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(54) **Title**: METHODS, COMPOUNDS AND COMPOSITIONS FOR TREATMENT AND PROPHYLAXIS IN THE RESPIRATORY TRACT

PF4 (**SEQ ID NO:2**):	^{47}NGRRICLDLQAPLYKKIIKKLLES^{70}
IL-8 (**SEQ ID NO:3**):	^{46}GRELCLDPKENWVQRVVEKFLKRAENS^{72}
ATIII (**SEQ ID NO:4**):	^{118}QIHFFFAKLNCRLYRKANKSSKLVSANRLFGDKS^{151}
ApoE (**SEQ ID NO:5**):	^{132}ELRVRLASHLRKLRKRLLRDADDLQKRLAVYQAG^{165}
AAMP (**SEQ ID NO:6**):	^{17}RRLRRMESESES^{25}
Amphiregulin (**SEQ ID NO: 7**):	^{125}KRKKKGGKNGKNRRNRKKKNP^{145}

FIG. 1

(57) **Abstract**: The present invention provides a method of reducing the quantity of mucus in the respiratory tract of a subject with elevated levels of mucus in said respiratory tract. The method includes administering to the subject a compound or composition containing a therapeutically effective amount of a fusion protein comprising a sialidase or an active portion thereof and an anchoring domain. The therapeutically effective amount comprises an amount of the fusion protein that results in a reduction of the quantity of mucus in the respiratory tract after administration of the compound or composition when compared to the quantity of mucus present prior to administration of the compound or composition.

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METHODS, COMPOUNDS AND COMPOSITIONS FOR TREATMENT AND PROPHYLAXIS IN THE RESPIRATORY TRACT

FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with Government support under contract number HHSN266200600015C awarded by the United States Department of Health and Human Services, National Institutes of Health. The Government has certain rights in the invention.

CLAIM OF PRIORITY

This application claims the benefit of U.S. Provisional Patent Application Serial No. 61/259,033, filed on November 6, 2009, and U.S. Provisional Patent Application Serial No. 61/259,055, filed on November 6, 2009, U.S. Provisional Patent Application Serial No. 61/322,813, filed on April 9, 2010, U.S. Provisional Patent Application Serial No. 61/322,063, filed on May 6, 2010, and U.S. Provisional Patent Application Serial No. 61/381,420 filed on September 9, 2010, the entire contents of each of which are hereby incorporated by reference.

BACKGROUND

Any discussion of the prior art throughout the specification should in no way be considered as an admission that such prior art is widely known or forms part of common general knowledge in the field.

Respiratory tract infections (RTIs) are among the most common, and potentially most severe, types of infectious diseases. Examples of RTIs include influenza, parainfluenza, RSV, sinusitis, otitis, laryngitis, bronchitis and pneumonia.

One common feature of agents that cause RTIs, such as respiratory pathogenic bacteria, is that they establish commensal colonization on the mucosal surface of the upper airway; such colonization precedes an infection and generally is prerequisite for infections. Bacterial colonization in a neonate occurs shortly after birth. During one's lifetime, the upper airway, specifically the nasopharynx and oropharynx, remains a dynamic ecological reservoir of microbial species with bacteria being acquired, eliminated and re-acquired continually. In most cases, the bacterial flora in the pharynx are harmless. However, when the condition of the host is altered, some microorganisms may invade adjacent tissues or bloodstream to cause diseases.

In addition to serving as the port of entry for mucosal and invasive infections by both bacteria and viruses, the nasopharynx and oropharynx are also the major source of spreading the pathogenic microorganisms between individuals, as well as the reservoir where antibiotic-resistant bacteria are selected (Garcia-Rodriguez and Martinez, *J Antimicrob Chemother*, (2002) 50(Suppl S2), 59-73; Soriano and Rodriguez-Cerrato, *J Antimicrob Chemother*, (2002) 50 Suppl S2, 51-58). It is well established clinically that individuals who are prone to RTIs tend to be persistent and recurrent carriers of pathogenic bacteria (Garcia-Rodriguez and Martinez, *J Antimicrob Chemother*, (2002) 50(Suppl S2), 59-73; Mbaki et al., Tohoku *J Exp. Med.*, (1987) 153(2), 111-121). For example, *Helicobacter pylori* is a human pathogen implicated in gastritis and peptic ulcer. The bacterium resides in the human stomach and binds to epithelial cells of the gastric antrum.

Other disorders of the respiratory tract (more broadly termed, RTDs) may not be caused by infectious agents, although they could arise as a consequence of infection. Examples of RTDs include a variety of obstructive lung diseases such as allergic and non-allergic asthma, COPD, bronchiectasis, vasculitis, mucous plugging, Wegener's granulomatosis and cystic fibrosis (CF). RTDs can have a genetic basis (for example, CF), can arise due to immunodeficiencies, can arise due to other deficiencies (for example, alpha-1-antitrypsin deficiency can make people more susceptible to bronchiectasis), can be caused by allergens and/or chemical pollutants, or can present as complications of other infectious diseases such as the RTIs described above or inflammatory diseases such as inflammatory bowel syndrome or Crohn's disease.

Common indications of RTIs and RTDs include inflammation and elevated levels of mucous in the respiratory tract. However, currently available drugs that are used to treat RTIs and RTDs often are unable to ameliorate these associated conditions. For example, Relenza® is a well-known treatment for influenza, but it is not recommended for patients who suffer from underlying airway disease, such as asthma and COPD. Thus, in addition to the need for drugs that reduce inflammation and/or reduce mucus in the respiratory tract or limit its increase are drugs that are capable of treating respiratory infectious diseases, such as influenza, parainfluenza and RSV, without aggravating underlying respiratory conditions, such as asthma, bronchitis, bronchiectasis, and COPD, of patients.

The present invention recognizes that drugs currently available for medical use have limited efficacy with respect to reducing inflammation, and/or reducing mucus in the respiratory tract or limiting its increase in the respiratory tract, and those that are available are associated with side effects. The present invention also recognizes that there is a need for drugs for treating respiratory infectious diseases in patients with underlying airway disease, such as asthma, bronchitis, bronchiectasis and COPD. Thus, there is a need for new drugs that are able to reduce inflammation, and/or drugs that reduce mucus in the respiratory tract or limit its increase in the respiratory tract. There is also a need for drugs that can treat respiratory infectious diseases while reducing inflammation, and/or while reducing mucus in the respiratory tract or limiting its increase in the respiratory tract.

SUMMARY

The compositions, components of compostions and methods provided below are characterized by a variety of component ingredients, steps of preparation, and biophysical, physical, biochemical or chemical parameters. As would be apparent to those of skill in the art, the compositions and methods provided herein include any and all permutations and combinations of the ingredients, steps and/or parameters described below.

The invention relates to the use of therapeutic compounds and compositions that have anti-inflammatory effects in the respiratory tract and to methods of treating respiratory inflammation and prophylaxis against respiratory inflammation. The invention also relates to therapeutic compounds and compositions that can be used to prevent or treat diseases that are caused by, cause, or are exacerbated by respiratory inflammation, including, but not limited to, inflammation not caused by allergies or allergic reactions.

The invention also relates to the use of therapeutic compounds and compositions to reduce the quantity of mucus in the respiratory tract of subjects with elevated levels of mucus in their respiratory tracts, and to corresponding methods of treatment. The

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invention also relates to the use of therapeutic compounds and compositions to limit an increase in the quantity of mucus in the respiratory tract of subjects above a baseline level of mucus in their respiratory tract and to corresponding methods of treatment. The invention also relates to therapeutic compounds and compositions that can be used to prevent or treat conditions and/or diseases that are caused by, cause, or are exacerbated by increased mucus in the respiratory tract, such as, both allergic and non-allergic asthma, chronic obstructive pulmonary disease (COPD), bronchitis (both acute and non-acute), bronchiectasis, cystic fibrosis (CF), vasculitis, mucus plugging, Wegener's granulomatosis, pneumonia, tuberculosis, cancers involving the lungs or the respiratory tract, Kartagener syndrome, Young's syndrome, chronic sinopulmonary infections, alpha 1-antitrypsin deficiency, primary immunodeficiencies, acquired immune deficiency syndrome, opportunistic infections, infectious and post infectious states, common cold, exercise-induced asthma, exercise induced hypersecretion of mucus, inflammatory bowel disease, ulcerative colitis, Crohn's disease, allergic reactions to inhaled fungus spores, respiratory infections, respiratory obstructions, inhalation or aspiration of ammonia and other toxic gases, pulmonary aspiration, alcoholism, various allergies, and any other disorder that causes increased mucus production in the respiratory tract or is caused by or exacerbated by increased mucus production in the respiratory tract. In some embodiments, the subject has more than one of the aforementioned conditions and/or diseases. In other embodiments, the subject having one or more of the aforementioned conditions and/or diseases does not have an accompanying infectious disease (RTI), such as influenza, parainfluenza or RSV. In other embodiments, the subject having one or more of the aforementioned conditions and/or diseases has one or more accompanying infectious diseases, such as influenza, parainfluenza or RSV. Thus, provided herein are methods, compounds and compositions for treating inflammatory and/or allergic responses associated with an RTI, an RTD, or combinations thereof.

According to a first aspect, the present invention provides a method of reducing the quantity or level of mucus or preventing an increase in the quantity or level of mucus in a respiratory tract of a subject, the method comprising:

determining a baseline level of mucus in the subject's respiratory tract; and

administering to the subject a compound or composition comprising a therapeutically effective amount of a fusion protein, wherein

the fusion protein comprises at least one catalytic domain of a sialidase, wherein the catalytic domain of the sialidase comprises the sequence of amino acids extending from amino acid 274 to amino acid 666 of SEQ ID NO:12, inclusive, and at least one anchoring domain, wherein the anchoring domain is a glycosaminoglycan (GAG)

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binding domain of human amphiregulin comprising the amino acid sequence of SEQ ID NO:7; and

the therapeutically effective amount comprises an amount of the fusion protein that results in a reduction of the quantity of mucus in the respiratory tract after administration of the compound or composition when compared to the quantity of mucus present prior to administration of the compound or composition.

According to a second aspect, the present invention provides a method of reducing the quantity of mucus in the respiratory tract of a subject with elevated levels of mucus in said respiratory tract, comprising:

determining a baseline level of mucus in the subject's respiratory tract; and

administering to the subject a compound or composition comprising a therapeutically effective amount of a peptide comprising a sialidase or an active portion thereof and, optionally, an anchoring domain, wherein

the therapeutically effective amount comprises an amount of the peptide that results in a reduction of the quantity of mucus in the respiratory tract after administration of the compound or composition when compared to the quantity of mucus present prior to administration of the compound or composition.

According to a third aspect, the present invention provides use of a fusion protein in the preparation of a medicament for reducing the quantity or level of mucus or preventing an increase in the quantity or level of mucus in a respiratory tract of a subject, wherein the fusion protein comprises at least one catalytic domain of a sialidase, wherein the catalytic domain of the sialidase comprises the sequence of amino acids extending from amino acid 274 to amino acid 666 of SEQ ID NO:12, inclusive, and at least one anchoring domain, wherein the anchoring domain is a GAG binding domain of human amphiregulin comprising the amino acid sequence of SEQ ID NO:7, wherein a baseline level of mucus in the subject's respiratory tract is determined prior to treatment.

According to a fourth aspect, the present invention provides use of a peptide comprising a sialidase or an active portion thereof and, optionally, an anchoring domain in the preparation of a medicament for reducing the quantity of mucus in the respiratory tract of a subject with elevated levels of mucus in said respiratory tract, wherein a baseline level of mucus in the subject's respiratory tract is determined prior to treatment.

Unless the context clearly requires otherwise, throughout the description and the claims, the words "comprise", "comprising", and the like are to be construed in an inclusive sense as opposed to an exclusive or exhaustive sense; that is to say, in the sense of "including, but not limited to".

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The compounds and compositions provided herein can reduce mucus production in the respiratory tract and/or reduce the levels of inflammatory cells that cause allergic or non-allergic types of inflammation, including, without limitation, monocytes, macrophages, dendritic cells, histiocytes, Kuppfer cells, mastocytes and neutrophiles.

The compounds and compositions provided herein include a sialidase or active portion thereof. Without being bound by any theory, sialic acids have been implicated in allergic and/or inflammatory responses associated with RTIs and RTDs. For example, siglecs (sialic acid binding Ig-like lectins) are members of the immunoglobulin (Ig) superfamily that bind to sialic acid and are mainly expressed by cells of the hematopoietic system. At least 11 siglecs have been discovered and they seem to exclusively recognize cell surface sialic acid as the ligand. It is believed that the binding of siglecs to sialic acid mediates cell-cell adhesion and interactions (Crocker and Varki, Trends Immunol., (2001) 22(6), 337-342; Angata and Brinkman-Van der Linden, Biochim. Biophys. Acta, (2002) 1572(2-3), 294-316). Siglec-8 (SAF-2) is an adhesion molecule that is highly restricted to the surface of eosinophils, basophils, and mast cells, which are the central effector cells in allergic conditions including allergic rhinitis, asthma and eczema. Siglec-8 (homologous to Siglec-F in mice) is considered to be responsible for mediating the recruitment of the three allergic cell types to the airway, the lungs and other sites of allergy. Siglec-1 (sialoadhesion) and siglec-2 (CD22) are the adhesion molecules on macrophages and B cells, both types of cells play central roles in immune reactions that lead to inflammation. Siglec-9 is predominantly expressed on neutrophils, which are known to be important effector cells in inflammation (von Gunten, Yousefi, Seitz, Jakob, Schaffner, Seger, Takala, Villiger, and Simon (2005) *Blood* 106:1423-1431). Further, without being bound by any particular theory, sialic acid residues have been implicated in the interaction of muscaranic receptors with agonists; thus, sialidases can affect the interecation of muscarinic receptors with their agonists.

The present invention relates to a method of reducing the quanitity of mucus in the respiratory tract of a subject with elevated levels of mucus in said respiratory tract. The method includes administering to the subject a compound or composition containing a therapeutically effective amount of a fusion protein having a sialidase or an active portion thereof and an anchoring domain. The therapeutically effective amount includes an amount of the fusion protein that results in a reduction of the quantity of mucus in the respiratory tract after administration of the compound or composition when compared to the quantity of mucus present prior to administration of the composition.

In another embodiment, another method of reducing the quantity of mucus in the respiratory tract of a subject with elevated levels of mucus in said respiratory tract is provided. The method includes administering to the subject a compound or composition containing a therapeutically effective amount of a fusion protein. The fusion protein has at least one catalytic domain of a sialidase, wherein the catalytic domain of the sialidase includes the sequence of amino acids extending from amino acid 274 to amino acid 666 of SEQ ID NO:12, inclusive, and at least one anchoring domain. The anchoring domain can be a glycosaminoglycan (GAG) binding domain of human amphiregulin including the amino acid sequence of SEQ ID NO:7. The therapeutically effective amount includes an amount of the fusion protein that results in a reduction of the quanitity of mucus in the respiratory tract after administration of the compound or composition when compared to the quantity of mucus present prior to administration of the composition.

In another embodiment, another method of reducing the quantity of mucus in the respiratory tract of a subject with elevated levels of mucus in said respiratory tract is provided. The method includes administering to the subject a compound or composition containing a therapeutically effective amount of a protein or peptide having a sialidase or an active portion thereof. The therapeutically effective amount includes an amount of the protein or peptide that results in a reduction of the quantity of mucus in the respiratory tract after administration of the compound or composition when compared to the quantity of mucus present prior to administration of the compound or composition.

In another embodiment, a method of treating or ameliorating the effects of chronic obstructive pulmonary disease (COPD), bronchitis, bronchiectasis, cystic fibrosis (CF), vasculitis, mucus plugging, Wegener's granulomatosis, pneumonia, tuberculosis, cancer involving the lungs or the respiratory tract, Kartagener syndrome, Young's syndrome, chronic sinopulmonary infection, alpha 1-antitrypsin deficiency, primary immunodeficiency, acquired immune deficiency syndrome, opportunistic infection, an infectious state, a post infectious state, common cold, exercise induced hypersecretion of mucus, inflammatory bowel disease, ulcerative colitis, Crohn's disease, respiratory infection, respiratory obstruction, inhalation or aspiration of a toxic gas, pulmonary aspiration, or alcoholism in a subject with an elevated level of mucus in his or her respiratory tract is provided. The method includes administering to the subject a compound or composition containing a therapeutically effective amount of a fusion protein. The fusion protein has at least one catalytic domain of a sialidase, wherein the catalytic domain of the sialidase includes the sequence of amino acids extending from amino acid 274 to amino acid 666 of SEQ ID NO:12, inclusive, and at least one anchoring domain. The anchoring domain can be a glycosaminoglycan (GAG) binding domain of human amphiregulin including the amino acid sequence of SEQ ID NO:7. The therapeutically effective amount includes an amount of the fusion protein that results in a reduction of the quanitity of mucus in the respiratory tract after administration of the compound or composition when compared to the quantity of mucus present prior to administration of the compound or composition.

In another embodiment, another method of treating or ameliorating the effects of chronic obstructive pulmonary disease (COPD), bronchitis, bronchiectasis, cystic fibrosis (CF), vasculitis, mucus plugging, Wegener's granulomatosis, pneumonia, tuberculosis, cancer involving the lungs or the respiratory tract, Kartagener syndrome, Young's syndrome, chronic sinopulmonary infection, alpha 1-antitrypsin deficiency, primary immunodeficiency, acquired immune deficiency syndrome, opportunistic infection, an infectious state, a post infectious state, common cold, exercise induced hypersecretion of mucus, inflammatory bowel disease, ulcerative colitis, Crohn's disease, respiratory infection, respiratory obstruction, inhalation or aspiration of a toxic gas, pulmonary aspiration, or alcoholism in a subject with an elevated level of mucus in his or her respiratory tract is provided. The method includes administering to the subject a compound or composition containing a therapeutically effective amount of a fusion protein. The fusion protein has a sialidase or an active portion thereof and an anchoring domain. The therapeutically effective amount includes an amount of the fusion protein that results in a reduction of the quanitity of mucus in the respiratory tract after administration of the compound or composition when compared to the quantity of mucus present prior to administration of the compound or composition.

In another embodiment, another method of treating or ameliorating the effects of chronic obstructive pulmonary disease (COPD), bronchitis, bronchiectasis, cystic fibrosis (CF), vasculitis, mucus plugging, Wegener's granulomatosis, pneumonia, tuberculosis, cancer involving the lungs or the respiratory tract, Kartagener syndrome, Young's syndrome, chronic sinopulmonary infection, alpha 1-antitrypsin deficiency, primary immunodeficiency, acquired immune deficiency syndrome, opportunistic infection, an infectious state, a post infectious state, common cold, exercise induced hypersecretion of mucus, inflammatory bowel disease, ulcerative colitis, Crohn's disease, respiratory infection, respiratory obstruction, inhalation or aspiration of a toxic gas, pulmonary aspiration, or alcoholism in a subject with an elevated level of mucus in his or her respiratory tract is provided. The method includes administering to the subject a compound or composition containing a therapeutically effective amount of a protein or peptide having a sialidase or an active portion thereof. The therapeutically effective amount includes an amount of the protein or peptide that results in a reduction of the quantity of mucus in the respiratory tract after administration of the compound or composition when compared to the quantity of mucus present prior to administration of the compound or composition.

In another embodiment, a method of limiting an increase in the quantity of mucus in the respiratory tract of a subject above a baseline level of mucus in said subject's respiratory tract is provided. The method includes administering to the subject a compound or composition containing a therapeutically effective amount of a fusion protein. The fusion protein has at least one catalytic domain of a sialidase, wherein the catalytic domain of the sialidase includes the sequence of amino acids extending from amino acid 274 to amino acid 666 of SEQ ID NO:12, inclusive, and at least one anchoring domain, wherein the anchoring domain is a glycosaminoglycan (GAG) binding domain of human amphiregulin comprising the amino acid sequence of SEQ ID NO:7. The therapeutically effective amount includes an amount of the fusion protein that limits an increase in the quanitity of mucus in the respiratory tract of said subject above a baseline level after administration of the compound or composition.

In another embodiment, another method of limiting an increase in the quantity of mucus in the respiratory tract of a subject above a baseline level of mucus in said subject's respiratory tract is provided. The method includes administering to the subject a compound or composition containing a therapeutically effective amount of a fusion protein having a sialidase or an active portion thereof and an anchoring domain. The therapeutically effective amount includes an amount of the fusion protein that limits an increase in the quanitity of mucus in the respiratory tract of said subject above a baseline level after administration of the compound or composition.

In yet another embodiment, another method of limiting an increase in the quantity of mucus in the respiratory tract of a subject above a baseline level of mucus in said subject's respiratory tract is provided. The method includes administering to the subject a compound or composition containing a therapeutically effective amount of a protein or peptide having a sialidase or an active portion thereof. The therapeutically effective amount includes an amount of the protein or peptide that limits an increase in the quanitity of mucus in the respiratory tract of the subject above a baseline level after administration of the compound or composition.

Also contemplated herein are methods of identifying sialidases or active portions thereof according to the compounds or compositions provided herein, where the sialidases or active portions thereof are effective at reducing the quantity of mucus in the respiratory tract of subjects. The reduction in mucus can be measured directly in standard assays known to those of skill in the art. For example, in some embodiments, a single compound or a library or collection of compounds or compositions comprising sialidase(s) and/or catalytically active portion(s) thereof are administered to an animal model of asthma having an associated inflammatory response, such as the guinea pig and the mouse as described in Example 1 and Example 2, respectively. An asthmatic or other inflammatory condition is created in the animal whereby the accumulation of mucus in the lung or respitatory tract is increased. The level of mucus is then quantitated and compared to the level after treatment with a sialidase or active portion thereof. If there is a reduction of the mucus level in the presence of the sialidase or active portion thereof, the sialidase or active portion thereof is identified or selected as one that can be used in the methods provided herein for treating inflammation, allergies and/or associated inflammatory/allergic responses, such as the overproduction of mucus.

In some embodiments, a sialidase or active portion thereof according to the compounds and compositions provided herein is identified as being suitable for treating inflammation, allergies or associated responses by measuring its ability to disrupt muscarinic receptor-agonist interactions according to standard methods known to those of skill in the art. For example, provided herein is a method of assessing whether a compound or composition comprising a sialidase and/or catalytically active portion thereof reduces the quantity of mucus in the respiratory tract of a subject, by

(a) contacting the muscarinic receptors of an animal subject with a compound or composition that includes a sialidase and/or a catalytically active portion thereof;

(b) administering a muscarinic receptor agonist to the subject;

(c) quantitating the airway resistance in the subject;

(d) comparing the airway resistance level measured in (c) with the airway resistance in the absence of contact with the compound or composition;

(e) identifying whether the compound or composition reduces the airway resistance relative to the airway resistance in the absence of contact with the compound or composition; and

(f) if the compound or composition reduces the airway resistance as determined in (e), assessing the compound or composition as one that reduces the quantity of mucus in the respiratory tract of the subject. Such a method is exemplified in Example 3.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Methods and materials are described herein for use in the present invention; other, suitable methods and materials known in the art can also be used. The materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, sequences, database entries, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

Other features and advantages of the invention will be apparent from the following detailed description and figures, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows GAG-binding sequences of four human genes: PF4, human platelet factor 4; IL8, human interleukin 8; AT III, human antithrombin III; ApoE, human apolipoprotein E; AAMP, human angio-associated migratory cell protein; human amphiregulin.

