ID NO:19 另外包含 N 末端甲硫氨酸。

[0298] 实施例 5

[0299] 在 TZM-bl 细胞中进行的荧光素酶报告基因检验是基于用分子克隆的包膜假型

病毒进行的单轮感染 (Single-Round Infection)。TZM-bl(JC53-bl) 是一种基因工程改造的 HeLa 细胞系，表达 CD4、CXCR4 和 CCR5 并且含有 Tat 诱导型 Luc 和 β-Gal 报告基因。该检验在单轮感染方面提供了高成功率，具有增加的检验能力 (2 天检验)、增加的精确度 (准确地测量 50％中和作用)、提高的标准化水平 (稳定细胞系) 以及经过优化并且验证的检验平台。Montefiori, D.C. (2004)Current Protocols in Immunology, John Wiley&Sons, 12.11.1-12.11.15(通过引用并入本文中) 描述了该检验。

[0300] 图 10 描绘了 TZM-BL 细胞 / “打开”中的包膜假型病毒。

[0301] 图 11 描绘了 TZM-BL 细胞 / “关闭”中的包膜假型病毒。

[0302] 图 12 描绘了 293T 细胞中 RSV- 融合假病毒的产生。

[0303] 图 13 示出了用于一项使用该检验的 RSV-F 中和研究中的免疫方案。

[0304] 图 14 示出了通过对不同靶细胞中 RSV-F 介导的感染进行定量得到的数据。带有 RSV-F 蛋白的假型病毒的感染性。使假型病毒聚结成粒，并且通过定量 HIV p24 抗原来估计颗粒量。用带有假型病毒或无包膜的一组含有 10ng 颗粒状 HIV p24 抗原的各式细胞系感染细胞。感染后 72 小时，细胞上清液并针对 p24 抗原进行检验。值为一式三份孔的平均值加标准偏差。

[0305] 图 15 示出了通过对 TZM-bl HeLa 细胞中的 RSV-F 中和活性进行定量得到的数据。数据显示了使用来自 DNA 疫苗接种的动物的 RSV-F 免疫的血清样品进行的荧光素酶检验得到的针对 RSV-F 的中和能力的比较结果，其可以阻止 HIV-Luc/FMDV 假病毒进入并感染靶细胞。滴度定义为产生 50％的病毒抑制作用的血浆的稀释度的倒数。来自接种疫苗的小鼠的血清样品中和并阻止 HIV-1Luc/RSV-F 的感染性的能力，在正常小鼠血清 (normal mouse serum, NMS) 中不存在此类阻止活性。

[0306] 图 16 示出了通过对 HeLa 细胞中由 RSV-F 假病毒引起的细胞死亡活性进行定量得到的数据。结果显示，在利用电穿孔法进行 3 次疫苗接种之后，共有 RSV-F DNA 质粒在小鼠中引起有效的体液和细胞免疫应答。仅仅利用 5ug DNA，早在第 2 次免疫后 1 周就观察到血清中的高 RSV-F 特异性 IgG 水平。在第 3 次免疫后 8 周才可检测到细胞介导的免疫应答，表明在 RSV-F DNA 疫苗接种之后免疫应答的长期持续性。增加 RSV-F DNA 浓度不会引起免疫应答增加。如通过假病毒中和检验所测量，在用 RSV-F DNA 进行 3 次疫苗接种加电穿孔之后产生的抗体能够中和感染。结果还显示 RSV-F 介导的靶细胞凋亡减少。

[0307] 实施例 6

[0308] 图 17 示出了所采取的 RSV-M2 剂量研究的免疫时程安排。从该研究收集的数据包括了在最后一次免疫之后 1 周，不同剂量 RSV-M2 疫苗的 IFN-g 产量的比较 (图 18)。结果显示，在利用电穿孔法进行 3 次疫苗接种之后，基于共有序列的 RSV-M2 DNA 质粒在小鼠中引起显著的细胞免疫应答。在脾和肺中都观察到对 RSV-M2 免疫优势或次优势表位具有特异性的高水平 IFN-g+T 细胞。利用 60ug DNA 观察到最大免疫应答 (然而，应答未远超过利用 10ug DNA 所观察到的应答)。

[0309] 实施例 7

[0310] 采取进一步研究来评价以 CCL20 作为潜在免疫佐剂来增强 DNA 免疫之后的粘膜免疫应答。CCL20(肝活化调控性趋化因子—LARC ; 巨噬细胞炎性蛋白 -3—MIP3a) 结合到 CCR6 并且在淋巴结、肝、阑尾、胎肺中表达 (在胸腺、睾丸、前列腺和肠中较低水平表达)。

[0311] 图 19 描绘了用于评价 RSV-F 疫苗与 CCL20 的组合的免疫时程安排。

[0312] 图 20 示出了从该研究得到的数据，包括通过比较在最后一次免疫之后 1 周，利用不同剂量 CCL20 的脾、肝和肺中的每一个的 IFN-g 产量得到的数据。

[0313] 图 21 示出了血清中 IgG 亚型 (IgG1 对比 IgG2a) 的比较数据。

[0314] 结果显示，用 CCL20 进行共免疫在 RSV-F DNA 免疫之后不会增强肺和肝中细胞介导的免疫应答。CCL20 看来使在 DNA 免疫之后产生的 RSV-F 特异性 IgG 抗体偏向 Th1 同型。与仅用 RSV-F 免疫相比较，在 CCL20+RSV-F 共免疫之后，在血清中观察到较少 IgG1 和较多 IgG2a。

[0315] 因此，提供了改良的疫苗、用于诱导所述免疫应答（包括粘膜免疫应答）的改良的方法及针对免疫原，特别是与病原体相关的免疫原和经由粘膜组织进入宿主的其它试剂相关的免疫原对个体进行预防性和 / 或治疗性免疫的方法。举例来说，感染原通常跨粘膜组织进入宿主中，所述粘膜组织如口腔粘膜以及消化管粘膜、呼吸道粘膜（包括嗅粘膜和结膜粘膜）、乳腺粘膜和泌尿生殖道粘膜。

[0316] 提供了包含编码免疫原的核酸序列以及编码 CCL20 和其功能片段的核酸序列的组合物。该组合物可以包含一种分离的核酸分子，该核酸分子编码免疫原以及 CCL20 和其功能片段，和 / 或组合物包含一种编码免疫原的分离的核酸分子与一种编码 CCL20 和其功能片段的分离的核酸分子的组合。提供的这些组合物可以呈可注射药物的形式。

[0317] 这些组合物可以用于诱导个体中针对免疫原的免疫应答的方法中，所述方法包括向所述个体施用此类组合物。

[0318] 提供了包含可操作地连接到调控元件的编码免疫原的核苷酸序列和编码 CCL20 或其功能片段的核苷酸序列的重组疫苗，以及诱导个体中针对免疫原的免疫应答的方法，包括诱导粘膜免疫应答的方法，所述方法包括向个体施用此类重组疫苗。

[0319] 还提供了包含编码 CCL20 或其功能片段的核苷酸序列的减毒活病原体，以及诱导个体中针对病原体的免疫应答的方法，包括诱导粘膜免疫应答的方法，所述方法包括向个体施用该减毒活病原体。

[0320] 还提供了诱导个体中针对免疫原的免疫应答的方法，所述方法包括向所述个体施用 CCL20 蛋白或其功能片段与编码免疫原的分离的核酸分子的组合，和 / 或编码免疫原的重组疫苗和 / 或包含免疫原的亚单位疫苗和 / 或减毒活疫苗和 / 或灭活疫苗。提供了包含 CCL20 蛋白或其功能片段与编码免疫原的一个或多个分离的核酸分子的组合；包含了可操作地连接到调控元件的编码免疫原的核苷酸序列的重组疫苗；减毒活病原体；及灭活病原体。如本文所使用，“CCL20 的功能片段”意图指相较于当在无该片段情况下传递免疫原时所诱导的免疫应答，当与免疫原一起传递时提供改进的免疫应答的 iCC120 蛋白的片段。片段一般为 10 个或更多氨基酸长度。在一些实施方案中，CCL20 的功能片段可以占特定全长 CCL20 蛋白的长度的 20％或更高，25％或更高，30％或更高，35％或更高，40％或更高，45％或更高，50％或更高，55％或更高，60％或更高，65％或更高，70％或更高，75％或更高，80％或更高，85％或更高，90％或更高，91％或更高，92％或更高，93％或更高，94％或更高，95％或更高，96％或更高，97％或更高，98％或更高，99％或更高百分比。

[0321] 当与免疫原一起传递时，CCL20 和其功能片段及其组合调节免疫应答。因此，这些蛋白质的组合可以作为基于 DNA 或蛋白质的疫苗的组分，以便诱导治疗性或预防性免疫应

答或以组合物形式用于诱导免疫应答。在一些实施方案中，传递免疫原的手段是DNA疫苗、重组疫苗、蛋白质亚单位疫苗、减毒疫苗或灭活疫苗。在一些实施方案中，传递CCL20和其功能片段中的一种或多种的手段是借助于在DNA疫苗、重组疫苗或减毒疫苗中包括编码序列的表达。

[0322] 免疫应答引起抗原特异性抗体和/或抗原特异性T细胞和B细胞的产生。抗原特异性抗体和/或细胞提供了防止感染、减轻或清除现有感染的手段。其还可以从个体分离并且被用于其它应用中，如被动免疫方案、免疫柱或作为试剂。

[0323] 在一些实施方案中，CCL20可用于诱导粘膜免疫应答，特别是使以IgG2a应答增加和IgG1应答减少为特征的Th1应答增加，甚至是在全身传递该组合物的方案中。与免疫原一起共免疫，如利用DNA疫苗或其它手段质粒加这些趋化因子中的一种或多种（如在DNA疫苗中或作为另一类型疫苗的编码序列的一部分），将提供独特的辅助特性。

[0324] CCL20蛋白和其编码核苷酸可以从许多来源（天然和合成来源）获得。在使用蛋白质的一些实施方案中，例如，本领域技术人员可以使用众所周知的技术，使用例如含有特异性结合到该蛋白质的抗体的免疫柱，从天然来源分离CCL20蛋白。作为替代方案，可以使用电泳法，从电泳基质分离出该蛋白质，并通过例如透析进行纯化，以得到基本上纯的蛋白质。可以采用其它众所周知的蛋白质纯化技术得到分离的基本上纯的蛋白质。在使用蛋白质的一些实施方案中，本领域技术人员可以使用众所周知的技术，将编码CCL20和其功能片段中任一种的DNA分子插入适用于众所周知的表达系统中的可商购的表达载体中。除从天然来源分离蛋白质或通过重组技术制造蛋白质外，还可以采用自动肽合成仪制造分离的基本上纯的蛋白质。

[0325] 根据本发明的一些方面，提供的组合物和方法针对如过敏原、病原体或异常的疾病相关细胞等免疫原对个体进行预防性和/或治疗性免疫。该疫苗可以是任何类型的疫苗，如减毒活疫苗、细胞疫苗、重组疫苗或核酸或DNA疫苗。通过传递编码免疫原以及CL20和其功能片段中一种或多种的核酸分子，可以调节由疫苗诱导的免疫应答。

[0326] 提供了用于传递免疫原和CCL20的组合物，特别是包含一个或多个包含可操作地连接到调控元件的编码CCL20和其功能片段中的一种或多种的核苷酸序列与可操作地连接到调控元件的编码免疫原的核苷酸序列的组合的核酸分子的那些。编码CCL20和免疫原的核酸序列可以在同一分子上或在单独分子上。

[0327] 这些核酸分子可以使用若干众所周知的技术中的任一种传递，包括DNA注射（又称为DNA疫苗接种）、重组载体（如重组腺病毒）、重组腺病毒相关性病毒和重组牛痘。在一些实施方案中，如本文所描述来构建DNA疫苗并使用如本文所描述的电穿孔法进行传递。有关本文所陈述的RSV疫苗的说明例如可以适用于CCL20与其它免疫原的使用。DNA疫苗描述于美国专利号5,593,972、5,739,118、5,817,637、5,830,876、5,962,428、5,981,505、5,580,859、5,703,055、5,676,594，以及其中所引用的优先权申请，各自通过引用并入本文中。除那些申请中描述的传递方案外，传递DNA的替代性方法描述于美国专利号4,945,050和5,036,006中，都通过引用并入本文中。施用途径包括但不限于，肌肉内、鼻内、腹膜内、真皮内、皮下、静脉内、动脉内、眼内及口服，以及局部、透皮、通过吸入或栓剂，或如通过阴道、直肠、尿道、颊和舌下组织灌洗施用到粘膜组织。优选的施用途径包括肌肉内、腹膜内、真皮内和皮下注射。在一些实施方案中，编码CCL20或其功能片段的

核苷酸序列可以连接到 IgE 信号肽。在一些实施方案中，免疫原是一种病原体抗原、癌症相关抗原或连接到与自体免疫疾病相关的细胞的抗原。本发明可以用于针对如病毒、原核生物和致病性真核生物体（如单细胞致病性生物体和多细胞寄生虫）等所有抗原对个体进行免疫。本发明特别适用于针对感染细胞并且未包覆壳体的那些病原体（如病毒，及原核生物，如淋球菌、李斯特氏菌属 (listeria) 和志贺氏菌属 (shigella)）对个体进行免疫。此外，本发明还可用于针对原生动物病原体对个体进行免疫，这些原生动物病原体包括在生命周期中的某一阶段，其中其为细胞内病原体。病原体的实例包括下述。小核糖核酸病毒科 (Picornavirus Family) 病毒属，如鼻病毒 (rhinovirus)（约 50％普通感冒病例由此引起）、肠道肠道病毒属 (Etherovirus)（包括脊髓灰质炎病毒 (poliovirus)、柯萨奇病毒 (coxsackievirus)、艾柯病毒 (echovirus) 及人肠道病毒，如甲型肝炎病毒 (hepatitis A virus)）及口蹄疫病毒属 (Apthovirus)（兽医学；靶抗原包括：VP1、VP2、VP3、VP4、VPG）。杯状病毒科 (Calcivirus Family) 病毒属包括诺瓦克病毒群 (Norwalk Group of Virus)（流行性胃肠炎的病原体）。披膜病毒科 (Togavirus Family) 病毒属包括 α 病毒属 (Alphavirus)：（实例包括 Senilis 病毒、罗斯河病毒 (RossRiver virus) 以及东方＆西方马脑炎病毒 (Eastern&Western,Equine encephalitis)）。呼肠孤病毒属 (Reovirus) 包括风疹病毒 (Rubella virus)。黄病毒科 (Flariviridue Family)，实例包括登革热、黄热病、日本脑炎、圣路易型脑炎 (St.Louis encephalitis) 和蜱媒脑炎病毒 (tick borne encephalitis virus)。西尼罗病毒 (West Nile virus)(Genbank NC001563、AF533540、AF404757、AF404756、AF404755、AF404754、AF404753、AF481864、M12294、AF317203、AF196835、AF260969、AF260968、AF260967、AF206518 及 AF202541)，代表性靶抗原：E NS5 C. 丙型肝炎病毒 (Hepatitis C Virus)。冠状病毒科 (Coronavirus Family) 包括感染性支气管炎病毒、猪传播性胃肠炎病毒、猪血细胞凝集性脑脊髓炎病毒、猫感染性腹膜炎病毒、猫肠冠状病毒、犬冠状病毒、SARS 相关性冠状病毒，人呼吸道冠状病毒（引起普通感冒中约 40％的病例）。EX.224E,OC43。靶抗原：E1—又称 M 或基质蛋白；E2—又称 S 或刺突蛋白；E3—又称 BE 或血细胞凝集素 -elterose 糖蛋白；N—核壳。棒状病毒科 (Rhabdovirus Family) 病毒属包括水泡性病毒属 (Vesiliovirus)、狂犬病病毒属 (Lyssavirus) 如狂犬病。靶抗原：G 蛋白、N 蛋白。丝状病毒科 (Filoviridue Family) 包括出血热病毒 (Hemorrhagic fever virus)，如（医学）马堡氏病毒 (Marburg) 和埃博拉病毒 (Ebola virus)。副粘病毒科 (Paramyxovirus) 包括副粘病毒属：如腮腺炎病毒、新城疫病毒；麻疹病毒属，如麻疹、犬瘟热；肺病毒属，如呼吸道合胞病毒；正粘病毒属。流感病毒科包括布尼亚病毒科 (Bungavirus Family) 属，如布尼亚病毒属、加利福尼亚脑炎、拉克罗斯脑炎 (LA Crosse)；白岭病毒属，如立夫特山谷热病毒 (Rift Valley Fever)；汉坦病毒 (Hantavirus) 属，如普马拉 (puremala；一种出血热病毒 (hemahagin fever virus))；纳伊罗病毒属 (Nairvirus)；未指定的布尼亚病毒；沙拉病毒科 (Arenavirus Family)LCM 及拉沙热病毒 (Lassi fever virus)。呼肠孤病毒科包括呼肠孤病毒；轮状病毒；环状病毒，如科罗拉多蜱传热、Lebombo、马器质性脑病、蓝舌病。逆转录病毒科亚科包括肿瘤病毒 (oncorivirinal) 亚科，如白血病病毒、HTLVI 和 HTLVII；慢病毒亚科，如 HIV、猫免疫缺陷性病毒、马感染及贫血症病毒。泡沫病毒亚科乳多空病毒科亚科包括多瘤病毒，如 BKU 和 JCU 病毒；亚科乳头状瘤病毒。腺病毒，如 EX AD7、ARD.、O.B.、275。细小病毒科包括猫细小病

毒、猫粒细胞缺乏症病毒、犬细小病毒、猪细小病毒。疱疹病毒科亚科包括 α 单纯疱疹病毒属 HSVI(Genbank X14112,NC001806)、HSVII(NC001798);水痘病毒属,假性狂犬病、水痘带状疱疹。亚科 -β- 疱疹病毒属包括巨细胞病毒属,如疱疹病毒科 HCMV。Muromegalovirus 亚科包括以下属:淋巴潜隐病毒属;γ-EBV-(伯基特氏淋巴瘤 (Burkitts lympho))、疱疹病毒科。猴病毒属痘病毒科亚科天花属包括(天花)、牛痘 (Chordopoxviridue Vaccinia/Cowpox)、(副痘病毒属)、禽痘病毒属、山羊痘病毒属、兔痘病毒属。猪痘病毒属亚科包括昆虫痘病毒 (Entemopoxviridue) 嗜肝 DNA 病毒。乙型肝炎病毒科;未分类的丁型肝炎病毒。细菌病原体包括病原性革兰氏阳性球菌,如肺炎球菌、葡萄球菌及链球菌。病原性革兰氏阴性球菌包括:脑膜炎球菌和淋球菌。病原性革兰氏阴性肠杆菌包括:肠杆菌科:假单胞菌、不动杆菌和艾肯氏菌 (eikenella)、类鼻疽;沙门氏菌;志贺氏菌病;嗜血菌属;软下疳;布鲁氏菌病;土拉菌病;耶尔森氏菌属 (yersinia)(巴斯德氏菌属);念珠状链球菌和螺菌属;;单核细胞增生李斯特氏菌;猪红斑丹毒丝菌;白喉;霍乱;炭疽;杜诺凡病 (donovanosis)(腹股沟肉芽肿);及巴尔通氏体病。病原性厌氧菌包括:破伤风;肉毒中毒;其它梭状芽孢杆菌;结核病;麻风病;和其它分枝杆菌。病原性螺旋体病包括:梅毒;密螺旋体病:雅氏病 (yaws)、品他病 (pinta) 和地方性梅毒;及钩端螺旋体病。由高级病原体细菌和致病真菌引起的其它感染包括:放线菌病;诺卡放线菌病;隐球菌病;芽生菌病;组织胞浆菌病和球孢子菌病;白假丝酵母病、曲霉病和毛霉病孢子丝菌病;副球孢子菌病、petriellidiosis、球拟酵母病、足分支菌病和着色真菌病;及皮肤真菌病。立克次氏体感染包括立克次氏体和立克次氏体病。支原体和衣原体感染的实例包括:支原体肺炎;性病淋巴颗粒病;鹦鹉热;和围产期衣原体感染。致病真核生物致病原生生物和蠕虫及其感染包括:阿米巴病;疟疾;利什曼病;锥虫病;弓形体病;卡氏肺囊虫;巴倍虫病;贾第虫病;旋毛虫病;丝虫病;血吸虫病;线虫;吸虫 (trematode/fluke);及绦虫 (cestode/tapeworm) 感染。制备的疫苗可以如包含了编码肽的 DNA 序列的 DNA 构建体,所述肽包含至少一个与病原体抗原上所展示的表位相同或基本上类似的表位。在一些实施方案中,针对病原体对个体进行免疫的方法是针对 RSV、HIV、HSV、HCV、WNV 或 HBV。

[0328] 赋予针对正在增殖的细胞的保护性免疫应答的方法利用了增生性疾病特有的免疫原蛋白。这些可用于疫苗和治疗罹患增生性疾病的个体的方法中。增生性疾病的实例包括所有形式的癌症和牛皮癣。针对增生性疾病的靶蛋白的实例包括由如 myb、myc、fyn 等肿瘤基因以及转位基因 bcr/abl、ras、src、P53、neu、trk 及 EGRF 所编码的那些蛋白质。除了以肿瘤基因产物作为靶抗原外,抗癌治疗和保护方案的靶蛋白包括由 B 细胞淋巴瘤产生的抗体的可变区和 T 细胞淋巴瘤的 T 细胞受体的可变区,在一些实施方案中,这些可变区也被用作自体免疫疾病的靶抗原。其它肿瘤相关蛋白可以用作靶蛋白,如在肿瘤细胞中所发现的较高水平的蛋白质,包括被单克隆抗体 17-IA 识别的蛋白质和叶酸结合蛋白或 PSA。还提供了治疗罹患自体免疫疾病和病症的个体的方法,所述方法是通过赋予针对于自体免疫性相关的靶(包括细胞受体和产生“自”导向抗体的细胞)广泛保护性免疫应答来实现。T 细胞介导的自体免疫疾病包括类风湿性关节炎 (RA)、多发性硬化 (MS)、修格连氏综合症 (Sjogren's syndrome)、肉状瘤病、胰岛素依赖性糖尿病 (1DDM)、自体免疫性甲状腺炎、反应性关节炎、强直性脊椎炎、硬皮病、多肌炎、皮肌炎、牛皮癣、血管炎、韦格纳氏肉芽肿 (Wegener's granulomatosis)、克罗恩氏病 (Crohn's disease) 和溃疡性结肠炎。这些

疾病各自以结合到内源抗原并且起始于自体免疫疾病相关的炎性级联的T细胞受体为特征。针对T细胞可变区进行的疫苗接种将引起包括CTL在内的免疫应答，从而消除这些T细胞。在RA中，已经对该疾病所涉及的T细胞受体(T cell receptor，TCR)的若干特异性可变区进行了表征。这些TCR包括Vβ-3、Vβ-14、20Vβ-17及Vα-17。因此，用编码这些蛋白质中至少一种的DNA构建体进行疫苗接种将引起会靶向RA中所涉及的T细胞的免疫应答。参见：Howell，M.D.等，1991Proc.Nat.Acad.Sci.USA 88:10921-10925；Piliard，X.等，1991 Science 253:325-329；Williams，W.V.等，1992 J Clin.Invest.90:326-333；各自通过引用并入本文中。在MS中，已经对该疾病所涉及的TCR的若干特异性可变区进行了表征。这些TCR包括VfP和Vα-10。因此，用编码这些蛋白质中至少一种的DNA构建体进行疫苗接种将引起会靶向MS中所涉及的T细胞的免疫应答。参见：Wucherpfennig，K.W.等，1990 Science 248:1016-1019；Oksenberg，J.R.等，1990 Nature 345:344-346；各自通过引用并入本文中。在硬皮病中，已经对该疾病所涉及的TCR的若干特异性可变区进行了表征。这些TCR包括Vβ-6、Vβ-g、Vβ-14和Vα-16、Vα-3C、Vα-7、Vα-14、Vα-15、Vα-16、Vα-28及Vα-12。因此，用编码这些蛋白质中至少一种的DNA构建体进行疫苗接种将引起会靶向硬皮病中所涉及的T细胞的免疫应答。为了治疗罹患T细胞介导的自体免疫疾病的患者，特别是TCR的可变区仍有待表征的那些，可以进行滑液活检。可以获取存在的T细胞的样品并且使用标准技术鉴别这些TCR的可变区。可以使用这一信息制备基因疫苗。B细胞介导的自体免疫疾病包括狼疮(SLE)、葛瑞夫兹氏病(Grave's disease)、重症肌无力、自体免疫性溶血性贫血、自体免疫性血小板减少性紫癜、哮喘、冷球蛋白血症、原发性胆汁性肝硬化及恶性贫血。这些疾病各自以结合到内源抗原并且起始于自体免疫疾病相关的炎性级联的抗体为特征。针对抗体可变区进行的疫苗接种将引起包括CTL在内的免疫应答，从而消除这些B细胞。

[0329] 为了治疗罹患B细胞介导的自体免疫疾病的患者，必须鉴别出自体免疫活性中所涉及的抗体的可变区。可以进行活检并且可以在炎症部位处获取存在的抗体的样品。这些抗体的可变区可以使用标准技术进行鉴别。可以使用这一信息制备基因疫苗。在SLE的情形中，相信一种抗原为DNA。因此，在针对SLE进行免疫的患者中，可以针对抗DNA抗体对其血清进行筛选并且可以制备一种疫苗，该疫苗包括了编码该血清中所见的这些抗DNA抗体的可变区的DNA构建体。TCR和抗体的可变区间的共同结构特征是众所周知的。编码特定TCR或抗体的DNA序列一般可以遵循众所周知的方法发现，如Kabat等人，1987 Sequence of Proteins of Immunological Interest U.S.Department of Health and Human Services，Bethesda Md.中所描述的那些方法，该文献通过引用并入本文中。此外，用于由抗体克隆功能性可变区的通用方法可以见于Chaudhary，V.K.等人，1990 Proc.Natl.Acad Sci.USA 87:1066中，该文献通过引用并入本文中。

[0330] 除使用CCL20蛋白质编码序列的可表达形式来改良DNA疫苗外，还提供了包含CCL20蛋白或其功能片段和/或编码CCL20或其功能片段的编码序列的改良的减毒活疫苗、改良的灭活疫苗和使用重组载体传递编码抗原的外来基因的改良的疫苗以及亚单位和糖蛋白疫苗。减毒活疫苗、使用重组载体传递外来抗原的疫苗、亚单位疫苗和糖蛋白疫苗的实例描述于以下美国专利号中：4,510,245、4,797,368、4,722,848、4,790,987、4,920,209、5,017,487、5,077,044、5,110,587、5,112,749、5,174,993、5,223,424、5,225,336、

5,240,703、5,242,829、5,294,441、5,294,548、5,310,668、5,387,744、5,389,368、5,424,065、5,451,499、5,453,364、5,462,734、5,470,734、5,474,935、5,482,713、5,591,439、5,643,579、5,650,309、5,698,202、5,955,088、6,034,298、6,042,836、6,156,319 及 6,589,529,各案通过引用并入本文中。

[0331] 实施例 8

[0332] 使用 RSV 免疫原 F、G(A) 和 G(B) 的共有序列提供了获得中和性免疫应答的基础。这些益处使共有 RSV DNA 疫苗比过去曾提出的任何其它方法更有益并且可能更有效。

[0333] 选出大多数 RSV 蛋白作为新 RSV 疫苗的靶 :融合蛋白 (F)、糖蛋白 (Ga) 和糖蛋白 (Ga)。如以上所提到的,F 蛋白和 G 蛋白在表面上表达并且是中和抗体的理想靶。制备混合 DNA 疫苗,其包含了具有编码并表达共有 RSV-F 的序列的质粒、具有编码并表达共有 RSV-G(A) 的序列的质粒及具有编码并表达共有 RSV-G(B) 的序列的质粒。个别质粒以及混合物均用于研究中。该混合疫苗被用于以兔和非人类灵长类动物进行的研究中并且这些研究的结果显示,诱导出高水平中和性免疫应答,表明该混合疫苗可以用于预防和治疗性应用中,并在防止 RSV 感染方面具有特定价值。

[0334] 如图 22A、图 22B 和图 22C 中所示,使用 pVAX 载体分别构建 RSV-F、RSV-G(A) 和 RSV-G(B) 的单一插入质粒。将每一对应质粒的共有序列克隆到 pVAX 载体中的 BamHI 与 XhoI 位点之间。所得质粒为 :pRSV-F,其包含 1813 个碱基对的插入序列,该插入序列编码共有 RSV-F,其被克隆到 pVAX 中以产生具有 4812 个碱基对的质粒 ;pRSV-G(A),其包含 945 个碱基对的插入序列,该插入序列编码共有 RSV-G(A),其被克隆到 pVAX 中以产生具有 3944 个碱基对的质粒 ;及 pRSV-G(B),其包含 930 个碱基对的插入序列,该插入序列编码共有 RSV-G(B),其被克隆到 pVAX 中以产生具有 3929 个碱基对的质粒。

[0335] 为了确定 RSV-F、RSV-G(A) 和 RSV-G(B) 构建体的表达,分别用 pRSV-F、pRSV-G(A) 和 pRSV-G(B) 转染 RD 细胞。使用经过免疫的汇集的兔血清进行免疫荧光评价。通过免疫荧光法,通过结合以化学方式与荧光素异硫氰酸酯 (fluorescein isothiocyanate, FITC) 偶联的抗 RSV 免疫原抗体来观察 RSV 抗原。使用 DAPI 作为核复染剂。图 23 中的结果显示,用 pVAX 转染的 RD 细胞显示未被 FITC 标记的抗 RSV 免疫原抗体免疫染色。

[0336] 使用小鼠进行 RSV-F 剂量研究。免疫时程安排示于图 24 中。使用了五组小鼠。第 1 组是注射 pVAX 的对照组。第 2 组注射 5μg pRSV-F 构建体。第 3 组注射 15μg pRSV-F 构建体。第 4 组注射 30μg pRSV-F 构建体。第 5 组注射 60μg pRSV-F 构建体。使用电穿孔法对动物免疫总计三次 :在第 0 周进行第一次免疫、在第 2 周进行第二次免疫并且在第 4 周进行第三次免疫。收集血清并测量 IgG 总计四次 :在第 2 周进行第一次收集,在第 4 周进行第二次收集,在第 6 周进行第三次收集,并且在第 12 周进行第四次收集。在第 12 周处死动物,采集脾并经由 ELISPOT 测量 IFN-γ。

[0337] 图 25 示出了通过比较血清中的总 RSV-F IgG 的 RSV-F 剂量研究得到的数据。所示数据是来自在第 1 次免疫之后、第 2 次免疫之后及第 3 次免疫之后收集的血清。在 10、100、1,000 和 10,000 的血清稀释度倒数下测量抗 RSV-F IgG。数据显示 pVAX 在任何免疫之后都不诱导抗体。数据显示 pRSV-F 构建体在第一次免疫之后不诱导抗体。数据显示 pRSV-F 构建体在第二次和第三次免疫之后诱导抗体。

[0338] 图 26 示出了通过比较在最后一次免疫之后约八周由 RSV-F 疫苗接种所诱导的

IFN-γ 产量的 RSV-F 剂量研究得到的数据。所示数据比较了来自注射 pVAX 与注射 15μg 平均剂量的 pRSV-F 构建体的动物的 IFN-γ 产量 (SFU/106 个脾细胞)。

