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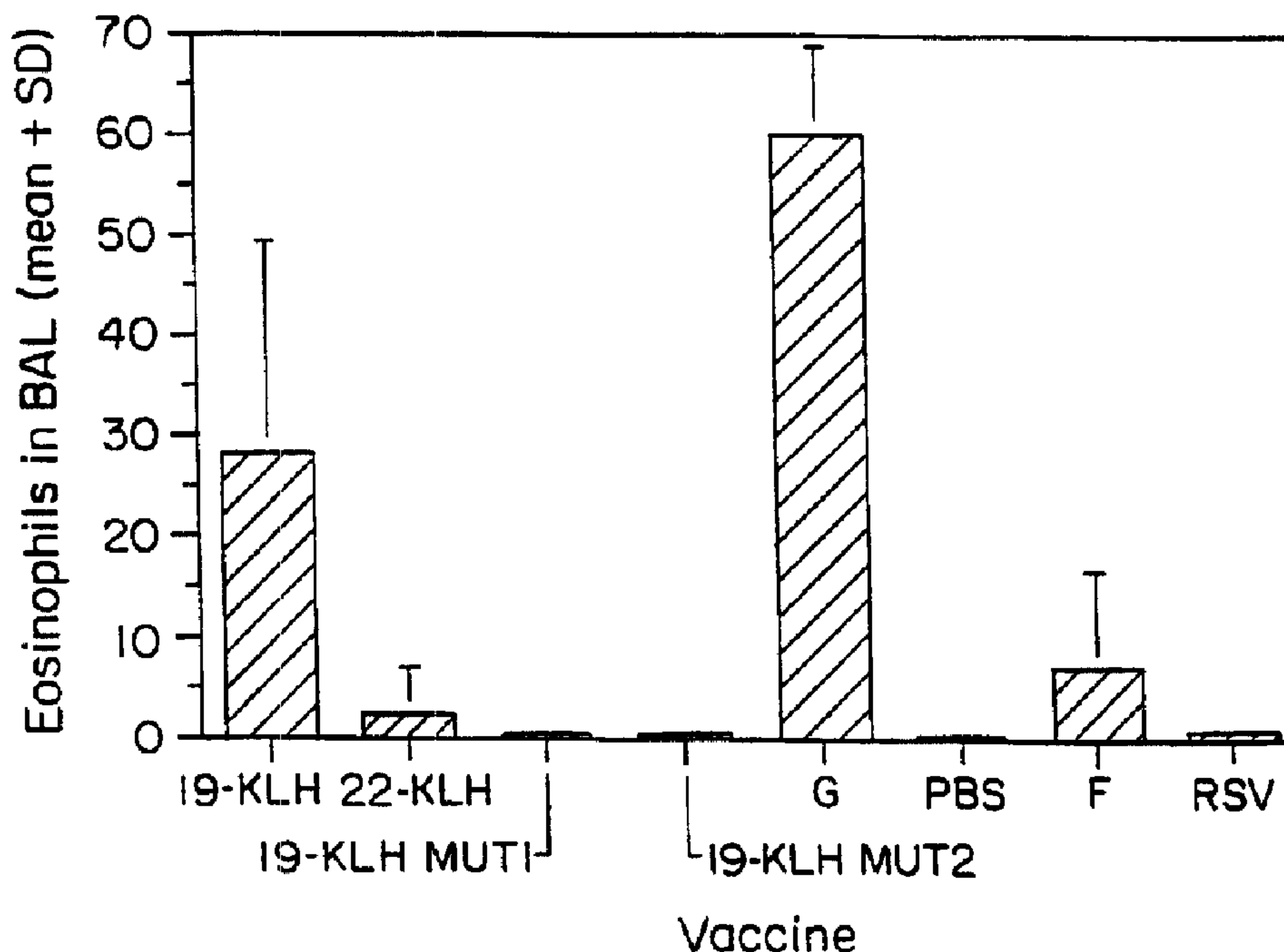
(54) Titre : PEPTIDES DERIVES DE LA PROTEINE D'ATTACHEMENT (G) DU VIRUS RESPIRATOIRE SYNCYTIAL BOVIN

(54) Title: PEPTIDES DERIVED FROM THE ATTACHMENT (G) PROTEIN OF RESPIRATORY SYNCYTIAL VIRUS



(57) Abrégé/Abstract:
An altered G protein or portion thereof of RSV which retains immunogenicity and which, when incorporated into an immunogenic composition or vaccine and administered to a vertebrate, does not induce enhanced disease (e.g., atypical pulmonary inflammation

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(57) Abrégé(suite)/Abstract(continued):
such as pulmonary eosinophilia) upon subsequent infection with RSV, is disclosed. In a particular embodiment, the altered G protein comprises an alteration in the region from amino acid 184 to amino acid 198. Immunogenic compositions and vaccines comprising the altered RSV G protein, and optionally comprising RSV F protein, are also disclosed.

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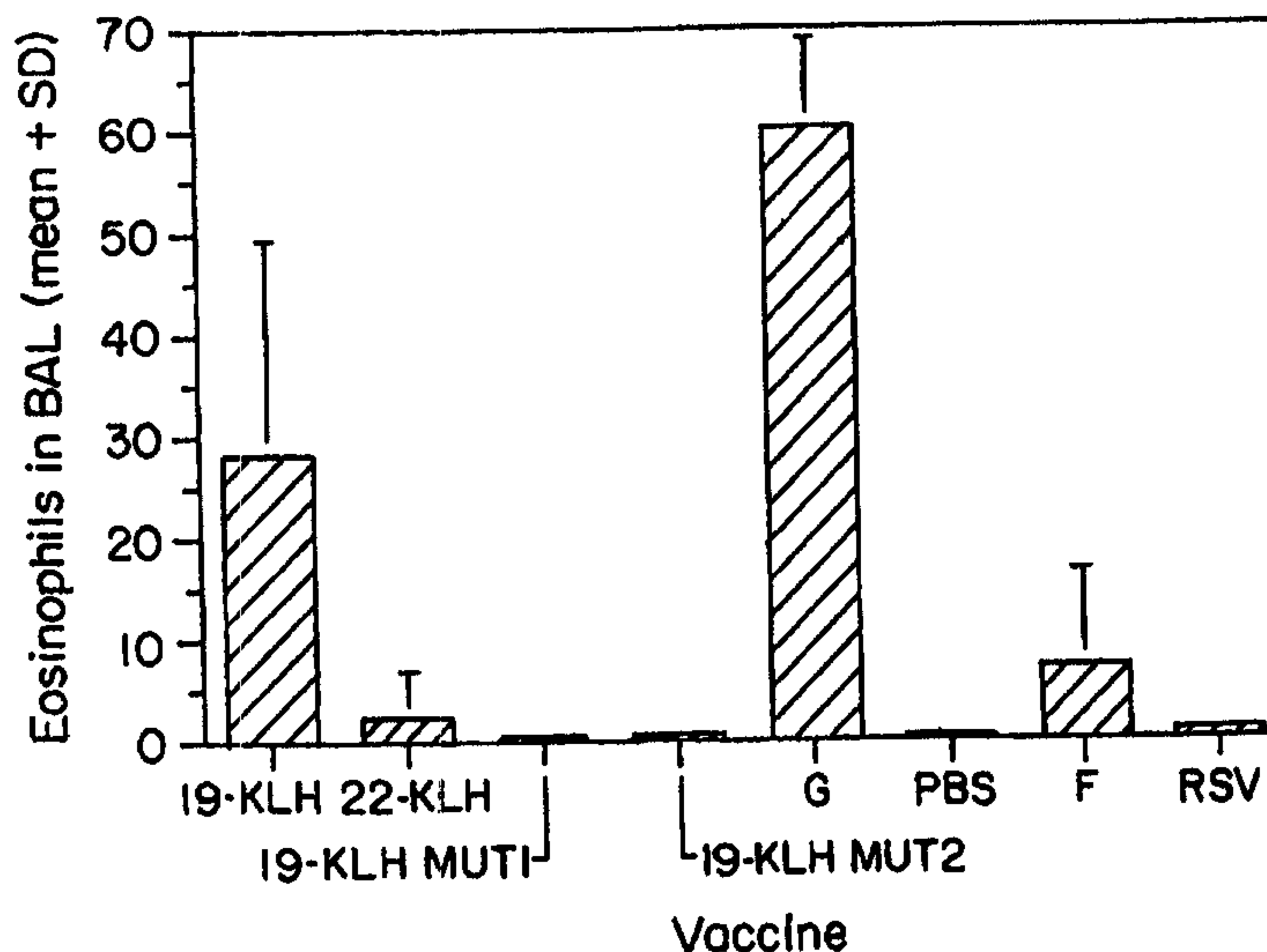

(57) Abstract

An altered G protein or portion thereof of RSV which retains immunogenicity and which, when incorporated into an immunogenic composition or vaccine and administered to a vertebrate, does not induce enhanced disease (e.g., atypical pulmonary inflammation such as pulmonary eosinophilia) upon subsequent infection with RSV, is disclosed. In a particular embodiment, the altered G protein comprises an alteration in the region from amino acid 184 to amino acid 198. Immunogenic compositions and vaccines comprising the altered RSV G protein, and optionally comprising RSV F protein, are also disclosed.

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PEPTIDES DERIVED FROM THE ATTACHMENT (G) PROTEIN OF RESPIRATORY SYNCYTIAL VIRUS

BACKGROUND OF THE INVENTION

Respiratory Syncytial Virus (RSV), a negative
5 strand virus of the *paramyxoviridae* family, is a major
cause of lower pulmonary tract disease, particularly in
young children and infants. The parenteral
administration of formalin-inactivated RSV (FI-RSV) as a
vaccine has been associated with enhanced disease in
RSV-naive recipients (seronegative) who subsequently
10 became infected with wild-type RSV. The enhanced
disease was characterized by an increased proportion of
eosinophils in both the peripheral blood and lungs of
affected individuals (Kim *et al.*, *Am. J. Epidemiol.*
89:422-434 (1969); Kim *et al.*, *Pediatric Res.* *10*:75-78
15 (1976)). Recent studies in rodents have indicated that
FI-RSV induces a T-helper 2 (TH2) immune response,

whereas live attenuated viral vaccine are preferentially associated with T-helper 1 (TH1) responses.

RSV contains two prominent outer envelope glycoproteins, fusion (F) protein and attachment (G) protein, that are important for viral infectivity and thus serve as reasonable targets for the design of a subunit vaccine to RSV. It has previously been shown that the generation of neutralizing antibodies to RSV by an F-protein-based vaccine can be greatly increased by the inclusion of G protein (Hancock *et al.*, *J. Virol.* *70*:7783-7791 (1996)). However, in attempting to understand the molecular basis for FI-RSV-induced enhanced disease, it has previously been shown that the native attachment (G) glycoprotein of RSV is sufficient to prime for atypical pulmonary inflammation characterized by pulmonary eosinophilia associated with high production levels of Interleukin-5 (IL-5), a TH2 cytokine (Hancock *et al.*, *J. Virol.* *70*:7783-7791 (1996)). In fact, the *in vivo* depletion of IL-5 significantly reduces the eosinophilic response in bronchoalveolar lavage cells of G protein-immunized mice challenged with RSV. The response to G protein was shown to be T cell mediated by transfer of G protein-specific CD4+ T cell lines into naive recipient mice, resulting in atypical pulmonary inflammatory responses upon subsequent challenge (Alwan *et al.*, *J. Exp. Med.* *179*:81-89 (1994)).

SUMMARY OF THE INVENTION

The immune responses elicited by native G protein and a series of overlapping peptides (shown in Figure 2) extending from amino acids 48 to 294 of G protein have been characterized as described herein. In stimulation assays of splenocytes from G protein-vaccinated mice, one peptide (19, spanning amino acids 184-198) was dominant in its ability to stimulate spleen cell

proliferation (Figure 3). In the absence of any similar effect from other G protein-derived peptides, the use of peptide 19 as an antigen resulted in a stimulation of spleen cell proliferation that was 15-fold above background levels. Peptide 19 was also found to be the major region of the G protein involved in cytokine release. Both IFN-γ and IL-5 were detected in the induction of supernatants from cultures of splenocytes derived from G protein-vaccinated BALB/c mice (Figures 4A and 4B). Peptide 19 (amino acids 184-198 of the RSV G protein) specifically induces pulmonary eosinophilia in BALB/c mice. Mice vaccinated with peptide 19 conjugated to keyhole limpet hemocyanin (KLH) showed significant pulmonary eosinophilia (39.5% of total bronchoalveolar lavage cells) upon subsequent challenge with live RSV (Figure 5). In contrast, mice immunized with a peptide containing amino acids 208-222 (peptide 22) conjugated to KLH exhibited minimal pulmonary eosinophilia (3.3%). Mutations in the amino acid sequence of peptide 19 abrogated the ability to predispose mice for pulmonary eosinophilia (Figure 6).

The *in vivo* depletion of CD4+ cells abrogated pulmonary eosinophilia in mice vaccinated with the peptide 19 conjugate, whereas the depletion of CD8+ cells had a negligible effect (Figure 8). These data indicate an association between peptide 19 of RSV G protein and the CD4+ T cell-mediated induction of pulmonary eosinophilia in response to live RSV challenge, suggesting that peptide 19-specific CD4+ T cells are the causative agent of pulmonary eosinophilia. In analyzing human peripheral blood cells from 43 donors, 6 showed reactivity to RSV G protein, 3 of which responded to peptide 19 (Figure 7). This data suggests that peptide 19 may be involved in the onset of bronchiolitis, atopy or asthma that is sometimes observed following RSV infection of seronegative infants

(Welliver and Welliver, *Pediatrics in Review 14*:134-139 (1993)).

Accordingly, the invention pertains to an altered G protein or polypeptide of RSV which retains immunogenicity and which, when incorporated into an immunogenic composition or vaccine and administered to a vertebrate, provides protection without inducing enhanced disease upon subsequent infection of the vertebrate with RSV. In a particular embodiment, the enhanced disease is atypical pulmonary inflammation, particularly pulmonary eosinophilia. In one embodiment, the alteration is in the region from amino acid 184 to amino acid 198 of the RSV G protein. In an alternate embodiment, the alteration results in inhibition of priming for IL-5 secretion by the altered G protein or polypeptide relative to wild type G protein.

The invention also pertains to a nucleic acid molecule encoding an altered G protein or polypeptide of RSV, where the altered protein or polypeptide retains immunogenicity and, when incorporated into an immunogenic composition or vaccine and administered to a vertebrate, does not induce enhanced disease upon subsequent infection of the vertebrate with RSV. In one embodiment, the alteration is in the region from amino acid 184 to amino acid 198 of the RSV G protein.

The invention also encompasses DNA constructs comprising a nucleic acid molecule described herein operably linked to a regulatory sequence. In a particular embodiment, the invention pertains to a chimeric DNA construct comprising: (a) a nucleic acid molecule encoding an altered G protein or polypeptide of RSV, where the altered protein or polypeptide retains immunogenicity and, when incorporated into an immunogenic composition or vaccine and administered to a vertebrate, does not induce enhanced disease upon subsequent infection of the vertebrate with RSV; (b) a

nucleic acid molecule encoding all or an immunogenic portion of F protein of RSV; and (c) a regulatory sequence operably linked to both the F and altered G proteins.

5 The invention also relates to a recombinant host cell comprising a DNA construct described herein, as well as to a method of producing an altered G protein or polypeptide of RSV which retains immunogenicity and which, when incorporated into an immunogenic composition 10 or vaccine and administered to a vertebrate, does not induce enhanced disease upon subsequent infection of the vertebrate with RSV, comprising maintaining a recombinant host cell of the invention under conditions suitable for expression of the altered G protein or 15 polypeptide.

The invention also pertains to a method of producing a chimeric polypeptide comprising an altered G protein or polypeptide of RSV which retains immunogenicity and which, when incorporated into an 20 immunogenic composition or vaccine and administered to a vertebrate, does not induce enhanced disease upon subsequent infection of the vertebrate with RSV, and all or an immunogenic portion of F protein of RSV.

The invention also pertains to the use of the 25 altered G protein or polypeptide, or recombinant host cell for expression thereof, for the manufacture of a medicament, such as a vaccine.

The invention further relates to an immunogenic composition comprising a physiologically acceptable 30 medium and an altered G protein or polypeptide of RSV which retains immunogenicity and which, when incorporated into an immunogenic composition and administered to a vertebrate, does not induce enhanced disease upon subsequent infection of the vertebrate with 35 RSV. In a particular embodiment, the immunogenic composition results in inhibition of priming for IL-5

secretion relative to an immunogenic composition comprising wild type G protein. In one embodiment, the alteration is in the region from amino acid 184 to amino acid 198 of the RSV G protein. The immunogenic composition can also comprise all or a portion of RSV F protein.

The invention also pertains to a vaccine composition comprising an immunologically effective amount of altered G protein or polypeptide of RSV which retains immunogenicity and which, when incorporated into a vaccine and administered to a vertebrate, provides protection without inducing enhanced disease upon subsequent infection of the vertebrate with RSV. In one embodiment, the alteration is in the region from amino acid 184 to amino acid 198. The vaccine composition can also comprise an immunologically effective amount of all or a portion of RSV F protein. In particular embodiments, the vaccine compositions further comprise an adjuvant.