FIG. 2 is a sequence comparison between human sialidases NEU2 and NEU4 (SEQ ID NOs: 8 & 9).

FIG. 3 is a table comparing substrate specificity of bacterial and fungal sialidases.

FIG. 4 depicts the nucleotide and amino acid sequence (SEQ ID NOs: 28 & 29) of a construct of the present invention encoding His6-AvCD. NcoI and HindIII sites used for cloning into pTrc99a are shown in bold.

FIG. 5 depicts the nucleotide and amino acid sequences (SEQ ID NOs: 18 & 19) of another construct of the present invention encoding AR-AvCD. NcoI and HindIII sites used for cloning into pTrc99a are shown in bold.

FIG. 6 depicts the nucleotide and amino acid sequences (SEQ ID NO: 36 & 37) of another construct of the present invention encoding AR-G_4S-AvCD. NcoI and HindIII sites used for cloning into pTrc99a are shown in bold.

FIGS. 7A-B are graphs showing that topical administration of recombinant AR-AvCD sialidase fusion protein reduces the inflammatory responses of ferrets infected with an influenza A (H1N1) virus. FIG. 7A shows the total number of inflammatory cells from nasal wash samples obtained from infected animals at the indicated times after infection. he protein concentration was determined in cell-free nasal wash samples of infected ferrets. Infected ferrets were vehicle-treated (squares) or were treated with recombinant AR-AvCD sialidase fusion protein made from Construct #2 (triangles). Uninfected animals were also treated with recombinant AR-AvCD sialidase fusion protein (diamonds). Statistically significant values are labeled with * ($p<0.05$) and ** ($p<0.01$).

FIG. 8 provides graphs showing formula and explanation of the Enhanced Pause (PENH), the parameter used for measuring bronchoconstriction in conscious unrestrained animals.

FIG. 9 provides a graph showing early asthmatic reaction in response to an OVA-aerosol. Results are expressed as arithmetic average ± SEM. *$p<0.05$, ***$p<0.001$ using student's t-test.

FIG. 10 provides a graph showing the total number of cells in guinea pigs on the day of section. Results are expressed as arithmetic average ± SEM. **$p<0.01$, ***$p<0.001$ using student's t-test.

FIG. 11 provides a graph showing the total number of macrophages recovered in guinea pig BAL fluid on the day of section. Results are expressed as arithmetic average ± SEM. **$p<0.01$.

FIG. 12 provides a graph showing the total number of lymphocytes recovered in guinea pig BAL fluid on the day of section. Results are expressed as arithmetic average ± SEM. *$p<0.05$.

FIG. 13 provides a graph showing the total number of neutrophils recovered in guinea pig BAL fluid on the day of section. Results are expressed as arithmetic average ± SEM. *$p<0.05$, ***$p<0.001$.

FIG. 14 provides a graph showing the total number of eosinophils recovered in guinea pig BAL fluid on the day of section. Results are expressed as arithmetic average ± SEM. *$p<0.05$, ***$p<0.001$.

FIG. 15 provides a graph showing the percent change in Penh at Mch 48 mg/mL in the effect of sialidase treatment on the early and late asthmatic reaction in guinea pigs.

FIG. 16 provides a graph showing the percent change in Pehn at a range of Mch concentrations in the effect of sialidase treatment on the early and late asthmatic reaction in guinea pigs.

FIG. 17 provides a graph showing blood Eosinophils in the effect of sialidase treatment on the early and late asthmatic reaction in guinea pigs.

FIG. 18 provides a graph showing PAS staining for lung mucus in the effect of sialidase treatment on the early and late asthmatic reaction in guinea pigs.

FIGS. 19A-F provide a PAS staining for lung mucus.

FIG. 20 provides a graph showing MBP immunostaining for eosinophils in the effect of sialidase treatment on the early and late asthmatic reaction in guinea pigs.

FIGS. 21A-B provide graphs showing reduced airway resistance in naïve mice treated intranasally with low doses of DAS181 (methacholine challenged).

FIG. 22 provides a graph showing reduced airway resistance in naïve mice treated intranasally with a low dose of DAS181 (methacholine challenged).

FIG. 23 provides a graph showing reduced airway resistance in naïve mice treated intranasally with DAS181 (carbachol challenged).

FIG. 24 provides a graph showing airway resistance in naïve mice treated intranasally with a low dose of DAS185 (methacholine challenged).

FIG. 25 provides graphsshowing time-course of DAS185 mediated reduction of airway resistance (methacholine challenged).

FIG. 26 provides a graph showing reduced airway resistance in naïve mice treated intranasally with very lose doses of DAS181 (methacholine challenged).

Like reference symbols in the various drawings indicate like elements.

DETAILED DESCRIPTION

The present disclosure provides, *inter alia*, novel methods of use for compounds described in U.S. Patent Application Serial Nos. 10/718,986 and 10/939,262 (both of which are hereby incorporated by reference in their entirety) to reduce mucus, e.g., in the respiratory tract of subjects with elevated levels of mucus in their respiratory tract. In some embodiments, the present disclosure provides compositions and methods for reducing mucus (e.g., mucus levels) in a subject in need of reduced mucus levels and that does not have influenza (e.g., is not infected with influenza at the time of treatment) or ashma.

In some embodiments, the compounds can include compounds made by NexBio, Inc. under the compound name DAS181 and under the trademark Fludase® (provided herein as **SEQ ID NO:21**). DAS181 is a fusion protein comprising a catalytic domain of a sialidase, and an anchoring domain. Several of the examples described herein use DAS181 or compositions containing DAS181.

<u>Definitions</u>

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Generally, the nomenclature used herein and the manufacture or laboratory procedures described below are well known and commonly employed in the art. Conventional methods are used for these procedures, such as those provided in the art and various general references. Where a term is provided in the singular, the inventors also contemplate the plural of that term. Where there are discrepancies in terms and definitions used in references that are incorporated by reference, the terms used in this application shall have the definitions given herein. As employed throughout the disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings:

As used herein, a "subject" includes any animal for whom diagnosis, screening, monitoring or treatment is contemplated. Animals include mammals such as primates and domesticated animals. An exemplary primate is human. A patient refers to a subject such as a mammal, primate, human or livestock subject afflicted with a disease condition or for which a disease condition is to be determined or risk of a disease condition is to be determined.

In some embodiments, the methods disclosed herein can include selecting a a subject in need of reduced mucus levels and that is not infected with one or more of influenza, parainfluenza, and/or respiratory syncytial virus (RSV). In some instances, the terms infected or infection can include the presence of a influenza and/or parainfluenza virus and/or RSV in a subject. In some instances, the terms infected or infection can include the presence of active or replicating influenza and/or parainfluenza virus and/or RSV in a subject. In some embodiments, a subject with an active or replicating influenza and/or parainfluenza virus and/or RSV infection can be selected based on the presence or detection of influenza and/or parainfluenza virus shedding and/or RSV shedding in the subject (e.g., in a sample from the subject). In some embodiments, the methods disclosed herein can include selecting a subject in need of reduced mucus levels, wherein the subject has a latent influenza, parainfluenza, and/or RSV infection.

An "animal model" as used herein means an animal that sufficiently mimics, resembles or reproduces a disease or condition of interest in its anatomy, physiology, or response (to a pathogen or allergen, e.g.) so as to be useful in medical research that can be extrapolated to the disease or condition of interest (*e.g.*, to screen for diagnostic or therapeutic agents; to measure therapeutic efficacy of a compound or composition, *etc.*). For example, the guinea pig and the mouse can be animal models to mimic imflammatory and/or allergic responses associated with asthma, as demonstrated in Examples 1 and 2, respectively. The mouse also can be an animal model to study the interaction of muscarinic receptors with their agonists, and the disruption thereof by agents such as the compounds and compositions provided herein (*see* Example 3).

A "pathogen" can be any virus or microorganism that can infect a cell, a tissue or an organism. A pathogen can be a virus, bacterium, or protozoan.

A "target cell" is any cell that can be infected by a pathogen or any cell that can interact with inflammatory cells, or a host cell that is the intended destination for an exogenous gene transferred by a recombinant virus.

"Inflammatory cells" are the cells that carry out or participate in inflammatory responses of the immune system. Inflammatory cells include include B lymphocytes, T lymphocytes, macrophages, basophils, eosinophils, mast cells, NK cells, monocytes, and neutrophils.

An "extracellular activity that can inhibit adhesion or function of inflammatory cells" is any activity that can prevent inflammatory cells from contacting the target cell and affecting the normal physiological status of the target cell.

A "domain that can anchor said at least one therapeutic domain to the membrane of a target cell", also called an "extracellular anchoring domain" or simply, "anchoring domain" refers to a chemical entity can that can stably bind a moiety that is at or on the exterior of a cell surface or is in close proximity to the surface of a cell. An extracellular anchoring domain can be reversibly or irreversibly linked to one or more moieties, such as one or more therapeutic domains, and thereby cause the one or more attached therapeutic moieties to be retained at or in close proximity to the exterior surface of a eukaryotic cell. An extracellular anchoring domain can bind at least one molecule on the surface of a target cell or at least one molecule found in close association with the surface of a target cell. For example, an extracellular anchoring domain can bind a molecule covalently or noncovalently associated with the cell membrane of a target cell, or can bind a molecule present in the extracellular matrix surrounding a target cell. An extracellular anchoring domain can be a peptide, polypeptide, or protein, and can also comprise any additional type of chemical entity, including one or more additional proteins, polypeptides, or peptides, a nucleic acid, peptide nucleic acid, nucleic acid analogue, nucleotide, nucleotide analogue, small organic molecule, polymer, lipids, steroid, fatty acid, carbohydrate, or a combination of any of these.

As used herein, a protein or peptide sequences is “substantially homologous” to a reference sequence when it is either identical to a reference sequence, or comprises one or more amino acid deletions, one or more additional amino acids, or more one or more conservative amino acid substitutions, and retains the same or essentially the same activity as the reference sequence. Conservative substitutions may be defined as exchanges within one of the following five groups:

I. Small, aliphatic, nonpolar or slightly polar residues: Ala, Ser, Thr, Pro, Gly

II. Polar, negatively charged residues and their amides: Asp, Asn, Glu, Gln

III. Polar, positively charged residues: His, Arg, Lys

IV. Large, aliphatic nonpolar residues: Met, Leu, Ile, Val, Cys

V. Large aromatic residues: Phe, Try, Trp

Within the foregoing groups, the following substitutions are considered to be "highly conservative": Asp/Glu, His/Arg/Lys, Phe/Tyr/Trp, and Met/Leu/Ile/Val. Semi-conservative substitutions are defined to be exchanges between two of groups (I)-(IV) above which are limited to supergroup (A), comprising (I), (II), and (III) above, or to supergroup (B), comprising (IV) and (V) above. In addition, where hydrophobic amino acids are specified in the application, they refer to the amino acids Ala, Gly, Pro, Met, Leu, Ile, Val, Cys, Phe, and Trp, whereas hydrophilic amino acids refer to Ser, Thr, Asp, Asn, Glu, Gln, His, Arg, Lys, and Tyr.

A “sialidase” is an enzyme that can remove a sialic acid residue from a substrate molecule. The sialidases (N-acylneuraminosylglycohydrolases, EC 3.2.1.18) are a group of enzymes that hydrolytically remove sialic acid residues from sialo-glycoconjugates. Sialic acids are alpha-keto acids with 9-carbon backbones that are usually found at the outermost positions of the oligosaccharide chains that are attached to glycoproteins and glycolipids. One of the major types of sialic acids is N-acetylneuraminic acid (Neu5Ac), which is the biosynthetic precursor for most of the other types. The substrate molecule can be, as nonlimiting examples, an oligosaccharide, a polysaccharide, a glycoprotein, a ganglioside, or a synthetic molecule. For example, a sialidase can cleave bonds having alpha(2,3)-Gal, alpha(2,6)-Gal, or alpha(2,8)-Gal linkages between a sialic acid residue and the remainder of a substrate molecule. A sialidase can also cleave any or all of the linkages between the sialic acid residue and the remainder of the substrate molecule. Two major linkages between Neu5Ac and the penultimate galactose residues of carbohydrate side chains are found in nature, Neu5Ac alpha (2,3)-Gal and Neu5Ac alpha (2,6)-Gal. Both Neu5Ac alpha (2,3)-Gal and Neu5Ac alpha (2,6)-Gal molecules can be recognized by influenza viruses as the receptor, although human viruses seem to prefer Neu5Ac alpha (2,6)-Gal, avian and equine viruses predominantly recognize Neu5Ac alpha (2,3)-Gal. A sialidase can be a naturally-occurring sialidase, an engineered sialidase (such as, but not limited to a sialidase whose amino acid sequence is based on the sequence of a naturally-occurring sialidase, including a sequence that is substantially homologous to the sequence of a naturally-occurring sialidase). As used herein, “sialidase” can also mean the active portion of a naturally-occurring sialidase, or a peptide or protein that comprises sequences based on the active portion of a naturally-occurring sialidase.

A “fusion protein” is a protein comprising amino acid sequences from at least two different sources. A fusion protein can comprise amino acid sequence that is derived from a naturally occurring protein or is substantially homologous to all or a portion of a naturally occurring protein, and in addition can comprise from one to a very large number of amino acids that are derived from or substantially homologous to all or a portion of a different naturally occurring protein. In the alternative, a fusion protein can comprise amino acid sequence that is derived from a naturally occurring protein or is substantially homologous to all or a portion of a naturally occurring protein, and in addition can comprise from one to a very large number of amino acids that are synthetic sequences.

A “sialidase catalytic domain protein” is a protein that comprises the catalytic domain of a sialidase, or an amino acid sequence that is substantially homologous to the catalytic domain of a sialidase, but does not comprises the entire amino acid sequence of the sialidase the catalytic domain is derived from, wherein the sialidase catalytic domain protein retains substantially the same activity as the intact sialidase the catalytic domain is derived from. A sialidase catalytic domain protein can comprise amino acid sequences that are not derived from a sialidase, but this is not required. A sialidase catalytic domain protein can comprise amino acid sequences that are derived from or substantially homologous to amino acid sequences of one or more other known proteins, or can comprise one or more amino acids that are not derived from or substantially homologous to amino acid sequences of other known proteins.

"Therapeutically effective amount" means an amount of a composition or compound that is needed for a desired therapeutic, prophylactic, or other biological effect or response when a composition or compound is administered to a subject in a single dosage form. The particular amount of the composition or compound will vary widely according to conditions such as the nature of the composition or compound, the nature of the condition being treated, the age and size of the subject.

"Treatment" means any manner in which one or more of the symptoms of a condition, disorder or disease are ameliorated or otherwise beneficially altered. Treatment also encompasses any pharmaceutical use of the composition or compound herein, such as for reducing mucus in the respiratory tract.

"Respiratory tract" means the air passages from the nose to the pulmonary alveoli, including the nose, throat, pharynx, larynx, trachea, and bronchi, and it also includes the lungs, and is sometimes referred to by medical practitioners as the respiratory system.

"Inhaler" means a device for giving medicines in the form of a spray or dry powder that is inhaled (breathed in either naturally or mechanically forced in to the lungs) through the nose or mouth, and includes without limitation, a passive or active ventilator (mechanical with or with an endotracheal tube), nebulizer, dry powder inhaler, metered dose inhaler, and pressureized metered dose inhaler.

"Inhalant" is any substance that is inhaled through the nose or mouth.

"Reducing the quantity of mucus" means diminishing all or some, generally more than by 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90% 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or more of the amount of mucus in the respiratory tract when compared with the amount prior to administration of the compositions or compounds described herein. "Reducing the quantity of mucus" can also mean reducing the amount of mucus in an amount that is observable by a healthcare practitioner using whatever medical implements are available for such observation, such as, e.g., by auscultation, by MRI or other radiographic study, by direct visualization with a bronchoscope or other visualization device, or by measuring patient mucus over time. "Reducing the quantity of mucus" can also mean reducing the amount of mucus in an amount that is observable by the patient or subject himself or herself with self-reporting or self-observation, such as, e.g., monitoring the amount of expectorated or swallowed mucus over time, or by subjectively observing the sense of congestion in his or her lungs over time.

"Limiting an increase in the quantity of mucus" means that the amount of mucus in the respiratory tract after administration of the compositions and compounds described herein does not increase more than if they had not been administered. "Limiting an increase in the quantity of mucus" also means that the amount of mucus in the respiratory tract after administration of the compositions and compounds described herein does not increase after their adminisation of the compositions and compounds. "Limiting an increase in the quantity of mucus" can also mean limiting an increase over the patient's baseline at the time of administration of the compounds or compositions in an amount that is observable or ascertainable by a healthcare practitioner using whatever medical implements and analytical systems are available for such observations, such as, e.g., by ausculation, by MRI or other radiographic study, by direct visualization with a bronchoscope or other visualization device, or by measuring patient sputum over time. "Limiting an increase in the quantity of mucus" can also mean limiting an increase over the patient's baseline at the time of administration of the compounds or compositions in an amount that is observable by the patient or subject himself or herself with self-reporting or self-observation, such as, e.g., monitoring the amount of expectorated or swallowed mucus over time, or by subjectively observing the sense of congestion in his or her lungs over time.

"Excipient" as used herein means one or more inactive substances or compounds that either alone or in combination are used as a carrier for the active ingredients of a medication. As used herein "excipient" can also mean one or more substances or compounds that are included in a pharmaceutical composition to improve its beneficial effects or that have a synergistic effect with the active ingredient.

Peptide or Protein Based Compounds

The present invention includes peptide or protein-based compounds that comprise at least one domain that can anchor the compound to the membrane of a eukaryotic cell and at least one additional domain that is a therapeutic domain. By "peptide or protein-based" compounds, it is meant that the two major domains of the compound have an amino acid framework, in which the amino acids are joined by peptide bonds. A peptide or protein-based compound can also have other chemical compounds or groups attached to the amino acid framework or backbone, including moieties that contribute to the anchoring activity of the anchoring domain, or moieties that contribute to the therapeutic activity of the therapeutic domain. For example, the protein-based therapeutics used in the present invention can comprise compounds and molecules such as but not limited to: carbohydrates, fatty acids, lipids, steroids, nucleotides, nucleotide analogues, nucleic acid molecules, nucleic acid analogues, peptide nucleic acid molecules, small organic molecules, or even polymers. The protein-based therapeutics of the present invention can also comprise modified or non-naturally occurring amino acids. Non-amino acid portions of the compounds can serve any purpose, including but not limited to: facilitating the purification of the compound, improving the solubility or distribution or the compound (such as in a therapeutic formulation), linking domains of the compound or linking chemical moieties to the compound, contributing to the two-dimensional or three-dimensional structure of the compound, increasing the overall size of the compound, increasing the stability of the compound, and contributing to the anchoring activity or therapeutic activity of the compound.

The peptide or protein-based compounds of the present invention can also include protein or peptide sequences in addition to those that comprise anchoring domains or therapeutic domains. The additional protein sequences can serve any purpose, including but not limited to any of the purposes outlined above (facilitating the purification of the compound, improving the solubility or distribution or the compound, linking domains of the compound or linking chemical moieties to the compound, contributing to the two-dimensional or three-dimensional structure of the compound, increasing the overall size of the compound, increasing the stability of the compound, or contributing to the anchoring activity or therapeutic activity of the compound). Any additional protein or amino acid sequences can be part of a single polypeptide or protein chain that includes the anchoring domain or domains and therapeutic domain or domains, but any feasible arrangement of protein sequences is within the scope of the present invention.

The anchoring domain and therapeutic domain can be arranged in any appropriate way that allows the compound to bind at or near a target cell membrane. The compound can have at least one protein or peptide-based anchoring domain and at least one peptide or protein-based therapeutic domain. In this case, the domains can be arranged linearly along the peptide backbone in any order. The anchoring domain can be N-terminal to the therapeutic domain, or can be C-terminal to the therapeutic domain. It is also possible to have one or more therapeutic domains flanked by at least one anchoring domain on each end. Alternatively, one or more anchoring domains can be flanked by at least one therapeutic domain on each end. Chemical or peptide linkers can optionally be used to join some or all of the domains of a compound.

It is also possible to have the domains in a nonlinear, branched arrangement. For example, the therapeutic domain can be attached to a derivatized side chain of an amino acid that is part of a polypeptide chain that also includes, or is linked to, the anchoring domain.

A compound of the present invention can have more than one anchoring domain. In cases in which a compound has more than one anchoring domain, the anchoring domains can be the same or different. A compound used in the present invention can have more than one therapeutic domain. In cases in which a compound has more than one therapeutic domain, the therapeutic domains can be the same or different. Where a compound comprises multiple anchoring domains, the anchoring domains can be arranged in tandem (with or without linkers) or on alternate sides of other domains, such as therapeutic domains. Where a compound comprises multiple therapeutic domains, the therapeutic domains can be arranged in tandem (with or without linkers) or on alternate sides of other domains, such as, but not limited to, anchoring domains.

A peptide or protein-based compound of the present invention can be made by any appropriate way, including purifying naturally occurring proteins, optionally proteolytically cleaving the proteins to obtain the desired functional domains, and conjugating the functional domains to other functional domains. Peptides can also be chemically synthesized, and optionally chemically conjugated to other peptides or chemical moieties. A peptide or protein-based compound of the present invention can be made by engineering a nucleic acid construct to encode at least one anchoring domain and at least one therapeutic domain together (with or without nucleic acid linkers) in a continuous polypeptide. The nucleic acid constructs, in some embodiments having appropriate expression sequences, can be transfected into prokaryotic or eukaryotic cells, and the therapeutic protein-based compound can be expressed by the cells and purified. Any desired chemical moieties can optionally be conjugated to the peptide or protein-based compound after purification. In some cases, cell lines can be chosen for expressing the protein-based therapeutic for their ability to perform desirable post-translational modifications (such as, but not limited to glycosylation).

A great variety of constructs can be designed and their protein products tested for desirable activities (such as, for example, binding activity of an anchoring domain, or a binding, catalytic, or inhibitory activity of a therapeutic domain).

Anchoring Domain

As used herein, an "extracellular anchoring domain" or "anchoring domain" is any moiety that can stably bind an entity that is at or on the exterior surface of a target cell or is in close proximity to the exterior surface of a target cell. An anchoring domain serves to retain a compound used in the present invention at or near the external surface of a target cell.