[0339] 使用小鼠进行 RSV-F PSEUDO 中和研究。免疫时程安排示于图 27 中。用 15μg pRSV-F 构建体对小鼠进行免疫。使用电穿孔法对动物免疫总计三次:在第 0 周进行第一次免疫、在第 2 周进行第二次免疫并且在第 4 周进行第三次免疫。收集血清并且在第 5 周进行中和检验。

[0340] 由 RSV-F PSEUDO 中和研究得到的数据示于图 28 中。对 TZM-bl HeLa 细胞中 RSV-F 假型中和活性进行定量。数据显示了使用来自 DNA 疫苗接种的动物的 RSV-F 免疫的血清样品进行的荧光素酶检验得到的中和 RSV-F 的能力的比较结果,其可以阻止 HIV-Luc/RSV-F 假病毒进入并感染靶细胞。滴度定义为产生 50%的病毒抑制作用的血浆的稀释度的倒数。注意到来自接种疫苗的小鼠的血清样品中和并阻止 HIV-Luc/RSV-F 的感染性的能力,在正常小鼠血清 (NMS) 中不存在此类阻止活性。

[0341] 如图 29 中所示,使用来自用 pRSV-F(RSV-F2 和 RSV-F5) 疫苗接种的两只动物的血清与来自 pVAX 疫苗接种的动物的血清进行两项比较以确定对 HeLa 细胞中由 RSV-F 假病毒引起的凋亡细胞死亡活性的抑制。对于每次比较,将血清以 1:1、1:10、1:100 和 1:1000 稀释。测定以百分比表示的细胞死亡水平,显示了来自用 pRSV-F 疫苗接种的动物的血清在血清存在下抑制暴露于 RSV-F 假病毒的 HeLa 细胞的凋亡细胞死亡。

[0342] 使用小鼠进行 RSV-G(A) 和 RSV-(B) 剂量研究。免疫时程安排示于图 30 中。使用了六组小鼠。第 1 组是注射 pVAX 的对照组。第 2 组注射 1μg pRSV-G(A) 或 pRSV-G(B) 构建体。第 3 组注射 5μg pRSV-G(A) 或 pRSV-G(B) 构建体。第 4 组注射 10μg pRSV-G(A) 或 pRSV-G(B) 构建体。第 5 组注射 20μg pRSV-G(A) 或 pRSV-G(B) 构建体。第 6 组注射 40μg pRSV-G(A) 或 pRSV-G(B) 构建体。使用电穿孔法对动物免疫总计三次:在第 0 周进行第一次免疫、在第 2 周进行第二次免疫并且在第 4 周进行第三次免疫。收集血清并测量 IgG 总计两次:在第 4 周进行第一次收集并且在第 6 周进行第二次收集。

[0343] 图 31 示出了在 RSV(A) 或 RSV-G(B) 剂量研究中在 RSV-G(A)DNA 免疫之后血清中的 IgG 数据。由该剂量研究测量血清中的总抗 RSV-G(A)IgG。所示数据来自在各种剂量下收集的血清,如图 31 中图表的 x 轴中所展示。以 1:100 的血清稀释液测量抗 RSV-G(A)IgG。

[0344] 图 32 示出了在 RSV(A) 或 RSV-G(B) 剂量研究中在 RSV-G(B)DNA 免疫之后血清中的 IgG 数据。由该剂量研究测量血清中的总抗 RSV-G(B)IgG。所示数据来自在各种剂量下收集的血清,如图 31 中图表的 x 轴中所展示。以 1:100 的血清稀释液测量抗 RSV-G(B)IgG。

[0345] 图 33 是示出了在小鼠研究中使用 Mono 疫苗的 RSV 初步中和结果的概述的图表。测量在免疫后 3 天所收集的血清的中和滴度。使用阴性血清作为阴性对照。阳性对照包括抗 RSV 单克隆抗体和抗 RSV 多克隆血清。测量对照物以及来自用 pRSV-F 疫苗接种的两只小鼠、用 pRSV-G(A) 疫苗接种的两只小鼠和用 pRSV-G(B) 疫苗接种的一只小鼠的血清的中和滴度。

[0346] 设计并采取 RSV 兔研究。RSV 兔研究设计示于图 34 中。用含有 pRSV-F、pRSV-G(A) 和 pRSV-G(B) 的混合液对四只雌性新西兰白色 (NZW) 兔进行免疫。每只动物接受 200μg/质粒,最终体积为 100μl。使用肌肉内 / 电穿孔法免疫对动物免疫总计四次:第一次免疫在第 0 周进行,第二次免疫在第 3 周进行,第三次免疫在第 6 周进行并且第四次免疫在第 9 周

进行。从动物收集血液，总计六次：第 -2 周（前期血液—第一次免疫前 2 周）、第 0 周（在第一次免疫前的前期血液）、第 2 周、第 5 周、第 8 周和第 13 周。使用 ELISA 和病毒中和检验分析血液。

[0347] 由该兔研究得到的数据示于图 35 中。测量针对 RSV-F 的体液免疫。在第 -2 周和第 0 周取得来自前期血液样品的抗 RSV-F IgG，并且在第 2 周、第 5 周、第 8 周和第 13 周取得来自样品的抗 RSV-F IgG。在第 2 周，收集样品的动物已免疫一次并且样品是在免疫后 2 周收集。在第 5 周，收集样品的动物已免疫两次并且样品是在第二次免疫后 2 周收集。在第 8 周，收集样品的动物已免疫三次并且样品是在第三次免疫后 2 周收集。在第 13 周，收集样品的动物已免疫四次并且样品是在第四次免疫后 4 周收集。

[0348] 由该兔研究得到的数据示于图 36 中。测量针对 RSV-G 的体液免疫。在第 -2 周和第 0 周取得来自前期血液样品的抗 RSV-G IgG，并且在第 2 周、第 5 周、第 8 周和第 13 周取得来自样品的抗 RSV-G IgG。在第 2 周，收集样品的动物已免疫一次并且样品是在免疫后 2 周收集。在第 5 周，收集样品的动物已免疫两次并且样品是在第二次免疫后 2 周收集。在第 8 周，收集样品的动物已免疫三次并且样品是在第三次免疫后 2 周收集。在第 13 周，收集样品的动物已免疫四次并且样品是在第四次免疫后 4 周收集。

[0349] 采取 RSV 非人类灵长类动物研究。该研究的目的是评价以混合物形式施用给非人类灵长类动物 (non-human primate，NHP) 的单一插入 pDNA RSV 疫苗的体液免疫原性。该研究还用于评价并确定如多部位疫苗传递、电穿孔法及在佐剂存在下进行传递等因素是否会增加体液反应的幅度。进行这些实验以确定针对 RSV-F、RSV-G(A) 和 RSV-G(B) 的合成 DNA 疫苗是否刺激高抗 RSV 抗体滴度，分析用 RSV 混合物疫苗接种的动物中的特异性免疫应答并且评价佐剂和 / 或多部位传递对所产生的免疫应答的影响。RSV 非人类灵长类动物研究设计示于图 37 中。所用非人类灵长类动物是恒河猕猴 (Rhesus macaques)。在研究中，将 15 只恒河猕猴分成 4 组：三组各含有四只动物并且一组含有三只动物。n = 4 的组是治疗组，即，其接受疫苗接种。n = 3 的组是未治疗组，用作对照组。这些组治疗如下。

[0350] 第 1 组 (n = 4)：每只动物施用 1mg RSV-F、1mg RSV-G(A)、1mg RSV-G(B) 的混合物，混合并且肌肉内传递到 1 个部位。

[0351] 第 2 组 (n = 4)：每只动物施用 1mg RSV-F、1mg RSV-G(A)、1mg RSV-G(B)、1mg rhMEC 的混合物，混合并且肌肉内传递到 1 个部位。

[0352] 第 3 组 (n = 4)：每只动物施用 1mg RSV-F、1mg RSV-G(A)、1mg RSV-G(B) 的混合物，混合并且真皮内传递到 3 个部位。

[0353] 第 4 组 (n = 3)：每只动物未进行治疗。

[0354] 使用电穿孔法对动物免疫总计三次。在第 1 周进行第一次免疫、在第 5 周进行第二次免疫并且在第 13 周进行第三次免疫。从动物收集血液，总计六次：第 0 周（前期血液，在第一次免疫之前）、第 3 周、第 7 周、第 15 周、第 17 周和第 18 周。使用 ELISA 和病毒中和检验分析血液。

[0355] 图 38A-38D 示出了由测量针对 RSV-F 的体液免疫的 RSV 非人类灵长类动物研究得到的数据。图 38A 示出了在原生动物中由测量针对 RSV-F 的体液免疫得到的数据。图 38B 示出了在一个部位接受混合疫苗 (IM) 的动物中由测量针对 RSV-F 的体液免疫得到的数据。图 38C 示出了在一个部位接受混合疫苗加 rhMEC 构建体 (IM) 的动物中由测量针对 RSV-F

的体液免疫得到的数据。图 38D 示出了在三个部位接受混合疫苗 (ID) 的动物中由测量针对 RSV-F 的体液免疫得到的数据。在第 0 周取得来自前期血液样品的抗 RSV-F IgG，并且在第 3 周、第 15 周和第 18 周取得来自样品的抗 RSV-F IgG。在第 3 周，收集样品的动物已免疫一次并且样品是在免疫后 2 周收集。在第 15 周，收集样品的动物已免疫三次并且样品是在第三次免疫后 2 周收集。在第 18 周，收集样品的动物已免疫三次并且样品是在第三次免疫后 5 周收集。

[0356] 图 39A-39D 示出了由测量针对 RSV-G 的体液免疫的 RSV 非人类灵长类动物研究得到的数据。图 39A 示出了在原生动物中由测量针对 RSV-G 的体液免疫得到的数据。图 39B 示出了在一个部位接受混合疫苗 (IM) 的动物中由测量针对 RSV-G 的体液免疫得到的数据。图 39C 示出了在一个部位接受混合疫苗加 rhMEC 构建体 (IM) 的动物中由测量针对 RSV-G 的体液免疫得到的数据。图 39D 示出了在三个部位接受混合疫苗 (ID) 的动物中由测量针对 RSV-G 的体液免疫得到的数据。在第 0 周取得来自前期血液样品的抗 RSV-G IgG，并且在第 3 周、第 15 周和第 18 周取得来自样品的抗 RSV-G IgG。在第 3 周，收集样品的动物已免疫一次并且样品是在免疫后 2 周收集。在第 15 周，收集样品的动物已免疫三次并且样品是在第三次免疫后 2 周收集。在第 18 周，收集样品的动物已免疫三次并且样品是在第三次免疫后 5 周收集。

[0357] 图 40 是示出了在兔非人类灵长类动物研究中使用混合疫苗的 RSV 初步中和结果的概述的图表。测量在免疫后 3 天所收集的血清的中和滴度。使用阴性血清作为阴性对照。阳性对照包括抗 RSV 单克隆抗体和抗 RSV 多克隆血清。测量对照组以及来自用混合疫苗进行疫苗接种的两只兔和用混合疫苗进行疫苗接种的两只非人类灵长类动物的血清的中和滴度。来自前期血液数据显示，在疫苗接种之后产生兔和非人类灵长类动物的中和滴度。

[0001]

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<120> 人呼吸道合胞病毒共有抗原、由其制备的核酸构建体和疫苗及其使用方法

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Thr Gly Trp Tyr Thr Ser Val Ile Thr Ile Glu Leu Ser Asn Ile Lys
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Glu Asn Lys Cys Asn Gly Thr Asp Ala Lys Val Lys Leu Ile Lys Gln
65 70 75 80

Glu Leu Asp Lys Tyr Lys Asn Ala Val Thr Glu Leu Gln Leu Leu Met
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Ser Ile Ile Lys Glu Glu Val Leu Ala Tyr Val Val Gln Leu Pro Leu
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Ile Phe Asn Pro Lys Tyr Asp Cys Lys Ile Met Thr Ser Lys Thr Asp
385 390 395 400

Val Ser Ser Ser Val Ile Thr Ser Leu Gly Ala Ile Val Ser Cys Tyr
405 410 415

Gly Lys Thr Lys Cys Thr Ala Ser Asn Lys Asn Arg Gly Ile Ile Lys
420 425 430

Thr Phe Ser Asn Gly Cys Asp Tyr Val Ser Asn Lys Gly Val Asp Thr
435 440 445

Val Ser Val Gly Asn Thr Leu Tyr Tyr Val Asn Lys Gln Glu Gly Lys
450 455 460

Ser Leu Tyr Val Lys Gly Glu Pro Ile Ile Asn Phe Tyr Asp Pro Leu
465 470 475 480

Val Phe Pro Ser Asp Glu Phe Asp Ala Ser Ile Ser Gln Val Asn Glu
485 490 495

Lys Ile Asn Gln Ser Leu Ala Phe Ile Arg Lys Ser Asp Glu Leu Leu
500 505 510

His Asn Val Asn Ala Gly Lys Ser Thr Thr Asn Ile Met Ile Thr Thr
515 520 525

Ile Ile Ile Val Ile Ile Val Ile Leu Leu Ser Leu Ile Ala Val Gly
530 535 540

Leu Leu Leu Tyr Cys Lys Ala Arg Ser Thr Pro Val Thr Leu Ser Lys
545 550 555 560

Asp Gln Leu Ser Gly Ile Ile Asn Asn Ile Ala Phe Ser Asn
565 570

<210> 3
<211> 577
<212> DNA
<213> 人工序列

<220>
<223> 合成序列

<400> 3
agcagacgga acccctgcaa gttcgagatc cggggccact gcctgaacgg caagcggtgc 60

cacttcagcc acaactactt cgagtggccc cctcacgccc tgctggtgcg ccagaacttc 120

atgctgaaca gaatcctgaa gtccatggac aagagcatcg acaccctgag cgagatcagc 180

[0005]

ggagccgccg agctggaccg gaccgaggaa tatgccctgg gcgtggtggg agtgctggaa 240

agctacatcg gcagcatcaa caacatcacc aagcagagcg cctgcgtggc catgagcaag 300

ctgctgaccg agctgaacag cgacgacatc aagaagctgc gggacaacga ggaacccaac 360

agccccaaga tccgggtgta caacaccgtg atcagctaca tcgagagcaa ccggaagaac 420

aacaagcaga ccatccatct gctgaagcgg ctgcccgccg acgtgctgaa gaaaaccatc 480

aagaacaccc tggacatcca caagtccatc accatcaaca accccaaaga aagcaccgtg 540

tccgacacca acgaccacgc caagaacaac gacacca 577

<210> 4
<211> 194
<212> PRT
<213> 人工序列

<220>
<223> 合成序列

<400> 4

Ser Arg Arg Asn Pro Cys Lys Phe Glu Ile Arg Gly His Cys Leu Asn
1 5 10 15

Gly Lys Arg Cys His Phe Ser His Asn Tyr Phe Glu Trp Pro Pro His
20 25 30

Ala Leu Leu Val Arg Gln Asn Phe Met Leu Asn Arg Ile Leu Lys Ser
35 40 45

Met Asp Lys Ser Ile Asp Thr Leu Ser Glu Ile Ser Gly Ala Ala Glu
50 55 60

Leu Asp Arg Thr Glu Glu Tyr Ala Leu Gly Val Val Gly Val Leu Glu
65 70 75 80

Ser Tyr Ile Gly Ser Ile Asn Asn Ile Thr Lys Gln Ser Ala Cys Val
85 90 95

Ala Met Ser Lys Leu Leu Thr Glu Leu Asn Ser Asp Asp Ile Lys Lys
100 105 110

Leu Arg Asp Asn Glu Glu Pro Asn Ser Pro Lys Ile Arg Val Tyr Asn
115 120 125

Thr Val Ile Ser Tyr Ile Glu Ser Asn Arg Lys Asn Asn Lys Gln Thr
130 135 140

Ile His Leu Leu Lys Arg Leu Pro Ala Asp Val Leu Lys Lys Thr Ile
145 150 155 160

[0006]

Lys Asn Thr Leu Asp Ile His Lys Ser Ile Thr Ile Asn Asn Pro Lys
165 170 175

Glu Ser Thr Val Ser Asp Thr Asn Asp His Ala Lys Asn Asn Asp Thr
180 185 190

Thr Gly

<210> 5
<211> 894
<212> DNA
<213> 人工序列

<220>
<223> 合成序列

<400> 5
tccaagaata aggatcagag gaccgcgaaa acgcttgaga ggacgtggga cacgctgaac 60

cacctcctgt tcatctcctc gtgtctctac aagctcaacc ttaagtccat cgcgcagatc 120

accttgagca ttctcgccat gatcatctcc accagcctta tcattgccgc aatcatcttc 180

atcgcatccg ccaaccataa ggtgacattg actacagcga ttatccaaga cgctactagc 240

cagatcaaga ataccacgcc gacctatttg acgcaaaatc ctcagttggg aattagcttc 300

tcgaatctct cggaaaccac gtcgcagccg actacaattc ttgcgtcaac gactccatcg 360

gccaaatcaa caccacaatc gactaccgta aaaacgaaga acacgactac aacacagatt 420

cagccttcaa agcccacgac caaacagaga cagaataagc cgcccaacaa gcccaacaat 480

gattttcact tcgaggtgtt taacttcgtg ccctgttcga tttgcagcaa taaccccacg 540

tgctgggcga tttgcaagcg aatcccgaat aagaagcccg ggaaaaagac cacgacgaaa 600

ccgacaaaga agccgacaat caagacaacg aaaaaggatc ttaaacctca gacgacaaag 660

cctaaggaag tcttgacaac gaagcctacg gaaaaaccca ctatcaatac taccaagact 720

aacatccgga caacactgct gacgagcaat accacgggaa acccggagct cacatcgcag 780

aaagagacac tccattcgac atcctccgag ggtaaccctt cgcccagcca ggtgtatacg 840

acgtcagaat accctagcca accctcatcg ccctcaaata cgacccggca atga 894

<210> 6
<211> 297
<212> PRT
<213> 人工序列

<220>
<223> 合成序列

<400> 6

Ser Lys Asn Lys Asp Gln Arg Thr Ala Lys Thr Leu Glu Arg Thr Trp

[0007]

1 5 10 15

Asp Thr Leu Asn His Leu Leu Phe Ile Ser Ser Cys Leu Tyr Lys Leu
20 25 30

Asn Leu Lys Ser Ile Ala Gln Ile Thr Leu Ser Ile Leu Ala Met Ile
35 40 45

Ile Ser Thr Ser Leu Ile Ile Ala Ala Ile Ile Phe Ile Ala Ser Ala
50 55 60

Asn His Lys Val Thr Leu Thr Thr Ala Ile Ile Gln Asp Ala Thr Ser
65 70 75 80

Gln Ile Lys Asn Thr Thr Pro Thr Tyr Leu Thr Gln Asn Pro Gln Leu
85 90 95

Gly Ile Ser Phe Ser Asn Leu Ser Glu Thr Thr Ser Gln Pro Thr Thr
100 105 110

Ile Leu Ala Ser Thr Thr Pro Ser Ala Lys Ser Thr Pro Gln Ser Thr
115 120 125

Thr Val Lys Thr Lys Asn Thr Thr Thr Thr Gln Ile Gln Pro Ser Lys
130 135 140

Pro Thr Thr Lys Gln Arg Gln Asn Lys Pro Pro Asn Lys Pro Asn Asn
145 150 155 160

Asp Phe His Phe Glu Val Phe Asn Phe Val Pro Cys Ser Ile Cys Ser
165 170 175

Asn Asn Pro Thr Cys Trp Ala Ile Cys Lys Arg Ile Pro Asn Lys Lys
180 185 190

Pro Gly Lys Lys Thr Thr Thr Lys Pro Thr Lys Lys Pro Thr Ile Lys
195 200 205

Thr Thr Lys Lys Asp Leu Lys Pro Gln Thr Thr Lys Pro Lys Glu Val
210 215 220

Leu Thr Thr Lys Pro Thr Glu Lys Pro Thr Ile Asn Thr Thr Lys Thr
225 230 235 240

Asn Ile Arg Thr Thr Leu Leu Thr Ser Asn Thr Thr Gly Asn Pro Glu
245 250 255

Leu Thr Ser Gln Lys Glu Thr Leu His Ser Thr Ser Ser Glu Gly Asn

[0008]

260 265 270

Pro Ser Pro Ser Gln Val Tyr Thr Thr Ser Glu Tyr Pro Ser Gln Pro
275 280 285

Ser Ser Pro Ser Asn Thr Thr Arg Gln
290 295

<210> 7
<211> 876
<212> DNA
<213> 人工序列

<220>
<223> 合成序列

<400> 7
agcaaaaaca aaaaccaaag gacggctcgg acgcttgaga aaacatggga cacgcttaat 60

caccttattg tgatctcatc gtgtttgtac cggttgaatc tcaagagcat cgcccagatt 120

gcgctgtcag tcctggccat gattatctcg acatcactca tcatcgcagc catcatcttt 180

atcatttcag cgaatcacaa ggtaacgctt acaacagtca cggtgcagac catcaagaat 240

cataccgaaa agaatatcac aacctacctc acccaagtca gcccggagag agtaagcccc 300

tcaaaacagc ctactacgac acctcccatc cacacgaact cggcgaccat ctcaccgaat 360

accaaatcag aaacgcatca tacgaccgca cagacaaagg gacgaaccac tacacccaca 420

cagaacaaca aacccagcac caagccgagg ccaaagaatc cgcccaagaa gccgaaagat 480

gactatcact ttgaagtgtt caacttcgta ccgtgttcga tttgcgggaa taatcagttg 540

tgcaaatcca tttgcaagac gatcccatcc aacaaaccga agaagaaacc taccatcaag 600

cccacaaaca agccaacgac aaaaacaacg aacaagcgcg atcccaaaac gctcgcgaaa 660

acgttgaaga aggaaacgac gacaaaccct acgaagaaac ccacgcccaa gaccactgag 720

agagacacct ccacctcgca atcgacggta cttgacacga ctacgagcaa gcacactatc 780

cagcaacagt ccctgcactc aaccacgccc gagaatacac caaactcaac acagactccg 840

acagcttcag agccttccac ttcgaattcc acatga 876

<210> 8
<211> 291
<212> PRT
<213> 人工序列

<220>
<223> 合成序列

<400> 8

Ser Lys Asn Lys Asn Gln Arg Thr Ala Arg Thr Leu Glu Lys Thr Trp
1 5 10 15

[0009]

Asp Thr Leu Asn His Leu Ile Val Ile Ser Ser Cys Leu Tyr Arg Leu
20 25 30

Asn Leu Lys Ser Ile Ala Gln Ile Ala Leu Ser Val Leu Ala Met Ile
35 40 45

Ile Ser Thr Ser Leu Ile Ile Ala Ala Ile Ile Phe Ile Ile Ser Ala
50 55 60

Asn His Lys Val Thr Leu Thr Thr Val Thr Val Gln Thr Ile Lys Asn
65 70 75 80

His Thr Glu Lys Asn Ile Thr Thr Tyr Leu Thr Gln Val Ser Pro Glu
85 90 95

Arg Val Ser Pro Ser Lys Gln Pro Thr Thr Thr Pro Pro Ile His Thr
100 105 110

Asn Ser Ala Thr Ile Ser Pro Asn Thr Lys Ser Glu Thr His His Thr
115 120 125

Thr Ala Gln Thr Lys Gly Arg Thr Thr Thr Pro Thr Gln Asn Asn Lys
130 135 140

Pro Ser Thr Lys Pro Arg Pro Lys Asn Pro Pro Lys Lys Pro Lys Asp
145 150 155 160

Asp Tyr His Phe Glu Val Phe Asn Phe Val Pro Cys Ser Ile Cys Gly
165 170 175

Asn Asn Gln Leu Cys Lys Ser Ile Cys Lys Thr Ile Pro Ser Asn Lys
180 185 190

Pro Lys Lys Lys Pro Thr Ile Lys Pro Thr Asn Lys Pro Thr Thr Lys
195 200 205

Thr Thr Asn Lys Arg Asp Pro Lys Thr Leu Ala Lys Thr Leu Lys Lys
210 215 220

Glu Thr Thr Thr Asn Pro Thr Lys Lys Pro Thr Pro Lys Thr Thr Glu
225 230 235 240

Arg Asp Thr Ser Thr Ser Gln Ser Thr Val Leu Asp Thr Thr Thr Ser
245 250 255

Lys His Thr Ile Gln Gln Gln Ser Leu His Ser Thr Thr Pro Glu Asn
260 265 270

[0010]

Thr Pro Asn Ser Thr Gln Thr Pro Thr Ala Ser Glu Pro Ser Thr Ser
275 280 285

Asn Ser Thr
290

<210> 9
<211> 840
<212> DNA
<213> 人工序列

<220>
<223> 合成序列

<400> 9
ctggcctacg tggtgcagct gcccctgtac ggcgtgatcg acacccccctg ctggaagctg 60

cacaccagcc ccctgtgcac caccaacacc aaagagggca gcaacatctg cctgacccgg 120

accgaccggg gctggtactg cgataatgcc ggcagcgtgt cattctttcc acaggccgag 180

acatgcaagg tgcagagcaa ccgggtgttc tgcgacacca tgaacagcct gaccctgccc 240

tccgaagtga acctgtgcaa catcgacatc ttcaacccta agtacgactg caagatcatg 300

acctccaaga ccgacgtgtc cagctccgtg atcacctccc tgggcgccat cgtgtcctgc 360

tacggcaaga ccaagtgcac cgccagcaac aagaaccggg gcatcatcaa gaccttcagc 420

aacggctgcg actacgtgtc caacaagggg gtggacaccg tgtccgtggg caacaccctg 480

tactacgtga acaaacagga aggcaagagc ctgtacgtga agggcgagcc catcatcaac 540

ttctacgacc ccctggtgtt ccccagcgac gagttcgacg ccagcatcag ccaggtgaac 600

gagaagatca accagagcct ggccttcatc agaaagagcg acgagctgct gcacaatgtg 660

aatgccggca agagcaccac caatatcatg atcaccacaa tcatcatcgt gatcattgtg 720

atcctgctgt ccctgatcgc cgtgggcctg ctgctgtact gcaaggcccg gtccaccccct 780

gtgaccctgt ccaaggacca gctgagcgga atcatcaaca atatcgcctt ctccaactga 840

<210> 10
<211> 592
<212> PRT
<213> 人工序列

<220>
<223> 合成序列

<400> 10

Met Asp Trp Thr Trp Ile Leu Phe Leu Val Ala Ala Ala Thr Arg Val
1 5 10 15

His Ser Glu Leu Pro Ile Leu Lys Thr Asn Ala Ile Thr Thr Ile Leu
20 25 30

[0011]

Ala Ala Val Thr Leu Cys Phe Ala Ser Ser Gln Asn Ile Thr Glu Glu
35 40 45

Phe Tyr Gln Ser Thr Cys Ser Ala Val Ser Lys Gly Tyr Leu Ser Ala
50 55 60

Leu Arg Thr Gly Trp Tyr Thr Ser Val Ile Thr Ile Glu Leu Ser Asn
65 70 75 80

Ile Lys Glu Asn Lys Cys Asn Gly Thr Asp Ala Lys Val Lys Leu Ile
85 90 95

Lys Gln Glu Leu Asp Lys Tyr Lys Asn Ala Val Thr Glu Leu Gln Leu
100 105 110

Leu Met Gln Ser Thr Pro Ala Ala Asn Asn Arg Ala Arg Arg Glu Leu
115 120 125

Pro Arg Phe Met Asn Tyr Thr Leu Asn Asn Thr Lys Asn Thr Asn Val
130 135 140

Thr Leu Ser Lys Lys Arg Lys Arg Arg Phe Leu Gly Phe Leu Leu Gly
145 150 155 160

Val Gly Ser Ala Ile Ala Ser Gly Ile Ala Val Ser Lys Val Leu His
165 170 175

Leu Glu Gly Glu Val Asn Lys Ile Lys Ser Ala Leu Leu Ser Thr Asn
180 185 190

Lys Ala Val Val Ser Leu Ser Asn Gly Val Ser Val Leu Thr Ser Lys
195 200 205

Val Leu Asp Leu Lys Asn Tyr Ile Asp Lys Gln Leu Leu Pro Ile Val
210 215 220

Asn Lys Gln Ser Cys Ser Ile Ser Asn Ile Glu Thr Val Ile Glu Phe
225 230 235 240

Gln Gln Lys Asn Asn Arg Leu Leu Glu Ile Thr Arg Glu Phe Ser Val
245 250 255

Asn Ala Gly Val Thr Thr Pro Val Ser Thr Tyr Met Leu Thr Asn Ser
260 265 270

Glu Leu Leu Ser Leu Ile Asn Asp Met Pro Ile Thr Asn Asp Gln Lys
275 280 285

[0012]

Lys Leu Met Ser Asn Asn Val Gln Ile Val Arg Gln Gln Ser Tyr Ser
290 295 300

Ile Met Ser Ile Ile Lys Glu Glu Val Leu Ala Tyr Val Val Gln Leu
305 310 315 320

Pro Leu Tyr Gly Val Ile Asp Thr Pro Cys Trp Lys Leu His Thr Ser
325 330 335

Pro Leu Cys Thr Thr Asn Thr Lys Glu Gly Ser Asn Ile Cys Leu Thr
340 345 350

Arg Thr Asp Arg Gly Trp Tyr Cys Asp Asn Ala Gly Ser Val Ser Phe
355 360 365

Phe Pro Gln Ala Glu Thr Cys Lys Val Gln Ser Asn Arg Val Phe Cys
370 375 380

Asp Thr Met Asn Ser Leu Thr Leu Pro Ser Glu Val Asn Leu Cys Asn
385 390 395 400

Ile Asp Ile Phe Asn Pro Lys Tyr Asp Cys Lys Ile Met Thr Ser Lys
405 410 415

Thr Asp Val Ser Ser Ser Val Ile Thr Ser Leu Gly Ala Ile Val Ser
420 425 430

Cys Tyr Gly Lys Thr Lys Cys Thr Ala Ser Asn Lys Asn Arg Gly Ile
435 440 445

Ile Lys Thr Phe Ser Asn Gly Cys Asp Tyr Val Ser Asn Lys Gly Val
450 455 460

Asp Thr Val Ser Val Gly Asn Thr Leu Tyr Tyr Val Asn Lys Gln Glu
465 470 475 480

Gly Lys Ser Leu Tyr Val Lys Gly Glu Pro Ile Ile Asn Phe Tyr Asp
485 490 495

Pro Leu Val Phe Pro Ser Asp Glu Phe Asp Ala Ser Ile Ser Gln Val
500 505 510

Asn Glu Lys Ile Asn Gln Ser Leu Ala Phe Ile Arg Lys Ser Asp Glu
515 520 525

Leu Leu His Asn Val Asn Ala Gly Lys Ser Thr Thr Asn Ile Met Ile
530 535 540

[0013]