The invention further relates to a method of inhibiting induction of enhanced disease after vaccination and subsequent infection of a vertebrate with RSV, comprising administering an altered RSV G protein or polypeptide, where said altered G protein or polypeptide retains immunogenicity and, when incorporated into a vaccine and administered to a vertebrate, provides protection without inducing enhanced disease upon subsequent infection of the vertebrate with RSV.

The invention also relates to a vaccine comprising a physiologically acceptable vehicle and an effective amount of a nucleic acid molecule encoding an altered G protein or polypeptide of RSV, where said altered G protein or polypeptide retains immunogenicity and, when incorporated into a vaccine and administered to a vertebrate, provides protection without inducing

enhanced disease upon subsequent infection of the vertebrate with RSV. In one embodiment, the vaccine further comprises a transfection-facilitating agent.

The invention also relates to a method of inducing an immune response in a vertebrate, comprising administering to said vertebrate an amount of DNA encoding an altered RSV G protein or polypeptide effective to induce an immune response, optionally with a transfection-facilitating agent, where said altered G protein or polypeptide retains immunogenicity and, when incorporated into a vaccine and administered to a vertebrate, provides protection without inducing enhanced disease upon subsequent infection of the vertebrate with RSV.

The invention also relates to a method of immunizing a vertebrate against RSV, comprising administering to the vertebrate a composition comprising an immunologically effective amount of altered G protein or polypeptide of RSV which retains immunogenicity and which, when incorporated into an immunogenic composition or vaccine and administered to a vertebrate, does not induce enhanced disease upon subsequent infection of the vertebrate with RSV.

The invention also pertains to a method of immunizing a vertebrate against RSV, comprising administering to the vertebrate a composition comprising an immunologically effective amount of a nucleic acid molecule encoding an altered G protein or polypeptide of RSV, where said altered G protein or polypeptide retains immunogenicity and, when incorporated into an immunogenic composition or vaccine and administered to a vertebrate, does not induce enhanced disease upon subsequent infection of the vertebrate with RSV.

In one embodiment, the composition further comprises an immunologically effective amount of all or a portion of RSV F protein, or a nucleic acid molecule

encoding an immunologically effective amount of all or a portion of RSV F protein, respectively. In another embodiment, the vertebrate is an RSV seronegative human.

In another aspect, the invention provides an isolated altered G protein or polypeptide of RSV, wherein the alteration is in one or more regions selected from the group consisting of the region from amino acid 159 to amino acid 198, the region from amino acid 159 to amino acid 174 as set out in SEQ ID NO: 15, the region from amino acid 171 to amino acid 187 as set out in SEQ ID NO: 17, the region from amino acid 176 to amino acid 190 as set out in SEQ ID NO: 18, and the region from amino acid 184 to amino acid 198 as set out in SEQ ID NO: 19, and where said isolated, altered G protein or polypeptide retains immunogenicity, and which isolated, altered G protein or polypeptide, when incorporated into an immunogenic composition or vaccine and administered to a vertebrate, does not induce enhanced RSV disease upon subsequent infection of the vertebrate with RSV.

In another aspect, the invention provides an isolated nucleic acid molecule encoding an altered G protein or polypeptide of RSV, wherein the alteration is in one or more regions selected from the group consisting of the region from amino acid 159 to amino acid 198, the region from amino acid 159 to amino acid 174 as set out in SEQ ID NO: 15, the region from amino acid 171 to amino acid 187 as set out in SEQ ID NO: 17, the region from amino acid 176 to amino acid 190 as set out in SEQ ID NO: 18, and the region from amino acid 184 to amino acid 198 as set out in SEQ ID NO: 19, wherein said altered G protein or polypeptide retains immunogenicity and, when said altered G protein or polypeptide is incorporated into an immunogenic

composition and administered to a vertebrate, does not induce enhanced RSV disease upon subsequent infection of the vertebrate with RSV.

In another aspect, the invention provides a 5 recombinant host cell comprising a nucleic acid construct as described herein.

In another aspect, the invention provides an immunogenic composition comprising a physiologically acceptable medium and an isolated altered G protein or 10 polypeptide of RSV, wherein the alteration is in one or more regions selected from the group consisting of the region from amino acid 159 to amino acid 198, the region from amino acid 159 to amino acid 174 as set out in SEQ ID NO: 15, the region from amino acid 171 to amino acid 187 as set out in 15 SEQ ID NO: 17, the region from amino acid 176 to amino acid 190 as set out in SEQ ID NO: 18, and the region from amino acid 184 to amino acid 198 as set out in SEQ ID NO: 19, and where said altered G protein or polypeptide retains immunogenicity, and which altered 20 G protein or polypeptide, when incorporated into an immunogenic composition or vaccine and administered to a vertebrate, does not induce enhanced RSV disease upon subsequent infection of the vertebrate with RSV.

In another aspect, the invention provides an 25 immunogenic composition comprising a physiologically acceptable medium, isolated F protein of RSV and an isolated altered G protein or polypeptide of RSV, wherein the alteration is in one or more regions selected from the group consisting of the region from amino acid 159 to amino 30 acid 198, the region from amino acid 159 to amino acid 174 as set out in SEQ ID NO: 15, the region from amino acid 171 to amino acid 187 as set out in SEQ ID NO: 17, the region

from amino acid 176 to amino acid 190 as set out in SEQ ID NO: 18, and the region from amino acid 184 to amino acid 198 as set out in SEQ ID NO: 19, and where said isolated, altered G protein or polypeptide retains
5 immunogenicity and which isolated, altered G protein or polypeptide, when incorporated into an immunogenic composition and administered to a vertebrate does not induce enhanced RSV disease upon subsequent infection of the vertebrate with RSV.

10 In another aspect, the invention provides the immunogenic composition as described herein, wherein the alteration is in the region from amino acid 184 to amino acid 198 of the G protein.

In another aspect, the invention provides an
15 immunogenic composition comprising a physiologically acceptable vehicle and an effective amount of an isolated nucleic acid molecule encoding an altered G protein or polypeptide of RSV, wherein the alteration is in one or more regions selected from the group consisting of the region
20 from amino acid 159 to amino acid 198, the region from amino acid 159 to amino acid 174 as set out in SEQ ID NO: 15, the region from amino acid 171 to amino acid 187 as set out in SEQ ID NO: 17, the region from amino acid 176 to amino acid 190 as set out in SEQ ID NO: 18, and the region from amino
25 acid 184 to amino acid 198 as set out in SEQ ID NO: 19, where said altered G protein or polypeptide retains immunogenicity and, when said altered G protein or polypeptide is incorporated into an immunogenic composition and administered to a vertebrate, provides protection
30 without inducing enhanced RSV disease upon subsequent infection of the vertebrate with RSV.

In another aspect, the invention provides use, for inducing an immune response in a vertebrate, of an effective amount of an isolated nucleic acid molecule encoding an altered RSV G protein or polypeptide effective to induce an immune response, and a transfection-facilitating agent, wherein the alteration is in one or more regions selected from the group consisting of the region from amino acid 159 to amino acid 198, the region from amino acid 159 to amino acid 174 as set out in SEQ ID NO: 15, the region from amino acid 171 to amino acid 187 as set out in SEQ ID NO: 17, the region from amino acid 176 to amino acid 190 as set out in SEQ ID NO: 18, and the region from amino acid 184 to amino acid 198 as set out in SEQ ID NO: 19, where said altered G protein or polypeptide retains immunogenicity and, when said altered G protein or polypeptide is incorporated into an immunogenic composition and administered to a vertebrate, provides protection without inducing enhanced RSV disease upon subsequent infection of the vertebrate with RSV.

In another aspect, the invention provides use, for inhibiting induction of enhanced disease after immunization and subsequent infection of a vertebrate with RSV, of an isolated altered RSV G protein or polypeptide, wherein the alteration is in one or more regions selected from the group consisting of the region from amino acid 159 to amino acid 198, the region from amino acid 159 to amino acid 174 as set out in SEQ ID NO: 15, the region from amino acid 171 to amino acid 187 as set out in SEQ ID NO: 17, the region from amino acid 176 to amino acid 190 as set out in SEQ ID NO: 18, and the region from amino acid 184 to amino acid 198 as set out in SEQ ID NO: 19, and where said isolated, altered G protein or polypeptide retains immunogenicity, and which isolated, altered G protein or polypeptide, when incorporated into an immunogenic

composition and administered to a vertebrate, provides protection without inducing enhanced RSV disease upon subsequent infection of the vertebrate with RSV.

In another aspect, the invention provides use, for
5 immunizing a vertebrate against RSV, of a composition comprising an immunologically effective amount of an isolated, altered G protein or polypeptide of RSV, wherein the alteration is in one or more regions selected from the group consisting of the region from amino acid 159 to amino
10 acid 198, the region from amino acid 159 to amino acid 174 as set out in SEQ ID NO: 15, the region from amino acid 171 to amino acid 187 as set out in SEQ ID NO: 17, the region from amino acid 176 to amino acid 190 as set out in SEQ ID NO: 18, and the region from amino acid 184 to amino
15 acid 198 as set out in SEQ ID NO: 19, and where said isolated, altered G protein or polypeptide retains immunogenicity, and which isolated, altered G protein or polypeptide, when incorporated into an immunogenic composition and administered to a vertebrate, does not
20 induce enhanced RSV disease upon subsequent infection of the vertebrate with RSV.

In another aspect, the invention provides an immunogenic composition comprising a physiologically acceptable vehicle and an immunologically effective amount
25 of a live attenuated pathogen which has inserted within it as a heterologous nucleic acid segment a nucleic acid sequence encoding an altered G protein or polypeptide of RSV, wherein the alteration is in one or more regions selected from the group consisting of the region from amino
30 acid 159 to amino acid 198, the region from amino acid 159 to amino acid 174 as set out in SEQ ID NO: 15, the region from amino acid 171 to amino acid 187 as set out in SEQ ID NO: 17, the region from amino acid 176 to amino

acid 190 as set out in SEQ ID NO: 18, and the region from amino acid 184 to amino acid 198 as set out in SEQ ID NO: 19, such that upon administration to the vertebrate, the altered G protein or polypeptide is
5 expressed and is immunogenic, but does not induce enhanced RSV disease upon subsequent infection of the vertebrate with RSV.

In another aspect, the invention provides use, for immunizing a vertebrate against RSV, of a composition
10 comprising a physiologically acceptable vehicle and an immunologically effective amount of a live attenuated pathogen which has inserted within it as a heterologous nucleic acid segment a nucleic acid sequence encoding an altered G protein or polypeptide of RSV, wherein the
15 alteration is in one or more regions selected from the group consisting of the region from amino acid 159 to amino acid 198, the region from amino acid 159 to amino acid 174 as set out in SEQ ID NO: 15, the region from amino acid 171 to amino acid 187 as set out in SEQ ID NO: 17, the region
20 from amino acid 176 to amino acid 190 as set out in SEQ ID NO: 18, and the region from amino acid 184 to amino acid 198 as set out in SEQ ID NO: 19, such that upon administration to the vertebrate, the altered G protein or polypeptide is expressed and is immunogenic, but does not
25 induce enhanced RSV disease upon subsequent infection of the vertebrate with RSV.

In another aspect, the invention provides an isolated, altered G protein or polypeptide of RSV which retains immunogenicity and which, when incorporated into an
30 immunogenic composition and administered to a vertebrate, does not induce enhanced disease upon subsequent infection of the vertebrate with RSV, said protein or polypeptide

having an amino acid sequence selected from the group consisting of SEQ ID NO: 32, SEQ ID NO: 33 and SEQ ID NO: 37.

In another aspect, the invention provides an immunogenic composition comprising a physiologically acceptable medium and an altered G protein or polypeptide of RSV which retains immunogenicity and which, when incorporated into an immunogenic composition and administered to a vertebrate, does not induce enhanced disease upon subsequent infection of the vertebrate with RSV, said protein or polypeptide having amino acid sequence selected from the group consisting of SEQ ID NO: 32, SEQ ID NO: 33 and SEQ ID NO: 37.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A and 1B are graphs illustrating the kinetics of leukocyte stimulation in BAL. BALB/c mice were vaccinated with either 1 µg G protein adjuvanted with Stimulon™ QS-21 (20 µg/mouse), native RSV A2 (1-2 x 10^6 PFU), or mock HEp-2 cell lysate. Two weeks post secondary vaccination, mice were challenged with RSV and thereafter 5 representatives from each vaccination group were sacrificed at days 3, 5, 7 and 10, and BAL cells isolated. Figure 1A shows total leukocyte counts which were performed by trypan blue exclusion. Figure 1B shows percent eosinophils in BAL which were determined by using the cell stain Diff-Quik. Data are presented as the mean count of 5 mice with error bars representing standard deviation.

Figure 2 is a table of synthetic peptides (SEQ ID NOS: 1-31) corresponding to overlapping regions of the G protein of RSV. A series of overlapping peptides were synthesized by Genosys Biotechnologies, Inc. (The Woodlands, TX). The peptides spanned the region from amino

acid 48 (which corresponds to the second translational start codon of G protein) to amino acid 294 of RSV A2 G protein. The purity of the peptides was determined by mass spectrometry. Lyophilized peptides were solubilized in
5 sterile water to a concentration of 2 mg/ml and stored at -20°C.

Figure 3 is a bar graph illustrating stimulation of G protein-primed splenocytes from BALB/c mice with G protein-derived peptides. BALB/c mice were vaccinated at
10 0 and 4 weeks with 1 μg G protein adjuvanted with Stimulon™ QS-21. Two weeks post-secondary vaccination, splenocytes from 5 mice were isolated, pooled and

cultured in the presence of antigen for 4 days. Each synthetic peptide was supplied at a concentration of 50 μg/ml. Native G protein was added at concentrations of 0.5 and 2.5 μg/ml. Concanavalin A (ConA) stimulation of splenocytes resulted in a mean cpm of 94,746 $\pm$ 8005. As controls, cultures were also stimulated with medium alone (Med) or CRM_{197} (CRM). Data are presented as the mean ($\pm$SD) of triplicate wells. The experiment is representative of five independent experiments, each of which showed qualitatively similar results.

Figures 4A and 4B are bar graphs showing an analysis of peptide-induced cytokines (IFN-γ and IL-5) in culture supernatants. Splenocytes from BALB/c mice vaccinated with native G protein and Stimulon™ QS-21 were cultured with the peptide antigens as described in the description of Figure 3. After 4 days of culture, 100 μl of supernatant was pooled from triplicate wells and subsequently assayed for IFN-γ (Figure 4A) and IL-5 (Figure 4B) by antigen capture ELISA. The data are presented as the mean OD_{490} of duplicate cytokine analyses.

Figure 5 is a bar graph showing the specific induction of pulmonary eosinophilia in BALB/c mice by peptide 19. Significant differences (*) are shown for G protein or 19-KLH vaccinated mice compared to control mice that received either PBS or KLH. The data are representative of three experiments in which similar results were obtained.

Figure 6 is a bar graph showing the identification of a T cell epitope in peptide 19 that facilitates the eosinophilic response. BALB/c mice (5 per group) were vaccinated intramuscularly at 0 and 4 weeks with either 1 μg of native purified RSV G protein in 20 μg Stimulon™ QS-21; 250 μg peptide 19-KLH; 250 μg peptide 22-KLH; 250 μg mutant peptide 19-1-KLH or 250 μg mutant peptide 19-2-KLH or intranasally with a 50 μl volume of live RSV

containing 10^6 pfu. Two weeks post-secondary vaccination, mice were challenged with live RSV and pulmonary eosinophilia quantitated by analysis of BAL 7 days thereafter. Data are presented as the mean percent of eosinophils in BAL (± standard deviation).

Figure 7 is a bar graph showing the proliferative responses of human PBMCs to G protein-derived peptides. PBMCs from 6 out of 43 donors that showed reactivity to RSV G protein were assayed for proliferation by culture in the presence of synthetic peptides 19 and 22. Each peptide was supplied at a concentration of 50 μg/ml. Purified G protein was added at a concentration of 3 μg/ml. PHA stimulation of PBMCs from all donors ranged from 22,945 to 55,619 cpm. PBMCs were also cultured in media alone and stimulation index calculated. Data are presented as the mean stimulation index obtained from triplicate cultures.

Figure 8 is a table showing that CD4 T cells mediate the eosinophilic response induced by RSV G protein and peptide 19-KLH. BALB/c mice (5 per group) were vaccinated intramuscularly at 0 and 4 weeks with either 1 μg of purified natural RSV G protein in 20 μg Stimulon™ QS-21; 250 μg KLH containing 18 μg peptide 19 adjuvanted with Stimulon™ QS-21; or intranasally with 50 μl of live RSV containing 10^6 pfu. In order to deplete T cell subsets, the indicated monoclonal antibodies (or rat Ig as a control) were administered intraperitoneally at 14 and 20 days post-final immunization, at doses of 750 μg and 250 μg per mouse, respectively. At day 21 post-final vaccination, mice were challenged with live RSV and pulmonary eosinophilia quantitated by analysis of BAL 7 days thereafter. FACS analysis was performed using anti-CD4 and anti-CD8 fluorescent antibodies. Data are presented as the mean percent of eosinophils in BAL (± standard deviation) and as the percent of CD4+ to CD8+ cells as a function of total splenic lymphocytes.

Significant differences (**) are indicated compared to similarly vaccinated control mice that received rat Ig.