An extracellular anchoring domain can bind 1) a molecule expressed on the surface of a target cell, or a moiety, domain, or epitope of a molecule expressed on the surface of a target cell, 2) a chemical entity attached to a molecule expressed on the surface of a target cell, or 3) a molecule of the extracellular matrix surrounding a target cell.

An anchoring domain can be a peptide or protein domain (including a modified or derivatized peptide or protein domain), or comprises a moiety coupled to a peptide or protein. A moiety coupled to a peptide or protein can be any type of molecule that can contribute to the binding of the anchoring domain to an entity at or near the target cell surface, and in some embodiments is an organic molecule, such as, for example, nucleic acid, peptide nucleic acid, nucleic acid analogue, nucleotide, nucleotide analogue, small organic molecule, polymer, lipids, steroid, fatty acid, carbohydrate, or any combination of any of these.

A molecule, complex, domain, or epitope that is bound by an anchoring domain may or may not be specific for the target cell. For example, an anchoring domain may bind an epitope present on molecules on or in close proximity to the target cell and that occur at sites other than the vicinity of the target cell as well. In many cases, however, localized delivery of a therapeutic compound of the present invention will restrict its occurrence primarily to the surface of target cells. In other cases, a molecule, complex, moiety, domain, or epitope bound by an anchoring domain may be specific to a target tissue or target cell type.

Target tissue or target cell type includes the sites in an animal or human body where a pathogen invades or amplifies. For example, a target cell can be an endothelial cell that can be infected by a pathogen. A composition used in the present invention can comprise an anchoring domain that can bind a cell surface epitope, for example, that is specific for the endothelial cell type. In another example, a target cell can be an epithelial cell and a composition of the present invention can bind an epitope present on the cell surface of many epithelial cell types, or present in the extracellular matrix of different types of epithelial cells. In this case localized delivery of the composition can restrict its localization to the site of the epithelial cells that are targets of the pathogen.

Compounds used in the present invention can have one or more anchoring domains that can bind at or near the surface of epithelial cells. For example, heparan sulfate, closely related to heparin, is a type of glycosaminoglycan (GAG) that is ubiquitously present on cell membranes, including the surface of respiratory epithelium. Many proteins specifically bind to heparin/heparan sulfate, and the GAG-binding sequences in these proteins have been identified (Meyer, FA, King, M and Gelman, RA. (1975) *Biochimica et Biophysica Acta* 392: 223-232; Schauer, S. ed., pp233. Sialic Acids Chemistry, Metabolism and Function. Springer-Verlag, 1982). For example, the GAG-binding sequences of human platelet factor 4 (PF4) (**SEQ ID NO:2**), human interleukin 8 (IL8) (**SEQ ID NO:3**), human antithrombin III (AT III) (**SEQ ID NO:4**), human apoprotein E (ApoE) (**SEQ ID NO:5**), human angio-associated migratory cell protein (AAMP) (**SEQ ID NO:6**), or human amphiregulin (**SEQ ID NO:7**) (FIG. 1) have been shown to have very high affinity (in the nanomolar range) towards heparin (Lee, MK and Lander, AD. (1991) Pro Natl Acad Sci USA 88:2768-2772; Goger, B, Halden, Y, Rek, A, Mosl, R, Pye, D. Gallagher, J and Kungl, AJ. (2002) Biochem. 41:1640-1646; Witt, DP and Lander AD (1994) Curr Bio 4:394-400; Weisgraber, KH, Rall, SC, Mahley, RW, Milne, RW and Marcel, Y. (1986) J Bio Chem 261:2068-2076). The GAG-binding sequences of these proteins are distinct from their receptor-binding sequences, so they will not induce the biological activities associated with the full-length proteins or the receptor-binding domains. These sequences, or other sequences that have been identified or are identified in the future as heparin/heparan sulfate binding sequences, or sequences substantially homologous to identified heparin/heparan sulfate binding sequences that have heparin/heparan sulfate binding activity, can be used as epithelium-anchoring-domains in compounds used in the present invention.

An anchoring domain can bind a moiety that is specific to the target cell type of a particular species or can bind a moiety that is found in the target cell type of more than one species.

Therapeutic Domain

A compound used in the present invention includes at least one therapeutic domain or active portion, those terms being used interchangeable herein. The therapeutic activity can be, as nonlimiting examples, a binding activity, a catalytic activity, or an inhibitory activity. A therapeutic domain can modify or inhibit a function of the target cell or target organism. An active portin of a compound has therapeutic activity. For example, the catalytic domain or active portion of a sialidase can be its therapeutic domain.

The therapeutic domain can act extracellularly, meaning that its infection-preventing, inflammatory response-modulating, or transduction-enhancing activity takes place at the target cell surface or in the immediate area surrounding the target cell, including sites within the extracellular matrix, intracellular spaces, or luminal spaces of tissues.

A therapeutic domain can be a peptide or protein domain (including a modified or derivatized peptide or protein domain), or comprises a moiety coupled to a peptide or protein. A moiety coupled to a peptide or protein can be any type of molecule, and is in some embodiments an organic molecule, such as, for example, nucleic acid, peptide nucleic acid, nucleic acid analogue, nucleotide, nucleotide analogue, small organic molecule, polymer, lipids, steroid, fatty acid, carbohydrate, or any combination of any of these.

A therapeutic domain can be a synthetic peptide or polypeptide, or can comprise a synthetic molecule that can be conjugated to a peptide or polypeptide, can be a naturally-occurring peptide or protein, or a domain of naturally-occurring protein. A therapeutic domain can also be a peptide or protein that is substantially homologous to a naturally-occurring peptide or protein.

Linkers

A compound used in the present invention can optionally include one or more linkers that can join domains of the compound. Linkers can be used to provide optimal spacing or folding of the domains of a compound. The domains of a compound joined by linkers can be therapeutic domains, anchoring domains, or any other domains or moieties of the compound that provide additional functions such as enhancing compound stability, facilitating purification, etc. A linker used to join domains of compounds of the present invention can be a chemical linker or an amino acid or peptide linker. Where a compound comprises more than one linker, the linkers can be the same or different. Where a compound comprises more than one linker, the linkers can be of the same or different lengths.

Many chemical linkers of various compositions, polarity, reactivity, length, flexibility, and cleavability are known in the art of organic chemistry. Preferred linkers include amino acid or peptide linkers. Peptide linkers are well known in the art. Some embodiments of linkers are between one and about one hundred amino acids in length, and between one and about thirty amino acids in length, although length is not a limitation in the linkers of the compounds of the present invention. The linkder amino acid sequences can be selected such that they do not interfere with the mucus-reducing and/or anti-inflammatory activity of the compounds and compositions used in the present invention. Some embodiments of linkers are those that include the amino acid glycine. For example, linkers having the sequence:

(GGGGS (**SEQ ID NO:10**))n, where n is a whole number between 1 and 20, or between 1 and 12, can be used to link domains of therapeutic compounds used in the present invention.

Composition comprising at least one anchoring domain and at least one catalytic activity

In some aspects, the present invention can use compounds that have a therapeutic domain that has an enzymatic activity. The enzymatic activity can be a catalytic activity that removes, degrades or modifies a host molecule or complex. In some embodiments the host molecule or complex can be removed, degraded, or modified by the enzymatic activity of a compound of the present invention is on, at, or near the surface of a target cell.

Compounds used in the present invention can have, for example, one of the following structures:

(Anchoring Domain)n-[linker]-(Enzymatic Activity)n (n=1,2, 3 or more)

or :

(Enzymatic Activity)n (n=1,2, 3 or more)-[linker]-(Anchoring Domain)n,

where the linkers are optional.

The enzymatic activity can be a monomeric form of a peptide or polypeptide or can be multiple copies of the same polypeptide that are either linked directly or with spacing sequence in between. The polypeptides or peptides can be linked directly or via a spacer composed of peptide linker sequence. The anchoring domain can be any peptide or polypeptide that can bind to or near the surface of target cells.

In one embodiment, a therapeutic domain comprises a sialidase that can eliminate or greatly reduce the level of sialic acid on the surface of epithelial cells. The therapeutic domain can comprise a complete sialidase protein, or an active portion thereof, wherein the active portion thereof retains the ability to perform the catalytic function(s) of the sialidase protein (*e.g.*, cleaving sialic acid residues).

Sialic acid mediates cell adhesion and interactions between inflammatory cells and target cells. Therefore, treating the surface of respiratory epithelial cells with a sialidase can prevent the recruitment of inflammatory cells to the airway surface, and therefore can treat allergic reactions including asthma and allergic rhinitis. It also unexpectedly results in reducing the quantity of mucus in the respiratory tract of subjects with elevated levels of mucus in their respiratory tract, and limiting increase in the quantity of mucus in the respiratory tract of subjects above a baseline of mucus in the respiratory tract of those subjects.

Among the sialidases contemplated for use in the methods described herein are the large bacterial sialidases that can degrade the receptor sialic acids Neu5Ac alpha(2,6)-Gal and Neu5Ac alpha(2,3)-Gal. For example, the bacterial sialidase enzymes from *Clostridium perfringens* (Genbank Accession Number X87369), *Actinomyces viscosus*, *Arthrobacter ureafaciens*, or *Micromonospora viridifaciens* (Genbank Accession Number D01045) can be used. Therapeutic domains of compounds of the present invention can comprise all or a portion of the amino acid sequence of a large bacterial sialidase or can comprise amino acid sequences that are substantially homologous to all or a portion of the amino acid sequence of a large bacterial sialidase. In one preferred embodiment, a therapeutic domain comprises a sialidase encoded by *Actinomyces viscosus*, such as that of **SEQ ID NO:12**, or such as sialidase sequence substantially homologous to **SEQ ID NO:12**. In yet another preferred embodiment, a therapeutic domain comprises the catalytic domain of the *Actinomyces viscosus* sialidase extending from amino acids 274-667 of **SEQ ID NO:12**, or a substantially homologous sequence.

Other sialidases contemplated for use in the methods described herein are the human sialidases such as those encoded by the genes NEU2 (**SEQ ID NO:8**; Genbank Accession Number Y16535; Monti, E, Preti, Rossi, E., Ballabio, A and Borsani G. (1999) *Genomics* 57:137-143) and NEU4 (**SEQ ID NO:9;** Genbank Accession Number NM080741; Monti, E, Preti, A, Venerando, B and Borsani, G. (2002) *Neurochem Res* 27:646-663) (FIG. 2). Therapeutic domains of compounds used in the present invention can comprise all or a portion of the amino acid sequences of a human sialidase or can comprise amino acid sequences that are substantially homologous to all or a portion of the amino acid sequences of a human sialidase. Where a therapeutic domain comprises a portion of the amino acid sequences of a naturally occurring sialidase, or sequences substantially homologous to a portion of the amino acid sequences of a naturally occurring sialidase, the portion can have essentially the same activity as the human sialidase.

A compound for reducing elevated levels of mucus in the respiratory tract can in some embodiments have one or anchoring domains that can bind at or near the surface of epithelial cells. In some embodiments, the epithelium-anchoring domain is a GAG-binding sequence from a human protein, such as, for example, the GAG-binding amino acid sequences of human platelet factor 4 (PF4) (**SEQ ID NO:2**), human interleukin 8 (IL8) (**SEQ ID NO:3**), human antithrombin III (AT III) (**SEQ ID NO:4**), human apoprotein E (ApoE) (**SEQ ID NO:5**), human angio-associated migratory cell protein (AAMP) (**SEQ ID NO:6**), and human amphiregulin (**SEQ ID NO:7**) (FIG.1). An epithelial anchoring domain can also be substantially homologous to a naturally occurring GAG-binding sequence, such as those listed in FIG. 1. Such compounds can be formulated for nasal, tracheal, bronchial, oral, or topical administration, or can be formulated as an injectable solution or as eyedrops, or formulated into a solution or dry powder and inhaled with inhalers.

A pharmaceutical composition comprising such compounds can be used to treat or prevent allergy or inflammatory response. In addition, such compounds have been shown herein to reduce the quantity of mucus in the respiratory tract of subjects with elevated levels of mucus in their respiratory tracts, and to limit increases in the quantity of mucus in the respiratory tract of subjects above a baseline of mucus in their respiratory tracts. Therefore, such compounds can be used to as therapeutic treatments to reduce the quantity of mucus in the respiratory tract of subjects with elevated levels of mucus in their respiratory tracts, or as prophylactic treatments to limit increases in the quantity of mucus in the respiratory tract of subjects above a baseline of mucus in their respiratory tracts. Due to their effect on mucus in the respiratory tract, these compounds can also be used to prevent, treat, or ameliorate the effects of chronic obstructive pulmonary disease (COPD), bronchitis, bronchiectasis, cystic fibrosis (CF), vasculitis, mucus plugging, Wegener's granulomatosis, pneumonia, tuberculosis, cancer involving the lungs or the respiratory tract, Kartagener syndrome, Young's syndrome, chronic sinopulmonary infection, alpha 1-antitrypsin deficiency, primary immunodeficiency, acquired immune deficiency syndrome, opportunistic infection, an infectious state, a post infectious state, common cold, exercise induced hypersecretion of mucus, inflammatory bowel disease, ulcerative colitis, Crohn's disease, respiratory infection, respiratory obstruction, inhalation or aspiration of a toxic gas, pulmonary aspiration, or alcoholism in subjects with elevated levels of mucus in their respiratory tract or who are at risk of having increased levels of mucus in their respiratory tract.

It is also within the scope of the present invention to use compounds or compositions comprising a human sialidase, such as any of those described herein, or an active portion thereof, or a compound with substantial homology to a sialidase, in the absence of an anchoring domain (a) to treat or prevent allergic and inflammatory responses in the respiratory tract, (b) to reduce the quantity of mucus in the respiratory tract of subjects with elevated levels of mucus in their respiratory tracts, (c) to limit increases in the quantity of mucus in the respiratory tract of subjects above a baseline of mucus in their respiratory tracts, and/or (d) to prevent, treat, or ameliorate the effects of chronic obstructive pulmonary disease (COPD), bronchitis, bronchiectasis, cystic fibrosis (CF), vasculitis, mucus plugging, Wegener's granulomatosis, pneumonia, tuberculosis, cancer involving the lungs or the respiratory tract, Kartagener syndrome, Young's syndrome, chronic sinopulmonary infection, alpha 1-antitrypsin deficiency, primary immunodeficiency, acquired immune deficiency syndrome, opportunistic infection, an infectious state, a post infectious state, common cold, exercise induced hypersecretion of mucus, inflammatory bowel disease, ulcerative colitis, Crohn's disease, respiratory infection, respiratory obstruction, inhalation or aspiration of a toxic gas, pulmonary aspiration, or alcoholism in subjects with elevated levels of mucus in their respiratory tract or who are at risk of having increased levels of mucus in their respiratory tract. The present invention recognizes that elevated levels of mucus in the respiratory tract can be reduced by the use of a sialidase or an active portion of a sialidase, and that such sialidases or active portions thereof can optionally be adapted, by genetic or chemical engineering, or by pharmaceutical formulation, to improve their half life or retention at the respiratory epithelium.

These compounds and pharmaceutical compositions can be delivered to the upper respiratory tract as a nasal spray, or delivered to the respiratory tract as an inhalant with inhalers.

The compounds described herein can be formulated into pharmaceutical compositions that include various additional compounds either alone or in various combinations, such as, Na_2SO_4, $MgSO_4$, $CaCl_2$, Histidine, Histine-HCl, and Trehalose or their analogs. These additional compounds can be included in the pharmaceutical compositions to act as excipients or as active ingredients that provide additional beneficial effects.

Therapeutic Composition Comprising at least one Sialidase Activity

The present invention includes methods that use therapeutic compounds and compositions that comprise at least one sialidase activity. The sialidase activity can be a sialidase isolated from any source, such as, for example, a bacterial or mammalian source, or can be a recombinant protein that is substantially homologous to at least a portion of a naturally occurring sialidase. In some embodiments sialidases are the large bacterial sialidases that can degrade the receptor sialic acids Neu5Ac alpha(2,6)-Gal and Neu5Ac alpha(2,3)-Gal. For example, the bacterial sialidase enzymes from *Clostridium perfringens* (Genbank Accession Number X87369), *Actinomyces viscosus* (Genbank Accession Number L06898), *Arthrobacter ureafaciens*, or *Micromonospora viridifaciens* (Genbank Accession Number D01045) or substantially homologous proteins can be used.

For example, therapeutic compounds and compositions used in the present invention can comprise a large bacterial sialidase or can comprise a protein with the amino acid sequence of a large bacterial sialidase or can comprise amino acid sequences that are substantially homologous to the amino acid sequence of a large bacterial sialidase. A pharmaceutical composition that can be used in the present invention comprises the *A. viscosus* sialidase (**SEQ ID NO:12**), or comprises a protein substantially homologous to the *A. viscosus* sialidase.

Other sialidases that can be used in the compositions, compounds and methods described herein are the human sialidases such as those encoded by the genes NEU2 (**SEQ ID NO:8**; Genbank Accession Number Y16535; Monti, E, Preti, Rossi, E., Ballabio, A and Borsani G. (1999) *Genomics* 57:137-143) and NEU4 (**SEQ ID NO:9**; Genbank Accession Number NM080741; Monti, E, Preti, A, Venerando, B and Borsani, G. (2002) *Neurochem Res* 27:646-663) (FIG. 2). Therapeutic domains of compounds of the present invention can comprise a human sialidase protein that is substantially homologous to the amino acid sequences of a human sialidase or can comprise amino acid sequences that are substantially homologous to all or a portion of the amino acid sequences of a human sialidase. Where a therapeutic domain comprises a portion of the amino acid sequences of a naturally occurring sialidase, or sequences substantially homologous to a portion of the amino acid sequences of a naturally occurring sialidase, the portion can have essentially the same activity as the human sialidase, e.g., an active portion of the sialidase.

Generally, sialidases that can effectively degrade on respiratory epithelial cells both receptor sialic acids Neu5Ac α(2,6)-Gal and Neu5Ac α(2,3)-Gal, can be used. Sialidases are found in higher eukaryotes, as well as in some mostly pathogenic microbes, including viruses, bacteria and protozoans. Viral and bacterial sialidases have been well characterized, and the three-dimensional structures of some of them have been determined (Crennell, SJ, Garman, E, Laver, G, Vimr, E and Taylor, G. (1994) *Structure* 2:535-544; Janakiraman, MN, White, CL, Laver, WG, Air, GM and Luo, M. (1994) *Biochemistry* 33:8172-8179; Pshezhetsky, A, Richard, C, Michaud, L, Igdoura, S, Wang, S, Elsliger, M, Qu, J, Leclerc, D, Gravel, R, Dallaire, L and Potier, M. (1997) *Nature Genet* 15: 316-320). Several human sialidases have also been cloned in the recent years (Milner, CM, Smith, SV, Carrillo MB, Taylor, GL, Hollinshead, M and Campbell, RD. (1997) *J Bio Chem* 272:4549-4558; Monti, E, Preti, A, Nesti, C, Ballabio, A and Borsani G. 1999. *Glycobiol* 9:1313-1321; Wada, T, Yoshikawa, Y, Tokuyama, S, Kuwabara, M, Akita, H and Miyagi, T. (1999) *Biochem Biophy Res Communi* 261:21-27; Monti, E, Bassi, MT, Papini, N, Riboni, M, Manzoni, M, Veneranodo, B, Croci, G, Preti, A, Ballabio, A, Tettamanti, G and Borsani, G. (2000) *Bichem J* 349:343-351). DAS181, which contains an active portion of a sialidase, has also been cloned.

All the sialidases characterized share a four amino acid motif in the amino terminal portion followed by the Asp box motif which is repeated three to five times depending on the protein. (Monti, E, Bassi, MT, Papini, N, Riboni, M, Manzoni, M, Veneranodo, B, Croci, G, Preti, A, Ballabio, A, Tettamanti, G and Borsani, G. (2000) *Bichem J* 349:343-351; Copley, RR, Russell, RB and Ponting, CP. (2001) *Protein Sci* 10:285-292). While the overall amino acid identity of the sialidase superfamily is relatively low at about 20-30%, the overall fold of the molecules, especially the catalytic amino acids, are remarkably similar (Wada, T, Yoshikawa, Y, Tokuyama, S, Kuwabara, M, Akita, H and Miyagi, T. (1999) *Biochem Biophy Res Communi* 261:21-27; Monti, E, Bassi, MT, Papini, N, Riboni, M, Manzoni, M, Veneranodo, B, Croci, G, Preti, A, Ballabio, A, Tettamanti, G and Borsani, G. (2000) *Bichem J* 349:343-351; Copley, RR, Russell, RB and Ponting, CP. (2001) *Protein Sci* 10:285-292).

The sialidases are generally divided into two families: "small" sialidases have molecular weight of about 42 kDa and do not require divalent metal ion for maximal activity; "large" sialidases have molecular weight above 65 kDa and may require divalent metal ion for activity (Wada, T, Yoshikawa, Y, Tokuyama, S, Kuwabara, M, Akita, H and Miyagi, T. (1999) *Biochem Biophy Res Communi* 261:21-27; Monti, E, Bassi, MT, Papini, N, Riboni, M, Manzoni, M, Veneranodo, B, Croci, G, Preti, A, Ballabio, A, Tettamanti, G and Borsani, G. (2000) *Bichem J* 349:343-351; Copley, RR, Russell, RB and Ponting, CP. (2001) *Protein Sci* 10:285-292).

Over fifteen sialidase proteins have been purified and they vary greatly from one another in substrate specificities and enzymatic kinetics. Large bacterial sialidases can effectively cleave sialic acid in both (α,2-6) linkage and (α,2-3) linkage in the context of most natural substrates (FIG. 4; Vimr, DR. (1994) *Trends Microbiol* 2: 271-277; Drzeniek, R. (1973) *Histochem J* 5:271-290; Roggentin, P, Kleineidam, RG and Schauer, R. (1995) *Biol Chem Hoppe-Seyler* 376:569-575; Roggentin, P, Schauer, R, Hoyer, LL and Vimr, ER. (1993) Mol Microb 9:915-921). Because of their broad substrate specificities, large bacterial sialidases make good candidates.

FIG. 4 shows several of the large bacterial sialidases with known substrate specificity. These enzymes have high specific activity (600 U/mg protein for *C. perfringens* (Corfield, AP, Veh, RW, Wember, M, Michalski, JC and Schauer, R. (1981) *Bichem J* 197:293-299) and 680 U/mg protein for *A. viscosus* (Teufel, M, Roggentin, P. and Schauer, R. (1989) *Biol Chem Hoppe Seyler* 370:435-443)), are fully active without divalent metal iron, and have been cloned and purified as recombinant proteins from *E. coli* (Roggentin, P, Kleineidam, RG and Schauer, R. (1995) *Biol Chem Hoppe-Seyler* 376:569-575, Teufel, M, Roggentin, P. and Schauer, R. (1989) *Biol Chem Hoppe Seyler* 370:435-443 , Sakurada, K, Ohta, T and Hasegawa, M. (1992) *J Bacteriol* 174: 6896-

6903). In addition, *C. perfringens* is stable in solution at 2-8^{0}C for several weeks, and at 4^{0}C in the presence of albumin for more than two years (Wang, FZ, Akula, SM, Pramod, NP, Zeng, L and Chandran, B. (2001) *J Virol* 75:7517-27). *A. viscosus* is labile towards freezing and thawing, but is stable at 4^{0}C in 0.1 M acetate buffer, pH 5 (Teufel, M, Roggentin, P. and Schauer, R. (1989) *Biol Chem Hoppe Seyler* 370:435-443).