Thr Thr Ile Ile Ile Val Ile Ile Val Ile Leu Leu Ser Leu Ile Ala
545 550 555 560

Val Gly Leu Leu Leu Tyr Cys Lys Ala Arg Ser Thr Pro Val Thr Leu
565 570 575

Ser Lys Asp Gln Leu Ser Gly Ile Ile Asn Asn Ile Ala Phe Ser Asn
580 585 590

<210> 11
<211> 631
<212> DNA
<213> 人工序列

<220>
<223> 合成序列

<400> 11
atggactgga cctggattct gttcctggtg gccgctgcta cccgggtgca cagcagcaga 60

cggaaccccc gcaagttcga gatccggggc cactgcctga acggcaagcg gtgccacttc 120

agccacaact acttcgagtg gccccctcac gccctgctgg tgcgccagaa cttcatgctg 180

aacagaatcc tgaagtccat ggacaagagc atcgacaccc tgagcgagat cagcggagcc 240

gccgagctgg accggaccga ggaatatgcc ctgggcgtgg tgggagtgct ggaaagctac 300

atcggcagca tcaacaacat caccaagcag agcgcctgcg tggccatgag caagctgctg 360

accgagctga acagcgacga catcaagaag ctgcgggaca acgaggaacc caacagcccc 420

aagatccggg tgtacaacac cgtgatcagc tacatcgaga gcaaccggaa gaacaacaag 480

cagaccatcc atctgctgaa gcggctgccc gccgacgtgc tgaagaaaac catcaagaac 540

accctggaca tccacaagtc catcaccatc aacaacccca aagaaagcac cgtgtccgac 600

accaacgacc acgccaagaa caacgacacc a 631

<210> 12
<211> 212
<212> PRT
<213> 人工序列

<220>
<223> 合成序列

<400> 12

Met Asp Trp Thr Trp Ile Leu Phe Leu Val Ala Ala Ala Thr Arg Val
1 5 10 15

His Ser Ser Arg Arg Asn Pro Cys Lys Phe Glu Ile Arg Gly His Cys
20 25 30

Leu Asn Gly Lys Arg Cys His Phe Ser His Asn Tyr Phe Glu Trp Pro

[0014]

35 40 45

Pro His Ala Leu Leu Val Arg Gln Asn Phe Met Leu Asn Arg Ile Leu
50 55 60

Lys Ser Met Asp Lys Ser Ile Asp Thr Leu Ser Glu Ile Ser Gly Ala
65 70 75 80

Ala Glu Leu Asp Arg Thr Glu Glu Tyr Ala Leu Gly Val Val Gly Val
85 90 95

Leu Glu Ser Tyr Ile Gly Ser Ile Asn Asn Ile Thr Lys Gln Ser Ala
100 105 110

Cys Val Ala Met Ser Lys Leu Leu Thr Glu Leu Asn Ser Asp Asp Ile
115 120 125

Lys Lys Leu Arg Asp Asn Glu Glu Pro Asn Ser Pro Lys Ile Arg Val
130 135 140

Tyr Asn Thr Val Ile Ser Tyr Ile Glu Ser Asn Arg Lys Asn Asn Lys
145 150 155 160

Gln Thr Ile His Leu Leu Lys Arg Leu Pro Ala Asp Val Leu Lys Lys
165 170 175

Thr Ile Lys Asn Thr Leu Asp Ile His Lys Ser Ile Thr Ile Asn Asn
180 185 190

Pro Lys Glu Ser Thr Val Ser Asp Thr Asn Asp His Ala Lys Asn Asn
195 200 205

Asp Thr Thr Gly
210

<210> 13
<211> 948
<212> DNA
<213> 人工序列

<220>
<223> 合成序列

<400> 13
atggactgga catggatctt gtttcttgta gcggcagcaa cacgcgtcca ctcctccaag 60

aataaggatc agaggaccgc gaaaacgctt gagaggacgt gggacacgct gaaccacctc 120

ctgttcatct cctcgtgtct ctacaagctc aaccttaagt ccatcgcgca gatcaccttg 180

agcattctcg ccatgatcat ctccaccagc cttatcattg ccgcaatcat cttcatcgca 240

[0015]

tccgccaacc ataaggtgac attgactaca gcgattatcc aagacgctac tagccagatc 300

aagaatacca cgccgaccta tttgacgcaa aatcctcagt tgggaattag cttctcgaat 360

ctctcggaaa ccacgtcgca gccgactaca attcttgcgt caacgactcc atcggccaaa 420

tcaacaccac aatcgactac cgtaaaaacg aagaacacga ctacaacaca gattcagcct 480

tcaaagccca cgaccaaaca gagacagaat aagccgccca acaagcccaa caatgatttt 540

cacttcgagg tgtttaactt cgtgccctgt tcgatttgca gcaataaccc cacgtgctgg 600

gcgatttgca agcgaatccc gaataagaag cccgggaaaa agaccacgac gaaaccgaca 660

aagaagccga caatcaagac aacgaaaaag gatcttaaac ctcagacgac aaagcctaag 720

gaagtcttga caacgaagcc tacggaaaaa cccactatca atactaccaa gactaacatc 780

cggacaacac tgctgacgag caataccacg ggaaacccgg agctcacatc gcagaaagag 840

acactccatt cgacatcctc cgagggtaac ccttcgccca gccaggtgta tacgacgtca 900

gaatacccta gccaacccтc atcgccctca aatacgaccc ggcaatga 948

<210> 14
<211> 315
<212> PRT
<213> 人工序列

<220>
<223> 合成序列

<400> 14

Met Asp Trp Thr Trp Ile Leu Phe Leu Val Ala Ala Ala Thr Arg Val
1 5 10 15

His Ser Ser Lys Asn Lys Asp Gln Arg Thr Ala Lys Thr Leu Glu Arg
20 25 30

Thr Trp Asp Thr Leu Asn His Leu Leu Phe Ile Ser Ser Cys Leu Tyr
35 40 45

Lys Leu Asn Leu Lys Ser Ile Ala Gln Ile Thr Leu Ser Ile Leu Ala
50 55 60

Met Ile Ile Ser Thr Ser Leu Ile Ile Ala Ala Ile Ile Phe Ile Ala
65 70 75 80

Ser Ala Asn His Lys Val Thr Leu Thr Thr Ala Ile Ile Gln Asp Ala
85 90 95

Thr Ser Gln Ile Lys Asn Thr Thr Pro Thr Tyr Leu Thr Gln Asn Pro
100 105 110

Gln Leu Gly Ile Ser Phe Ser Asn Leu Ser Glu Thr Thr Ser Gln Pro

[0016]

115 120 125

Thr Thr Ile Leu Ala Ser Thr Thr Pro Ser Ala Lys Ser Thr Pro Gln
130 135 140

Ser Thr Thr Val Lys Thr Lys Asn Thr Thr Thr Thr Gln Ile Gln Pro
145 150 155 160

Ser Lys Pro Thr Thr Lys Gln Arg Gln Asn Lys Pro Pro Asn Lys Pro
165 170 175

Asn Asn Asp Phe His Phe Glu Val Phe Asn Phe Val Pro Cys Ser Ile
180 185 190

Cys Ser Asn Asn Pro Thr Cys Trp Ala Ile Cys Lys Arg Ile Pro Asn
195 200 205

Lys Lys Pro Gly Lys Lys Thr Thr Thr Lys Pro Thr Lys Lys Pro Thr
210 215 220

Ile Lys Thr Thr Lys Lys Asp Leu Lys Pro Gln Thr Thr Lys Pro Lys
225 230 235 240

Glu Val Leu Thr Thr Lys Pro Thr Glu Lys Pro Thr Ile Asn Thr Thr
245 250 255

Lys Thr Asn Ile Arg Thr Thr Leu Leu Thr Ser Asn Thr Thr Gly Asn
260 265 270

Pro Glu Leu Thr Ser Gln Lys Glu Thr Leu His Ser Thr Ser Ser Glu
275 280 285

Gly Asn Pro Ser Pro Ser Gln Val Tyr Thr Thr Ser Glu Tyr Pro Ser
290 295 300

Gln Pro Ser Ser Pro Ser Asn Thr Thr Arg Gln
305 310 315

<210> 15
<211> 926
<212> DNA
<213> 人工序列

<220>
<223> 合成序列

<400> 15
atggattgga cttggatttt gttcttggtg gcagcggcca ctcgcgtcca ttccagcaaa 60

aacaaaaacc aaaggacggc tcggacgctt gagaaaacat gggacacgct taatcacctt 120

[0017]

attgtgatct catcgtgttt gtaccggttg aatctcaaga gcatcgccca gattgcgctg 180

tcagtcctgg ccatgattat ctcgacatca ctcatcatcg cagccatcat ctttatcatt 240

tcagcgaatc acaaggtaac gcttacaaca gtcacggtgc agaccatcaa gaatcatacc 300

aaaagaatat cacaacctac ctcacccaag tcagcccgga gagagtaagc ccctcaaaac 360

agcctactac gacacctccc atccacacga actcggcgac catctcaccg aataccaaac 420

agaaacgcat catacgaccg cacagacaaa gggacgaacc actacaccca cacagaacac 480

aaacccagca ccaagccgag gccaaagaat ccgcccaaga agccgaaaga tgactatcac 540

tttgaagtgt tcaacttcgt accgtgttcg atttgcggga ataatcagtt gtgcaaatcc 600

atttgcaaga cgatcccatc caacaaaccg aagaagaaac ctaccatcaa gcccacaaac 660

aagccaacga caaaaacaac gaacaagcgc gatcccaaaa cgctcgcgaa aacgttgaga 720

aggaaacgac gacaaaccct acgaagaaac ccacgcccaa gaccactgag agagacacct 780

ccacctcgca atcgacggta cttgacacga ctacgagcaa gcacactatc cagcaacagt 840

ccctgcactc aaccacgccc gagaatacac caaactcaac acagactccg acagcttcag 900

agccttccac ttcgaattcc acatga 926

<210> 16
<211> 309
<212> PRT
<213> 人工序列

<220>
<223> 合成序列

<400> 16

Met Asp Trp Thr Trp Ile Leu Phe Leu Val Ala Ala Ala Thr Arg Val
1 5 10 15

His Ser Ser Lys Asn Lys Asn Gln Arg Thr Ala Arg Thr Leu Glu Lys
20 25 30

Thr Trp Asp Thr Leu Asn His Leu Ile Val Ile Ser Ser Cys Leu Tyr
35 40 45

Arg Leu Asn Leu Lys Ser Ile Ala Gln Ile Ala Leu Ser Val Leu Ala
50 55 60

Met Ile Ile Ser Thr Ser Leu Ile Ile Ala Ala Ile Ile Phe Ile Ile
65 70 75 80

Ser Ala Asn His Lys Val Thr Leu Thr Thr Val Thr Val Gln Thr Ile
85 90 95

Lys Asn His Thr Glu Lys Asn Ile Thr Thr Tyr Leu Thr Gln Val Ser

[0018]

100 105 110

Pro Glu Arg Val Ser Pro Ser Lys Gln Pro Thr Thr Thr Pro Pro Ile
115 120 125

His Thr Asn Ser Ala Thr Ile Ser Pro Asn Thr Lys Ser Glu Thr His
130 135 140

His Thr Thr Ala Gln Thr Lys Gly Arg Thr Thr Thr Pro Thr Gln Asn
145 150 155 160

Asn Lys Pro Ser Thr Lys Pro Arg Pro Lys Asn Pro Pro Lys Lys Pro
165 170 175

Lys Asp Asp Tyr His Phe Glu Val Phe Asn Phe Val Pro Cys Ser Ile
180 185 190

Cys Gly Asn Asn Gln Leu Cys Lys Ser Ile Cys Lys Thr Ile Pro Ser
195 200 205

Asn Lys Pro Lys Lys Lys Pro Thr Ile Lys Pro Thr Asn Lys Pro Thr
210 215 220

Thr Lys Thr Thr Asn Lys Arg Asp Pro Lys Thr Leu Ala Lys Thr Leu
225 230 235 240

Lys Lys Glu Thr Thr Thr Asn Pro Thr Lys Lys Pro Thr Pro Lys Thr
245 250 255

Thr Glu Arg Asp Thr Ser Thr Ser Gln Ser Thr Val Leu Asp Thr Thr
260 265 270

Thr Ser Lys His Thr Ile Gln Gln Gln Ser Leu His Ser Thr Thr Pro
275 280 285

Glu Asn Thr Pro Asn Ser Thr Gln Thr Pro Thr Ala Ser Glu Pro Ser
290 295 300

Thr Ser Asn Ser Thr
305

<210> 17
<211> 18
<212> PRT
<213> 人类

<400> 17

Met Asp Trp Thr Trp Ile Leu Phe Leu Val Ala Ala Ala Thr Arg Val
1 5 10 15

[0019]

His Ser

<210> 18
<211> 291
<212> DNA
<213> 人类

<400> 18
gcctgcggcg gcaagagact gctgttcctg gccctggcct gggtgctgct ggcccacctg 60

tgttctcagg ccgaggccag caactacgac tgctgcctga gctacatcca gacccccctg 120

cccagcagag ccatcgtggg cttcaccaga cagatggccg acgaggcctg cgacatcaac 180

gccatcatct tccacaccaa gaaacgcaag agcgtgtgcg ccgaccccaa gcagaactgg 240

gtcaagagag ccgtgaacct gctgagcctg agagtgaaga aaatgtgatg a 291

<210> 19
<211> 95
<212> PRT
<213> 人类

<400> 19

Ala Cys Gly Gly Lys Arg Leu Leu Phe Leu Ala Leu Ala Trp Val Leu
1 5 10 15

Leu Ala His Leu Cys Ser Gln Ala Glu Ala Ser Asn Tyr Asp Cys Cys
20 25 30

Leu Ser Tyr Ile Gln Thr Pro Leu Pro Ser Arg Ala Ile Val Gly Phe
35 40 45

Thr Arg Gln Met Ala Asp Glu Ala Cys Asp Ile Asn Ala Ile Ile Phe
50 55 60

His Thr Lys Lys Arg Lys Ser Val Cys Ala Asp Pro Lys Gln Asn Trp
65 70 75 80

Val Lys Arg Ala Val Asn Leu Leu Ser Leu Arg Val Lys Lys Met
85 90 95

<210> 20
<211> 345
<212> DNA
<213> 人工序列

<220>
<223> 合成序列

<400> 20
atggactgga cctggattct gttcctggtg gccgctgcta caagagtgca ctctgcctgc 60

[0020]

ggcggcaaga gactgctgtt cctggccctg gcctgggtgc tgctggccca cctgtgttct 120

caggccgagg ccagcaacta cgactgctgc ctgagctaca tccagacccc cctgcccagc 180

agagccatcg tgggcttcac cagacagatg gccgacgagg cctgcgacat caacgccatc 240

atcttccaca ccaagaaacg caagagcgtg tgcgccgacc ccaagcagaa ctgggtcaag 300

agagccgtga acctgctgag cctgagagtg aagaaaatgt gatga 345

<210> 21
<211> 113
<212> PRT
<213> 人工序列

<220>
<223> 合成序列

<400> 21

Met Asp Trp Thr Trp Ile Leu Phe Leu Val Ala Ala Ala Thr Arg Val
1 5 10 15

His Ser Ala Cys Gly Gly Lys Arg Leu Leu Phe Leu Ala Leu Ala Trp
20 25 30

Val Leu Leu Ala His Leu Cys Ser Gln Ala Glu Ala Ser Asn Tyr Asp
35 40 45

Cys Cys Leu Ser Tyr Ile Gln Thr Pro Leu Pro Ser Arg Ala Ile Val
50 55 60

Gly Phe Thr Arg Gln Met Ala Asp Glu Ala Cys Asp Ile Asn Ala Ile
65 70 75 80

Ile Phe His Thr Lys Lys Arg Lys Ser Val Cys Ala Asp Pro Lys Gln
85 90 95

Asn Trp Val Lys Arg Ala Val Asn Leu Leu Ser Leu Arg Val Lys Lys
100 105 110

Met

<210> 22
<211> 9
<212> PRT
<213> 呼吸道合胞病毒

<400> 22

Lys Tyr Lys Asn Ala Val Thr Glu Leu
1 5

[0021]

<210> 23
<211> 18
<212> PRT
<213> 呼吸道合胞病毒

<400> 23

Ile Glu Thr Val Ile Glu Phe Gln Gln Lys Asn Asn Arg Leu Leu Glu
1 5 10 15

Ile Thr

<210> 24
<211> 9
<212> PRT
<213> 呼吸道合胞病毒

<400> 24

Tyr Met Leu Thr Asn Ser Glu Leu Leu
1 5

<210> 25
<211> 70
<212> PRT
<213> 呼吸道合胞病毒

<400> 25

Ser Val Gly Asn Thr Leu Tyr Tyr Val Asn Lys Gln Glu Gly Lys Ser
1 5 10 15

Leu Tyr Val Lys Gly Glu Pro Ile Ile Asn Phe Tyr Asp Pro Leu Val
20 25 30

Phe Pro Ser Asp Glu Phe Asp Ala Ser Ile Ser Gln Val Asn Glu Lys
35 40 45

Ile Asn Gln Ser Leu Ala Phe Ile Arg Lys Ser Asp Glu Leu Leu His
50 55 60

Asn Val Asn Ala Gly Lys
65 70

<210> 26
<211> 5
<212> PRT
<213> 呼吸道合胞病毒

<400> 26

Arg Ala Arg Arg Glu
1 5

[0022]

<210> 27
<211> 7
<212> PRT
<213> 呼吸道合胞病毒

<400> 27

Lys Lys Arg Lys Arg Arg Phe
1 5

<210> 28
<211> 15
<212> PRT
<213> 呼吸道合胞病毒

<400> 28

Leu Leu Gly Val Gly Ser Ala Ile Ala Ser Gly Ile Ala Val Ser
1 5 10 15

<210> 29
<211> 42
<212> PRT
<213> 呼吸道合胞病毒

<400> 29

Ser Leu Ile Asn Asp Met Pro Ile Thr Asn Asp Gln Lys Lys Leu Met
1 5 10 15

Ser Asn Asn Val Gln Ile Val Arg Gln Gln Ser Tyr Ser Ile Met Ser
20 25 30

Ile Ile Lys Glu Glu Val Leu Ala Tyr Val
35 40

<210> 30
<211> 9
<212> PRT
<213> 呼吸道合胞病毒

<400> 30

Ser Tyr Ile Gly Ser Ile Asn Asn Ile
1 5

<210> 31
<211> 9
<212> PRT
<213> 呼吸道合胞病毒

<400> 31

Asn Tyr Phe Glu Trp Pro Pro His Ala
1 5

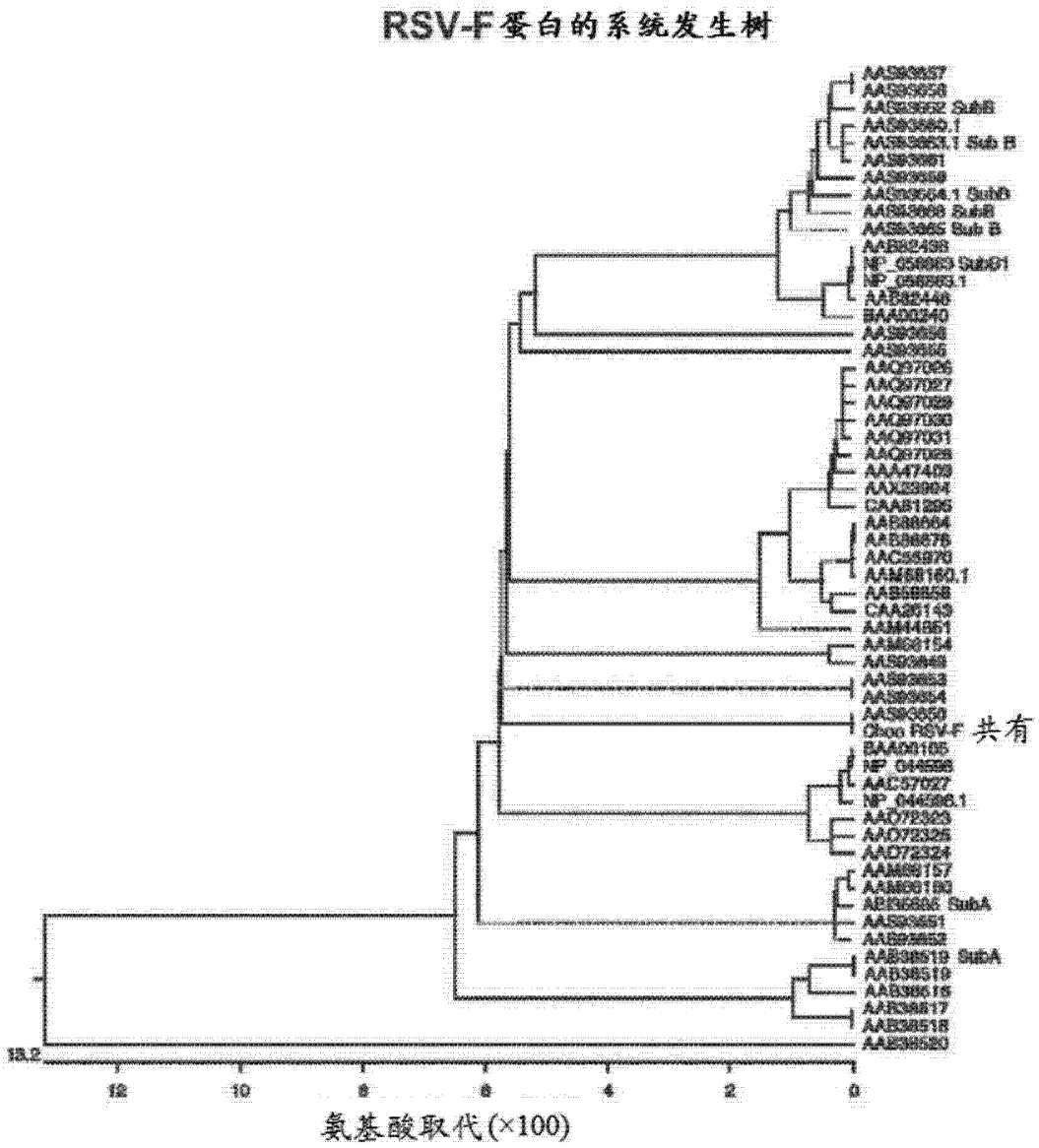

RSV-F蛋白的系统发生树
AA593657
AA593658
AA593662 SubB
AA593663.1
AA593663.1 Sub B
AA593661
AA593659
AA593664.1 SubB
AA593666 SubB
AA593665 Sub B
AAB82438
NP_056863 SubG1
NP_056863.1
AAB82446
BAA00240
AA593656
AA593655
AAQ97026
AAQ97027
AAQ97029
AAQ97030
AAQ97031
AAQ97028
AAA47409
AAX23904
CAA81295
AAB58664
AAB58676
AAC55970
AAM68160.1
AAB58658
CAA26143
AAM44861
AAM68154
AA593648
AA593653
AA593654
AA593650
Choo RSV-F 共有
BAA00185
NP_044598
AAC57027
NP_044596.1
AAD72323
AAD72325
AAD72324
AAM68157
AAM68160
AB105686 SubA
AA593651
AA593652
AAB38519 SubA
AAB38519
AAB38515
AAB38517
AAB38518
AAB38520
18.2
12
10
8
6
4
2
0
氨基酸取代(×100)

图 1

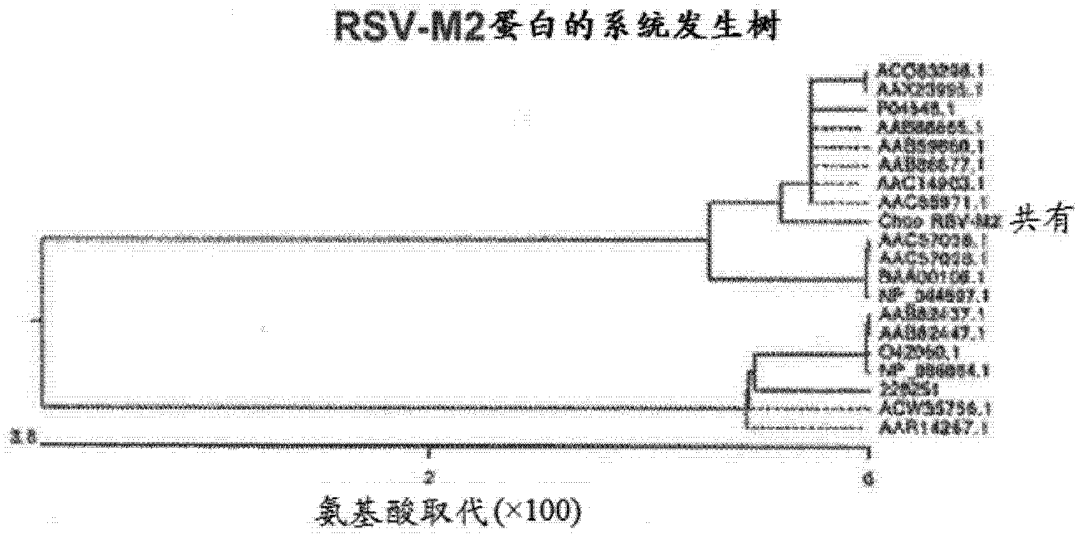


图 2

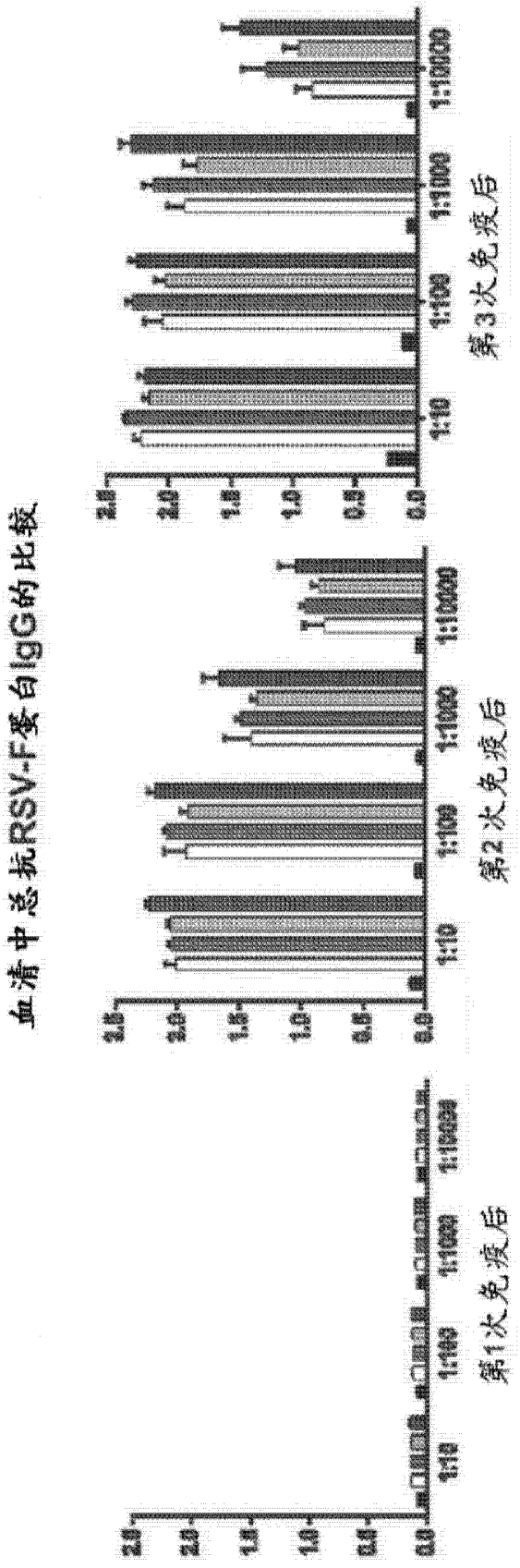

血清中总抗RSV-F蛋白IgG的比较
第1次免疫后
第2次免疫后
第3次免疫后
1:10
1:100
1:1000
1:10000
1:100000
0.0
0.5
1.0
1.5
2.0
2.5

图 3

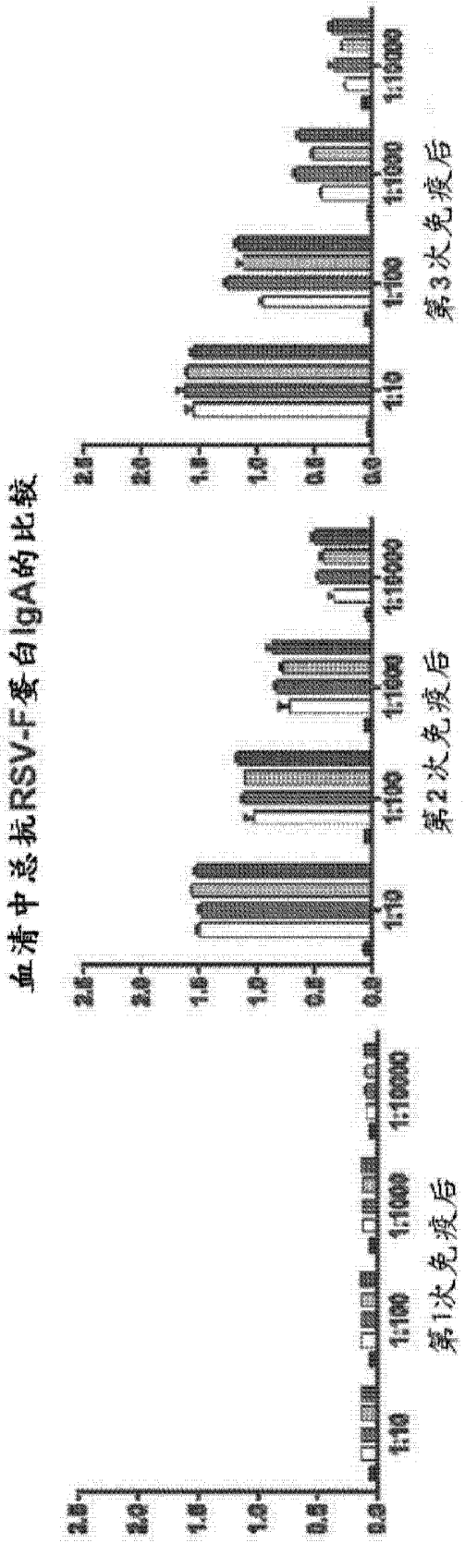

血清中总抗RSV-F蛋白IgA的比较
第1次免疫后
第2次免疫后
第3次免疫后
1:10
1:100
1:1000
1:10000
1:100000
0.0
0.5
1.0
1.5
2.0
2.5