DETAILED DESCRIPTION OF THE INVENTION

RSV G protein substantially augments the ability of F protein to protect BALB/c mice against challenge (Table). This suggests the inclusion of G protein in a subunit vaccine to RSV. However, the priming for pulmonary eosinophilia by G protein is both persistent and extensive, making it generally unsuitable for vaccine use. In quantitating the kinetics of influx of white blood cells into the BAL of vaccinated mice after challenge, it can be seen that the greatest cellular infiltrate (1.42 x 10^6 cells) occurs at day 7 in mice vaccinated with G protein (Figure 1A). Eosinophils were seen in response to vaccination with G protein throughout the 10-day time course, reaching a maximum of 65% of total white blood cells at day 7 (Figure 1B).

The present invention relates to the synthesis of RSV G protein-derived proteins and/or polypeptides that do not result in the stimulation of pulmonary eosinophilia upon subsequent RSV infection. Specifically, the work described herein is directed to compositions and methods of preparation of proteins and/or polypeptides comprising altered G proteins or polypeptides that can be used as immunogens in vaccine formulations, including multivalent vaccines, and which can be used for active immunization. The strategy involves alteration of one or more amino acids in a specific region of the G protein sequence, resulting in a protein or polypeptide derived from RSV G protein that is immunogenic without priming for atypical pulmonary inflammation (e.g., pulmonary eosinophilia) or any form of enhanced RSV disease.

The wild type (native) nucleotide and amino acid sequences of the RSV G protein are known in the art

(Wertz *et al.*, *Proc. Natl. Acad. Sci. USA 92*:4075-4079 (1985); Satake *et al.*, *Nucl. Acids Res. 13*(21): 7795-7810 (1985)). As used herein, "alteration" and its derivatives is intended to mean an amino acid sequence which is different from the wild type sequence, as well as a nucleotide sequence which encodes an amino acid sequence which is different from the wild type amino acid sequence. Alteration includes insertion, deletion and/or substitution of one or more nucleotides or amino acids.

For example, the alteration can be the insertion or deletion of a single nucleotide, or of more than one nucleotide, resulting in a frame shift mutation; the change of at least one nucleotide, resulting in a change in one or more encoded amino acids; the change of at least one nucleotide, resulting in the generation of a premature stop codon; the deletion of several nucleotides, resulting in a deletion of one or more amino acids encoded by the nucleotides; the insertion of one or several nucleotides, resulting in an interruption of the coding sequence of the gene; duplication of all or a part of the gene; transposition of all or a part of the gene; or rearrangement of all or a part of the gene. More than one such mutation may be present in a single gene. Such sequence changes cause an alteration in the G protein encoded by the gene. For example, if the alteration is a frame shift mutation, the frame shift can result in a change in the encoded amino acids, and/or can result in the generation of a premature stop codon, causing generation of a truncated protein.

For example, the alteration(s) can preferably preserve the three-dimensional configuration of the native G protein. Moreover, amino acids which are essential for the function of the G protein, particularly for immunogenicity, can be identified by methods known in the art. Particularly useful methods

include identification of conserved amino acids, site-directed mutagenesis and alanine-scanning mutagenesis (for example, Cunningham and Wells, *Science 244*:1081-1085 (1989)), crystallization and nuclear magnetic resonance. The altered polypeptides produced by these methods can be tested for particular biologic activities, including immunogenicity, reduction in pulmonary eosinophilia and antigenicity.

Specifically, appropriate amino acid alterations can be made on the basis of several criteria, including hydrophobicity, basic or acidic character, charge, polarity, size, the presence or absence of a functional group (e.g., -SH or a glycosylation site), and aromatic character. Assignment of various amino acids to similar groups based on the properties above will be readily apparent to the skilled artisan; further appropriate amino acid changes can also be found in Bowie *et al.* (*Science 247*:1306-1310(1990)).

For example, with reference to the region of amino acid 184 to 198, the alteration can take the form of conservative (e.g., glycine for alanine; valine for isoleucine; asparagine for glutamine) site-directed mutation of the region 184 to 198 (amino acid sequence AICKRIPNKKPGKKT; SEQ ID NO: 19) which retains attributes of the region of the G protein involved in protective immune responses but deletes or modifies epitopes involved in the stimulation of pulmonary eosinophilia (i.e., a biological equivalent). The alteration can also take the form of non-conservative mutations (e.g., lysine for threonine; alanine for proline) wherein the deleterious stimulation of eosinophilia is reduced or abolished. The alteration can also take the form of complete deletion of the region 184-198 or any part thereof, with continued use of the remaining RSV G protein derived moiety. Deletions can be replaced by linker regions which retain the spatiality of the

remaining G protein or polypeptide in order for optimal translation and/or immunogenicity. Alterations can be made using any standard mutagen or mutagenic process, such as site-directed mutation involving phages (e.g., 5 M13) or use of polymerase chain reaction (PCR) technology involving synthetic oligonucleotides.

Accordingly, the invention pertains to a nucleotide sequence encoding an altered G protein of RSV, or portion thereof, wherein the altered G protein or 10 portion thereof retains immunogenicity. As used herein, the term "altered G protein" is intended to mean a G protein (or portion thereof) of RSV which retains immunogenicity and which, when incorporated into an immunogenic composition or vaccine and administered to a 15 vertebrate, does not induce enhanced disease (e.g., atypical pulmonary inflammation, such as pulmonary eosinophilia) upon subsequent infection with RSV. In a particular embodiment, the altered G protein comprises an alteration in the region from amino acid 184 to amino 20 acid 198.

Although the invention is specifically described with relation to the region of RSV G protein comprising amino acid 184-198, it is intended that the methodologies described herein used to identify the 184- 25 198 region can be applied to additional regions of the wild type G protein to identify additional regions for alteration. For example, the regions upstream (toward the amino-terminus) and downstream (toward the carboxy-terminus) of the studied amino acid region (48 to 294) 30 can be analyzed for additional domains in which alteration will produce beneficial effects. Alternatively, the region of amino acids from 48 to 294 can be re-analyzed with peptides having different overlaps to identify other domains in which alteration 35 would be beneficial.

As appropriate, nucleic acid molecules of the present invention can be RNA, for example, mRNA, or DNA, such as cDNA and genomic DNA. DNA molecules can be double-stranded or single-stranded; single stranded RNA or DNA can be either the coding, or sense, strand or the non-coding, or antisense, strand. Preferably, the nucleic acid molecule comprises at least about 14 nucleotides, more preferably at least about 50 nucleotides, and even more preferably at least about 200 nucleotides. The nucleotide sequence can be only that which encodes at least a fragment of the amino acid sequence of the altered G protein; alternatively, the nucleotide sequence can include at least a fragment of the altered G protein amino acid coding sequence along with additional non-coding sequences such as introns and non-coding 3' and 5' sequences (including regulatory sequences, for example). Additionally, the nucleotide sequence can be fused to a marker sequence, for example, a sequence which encodes a polypeptide to assist in isolation or purification of the polypeptide. Such sequences include, but are not limited to, those which encode a glutathione-S-transferase (GST) fusion protein and those which encode a hemagglutinin A (HA) peptide marker from influenza.

The term "nucleotide sequence" can include a nucleotide sequence which is synthesized chemically or by recombinant means. Thus, recombinant DNA contained in a vector is included in the invention. Also, nucleotide sequences include recombinant DNA molecules in heterologous host cells, as well as partially or substantially purified DNA molecules in solution. *In vivo* and *in vitro* RNA transcripts of the DNA molecules of the present invention are also encompassed by nucleotide sequences of the invention. Such nucleotide sequences are useful, e.g., in the manufacture of the encoded altered G protein.

The invention also encompasses variations of the nucleotide sequences of the invention, such as those encoding portions, analogues or derivatives of the altered G protein, provided the portion, analogue or derivative comprises the altered G protein. Such variations can be naturally-occurring variations in the unaltered portion of the nucleotide sequence, such as in the case of allelic variation, or non-naturally-occurring, such as those induced by various mutagens and mutagenic processes. Intended variations include, but are not limited to, addition, deletion and substitution of one or more nucleotides which can result in conservative or non-conservative amino acid changes, including additions and deletions.

The invention described herein also relates to fragments of the nucleic acid molecules described above. The term "fragment" is intended to encompass a portion of a nucleotide sequence described herein which is from at least about 14 contiguous nucleotides to at least about 50 contiguous nucleotides or longer in length, providing that such fragments encode an altered G polypeptide; such fragments are useful as primers. Particularly preferred primers and probes selectively hybridize to the nucleic acid molecule encoding the altered G protein described herein. For example, fragments which encode antigenic portions of the altered G protein described herein are useful.

The invention also pertains to nucleotide sequences which hybridize under medium, and, more preferably, high stringency hybridization conditions (e.g., for selective hybridization) to a nucleotide sequence described herein. Appropriate stringency conditions are known to those skilled in the art or can be found in standard texts such as *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6.

Accordingly, the invention pertains to nucleotide sequences which have a substantial identity with the altered nucleotide sequences described herein; particularly preferred are nucleotide sequences which have at least about 90%, and more preferably at least about 95% identity with nucleotide sequences described herein. Particularly preferred in this instance are nucleotide sequences encoding polypeptides having substantially similar immunogenic activity as the altered G protein described herein.

This invention also pertains to an altered G protein or polypeptide of RSV. The altered G protein or polypeptide is a G protein (or portion thereof) of RSV which retains immunogenicity and which, when incorporated into an immunogenic composition or vaccine and administered to a vertebrate, does not induce enhanced disease (e.g., atypical pulmonary inflammation such as pulmonary eosinophilia) upon subsequent infection with RSV. In a particular embodiment, the altered G protein comprises at least one alteration in the region from amino acid 184 to amino acid 198. The altered G protein of the invention can be partially or substantially purified (e.g., purified to homogeneity), and/or is substantially free of other proteins.

The altered G protein or polypeptide can also be a fusion protein comprising all or a portion of the altered G protein amino acid sequence fused to an additional component. Additional components, such as radioisotopes and antigenic tags, can be selected to assist in the isolation or purification of the polypeptide or to extend the half life of the polypeptide; for example, a hexahistidine tag would permit ready purification by nickel chromatography. Alternatively, the altered G protein or polypeptide can be a fusion protein comprising all or a portion of the altered G protein amino acid sequence fused to all or a

portion of the RSV F protein amino acid sequence (Collins *et al.*, *Proc. Natl. Acad. Sci (USA) 81*:7683-7687 (1984); U.S. Patent No. 5,639,853; U.S. Patent No. 5,723,130).

5 The invention also includes altered G proteins and polypeptides which comprise additional amino acid alterations beyond those alterations necessary to prevent production of enhanced disease in a vertebrate to which the altered protein or polypeptide is
10 administered. For example, amino acid alterations, e.g., conservative amino acid changes which do not impact on the disease characteristics resulting from administration of the altered protein are included in the invention. Also included in the invention are
15 polypeptides which are at least about 40% identical to the altered G protein or polypeptide described herein. However, polypeptides exhibiting lower levels of identity are also useful, particular if they exhibit high, e.g., at least about 40%, identity over one or
20 more particular domains of the protein. For example, altered polypeptides sharing high degrees of identity over domains necessary for particular activities, including immunogenic function and receptor binding activity, are included herein. Polypeptides described
25 herein can be chemically synthesized or recombinantly produced.

To determine the percent identity of two polypeptide sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be
30 introduced in the sequence of a first amino acid sequence). The amino acid residues at corresponding amino acid positions are then compared. When a position in the first sequence is occupied by the same amino acid residue as the corresponding position in the second
35 sequence, then the molecules are identical at that position. The percent identity between the two

sequences is a function of the number of identical positions shared by the sequences (i.e., % identity = # of identical positions/total # of positions x 100).

The determination of percent identity between two
5 sequences can be accomplished using a mathematical algorithm. A preferred, non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin *et al.*, *Proc. Natl. Acad. Sci. USA*, *90*:5873-5877 (1993). Such an
10 algorithm is incorporated into the NBLAST and XBLAST program, score = 50, wordlength = 3, to obtain amino acid sequences having the desired identity to polypeptide or protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped
15 BLAST can be utilized as described in Altschul *et al.*, *Nucleic Acids Res*, *25*:3389-3402 (1997). When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See http://www.ncbi.nlm.nih.gov. Another
20 preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers *et al.*, CABIOS (1989). Such an algorithm is incorporated into the ALIGN program (version 2.0) which is part of the GCG sequence
25 alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used.

The percent of identity between two sequences can be
30 determined using techniques similar to those described above, with or without allowing gaps. In calculating percent identity, only exact matches are counted.

The invention also provides expression vectors, e.g., nucleic acid constructs, containing a nucleic acid
35 sequence encoding an altered G protein or polypeptide, operably linked to at least one regulatory sequence.

Many such vectors are commercially available, and other suitable vectors can be readily prepared by the skilled artisan. "Operably linked" is intended to mean that the nucleotide sequence is linked to a regulatory sequence in a manner which allows expression of the nucleic acid sequence; this term is intended to include both direct physical linkage and linkage by means of a linker or intervening sequence. Regulatory sequences are art-recognized and are selected to produce a polypeptide which is an altered G protein or polypeptide. Accordingly, the term "regulatory sequence" includes promoters, enhancers, and other expression control elements which are described in Goeddel, *Gene Expression Technology: Methods in Enzymology 185*, Academic Press, San Diego, CA (1990). For example, the native regulatory sequences or regulatory sequences native to the transformed host cell can be employed. It should be understood that the design of the expression vector may depend on such factors as the choice of the host cell to be transformed and/or the type of protein desired to be expressed.

For instance, the altered G proteins and polypeptides of the present invention can be produced by ligating the nucleic acid molecule, or a portion thereof, into a vector suitable for expression in either prokaryotic cells, eukaryotic cells or both (see, for example, Broach, *et al.*, *Experimental Manipulation of Gene Expression*, ed. M. Inouye (Academic Press, 1983) p. 83; *Molecular Cloning: A Laboratory Manual*, 2nd Ed., ed. Sambrook *et al.* (Cold Spring Harbor Laboratory Press, 1989) Chapters 16 and 17). Typically, expression constructs will contain one or more selectable markers, including, but not limited to, the gene that encodes dihydrofolate reductase and the genes that confer resistance to neomycin, tetracycline, ampicillin, chloramphenicol, kanamycin and streptomycin resistance.

The expression construct can comprise a regulatory sequence operably linked to a nucleic acid molecule encoding an altered G protein or polypeptide, optionally linked, either directly or by means of a polynucleotide linker, to a nucleic acid molecule encoding all or a portion of the RSV F protein. Expression of such an expression construct will result in a chimera comprising an altered G protein or polypeptide and all or a portion of an F protein or polypeptide; if a polynucleotide linker is utilized in the construct, the F and altered G polypeptides will be linked by one or more amino acids. Methods for preparing and expressing F/G chimeras in general are taught, e.g., in U.S. Patent No. 5,194,595 (Wathen).

Prokaryotic and eukaryotic host cells transfected by the described vectors are also provided by this invention. For instance, cells which can be transfected with the vectors of the present invention include, but are not limited to, bacterial cells such as *E. coli* (e.g., *E. coli* K12 strains), *Streptomyces*, *Pseudomonas*, *Serratia marcescens* and *Salmonella typhimurium*, insect cells (baculovirus), including *Drosophila*, fungal cells, such as yeast cells, plant cells and mammalian cells, such as thymocytes, Chinese hamster ovary cells (CHO), HEp-2 cells, Vero cells and COS cells.

Thus, a nucleotide sequence encoding the altered G protein or polypeptide described herein can be used to produce a recombinant form of the protein via microbial or eukaryotic cellular processes. Ligating the polynucleotide sequence into a gene construct, such as an expression vector, and transforming or transfecting into hosts, either eukaryotic (yeast, avian, insect, plant or mammalian) or prokaryotic (bacterial cells), are standard procedures used in producing other well known proteins. Viral vectors include, but are not

limited to, adenoviruses and Venezuelan equine encephalitis vector. In addition, Vaccinia virus (VV) has been used to express in mammalian cell lines, or deliver to animal models, various proteins of RSV
5 (Olmstead *et al.*, *PNAS 83*:7462-7466 (1986); Wertz *et al.*, *J. Virol 63*:4767-4776 (1989)). Likewise, similar constructs with the altered cDNA for RSV G protein inserted into the thymidine kinase gene of VV may be utilized to synthesize the altered G protein or
10 polypeptide. For example, the methods detailed by Ball *et al.*, (*Proc. Natl. Acad. Sci. USA 83*:246-250 (1986)) or Olmstead *et al.*, (*Proc. Natl. Acad. Sci. USA 83*:7462-7466 (1986)) can be used to express the altered G protein or the F protein/altered G protein chimera from
15 vaccinia virus vectors. Similar procedures, or modifications thereof, can be employed to prepare recombinant proteins according to the present invention by microbial means or tissue-culture technology. Accordingly, the invention pertains to the production of
20 altered G proteins or polypeptides by recombinant technology.