A pharmaceutical composition comprising a sialidase can include other compounds, including but not limited to other proteins, that can also have therapeutic activity. A pharmaceutical composition comprising a sialidase can include other compounds that can enhance the stability, solubility, packaging, delivery, consistency, taste, or fragrance of the composition.

Compounds comprising a sialidase can be formulated for nasal, tracheal, bronchial, oral, or topical administration, or can be formulated as an injectable solution or as eyedrops, or formulated into a solution or dry powder and inhaled with inhalers. The sialidases described herein can be formulated into pharmaceutical compositions that include various additional compounds such as, $MgSO_4$, $CaCl_2$, Histidine, Histine-HCl, and Trehalose or their analogs.

These sialidases or pharmaceutical compositions containing them can be used (a) to treat or prevent allergic and inflammatory responses in the respiratory tract, (b) to reduce the quantity of mucus in the respiratory tract of subjects with elevated levels of mucus in their respiratory tracts, (c) to limit increases in the quantity of mucus in the respiratory tract of subjects above a baseline of mucus in their respiratory tracts, and/or (d) to prevent, treat, or ameliorate the effects of chronic obstructive pulmonary disease (COPD), bronchitis, bronchiectasis, cystic fibrosis (CF), vasculitis, mucus plugging, Wegener's granulomatosis, pneumonia, tuberculosis, cancer involving the lungs or the respiratory tract, Kartagener syndrome, Young's syndrome, chronic sinopulmonary infection, alpha 1-antitrypsin deficiency, primary immunodeficiency, acquired immune deficiency syndrome, opportunistic infection, an infectious state, a post infectious state, common cold, exercise induced hypersecretion of mucus, inflammatory bowel disease, ulcerative colitis, Crohn's disease, respiratory infection, respiratory obstruction, inhalation or aspiration of a toxic gas, pulmonary aspiration, or alcoholism in subjects with elevated levels of mucus in their respiratory tract or who are at risk of having increased levels of mucus in their respiratory tract. In some embodiments, subjects with elevated levels of mucus in their respiratory tract do not include subjects with one or more of influenza, parainfluenza, and/or respiratory syncytial virus (RSV).

Sialidase Catalytic Domain Proteins or Peptides

As used herein a "sialidase catalytic domain protein or peptide" comprises a catalytic domain of a sialidase but does not comprise the entire amino acid sequence of the sialidase from which the catalytic domain is derived. A sialidase catalytic domain protein or peptide has sialidase activity. A sialidase catalytic domain protein or peptide can have at least 10%, at least 20%, at least 50%, at least 70% of the activity of the sialidase from which the catalytic domain sequence is derived. A sialidase catalytic domain protein or peptide can have at least 90% of the activity of the sialidase from which the catalytic domain sequence is derived.

A sialidase catalytic domain protein or peptide can include other amino acid sequences, such as but not limited to additional sialidase sequences, sequences derived from other proteins, or sequences that are not derived from sequences of naturally-occurring proteins. Additional amino acid sequences can perform any of a number of functions, including contributing other activities to the catalytic domain protein, enhancing the expression, processing, folding, or stability of the sialidase catalytic domain protein, or even providing a desirable size or spacing of the protein or peptide.

A preferred sialidase catalytic domain protein or peptide is a protein that comprises the catalytic domain of the *A. viscosus* sialidase. An *A. viscosus* sialidase catalytic domain protein or peptide can include amino acids 270-667 of the *A. viscosus* sialidase sequence (**SEQ ID NO:12**). An *A. viscosus* sialidase catalytic domain protein or peptide can include amino acid sequence that begins at any of the amino acids from amino acid 270 to amino acid 290 of the *A. viscosus* sialidase sequence (**SEQ ID NO:12**) and ends at any of the amino acids from amino acid 665 to amino acid 901 of said *A. viscosus* sialidase sequence (**SEQ ID NO:12**), and lacks any *A. viscosus* sialidase protein sequence extending from amino acid 1 to amino acid 269. (As used herein "lacks any *A. viscosus* sialidase protein sequence extending from amino acid 1 to amino acid 269" means lacks any stretch of four or more consecutive amino acids as they appear in the designated protein or amino acid sequence.)

In some embodiments, an *A. viscosus* sialidase catalytic domain protein or peptide comprises amino acids 274-681 of the *A. viscosus* sialidase sequence (**SEQ ID NO:12**) and lacks other *A. viscosus* sialidase sequence. In other embodiments, an *A. viscosus* sialidase catalytic domain protein comprises amino acids 290-666 or 290-667 of the *A. viscosus* sialidase sequence (**SEQ ID NO:12**) and lacks any other *A. viscosus* sialidase sequence. In yet other embodiments, an *A. viscosus* sialidase catalytic domain protein or peptide comprises amino acids 274-666 of the *A. viscosus* sialidase sequence (**SEQ ID NO:12**) and lacks any other *A. viscosus* sialidase sequence. In yet other embodiments, an *A. viscosus* sialidase catalytic domain protein or peptide comprises amino acids 290-666 or 290-667 of the *A. viscosus* sialidase sequence (**SEQ ID NO:12**) and lacks any other *A. viscosus* sialidase sequence. In yet other embodiments, an *A. viscosus* sialidase catalytic domain protein or peptide comprises amino acids 290-681 of the *A. viscosus* sialidase sequence (**SEQ ID NO:12**) and lacks any other *A. viscosus* sialidase sequence.

Such sialidase catalytic domain proteins or peptides can be formulated for nasal, tracheal, bronchial, oral, or topical administration, or can be formulated as an injectable solution or as eyedrops, or formulated into a solution or dry powder and inhaled with an inhaler. The sialidase catalytic domain proteins or peptides described herein can be formulated into pharmaceutical compositions that include various additional compounds, such as, $MgSO_4$, $CaCl_2$, Histidine, Histine-HCl, and Trehalose or their analogs. These additional compounds can be included in the pharmaceutical compositions either alone or in various combinations, such as, Na_2SO_4, $MgSO_4$, $CaCl_2$, Histidine, Histine-HCl, and Trehalose or their analogs. These additional compounds can be included in the pharmaceutical compositions to act as excipients or as active ingredients that provide additional beneficial effects.

Such sialidase catalytic domain proteins or peptides or pharmaceutical compositions containing them can be used (a) to treat or prevent allergic and inflammatory responses in the respiratory tract, (b) to reduce the quantity of mucus in the respiratory tract of subjects with elevated levels of mucus in their respiratory tracts, (c) to limit increases in the quantity of mucus in the respiratory tract of subjects above a baseline of mucus in their respiratory tracts, and/or (d) to prevent, treat, or ameliorate the effects of chronic obstructive pulmonary disease (COPD), bronchitis, bronchiectasis, cystic fibrosis (CF), vasculitis, mucus plugging, Wegener's granulomatosis, pneumonia, tuberculosis, cancer involving the lungs or the respiratory tract, Kartagener syndrome, Young's syndrome, chronic sinopulmonary infection, alpha 1-antitrypsin deficiency, primary immunodeficiency, acquired immune deficiency syndrome, opportunistic infection, an infectious state, a post infectious state, common cold, exercise induced hypersecretion of mucus, inflammatory bowel disease, ulcerative colitis, Crohn's disease, respiratory infection, respiratory obstruction, inhalation or aspiration of a toxic gas, pulmonary aspiration, or alcoholism in subjects with elevated levels of mucus in their respiratory tract or who are at risk of having increased levels of mucus in their respiratory tract.

Fusion Proteins

Sialidase catalytic domain proteins can be fusion proteins, in which the fusion protein comprises at least one sialidase catalytic domain and at least one other protein domain, including but not limited to: a purification domain, a protein tag, a protein stability domain, a solubility domain, a protein size-increasing domain, a protein folding domain, a protein localization domain, an anchoring domain, an N-terminal domain, a C-terminal domain, a catalytic activity domain, a binding domain, or a catalytic activity-enhancing domain. The at least one other protein domain can be derived from another source, such as, but not limited to, sequences from another protein. The at least one other protein domain need not be based on any known protein sequence, but can be engineered and empirically tested to perform any function in the fusion protein.

Purification domains can include, as nonlimiting examples, one or more of a his tag, a calmodulin binding domain, a maltose binding protein domain, a streptavidin domain, a streptavidin binding domain, an intein domain, or a chitin binding domain. Protein tags can comprise sequences that can be used for antibody detection of proteins, such as, for example, the myc tag, the hemagglutinin tag, or the FLAG tag. Protein domains that enhance protein expression, modification, folding, stability, size, or localization can be based on sequences of know proteins or engineered. Other protein domains can have binding or catalytic activity or enhance the catalytic activity of the sialidase catalytic domain.

Fusion proteins used in the compositions, compounds and methods of the present invention comprise at least one sialidase catalytic domain and at least one anchoring domain. In some embodiments, anchoring domains include GAG-binding domains, such as the GAG-binding domain or human amphiregulin (**SEQ ID NO:7**).

Sialidase catalytic domains and other domains of a fusion protein used in the present invention can optionally be joined by linkers, such as but not limited to peptide linkers. A variety of peptide linkers are known in the art. In one embodiment a linker can be a peptide linker comprising glycine, such as G-G-G-G-S (**SEQ ID NO:10**).

Such fusion proteins can be formulated for nasal, tracheal, bronchial, oral, or topical administration, or can be formulated as an injectable solution or as eyedrops or formulated into a solution or dry powder and inhaled with an inhaler. These fusion proteins can be formulated into pharmaceutical compositions that include various additional compounds either alone or in various combinations, such as, Na_2SO_4, $MgSO_4$, $CaCl_2$, Histidine, Histine-HCl, and Trehalose or their analogs. These additional compounds can be included in the pharmaceutical compositions to act as excipients or as active ingredients that provide additional beneficial effects.

Such fusion proteins or pharmaceutical compositions containing them can be used (a) to treat or prevent allergic and inflammatory responses in the respiratory tract, (b) to reduce the quantity of mucus in the respiratory tract of subjects with elevated levels of mucus in their respiratory tracts, (c) to limit increases in the quantity of mucus in the respiratory tract of subjects above a baseline of mucus in their respiratory tracts, and/or (d) to prevent, treat, or ameliorate the effects of chronic obstructive pulmonary disease (COPD), bronchitis, bronchiectasis, cystic fibrosis (CF), vasculitis, mucus plugging, Wegener's granulomatosis, pneumonia, tuberculosis, cancer involving the lungs or the respiratory tract, Kartagener syndrome, Young's syndrome, chronic sinopulmonary infection, alpha 1-antitrypsin deficiency, primary immunodeficiency, acquired immune deficiency syndrome, opportunistic infection, an infectious state, a post infectious state, common cold, exercise induced hypersecretion of mucus, inflammatory bowel disease, ulcerative colitis, Crohn's disease, respiratory infection, respiratory obstruction, inhalation or aspiration of a toxic gas, pulmonary aspiration, or alcoholism in subjects with elevated levels of mucus in their respiratory tract or who are at risk of having increased levels of mucus in their respiratory tract.

Various constructs of fusion proteins are shown in FIGS. 4-6, as well as in the sequences provided in the sequence listing provided herein.

Methods for testing the compounds and compositions and/or for screening to identify sialidases and/or active portions thereof to treat diseases accompanied by inflammation

The compounds and compositions provided herein can be tested for their activity in reducing inflammation, allergies or associated responses, such as mucus overproduction, using standard assays known to those of skill in the art. Several cell-based (*e.g.*, tracheal cell cultures) and animal-based assays (mouse models, guinea pig models) for measuring inflammation or mucus overproduction are known (*see*, *e.g.*, Nakao *et al.*, *J. Immunol.*, 180:6262-6269 (2008); Westerhof *et al.*, *Mediators Inflamm.*, 10(3):143-154 (2001); Miller *et al.*, *J. Immunol.*, 170:3348-3356 (2003); Nakanishi *et al.*, *Proc. Natl. Acad. Sci. USA*, 98(9):5175-5180 (2001); and DuBuske, *Allergy Proc.*, 16(2):55-58 (1995), the contents of each of which are incorporated in their entirety by reference herein). The compounds and compositions provided herein can be tested for their ability to reduce inflammation or mucus overproduction in any of these assays or other standard assays known to those of skill in the art. In addition, sialidases or active portions thereof can be identified and/or selected for their anti-inflammatory activity and/or ability to reduce associated responses, such as mucus overproduction, using such assays. Exemplary assays and protocols are described herein in Example 1 and Example 2.

In addition to assays that measure inflammation or associated responses, such as mucus overproduction, the compounds and compositions provided herein can be tested for their activity by assessing their ability to disrupt muscarinic receptor –mediated signaling in the presence of an agonist. Muscarinic receptors, or mAChRs, are G protein-coupled acetylcholine receptors found in the plasma membranes of certain neurons and other cells. They play several roles, including acting as the main end-receptor stimulated by acetylcholine released from postganglionic fibers in the parasympathetic nervous system.

Muscarinic receptor-agonist interactions, and the resulting signaling, is believed to play a role in diseases that have associated inflammatory and/or allergic responses, such as asthma and COPD (*see*, *e.g.*, "Muscarinic Receptors in Airways Diseases," Birkhauser-Verlag publ., Zangsma et al., Eds.).

More specifically, acetylcholinergic mechanisms are recognized to influence the following normal and pathogenic respiratory functions:

1. secretion of mucus,
2. active transport of ions across the respiratory epithelium and during mucociliary transport,
3. smooth muscle tone of the airways,
4. immunologic and inflammatory response of the airways,
5. reflex regulation of the airways,
6. respiratory responses of the airways in asthma and in other hypersensitivity states of the respiratory tract.

Consequently, certain anti-muscarinic agents have been effective against: (a) acetylcholinergically induced bronchoconstriction; (b) iatrogenic airway spasms induced by beta blockers; and (c) psychogenic bronchospasm. The two main pulmonary applications of anti-muscarinic agents has been chronic bronchitis and bronchial asthma (Pharmacology of Anti-Muscarinic Agents, Laszlo Gyermek (1998)).

There are five broad classes of muscarinic receptors, based on their physiological roles, and agonists for each of these receptors are known to those of skill in the art:

M1 receptor – exemplary agonists include acetylcholine, oxotremorine, muscarine, carbachol and McNA343

M2 receptor – exemplary agonists include acetylcholine, methacholine, carbachol, oxotremorine and muscarine

M3 receptor – exemplary agonists include acetylcholine, bethanechol, carbachol, oxotremorine and pilocarpine

M4 receptor – exemplary agonists include acetylcholine, carbachol and oxotremorine

M5 receptor – exemplary agonists include acetylcholine, carbachol and oxotremorine

In some embodiments, the compounds and compositions provided herein can be tested for the ability to reduce inflammation and/or allergic responses, including mucus overproduction, associated with RTIs or RTDs by assessing their ability to disrupt muscarinic receptor-agonist interactions. Further, sialidases and/or active portions thereof can be screened, identified and selected for their ability to reduce inflammation, allergies, and/or associated responses such as mucus overproduction by assessing their ability to disrupt muscarinic receptor – agonist interactions. These tests and screens can be performed using standard assays known to those of skill in the art (*see*, *e.g*, Armstrong *et al.*, *Curr. Protocols in Pharmacol.*, UNIT 12-13 (2010), the contents of which are incorporated in their entirety by reference herein). An exemplary assay and protocol is provided herein in Example 3.

Pharmaceutical Compositions

The present invention includes compounds of the present invention formulated as pharmaceutical compositions. The pharmaceutical compositions comprise a pharmaceutically acceptable carrier prepared for storage and subsequent administration, which have a pharmaceutically effective amount of the compound in a pharmaceutically acceptable carrier or diluent. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art, and are described, for example, in Remington's Pharmaceutical Sciences, 18th Ed., Mack Publishing Co., Easton, PA (1990)). Preservatives, stabilizers, dyes and even flavoring agents can be provided in the pharmaceutical composition. For example, sodium benzoate, sorbic acid and esters of p-hydroxybenzoic acid can be added as preservatives. In addition, antioxidants and suspending agents can be used.

Depending on the target cell, the compounds of the present invention can be formulated and used as tablets, capsules or elixirs for oral or inhaled administration; salves or ointments for topical application; suppositories for rectal administration; sterile solutions, suspensions, and encapsulated powders and the like for use as inhalants or nasal sprays. Injectables can also be prepared in conventional forms either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions.

Suitable excipients are, for example, water, saline, dextrose, mannitol, lactose, lecithin, albumin, sodium glutamate, cysteine hydrochloride and the like. In addition to those excipients, additional compounds that can be included in the pharmaceutical compositions described herein either alone or in various combinations include Na_2SO_4, $MgSO_4$, $CaCl_2$, Histidine, Histine-HCl, and Trehalose or their analogs or Mg salts and/or Ca salts. These additional compounds can be included in the pharmaceutical compositions to act as excipients or as active ingredients that provide additional beneficial effects. In addition, if desired, the injectable pharmaceutical compositions can contain minor amounts of nontoxic auxiliary substances, such as wetting agents, pH buffering agents and the like.

The pharmaceutically effective amount of a test compound required as a dose will depend on the route of administration, the type of animal or patient being treated, and the physical characteristics of the specific animal under consideration. The dose can be tailored to achieve a desired effect, such as reduction of elevated levels of mucus in the respiratory tract, but will depend on such factors as weight, diet, concurrent medication and other factors which those skilled in the medical arts will recognize. In practicing the methods of the present invention, the pharmaceutical compositions can be used alone or in combination with one another, or in combination with other therapeutic or diagnostic agents. These products can be utilized *in vivo* in a non-human animal subject, in a mammalian subject, in a human subject, or *in vitro*. In employing them *in vivo*, the pharmaceutical compositions can be administered to the patient or subject in a variety of ways, including topically, parenterally, intravenously, subcutaneously, intramuscularly, colonically, rectally, nasally or intraperitoneally, employing a variety of dosage forms. Such methods can also be used in testing the activity of test compounds *in vivo*.

In some embodiments, these pharmaceutical compositions may be in the form of orally-administrable suspensions, solutions, tablets or lozenges; nasal sprays; inhalants; injectables, topical sprays, ointments, powders, or gels, or formulated into a solutions or dry powders and inhaled with an inhaler.

When administered orally as a suspension, compositions of the present invention are prepared according to techniques well-known in the art of pharmaceutical formulation and may contain microcrystalline cellulose for imparting bulk, alginic acid or sodium alginate as a suspending agent, methylcellulose as a viscosity enhancer, and sweeteners/flavoring agents known in the art. As immediate release tablets, these compositions may contain microcrystalline cellulose, dicalcium phosphate, starch, magnesium stearate and lactose and/or other excipients, binders, extenders, disintegrants, diluents and lubricants known in the art. Components in the formulation of a mouthwash or rinse include antimicrobials, surfactants, cosurfactants, oils, water and other additives such as sweeteners/flavoring agents known in the art.

When administered by a drinking solution, the composition comprises one or more of the compounds of the present invention, dissolved in water, with appropriate pH adjustment, and with carrier. The compound can be dissolved in distilled water, tap water, spring water, and the like. The pH can in some embodiments be adjusted to between about 3.5 and about 8.5. Sweeteners can be added, e.g., 1% (w/v) sucrose.

Lozenges can be prepared according to U.S. Patent No. 3,439,089, herein incorporated by reference for these purposes.

When administered by nasal aerosol or inhalation, the pharmaceutical compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and can be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other solubilizing or dispersing agents known in the art. See, for example, Ansel, H. C. et al., Pharmaceutical Dosage Forms and Drug Delivery Systems, Sixth Ed. (1995). Inhaled powders can also be prepared using techniques described in U.S. Patent Application Serial Nos. 11/657,813 and 12/179,520, both of which are incorporated herein by reference in their entirety. These compositions and formulations can generally be prepared with suitable nontoxic pharmaceutically acceptable ingredients. These ingredients are known to those skilled in the preparation of nasal dosage forms and some of these can be found in Remington's Pharmaceutical Sciences, 18th Ed., Mack Publishing Co., Easton, PA (1990, a standard reference in the field. The choice of suitable carriers is highly dependent upon the exact nature of the nasal dosage form desired, e.g., solutions, suspensions, ointments, or gels. Nasal dosage forms generally contain large amounts of water in addition to the active ingredient. Minor amounts of other ingredients such as pH adjusters, emulsifiers or dispersing agents, preservatives, surfactants, jelling agents, or buffering and other stabilizing and solubilizing agents can also be present. Generally, the nasal dosage form can be isotonic with nasal secretions.

Nasal formulations can be administers as drops, sprays, aerosols or by any other intranasal dosage form. Optionally, the delivery system can be a unit dose delivery system. The volume of solution or suspension delivered per dose can be anywhere from about 5 to about 2000 microliters, from about 10 to about 1000 microliters, or from about 50 to about 500 microliters. Delivery systems for these various dosage forms can be dropper bottles, plastic squeeze units, atomizers, nebulizers or pharmaceutical aerosols in either unit dose or multiple dose packages.

The formulations of this invention can be varied to include; (1) other acids and bases to adjust the pH; (2) other tonicity imparting agents such as sorbitol, glycerin and dextrose; (3) other antimicrobial preservatives such as other parahydroxy benzoic acid esters, sorbate, benzoate, propionate, chlorbutanol, phenylethyl alcohol, benzalkonium chloride, and mercurials; (4) other viscosity imparting agents such as sodium carboxymethylcellulose, microcrystalline cellulose, polyvinylpyrrolidone, polyvinyl alcohol and other gums; (5) suitable absorption enhancers; (6) stabilizing agents such as antioxidants, like bisulfite and ascorbate, metal chelating agents such as sodium edetate and drug solubility enhancers such as polyethylene glycols.