图 4

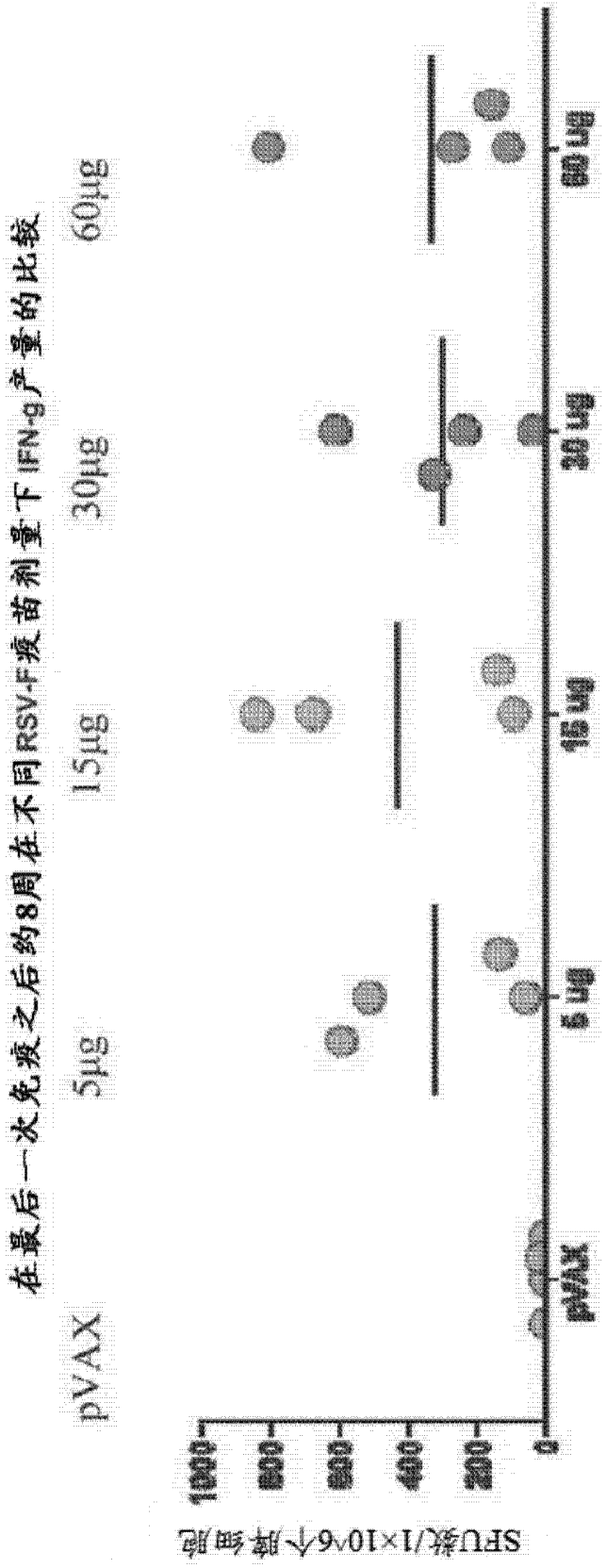

在最后一次免疫之后约8周在不同RSV-F疫苗剂量下IFN-g产量的比较
pVAX
5μg
15μg
30μg
60μg
SFU数/1×10^6个脾细胞
1000
800
600
400
200
0
pVAX
5 ug
15 ug
30 ug
60 ug

图 5

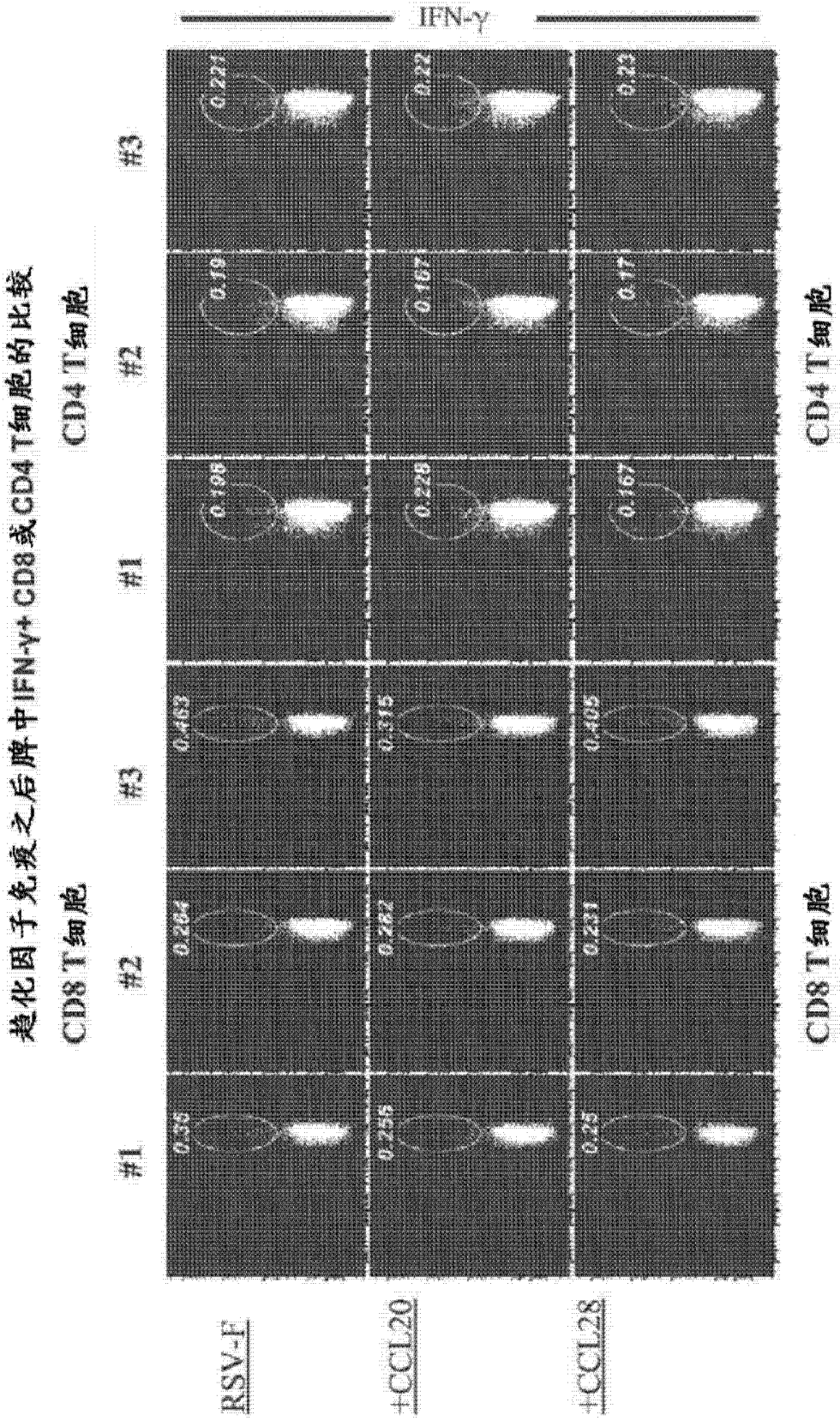

趋化因子免疫之后脾中IFN-γ+ CD8或CD4 T细胞的比较
IFN-γ
CD8 T细胞
CD4 T细胞
#1
#2
#3
RSV-F
+CCL20
+CCL28
0.36
0.284
0.463
0.198
0.198
0.221
0.255
0.282
0.315
0.228
0.167
0.22
0.25
0.231
0.405
0.167
0.17
0.23

图 6

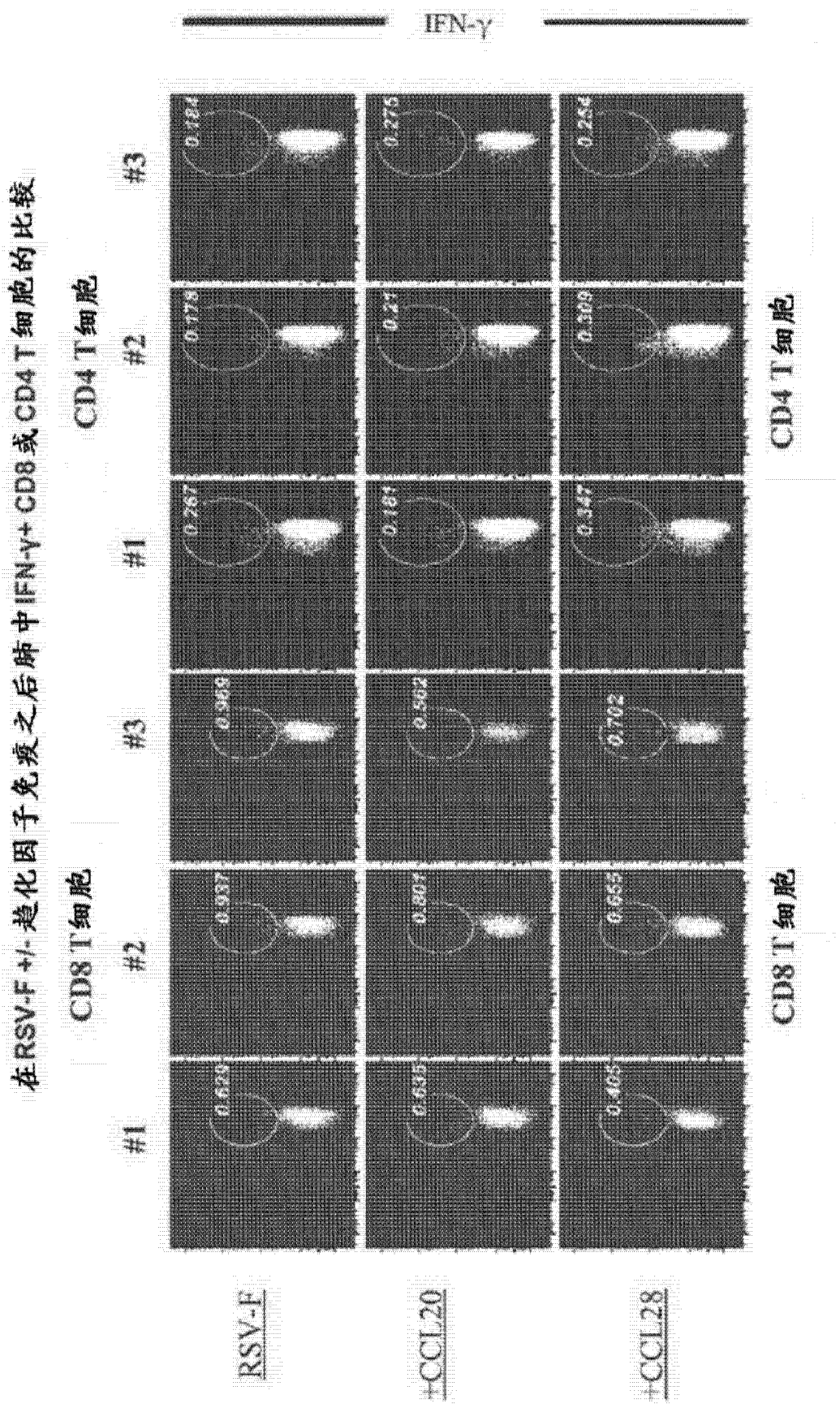

在RSV-F +/- 趋化因子免疫之后肺中IFN-γ+ CD8或CD4 T细胞的比较
IFN-γ
CD8 T细胞
CD4 T细胞
#1
#2
#3
RSV-F
+CCL20
+CCL28

图 7

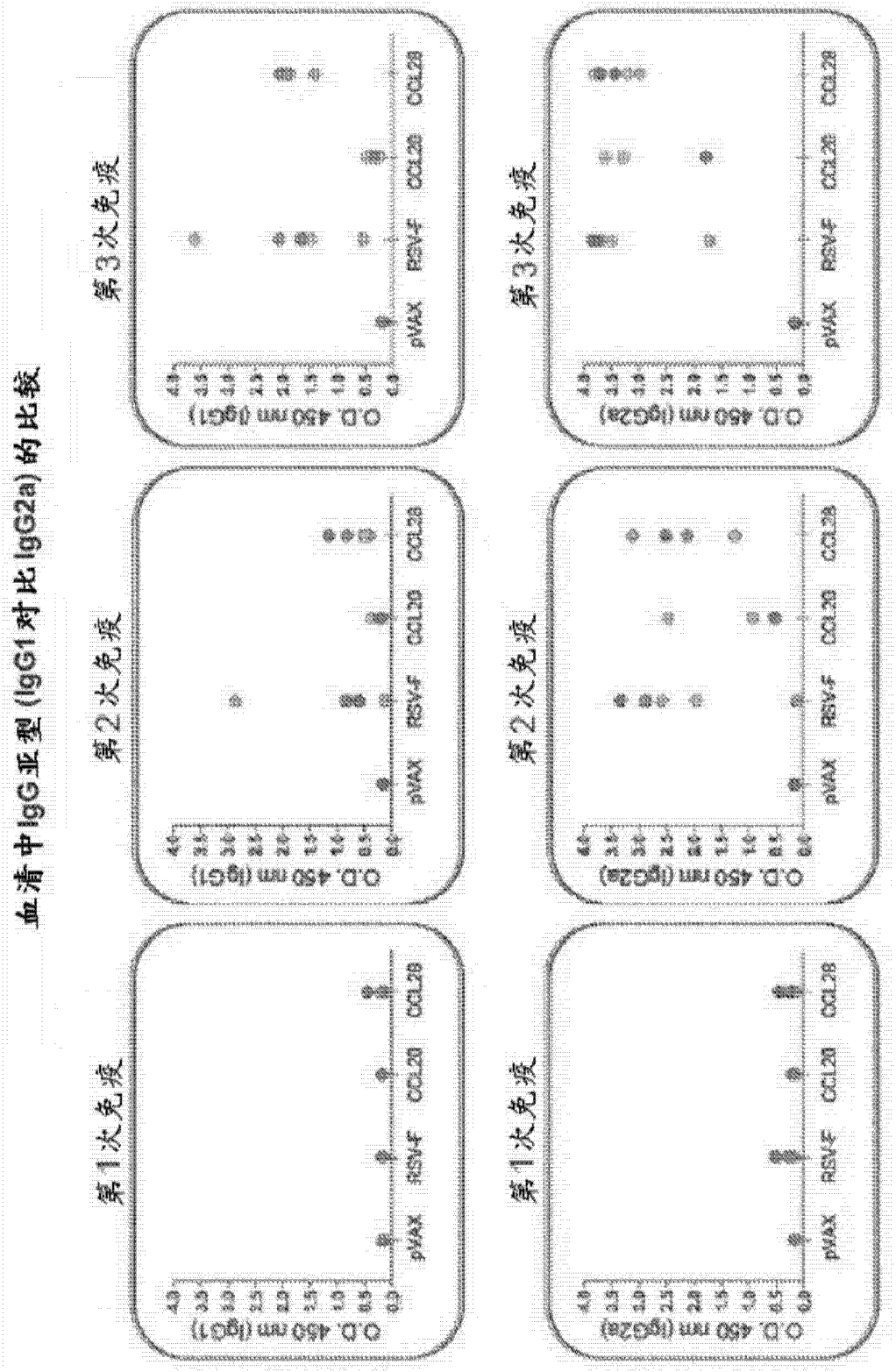

血清中IgG亚型(IgG1对比IgG2a)的比较
第1次免疫
第2次免疫
第3次免疫
O.D. 450 nm (IgG1)
O.D. 450 nm (IgG2a)
pVAX
RSV-F
CCL28

图 8

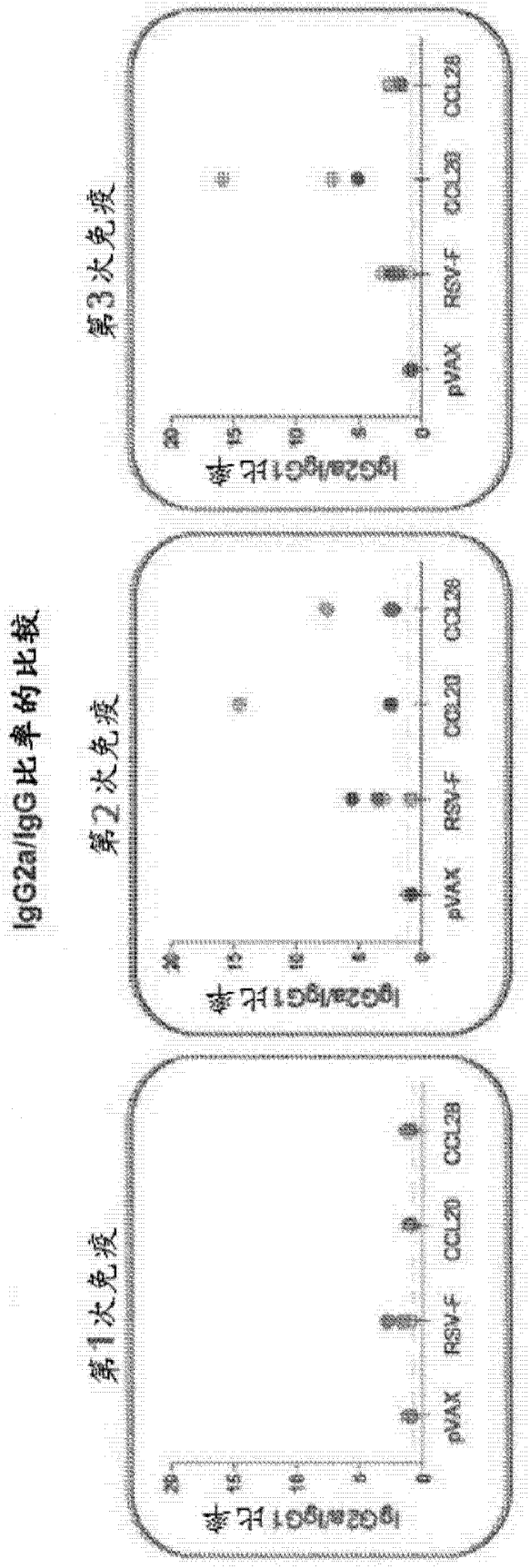

IgG2a/IgG比率的比较
第1次免疫
第2次免疫
第3次免疫
IgG2a/IgG1比率
pVAX
RSV-F
CCL20
CCL28
0
5
10
15
20

图 9

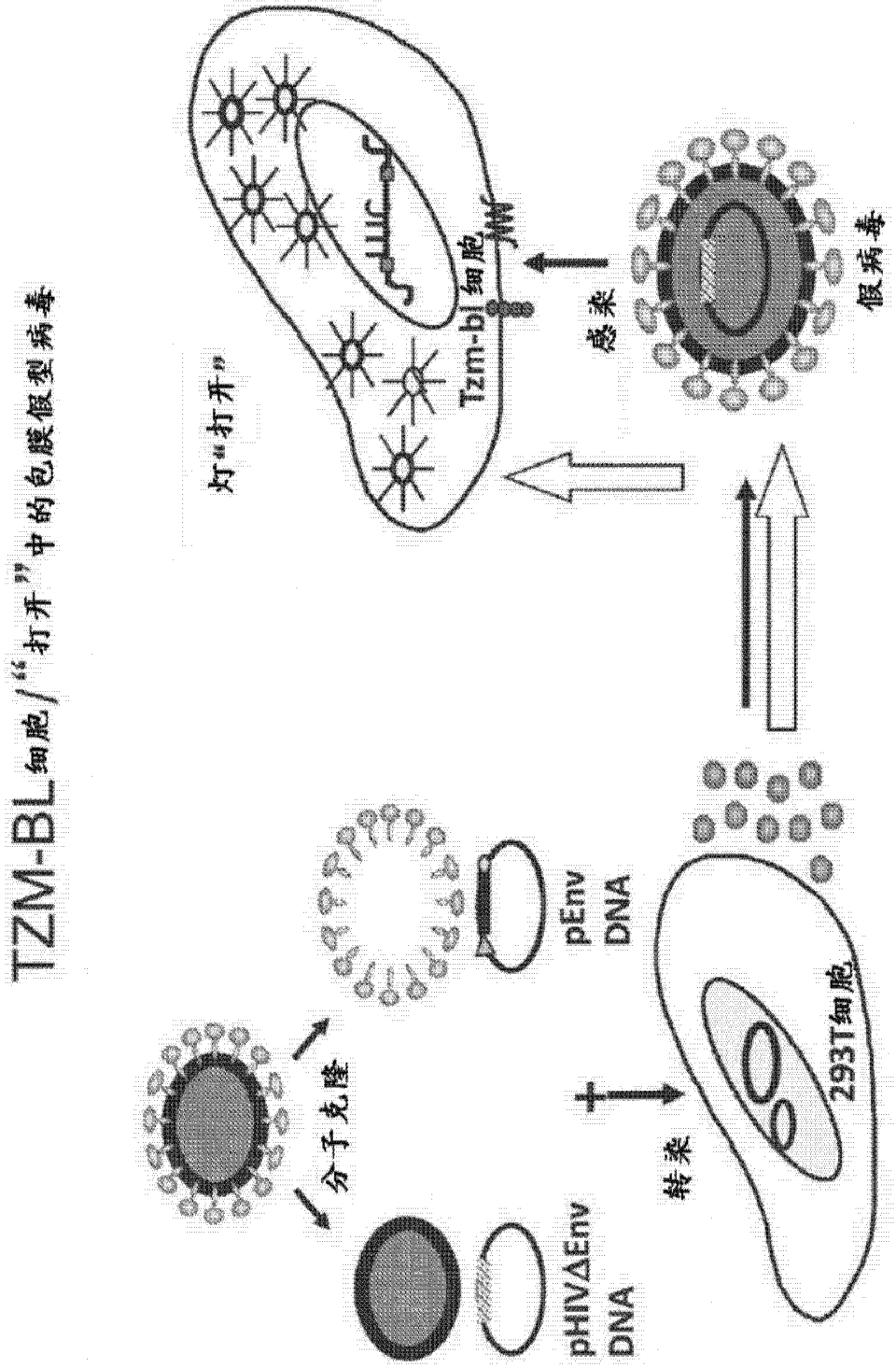

TZM-BL细胞/“打开”中的包膜假型病毒
灯“打开”
Tzm-bl细胞
感染
假病毒
pEnv
DNA
293T细胞
分子克隆
转染
pHIVΔEnv
DNA

图 10

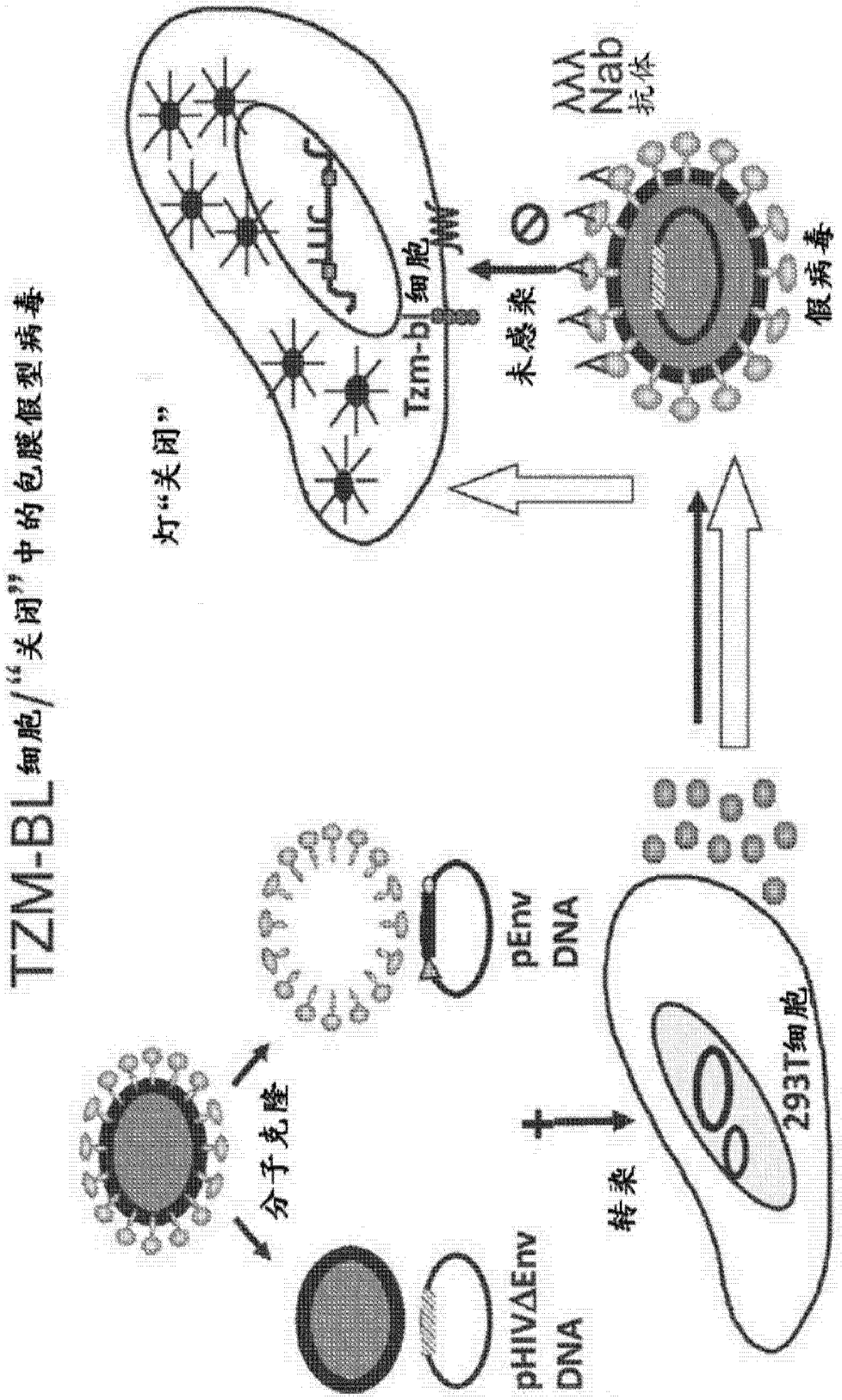

TZM-BL细胞/“关闭”中的包膜假型病毒
灯“关闭”
Tzm-bl细胞
未感染
MAb Nab抗体
假病毒
pEnv DNA
293T细胞
转染
分子克隆
pHIVΔEnv DNA

图 11

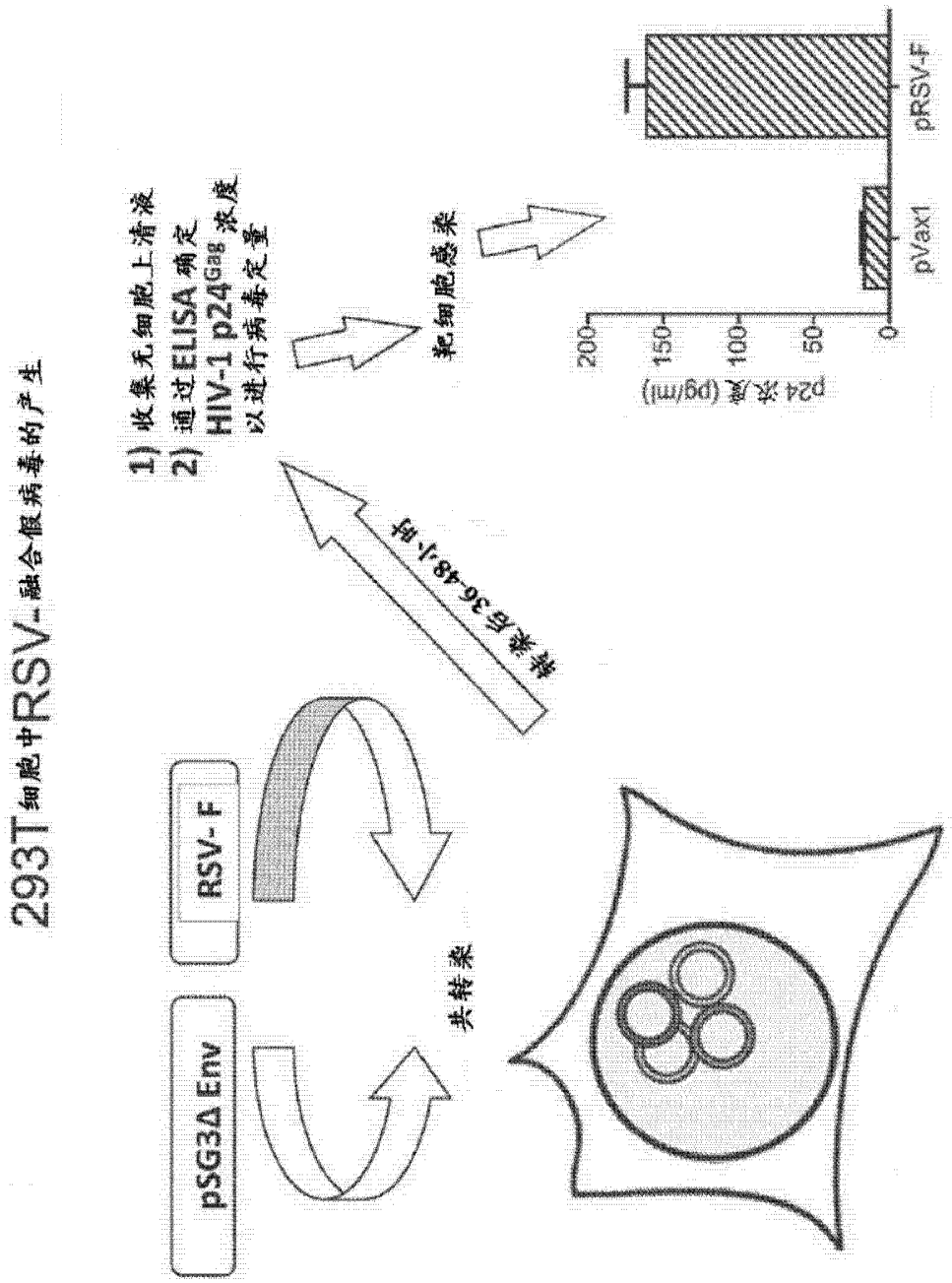

293T细胞中RSV-融合假病毒的产生
pSG3Δ Env
RSV- F
共转染
转染后36-48小时
1) 收集无细胞上清液
2) 通过ELISA确定
HIV-1 p24Gag 浓度
以进行病毒定量
靶细胞感染
pVax1
pRSV-F
0
50
100
150
200
p24水平 (pg/ml)