In addition to the foregoing host cell systems in which the altered G proteins or polypeptides of this invention are produced *in vitro*, a variety of systems
25 are appropriate for expression and delivery of such altered G proteins and polypeptides *in vivo*. These systems utilize attenuated pathogens such as bacteria or viruses as delivery agents. These live attenuated pathogens have inserted within them as a heterologous
30 nucleic acid segment the nucleic acid sequence encoding the desired altered G proteins or polypeptides of this invention. Using these systems, the desired altered G proteins or polypeptides are expressed by a live, attenuated bacterium or virus within the body of a
35 vertebrate.

Examples of such live attenuated pathogens include, but are not limited to, the live attenuated bacteria such as Salmonella which are described in U.S. Patent Number 4,837,151, which is particularly suitable for oral delivery, and the live attenuated Venezuelan Equine Encephalitis virus described in U.S. Patent Number 5,643,576, which is particularly suitable for intranasal or inhalation delivery.

The proteins and polypeptides of the present invention can be isolated or purified (e.g., to homogeneity) from recombinant cell culture by a variety of processes. These include, but are not limited to, anion or cation exchange chromatography, ethanol precipitation, affinity chromatography and high performance liquid chromatography (HPLC). The particular method used will depend upon the properties of the polypeptide and the selection of the host cell; appropriate methods will be readily apparent to those skilled in the art.

The present invention also relates to antibodies which bind an altered G protein or polypeptide. For instance, polyclonal and monoclonal antibodies, including non-human and human antibodies, humanized antibodies, chimeric antibodies and antigen-binding fragments thereof (*Current Protocols in Immunology*, John Wiley & Sons, N.Y. (1994); EP Application 173,494 (Morrison); International Patent Application WO86/01533 (Neuberger); and U.S. Patent No. 5,225,539 (Winters)) which bind to the described altered G protein are within the scope of the invention. A mammal, such as a mouse, rat, hamster or rabbit, can be immunized with an immunogenic form of the altered G protein or polypeptide. Techniques for conferring immunogenicity on a protein or peptide include conjugation to carriers or other techniques well known in the art. The protein or polypeptide can be administered in the presence of an

adjuvant. The progress of immunization can be monitored by detection of antibody titers in plasma or serum. Standard ELISA or other immunoassays can be used with the immunogen as antigen to assess the levels of antibody.

Following immunization, anti-peptide antisera can be obtained, and if desired, polyclonal antibodies can be isolated from the serum. Monoclonal antibodies can also be produced by standard techniques which are well known in the art (Kohler and Milstein, *Nature 256*:495-497 (1975); Kozbar et al., *Immunology Today 4*:72 (1983); and Cole et al., *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96 (1985)). The term "antibody" as used herein is intended to include fragments thereof, such as Fab and $F(ab)_2$. Antibodies described herein can be used to inhibit the activity of the altered G protein described herein, particularly *in vitro* and in cell extracts, using methods known in the art. As used herein, "inhibition" is intended to mean any reduction in quantity or quality, including complete absence. Additionally, such antibodies, in conjunction with a label, such as a radioactive label, can be used to assay for the presence of the expressed protein in a cell from, e.g., a tissue sample or cell culture, and can be used in an immunoabsorption process, such as an ELISA, to isolate the altered G protein or polypeptide. Tissue samples which can be assayed include human tissues, e.g., differentiated and non-differentiated cells. Examples include lung, bone marrow, thymus, kidney, liver, brain, pancreas, fibroblasts and epithelium.

The present invention also pertains to pharmaceutical compositions comprising altered G proteins and polypeptides described herein. For instance, an altered G polypeptide or protein, or prodrug thereof, of the present invention can be

formulated with a physiologically acceptable medium to prepare a pharmaceutical composition (e.g., an immunogenic composition). The particular physiological medium may include, but is not limited to, water, buffered saline, polyols (e.g., glycerol, propylene glycol, liquid polyethylene glycol) and dextrose solutions. The optimum concentration of the active ingredient(s) in the chosen medium can be determined empirically, according to well known procedures, and will depend on the ultimate pharmaceutical formulation desired. Methods of introduction of exogenous peptides at the site of treatment include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, oral and intranasal. Other suitable methods of introduction can also include gene therapy, rechargeable or biodegradable devices, aerosols and slow release polymeric devices. The altered G protein can be administered in conjunction with additional immunogens, including all or a portion of RSV F protein; the altered G protein or polypeptide can be administered separately, sequentially or concurrently with the additional immunogen. For example, the altered G protein or polypeptide can be given in an admixture with all or a portion of RSV F protein.

The altered G protein or polypeptide (or admixture, fusion protein or chimera thereof) can be used as antigen to elicit an immune response to the antigen in a vertebrate, such as a mammalian host. For example, the antigen can be all or an immunogenic portion of the altered G protein or a chimera of the altered G protein or polypeptide and all or an immunogenic portion of the RSV F protein. The descriptions herein relating to compositions comprising an altered G protein or polypeptide are intended to include compositions comprising an altered G protein or polypeptide along with all or a portion of the RSV F protein.

The method of the present invention comprises administering to the vertebrate an immunologically effective dose of a vaccine composition comprising a mixture of an altered G protein or polypeptide and any suitable adjuvant. As used herein, an "adjuvant" is intended to mean any agent which is sufficient to enhance or modify the immune response to the vaccine antigen. As used herein, an "immunologically effective" dose of the vaccine composition is a dose which is suitable to elicit an immune response. The particular dosage will depend upon the age, weight and medical condition of the vertebrate to be treated, as well as on the method of administration. Suitable doses will be readily determined by the skilled artisan. The vaccine composition can be optionally administered in a pharmaceutically or physiologically acceptable vehicle, such as physiological saline or ethanol polyols such as glycerol or propylene glycol.

Suitable adjuvants include vegetable oils or emulsions thereof, surface active substances, e.g., hexadecylamin, octadecyl amino acid esters, octadecylamine, lysolecithin, dimethyl-dioctadecylammonium bromide, N,N-dicoctadecyl-N'-N'bis (2-hydroxyethyl-propane diamine), methoxyhexadecylglycerol, and pluronic polyols; polyamines,e.g., pyran, dextransulfate, poly IC, carbopol; peptides, e.g., muramyl dipeptide, dimethylglycine, tuftsin; immune stimulating complexes; oil emulsions; mineral gels; aluminum compounds such as aluminum hydroxide and aluminum phosphate; MPL™ (3-O-deacylated monophosphoryl lipid A, RIBI ImmunoChem Research, Inc., Hamilton, MT); detoxified mutants of Cholera toxin and E. coli heat labile toxin; naked DNA CpG motifs; and Stimulon™ QS-21 (Aquila Biopharmaceuticals, Inc., Framingham, MA). The altered G protein or polypeptide of this invention can also be

incorporated into liposomes or ISCOMS (immunostimulating complexes), and supplementary active ingredients may also be employed. The antigens of the present invention can also be administered in combination with lymphokines, including, but not limited to, IL-2, IL-3, IL-12, IL-15, IFN-γ and GM-CSF.

The compositions and vaccines of this invention can be administered to a human or animal by a variety of routes, including parenteral, intrarterial, intradermal, transdermal (such as by the use of slow release polymers), intramuscular, intraperitoneal, intravenous, subcutaneous, oral and intranasal routes of administration. The amount of altered G protein employed in such vaccines will vary depending upon the route of administration and physical characteristics of the subject vertebrate. Adjustment and manipulation of established dosage ranges used with traditional carrier antigens for adaptation to the present vaccine is well within the ability of those skilled in the art. The vaccines of the present invention are intended for use in the treatment of both immature and adult vertebrates, and, in particular, humans.

The altered G protein or polypeptide of the present invention can be coupled to a carrier molecule in order to modulate or enhance the immune response. Suitable carrier proteins include bacterial toxins which are safe for administration to vertebrates and immunologically effective as carriers. Examples include pertussis, diphtheria, and tetanus toxoids and non-toxic mutant proteins (cross-reacting materials (CRM)), such as the non-toxic variant of diphtheria toxoid, CRM_{197}. Fragments of the native toxins or toxoids, which contain at least one T-cell epitope, are also useful as carriers for antigens. Methods for preparing conjugates of antigens and carrier molecules are well known in the art and can be found, for example, in Wong, *Chemistry of*

Protein Conjugation (CRC Press Inc., Ann Arbor, MI (1991)); Bernatowicz and Matsueda, *Analytical Biochemistry* *155*:95-102 (1986); Frisch *et al.*, *Bioconjugate Chem.* 7:180-186 (1996); and Boeckler *et al.*, *J. Immunological Methods* *191*:1-10 (1996).

In addition, if the entire peptide 19 region (amino acids 184-198) is deleted, one or more epitopes from an antigen from another organism, including, but not limited to, parainfluenza virus type 3, can be inserted into the deleted region, in order to create a bivalent vaccine.

The invention also relates to a vaccine comprising a nucleic acid molecule encoding an altered G protein or polypeptide of RSV, wherein said altered G protein or polypeptide retains immunogenicity and, when incorporated into an immunogenic composition or vaccine and administered to a vertebrate, provides protection without inducing enhanced disease upon subsequent infection of the vertebrate with RSV, and a physiologically acceptable vehicle. Such a vaccine is referred to herein as a nucleic acid vaccine or DNA vaccine and is useful for the genetic immunization of vertebrates.

The term, "genetic immunization", as used herein, refers to inoculation of a vertebrate, particularly a mammal, with a nucleic acid vaccine directed against a pathogenic agent, particularly RSV, resulting in protection of the vertebrate against RSV. A "nucleic acid vaccine" or "DNA vaccine" as used herein, is a nucleic acid construct comprising a nucleic acid molecule encoding a polypeptide antigen, particularly an altered G protein or polypeptide of RSV described herein. The nucleic acid construct can also include transcriptional promoter elements, enhancer elements, splicing signals, termination and polyadenylation signals, and other nucleic acid sequences.

"Protection against RSV" as used herein refers to generation of an immune response in the vertebrate, the immune response being protective (partially or totally) against manifestations of the disease caused by RSV. A vertebrate that is protected against disease caused by the RSV virus may be infected with RSV, but to a lesser degree than would occur without immunization; may be infected with RSV, but does not exhibit disease symptoms; or may be infected with RSV, but exhibits fewer disease symptoms than would occur without immunization. Alternatively, the vertebrate that is protected against disease caused by RSV may not become infected with the RSV virus at all, despite exposure to the virus. In all cases, however, the nucleic acid vaccine does not induce enhanced disease upon subsequent infection of the vertebrate with RSV.

The nucleic acid vaccine can be produced by standard methods. For example, using known methods, a nucleic acid (e.g., DNA) encoding an altered G protein or polypeptide of RSV, can be inserted into an expression vector to construct a nucleic acid vaccine (see Maniatis *et al.*, *Molecular Cloning, A Laboratory Manual*, 2nd edition, Cold Spring Harbor Laboratory Press (1989)).

The individual vertebrate is inoculated with the nucleic acid vaccine (i.e., the nucleic acid vaccine is administered), using standard methods. The vertebrate can be inoculated subcutaneously, intravenously, intraperitoneally, intradermally, intramuscularly, topically, orally, rectally, nasally, buccally, vaginally, by inhalation spray, or via an implanted reservoir in dosage formulations containing conventional non-toxic, physiologically acceptable carriers or vehicles. Alternatively, the vertebrate is inoculated with the nucleic acid vaccine through the use of a particle acceleration instrument (a "gene gun"). The

form in which it is administered (e.g., capsule, tablet, solution, emulsion) will depend in part on the route by which it is administered. For example, for mucosal administration, nose drops, inhalants or suppositories can be used.

The nucleic acid vaccine can be administered in conjunction with any suitable adjuvant. The adjuvant is administered in a sufficient amount, which is that amount that is sufficient to generate an enhanced immune response to the nucleic acid vaccine. The adjuvant can be administered prior to (e.g., 1 or more days before) inoculation with the nucleic acid vaccine; concurrently with (e.g., within 24 hours of) inoculation with the nucleic acid vaccine; contemporaneously (simultaneously) with the nucleic acid vaccine (e.g., the adjuvant is mixed with the nucleic acid vaccine, and the mixture is administered to the vertebrate); or after (e.g., 1 or more days after) inoculation with the nucleic acid vaccine. The adjuvant can also be administered at more than one time (e.g., prior to inoculation with the nucleic acid vaccine and also after inoculation with the nucleic acid vaccine). As used herein, the term "in conjunction with" encompasses any time period, including those specifically described herein and combinations of the time periods specifically described herein, during which the adjuvant can be administered so as to generate an enhanced immune response to the nucleic acid vaccine (e.g., an increased antibody titer to the antigen encoded by the nucleic acid vaccine, or an increased antibody titer to RSV). The adjuvant and the nucleic acid vaccine can be administered at approximately the same location on the vertebrate; for example, both the adjuvant and the nucleic acid vaccine are administered at a marked site on a limb of the vertebrate.

In a particular embodiment, the nucleic acid construct is co-administered with a transfection-

facilitating agent. In a preferred embodiment, the transfection-facilitating agent is dioctylglycylspermine (DOGS) (published PCT application publication no. WO 96/21356). In another embodiment, the transfection-facilitating agent is bupivicaine (U.S. Patent 5,593,972).

The invention also provides a method of inducing an immune response in a vertebrate, comprising administering to the vertebrate an immunogenic composition, vaccine or nucleic acid vaccine described herein in an amount effective to induce an immune response. In a particular embodiment, the vertebrate is a seronegative vertebrate, e.g., a seronegative human. The invention also provides a method of immunizing a vertebrate, e.g., an RSV seronegative human, against RSV, comprising administering to the vertebrate a composition comprising an immunologically effective amount of altered G protein or polypeptide of RSV which retains immunogenicity and which, when incorporated into an immunogenic composition or vaccine and administered to a vertebrate, does not induce enhanced disease upon subsequent infection of the vertebrate with RSV. Alternatively, the composition comprises a nucleic acid molecule encoding an immunologically effective amount of altered G protein or polypeptide of RSV which retains immunogenicity and which, when incorporated into an immunogenic composition or vaccine and administered to a vertebrate, does not induce enhanced disease upon subsequent infection of the vertebrate with RSV. The invention also relates to a method of vaccinating a vertebrate, comprising administering to the vertebrate a vaccine or nucleic acid vaccine described herein.

Collectively, the data described herein show that peptide 19 (AICKRIPNKKPGKKT) (SEQ ID NO: 19) primes for pulmonary eosinophilia by stimulating the expansion of $CD4^+$ T cells destined to secrete IL-5, a cytokine

associated with the induction and recruitment of eosinophils, upon restimulation (Coffman *et al.*, *Science* *245*:308-310 (1989)).

Without wishing to be bound by theory, a model of immune priming is suggested in which one or more Th cell epitopes within peptide 19 control the qualitative nature of subsequent immune responses, resulting in a profound skewing toward the Th2 phenotype. That the peptide component of the T cell receptor (TCR)-MHC interaction can modulate the quality of the immune response between Th1 and Th2 phenotypes has previously been shown by altering peptide sequences (Pfieffer *et al.*, *J. Exp. Med.* *181*:1569-1574 (1995); Murray *et al.*, *Eur. J. Immunol.* *24*:2337-2344 (1994)). However, it remains possible that the 15 amino acids that comprise peptide 19 contain more than one T cell epitope, each with a discrete ability to stimulate a Th1 versus Th2 response. In favor of this hypothesis, an analysis of the sequence of peptide 19 indicates that it contains three potential T cell epitopes restricted to MHC class II I-E^d which align closely at the critical 1, 4, 6 and 9 anchor residues (I, K or R, I, and K, respectively) (amino acids 187, 189 and 192 of peptide 19)(Rammensee *et al.*, *Immunogenetics* *41*:178-228 (1995)). Each of these putative sequences is consistent with class II binding based upon the publication of known ligands generated by the biochemical isolation of MHC-associated peptides or by peptide binding assays (Rammensee *et al.*, *Immunogenetics* *41*:178-228 (1995)). The mutation of peptide 19 (AICKRIPNKKPGKKT) (SEQ ID NO. 19) to a sequence which disrupts the critical MHC-binding anchor regions of the potential T cell epitopes (AICGRGPNGKPGKKT (mutant 1; SEQ ID NO. 32) or AGCGRGPGGKPGKGT (mutant 2; SEQ ID NO: 33)) completely abrogated the ability of this peptide to predispose mice for pulmonary eosinophilia (Figure 6).

The data presented herein provide a positive correlation between the peptide encompassing amino acids 184 to 198 of G protein and the predisposition for pulmonary eosinophilia. Thus, for seronegative populations, the results argue for the construction of a vaccine for RSV that is genetically modified in the region of amino acids 184 to 198 of G protein. This vaccine would not bias recipients for atypical pulmonary disease, but would retain an ability to protect against subsequent RSV challenge. The alignment of HLA type with reactivity to peptide 19 may provide a more profound understanding of the role of this amino acid sequence in the onset of bronchiolitis, atopy or asthma that is sometimes observed following RSV infection of seronegative infants (Welliver and Welliver, *Pediatrics in Review 14*:134-139 (1993)). Thus, the most favorable RSV vaccine strategy for seronegative populations would consist of components that, while not priming for immunopathological sequelae, achieve a balanced immune response resulting in the stimulation of protective $CD4^+$ and $CD8^+$ cell types. The data presented in Figures 4A and 4B identify a number of peptides which stimulate IFN-γ secretion and may play this role (viz: peptides 10, 14, 16 and 18). Similarly, the data suggested a number of peptides (e.g., for donor 100; peptides 2, 4, 9, 15, and 29) which were able to stimulate proliferation of PBMCs and which may be sufficient for protection against RSV challenge in the absence of the sequence occupying peptide 19.