One embodiment of the invention includes pharmaceutical compositions that at various dosage levels, such as dosage levels between about .01 mg and about 100 mg, reduce the quantity of mucus in the respiratory tract of subjects with elevated levels of mucus in their respiratory tracts, and/or that limit increases in the quantity of mucus in the respiratory tract of subjects above a baseline of mucus in their respiratory tracts. Examples of such dosage levels include doses of about .05 mg, .06 mg, 0.1 mg, 0.5 mg, 1 mg, 5 mg, 10 mg, 20 mg, 50 mg, or 100 mg. Another embodiment of the invention includes pharameutical compositions that at various dosage levels, such as dosage levels between about .01 mg and about 100 mg, reduce inflammation in the respiratory tract or prevent worsening of inflammation in the respiratory tract. Examples of such dosage levels include doses of about .05 mg, .06 mg, 0.1 mg, 0.5 mg, 1 mg, 5 mg, 10 mg, 20 mg, 50 mg, or 100 mg. The foregoing doses can be administered one or more times per day, for one day, two days, three days, four days, five days, six days, seven days, eight days, nine days, ten days, eleven days, twelve days, thirteen days, or fourteen or more days. Higher doses or lower doses can also be administered. Typically, dosages can be between about 1 ng/kg and about 10 mg/kg, between about 10 ng/kg and about 1 mg/kg, and between about 100 ng/kg and about 100 micrograms/kg. In various examples described herein, mice were treated with various dosages of the compositions described herein, including dosages of .0008 mg/kg, .004 mg/kg, .02 mg/kg, .06 mg/kg, 0.1 mg/kg, 0.3 mg/kg, 0.6 mg/kg, and 1.0 gm/kg.

In one embodiment a pharmaceutical composition includes DAS181, $MgSO_4$ 1.446mg/ml, $CaCl_2$ 0.059mg/ml, Histidine 1.427mg/ml, Histidine-HCl 1.943mg/ml, and Trehalose 3.000mg/ml.

In another embodiment a pharameutical composition includes DAS181, $MgSO_4$, $CaCl_2$, Histidine, Histidine-HCl, and Trehalose.

In another embodiment a pharameutical composition includes DAS181, Na_2SO_4, and $CaCl_2$.

In another embodiment a pharmaceutical composition includes DAS181 and any combination of one or more of the following: Na_2SO_4, $MgSO_4$, $CaCl_2$, Histidine, Histidine-HCl, and Trehalose.

In another embodiment a pharmaceutical composition includes (a) a naturally occurring sialidase protein or peptide or an active portion thereof, or a recombinant protein substantially homologous to at least a portion of a naturally occurring sialidase, (b) $MgSO_4$ 1.446mg/ml, (c) $CaCl_2$ 0.059mg/ml, (d) Histidine 1.427mg/ml, (e) Histidine-HCl 1.943mg/ml, and (f) Trehalose 3.000mg/ml. In one embodiment, the protein or peptide is a sialidase with substantial homology to the *A. viscosus* sialidase (**SEQ ID NO:12**) or substantial homology to an active portion thereof, such as amino acids 274-666, 274-667, 270-667, 274-681, or 290-681 of SEQ ID NO:12, or any other catalytic domain of *Actinomyces viscosis* sialidase. In other embodiments, the protein or peptide is from one of the large bacterial sialidases that can degrade the receptor sialic acids Neu5Ac alpha(2,6)-Gal and Neu5Ac alpha(2,3)-Gal. For example, the bacterial sialidase enzymes from *Clostridium perfringens* (Genbank Accession Number X87369), *Arthrobacter ureafaciens*, or *Micromonospora viridifaciens* (Genbank Accession Number D01045) or proteins or peptides that are substantially homologous to those sialidases or their active portions. In other embodiments, the protein or peptide is from other sialidases, such as those encoded by the genes NEU2 (**SEQ ID NO:8**; Genbank Accession Number Y16535; Monti, E, Preti, Rossi, E., Ballabio, A and Borsani G. (1999) *Genomics* 57:137-143) and NEU4 (**SEQ ID NO:9;** Genbank Accession Number NM080741; Monti, E, Preti, A, Venerando, B and Borsani, G. (2002) *Neurochem Res* 27:646-663) (FIG. 2), or active portions of those sialidases.

In another embodiment a pharmaceutical composition includes (a) a naturally occurring sialidase protein or peptide or an active portion thereof, or a recombinant protein substantially homologous to at least a portion of a naturally occurring sialidase, (b) $MgSO_4$, (c) $CaCl_2$, (d) Histidine, (e) Histidine-HCl, and (f) Trehalose. . In one embodiment, the protein or peptide is a sialidase with substantial homology to the *A. viscosus* sialidase (**SEQ ID NO:12**) or substantial homology to an active portion thereof, such as amino acids 274-666, 274-667, 270-667, 274-681, or 290-681 of SEQ ID NO:12, or any other catalytic domain of *Actinomyces viscosis* sialidase. In other embodiments, the protein or peptide is from one of the large bacterial sialidases that can degrade the receptor sialic acids Neu5Ac alpha(2,6)-Gal and Neu5Ac alpha(2,3)-Gal. For example, the bacterial sialidase enzymes from *Clostridium perfringens* (Genbank Accession Number X87369), *Arthrobacter ureafaciens*, or *Micromonospora viridifaciens* (Genbank Accession Number D01045) or proteins or peptides that are substantially homologous to those sialidases or their active portions. In other embodiments, the protein or peptide is from other sialidases, such as those encoded by the genes NEU2 (**SEQ ID NO:8**; Genbank Accession Number Y16535; Monti, E, Preti, Rossi, E., Ballabio, A and Borsani G. (1999) *Genomics* 57:137-143) and NEU4 (**SEQ ID NO:9;** Genbank Accession Number NM080741; Monti, E, Preti, A, Venerando, B and Borsani, G. (2002) *Neurochem Res* 27:646-663) (FIG. 2), or active portions of those sialidases.

In another embodiment a pharmaceutical composition includes (a) a naturally occurring sialidase protein or peptide or an active portion thereof, or a recombinant protein substantially homologous to at least a portion of a naturally occurring sialidase, (b) Na_2SO_4, and (c) $CaCl_2$. In one embodiment, the protein or peptide is a sialidase with substantial homology to the *A. viscosus* sialidase (**SEQ ID NO:12**) or substantial homology to an active portion thereof, such as amino acids 274-666, 274-667, 270-667, 274-681, or 290-681 of SEQ ID NO:12, or any other catalytic domain of *Actinomyces viscosis* sialidase. In one embodiment, the protein or peptide is a sialidase with substantial homology to the *A. viscosus* sialidase (**SEQ ID NO:12**) or substantial homology to an active portion thereof, such as amino acids 274-666, 274-667, 270-667, 274-681, or 290-681 of SEQ ID NO:12, or any other catalytic domain of *Actinomyces viscosis* sialidase. In other embodiments, the protein or peptide is from one of the large bacterial sialidases that can degrade the receptor sialic acids Neu5Ac alpha(2,6)-Gal and Neu5Ac alpha(2,3)-Gal. For example, the bacterial sialidase enzymes from *Clostridium perfringens* (Genbank Accession Number X87369), *Arthrobacter ureafaciens*, or *Micromonospora viridifaciens* (Genbank Accession Number D01045) or proteins or peptides that are substantially homologous to those sialidases or their active portions. In other embodiments, the protein or peptide is from other sialidases, such as those encoded by the genes NEU2 (**SEQ ID NO:8**; Genbank Accession Number Y16535; Monti, E, Preti, Rossi, E., Ballabio, A and Borsani G. (1999) *Genomics* 57:137-143) and NEU4 (**SEQ ID NO:9;** Genbank Accession Number NM080741; Monti, E, Preti, A, Venerando, B and Borsani, G. (2002) *Neurochem Res* 27:646-663) (FIG. 2), or active portions of those sialidases.

In another embodiment a pharmaceutical composition includes (a) a naturally occurring sialidase protein or peptide or an active portion thereof, or a recombinant protein substantially homologous to at least a portion of a naturally occurring sialidase, and any combination of one or more of the following: Na_2SO_4, $MgSO_4$, $CaCl_2$, Histidine, Histidine-HCl, and Trehalose. In one embodiment, the protein or peptide is a sialidase with substantial homology to the *A. viscosus* sialidase (**SEQ ID NO:12**) or substantial homology to an active portion thereof, such as amino acids 274-666, 274-667, 270-667, 274-681, or 290-681 of SEQ ID NO:12, or any other catalytic domain of *Actinomyces viscosis* sialidase. In other embodiments, the protein or peptide is from one of the large bacterial sialidases that can degrade the receptor sialic acids Neu5Ac alpha(2,6)-Gal and Neu5Ac alpha(2,3)-Gal. For example, the bacterial sialidase enzymes from *Clostridium perfringens* (Genbank Accession Number X87369), *Arthrobacter ureafaciens*, or *Micromonospora viridifaciens* (Genbank Accession Number D01045) or proteins or peptides that are substantially homologous to those sialidases or their active portions. In other embodiments, the protein or peptide is from other sialidases, such as those encoded by the genes NEU2 (**SEQ ID NO:8**; Genbank Accession Number Y16535; Monti, E, Preti, Rossi, E., Ballabio, A and Borsani G. (1999) *Genomics* 57:137-143) and NEU4 (**SEQ ID NO:9**; Genbank Accession Number NM080741; Monti, E, Preti, A, Venerando, B and Borsani, G. (2002) *Neurochem Res* 27:646-663) (FIG. 2), or active portions of those sialidases.

In another embodiment a pharmaceutical composition includes (a) a fusion protein that has at least one catalytic domain of a sialidase, wherein the catalytic domain of the sialidase includes the sequence of amino acids extending from amino acid 274 to amino acid 666 of SEQ ID NO:12 (alternatively, 274 to 666, 270-667, 274-681, 290-681 of SEQ ID NO:12, or any other catalytic domain of *Actinomyces viscosis*), inclusive, and at least one anchoring domain, wherein the anchoring domain is a glycosaminoglycan (GAG) binding domain of human amphiregulin including the amino acid sequence of SEQ ID NO:7, (b) $MgSO_4$ 1.446mg/ml, (c) $CaCl_2$ 0.059mg/ml, (d) Histidine 1.427mg/ml, (e) Histidine-HCl 1.943mg/ml, and (f) Trehalose 3.000mg/ml.

In another embodiment a pharmaceutical composition includes (a) a fusion protein that has at least one catalytic domain of a sialidase, wherein the catalytic domain of the sialidase includes the sequence of amino acids extending from amino acid 274 to amino acid 666 of SEQ ID NO:12 (alternatively, 274 to 666, 270-667, 274-681, 290-681 of SEQ ID NO:12, or any other catalytic domain of *Actinomyces viscosis*), inclusive, and at least one anchoring domain, wherein the anchoring domain is a glycosaminoglycan (GAG) binding domain of human amphiregulin including the amino acid sequence of SEQ ID NO:7, (b) $MgSO_4$, (c) $CaCl_2$, (d) Histidine, (e) Histidine-HCl, and (f) Trehalose.

In another embodiment a pharmaceutical composition includes (a) a fusion protein that has at least one catalytic domain of a sialidase, wherein the catalytic domain of the sialidase includes the sequence of amino acids extending from amino acid 274 to amino acid 666 of SEQ ID NO:12 (alternatively, 274 to 666, 270-667, 274-681, 290-681 of SEQ ID NO:12, or any other catalytic domain of *Actinomyces viscosis*), inclusive, and at least one anchoring domain, wherein the anchoring domain is a glycosaminoglycan (GAG) binding domain of human amphiregulin including the amino acid sequence of SEQ ID NO:7, (b) Na_2SO_4, and (c) $CaCl_2$.

In another embodiment a pharmaceutical composition includes (a) a fusion protein that has at least one catalytic domain of a sialidase, wherein the catalytic domain of the sialidase includes the sequence of amino acids extending from amino acid 274 to amino acid 666 of SEQ ID NO:12 (alternatively, 274 to 666, 270-667, 274-681, 290-681 of SEQ ID NO:12, or any other catalytic domain of *Actinomyces viscosis*), inclusive, and at least one anchoring domain, wherein the anchoring domain is a glycosaminoglycan (GAG) binding domain of human amphiregulin including the amino acid sequence of SEQ ID NO:7, and (b) any combination of one or more of the following: Na_2SO_4, $MgSO_4$, $CaCl_2$, Histidine, Histidine-HCl, and Trehalose.

In another embodiment a pharmaceutical composition includes (a) a fusion protein having a sialidase or an active portion thereof and an anchoring domain, (b) $MgSO_4$ 1.446mg/ml, (c) $CaCl_2$ 0.059mg/ml, (d) Histidine 1.427mg/ml, (e) Histidine-HCl 1.943mg/ml, and (f) Trehalose 3.000mg/ml.

In another embodiment a pharmaceutical composition includes (a) a fusion protein having a sialidase or an active portion thereof and an anchoring domain, (b) $MgSO_4$, (c) $CaCl_2$, (d) Histidine, (e) Histidine-HCl, and (f) Trehalose.

In another embodiment a pharmaceutical composition includes (a) a fusion protein having a sialidase or an active portion thereof and an anchoring domain, (b) Na_4SO_4, and (c) $CaCl_2$.

In another embodiment a pharmaceutical composition includes (a) a fusion protein having a sialidase or an active portion thereof and an anchoring domain, and (b) any combination of one or more of the following: Na_2SO_4, $MgSO_4$, $CaCl_2$, Histidine, Histidine-HCl, and Trehalose.

Another representative example of a pharmaceutical composition of the present invention and that can be used in the methods described herein includes the following: DAS181, histidine, magnesium sulfate (or citrate salt), calcium chloride, trehalose, water, Na-Acetate, and acetic acid.

Yet another representative example of a pharmaceutical composition of the present invention and that can be used in the methods described herein includes DAS181 (in any concentration between about 0.01% and about 100% w/w, between about 1.00% and about 90.0% w/w, between about 5.00% and about 80.0% w/w, between about 10.0%

and about 70.0% w/w, between about 20.0% and about 70% w/w, between about 30.0% and about 70.0% w/w, between about 40.0% and about 70.0% w/w, between about 50.0% and about 70% w/w, between about 60.0% and about 70.0% w/w) in combination with any of the following: histidine or histidine-HCl (in any concentration between about 0.00% and about 90.0% w/w, between about 0.01% and about 80.0% w/w, between about 1.00% and about 75.0% w/w, between about 2.00% and about 70.0% w/w, between about 3.00% and about 60% w/w, between about 4.00% and about 50.0% w/w, between about 5.00% and about 40.0% w/w, between about 6.00% and about 30% w/w, between about 7.00% and about 20.0% w/w), magnesium sulfate (or citrate salt or sodium sulfate)(in any concentration between about 0.00% and about 90.0% w/w, between about 0.01% and about 80.0% w/w, between about 1.00% and about 75.0% w/w, between about 2.00% and about 70.0% w/w, between about 3.00% and about 60% w/w, between about 4.00% and about 50.0% w/w, between about 5.00% and about 40.0% w/w, between about 6.00% and about 30% w/w, between about 7.00% and about 20.0% w/w) , calcium chloride (in any concentration between about 0.00% and about 90.0% w/w, between about 0.01% and about 80.0% w/w, between about 0.01% and about 75.0% w/w, between about 0.01% and about 70.0% w/w, between about 0.01% and about 60% w/w, between about 0.01% and about 50.0% w/w, between about 0.01% and about 40.0% w/w, between about 0.01% and about 30% w/w, between about 0.10% and about 20.0% w/w), trehalose (in any concentration between about 0.00% and about 90.0% w/w, between about 0.01% and about 80.0% w/w, between about 1.00% and about 75.0% w/w, between about 2.00% and about 70.0% w/w, between about 3.00% and about 60% w/w, between about 4.00% and about 50.0% w/w, between about 5.00% and about 40.0% w/w, between about 6.00% and about 30% w/w, between about 7.00% and about 20.0% w/w), water (in any concentration between about 0.00% and about 90.0% w/w, between about 0.01% and about 80.0% w/w, between about 1.00% and about 75.0% w/w, between about 2.00% and about 70.0% w/w, between about 3.00% and about 60% w/w, between about 4.00% and about 50.0% w/w, between about 5.00% and about 40.0% w/w, between about 6.00% and about 30% w/w, between about 7.00% and about 20.0% w/w), Na-Acetate (in any concentration between about 0.00% and about 90.0% w/w, between about 0.01% and about 80.0% w/w, between about 0.01% and about 75.0% w/w, between about 0.01% and about 70.0% w/w, between about 0.01% and about 60% w/w, between about 0.01% and about 50.0% w/w, between about 0.01% and about 40.0% w/w, between about 0.01% and about 30% w/w, between about 0.10% and about 20.0% w/w), and acetic acid (in any concentration between about 0.00% and about 90.0% w/w, between about 0.01% and about 80.0% w/w, between about 0.01% and about 75.0% w/w, between about 0.01% and about 70.0% w/w, between about 0.01% and about 60% w/w, between about 0.01% and about 50.0% w/w, between about 0.01% and about 40.0% w/w, between about 0.01% and about 30% w/w, between about 0.10% and about 20.0% w/w).

Any of the above pharmaceutical compositions may in addition include $MgCl_2$ in various concentrations ranging from about 0% to about 75% w/w.

Reducing mucus in the respiratory tract and limiting its increase

Accumulation or elevated levels of mucus in the respiratory airway tree can be caused by an increased volume of mucus produced, and also by decreased clearance due to defects in the ciliary clearance apparatus in the respiratory tract. Hypersecretion of mucus can be chronic, but increased volumes are produced in exacerbations of COPD, during attacks of asthma, and in bronchiectatic and cystic fibrosis patients (W.D. Kim, Eur Respir. J. 1997, 10:1914-1917). Intraluminal mucus accumulation (i.e., elevated levels of mucus) in the airways associated with hypersecretion of mucus or decreased clearance thereof creates a clinical problem in almost all pulmonary diseases and diseases that have an affect on the respiratory tract, including without limitation chronic obstructive pulmonary disease (COPD), bronchitis, bronchiectasis, cystic fibrosis (CF), vasculitis, mucus plugging, Wegener's granulomatosis, pneumonia, tuberculosis, cancer involving the lungs or the respiratory tract, Kartagener syndrome, Young's syndrome, chronic sinopulmonary infection, alpha 1-antitrypsin deficiency, primary immunodeficiency, acquired immune deficiency syndrome, opportunistic infection, an infectious state, a post infectious state, common cold, exercise induced hypersecretion of mucus, inflammatory bowel disease, ulcerative colitis, Crohn's disease, respiratory infection, respiratory obstruction, inhalation or aspiration of a toxic gas, pulmonary aspiration, or alcoholism. Elevated levels of mucus in the respiratory tract are an important determinant in the prognosis and clinical feastures of various pulmonary diseases, such as chronic bronchitis, bronchiectasis and bronchial asthma, in addition to cystic fibrosis and COPD (W.D. Kim, Eur Respir. J. 1997, 10:1914-1917). Accordingly, in some embodiments, the present disclosure include methods in which a subject with one or more of these conditions or diseases is selected for treatment. In some embodiments, the methods can include selecting a subject with one or more of the conditions or diseases provided herein and that is not infected with one or more of influenza, parainfluenza, and/or respiratory syncytial virus (RSV). Following selection, the subject can be treated by administration of one or more of the compositions disclosed herein.

Provided herein are methods that include the administration of the compounds described herein and in U.S. Application Serial Nos. 10/718,986 and 10/939,262, or compositions containing them, to reduce the quantity of mucus in the respiratory tract of subjects with elevated levels of mucus in their respiratory tracts and to limit increases in the quantity of mucus in the respiratory tract of subjects above a baseline of mucus in their respiratory tracts. Thus, the invention relates to method of using the therapeutic compounds and/or compositions described herein to prevent or treat diseases that are caused by, cause, or are exacerbated by respiratory inflammation or increased mucus production, such as, both allergic and non-allergic asthma, chronic obstructive pulmonary disease (COPD), bronchitis (both acute and non-acute), bronchiectasis, cystic fibrosis (CF), vasculitis, mucuous plugging, Wegener's granulomatosis, and any other disorder that causes inflammation or increased mucus production in the respiratory tract or is caused by or exacerbated by inflammation or increased mucus production in the respiratory tract. The invention also includes methods of using the therapeutic compounds and/or compositions described herein to reduce the quantity of mucus in the respiratory tract of subjects with elevated levels of mucus in their respiratory tracts and limit increases in the quantity of mucus in the respiratory tract of subjects above a baseline of mucus in their respiratory tracts.

In some embodiments, the methods include administering a composition or compound containing a therapeutically effective amount of a protein or peptide having a sialidase or an active portion thereof to a subject. The protein or peptide can be an isolated naturally occurring sialidase protein, or a recombinant protein substantially homologous to at least a portion of a naturally occurring sialidase. In one embodiment, a pharmaceutical composition or compound contains a sialidase with substantial homology to the *A. viscosus* sialidase (**SEQ ID NO:12**) or substantial homology to an active portion thereof, such as amino acids 274-666, 274-667, 270-667, 274-681, or 290-681 of SEQ ID NO:12, or any other catalytic domain of *Actinomyces viscosis* sialidase. The therapeutically effective amount includes an amount of the protein or peptide that results in a reduction of the quantity of mucus in the respiratory tract after administration of the composition or compound when compared to the quantity of mucus present prior to administration of the composition.

In other embodiments, the methods include administering a composition or compound containing a therapeutically effective amount of a fusion protein, wherein the fusion protein has at least one catalytic domain of a sialidase, wherein the catalytic domain of the sialidase includes the sequence of amino acids extending from amino acid 274 to amino acid 666 of SEQ ID NO:12 (alternatively, 274 to 666, 270-667, 274-681, 290-681 of SEQ ID NO:12, or any other catalytic domain of *Actinomyces viscosis*), inclusive, and at least one anchoring domain, wherein the anchoring domain is a glycosaminoglycan (GAG) binding domain of human amphiregulin including the amino acid sequence of SEQ ID NO:7. The therapeutically effective amount includes an amount of the fusion protein that results in a reduction of the quanitity of mucus in the respiratory tract after administration of the composition or compound when compared to the quantity of mucus present prior to administration of the composition.

In yet other embodiments, the methods include administering a composition containing a therapeutically effective amount of a fusion protein having a sialidase or an active portion thereof and an anchoring domain. The therapeutically effective amount includes an amount of the fusion protein that results in a reduction of the quanitity of mucus in the respiratory tract after administration of the composition or compound when compared to the quantity of mucus present prior to administration of the composition.