图 12

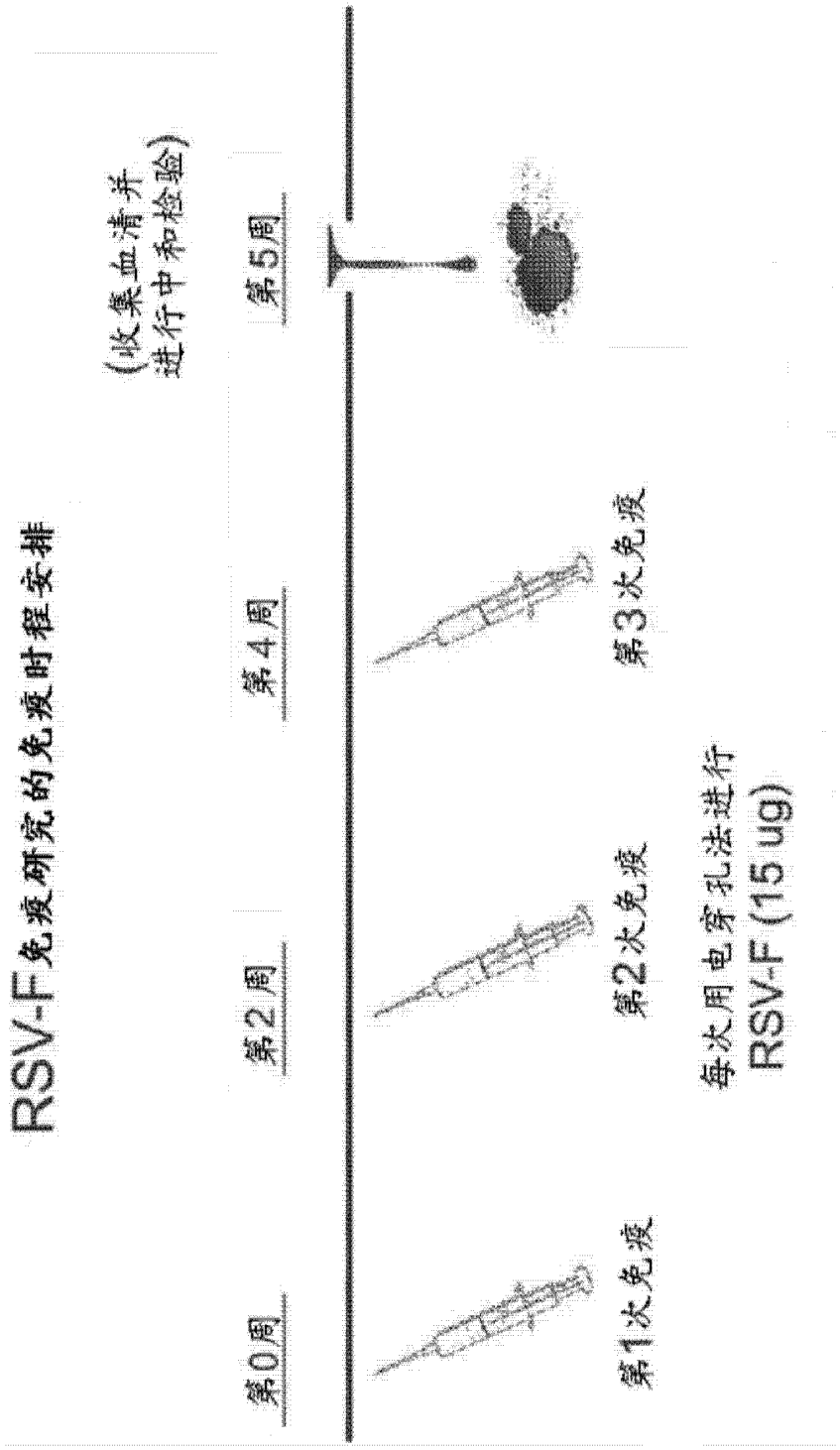

RSV-F免疫研究的免疫时程安排
第0周
第2周
第4周
第5周
(收集血清并
进行中和检验)
第1次免疫
第2次免疫
第3次免疫
每次用电穿孔法进行
RSV-F (15 ug)

图 13

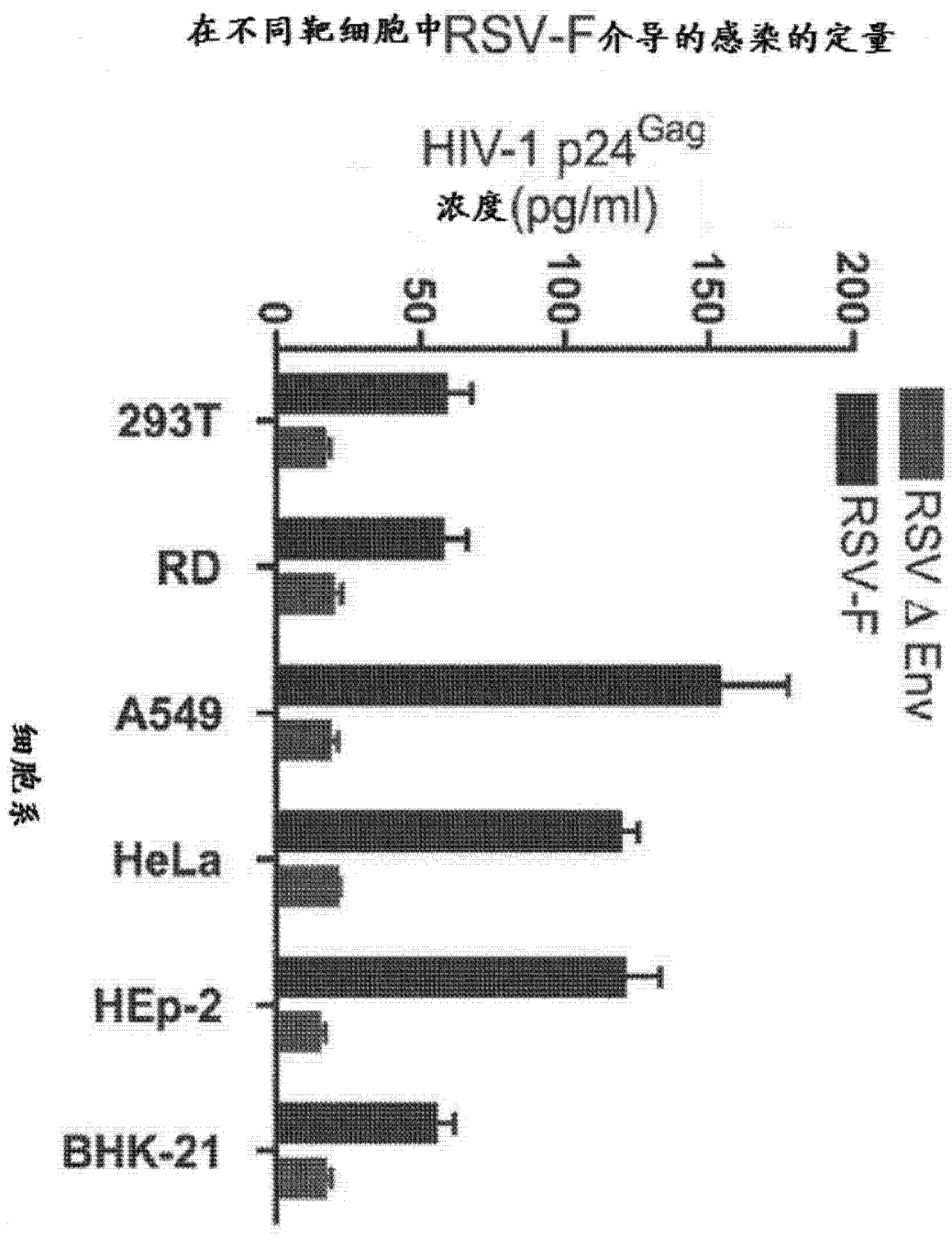
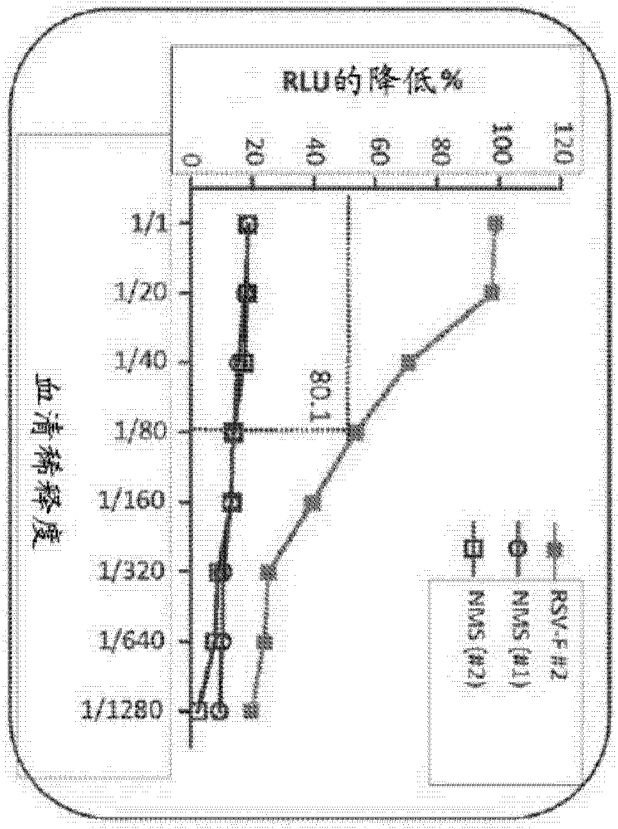

在不同靶细胞中RSV-F介导的感染的定量
HIV-1 p24Gag
浓度(pg/ml)
0
50
100
150
200
293T
RD
A549
HeLa
HEp-2
BHK-21
细胞系
RSV-F
RSV A Env

图 14

在TZM-bl HeLa细胞中RSV-F中和活性的定量



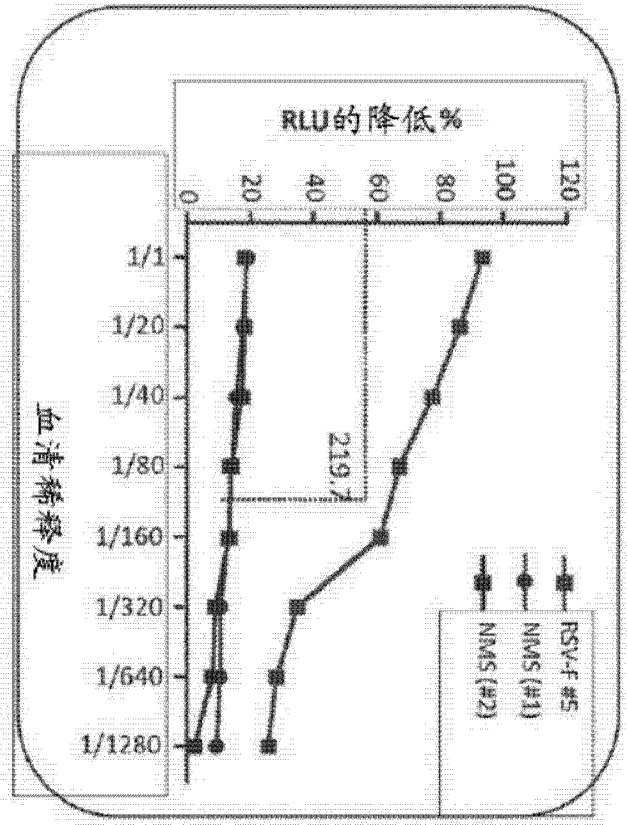


图 15

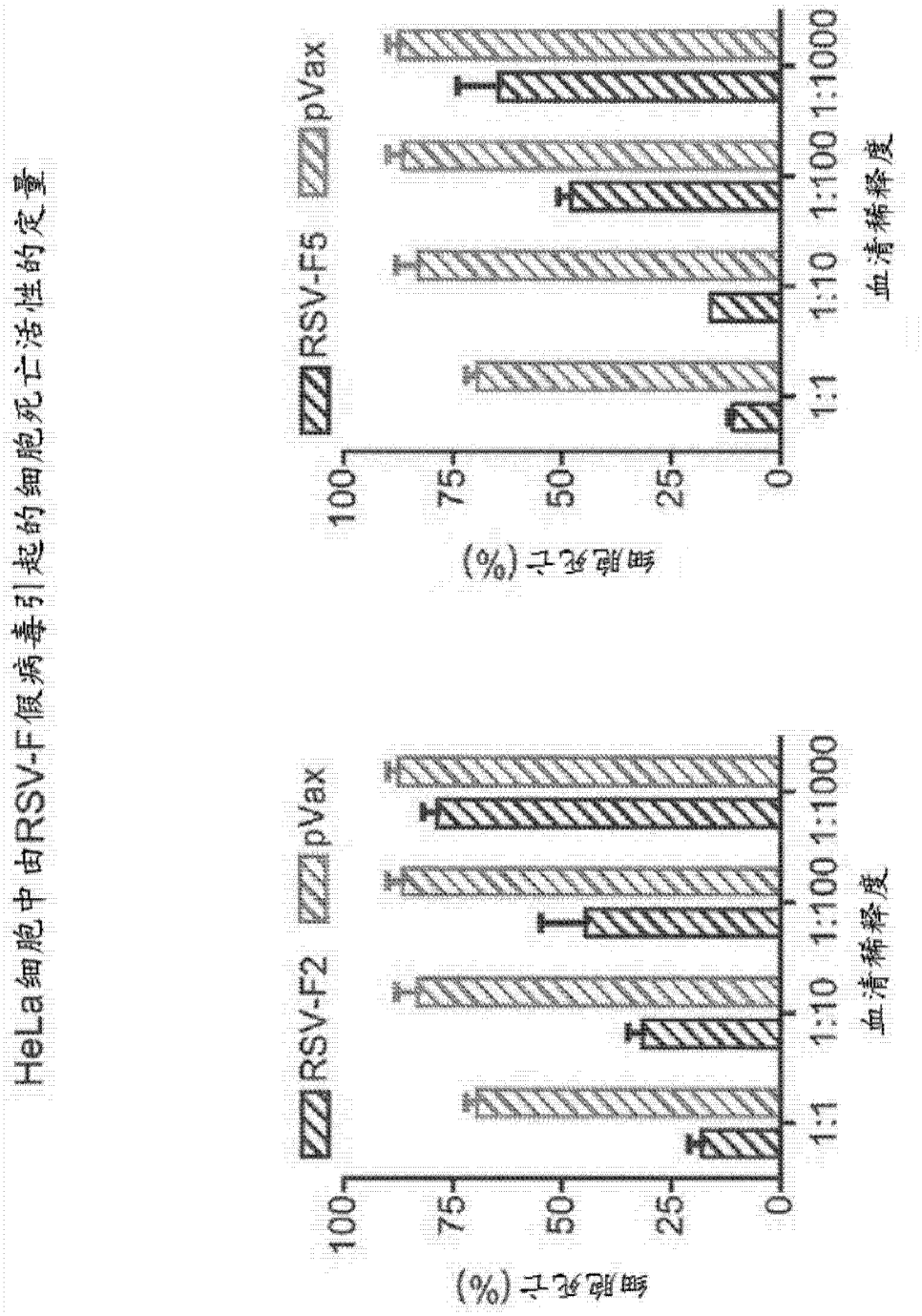

HeLa细胞中由RSV-F假病毒引起的细胞死亡活性的定量
RSV-F2
pVax
RSV-F5
pVax
细胞死亡 (%)
100
75
50
25
0
1:1
1:10
1:100
1:1000
血清稀释度

图 16

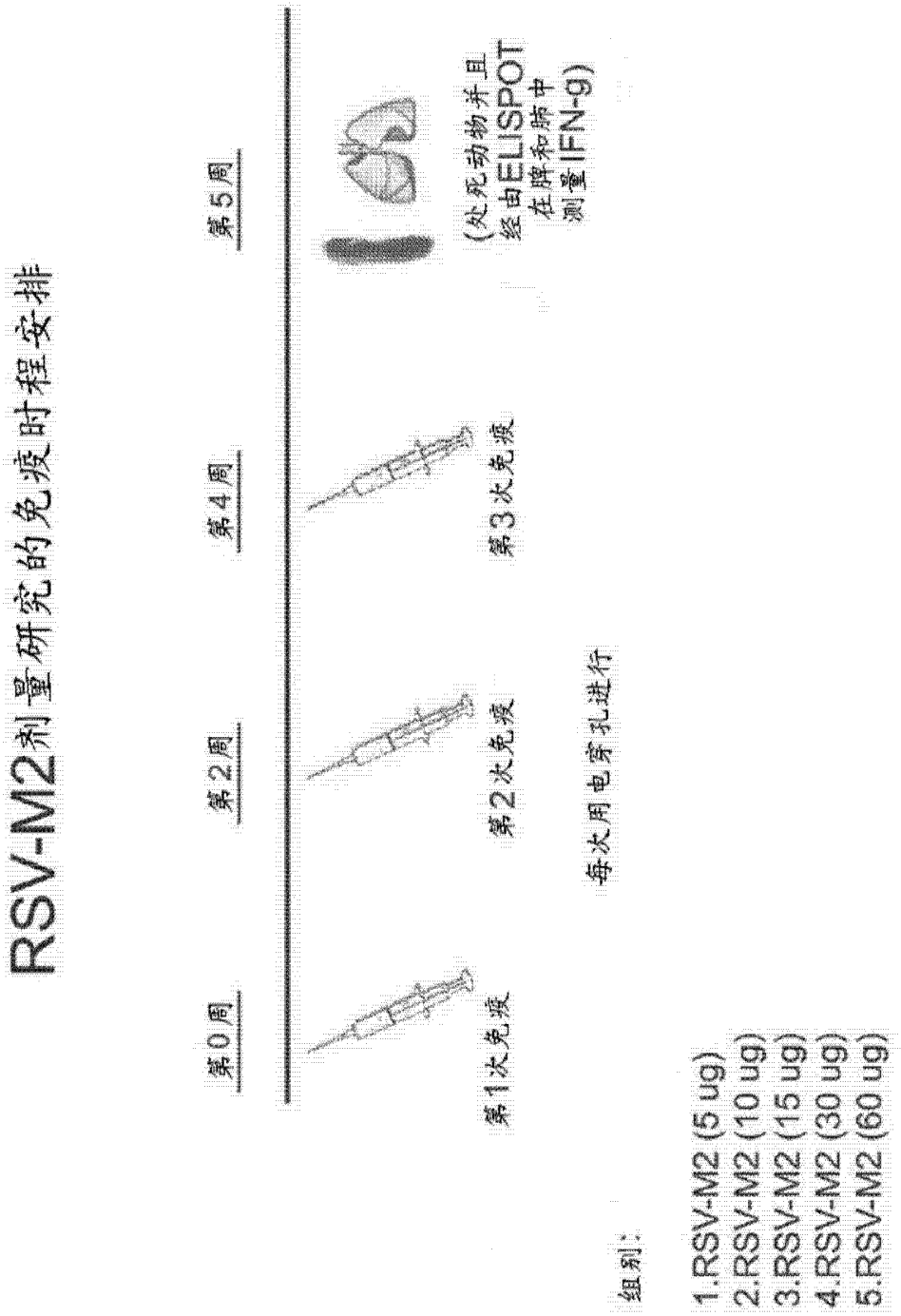

RSV-M2剂量研究的免疫时程安排
第0周
第2周
第4周
第5周
第1次免疫
第2次免疫
第3次免疫
每次用电穿孔进行
(处死动物并且
经由ELISPOT
在脾和肺中
测量IFN-g)
组别:
1.RSV-M2 (5 ug)
2.RSV-M2 (10 ug)
3.RSV-M2 (15 ug)
4.RSV-M2 (30 ug)
5.RSV-M2 (60 ug)

图 17

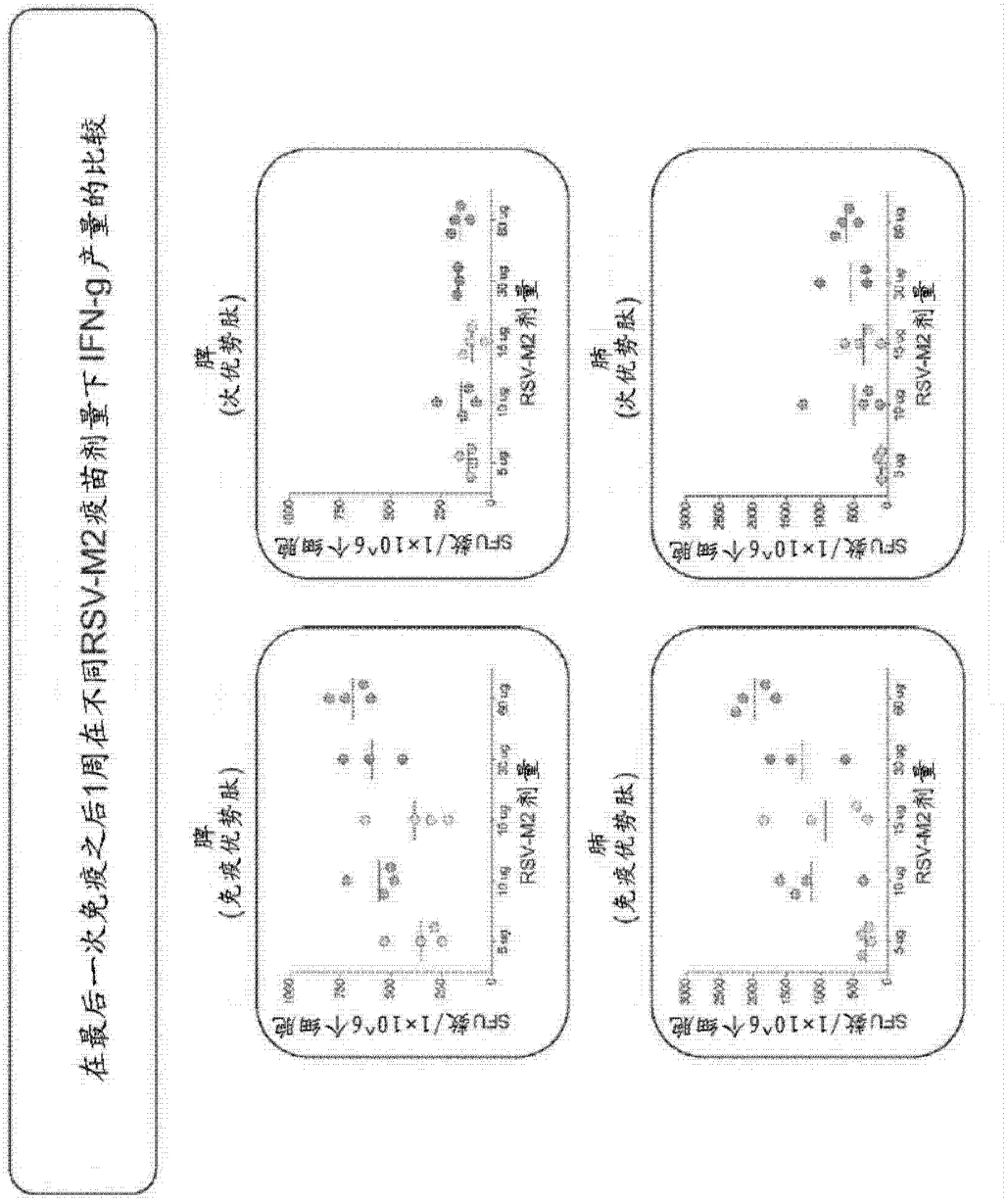

在最后一次免疫之后1周在不同RSV-M2疫苗剂量下IFN-g产量的比较
脾
(次优势肽)
肺
(次优势肽)
脾
(免疫优势肽)
肺
(免疫优势肽)
SFU数/1×10^6个细胞
RSV-M2剂量

图 18

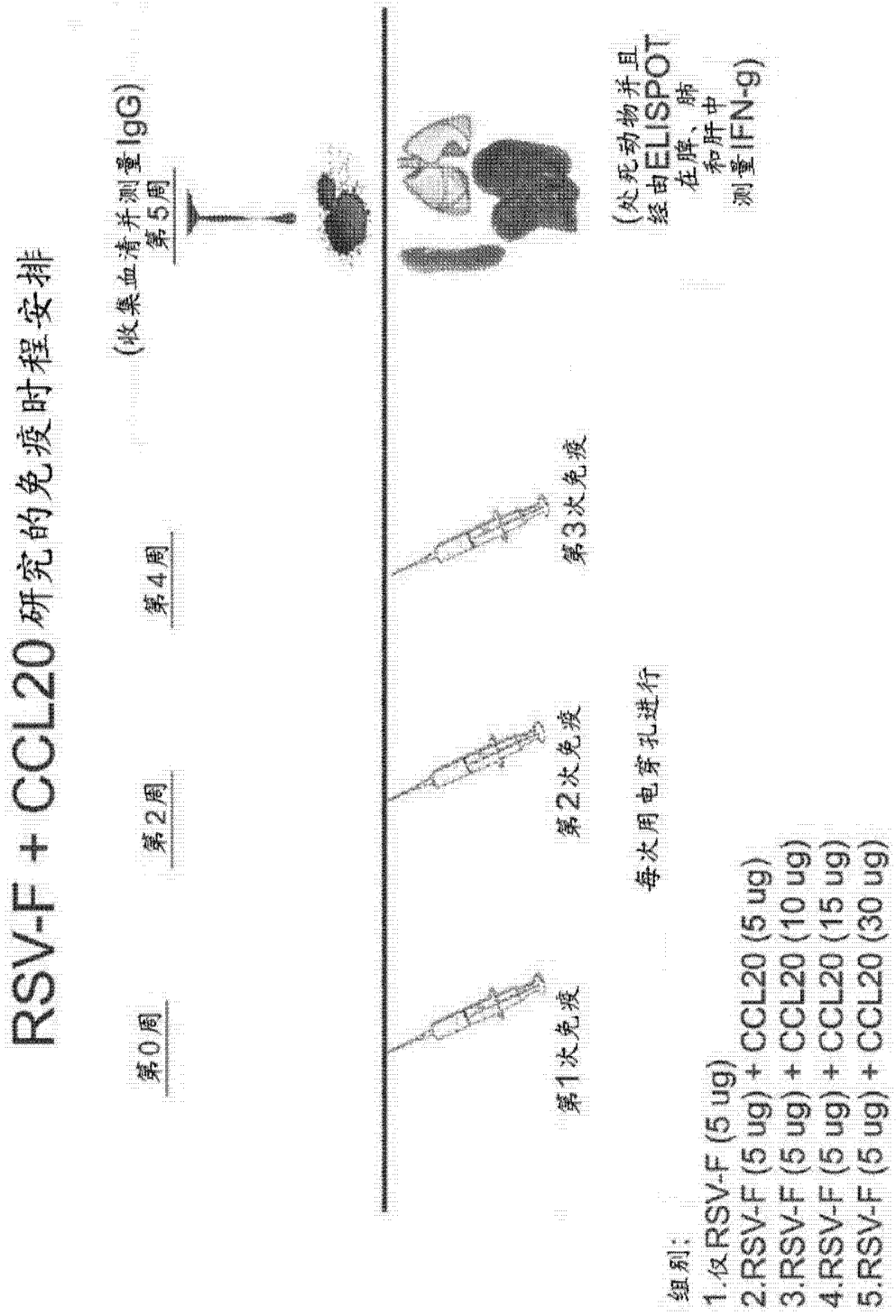

RSV-F + CCL20 研究的免疫时程安排
第0周
第2周
第4周
第5周
(收集血清并测量IgG)
第1次免疫
第2次免疫
第3次免疫
每次用电穿孔进行
(处死动物并且
经由ELISPOT
在脾、肺
和肝中
测量IFN-g)
组别:
1.仅RSV-F (5 ug)
2.RSV-F (5 ug) + CCL20 (5 ug)
3.RSV-F (5 ug) + CCL20 (10 ug)
4.RSV-F (5 ug) + CCL20 (15 ug)
5.RSV-F (5 ug) + CCL20 (30 ug)

图 19

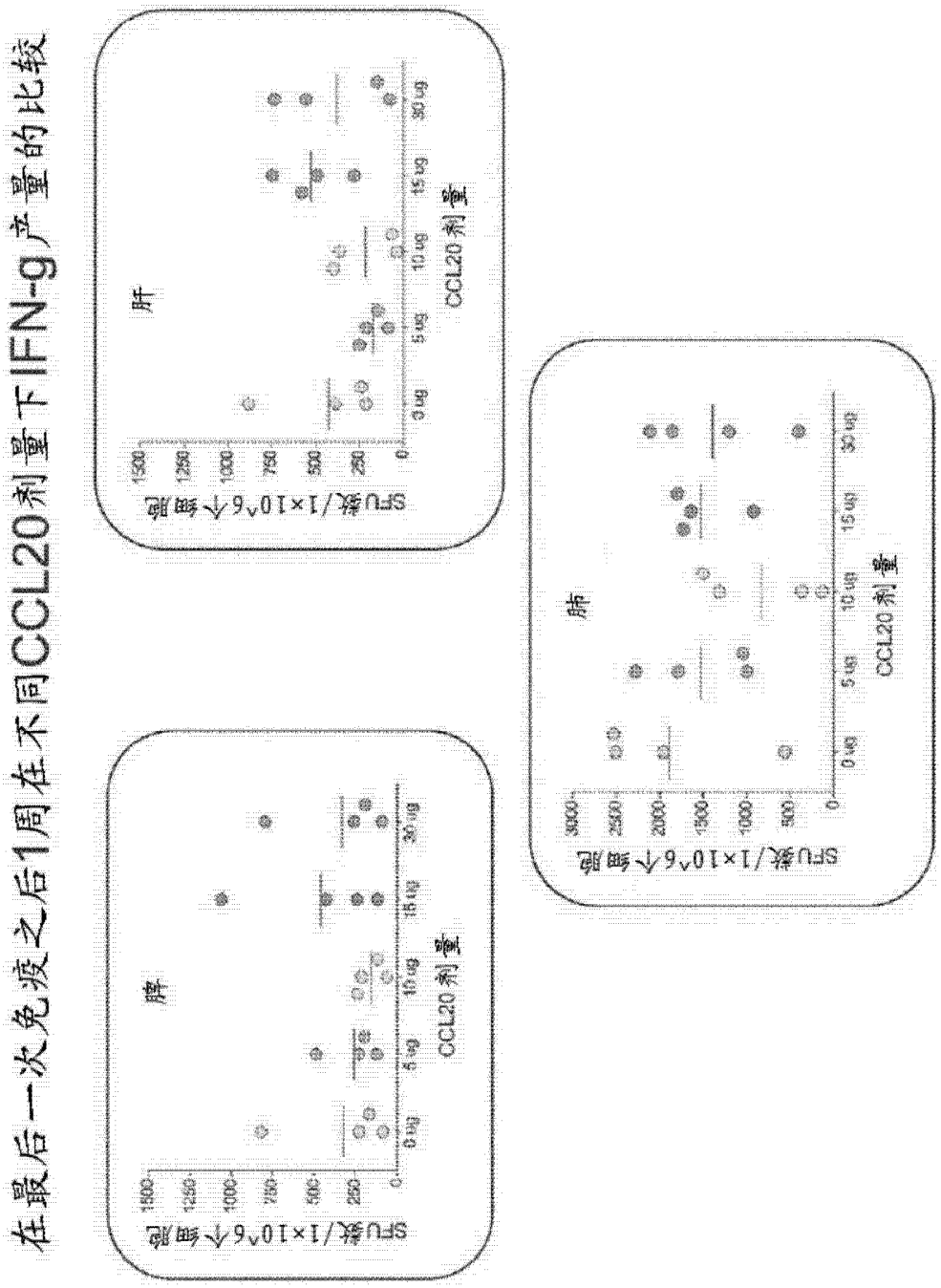

在最后一次免疫之后1周在不同CCL20剂量下IFN-g产量的比较
肝
脾
肺
CCL20 剂量
SFU数/1×10^6个细胞
0 ug
5 ug
10 ug
15 ug
30 ug