The following Examples are offered for the purpose of illustrating the present invention and are not to be construed to limit the scope of this invention.

EXAMPLES

MATERIALS AND METHODS

Mice

Female BALB/c (H-2^d) mice, aged 7-9 weeks, were purchased from Taconic Farms, Inc. (Germantown, NY). All mice were housed in a facility designated by the American Association for Accreditation of Laboratory Animal Care.

Preparation and Use of Vaccine Antigens:

Viral particles from strain A2 of RSV were produced by infecting HEp-2 cells (ATCC CCL 23) and subsequently clarifying the virus by centrifugation of culture supernatants to remove cell debris. RSV F and G proteins were purified from the A2 strain of RSV that had been grown in Vero cells (ATCC CCL 81). G protein was isolated using immunoaffinity chromatography with the G protein-specific monoclonal antibody L7 (hybridoma deposited as ATCC HB10233) as previously described (Hancock *et al.*, *J. Virol.* *70*:7783-7791 (1996)). The resultant G protein was determined, by ELISA and SDS-PAGE, to be >95% pure. For immunizations, each mouse was vaccinated intramuscularly at 0 and 4 weeks with 0.1 ml of PBS containing 1 µg of purified G protein and 20 µg Stimulon™ QS-21 (Aquila Biopharmaceuticals, Inc., Framingham, MA) as an adjuvant, unless otherwise stated. F protein was purified by ion-exchange chromatography and 3 µg used in vaccinations adjuvanted with 100 µg aluminum hydroxide (AlOH). For RSV vaccinations and challenges, 1-2 x 10^6 plaque forming units (pfu) of infectious RSV A2 was administered intranasally in a 50 µl volume. An equal volume of HEp-2 cell lysate was utilized as a mock vaccination.

Preparation and Use of Peptide Antigens

A series of synthetic peptides corresponding to overlapping regions of the G proteinof RSV were synthesized by Genosys Biotechnologies, Inc. (The Woodlands, TX) (Figure 2). The resultant series encompassed amino acids 48-294 of RSV A2 G protein (Wertz *et al.*, *Proc. Natl. Acad. Sci. USA 82*:4075-4079 (1985)). The purity of the peptides was determined by mass spectometry to be above 90%. Lyophilized peptides were dissolved in sterile water to a concentration of 2 mg/ml and stored at -20°C. Peptides were used at a concentration of 50 μg/ml to stimulate human peripheral blood mononuclear cells (PBMCs) or G protein-primed murine spleen cells *in vitro*.

Selected peptides were conjugated to maleimide-activated (Partis *et al.*, *J. Prot. Chem. 2*:263-277 (1983)) keyhole limpet hemocyanin (KLH) using an Imject® activated conjugation kit (Pierce Chemical, Rockford, IL). Since the mechanism of conjugation was dependent upon a chemical reaction between maleimide groups in KLH and SH groups in the peptide, a cysteine was added to the carboxy-terminus end of peptide 22. The degree to which the various peptides were conjugated was quantitated by determining the loss of thiol groups in the peptide, using Ellman's reagent (Pierce Chemical). The extent of conjugation (typically 50-80 μg peptide per mg of KLH) seen in these reactions compared favorably with that previously seen for the attachment of peptides to KLH by this technique (Tsao *et al.*, *Anal. Biochemistry 197*:137-142 (1991)). For study of the induction of eosinophilia by various peptides, 250 μg of KLH conjugated to the respective peptides were adjuvanted with 20 μg Stimulon™ QS-21 in 0.1 ml of PBS and used to immunize each mouse, intramuscularly, at 0 and 4 weeks. Two weeks post secondary vaccination, mice were challenged with 1-2 x 10^6 PFU of infectious RSV A2,

administered intranasally in a 50 μl volume (Hancock *et al., J. Virol* 70:7783-7791 (1996)). Seven days later, mice were sacrificed by cervical dislocation and bronchoalveolar lavage (BAL) was performed.

5 Titration of Infectious RSV:

Supernatants derived from the homogenized lungs of RSV-infected mice were serially diluted and permitted to infect monolayers of HEp-2 cells. After a 2-hour incubation, the inoculum was aspirated and each well was 10 overlayed with 1% Sephadex G-75 in media. After a further 3 day incubation, the gel overlay was removed and the wells were fixed in 80% methanol. RSV plaques were identified using a monoclonal antibody to RSV G protein and a secondary mAb of goat anti-mouse 15 conjugated to horseradish peroxidase. Color was developed by addition of the substrate, 0.05% 4-chloronaphthol/0.09% hydrogen peroxide in phosphate buffered saline (PBS). RSV plaque forming units (pfu) were enumerated and the titers expressed as pfu per gram 20 of lung tissue.

BALB/c mice were primed intramuscularly with one of several vaccines composed of the native fusion (F) and/or attachment (G) proteins purified from the A2 strain of RSV. Three groups of mice were primed with 25 either 3,000, 300, or 30 ng F protein/dose. Three separate groups of mice were primed with 1,000, 100, or 10 ng G protein/dose. Also 3 groups of mice were primed with a combination vaccine containing 3,000 + 1,000; 300 + 100: or 30 + 10 ng F and G protein, respectively. All 30 vaccines were formulated with aluminum hydroxide (AlOH, 100 μg/dose). Control mice were either infected with the A2 strain of RSV or received an intramuscular injection of PBS/AlOH. Four weeks after primary immunization all mice were challenged with the A2 strain 35 of RSV. Four days later the mice were sacrificed and

the pulmonary tissues were processed for the quantitation of infectious virus (Table).

Table: Determination of RSV titers in Mouse Lung

ANTIGEN (ng)	GMT RSV[a]
F(3000)+G(1000)	<1.6±0.2
F(300)+G(100)	<2.1±0.7
F(30)+G(10)	<2.8±0.9[b]
F(3000)	<2.1±0.5
F(300)	<2.5±1.0
F(30)	4.1±0.8
G(1000)	3.5±0.5
G(100)	4.7±0.2
G(10)	4.7±0.2
PBS	5.1±0.2
RSV	<1.6±0.1

[a] GMT is the geometric means titer ($\log_{10}$) ± 1 standard deviation of RSV per gram of pulmonary tissue. The GMT RSV was determined 4 days after intranasal challenge.

[b] $P<0.05$ vs. groups vaccinated with F (30) alone, G (10) alone, or PBS.

Bronchoalveolar Lavages:

Bronchoalveolar lavages (BAL) were performed by infusing into the trachea, and withdrawing, for a minimum of five repetitions, a solution containing 1 ml of ice-cold 12 mM Lidocaine HCl in RPMI (Hancock *et al.*, *Vaccine 13*:391-400 (1995)). The BAL suspension was then centrifuged to pellet the cells. Leukocytes were quantified by staining an aliquot of the cells with 0.2% trypan blue in PBS. Subsequently, cells were cytospun onto glass slides, fixed and stained with Diff-Quik (Dade International Inc., Miami, FL)). Individual leukocyte populations were enumerated by analyzing a minimum of 400 cells per slide. The results are expressed as mean percent (+SD) of five mice per group.

In Vivo Depletion of T Cell Subsets

Monoclonal antibodies (mAbs) to murine CD4, GK1.5 (ATCC TIB 207) and murine CD8, 53-6.72 (ATCC TIB 105) were purified from hybridoma culture supernatants over a recombinant protein G column (Pharmacia, Piscataway, NJ). As a control, purified rat IgG was purchased from Calbiochem (San Diego, CA). MAbs were administered at 14 and 20 days post final immunization in doses of 750 μg and 250 μg per mouse, respectively. Mice were subsequently challenged with live RSV and pulmonary eosinophilia quantitated by analysis of BAL-derived cells 7 days thereafter. Flow cytometry was performed on a FACScan (Becton Dickinson, Mountain View, CA) to assess the effectiveness of the depletion regime. Standard flow cytometric techniques were used using PE anti-mouse CD4 (L3T4) and FITC anti-mouse CD8 (Ly-2) purchased from Pharmingen (San Diego, CA).

In Vitro Expansion of Splenic Immunocytes:

Spleens were isolated from groups of five mice two weeks post-secondary vaccination with native G protein

and Stimulon™ QS-21 and were converted to single cell suspensions as previously described (Hancock *et al.*, *J. Virol.* *70*:7783-7791 (1996)). Erythrocytes were removed using ammonium chloride lysis and the resultant spleen
5 cells quantified by trypan blue exclusion. Cells were cultured, in triplicate, in 96-well flat bottomed plates at a concentration of 2.5 x 10^5 cells per well in a medium containing RPMI 1640 supplemented with 2 mM glutamine; 100 U of penicillin and 50 μg streptomycin
10 per ml; 5 x 10^{-4} M β-mercaptoethanol; 10 mM HEPES; 1% normal mouse serum (Biocell Labs, Inc., Rancho Dominguez, CA). Peptide antigens were added to the culture medium at a concentration of 50 μg per ml. As controls, purified G protein, diphtheria toxoid cross-
15 reactive protein (CRM_{197}) and Concanavalin A (ConA) were added at final concentrations of 0.5 μg/ml, 10 μg/ml and 1 μg/ml, respectively. After 4 days in culture at 37°C and 5% CO_2, cells were pulsed with 1 μCi of ^{3}H-thymidine for a further 18 hours. Cells were subsequently
20 harvested and ^{3}H-thymidine incorporated into DNA was determined by liquid scintillation counting.

Heparinized human blood was collected from normal adult donors and separated using Ficoll-Hypaque (Pharmacia, Piscataway, NJ) centrifugation. Cells were
25 cultured with peptides as described above in RPMI 1640 medium containing 10% AB^- serum (Biocell). As controls, cells were cultured with CRM_{197} (30 μg/ml), PHA (5 μg/ml) or in medium alone.

Cytokine Assays:

30 Pooled supernatants from triplicate wells were assayed for IFN-γ and IL-5 by antigen-capture ELISA. Briefly, maxisorb plates (Nunc) were coated with 50 μl of carbonate-bicarbonate buffer (pH 9.6) containing either R4-6A2 (3 μg/ml) or TRFK.5 (2.5 μg/ml) monoclonal
35 antibodies for the capture of IFN-γ and IL-5,

respectively. Non-specific binding sites were blocked using Tris-buffered saline containing 5% FBS and 10% milk powder (wt/vol). Culture supernatants were added to the wells in duplicate and allowed to incubate at room temperature for 2 hours. To detect bound cytokine, biotinylated antibodies XMG1.2 (IFN-γ) and TRFK.4 (IL-5) were used at concentrations of 1 μg/ml and 2 μg/ml, respectively. All four monoclonal antibodies used in the cytokine assays were obtained from Pharmingen (San Diego, CA). Cytokines were quantitated using streptavidin-alkaline phosphatase with a substrate system consisting of NADP, diaphorase, alcohol dehydrogenase and INT violet. Substrate color development proceeded by adding 0.3 M sulfuric acid and optical density determined at 490 nm (OD_{490}) on a Dynatech (Chantilly, VA) ELISA reader. Standard curves were generated for each cytokine using recombinant IFN-γ (Genzyme, Cambridge, MA) and IL-5 (Pharmingen, San Diego, CA) in order to ensure linearity. Data are presented as mean OD_{490} for each antigen.

Induction of Pulmonary Eosinophilia:

Female BALB/c (H-2^d) mice, aged 7 to 9 weeks (5 mice per group) were vaccinated intramuscularly at 0 and 4 weeks with 0.1 ml of PBS adjuvanted with 20 μg Stimulon™ QS-21 and containing either 1 μg of purified G protein; 250 μg KLH; 250 μg KLH conjugated to peptides 19 or 22 or 250 μg free peptide 19 or 22. Peptides were conjugated to maleimide-activated KLH using an Imject® activated conjugation kit (product no. 77111) purchased from Pierce Chemical, Rockford, IL. Typically, for each conjugation reaction, 80-100 μg of peptide was bound to 1 mg of KLH. Thus, since each mouse received 250 μg KLH per vaccination, this corresponded to 20-25 μg of the relevant peptide. Two weeks post-secondary vaccination, mice were challenged with 1-2 x 10^6 PFU of infectious RSV

A2 by intranasal instillation in a 50 μl volume. Seven days later mice were sacrificed by cervical dislocation and bronchoalveolar lavage was performed. Cells were cytospun onto glass slides, fixed and stained with Diff-Quik (Dade Diagnostics). The relative proportion of eosinophils, as a function of total white cells, was enumerated by analyzing a minimum of 400 cells per slide. The results (Figure 5) are expressed as mean percent (+SD) of five mice per group.

Statistical Analyses

Significant differences between groups were determined by the Tukey-Kramer HSD multiple comparisons test using JMP® statistical discovery software (SAS Institute Inc., Cary, NC).

RESULTS

The immune responses elicited by native G protein and a series of overlapping peptides (shown in Figure 2) extending from amino acids 48 to 294 of G protein have been characterized as a result of work described herein. Amino acid 48 corresponds to the second translational start codon of RSV G protein. BALB/c mice that received an intramuscular (IM) vaccination of native G protein at 0 and 4 weeks exhibited maximal bronchoalveolar lavage (BAL) eosinophilia (65% of total white cells) at 7 days post-intranasal (IN) challenge with live RSV. In contrast, the BAL fluids of mice vaccinated by experimental infection or experiencing primary infection contained less than 2% eosinophils (Figure 1B).

In vitro stimulation assays of spleen cells from G protein-vaccinated BALB/c mice showed a dominant proliferative response to a peptide encompassing amino acids 184-198 of G protein (Figure 3). Proliferation was 16-fold above background levels and far exceeded that attained by other G protein-derived peptides. In

addition, the magnitude of the response to peptide 19 was comparable to that attained with purified native G protein. The data therefore indicate that the ability of RSV G protein to stimulate proliferation of primed splenocytes from BALB/c mice is entirely contained within the segment of protein occupying amino acids 184-198.

Analysis of culture supernatants for cytokines associated with helper T cell subsets indicated that the highest levels of IFN-γ and IL-5 were observed after stimulation with peptide 19. Moreover, the levels were equivalent to, or greater than, those obtained after restimulation with native G protein (Figures 4A and 4B). This data indicates that a region spanning amino acids 184-198 contains the dominant epitope(s) in RSV G protein recognized by T cells in BALB/c mice.

Preliminary studies demonstrated that the pulmonary eosinophilia in BALs of BALB/c mice vaccinated with G protein peaked at 65 ± 5.4%, 7 days post-challenge. To confirm the direct role of peptide 19 in priming for pulmonary eosinophilia in mice, peptide 19 was compared to peptide 22. The latter peptide appeared to stimulate IFN-γ production, without the stimulation of IL-5 (Figures 4A and 4B).

To ensure sufficient immunogenicity, the peptides were conjugated to maleimide-activated KLH. Statistically significant pulmonary eosinophilia was observed in mice that had been primed with peptide 19-KLH (39.5 ± 8.0%) or G protein (63 ± 1.9%) compared to mice vaccinated with adjuvant alone (2.5 ± 2.0%) (Figure 5). In contrast, the level of eosinophilia associated with peptide 22-KLH (4.9 ± 3.3%) was at background levels, despite data showing that peptide 22 was immunogenic. Two weeks post final vaccination, the geometric mean anti-RSV G protein IgG titers of mice vaccinated with peptide 19-KLH or peptide 22-KLH were

1517 and 5611, respectively. Thus, although humoral immune responses were generated to each of the peptide-conjugates, the induction of aberrant eosinophilia was limited to those mice that received peptide 19.
5 Immunization with unconjugated peptides 19 or 22 did not elicit a detectable humoral immune response and yielded relative percentages of eosinophilia (6.5 ± 5.2% and 2.5 ± 2.5, respectively) that were not significantly different from PBS/Stimulon™ QS-21 controls.

10 In order to assess peptide 19 as the causative agent of pulmonary eosinophilia, mutants of peptide 19 with amino acid substitutions at the critical 1, 4, 6 and 9 anchor regions of the MHC class II binding site were assessed. These mutations abrogated the ability to
15 predispose mice for pulmonary eosinophilia (Figure 6). In assoication with Figure 5, this data indicates a direct relationship between the amino acid sequence of peptide 19 and the induction of pumonary eosinophilia in mice upon subsequent challenge.

20 To determine whether the peptide 19-KLH associated eosinophilia was mediated by $CD4^+$ cells, a series of depletion experiments were performed using mAbs to CD4 or CD8 surface molecules. FACS analysis was performed on gated populations of lymphocytes from the spleens of
25 vaccinated mice 7 days post-challenge (Figure 8).

The depletion of $CD4^+$ cells resulted in a significant reduction in BAL eosinophilia in mice vaccinated with either G/Stimulon™ QS-21 or peptide 19-KLH/Stimulon™ QS-21 (Figure 8). Specifically, treatment
30 with anti-CD4 mAb significantly reduced pulmonary eosinophilia from 67.2 ± 8.5% and 29.6 ± 13.3% to 8.1 ± 4.7% and 0.75 ± 0.6%, respectively. The corresponding effect of anti-CD8 mAb treatment had minimal impact on the induction of eosinophilia, since levels persisted at
35 63.8 ± 6.4% and 32.8 ± 10.3% for G and peptide 19-KLH vaccinated mice, respectively. As shown in Figure 8,

only 0.7% eosinophilia was observed in the BAL of mice experimentally infected with RSV. Thus, the data demonstrate that $CD4^+$ cells are required for the pulmonary eosinophilic response seen in G protein or
5 peptide 19 immunized BALB/c mice upon challenge.