Other embodiments include methods of preventing, treating or ameliorating the effects of chronic obstructive pulmonary disease (COPD), bronchitis, bronchiectasis, cystic fibrosis (CF), vasculitis, mucus plugging, Wegener's granulomatosis, pneumonia, tuberculosis, cancer involving the lungs or the respiratory tract, Kartagener syndrome, Young's syndrome, chronic sinopulmonary infection, alpha 1-antitrypsin deficiency, primary immunodeficiency, acquired immune deficiency syndrome, opportunistic infection, an infectious state, a post infectious state, common cold, exercise induced hypersecretion of mucus, inflammatory bowel disease, ulcerative colitis, Crohn's disease, respiratory infection, respiratory obstruction, inhalation or aspiration of a toxic gas, pulmonary aspiration, or alcoholism in a subject with an elevated level of mucus in his or her respiratory tract. The methods include administering (a) a composition containing a therapeutically effective amount of a protein or peptide having a sialidase or an active portion thereof to a subject, (b) a composition containing a therapeutically effective amount of a fusion protein, wherein the fusion protein has at least one catalytic domain of a sialidase, wherein the catalytic domain of the sialidase includes the sequence of amino acids extending from amino acid 274 to amino acid 666 of SEQ ID NO:12 (alternatively, 274 to 666, 270-667, 274-681, 290-681 of SEQ ID NO:12, or any other catalytic domain of *Actinomyces viscosis*), inclusive, and at least one anchoring domain, wherein the anchoring domain is a glycosaminoglycan (GAG) binding domain of human amphiregulin including the amino acid sequence of SEQ ID NO:7, or (c) a composition or compound containing a therapeutically effective amount of a fusion protein having a sialidase or an active portion thereof and an anchoring domain. The therapeutically effective amount of these compositions or compounds includes an amount that results in a reduction of the quanitity of mucus in the respiratory tract after administration of the composition when compared to the quantity of mucus present prior to administration of the composition or compound.

Yet other embodiments include methods of limiting an increase in the quantity of mucus in the respiratory tract of a subject above a baseline level of mucus in said subject's respiratory tract. The methods include administering (a) a composition or compound containing a therapeutically effective amount of a protein or peptide having a sialidase or an active portion thereof to a subject, (b) a composition or compound containing a therapeutically effective amount of a fusion protein, wherein the fusion protein has at least one catalytic domain of a sialidase, wherein the catalytic domain of the sialidase includes the sequence of amino acids extending from amino acid 274 to amino acid 666 of SEQ ID NO:12 (alternatively, 274 to 666, 270-667, 274-681, 290-681 of SEQ ID NO:12, or any other catalytic domain of *Actinomyces viscosis*), inclusive, and at least one anchoring domain, wherein the anchoring domain is a glycosaminoglycan (GAG) binding domain of human amphiregulin including the amino acid sequence of SEQ ID NO:7, or (c) a composition or compound containing a therapeutically effective amount of a fusion protein having a sialidase or an active portion thereof and an anchoring domain. The therapeutically effective amount of these compositions or compounds includes an amount that limits an increase in the quanitity of mucus in the respiratory tract of the subject above a baseline level after administration of the composition.

In some embodiments, the compositions or compounds used can include additional compounds, including, without limitation, any one or more of the following either alone or in various combinations: Na_2SO_4, $MgSO_4$, $CaCl_2$, Histidine, Histine-HCl, and Trehalose or their analogs, Mg salts and/or Ca salts. These additional compounds can be included in the pharmaceutical compositions to act as excipients or as active ingredients that provide additional beneficial effects.

The subjects to be treated with the foregoing methods can be human subjects or non-human animal subjects. The compounds and compositions described herein can be administered to epithelial cells of the subject through various routes of administration, including, without limitation, by using inhalers to introduce the compounds or compositions into the respiratory tract of the subject.

In some preferred embodiments, compounds described herein can be delivered asan inhalant with an inhaler or as a nasal spray. They can also be administered as eye drops, ear drops, or sprays, ointments, lotions, or gels to be applied to the skin. They can also be administered intravenously or as a local injection.

<u>Reducing or preventing inflammation in the respiratory tract</u>

The present invention involves the unexpected discovery that administration of the compounds described in U.S. Application Serial Nos. 10/718,986 and 10/939,262, or compositions containing them, to reduce the amount of inflammatory cells in the respiratory tract. Thus, the invention relates to therapeutic compositions or compounds that can be used to reduce inflammation in the respiratory tract or prevent worsening of inflammation in the respiratory tract. The invention also includes methods of reducing inflammation in the respiratory tract or preventing worsening of inflammation in the respiratory tract. In addition, the invention relates to therapeutic compositions or compounds that can be used to prevent or treat diseases that are caused by, cause, or are exacerbated by respiratory inflammation, such as, both allergic and non-allergic asthma, allergic rhinitis, eczema, psoriasis, reactions to plant or animal toxins, autoimmune conditions, and any other disorder, disease or condition that causes inflammation in the respiratory tract or is caused by or exacerbated by inflammation in the respiratory tract.

In some preferred embodiments, the methods include administering a composition or compound containing a therapeutically effective amount of a protein or peptide having a sialidase or an active portion thereof to a subject. The protein or peptide can be an isolated naturally occurring sialidase protein, or a recombinant protein substantially homologous to at least a portion of a naturally occurring sialidase. A preferred pharmaceutical composition contains a sialidase with substantial homology to the *A. viscosus* sialidase (**SEQ ID NO:12**) or substantial homology to an active portion thereof, such as amino acids 274-666, 274-667, 270-667, 274-681, or 290-681 of SEQ ID NO:12, or any other catalytic domain of *Actinomyces viscosis* sialidase. The therapeutically effective amount includes an amount of the protein or peptide that prevents or reduces an allergic or inflammatory response in the respiratory tract the respiratory tract after administration of the composition or compound.

In other embodiments, the methods include administering a composition or compound containing a therapeutically effective amount of a fusion protein, wherein the fusion protein has at least one catalytic domain of a sialidase, wherein the catalytic domain of the sialidase includes the sequence of amino acids extending from amino acid 274 to amino acid 666 of SEQ ID NO:12 (alternatively, 274 to 666, 270-667, 274-681, 290-681 of SEQ ID NO:12, or any other catalytic domain of *Actinomyces viscosis*), inclusive, and at least one anchoring domain, wherein the anchoring domain is a glycosaminoglycan (GAG) binding domain of human amphiregulin including the amino acid sequence of SEQ ID NO:7. The therapeutically effective amount includes an amount of the fusion protein that prevents or reduces an allergic or inflammatory response in the respiratory tract the respiratory tract after administration of the composition or compound.

In yet other embodiments, the methods include administering a composition or compound containing a therapeutically effective amount of a fusion protein having a sialidase or an active portion thereof and an anchoring domain. The therapeutically effective amount includes an amount of the fusion protein that prevents or reduces an allergic or inflammatory response in the respiratory tract the respiratory tract after administration of the composition or compound.

In some embodiments, the compositions or compounds used can include additional compounds, including, without limitation, any one or more of the following either alone or in various combinations: Na_2SO_4, $MgSO_4$, $CaCl_2$, Histidine, Histine-HCl, and Trehalose or their analogs. These additional compounds can be included in the pharmaceutical compositions to act as excipients or as active ingredients that provide additional beneficial effects.

The subjects to be treated with the foregoing methods can be human subjects or non-human animal subjects. The compositions and compounds described herein can be administered to epithelial cells of the subject through various routes of administration, including, without limitation, by using inhalers to introduce the compounds or compositions into the respiratory tract of the subject.

In some preferred embodiments, compositions or compounds described herein can be delivered as an inhalant with an inhaler or as a nasal spray. They can also be administered as eye drops, ear drops, or sprays, ointments, lotions, or gels to be applied to the skin. They can also be administered intravenously or as a local injection.

FIGS. 7A-B show the results of the effect of the use of one of the fusion protein construct depicted in FIG. 5 on inflammatory cells of ferrets infected with human unadapted influenza. In ferrets that shed the virus despite treatment with fusion protein (n=8), the inflammatory response was reduced and animals appeared to be more alert and active compared to the untreated ferrets that were invariably lethargic and feverish. For this group of 8 infected, fusion-protein treated animals, the mean AUC (area under the curve) value calculated for the nasal protein concentrations was reduced by approximately 40% (2.68 vs. 4.48, arbitrary units) compared to the vehicle-treated (phosphate buffer saline) infected animals (FIG. 7B). In vehicle-treated infected animals, the number of inflammatory cells in nasal washes was increased to approximately 100-

fold above those in uninfected animals on day 2 post challenge. These levels were sustained for 4 additional days. The fusion protein-treated animals exhibited a significant reduction in the number of inflammatory cells in the nasal washes. Specifically, the AUC value for cell counts was reduced by approximately 3-fold in the fusion protein-treated animals compared to the vehicle-treated infected animals (1965 vs. 674, arbitrary units, (FIG. 7B). The observed reduction in the inflammatory response indicates the importance of inhibiting viral replication at the early stage of infection.

Dosage

As will be readily apparent to one skilled in the art, the useful *in vivo* dosage to be administered and the particular mode of administration will vary depending upon the age, weight and type of patient being treated, the particular pharmaceutical composition employed, and the specific use for which the pharmaceutical composition is employed. The determination of effective dosage levels, that is the dose levels necessary to achieve the desired result, can be accomplished by one skilled in the art using routine methods as discussed above. In non-human animal studies, applications of the pharmaceutical compositions are commenced at higher dose levels, with the dosage being decreased until the desired effect is no longer achieved or adverse side effects are reduced or disappear. The dosage for a compound of the present invention can range broadly depending upon the desired affects, the therapeutic indication, route of administration and purity and activity of the compound. Typically, human clinical applications of products are commenced at lower dosage levels, with dosage level being increased until the desired effect is achieved. Alternatively, acceptable *in vitro* studies can be used to establish useful doses and routes of administration of the test compound. Typically, dosages can be between about 1 ng/kg and about 10 mg/kg, between about 10 ng/kg and about 1 mg/kg, and between about 100 ng/kg and about 100 micrograms/kg. In various examples described herein, mice were treated with various dosages of the compositions described herein, including dosages of .0008 mg/kg, .004 mg/kg, .02 mg/kg, .06 mg/kg, 0.1 mg/kg, 0.3 mg/kg, 0.6 mg/kg, and 1.0 gm/kg. As nonlimiting examples, the compositions described herein can be administered to humans in doses of between about .01 mg and about 100 mg, such as about .05 mg, .06 mg, 0.1 mg, 0.5 mg, 1 mg, 5 mg, 10 mg, 20 mg, 50 mg, or 100 mg, and can be administered one or more times per day, for one day, two days, three days, four days, five days, six days, seven days, eight days, nine days, ten days, eleven days, twelve days, thirteen days, or fourteen or more days. Higher doses or lower doses can also be administered. In one embodiment, as shown in Example 3 below, a dose of .06 mg/kg of a sialidase compound is sufficient to desialylate muscarinic receptors resulting in reduced airway responsiveness to muscarinic receptor agonists, and thus potentially resulting in reducing airway constriction, airway hypersensitivity, inflammation, allergies or associated responses, such as bronchoconstriction, asthma, and mucus overproduction. Efficacy in low doses, such as .06 mg/kg (translating in adult humans into a dose of about 4 or 5 mg), or .02 mg/kg (translating in adult humans into a dose of about 1 or 2 mg), makes the sialidase-based compounds described herein good candidates for use in chronic diseases that require repeated long-term administration.

A treatment regimen can include administration of the compounds and compositions described herein from once per day to ten times per day, from once per day to six times per day, from once per day to five times per day, from once per day to four times per day, from once per day to three times per day, from once per day to twice per day, and just once per day. The treatment can last from just one day to daily, weekly, monthly, or other periodic use for a predetermined period of time or for the remainder of the subject's life.

The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition (see, Fingle et al., in The Pharmacological Basis of Therapeutics (1975)). It should be noted that the attending physician would know how to and when to terminate, interrupt or adjust administration due to toxicity, organ dysfunction or other adverse effects. Conversely, the attending physician would also know to adjust treatment to higher levels if the clinical response were not adequate. The magnitude of an administrated does in the management of the disorder of interest will vary with the severity of the condition to be treated and to the route of administration. The severity of the condition may, for example, be evaluated, in part, by standard prognostic evaluation methods. Further, the dose and perhaps dose frequency, will also vary according to the age, body weight and response of the individual patient, including those for veterinary applications.

In some preferred regimens, appropriate dosages are administered to each patient by either inhaler, nasal spray, or by topical application. It will be understood, however, that the specific dose level and frequency of dosage for any particular patient may be varied and will depend upon a variety of factors including the activity of the specific salt or other form employed, the metabolic stability and length of action of that compound, the age, body weight, general health, sex, diet, mode and time of administration, rate of excretion, drug combination, the severity of the particular condition, and the host undergoing therapy.

In some embodiments, the present disclosure provides methods for using any one or more of the compositions (indicated below as 'X') disclosed herein in the following methods:

Substance X for use as a medicament in the treatment of excess mucus or abnormal (e.g., above normal mucus levels as compared to one or more healthy subjects (e.g., of the same ethnicity and/or in the same or similar geographical location) and/or as indicated by a health care practitioner), elevated mucus production, and/or any one or more of the diseases/conditions disclosed herein; (each of which is collectively referred to in the following examples as 'Y.'

Use of substance X for the manufacture of a medicament for the treatment of Y; and

Substance X for use in the treatment of Y.

EXAMPLES

The invention is further described in the following examples, which do not limit the scope of the invention described in the claims.

Example 1. Effect of Sialidase Treatment on the Early and Late Asthmatic Reaction in Guinea Pigs

1. Overview

In this study Fludase® was tested in a guinea pig model of allergic asthma. Guinea pigs were sensitised with ovabumin (OVA) or saline and after 15 or 20 days they were treated with Fludase® or sodium sulfate. On day 21, all the animals were challenged with OVA to measure the early asthmatic reaction. Airway compliance/resistance were determined and broncho-alveolar lavage (BAL) fluid was taken from the left lung to count the total number of cells and to differentiate them.

2. Introduction

The main purpose of this study was to achieve a characterization of the effect of Fludase® on the early and late reactions in a guinea pig model for asthma. The guinea pigs involved in the study were naïve, and thus not infected with influenza or other infectious agent as part of the experiment. Asthma was induced by sensitising the guinea pigs on day 0 with OVA. After 15 or 20 days the guinea pigs were treated intatracheal with Fludase® (0.3mg/kg) or sodium sulfate (0.143mM, pH 5.0). On day 21 the guinea pigs received an OVA aerosol and the airway responsiveness (PenH) was measured. On day 22 the pulmonary resistance and compliance were determined. At time intervals of 2 minutes doses of histamine from 0.2-2 µg/kg were administered by intravenously injection. At the end of the experiment guinea pigs were sacrificed and the left lung was lavaged and the isolated BAL cells were washed, counted and differentiated into macrophages, lymphocytes, neutrophils and eosinophils.

3. Materials & Methods

Animals

Male Hartley-strain guinea pigs (HSD Poc: DH, weighing 400-500) of specific pathogen free quality were obtained from Harlan-CPB (Zeist, The Netherlands). They were used after 1 week of acclimatisation to their housing conditions. Water and commercial chow were allowed ad libitum. The experiments were approved by the Animal Ethics Committee of the Utrecht University (Utrecht, The Netherlands).

Sensitisation, Pre-treatment & Challenge

Guinea pigs were sensitised with saline (solutions contains 100 mg/ml $Al(OH)_3$) or OVA (solution contains 20 µg/ml OVA and 100 mg/ml $Al(OH)_3$), administered intraperitoneally 0.5 ml and subcutanously 5 x 0.1 ml, total injection volume 1 ml.

After 15 or 20 days animals were treated once with 0.3 mg/kg Fludase® and the control animals were treated with sodium sulfate (0.143 mM, pH 5.0) on day 20 by tracheal instillation. A laryngoscope was used to facilitate the location of the epiglottis. Then the Fludase® or sodium sulfate was given with a liquid aerosol using the IA-1C MicroSprayer™ (Penn Century, Inc, Philadelphia, USA). The guinea pig was in an upright position during the tracheal instillation.

During this tracheal instillation the guinea pigs were anaesthetized with 150 μl of a mixture of Ketamine®, Xylazin®, Atropin and saline (3.5:3:1:3), injected intra muscular in the hind paw.

On day 21 the guinea pigs were challenged by exposure to an aerosol OVA (0.1% wt/vol in sterile saline). The aerosol was generated into a 3 liter perspex chamber in which the guinea pigs were placed. First the basal bronchoconstriction (PenH) was measured. The guinea pigs were provoked with OVA aerosol for 10 seconds. Directly after the challenge the early asthmatic reaction was (PenH) was measured.

Allergen-induced early asthmatic reaction in conscious unrestrained guinea pigs

Airway function of the animals was measured directly after exposure to aerosolised OVA in a ventilated bias flow whole body plethysmograph (Buxco Electronics, Sharon CT, USA). The plethysmograph consists of a reference chamber and an animal chamber. The animal chamber is attached to the outside via a pneumotachograph in the top of the plethysmograph. An aerosol inlet to the animal chamber is centrically located in the roof of the animal chamber. When an animal is placed in the animal chamber and is breathing quietly, it creates pressure between tidal volume and thoracic movement during respiration. The differential pressure transducer measures the changes in pressure between animal chamber and the reference chamber and brings these data to a preamplifier. Thereafter, data is sent to a computer where several parameters are calculated, which represents animal's lung function. All guinea pigs used were measured basal for 5 minutes and after the aerosol for 15 minutes in the whole body plethysmograph. Besides known lung function parameters as peak expiratory flow (PEF) and tidal volume (TV), the enhanced pause (PenH) was also measured. The formula and explanation of the PenH is shown in FIG. 8. During bronchoconstriction peak expiratory flow and peak inspiratory flow are increased, while relaxation time and expiratory time are decreased. This results in an increased PenH. Data from bronchoconstriction in conscious unrestrained guinea pigs are presented in PenH (FIG. 8).

Airway responsiveness in vivo

On day 22 the guinea pigs were anaesthetized with urethane 2 g/kg intra peritoneally. The animals were allowed to breathe spontaneously. An anaesthesia-induced fall in body temperature was avoided by placing the animals in a heated chamber, which kept the body temperature at 37°C. The guinea pigs were prepared for the measurement of pulmonary resistance (R_L) and compliance (C) as follows. A small polyethylene catheter (PE-50) was placed in the right jugular vein for intra venous administration of increasing doses of histamine (0.2-2 µg/kg). First the basal R_L and C were measured for 5 minutes. Thereafter an increasing dose of histamine was injected and R_L and C were measured for 2 minutes. Airflow and tidal volume were determined by cannulating and connecting the trachea with Fleisch flow head (nr 000; Meijnhart, Bunnik, The Netherlands) to a pneumotachograph. A pressure transducer (model MP45-2; Validyne Engineering Corp., Northridge, CA) measured the transpulmonary pressure by determing pressure differences between the tracheal cannula and a cannula filled with saline inserted in the oesophagus. R_L and C were determined breath by breath with a respiratory analyser. R_L was yielded by dividing transpulmonary pressure by airflow at isovolume points. C was determined by dividing volume by transpulmonary pressure at isoflow points. Data are presented as maximal R_L and minimal C in cm H_2O/ml*sec^{-1} an ml/cm H_2O, respectively.

Collection of broncho-alveolar lung lavage cells

Broncho-alveolar lavage cells were obtained as follows. The trachea was trimmed free of connective tissue and blood vessels and a small incision was made for insertion of a cannula into the trachea. The right lung was tied up so only the left lung was lavaged. The left lung was filled with 5 ml saline (0.9% NaCl) of 37°C in situ. Fluid was withdrawn from the lung after gentle massage and collected in a plastic tube on ice (4°C).

This procedure was repeated 3 times (total 15 ml) and the cell suspensions recovered from each animal were pooled. Thereafter, cells were sedimented by centrifugation at 1500 rpm for 5 minutes at 4°C. The supernatant solution was thrown away and the pellet was resuspended in 1 ml saline. Only plastic tubes were used throughout the isolation procedure in order to minimize adherence of the cells to the walls of the tubes.

Cell count and differentiation

The cells were stained with Türk solution and counted in a Bürker-Türk bright-line counting chamber (microscope, magnification 100x). For differential BAL cell counts cytospin preparations were made and stained with Diff-Quick (Merz & Dade A.G., Düdingen, Switzerland). After coding all cytospin preparations were evaluated by one observer using oil immersion microscopy (magnification 1000x). Cells were differentiated into macrophages, lymphocytes, neutrophils and eosinophils by standard morphology. At least 200 cells per cytospin preparation were counted and the absolute number of each cell type was calculated.

Statistical analysis

Unless stated otherwise, data are expressed as arithmetic average ± standard error of mean and comparisons between groups were made using Student's t-test. A probability value $p<0.05$ was considered significant.

4. Results

Airway responsiveness in conscious unrestrained guinea pigs

As shown in FIG. 9, basal airway resistance was not different between the saline guinea pigs treated with sodium sulfate or DAS181 on day 15 or day 20 (PenH = 0.24 ± 0.009 sal-sodium sulfate, 0.25 ± 0.01 saline-Flu day 15, 0.28 ± 0.01 sal-Flu day 20 treated guinea pigs). There was also no difference between the OVA guinea pigs treated with sodium sulfate or Fludase® on day 15 or day 20 at basal level (PenH = 0.25 ± 0.01 OVA-sodium sulfate, 0.27 ± 0.02 OVA-Flu day 15, 0.31 ± 0.03 OVA-Flu day 20 treated guinea pigs).

Ova challenge slightly increased the basal airway resistance in saline sensitized animals (SOD, Flu 15 and 20, FIG. 2). However, the early asthmatic reaction in response to the OVA aerosol was strongly increased in the OVA-sodium sulfate treated guinea pigs (PenH = 1.57 ± 0.45). After treatment with Fludase® the early asthmatic reaction was decreased by nearly 30 % on day 15, but not on day 20.

Cell count in broncho-alveolar lavage fluid

As shown in FIG. 10, Fludase® decreases the total number of cells both in saline and OVA guinea pigs.

Differential cell count in the broncho-alveolar lavage fluid

As shown in FIG. 11, the number of macrophages was enhanced by (30 %) in the OVA guinea pigs compared to the saline treated group. Total number of macrophages was strongly decreased after treatment with Fludase® on day 15 and 20, both in saline and OVA treated guinea pigs.

As shown in FIG. 12, a similar pattern was observed with the number of lymphocytes. The number of lymphocytes is decreased after Fludase® treatment.

As shown in FIG. 13, compared to historical controls, SOD induces a strong increase in the number of neutrophils into the lungs. Interestingly, Fludase® completely prevented this influx in both the saline and OVA groups.

As shown in FIG. 14, compared to historical controls SOD induces also an eosinophil influx into the airways, which is further increased by OVA challenge. Interestingly, Fludase® treatment can restore the number of eosinophils up to historical control levels.

5. Discussion / conclusion

OVA-sensitised and challenged animals demonstrate an early and late asthmatic reaction as measured by an increase in PENH up on OVA-challenge, and an increase in the number of inflammatory cells in the BAL-fluid.

Interestingly, Fludase® (Flu15) reduced the early asthmatic response by nearly 30% (FIG. 9), suggesting an effect of this compound on mast cell stimulation. Moreover, Fludase® had a tremendous effect on the inflammation caused by SOD or OVA. The number of all inflammatory cells (macrophages, lymphocytes, neutrophils and eosinophils) was significantly decreased by this compound.

In conclusion, Fludase® demonstrated to be effective in both the SOD-induced inflammation and the OVA-induced inflammation.