图 20

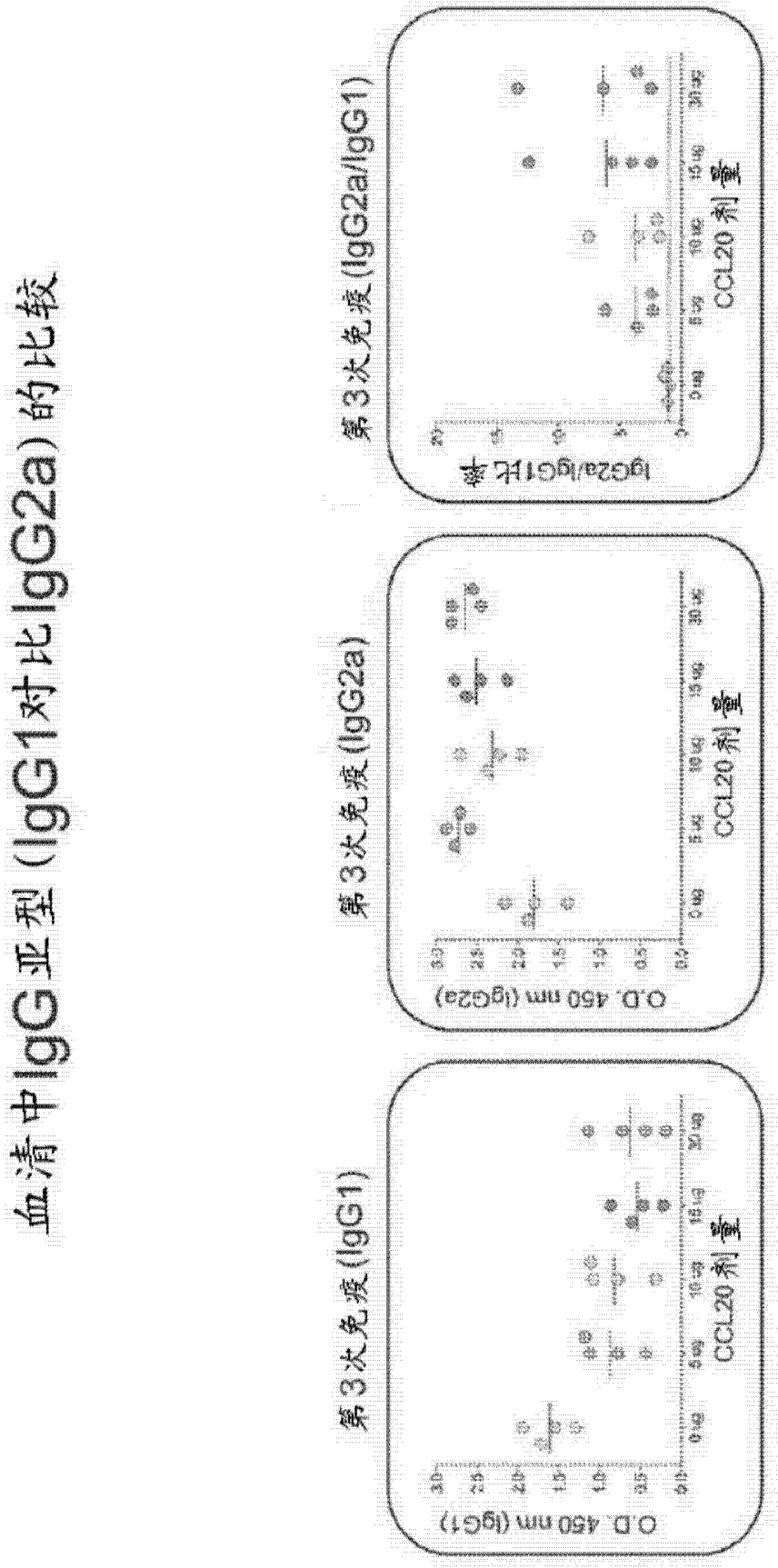

血清中IgG亚型(IgG1对比IgG2a)的比较
第3次免疫(IgG1)
O.D. 450 nm (IgG1)
CCL20 剂量
第3次免疫(IgG2a)
O.D. 450 nm (IgG2a)
CCL20 剂量
第3次免疫(IgG2a/IgG1)
IgG2a/IgG1比率
CCL20 剂量

图 21

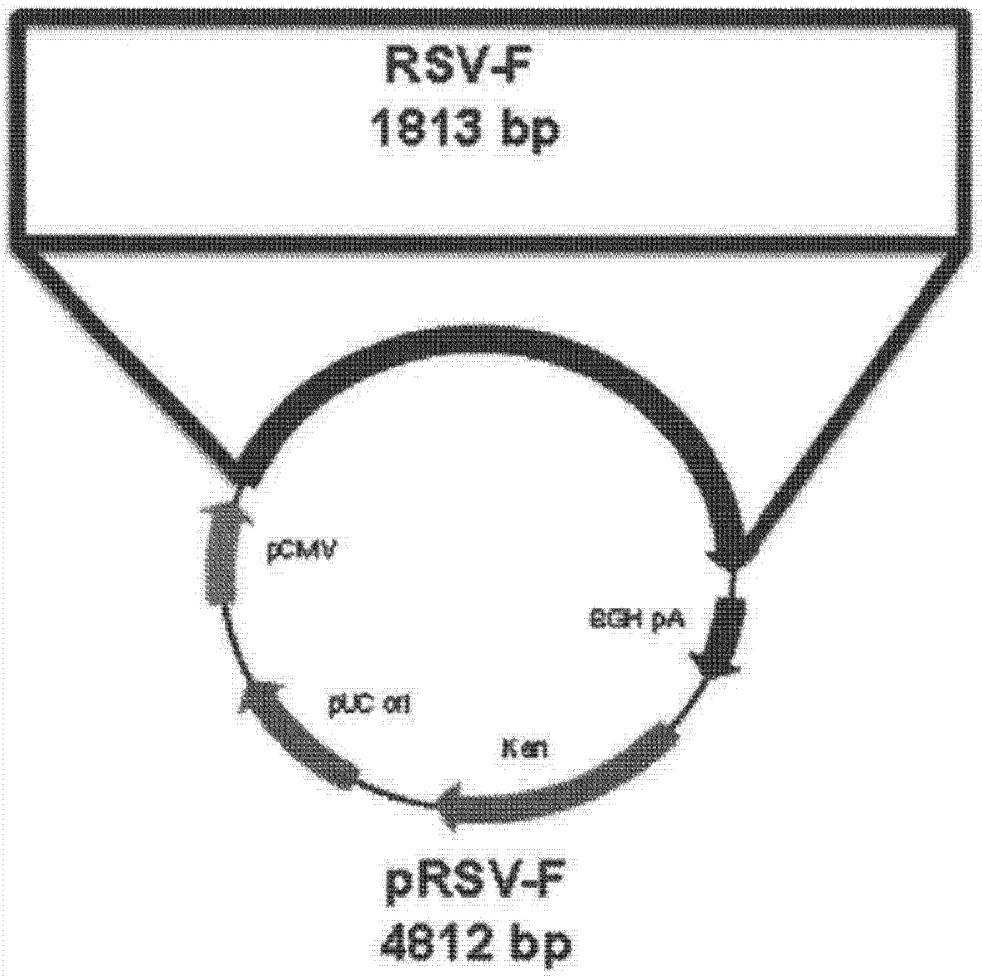


图 22A

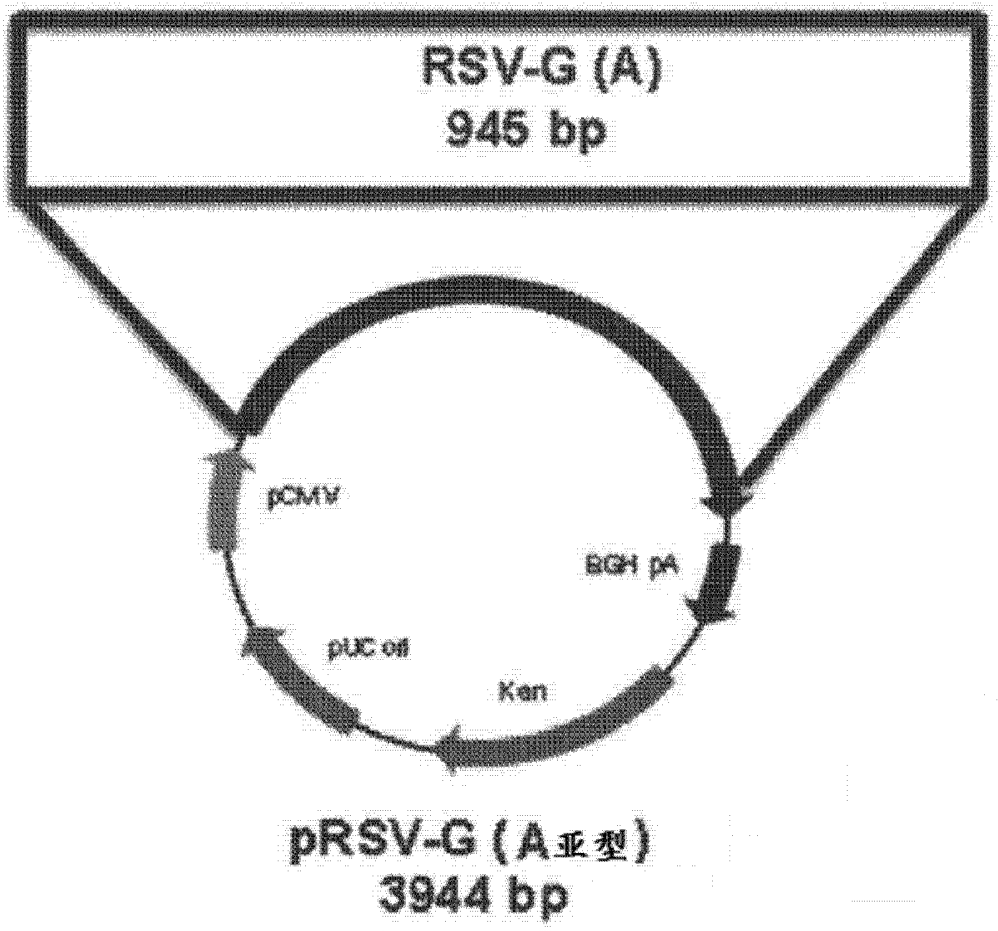


图 22B

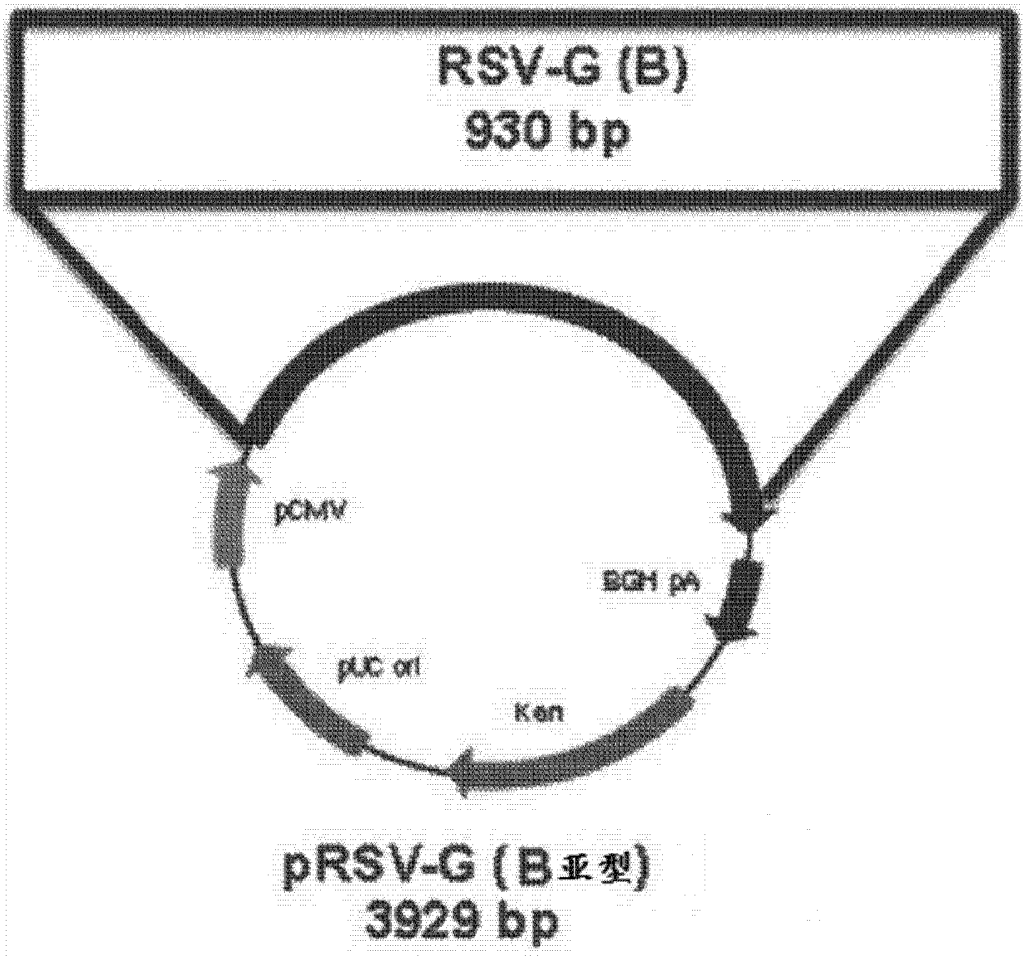


图 22C

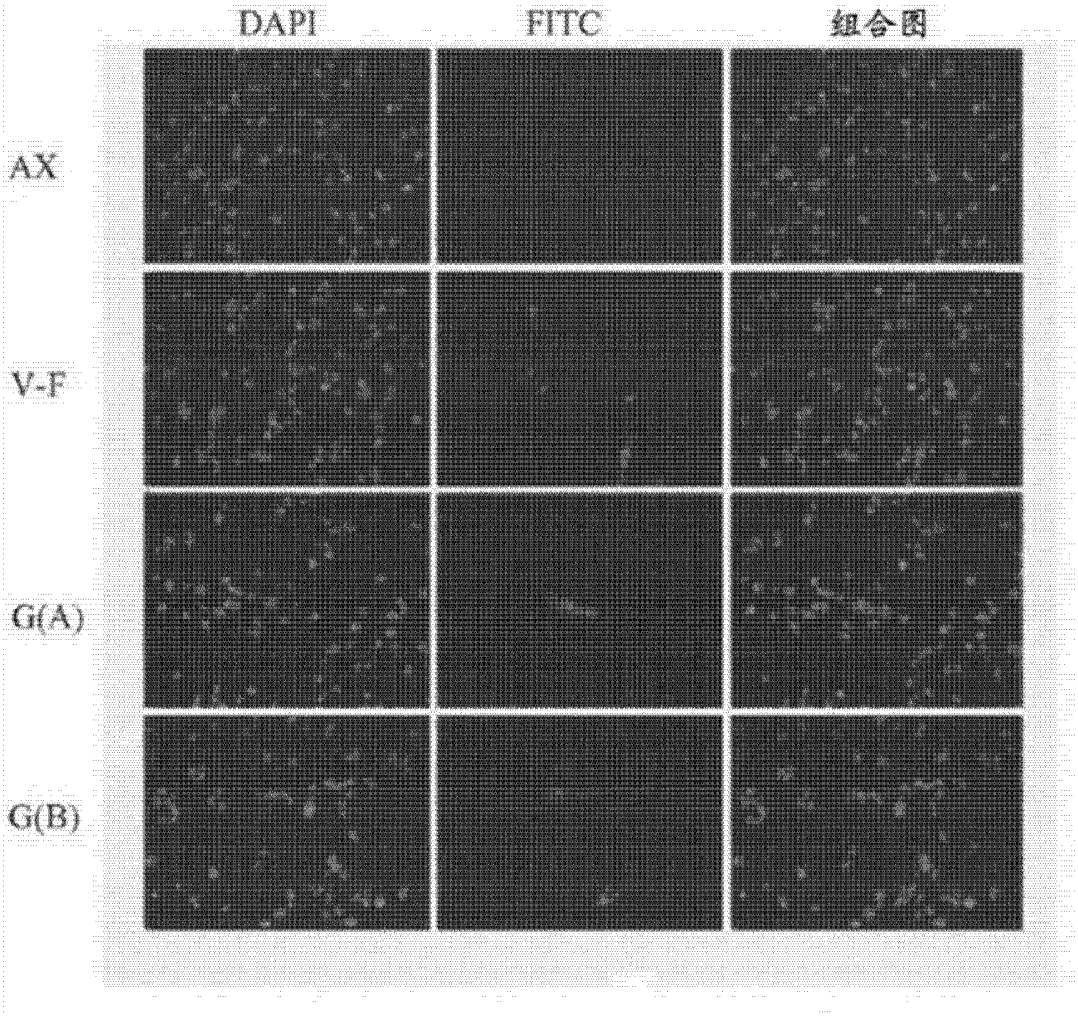


图 23

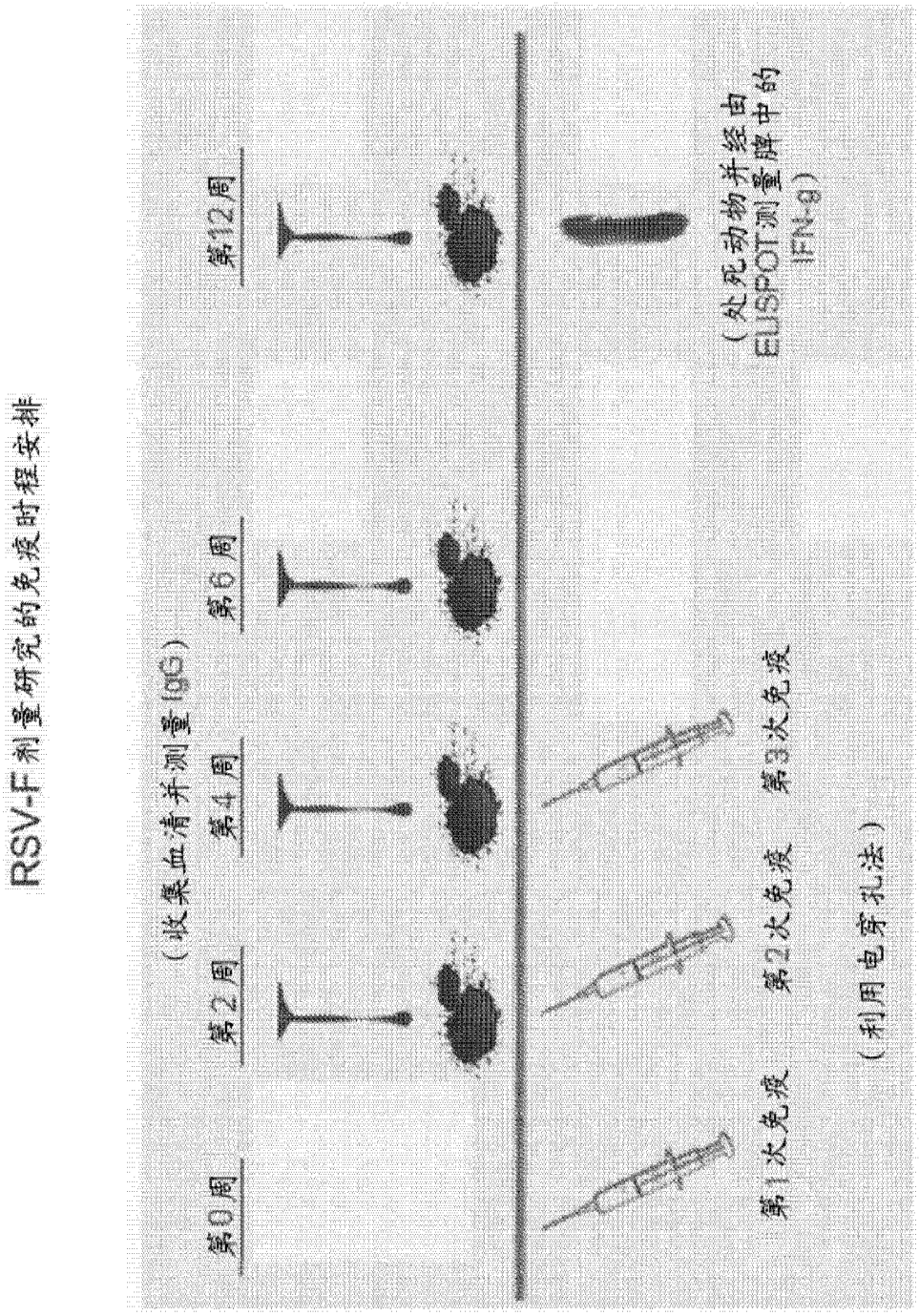

RSV-F 刺拿研究的免疫时程安排
第0周
第2周
第4周
第6周
第12周
（收集血清并测量 IgG）
第1次免疫
第2次免疫
第3次免疫
（利用电穿孔法）
（处死动物并经由
ELISPOT 测量脾中的
IFN-g）

图 24

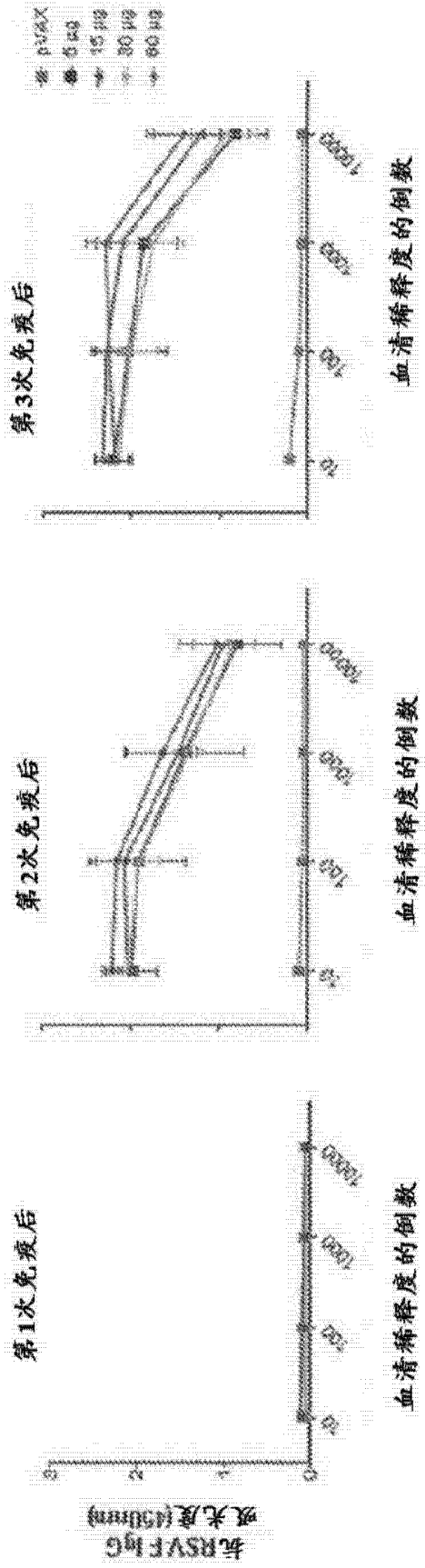

抗RSV F IgG
灵长类(45dmn)
第1次免疫后
血清稀释度的倒数
第2次免疫后
血清稀释度的倒数
第3次免疫后
血清稀释度的倒数

图 25

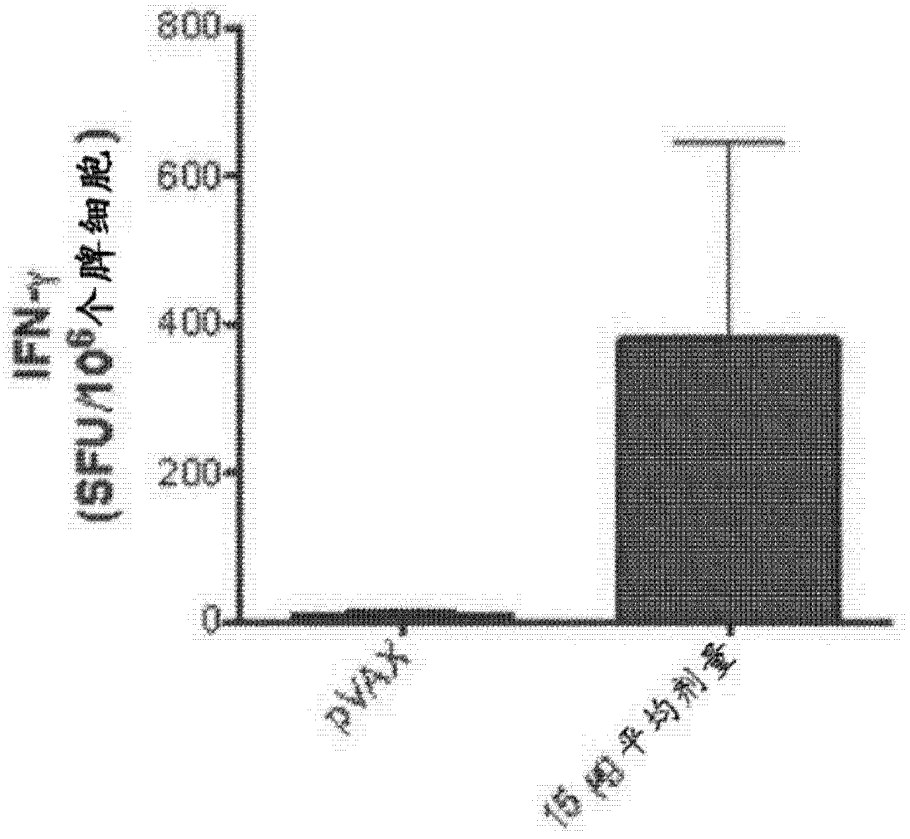


图 26

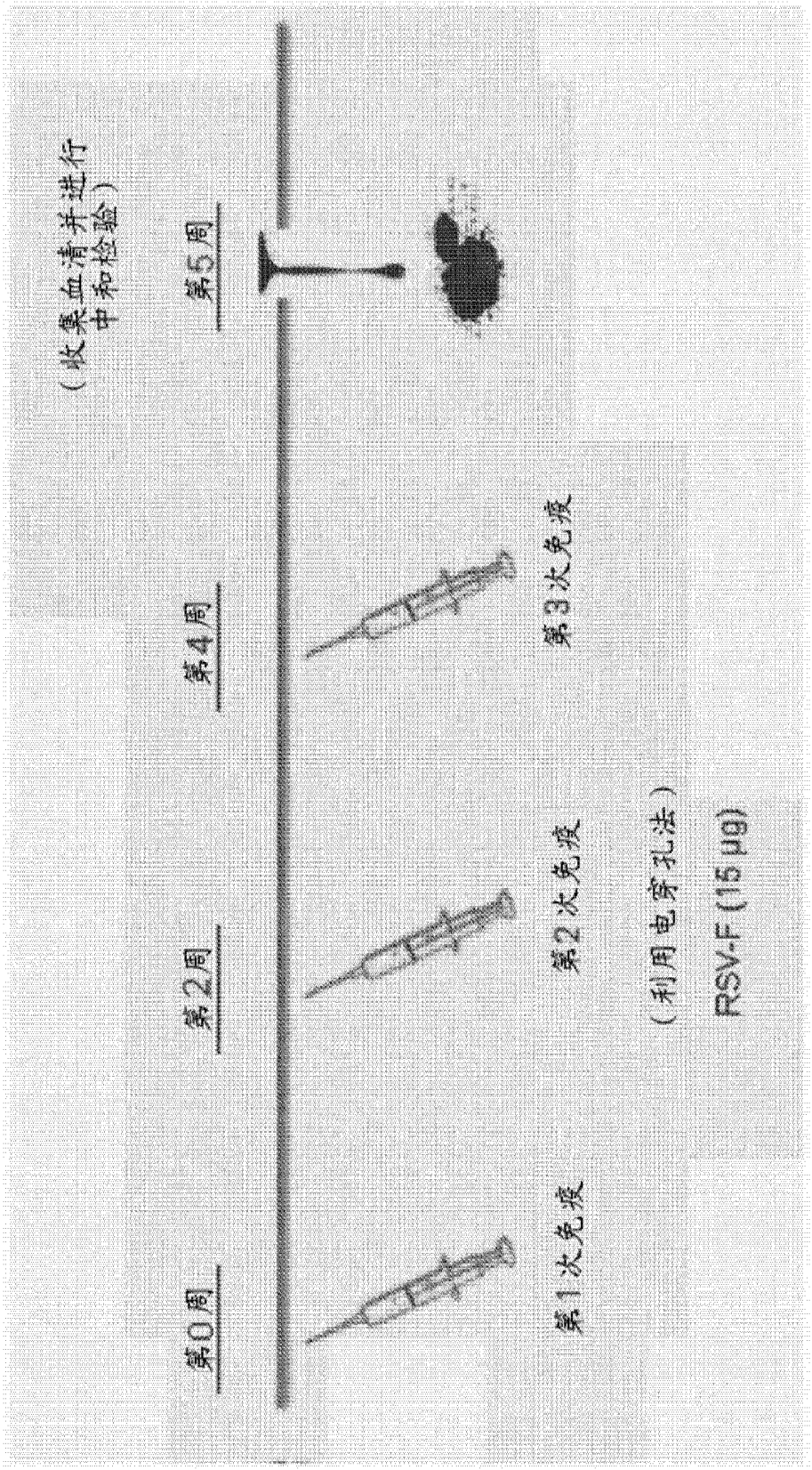

第0周
第2周
第4周
第5周
第1次免疫
第2次免疫
第3次免疫
RSV-F (15 μg)
(利用皮内孔法)
(收集血清并进行
中和检验)

图 27

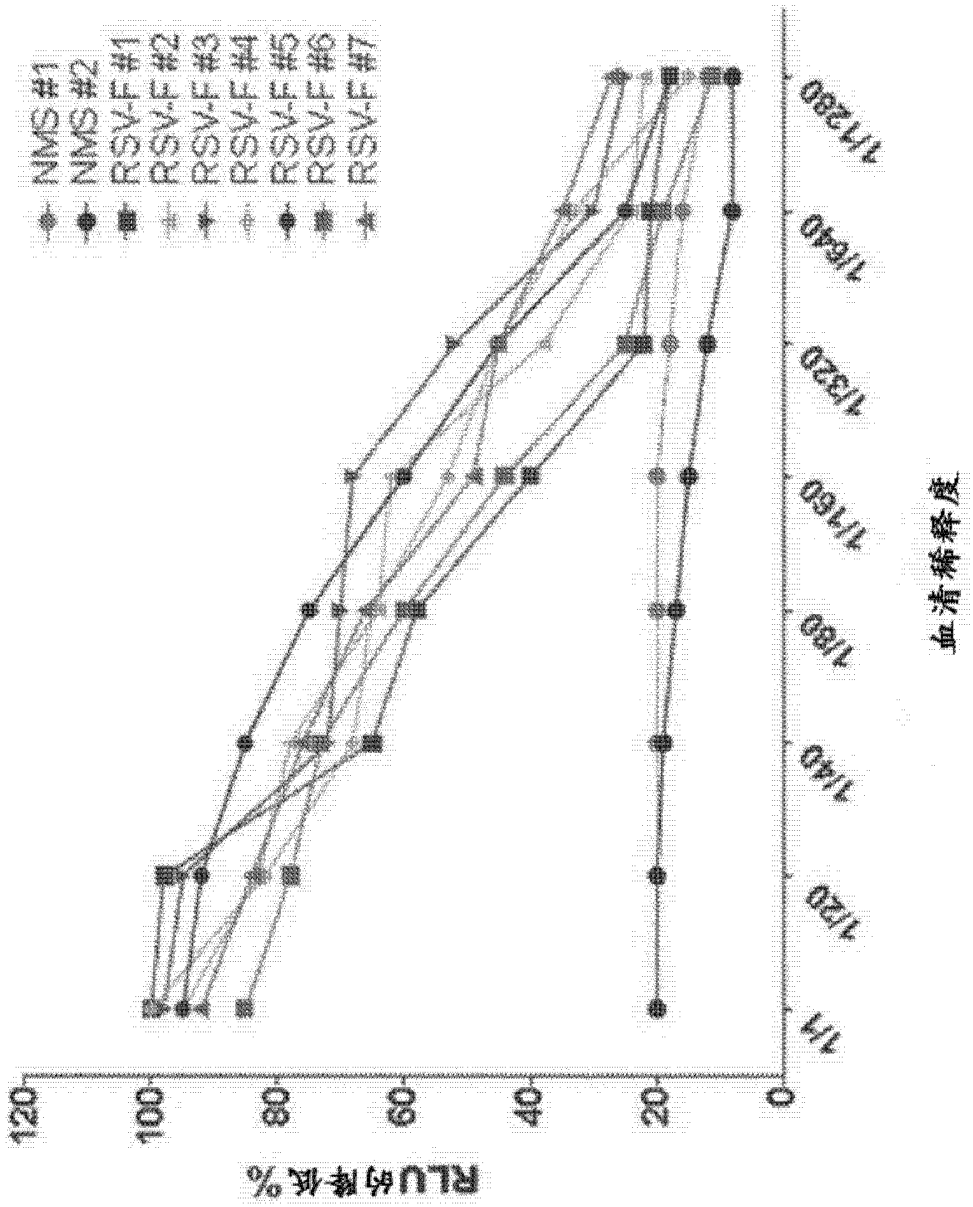

NMS #1
NMS #2
SV-EE #1
SV-EE #2
SV-EE #3
SV-EE #4
SV-EE #5
SV-EE #6
SV-EE #7
RLU的降低%
120
100
80
60
40
20
0
血清稀释度
1/1
1/20
1/40
1/80
1/160
1/320
1/640
1/1280