The peripheral blood mononuclear cells from a panel of donors were tested for reactivity to purified G protein to identify individuals which had undergone a recent infection. Subsequently, the cells from six
10 donors that showed a proliferative response to G protein were cultured in the presence of the synthetic peptides described in Figure 2 (Figure 7). A strong proliferative response to peptide 19 was observed for donor 100 (mean stimulation index, 8.5). In addition,
15 proliferative responses to peptide 19 were apparent in donors 9 and 20 with mean stimulation indices of 3-fold in each case.

EQUIVALENTS

While this invention has been particularly shown
20 and described with reference to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the spirit and scope of the invention as defined by the appended claims.

SEQUENCE LISTING

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Hancock, Gerald E.

Tebbey, Paul W.

<120> ENHANCED IMMUNE RESPONSE TO ATTACHMENT

(G) PROTEIN OF RESPIRATORY SYNCYTIAL VIRUS

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<141> 1998-09-17

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CLAIMS:

1. An isolated altered G protein or polypeptide of RSV, wherein the alteration is in one or more regions selected from the group consisting of the region from amino
5 acid 159 to amino acid 198, the region from amino acid 159 to amino acid 174 as set out in SEQ ID NO: 15, the region from amino acid 171 to amino acid 187 as set out in SEQ ID NO: 17, the region from amino acid 176 to amino acid 190 as set out in SEQ ID NO: 18, and the region from
10 amino acid 184 to amino acid 198 as set out in SEQ ID NO: 19, and where said isolated, altered G protein or polypeptide retains immunogenicity, and which isolated, altered G protein or polypeptide, when incorporated into an immunogenic composition or vaccine and administered to a
15 vertebrate, does not induce enhanced RSV disease upon subsequent infection of the vertebrate with RSV.

2. The isolated, altered G protein or polypeptide according to Claim 1, wherein the enhanced disease is atypical pulmonary inflammation.

20 3. The isolated, altered G protein or polypeptide according to Claim 2, wherein the atypical pulmonary inflammation is pulmonary eosinophilia.

4. The isolated, altered G protein or polypeptide according to Claim 1, wherein the alteration results in
25 inhibition of priming for IL-5 secretion by the isolated, altered G protein or polypeptide relative to wild type G protein.

5. The isolated, altered G protein of polypeptide according to Claim 1, wherein the alteration results in
30 enhancement of priming for IFN-γ secretion by the isolated,

altered G protein or polypeptide relative to wild type G protein.

6. An isolated nucleic acid molecule encoding an altered G protein or polypeptide of RSV, wherein the
5 alteration is in one or more regions selected from the group consisting of the region from amino acid 159 to amino acid 198, the region from amino acid 159 to amino acid 174 as set out in SEQ ID NO: 15, the region from amino acid 171 to amino acid 187 as set out in SEQ ID NO: 17, the region
10 from amino acid 176 to amino acid 190 as set out in SEQ ID NO: 18, and the region from amino acid 184 to amino acid 198 as set out in SEQ ID NO: 19, wherein said altered G protein or polypeptide retains immunogenicity and, when said altered G protein or polypeptide is incorporated into
15 an immunogenic composition and administered to a vertebrate, does not induce enhanced RSV disease upon subsequent infection of the vertebrate with RSV.

7. The isolated nucleic acid molecule according to Claim 6, wherein the alteration is in the region from amino
20 acid 184 to amino acid 198 as set out in SEQ ID NO: 19.

8. The nucleic acid construct comprising an isolated nucleic acid molecule according to Claim 6 operably linked to a regulatory sequence.

9. A chimeric nucleic acid construct comprising:

25 a) an isolated nucleic acid molecule encoding an altered G protein or polypeptide of RSV, wherein the alteration is in one or more regions selected from the group consisting of the region from amino acid 159 to amino acid 198, the region from amino acid 159 to amino acid 174
30 as set out in SEQ ID NO: 15, the region from amino acid 171 to amino acid 187 as set out in SEQ ID NO: 17, the region

from amino acid 176 to amino acid 190 as set out in SEQ ID NO: 18, and the region from amino acid 184 to amino acid 198 as set out in SEQ ID NO: 19, wherein said altered G protein or polypeptide retains immunogenicity and, when 5 said altered G protein or polypeptide is incorporated into an immunogenic composition and administered to a vertebrate, does not induce enhanced RSV disease upon subsequent infection of the vertebrate with RSV;

b) an isolated nucleic acid molecule encoding all 10 or an immunogenic portion of F protein of RSV; and

c) a regulatory sequence operably linked to both (a) and (b).

10. A recombinant host cell comprising a nucleic acid construct according to Claim 8.

15 11. A recombinant host cell comprising a nucleic acid construct according to Claim 9.

12. A method of producing an altered G protein or polypeptide of RSV, wherein the alteration is in one or more regions selected from the group consisting of the region 20 from amino acid 159 to amino acid 198, the region from amino acid 159 to amino acid 174 as set out in SEQ ID NO: 15, the region from amino acid 171 to amino acid 187 as set out in SEQ ID NO: 17, the region from amino acid 176 to amino acid 190 as set out in SEQ ID NO: 18, and the region from 25 amino acid 184 to amino acid 198 as set out in SEQ ID NO: 19, which retains immunogenicity and which, when incorporated into an immunogenic composition and administered to a vertebrate, does not induce enhanced RSV disease upon subsequent infection of the vertebrate with 30 RSV, comprising maintaining a recombinant host cell

according to Claim 10 under conditions suitable for expression of the altered G protein or polypeptide.

13. A method of producing a chimeric polypeptide comprising an altered G protein or polypeptide of RSV, 5 wherein the alteration is in one or more regions selected from the group consisting of the region from amino acid 159 to amino acid 198, the region from amino acid 159 to amino acid 174 as set out in SEQ ID NO: 15, the region from amino acid 171 to amino acid 187 as set out in SEQ ID NO: 17, the 10 region from amino acid 176 to amino acid 190 as set out in SEQ ID NO: 18, and the region from amino acid 184 to amino acid 198 as set out in SEQ ID NO: 19, which retains immunogenicity and which, when incorporated into an immunogenic composition and administered to a vertebrate, 15 does not induce enhanced RSV disease upon subsequent infection of the vertebrate with RSV, and all or an immunogenic portion of F protein of RSV, comprising maintaining a recombinant host cell according to Claim 11 under conditions suitable for expression of the encoded 20 chimeric protein.

14. An immunogenic composition comprising a physiologically acceptable medium and an isolated altered G protein or polypeptide of RSV, wherein the alteration is in one or more regions selected from the group consisting of 25 the region from amino acid 159 to amino acid 198, the region from amino acid 159 to amino acid 174 as set out in SEQ ID NO: 15, the region from amino acid 171 to amino acid 187 as set out in SEQ ID NO: 17, the region from amino acid 176 to amino acid 190 as set out in SEQ ID NO: 18, and 30 the region from amino acid 184 to amino acid 198 as set out in SEQ ID NO: 19, and where said altered G protein or polypeptide retains immunogenicity, and which altered G protein or polypeptide, when incorporated into an

immunogenic composition or vaccine and administered to a vertebrate, does not induce enhanced RSV disease upon subsequent infection of the vertebrate with RSV.

15. The immunogenic composition according to Claim 14, wherein the immunogenic composition results in inhibition of priming for IL-5 secretion relative to an immunogenic composition comprising wild type G protein.

16. The immunogenic composition according to Claim 14, wherein the immunogenic composition results in enhancement of priming for IFN-γ secretion relative to an immunogenic composition comprising wild type G protein.

17. An immunogenic composition comprising a physiologically acceptable medium, isolated F protein of RSV and an isolated altered G protein or polypeptide of RSV, wherein the alteration is in one or more regions selected from the group consisting of the region from amino acid 159 to amino acid 198, the region from amino acid 159 to amino acid 174 as set out in SEQ ID NO: 15, the region from amino acid 171 to amino acid 187 as set out in SEQ ID NO: 17, the region from amino acid 176 to amino acid 190 as set out in SEQ ID NO: 18, and the region from amino acid 184 to amino acid 198 as set out in SEQ ID NO: 19, and where said isolated, altered G protein or polypeptide retains immunogenicity and which isolated, altered G protein or polypeptide, when incorporated into an immunogenic composition and administered to a vertebrate does not induce enhanced RSV disease upon subsequent infection of the vertebrate with RSV.

18. The immunogenic composition according to Claim 17, wherein the immunogenic composition results in inhibition of priming for IL-5 secretion relative to wild-type G protein.

19. The immunogenic composition according to Claim 17, wherein the immunogenic composition results in enhancement of priming for IFN-γ secretion relative to wild-type G protein.

5 20. The immunogenic composition according to Claim 17, wherein the alteration is in the region from amino acid 184 to amino acid 198 of the G protein.

21. A vaccine composition comprising an immunologically effective amount of an isolated altered 10 G protein or polypeptide of RSV, wherein the alteration is in one or more regions selected from the group consisting of the region from amino acid 159 to amino acid 198, the region from amino acid 159 to amino acid 174 as set out in SEQ ID NO: 15, the region from amino acid 171 to amino 15 acid 187 as set out in SEQ ID NO: 17, the region from amino acid 176 to amino acid 190 as set out in SEQ ID NO: 18, and the region from amino acid 184 to amino acid 198 as set out in SEQ ID NO: 19, and where said isolated, altered G protein or polypeptide retains immunogenicity, and which isolated, 20 altered G protein or polypeptide, when incorporated into an immunogenic composition or vaccine and administered to a vertebrate, does not induce enhanced RSV disease upon subsequent infection of the vertebrate with RSV.

22. The vaccine composition according to Claim 21, 25 wherein the alteration is in the region from amino acid 184 to amino acid 198.

23. The vaccine composition according to Claim 22, further comprising an adjuvant.

24. An immunogenic composition comprising a 30 physiologically acceptable vehicle and an effective amount of an isolated nucleic acid molecule encoding an altered

G protein or polypeptide of RSV, wherein the alteration is in one or more regions selected from the group consisting of the region from amino acid 159 to amino acid 198, the region from amino acid 159 to amino acid 174 as set out in
5 SEQ ID NO: 15, the region from amino acid 171 to amino acid 187 as set out in SEQ ID NO: 17, the region from amino acid 176 to amino acid 190 as set out in SEQ ID NO: 18, and the region from amino acid 184 to amino acid 198 as set out in SEQ ID NO: 19, where said altered G protein or polypeptide
10 retains immunogenicity and, when said altered G protein or polypeptide is incorporated into an immunogenic composition and administered to a vertebrate, provides protection without inducing enhanced RSV disease upon subsequent infection of the vertebrate with RSV.

15 25. The immunogenic composition according to Claim 24, further comprising a transfection-facilitating agent.

26. Use, for inducing an immune response in a vertebrate, of an effective amount of an isolated nucleic acid molecule encoding an altered RSV G protein or
20 polypeptide effective to induce an immune response, and a transfection-facilitating agent, wherein the alteration is in one or more regions selected from the group consisting of the region from amino acid 159 to amino acid 198, the region from amino acid 159 to amino acid 174 as set out in
25 SEQ ID NO: 15, the region from amino acid 171 to amino acid 187 as set out in SEQ ID NO: 17, the region from amino acid 176 to amino acid 190 as set out in SEQ ID NO: 18, and the region from amino acid 184 to amino acid 198 as set out in SEQ ID NO: 19, where said altered G protein or
30 polypeptide retains immunogenicity and, when said altered G protein or polypeptide is incorporated into an immunogenic composition and administered to a vertebrate, provides

protection without inducing enhanced RSV disease upon subsequent infection of the vertebrate with RSV.

27. Use, for inhibiting induction of enhanced disease after immunization and subsequent infection of a vertebrate
5 with RSV, of an isolated altered RSV G protein or polypeptide, wherein the alteration is in one or more regions selected from the group consisting of the region from amino acid 159 to amino acid 198, the region from amino acid 159 to amino acid 174 as set out in SEQ ID NO: 15, the
10 region from amino acid 171 to amino acid 187 as set out in SEQ ID NO: 17, the region from amino acid 176 to amino acid 190 as set out in SEQ ID NO: 18, and the region from amino acid 184 to amino acid 198 as set out in SEQ ID NO: 19, and where said isolated, altered G protein or
15 polypeptide retains immunogenicity, and which isolated, altered G protein or polypeptide, when incorporated into an immunogenic composition and administered to a vertebrate, provides protection without inducing enhanced RSV disease upon subsequent infection of the vertebrate with RSV.

20 28. Use, for immunizing a vertebrate against RSV, of a composition comprising an immunologically effective amount of an isolated, altered G protein or polypeptide of RSV, wherein the alteration is in one or more regions selected from the group consisting of the region from amino acid 159
25 to amino acid 198, the region from amino acid 159 to amino acid 174 as set out in SEQ ID NO: 15, the region from amino acid 171 to amino acid 187 as set out in SEQ ID NO: 17, the region from amino acid 176 to amino acid 190 as set out in SEQ ID NO: 18, and the region from amino acid 184 to amino
30 acid 198 as set out in SEQ ID NO: 19, and where said isolated, altered G protein or polypeptide retains immunogenicity, and which isolated, altered G protein or polypeptide, when incorporated into an immunogenic

composition and administered to a vertebrate, does not induce enhanced RSV disease upon subsequent infection of the vertebrate with RSV.

29. The use according to Claim 28, wherein the composition further comprises an immunologically effective amount of isolated RSV F protein.

30. The use according to Claim 28, wherein the vertebrate is a seronegative human.

31. An immunogenic composition comprising a physiologically acceptable vehicle and an immunologically effective amount of a live attenuated pathogen which has inserted within it as a heterologous nucleic acid segment a nucleic acid sequence encoding an altered G protein or polypeptide of RSV, wherein the alteration is in one or more regions selected from the group consisting of the region from amino acid 159 to amino acid 198, the region from amino acid 159 to amino acid 174 as set out in SEQ ID NO: 15, the region from amino acid 171 to amino acid 187 as set out in SEQ ID NO: 17, the region from amino acid 176 to amino acid 190 as set out in SEQ ID NO: 18, and the region from amino acid 184 to amino acid 198 as set out in SEQ ID NO: 19, such that upon administration to the vertebrate, the altered G protein or polypeptide is expressed and is immunogenic, but does not induce enhanced RSV disease upon subsequent infection of the vertebrate with RSV.

32. The immunogenic composition according to Claim 31, wherein the live attenuated pathogen is an attenuated bacterium.

33. The immunogenic composition according to Claim 32, wherein the live attenuated bacterium is Salmonella.

34. The immunogenic composition according to Claim 31, wherein the live attenuated pathogen is an attenuated virus.

35. The immunogenic composition according to Claim 34, wherein the live attenuated virus is an attenuated
5 Venezuelan Equine Encephalitis virus.

36. Use, for immunizing a vertebrate against RSV, of a composition comprising a physiologically acceptable vehicle and an immunologically effective amount of a live attenuated pathogen which has inserted within it as a heterologous
10 nucleic acid segment a nucleic acid sequence encoding an altered G protein or polypeptide of RSV, wherein the alteration is in one or more regions selected from the group consisting of the region from amino acid 159 to amino acid 198, the region from amino acid 159 to amino acid 174
15 as set out in SEQ ID NO: 15, the region from amino acid 171 to amino acid 187 as set out in SEQ ID NO: 17, the region from amino acid 176 to amino acid 190 as set out in SEQ ID NO: 18, and the region from amino acid 184 to amino acid 198 as set out in SEQ ID NO: 19, such that upon
20 administration to the vertebrate, the altered G protein or polypeptide is expressed and is immunogenic, but does not induce enhanced RSV disease upon subsequent infection of the vertebrate with RSV.

37. The use according to Claim 36, wherein the live
25 attenuated pathogen is an attenuated bacterium.

38. The use according to Claim 37, wherein the live attenuated bacterium is Salmonella.

39. The use according to Claim 36, wherein the live attenuated pathogen is an attenuated virus.

40. The use according to Claim 39, wherein the live attenuated virus is an attenuated Venezuelan Equine Encephalitis virus.

41. An isolated, altered G protein or polypeptide of
5 RSV which retains immunogenicity and which, when incorporated into an immunogenic composition and administered to a vertebrate, does not induce enhanced disease upon subsequent infection of the vertebrate with RSV, said protein or polypeptide having an amino acid
10 sequence selected from the group consisting of SEQ ID NO: 32, SEQ ID NO: 33 and SEQ ID NO: 37.

42. An immunogenic composition comprising a physiologically acceptable medium and an altered G protein or polypeptide of RSV which retains immunogenicity and
15 which, when incorporated into an immunogenic composition and administered to a vertebrate, does not induce enhanced disease upon subsequent infection of the vertebrate with RSV, said protein or polypeptide having amino acid sequence selected from the group consisting of SEQ ID NO: 32,
20 SEQ ID NO: 33 and SEQ ID NO: 37.