Example 2. The Effect of Fludase in a Mouse Model of Acute OVA Induced Asthma

1. Introduction

The aim of the study was to investigate whether Fludase® (also referred to as DAS181) (1) inhibits allergen induced airway inflammation and airway hyperreactivity in an acute OVA challenge mouse model of asthma. In addition to studying the effect of DAS 181 alone as an intervention, three other interventions in the mouse asthma model were studied including a) DAS181 + an excipient (used as the dry powder formulation to deliver DAS181 in vivo), b) the excipient alone, and c) dexamethasone (as a comparator).

2. Materials and Methods

Mice

Female BALB/c mice age 12 weeks were purchased from Charles River and housed in the UCSD vivarium. They were used after approximately one week of acclimatization in the UCSD vivarium.

OVA sensitization and OVA challenge

Mice were immunized s.c. on days 0, 7, 14, and 21 with 25 ug of OVA adsorbed to 1 mg of alum in 200 ul normal saline to induce a predominant Th2 immune response. Intranasal OVA challenges (20 ug/50 ul) were started on day 26 and then repeated on day 28 and day 30. In the no OVA group, mice were sensitized to OVA but not challenged intranasally with OVA. Mice had airway responsiveness to methacholine (Mch) measurements performed 24 hours after the final OVA challenge by Penh on day 31. The mice involved in the study were naïve and thus not infected with influenza or other infectious agent as part of the experiment. Mice were then immediately sacrificed. Bronchoalveolar lavage fluid (BAL), blood, and lung tissue were processed for outcomes detailed below.

Administration of test compounds to mice

1. Compounds tested

The following compounds were studied in the mouse model of asthma

a) DAS181 with excipient (0.6 mg/kg intranasal): DAS181-F02 (NexBio, Inc. part # 43-071, lot# 47-034) was prepared in PBS to 20mgDAS/ml. Before each dosing, it was freshly diluted in PBS to 0.6mgDAS181/kg with dosing volume of 50ul, for mice with an average body weight of 21g.

b) DAS181 (0.6 mg/kg intranasal)

c) Excipient (50 μL/mouse): Excipient (416TL022A) was supplied by NexBio, Inc. as a solution, and the concentration of each of the excipient component is equivalent to that in 20mgDAS181/ml ($MgSO_4$ 1.446mg/ml, $CaCl_2$ 0.059mg/ml, Histidine 1.427mg/ml, Histidine-HCl 1.943mg/ml, and Trehalose 3.000mg/ml). It was freshly diluted in PBS for dosing as in a). The final concentration of each excipient component is equivalent to that in 0.6mgDAS181/kg, in 50ul, for mice with average body weight of 21g.

d) Dexamethasone (1.0 mg/kg intraperitoneally)

Excipient solution was prepared in the following manner. The target final concentration for each excipient was calculated to reach equivalent concentration when DAS181-F02 bulk dry powder is reconstituted at a 20 mg protein/mL. The 10x stock solutions (100x for Calcium Chloride) for each excipient (10 mL each) were then prepared. Materials used to prepare the stock solutions are listed in Table 1. All materials are USP grade, or equivalent. Appropriate amounts of each excipient were weighed into a 15 mL conical tube according to Table 2, and water was added to bring the total weight to 10 grams and vortex to dissolve the material completely. The final 1x excipient solution was prepared by adding 1 mL of each stock solution, and then bringing the volume to 10 mL using water. All sample preparation was preformed gravimetrically, assuming solution density of 1 gram/mL.

Although referred to as "excipient" or "excipient" solution in these examples, these additional compounds can have additional beneficial effects with respect to reduction of mucus and reduction of inflammation and inflammatory cells. These excpients can also have a synergistic effect with DAS181.

Table 1. Material Information

Description	Manufacturer	Mfg. Part #	Mfg. Lot #	Expiry
L-Histidine	Sigma	H6034-100g	078K0179	Sept 2012
L-Histidine monohydrochloride monohydrate	Sigma	H4036-1kg	068K8310	Jan 2012
a,a – Trehalose, Dihydrate	J.T. Baker	4226-04	G47596	No. 2010
Magnesium Sulfate Heptahydrate	EMD	1.05882.0500	K38528682 R	Feb 2013
Calcium Chloride, Dihydrate	Mallinckrodt	4616-04	G24475	Sept 2009
WFI	B. Braun	S9200-SS	J8K015	Aug 2010

Table 2. Stock Solution Preparation Sheet (Theoretical)

Composition	Final conc. (mg/mL)	Dilution Factor	Stock conc. (mg/mL)	Wt. of Salt for 10 mL (mg)
$MgSO_4$	1.446	10	14.457	**296**
$CaCl_2$	0.059	100	5.943	**79**
Histidine	1.427	10	14.266	**143**
Histidine·HCl	1.943	10	19.431	**213**
Trehalose	3.000	10	30.000	**332**

2. Mouse model of asthma

The following groups of Balb/c mice (n=10 female mice/group) were studied.

a) No OVA

b) OVA

c) OVA + DAS181 with excipient

d) OVA+ DAS181

e) OVA+ excipient

f) OVA+ dexamethasone

3. Timing of administration of test compounds

The test compounds were administered one hour prior to each of the three intranasal OVA challenges on days 26, 28, and 30.

Timing of end-points studied

Mice were sacrificed 24 hours after the final OVA challenge and blood, BAL, and lungs were analyzed (27).

4. End-points studied

a) Penh

Airway responsiveness was assessed on day 31, twenty four hours after the final OVA inhalation, using a single chamber whole body plethysmograph obtained from Buxco (Troy, NY). In this system, an unrestrained, spontaneously breathing mouse is placed into the main chamber of the plethysmograph, and pressure differences between this chamber and a reference chamber are recorded. The resulting box pressure signal is caused by volume and resultant pressure changes during the respiratory cycle of the mouse. A low pass filter in the wall of the main chamber allows thermal compensation. From these box pressure signals, the phases of the respiratory cycle, tidal volumes, and the enhanced pause (Penh) can be calculated. Penh is a dimensionless value that represents a function of the proportion of maximal expiratory to maximal inspiratory box pressure signals and of the timing of expiration. It correlates closely with pulmonary resistance measured by conventional two-chamber plethysmography in ventilated mice. In the plethysmograph, mice were exposed for 3 min to nebulized PBS and subsequently to increasing concentrations of nebulized metacholine (MCh)(3, 6, 12, 24, 48 mg/ml Mch) (Sigma, St. Louis, MO) in PBS using an Aerosonic ultrasonic nebulizer (DeVilbiss). After each nebulization, recordings were taken for 3 min. The Penh values measured during each 3-min sequence were averaged and are expressed for each MCh concentration as the percentage of baseline Penh values following PBS exposure.

b) Blood eosinophil counts

Peripheral blood was collected from mice by cardiac puncture into EDTA-containing tubes. Erythrocytes were lysed using a 1:10 solution of 100 mM potassium carbonate–1.5 M ammonium chloride. The remaining cells were resuspended in 1 mL PBS. To perform differential cell counts, 200 μL resuspended peripheral-blood leukocyte suspensions were cytospun onto microscope slides and air-dried. Slides were stained with Wright-Giemsa and the % of eosinophils in the total number of white blood cells were assessed under a light microscope.

c) PAS staining for lung mucus

To quantitate the level of mucus expression in the airway, the number of periodic acid Schiff (PAS)-positive and PAS-negative epithelial cells in individual bronchioles were counted as previously described (Zhang, M., T. Angata, J.Y. Cho, M. Miller, D.H. Broide, A. Varki. 2007 *Blood.* 109:4280-4287). At least ten bronchioles were counted in each slide. Results are expressed as the percentage of PAS-positive cells per bronchiole, which is calculated from the number of PAS-positive epithelial cells per bronchus divided by the total number of epithelial cells of each bronchiole. Slides of lung tissue with no OVA, OVA and OVA + DAS181 were also taken and observed.

d) MBP staining of lungs for peribronchial eosinophils

Lungs from the different experimental groups were processed as a batch for either histologic staining or immunostaining under identical conditions as described in Zhang et al. Stained and immunostained slides were all quantified under identical light microscope conditions, including magnification (20 X), gain, camera position, and background illumination. Lung sections were processed for MBP immunohistochemistry as described above, using an anti-mouse MBP (Major Basic Protein) Ab (kindly provided by James Lee PhD, Mayo Clinic, Scottsdale, Arizona) and the immunoperoxidase method as previously described in Zhang et al. Major Basic Protein is an eosinophil cytoplasmic granule protein which serves as a marker of eosinophils in tissues. The number of individual cells staining positive for MBP in the peribronchial space were counted using a light microscope. Results are expressed as the number of peribronchial cells staining positive for MBP per bronchiole with 150-200 μm of internal diameter. At least ten bronchioles were counted in each slide.

5. Statistical Analysis

Results in the different groups of mice were compared by Mann Whitney non-parametric T test. All results are presented as mean ± SEM. A statistical software package (Graph Pad Prism, San Diego, CA) was used for the analysis. P values of < 0.05 were considered statistically significant.

6. Results

a) Penh

As shown in FIG. 15, OVA challenge induced a significant increase in airway responsiveness as assessed by changes in Penh (OVA vs no OVA; $p<0.0001$).

OVA challenged mice pre-treated with DAS181+ excipient had a significant reduction in Penh compared to OVA challenged mice (OVA vs OVA+ DAS181+ excipient; $p<0.01$).

OVA challenged mice pre-treated with DAS181 resulted in a reduction in Penh compared to OVA challenged mice (OVA vs OVA+ DAS181; $p<0.005$).

As shown in FIG. 16, measurement of Penh at 48 mg/ml Mch provides the largest difference between positive and negative controls (no OVA vs OVA) and is why this dose of Mch is used to assess the effect of an intervention such as DAS181.

b) Blood Eosinophils

As shown in FIG. 17, OVA challenge induced a significant increase in blood eosinophils (OVA vs no OVA; $p<0.0005$).

DAS 181 significantly reduced blood eosinophils (OVA vs OVA+ DAS, $p=0.04$)

c) PAS staining for lung mucus

As shown in FIGS. 18-19A-F, OVA challenge induced a significant increase in the % of airway epithelium staining positive for PAS (OVA vs no OVA; $p<0.0001$).

OVA challenged mice pre-treated with DAS181+ excipient had a significant reduction in PAS staining compared to OVA challenged mice (OVA vs OVA+ DAS181+ excipient; $p<0.0001$).

OVA challenged mice pre-treated with DAS181 had a statistically significant reduction in PAS staining compared to OVA challenged mice (OVA vs OVA+ DAS181; $p<0.0001$).

Effect on mucus

DAS181 with excipient as well as DAS181 alone significantly reduced PAS staining showing that there is an inhibitory effect of DAS 181 on PAS staining. This shows that DAS181 with excipient or DAS181 alone reduces mucus in the respiratory tract.

d) MBP immunostaining of lungs

As shown in FIG. 20, OVA challenge induced a significant increase in the number of peribronchial MBP+ eosinophils (OVA vs no OVA; $p<0.0001$).

OVA challenged mice pre-treated with DAS181+ excipient had a significant reduction in the number of peribronchial MBP+ eosinophils compared to OVA challenged mice (OVA vs OVA+ DAS181+ excipient; $p=0.02$).

Example 3. Reduced Airway Resistance in Naïve Mice Treated Intranasally with Low Doses of DAS181 (Methacholine Challenged)

The objective of the study was to test the effect of different dose levels of DAS181 on muscarinic receptor mediated airway resistance in naive mice. BALB/c mice (N=4) were treated intranasally with PBS or DAS181 at 0.06, 0.1 or 0.6 mg/kg once daily for three days. Eight hours post the final treatment animals were challenged with increasing doses of muscarinic receptor agonist Methacholine (Mch). Airway responsiveness was assessed using whole body plethysmography. Changes in airway resistance were expressed as the enhanced pause (Penh), a dimensionless value that represents a function of the proportion of maximal expiratory to maximal inspiratory box pressure signals and of the timing of expiration. Mice were exposed to nebulized PBS and subsequently to increasing concentrations of nebulized MCh (12, 24, 48 mg/ml Mch) for 2 min in PBS. Recordings were performed for 3 min following each exposure. The obtained Penh values were averaged and expressed as the percentage of baseline following PBS exposure.

Results: 24 and 48 mg/ml of the muscarinic receptor agonist methacholine increased airway resistance above baseline. All animals treated with DAS181 had significantly reduced airway resistance at 48 mg/ml of Mch (Fig). No difference was observed between the different dose groups.

Conclusions: Consistent with previous data, intranasal treatment with DAS181 reduced bronchoconstriction in response to the muscarinic receptor agonist Mch, further supporting the hypothesis that DAS181 dependent desialylation causes a reduction in muscarinic receptor signaling. Surprisingly, the two higher dose levels did not exert any greater effect than the lowest dose, suggesting that a dosage level as low as 0.06 mg/kg of intranasal DAS181 is sufficient to desialylate muscarinic receptors resulting in reduced airway responsiveness to muscarinic receptor agonists, and thus potentially resulting in reducing inflammation, allergies or acetylcholine-associated responses, such as bronchoconstriction, asthma, and mucus overproduction. These results are depicted in FIGS. 21A and 21B.

Example 4. Reduced Airway Resistance in Naïve Mice Treated Intranasally with a Low Dose of DAS181 (Methacholine Challenged)

The objective of the study was to test the effect of a 0.6 mg/kg once daily dose of DAS181 on muscarinic receptor mediated airway resistance in naive mice. BALB/c mice (N=4) were treated intranasally with PBS or DAS181 at 0.6 mg/kg once daily for three days. Eight hours post the final treatment animals were challenged with increasing doses of muscarinic receptor agonist Methacholine (Mch). Airway responsiveness was assessed using whole body plethysmography. Changes in airway resistance were expressed as the enhanced pause (Penh), a dimensionless value that represents a function of the proportion of maximal expiratory to maximal inspiratory box pressure signals and of the timing of expiration. Mice were exposed to nebulized PBS and subsequently to increasing concentrations of nebulized MCh (3, 6, 12, 24, 48 mg/ml Mch) for 2 min in PBS. Recordings were performed for 3 min following each exposure. The obtained Penh values were averaged and expressed as the percentage of baseline following PBS exposure.

Results: 3, 6, 12, 24 and 48 mg/ml of the muscarinic receptor agonist methacholine increased airway resistance above baseline in PBS treated animals, and 12, 24 and 48 mg/ml of methacholine increased airway resistance above baseline in DAS181 treated animals, while 3 and 6 mg/ml of methacholine did not increase airway resistance above baseline in DAS181 treated animals. All animals treated with DAS181 had significantly reduced airway resistance at 6, 12, 24 and 48 mg/ml of Mch compared to the control.

Conclusions: Consistent with previous data, intranasal treatment with DAS181 reduced bronchoconstriction in response to the muscarinic receptor agonist Mch, further supporting the hypothesis that DAS181 dependent desialylation causes a reduction in muscarinic receptor signaling. These results are depicted in FIG. 22.

Example 5. Reduced Airway Resistance in Naïve Mice Treated Intranasally with DAS181 (Carbachol Challenged)

The objective of the study was to test the effect of a 0.6 mg/kg once daily dose of DAS181 on muscarinic receptor mediated airway resistance in naive mice. BALB/c mice (N=4) were treated intranasally with PBS or DAS181 at 0.6 mg/kg once daily for three days. Eight hours post the final treatment animals were challenged with increasing doses of muscarinic receptor agonist carbachol. Airway responsiveness was assessed using whole body plethysmography. Changes in airway resistance were expressed as the enhanced pause (Penh), a dimensionless value that represents a function of the proportion of maximal expiratory to maximal inspiratory box pressure signals and of the timing of expiration. Mice were exposed to nebulized PBS and subsequently to increasing concentrations of nebulized Carbachol (1.25, 2.5, 5, 10, 20 mg/ml carbachol) for 2 min in PBS. Recordings were performed for 3 min following each exposure. The obtained Penh values were averaged and expressed as the percentage of baseline following PBS exposure.

Results: 5, 10, and 20 mg/ml of the muscarinic receptor agonist carbachol increased airway resistance above baseline in both PBS treated and DAS181 animals. All animals treated with DAS181 had significantly reduced airway resistance at 5, 10, and 20 mg/ml of carbachol.

Conclusions: Consistent with previous data, intranasal treatment with DAS181 reduced bronchoconstriction in response to the muscarinic receptor agonist carbachol, further supporting the hypothesis that DAS181 dependent desialylation causes a reduction in muscarinic receptor signaling. These results are depicted in FIG. 23.

Example 6. Airway Resistance in Naïve Mice Treated Intranasally with a Low Dose of DAS185 (Methacholine Challenged)

The objective of the study was to test the effect of a 0.6 mg/kg once daily dose of DAS185 on muscarinic receptor mediated airway resistance in naive mice. DAS185 is an enzymatically inactive version of DAS181, in which a mutation in the sialidase portion renders the sialidase inactive. BALB/c mice (N=4) were treated intranasally with PBS or DAS185 at 0.6 mg/kg once daily for three days. Eight hours post the final treatment animals were challenged with increasing doses of muscarinic receptor agonist Methacholine (Mch). Airway responsiveness was assessed using whole body plethysmography. Changes in airway resistance were expressed as the enhanced pause (Penh), a dimensionless value that represents a function of the proportion of maximal expiratory to maximal inspiratory box pressure signals and of the timing of expiration. Mice were exposed to nebulized PBS and subsequently to increasing concentrations of nebulized MCh (3, 6, 12, 24, 48 mg/ml Mch) for 2 min in PBS. Recordings were performed for 3 min following each exposure. The obtained Penh values were averaged and expressed as the percentage of baseline following PBS exposure.

Results: There was no difference in airway resistance in response to the Mch challenge between the DAS185 treated and PBS treated animals.

Conclusions: Whereas there was a difference in airway resistance between DAS181 and PBS treated animals in example 4 above, there was no difference when DAS181 was replaced with enzymatically inactive DAS185. This experiment shows that reduction in airway resistance in response to DAS181 is sialidase dependent. These results are depicted in FIG. 24.

Example 7. Time-Course of DAS181 Mediated Reduction of Airway Resistance (Methacholine Challenged)

The objective of the study was to test the effect of a 0.6 mg/kg once daily dose of DAS181 for one, two or three days on muscarinic receptor mediated airway resistance in naive mice. BALB/c mice (N=4) were treated intranasally with PBS or DAS181 at 0.6 mg/kg once daily for one, two or three days. Eight hours post the final treatment animals were challenged with increasing doses of muscarinic receptor agonist Methacholine (Mch). Airway responsiveness was assessed using whole body plethysmography. Changes in airway resistance were expressed as the enhanced pause (Penh), a dimensionless value that represents a function of the proportion of maximal expiratory to maximal inspiratory box pressure signals and of the timing of expiration. Mice were exposed to nebulized PBS and subsequently to increasing concentrations of nebulized MCh (3, 6, 12, 24, 48 mg/ml Mch) for 2 min in PBS. Recordings were performed for 3 min following each exposure. The obtained Penh values were averaged and expressed as the percentage of baseline following PBS exposure.

Results: For one day of treatment, there was no difference in airway resistance in response to the Mch challenge between the DAS181 treated and PBS treated animals. For two days of treatment, DAS181 appears to reduce airway resistance relative to PBS at 12 and 24 mg/ml methacholine, but not at 3, 6 and 48 mg/ml of methacholine. At three days of treatment, DAS181 had significantly reduced airway resistance at 24 and 48 mg/ml of Mch.

Conclusions: Consistent with previous data, there was a difference in airway resistance between DAS181 and PBS treated animals on day 3. There was no difference when following one treatment dose, and partial reduction following two days of treatment with DAS181. This experiment shows that 2-3 days of treatment is optimal to achieve a reduction in airway resistance. These results are depicted in FIG. 25.

Example 8. Reduced Airway Resistance in Naïve Mice Treated Intranasally with Very Low Doses of DAS181 (Methacholine Challenged)

The objective of the study was to test the effect of different low-dose levels of DAS181 on muscarinic receptor mediated airway resistance in naive mice. BALB/c mice (N=4) were treated intranasally with PBS or DAS181 at 0.0008, 0.004, 0.02, or 0.1 mg/kg once daily for three days. Eight hours post the final treatment animals were challenged with increasing doses of muscarinic receptor agonist Methacholine (Mch). Airway responsiveness was assessed using whole body plethysmography. Changes in airway resistance were expressed as the enhanced pause (Penh), a dimensionless value that represents a function of the proportion of maximal expiratory to maximal inspiratory box pressure signals and of the timing of expiration. Mice were exposed to nebulized PBS and subsequently to increasing concentrations of nebulized MCh (12, 24, 48 mg/ml Mch) for 2 min in PBS. Recordings were performed for 3 min following each exposure. The obtained Penh values were averaged and expressed as the percentage of baseline following PBS exposure.

Results: 24 and 48 mg/ml of the muscarinic receptor agonist methacholine increased airway resistance above baseline. All animals treated with DAS181 had significantly reduced airway resistance at 24 and 48 mg/ml of Mch. No difference was observed between the different dose groups of DAS181.

Conclusions: Intranasal treatment with very low doses of DAS181 reduced bronchoconstriction in response to the muscarinic receptor agonist Mch, further supporting the hypothesis that DAS181 dependent desialylation causes a reduction in muscarinic receptor signaling even at very low doses of DAS181. This experiment shows that dosage levels as low as 0.0008 mg/kg of intranasal DAS181 is sufficient to desialylate muscarinic receptors resulting in reduced airway responsiveness to muscarinic receptor agonists, and thus potentially resulting in reducing inflammation, allergies or acetylcholine-associated responses, such as bronchoconstriction, asthma, and mucus overproduction. These results are depicted in FIG. 26.

Example 9. The Reduced Airway Resistance in Naïve Mice Treated Intranasally with DAS181 is Dose-Dependent (Methacholine Challenged)

The objective of the study was to test the effect of different dose levels of DAS181 on muscarinic receptor mediated airway resistance in naive mice. BALB/c mice (N=4) were treated intranasally with PBS or DAS181 at 10 ng/kg, 0.1 μg/kg, 1 μg/kg, 10 μg/kg or 0.1 mg/kg once daily for three days followed by increasing doses of muscarinic receptor agonist methacholine (Mch). Airway responsiveness was assessed using whole body plethysmography. Changes in airway resistance were expressed as the enhanced pause (Penh), a dimensionless value that represents a function of the proportion of maximal expiratory to maximal inspiratory box pressure signals and of the timing of expiration. Mice were exposed to nebulized PBS and subsequently to increasing concentrations of nebulized MCh (12, 24, 48 mg/ml Mch) for 2 min in PBS. Recordings were performed for 3 min following each exposure. The obtained Penh values were averaged and expressed as the percentage of baseline following PBS exposure.