图 28

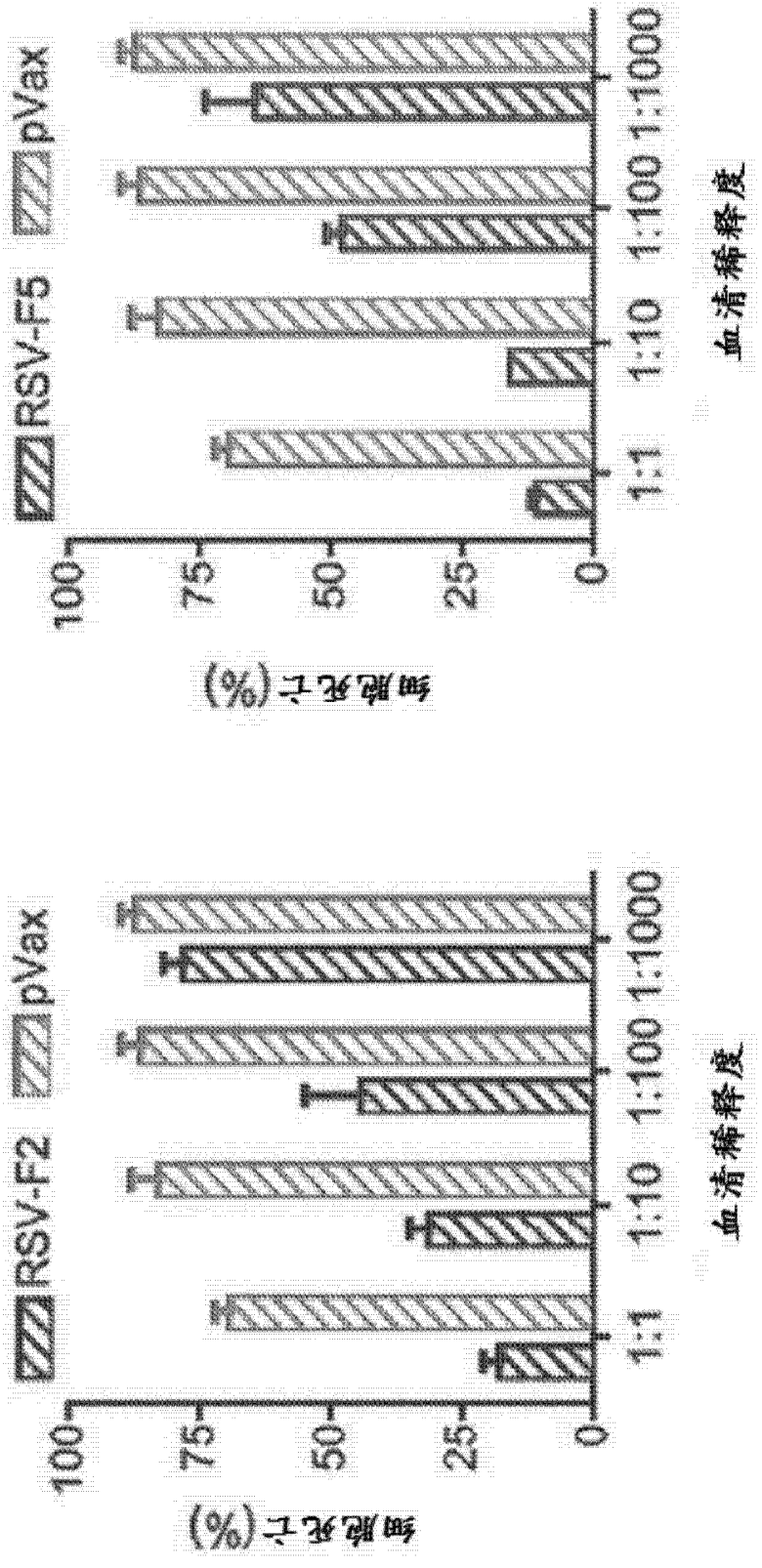

RSV-F5
pVax
100
75
50
25
0
细胞死亡（%）
1:1
1:10
1:100
1:1000
血清稀释度
RSV-F2
pVax
100
75
50
25
0
细胞死亡（%）
1:1
1:10
1:100
1:1000
血清稀释度

图 29

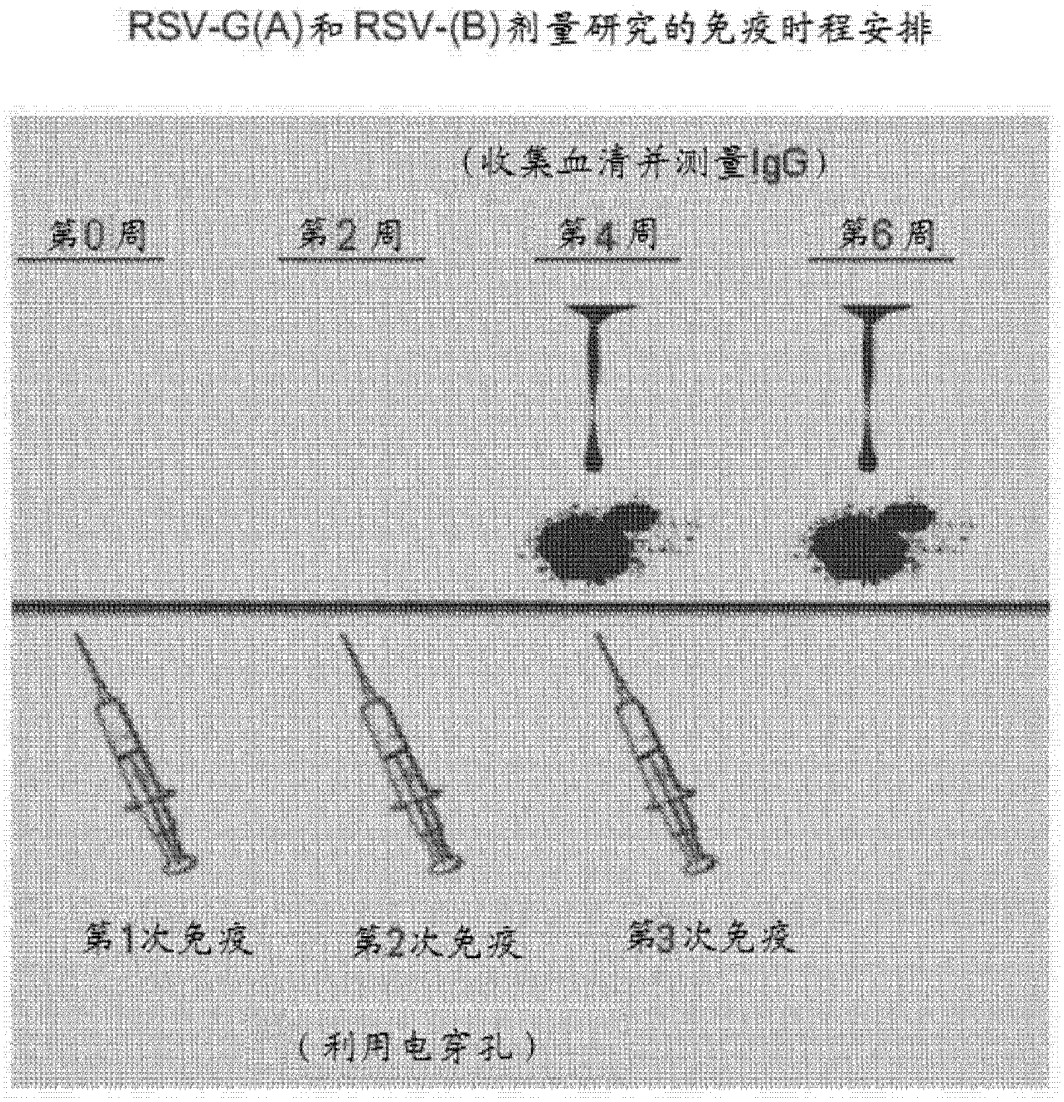

RSV-G(A)和RSV-(B)剂量研究的免疫时程安排
（收集血清并测量IgG）
第0周
第2周
第4周
第6周
第1次免疫
第2次免疫
第3次免疫
（利用电穿孔）

图 30

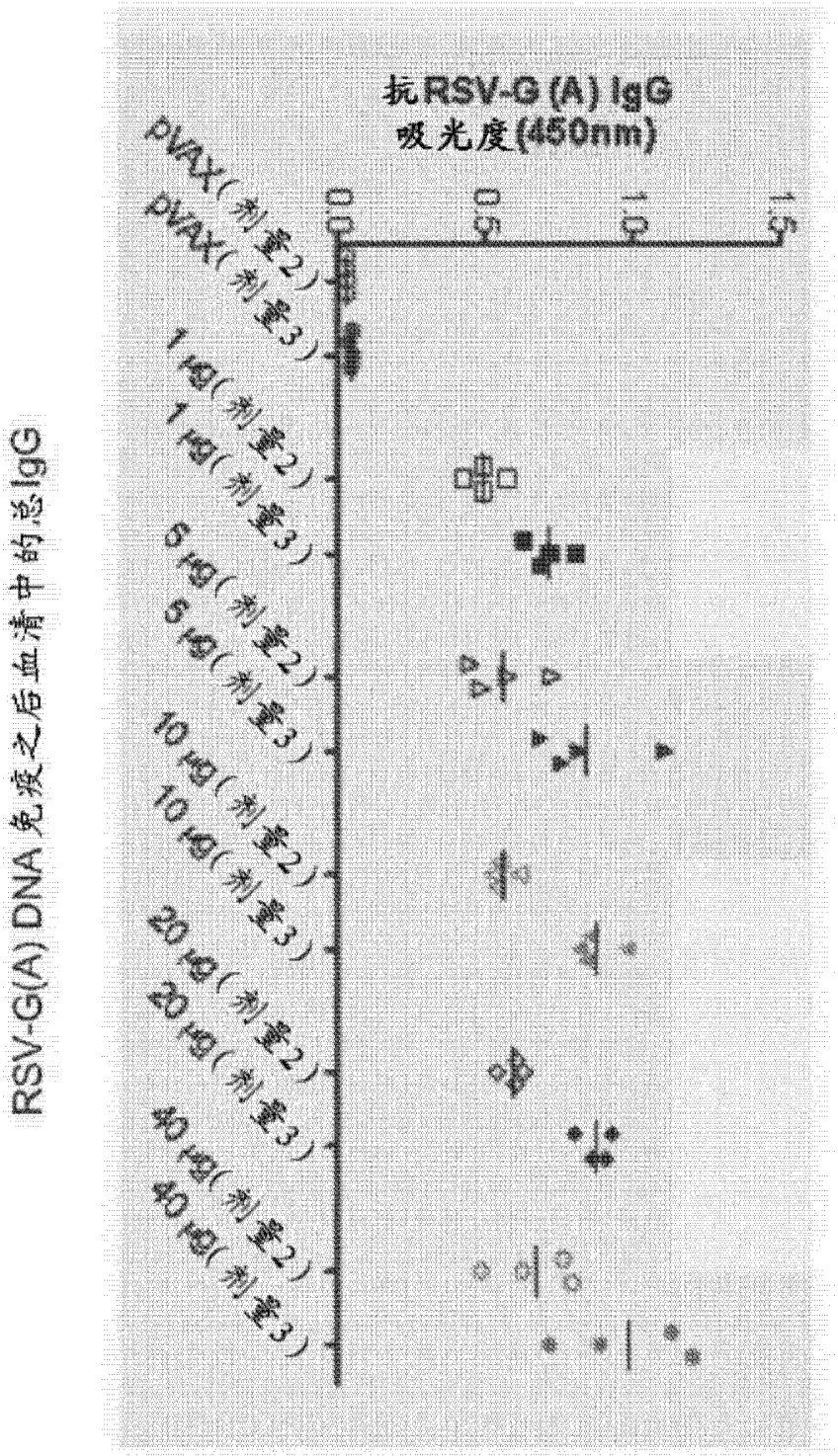

抗RSV-G (A) IgG
吸光度(450nm)
0.0
0.5
1.0
1.5
pVAX(剂量2)
pVAX(剂量3)
1 μg(剂量2)
1 μg(剂量3)
5 μg(剂量2)
5 μg(剂量3)
10 μg(剂量2)
10 μg(剂量3)
20 μg(剂量2)
20 μg(剂量3)
40 μg(剂量2)
40 μg(剂量3)
RSV-G(A) DNA免疫之后血清中的总IgG

图 31

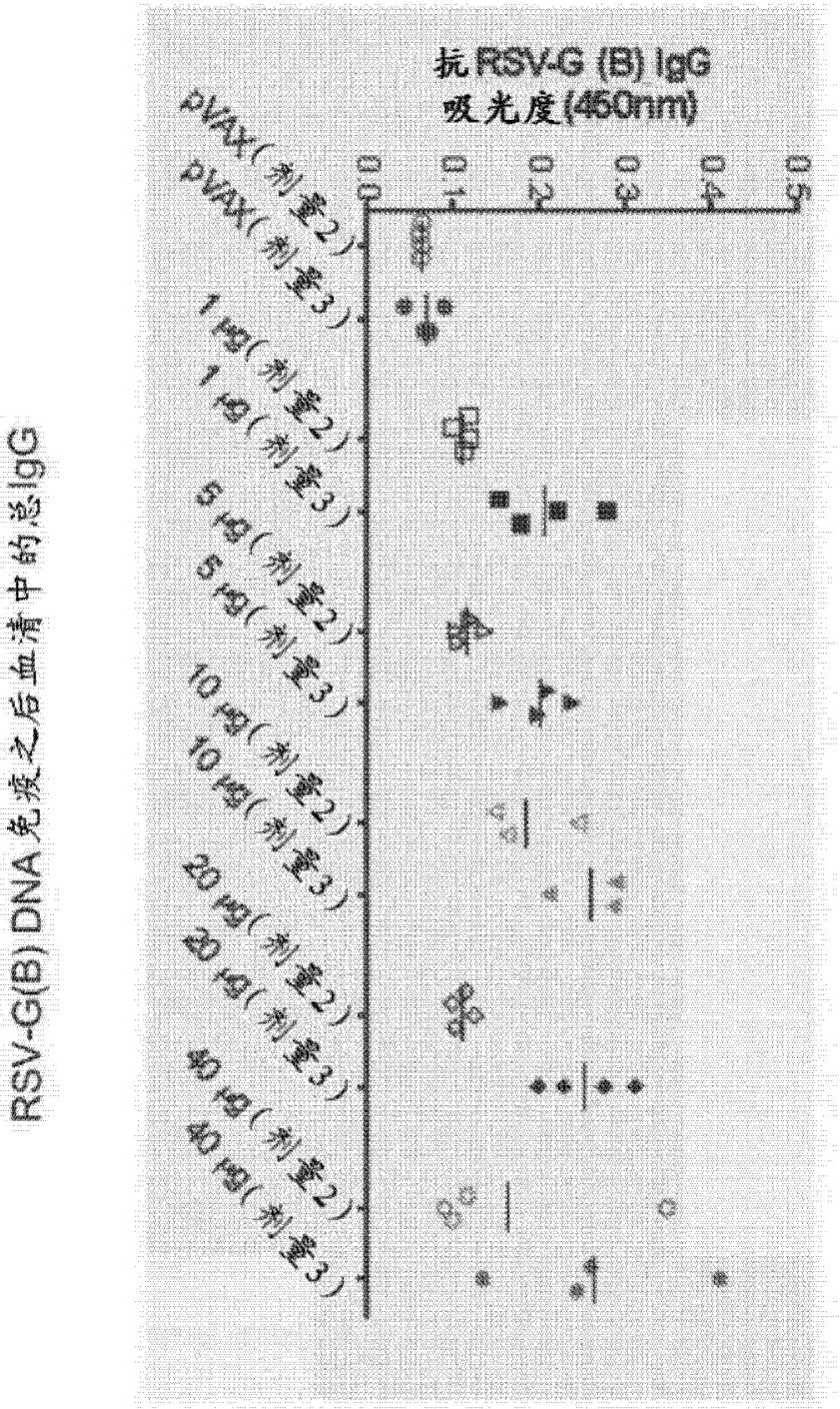

RSV-G(B) DNA 免疫之后血清中的总IgG
抗 RSV-G (B) IgG
吸光度(450nm)
0.0
0.1
0.2
0.3
0.4
0.5
PVAX
PVAX（剂量2）
PVAX（剂量3）
1 μg（剂量2）
1 μg（剂量3）
5 μg（剂量2）
5 μg（剂量3）
10 μg（剂量2）
10 μg（剂量3）
20 μg（剂量2）
20 μg（剂量3）
40 μg（剂量2）
40 μg（剂量3）

图 32

由 Mono 疫苗小鼠研究得到的初步中和结果

测试的样品	第3次免疫后的中和滴度
阴性血清	0
Mab 抗 RSV	40
多克隆血清	160
小鼠 1-F Ag	320
小鼠 2-F Ag	160
小鼠 3- GA	80
小鼠 a - GA	320
小鼠 14 - GB	160

图 33

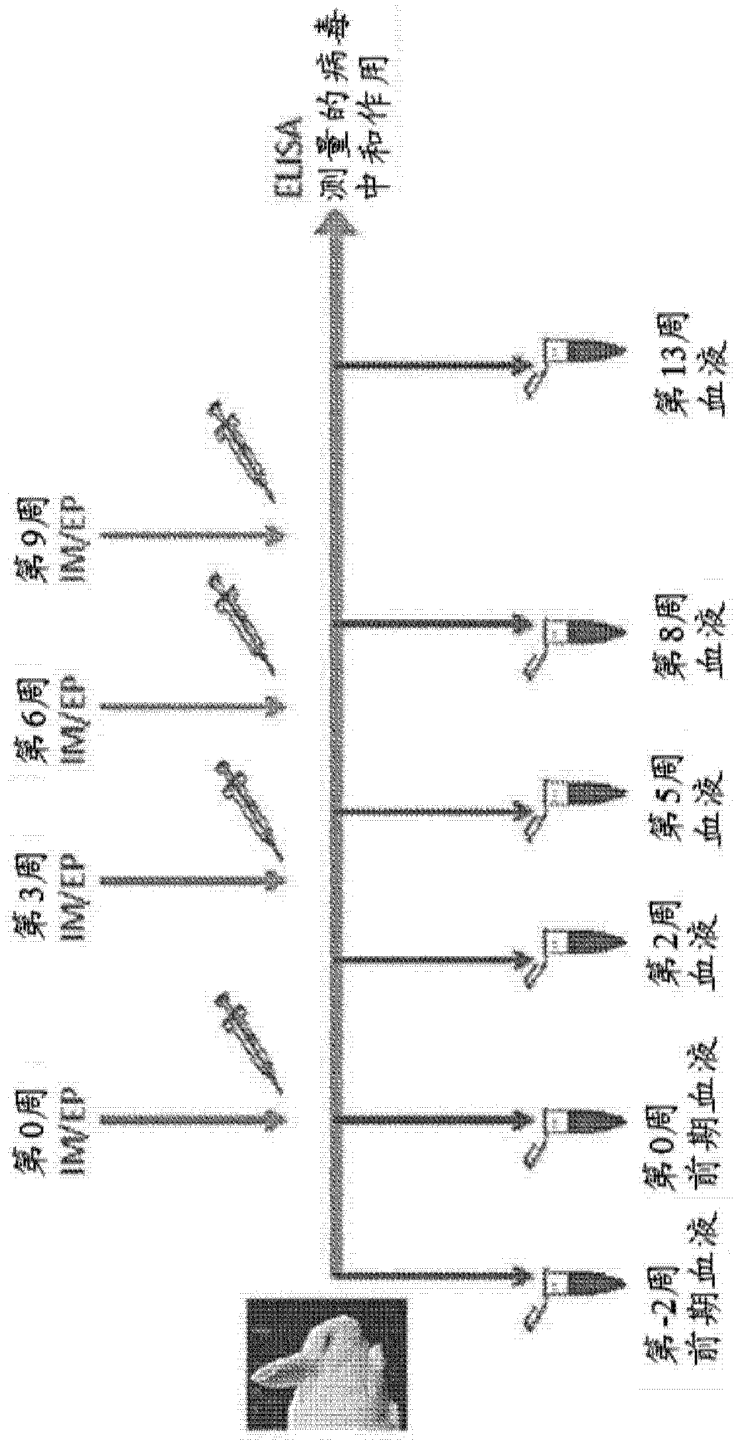

ELISA
测量的病毒
中和作用
第0周
IM/EP
第3周
IM/EP
第6周
IM/EP
第9周
IM/EP
第-2周
前期血液
第0周
前期血液
第2周
血液
第5周
血液
第8周
血液
第13周
血液

图 34

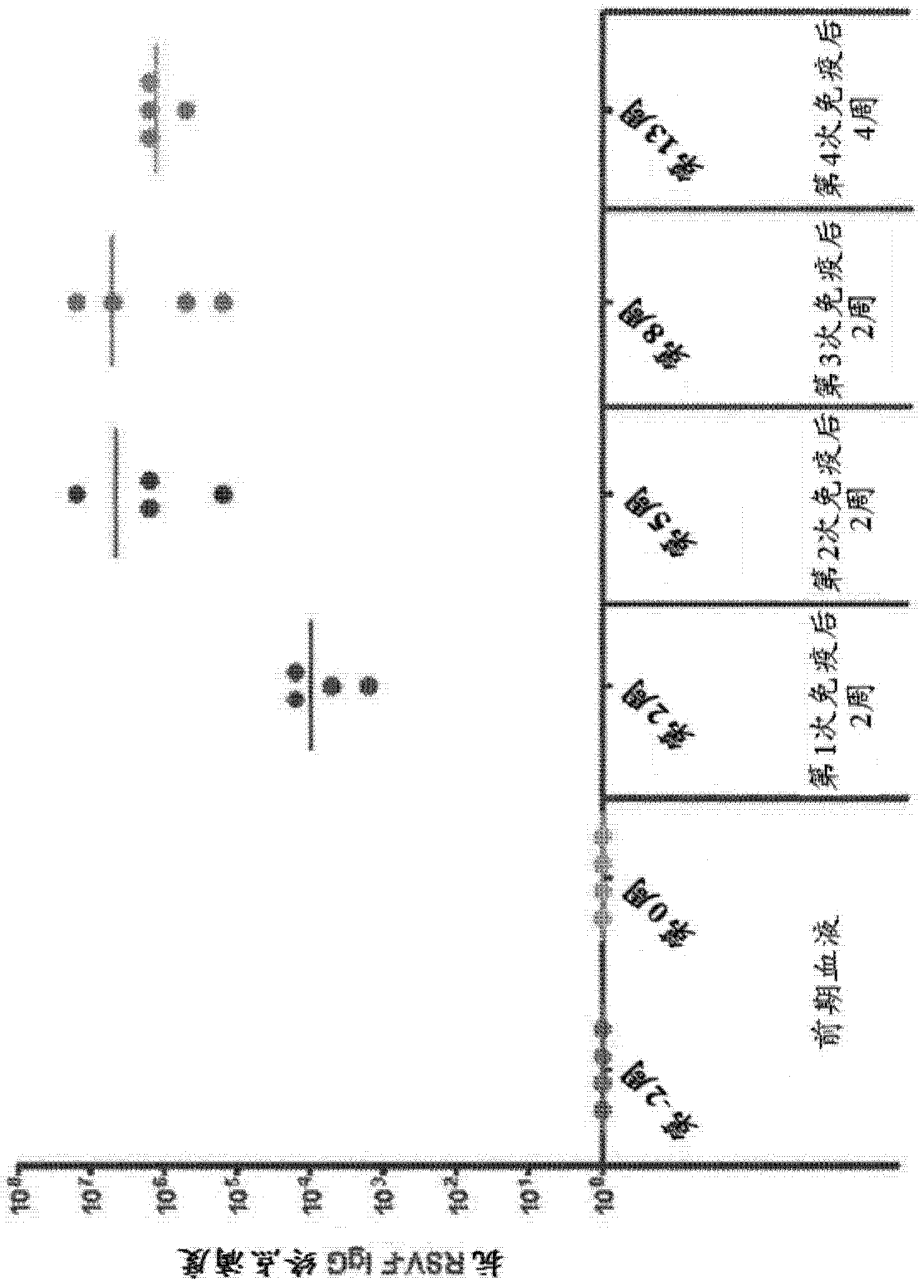

抗 RSVF log 终点滴度
第 -2 周
第 0 周
第 2 周
第 5 周
第 8 周
第 13 周
前期血液
第1次免疫后 2周
第2次免疫后 2周
第3次免疫后 2周
第4次免疫后 4周

图 35

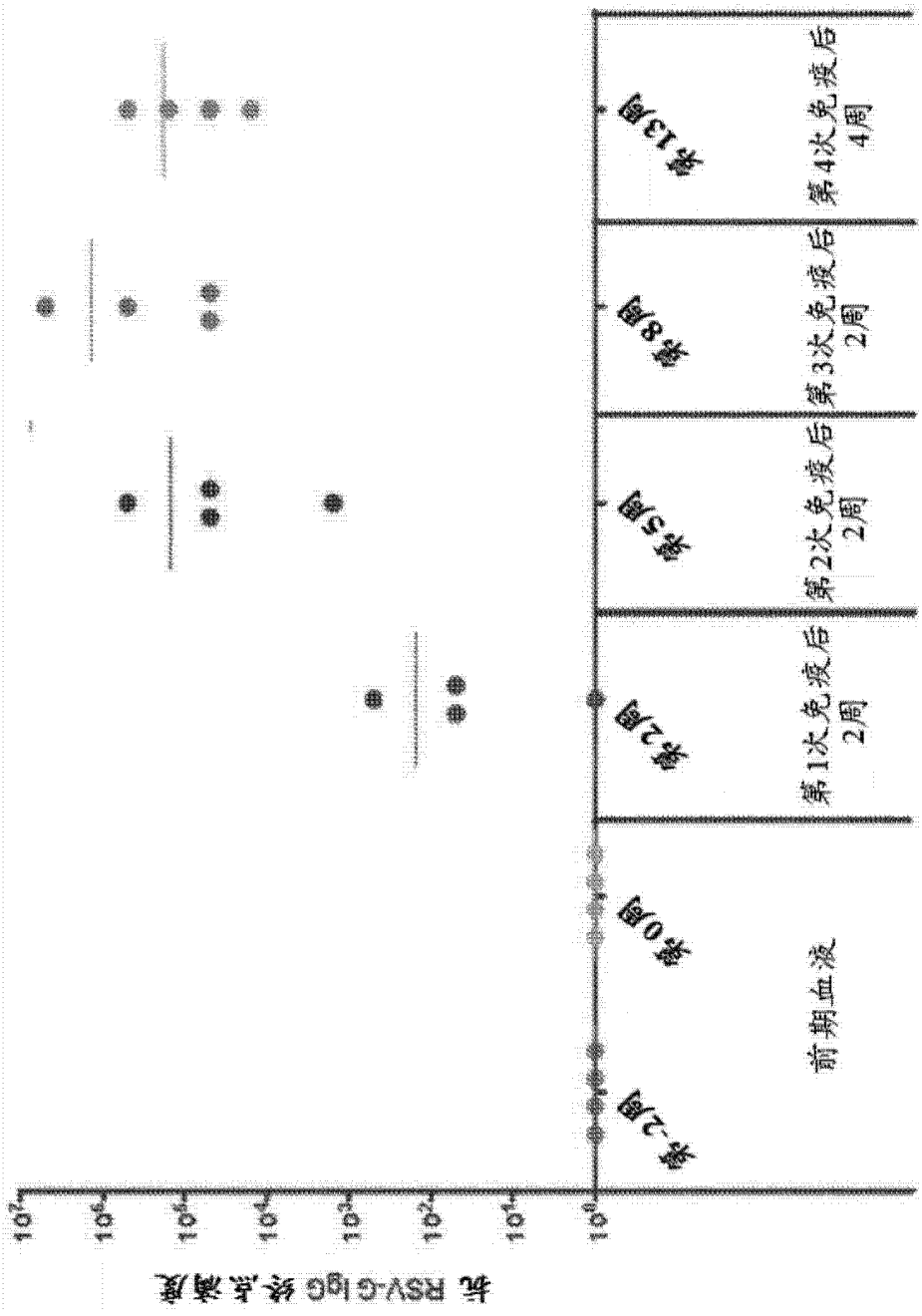

抗 RSV-G IgG 终点滴度
10^{7}
10^{6}
10^{5}
10^{4}
10^{3}
10^{2}
10^{1}
10^{0}
第-2周
第0周
第2周
第5周
第8周
第13周
前期血液
第1次免疫后 2周
第2次免疫后 2周
第3次免疫后 2周
第4次免疫后 4周