43. The isolated altered G protein or polypeptide according to claim 1, wherein said alteration is in the region from amino acid 184 to amino acid 198.

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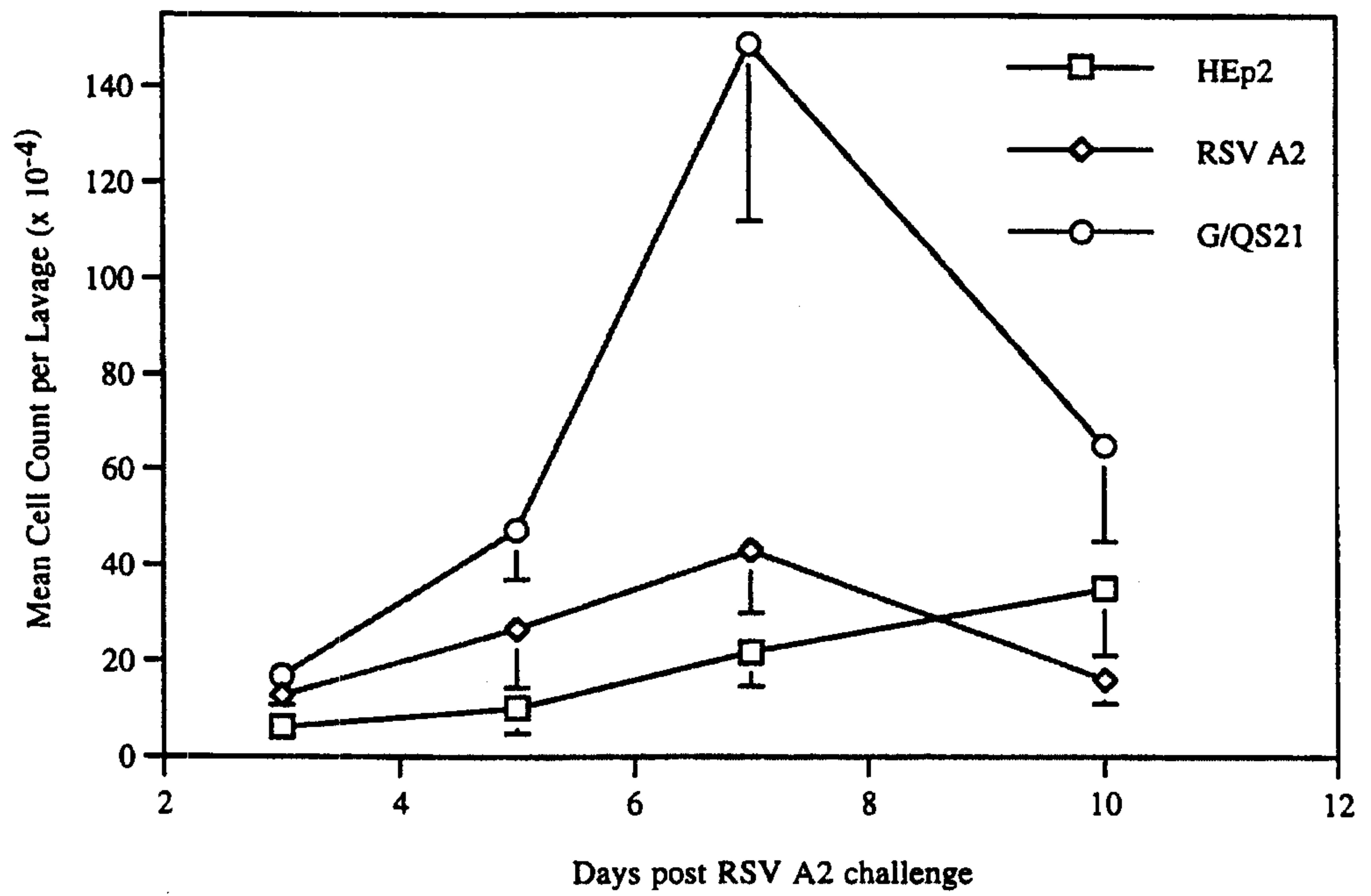

Figure 1A

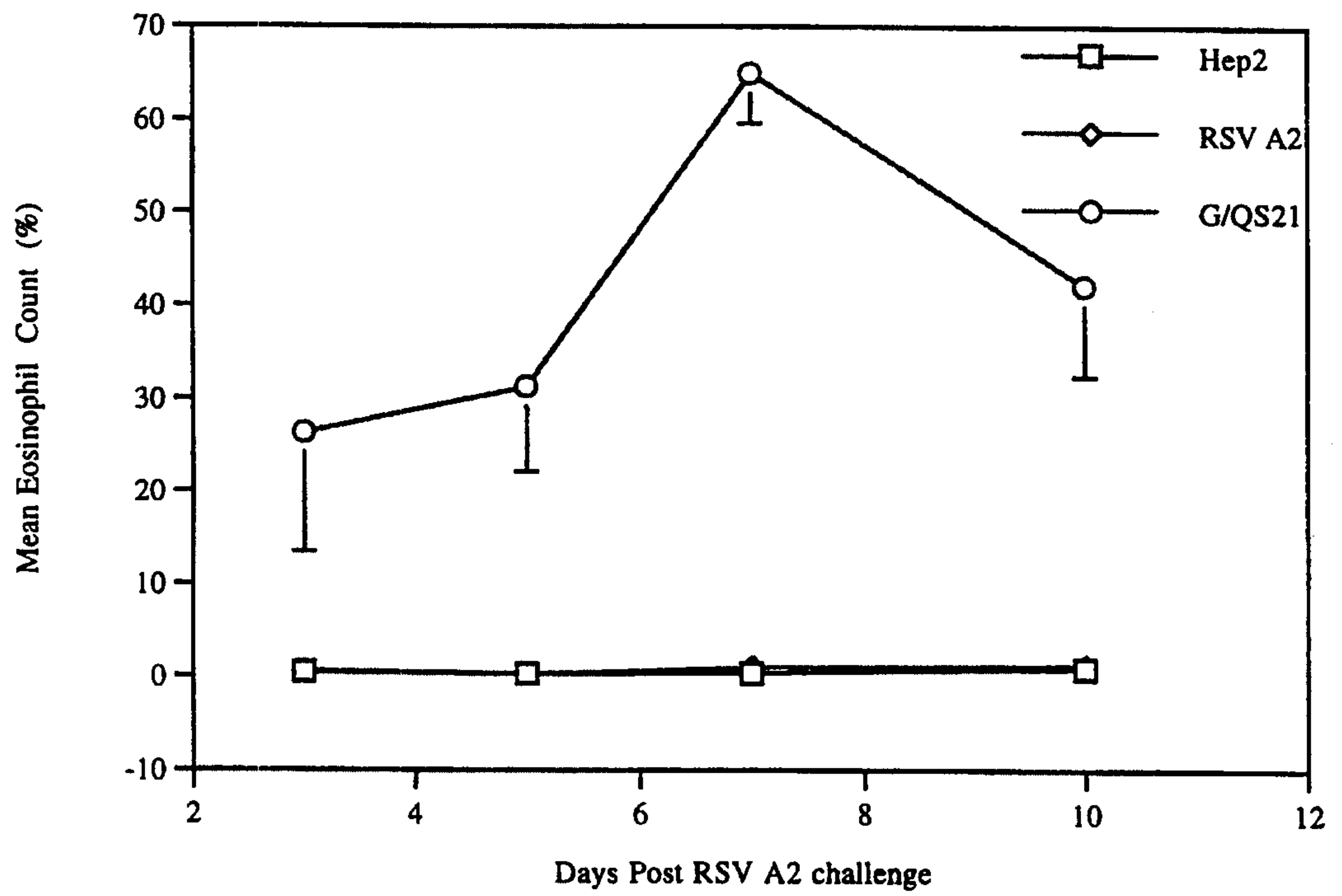

Figure 1B

Peptide	Amino Acids	Sequence	
1	48-62	MIISTSLIIAAIIFI	SEQ ID NO: 1
2	56-70	IAAIIFIASANHKVT	SEQ ID NO: 2
3	64-78	SANHKVTPTTAIIQD	SEQ ID NO: 3
4	72-86	TTAIIQDATSQIKNT	SEQ ID NO: 4
5	80-94	TSQIKNTTPTYLTQN	SEQ ID NO: 5
6	88-102	PTYLTQNPQLGISPS	SEQ ID NO: 6
7	95-110	PQLGISPSNPSEITSQ	SEQ ID NO: 7
8	104-118	PSEITSQITTILAST	SEQ ID NO: 8
9	112-126	TTILASTTPGVKSTL	SEQ ID NO: 9
10	120-134	PGVKSTLQSTTVKTK	SEQ ID NO: 10
11	128-142	STTVKTKNTTTTQTQ	SEQ ID NO: 11
12	136-150	TTTTQTQPSKPTTKQ	SEQ ID NO: 12
13	144-158	SKPTTKQRQNKPPSK	SEQ ID NO: 13
14	151-166	RQNKPPSKPNNDFHFE	SEQ ID NO: 14
15	159-174	PNNDFHFEVFNFVPCS	SEQ ID NO: 15
16	168-181	FNFVPCSICSNNPT	SEQ ID NO: 16
17	171-187	VPCSICSNNPTCWAICK	SEQ ID NO: 17
18	176-190	CSNNPTCWAICKRIP	SEQ ID NO: 18
19	184-198	AICKRIPNKKPGKKT	SEQ ID NO: 19
20	192-206	KKPGKKTTTKPTKKP	SEQ ID NO: 20
21	200-214	TKPTKKPTLKTTKKD	SEQ ID NO: 21
22	208-222	LKTTKKDPKPQTTKS	SEQ ID NO: 22
23	216-230	KPQTTKSKEVPTTKP	SEQ ID NO: 23
24	224-238	EVPTTKPTEEPTINT	SEQ ID NO: 24
25	232-246	EEPTINTTKTNIITT	SEQ ID NO: 25
26	240-254	KTNIITTLLTSNTTG	SEQ ID NO: 26
27	248-262	LTSNTTGNPELTSQM	SEQ ID NO: 27
28	256-270	PELTSQMETFHSTSS	SEQ ID NO: 28
29	264-278	TFHSTSSEGNPSPSQ	SEQ ID NO: 29
30	272-286	GNPSPSQVSTTSEYP	SEQ ID NO: 30
31	280-294	STTSEYPSQPSSPPN	SEQ ID NO: 31