图 36

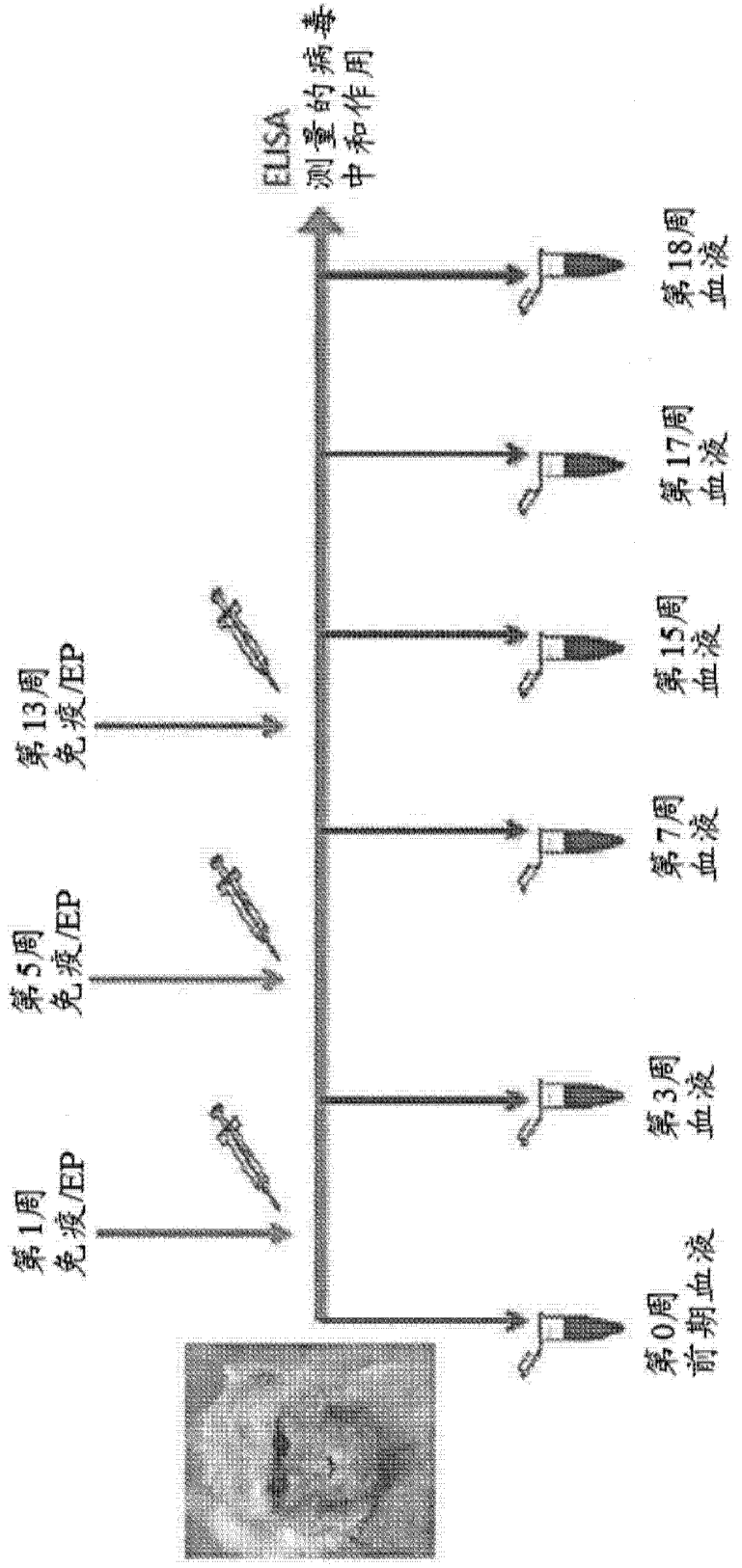

ELISA
测量的病毒
中和作用
第0周
前期血液
第1周
免疫/EP
第3周
血液
第5周
免疫/EP
第7周
血液
第13周
免疫/EP
第15周
血液
第17周
血液
第18周
血液

图 37

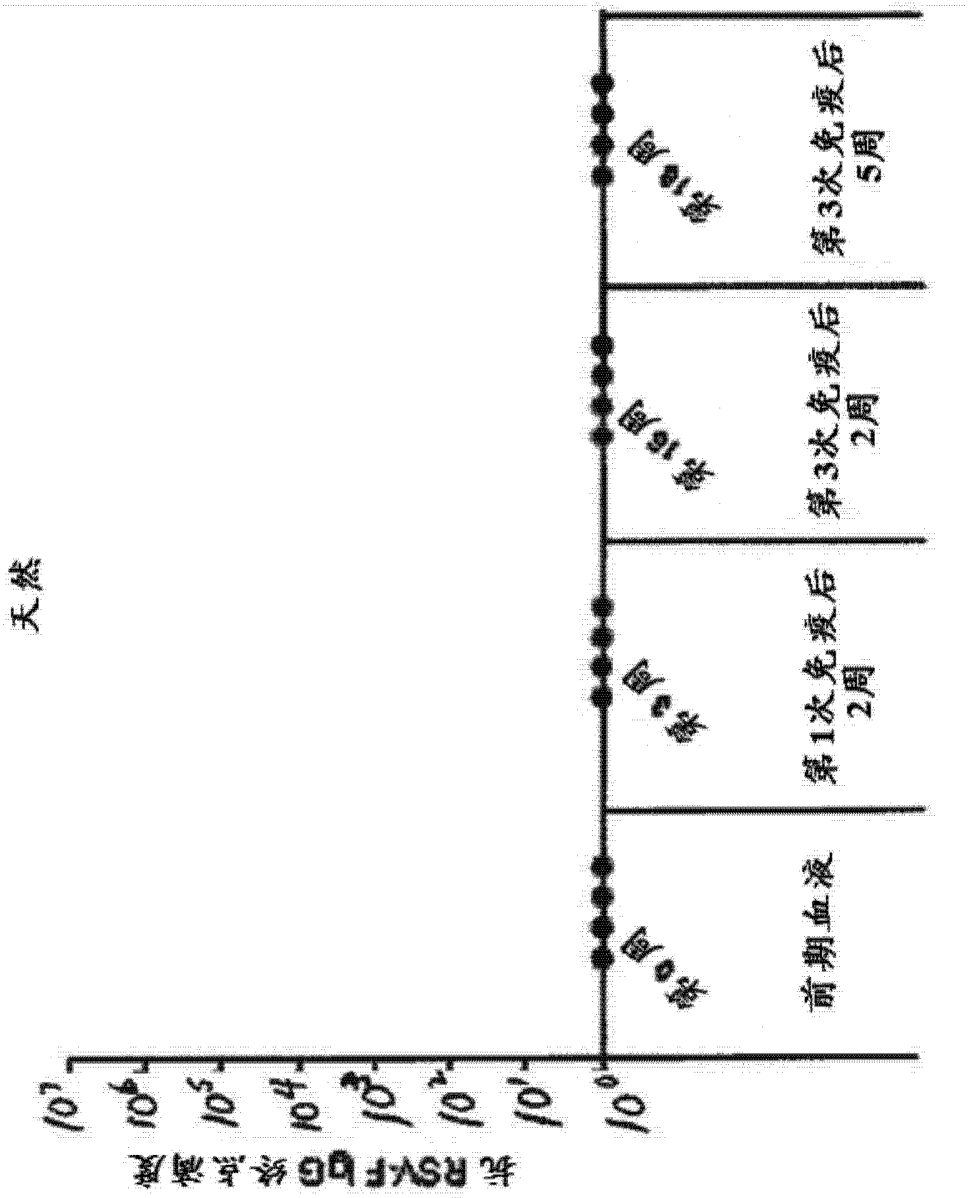

抗 RSV-F IgG 终点滴度
10^0
10^1
10^2
10^3
10^4
10^5
10^6
10^7
前期血液
第0周
第1次免疫后
2周
第3周
第3次免疫后
2周
第16周
第3次免疫后
5周
第16周
天然

图 38A

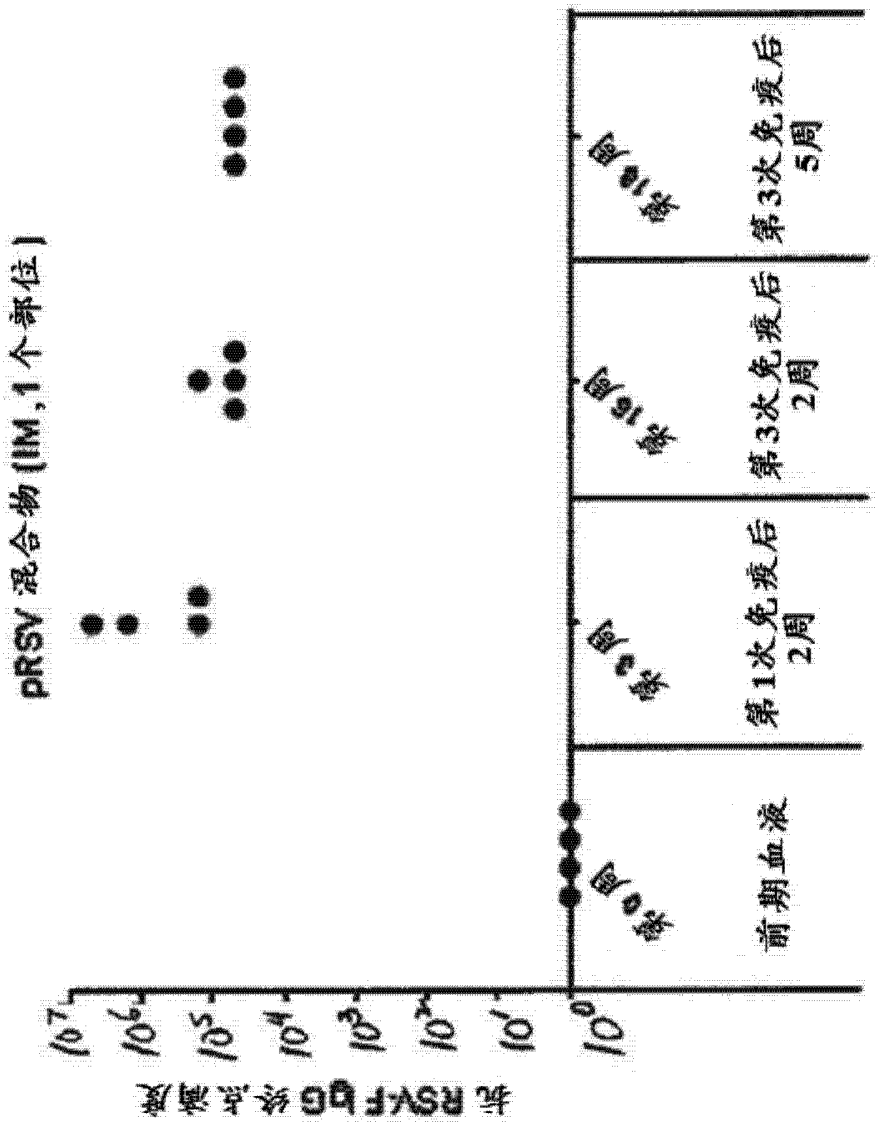

抗 RSV-F IgG 终点滴度
10^0
10^1
10^2
10^3
10^4
10^5
10^6
10^7
pRSV 混合物 (IM，1 个部位)
第 0 周
第 2 周
第 16 周
第 28 周
前期血液
第1次免疫后 2周
第3次免疫后 2周
第3次免疫后 5周

图 38B

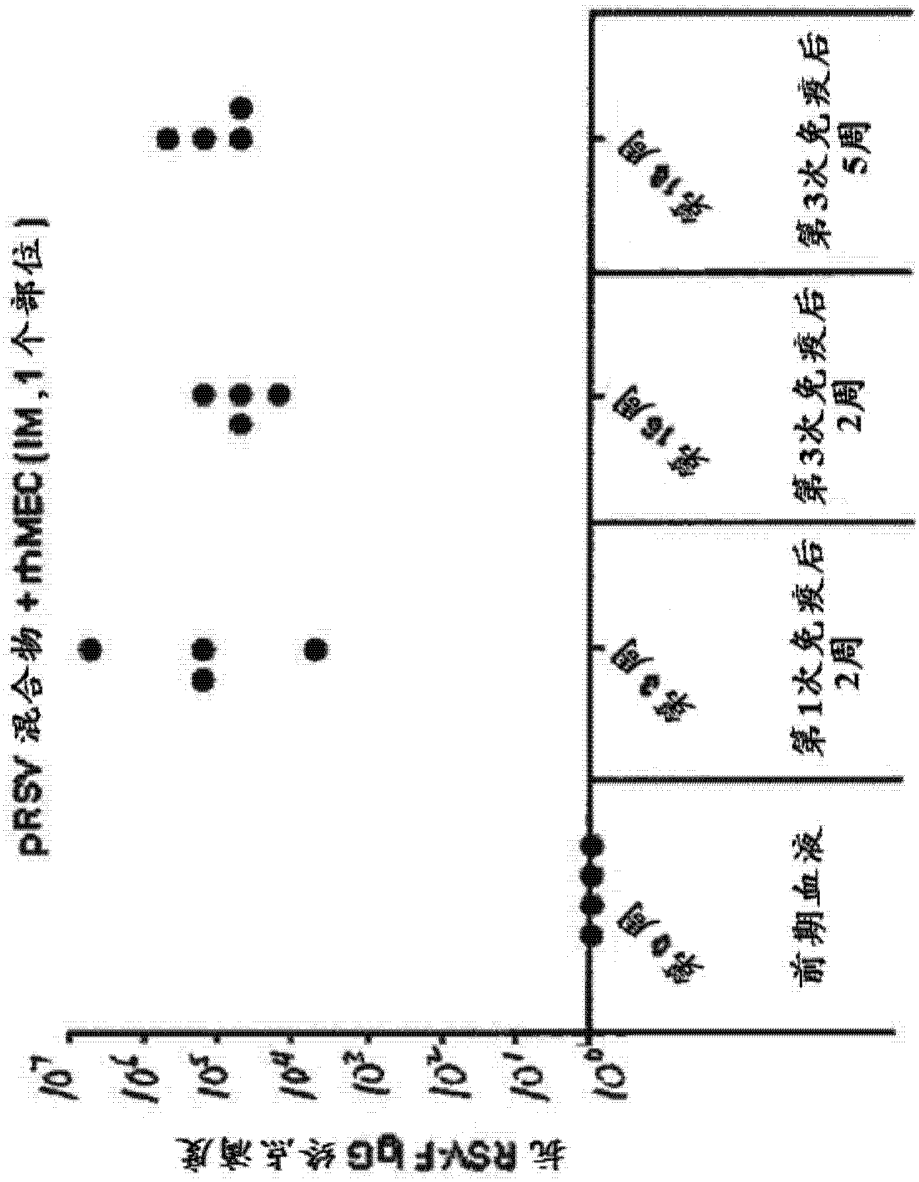

抗 RSV-F IgG 终点滴度
10^0
10^1
10^2
10^3
10^4
10^5
10^6
10^7
第0周
第6周
第16周
第16周
前期血液
第1次免疫后 2周
第3次免疫后 2周
第3次免疫后 5周
PRSV 混合物 + rhMEC (IM, 1 个部位)

图 38C

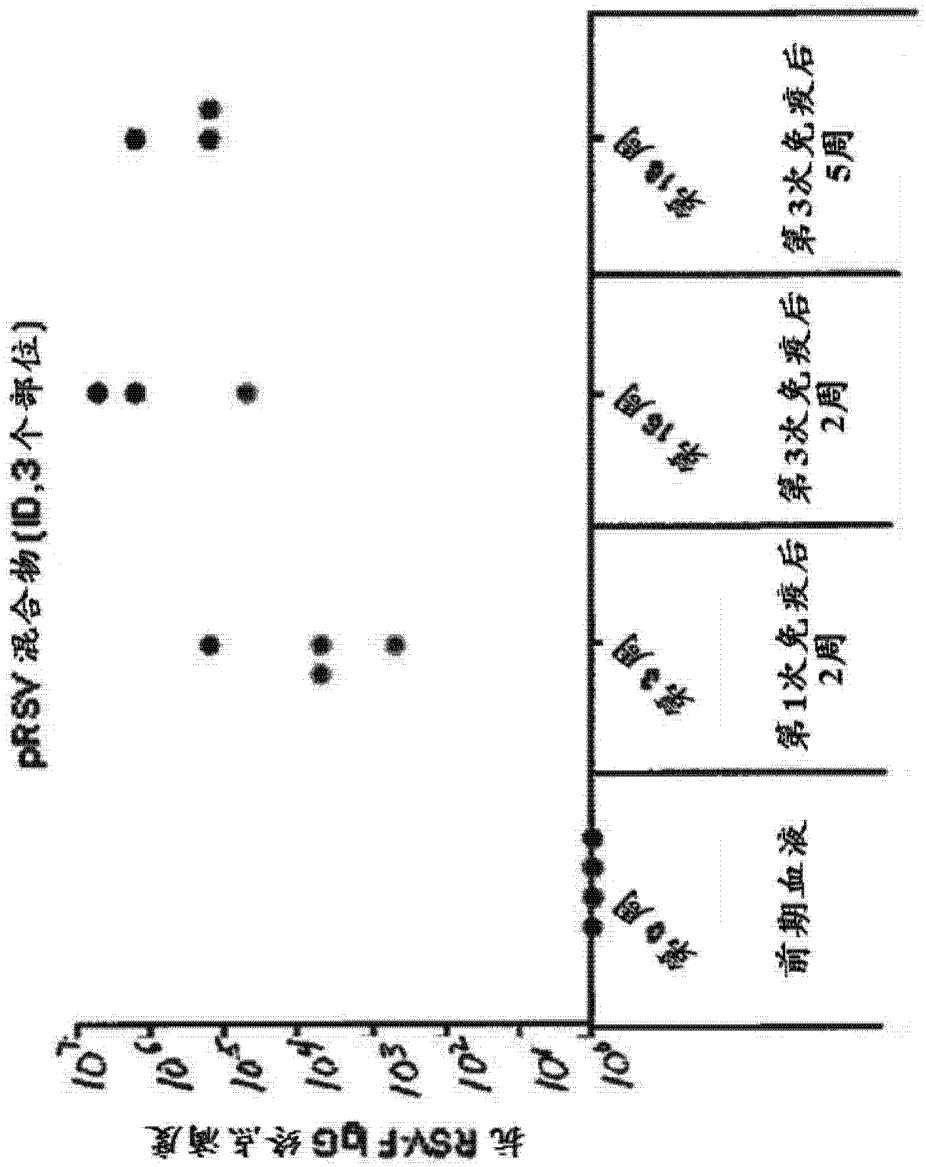

抗 RSV F IgG 终点滴度
10^1
10^2
10^3
10^4
10^5
10^6
10^7
pRSV 混合物(ID,3个部位)
前期血液
第1次免疫后 2周
第3次免疫后 2周
第3次免疫后 5周

图 38D

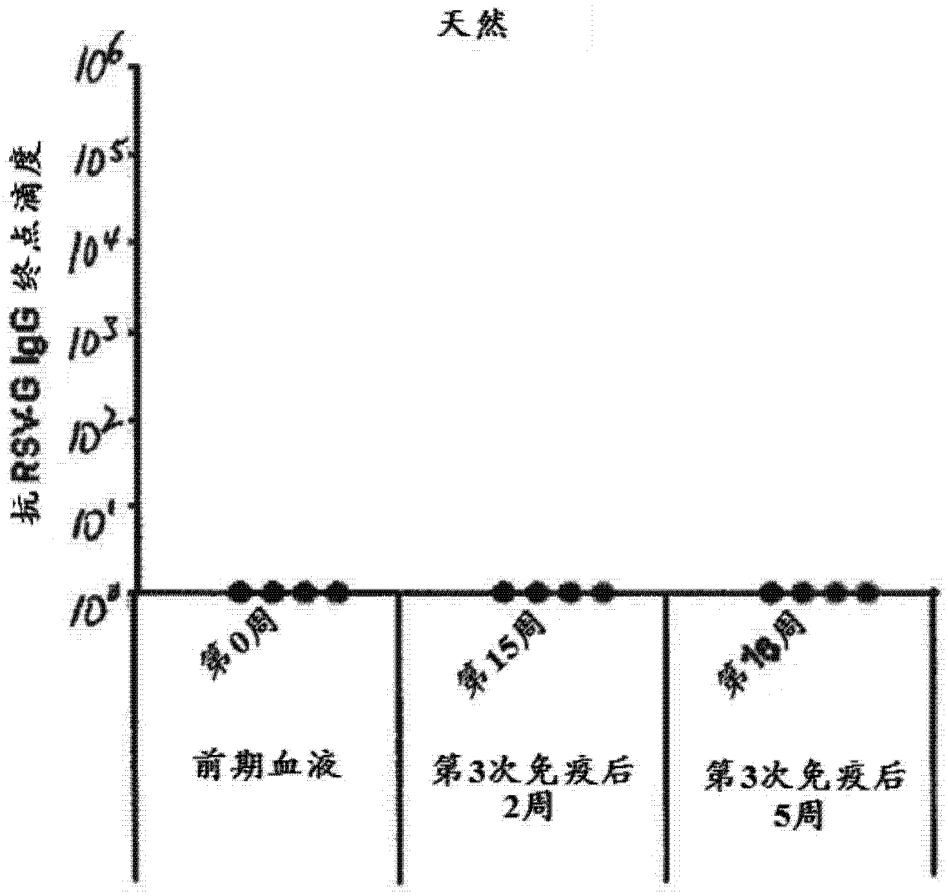

天然
抗RSVG IgG 终点滴度
10^6
10^5
10^4
10^3
10^2
10^1
10^0
第0周
第15周
第16周
前期血液
第3次免疫后
2周
第3次免疫后
5周

图 39A

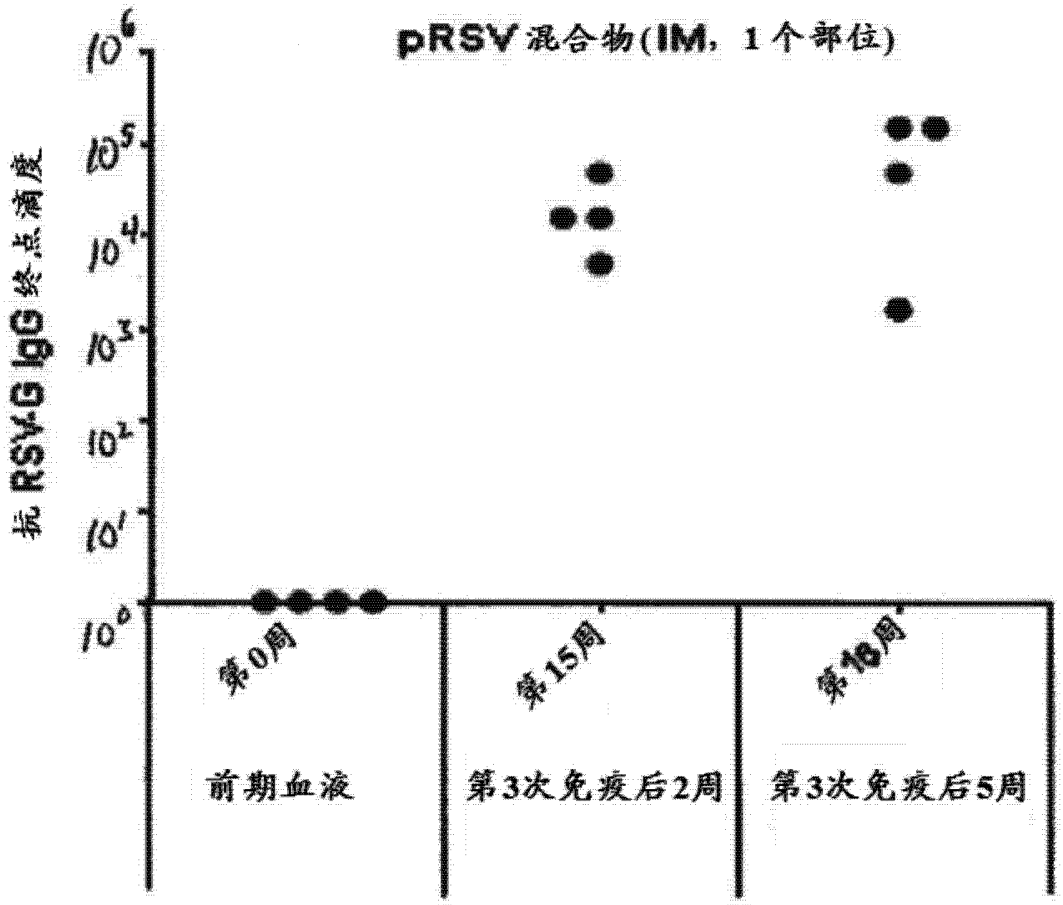

pRSV混合物(IM，1个部位)
抗RSV-G IgG终点滴度
10^6
10^5
10^4
10^3
10^2
10^1
10^0
第0周
第15周
第18周
前期血液
第3次免疫后2周
第3次免疫后5周

图 39B

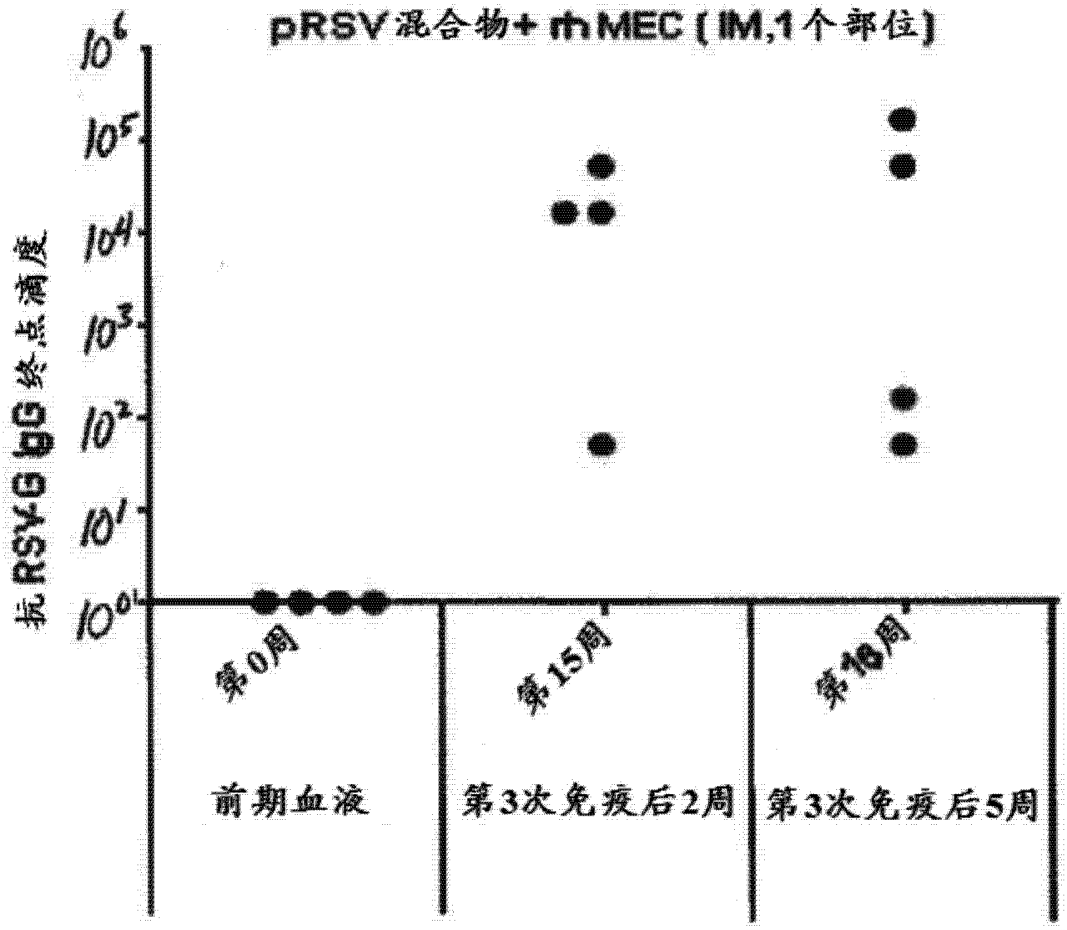

pRSV混合物+mMEC(IM,1个部位)
抗RSV-G IgG终点滴度
10^6
10^5
10^4
10^3
10^2
10^1
$10^{0.1}$
第0周
第15周
第18周
前期血液
第3次免疫后2周
第3次免疫后5周

图 39C

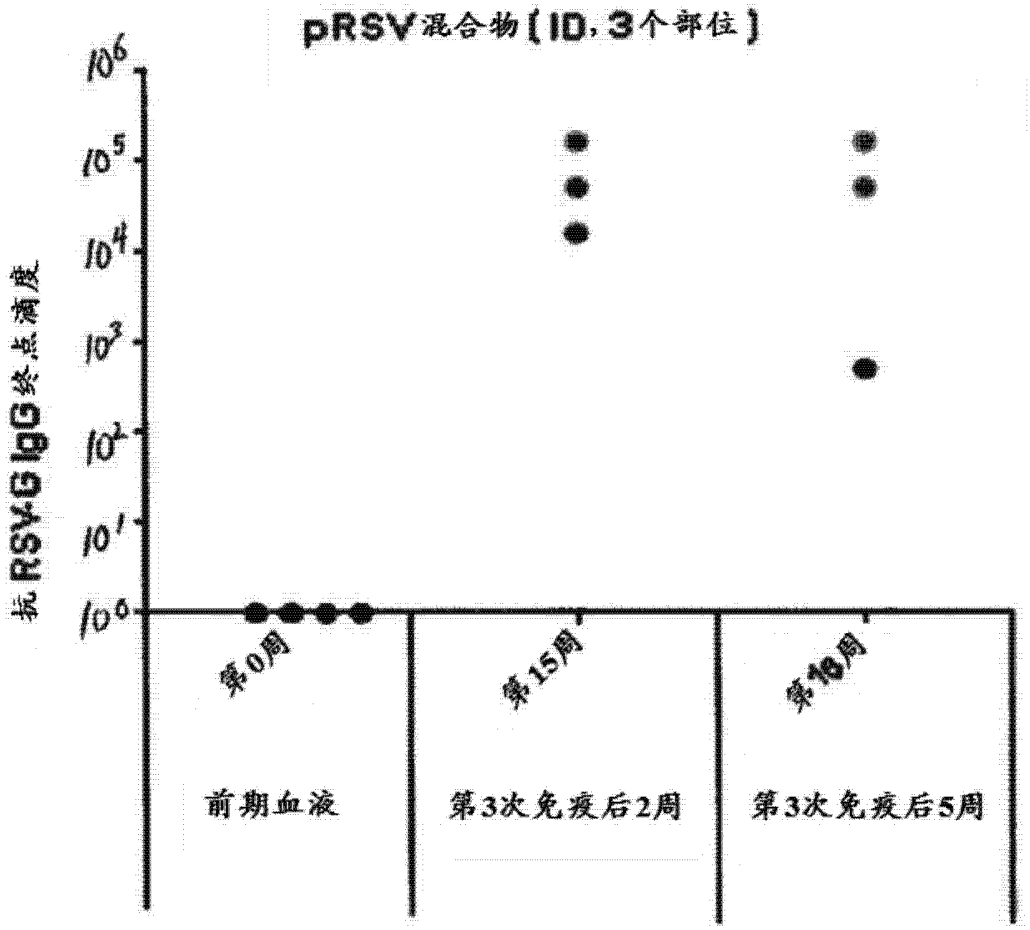

pRSV混合物(ID,3个部位)
抗RSV-G IgG终点滴度
10^6
10^5
10^4
10^3
10^2
10^1
10^0
第0周
第15周
第19周
前期血液
第3次免疫后2周
第3次免疫后5周

图 39D

在兔和非人类灵长类动物研究中由混合疫苗得到的初步中和结果

测试的样品	前期血液	第3次免疫后的中和滴度
阴性血清	0	
Mab 抗 RSV		40
多克隆血清		160
兔 71	0	640
兔 72	0	320
NHP IM-1	0	640
NHP IM-2	0	320

图 40