Figure 2

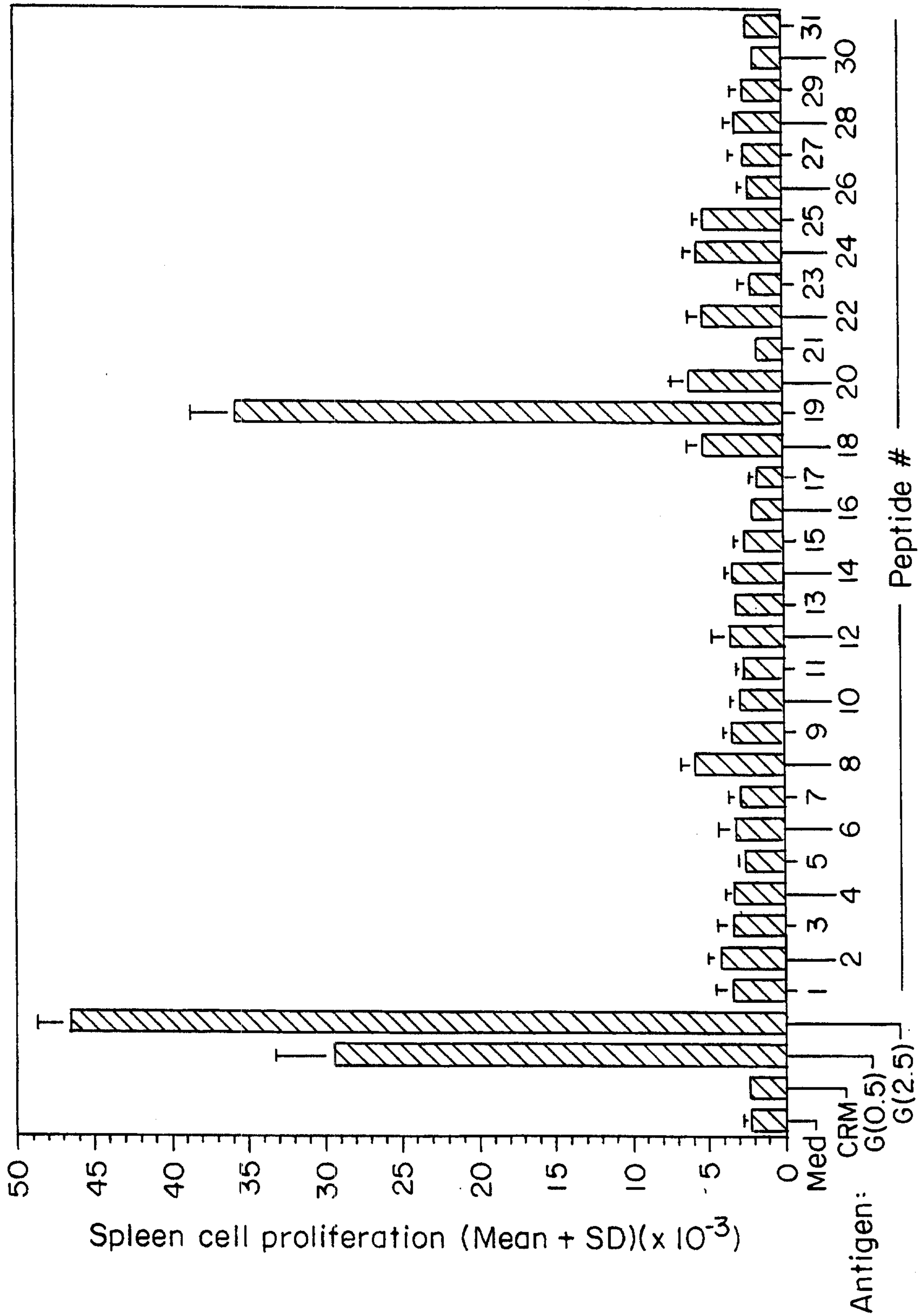

FIG. 3

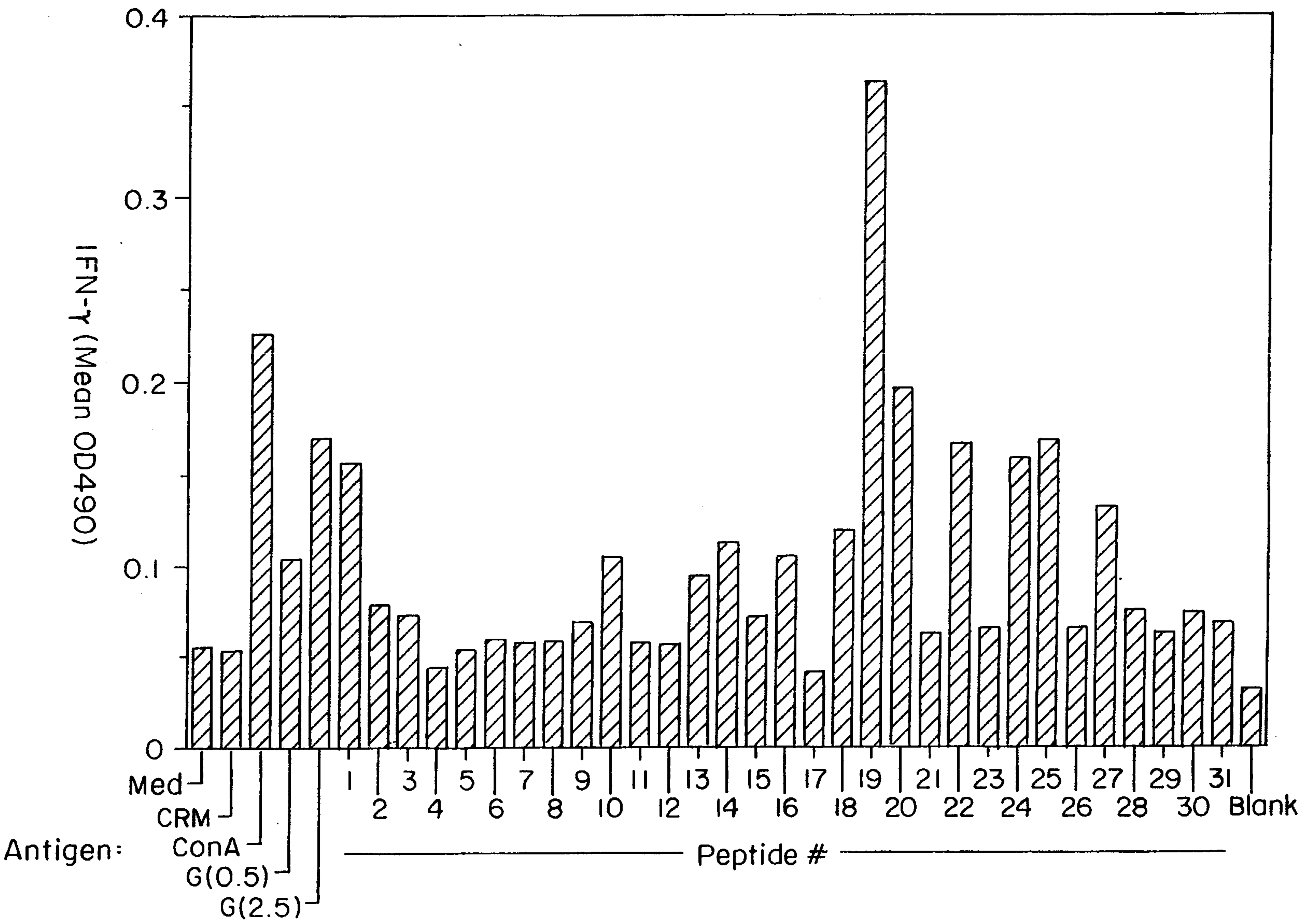

FIG. 4A

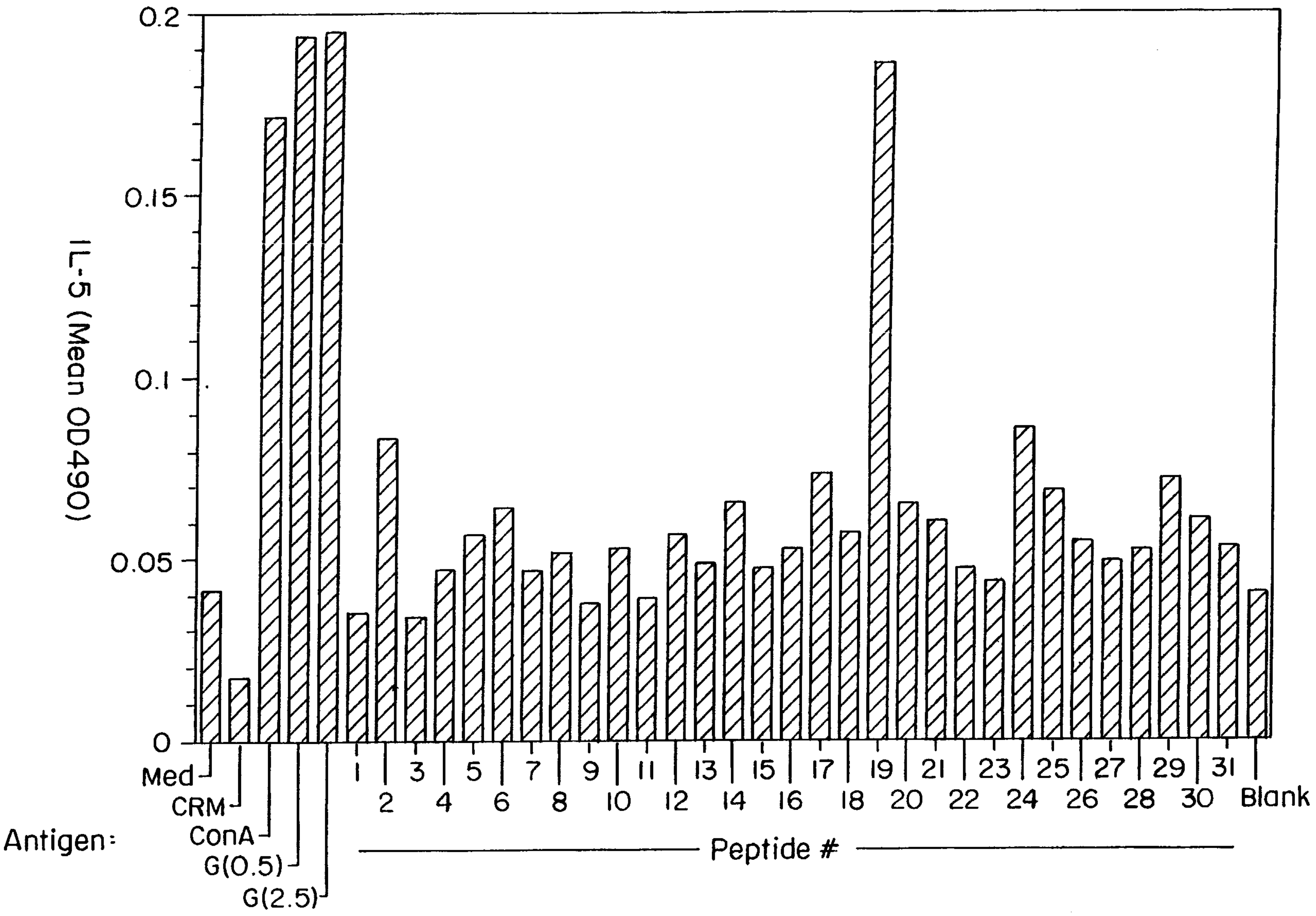

FIG. 4B

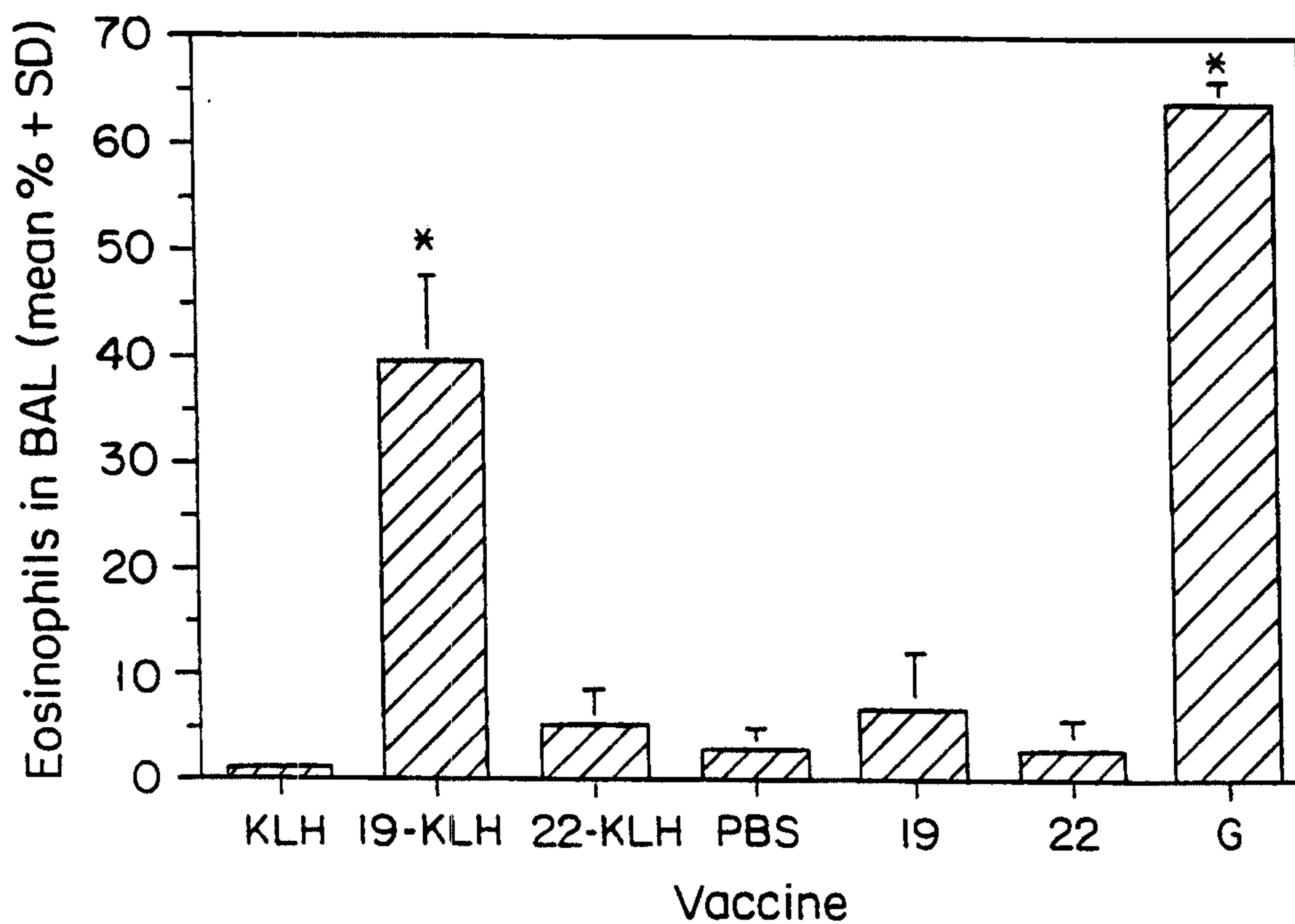

FIG. 5

Peptide 19: AICKRIPNKKPGKKT (SEQ ID NO: 19)
Peptide 19 mutant 1: AICGRGPNGKPGKKT (SEQ ID NO: 32)
Peptide 19 mutant 2: AGCGRGPGGKPGKGT (SEQ ID NO: 33)

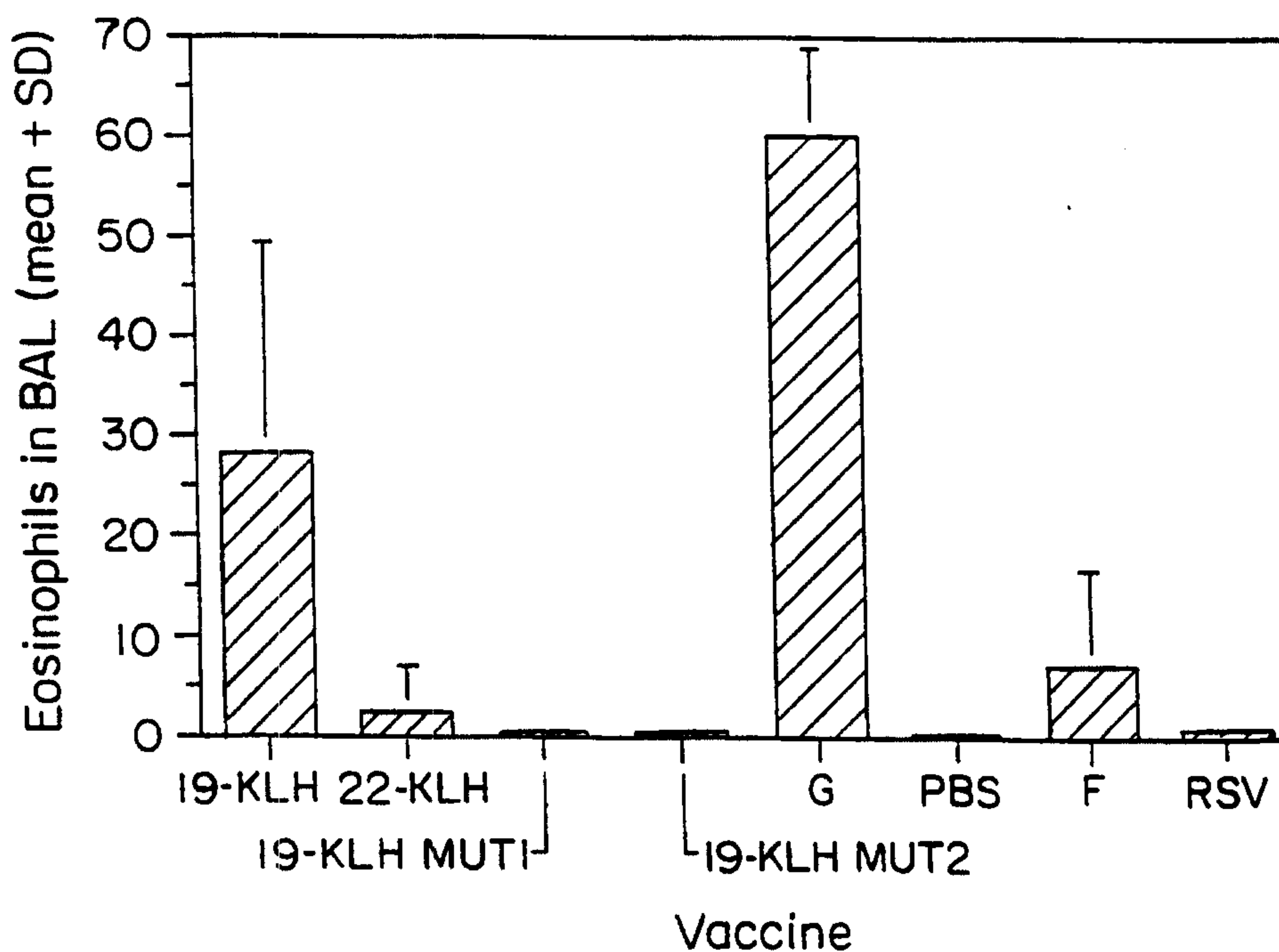

FIG. 6

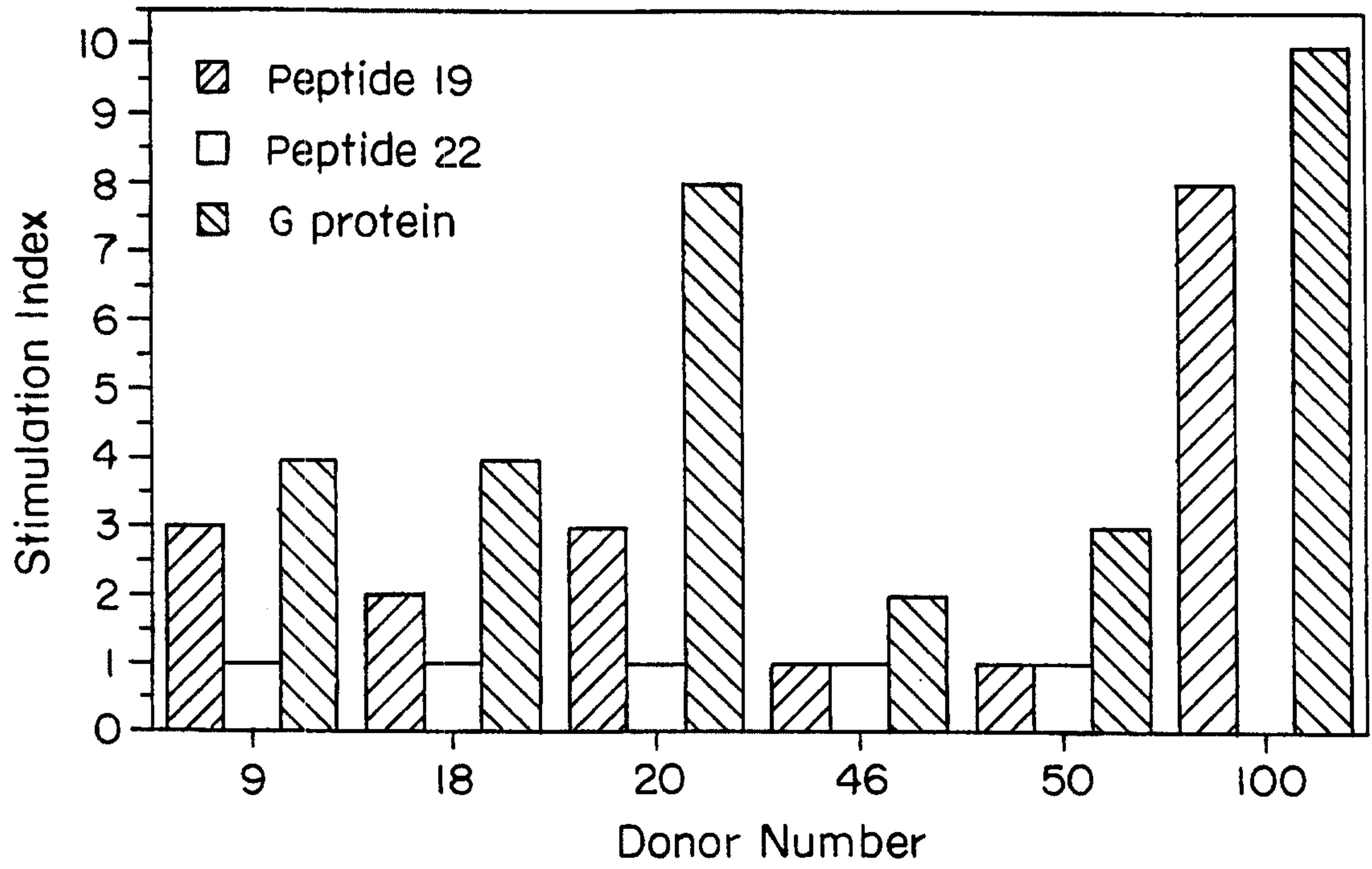

FIG. 7

Vaccine	Antibody Treatment	% $CD4^+$ Cells	% $CD8^+$ Cells	% BAL Eosinophils
G/QS21	rat Ig	21.2	8.5	67.2 ± 8.5
G/QS21	anti-CD4	1.5	15.0	8.1 ± 4.7**
G/QS21	anti-CD8	24.4	2.7	63.8 ± 6.4
Peptide 19-KLH/QS21	rat Ig	19.0	7.7	29.6 ± 13.3
Peptide 19-KLH/QS21	anti-CD4	0.3	20.0	0.75 ± 0.6**
Peptide 19-KLH/QS21	anti-CD8	27.4	2.8	32.8 ± 10.3
RSV	none	25.8	11.2	0.7 ± 1.0

Figure 8

Peptide 19: AICKRIPNKKPGKKT (SEQ ID NO: 19)
Peptide 19 mutant 1: AICGRGPNGKPGKKT (SEQ ID NO: 32)
Peptide 19 mutant 2: AGCGRGPGGKPGKGT (SEQ ID NO: 33)