Results: 24 mg/ml and higher concentrations of the muscarinic receptor agonist methacholine increased airway resistance above baseline. The airway responsiveness was assessed 8 hours post-treatment on Day 3. All animals treated with DAS181 showed reduced airway resistance at 24 mg/ml of Mch, with average percentage change Penh values (representing increasein airway resistance over baseline) as follows:

Control (PBS):	550-560%
10 ng/kg DAS181:	525-530%
0.1 μg/kg DAS181:	450%
1 μg/kg DAS181:	525%
10 μg/kg DAS181:	225%
0.1 mg/kg DAS181:	200%

As seen from the above results, dose levels of 0.01 or 0.1 mg/kg of DAS181 significantly reduced airway resistance in response to Mch.

Conclusion: The results demonstrate that reduced airway resistance in response to intranasal treatment with DAS181 is dose-dependent.

Example 10. Effect of DAS181 on M2 and M3 Muscarinic Receptor Signaling In Vitro

The objective of the study was to assess muscarinic receptor desialylation as a potential mechanism for airway protection, using an *in vitro* model. Human chem-1 cells (Millipore) stably transfected with M2 or M3 muscarinic receptor were treated with 0.4, 2 or 10 μM of DAS181 for 30 min at 37°C prior to the addition of a receptor agonist (acetylcholine; ACh). Receptor signaling was determined using a fluorescence reporter for intracellular calcium.

Results: At all doses of DAS181 tested, treatment of human chem-1 cells with DAS181 increased the potency of agonist-mediated M2 receptor signaling and decreased the potency of agonist-mediated M3 receptor signaling. The results are shown below:

A. M2 Receptor

Treatment	Predicted EC50 Potency Value (M)
ACh alone (no DAS181)	390 nM
ACh + 10 μM DAS181	140 nM
ACh + 2 μM DAS181	130 nM
ACh + 0.4 μM DAS181	140 nM

B. M3 Receptor

Treatment	Predicted EC50 Potency Value (M)
ACh alone (no DAS181)	6.3 nM
ACh + 10 μM DAS181	270 nM
ACh + 2 μM DAS181	51 nM
ACh + 0.4 μM DAS181	110 nM

Conclusion: The results demonstrate that DAS181 increases signaling through the M2 muscarinic receptor and decreases signaling through the M3 muscarinic receptor. Thus, the DAS181-mediated responses could be indicative of positive allosteric modulation of the M2, and either antagonist or negative modulation M3. Desialylation may offer airway protection by reducing the stimulatory signal as well as enhancing the inhibitory signal mediated by muscarinic receptors.

Example 11. Therapeutic Efficacy of DAS181 Microparticle Formulations against Parainfluenza

The efficacy of DAS181 was tested against parainfluenza virus (PIV), which is an acute respiratory infection. A 63 year old female patient tested positive for PIV on July 14, 2010 and July 21, 2010; shedding of the PIV antigen in nasal swabs and sputum samples collected from the patient on those days was detected by PCR.

The patient was treated on July 23, 24 and 25, 2010 with one capsule (10 mg delivered dose) a day of a dry powder formulation of DAS181 whose components and wt/wt% in the composition are as follows: DAS181: 64.54-64.69%; Histidine free base: 4.32-4.60%; Histidine HCl: 5.85-6.27%; Trehalose: 9.06-9.68%; Magnesium sulfate:

4.66-5.84%; Calcium chloride: 0.19%; Sodium acetate: 0.04-0.05%; Acetic acid: 0.02%; Water: 10%; Isopropanol: trace amounts. Each capsule contained 13 mg of the dry powder in a type 3 clear HPMC capsule (Capsugel), giving a delivered dose of 10 mg. The patient was administered one capsule a day for three days (July 23, July 24 and July 25, 2010) by inhalation. The patient tested positive for PIV on the day after completion of the treatment (July 26), and tested negative for PIV on the fifth day following treatment (July 30, 2010). The results demonstrate the effectiveness of DAS181 against parainfluenza, *i.e.*, a respiratory infection of the upper respiratory tract.

Example 12. Therapeutic Efficacy of DAS181 Microparticle Formulations against Asthma

The efficacy of the DAS181 microparticle formulation used in Example 9 above was tested against asthma. A 20 year old male Caucasian asthma patient was tested for changes in airflow prior to and 1 hour after oral administration of a 10 mg delivered dose (13 mg capsule) of Formulation A, as measured by FEV1 (forced expiratory volume of air in 1 second). Prior to administration of the drug, the FEV1 of the patient was at 82% of the predicted normal lung function. One hour after administration of the drug, the FEV1 of the patient indicated a clinically significant improvement in lung function, with a 10% increase in value to 92%. The results demonstrate that a DAS181 formulation can be effective against asthma, i.e., a non-infectious respiratory disorder affecting the central to upper respiratory tract.

OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

2010315024 25 Nov 2014

CLAIMS

1. A method of reducing the quantity or level of mucus or preventing an increase in the quantity or level of mucus in a respiratory tract of a subject, the method comprising:

determining a baseline level of mucus in the subject’s respiratory tract; and

administering to the subject a compound or composition comprising a therapeutically effective amount of a fusion protein, wherein

the fusion protein comprises at least one catalytic domain of a sialidase, wherein the catalytic domain of the sialidase comprises the sequence of amino acids extending from amino acid 274 to amino acid 666 of SEQ ID NO:12, inclusive, and at least one anchoring domain, wherein the anchoring domain is a glycosaminoglycan (GAG) binding domain of human amphiregulin comprising the amino acid sequence of SEQ ID NO:7; and

the therapeutically effective amount comprises an amount of the fusion protein that results in a reduction of the quantity of mucus in the respiratory tract after administration of the compound or composition when compared to the quantity of mucus present prior to administration of the compound or composition.

2. The method of claim 1, wherein the subject has one or more of the following conditions: chronic obstructive pulmonary disease (COPD), bronchitis, bronchiectasis, cystic fibrosis (CF), vasculitis, mucus plugging, Wegener’s granulomatosis, pneumonia, tuberculosis, cancer involving the lungs or the respiratory tract, Kartagener syndrome, Young’s syndrome, chronic sinopulmonary infection, alpha 1-antitrypsin deficiency, primary immunodeficiency, acquired immune deficiency syndrome, opportunistic infection, an infectious state, a post infectious state, common cold, exercise induced hypersecretion of mucus, inflammatory bowel disease, ulcerative colitis, Crohn’s disease, respiratory infection, respiratory obstruction, inhalation or aspiration of a toxic gas, pulmonary aspiration, and alcoholism.

3. The method of claim 1 or claim 2, wherein the fusion protein has the sequence set forth in SEQ ID NO:21.

4. A method of reducing the quantity of mucus in the respiratory tract of a subject with elevated levels of mucus in said respiratory tract, comprising:

determining a baseline level of mucus in the subject’s respiratory tract; and

administering to the subject a compound or composition comprising a therapeutically effective amount of a peptide comprising a sialidase or an active portion thereof and, optionally, an anchoring domain, wherein the therapeutically effective amount comprises an amount of the peptide that results in a reduction of the quantity of mucus in the respiratory tract after administration of the compound or composition when compared to the quantity of mucus present prior to administration of the compound or composition.

5. The method of any one of claims 1 to 4, wherein the subject is not infected with one or more of influenza virus, parainfluenza virus, and/or respiratory syncytial virus (RSV).

6. The method of any one of claims 1 to 4, wherein the subject is not suffering from asthma.

7. The method of any one of claims 1 to 4, wherein the subject is suffering from asthma.

8. Use of a fusion protein in the preparation of a medicament for reducing the quantity or level of mucus or preventing an increase in the quantity or level of mucus in the respiratory tract of a subject, wherein the fusion protein comprises at least one catalytic domain of a sialidase, wherein the catalytic domain of the sialidase comprises the sequence of amino acids extending from amino acid 274 to amino acid 666 of SEQ ID NO:12, inclusive, and at least one anchoring domain, wherein the anchoring domain is a GAG binding domain of human amphiregulin comprising the amino acid sequence of SEQ ID NO:7, wherein a baseline level of mucus in the subject's respiratory tract is determined prior to treatment.

9. The use of claim 8, wherein the subject has one or more of the following conditions: COPD, bronchitis, bronchiectasis, CF, vasculitis, mucus plugging, Wegener's granulomatosis, pneumonia, tuberculosis, cancer involving the lungs or the respiratory tract, Kartagener syndrome, Young's syndrome, chronic sinopulmonary infection, alpha 1-antitrypsin deficiency, primary immunodeficiency, acquired immune deficiency syndrome, opportunistic infection, an infectious state, a post infectious state, common cold, exercise induced hypersecretion of mucus, inflammatory bowel disease, ulcerative colitis, Crohn's disease, respiratory infection, respiratory obstruction, inhalation or aspiration of a toxic gas, pulmonary aspiration, and alcoholism.

10. The use of claim 8 or claim 9, wherein the fusion protein has the sequence set forth in SEQ ID NO:21.

11. Use of a peptide comprising a sialidase or an active portion thereof and, optionally, an anchoring domain in the preparation of a medicament for reducing the quantity of mucus in the respiratory tract of a subject with elevated levels of mucus in said respiratory tract, wherein a baseline level of mucus in the subject's respiratory tract is determined prior to treatment.

12. The use of any one of claims 8 to 11, wherein the subject is not infected with one or more of influenza virus, parainfluenza virus, and/or RSV.

13. The use of any one of claims 8 to 11, wherein the subject is not suffering from asthma.

14. The use of any one of claims 8 to 11, wherein the subject is suffering from asthma.

15. A method according to claim 1, substantially as herein described with reference to any one or more of the examples, but excluding comparative examples.

16. A method according to claim 4, substantially as herein described with reference to any one or more of the examples, but excluding comparative examples.

17. Use according to claim 8, substantially as herein described with reference to any one or more of the examples, but excluding comparative examples.

18. Useaccording to claim 11, substantially as herein described with reference to any one or more of the examples, but excluding comparative examples.

PF4 (**SEQ ID NO:2**):	^{47}NGRRICLDLQAPLYKKIIKKLLES^{70}
IL-8 (**SEQ ID NO:3**):	^{46}GRELCLDPKENWVQRVVEKFLKRAENS^{72}
ATIII (**SEQ ID NO:4**):	^{118}QIHFFFAKLNCRLYRKANKSSKLVSANRLFGDKS^{151}
ApoE (**SEQ ID NO:5**):	^{132}ELRVRLASHLRKLRKRLLRDADDLQKRLAVYQAG^{165}
AAMP (**SEQ ID NO:6**):	^{17}RRLRRMESESES^{25}
Amphiregulin (**SEQ ID NO: 7**):	^{125}KRKKKGGKNGKNRRNRKKKNP^{145}

FIG. 1

```
NEU2(SEQ ID NO:8): 1 MASLPVLQKE SVFQSGAHA- -YRIPALLYL PGQQSLLAFA EQRASKKDEH
                                              YR+P+LL + P     +LLAF EQR S   D H
NEU4(SEQ ID NO:9): 1 MGVPRTPSRT VLFERERTGL TYRVPSLLPV PPGPTLLAFV EQRLSPDDSH

NEU2:  49  AELIVLRRGD YDAPTHQVQW QAQEVVAQAR LDGHRSMNPC PLYDAQTGTL FLFFIAIPGQ
           A   +VLRRG          +W  A  ++  A     HRSMNPC P++DA TGT+ FLFFIA+ G
NEU4:  51  AHRLVLRRGT LAGGSV---RW GALHVLGTAA LAEHRSMNPC PVHDAGTGTV FLFFIAVLGH

NEU2: 110 VTEQQQLQTR ANVTRLCQVT STDHGRTWSS PRDLTDAAIG PAYREWSTFA VGPGHCLQLN
             E  Q+ T    N  RLC V  S D G +W S  RDLT+ AIG  A ++W+TFA VGPGH +QL
NEU4: 109 TPEAVQIATG RNAARLCCVA SRDAGLSWGS ARDLTEEAIG GAVQDWATFA VGPGHGVQLP

NEU2: 170 DRARSLVVPA YAYRKLHP-- ---IQRPIPS AFCFLSHDHG RTWARGHFVA QD-TLECQVA
            R L+VPA Y YR           I R  P  +F F S DHG RTW  G  V      + ECQ+A
NEU4: 169 S-GR-LLVPA YTYRVDRLEC FGKICRTSPH SFAFYSDDHG RTWRCGGLVP NLRSGECQLA

NEU2: 224  EVETGEQRVV TL-NARSHLR ARVQAQSTND GLDFQESQLV KKLVEPPPQG CQGSVISFPS
             V+ G+        NARS L  +RVQA  ST++ G F  ++ V  L E    G CQGS++ FP
NEU4: 227  AVUGGQAGSF LYCNARSPLG SRVQALSTDE GTSFLPAERV ASLPETAW-G CQGSIVGFPA

NEU2: 283  P--------- ---------- ---------- ---------- ---------  ---------

NEU4: 286  PAPNRPRODS NSVGPRSPLQ PPLLGPGVHE PPEEAAVDPR GGQVPGGPFS RLQPRGDGP

NEU2: 284 ---------- ---------- ---RSGPGSP QWLLYTHPTH SWQRADLGAY LNPRPPAPEA
                                   WLLY+HP       R  +G   L+  P  P +
NEU4: 346 RQPGPRPGVSG DVGSWTLALP MPPAAPFQSP TWLLYSHPVG RRARLHMGIR LSQSPLDPRS

NEU2: 321  WSEPVLLAKG SCAYSDLQSM GTGPDGSPLF GCLYEANDY- --EEIVFLMF TLKQAFPAEY
            W+EP ++ +      YSDL S+ G   P+G  +F +CLYE                 +L++
NEU4: 405  WTEPWVIYEG PSGYSDLASI GPAPEGGLVF ACLYESGART SYDEISFCTF SLREVLENVP

NEU2: 378 LPQ

NEU4: 466 ASPKPPNLGD KPRGCCWPS
```

FIG. 2

Substrate Specificity of Bacteria and Fungal Sialidases

Substrates	Vibrio Cholerae	Clostridium perfringens (71Kd)	Clostridium perfringens (43Kd)	Arthrobacter ureafaciens	Salmonella typhimurium	Actinomyces viscosus
Oligo- and polysaccharides						
II^3Neu5AcLac	100	100	100	100	100	100
II^6Neu5AcLac	53	44	19	157	0.4	462
Colominic acid (α2-8)	30	33	4.0	63	0.1	300
Glycoproteins						
Fetuin (α2-3>α2-6)	340	272	6.6	59	17	---
α1-Acid glycoprotein (α2-6>α2-3)	1000	555	---	---	---	761
Submandibular gland mucin (α2-6)	400	139	5.1	---	---	123
Submaxillary gland mucin (α2-6)	---	---	---	56	---	---
Gangliosides						
Gangliosides mixtures	(360)	(350)	1.6	78	34	285
Synthetic						
4MU-Neu5Ac	1580	605	58	---	1050	---

*Each value represents a relative sialidase activity when the activity directed toward II^3Neu5AcLac is regard as 100

FIG. 3

SEQ ID NOs: 28 & 29

ccatggggcatcaccatcaccatcatctagagggagatcatccacaagctacaccagcacct
M G H H H H H H L E G D H P Q A T P A P
gcaccagatgctagcactgagctgccagcaagcatgtctcaggctcagcatcttgcagca
A P D A S T E L P A S M S Q A Q H L A A
aatacggctactgataattatcgcattccagcgattacaaccgctccgaatggtgattta
N T A T D N Y R I P A I T T A P N G D L
ctgattagctatgatgaacggccgaaggacaatggaaatggtggttccgatgcccctaac
L I S Y D E R P K D N G N G G S D A P N
ccgaatcatattgttcagcgtcgctccacagatggcggtaaaacttggagcgcgccaacc
P N H I V Q R R S T D G G K T W S A P T
tatattcatcagggtacggagactggcaagaaagtgggatattccgacccctcttatgtg
Y I H Q G T E T G K K V G Y S D P S Y V
gtggatcatcaaaccggtacaatcttcaattttcatgtgaaatcatacgatcagggctgg
V D H Q T G T I F N F H V K S Y D Q G W
ggaggtagccgtgggggaacagacccggaaaaccgcgggattattcaggcagaggtgtct
G G S R G G T D P E N R G I I Q A E V S
acgagcacggataatggatggacgtggacacatcgcaccatcaccgcggatattacgaaa
T S T D N G W T W T H R T I T A D I T K
gataaaccgtggaccgcgcgttttgcggcgtccggccaaggcattcagatccagcatggg
D K P W T A R F A A S G Q G I Q I Q H G
ccgcatgccggccgtctggtgcaacagtataccattcgtacggccggtggagcggtgcag
P H A G R L V Q Q Y T I R T A G G A V Q
gctgtatcggtttattccgatgatcatgggaaaacgtggcaggctggcaccccgattggg
A V S V Y S D D H G K T W Q A G T P I G
acgggtatggatgaaaacaaagttgtagagctgtctgacggctctctgatgctgaacagt
T G M D E N K V V E L S D G S L M L N S
cgtgcgtcggacgggagcggctttcgtaaggttgcgcatagcactgatggtgggcagacc
R A S D G S G F R K V A H S T D G G Q T
tggtccgaaccggtttcggacaaaaatttgccggattcggttgataatgcccagataatt
W S E P V S D K N L P D S V D N A Q I I
cgtgcgtttcctaatgctgcccccgatgacccgcgcgcgaaagtacttcttctgagtcat
R A F P N A A P D D P R A K V L L L S H
tccccaaatccacgtccgtggtcccgggatcgtggtacgataagcatgtcatgtgatgac
S P N P R P W S R D R G T I S M S C D D
ggggcctcatggaccacttccaaagtttttcacgaaccgtttgtgggctacacgactatt
G A S W T T S K V F H E P F V G Y T T I
gcagttcagagtgatggaagcatcggtctgctgtcggaggacgcgcacaatggcgctgat
A V Q S D G S I G L L S E D A H N G A D
tatggtggcatctggtatcgtaattttacgatgaactggctgggagaacaatgtggacaa
Y G G I W Y R N F T M N W L G E Q C G Q
aaacccgcggaat**aagctt**
K P A E

FIG. 4

SEQ ID NOs: 18 & 19

```
ccatggttaagcgcaaaaaaaaaggcggcaaaaacggtaaaaatcgtcgtaaccgtaagaa
  M   V   K   R   K   K   K   G   G   K   N   G   K   N   R   R   N   R   K
aaaaatcctggagatcatccacaagctacaccagcacctgcaccagatgctagcactgag
 K   N   P   G   D   H   P   Q   A   T   P   A   P   A   P   D   A   S   T   E
ctgccagcaagcatgtctcaggctcagcatcttgcagcaaatacggctactgataattat
 L   P   A   S   M   S   Q   A   Q   H   L   A   A   N   T   A   T   D   N   Y
cgcattccagcgattacaaccgctccgaatggtgatttactgattagctatgatgaacgg
 R   I   P   A   I   T   T   A   P   N   G   D   L   L   I   S   Y   D   E   R
ccgaaggacaatggaaatggtggttccgatgcccctaacccgaatcatattgttcagcgt
 P   K   D   N   G   N   G   G   S   D   A   P   N   P   N   H   I   V   Q   R
cgctccacagatggcggtaaaacttggagcgcgccaacctatattcatcagggtacggag
 R   S   T   D   G   G   K   T   W   S   A   P   T   Y   I   H   Q   G   T   E
actggcaagaaagtgggatattccgacccctcttatgtggtggatcatcaaaccggtaca
 T   G   K   K   V   G   Y   S   D   P   S   Y   V   V   D   H   Q   T   G   T
atcttcaatttttcatgtgaaatcatacgatcagggctggggaggtagccgtgggggaaca
 I   F   N   F   H   V   K   S   Y   D   Q   G   W   G   G   S   R   G   G   T
gacccggaaaaccgcgggattattcaggcagaggtgtctacgagcacggataatggatgg
 D   P   E   N   R   G   I   I   Q   A   E   V   S   T   S   T   D   N   G   W
acgtggacacatcgcaccatcaccgcggatattacgaaagataaaccgtggaccgcgcgt
 T   W   T   H   R   T   I   T   A   D   I   T   K   D   K   P   W   T   A   R
tttgcggcgtccggccaaggcattcagatccagcatgggccgcatgccggccgtctggtg
 F   A   A   S   G   Q   G   I   Q   I   Q   H   G   P   H   A   G   R   L   V
caacagtataccattcgtacggccggtggagcggtgcaggctgtatcggtttattccgat
 Q   Q   Y   T   I   R   T   A   G   G   A   V   Q   A   V   S   V   Y   S   D
gatcatgggaaaacgtggcaggctggcaccccgattgggacgggtatggatgaaaacaaa
 D   H   G   K   T   W   Q   A   G   T   P   I   G   T   G   M   D   E   N   K
gttgtagagctgtctgacggctctctgatgctgaacagtcgtgcgtcggacgggagcggc
 V   V   E   L   S   D   G   S   L   M   L   N   S   R   A   S   D   G   S   G
tttcgtaaggttgcgcatagcactgatggtgggcagacctggtccgaaccggtttcggac
 F   R   K   V   A   H   S   T   D   G   G   Q   T   W   S   E   P   V   S   D
aaaaatttgccggattcggttgataatgcccagataattcgtgcgtttcctaatgctgcc
 K   N   L   P   D   S   V   D   N   A   Q   I   I   R   A   F   P   N   A   A
cccgatgacccgcgcgcgaaagtacttcttctgagtcattccccaaatccacgtccgtgg
 P   D   D   P   R   A   K   V   L   L   L   S   H   S   P   N   P   R   P   W
tcccgggatcgtggtacgataagcatgtcatgtgatgacggggcctcatggaccacttcc
 S   R   D   R   G   T   I   S   M   S   C   D   D   G   A   S   W   T   T   S
aaagtttttcacgaaccgtttgtgggctacacgactattgcagttcagagtgatggaagc
 K   V   F   H   E   P   F   V   G   Y   T   T   I   A   V   Q   S   D   G   S
atcggtctgctgtcggaggacgcgcacaatggcgctgattatggtggcatctggtatcgt
 I   G   L   L   S   E   D   A   H   N   G   A   D   Y   G   G   I   W   Y   R
aatttacgatgaactggctgggagaacaatgtggacaaaaacccgcggaataagctt
 N   F   T   M   N   W   L   G   E   Q   C   G   Q   K   P   A   E   -   A
```

FIG. 5

SEQ ID NO:36 & 37

ccatggttaagcgcaaaaaaaaaggcggcaaaaacggtaaaaatcgtcgtaaccgtaagaaa

M V K R K K K G G K N G K N R R N R K K

aaaaatcctggtggtggtggttctggagatcatccacaagctacaccagcacctgcacca

K N P G G G G S G D H P Q A T P A P A P

gatgctagcactgagctgccagcaagcatgtctcaggctcagcatcttgcagcaaatacg

D A S T E L P A S M S Q A Q H L A A N T

gctactgataattatcgcattccagcgattacaaccgctccgaatggtgatttactgatt

A T D N Y R I P A I T T A P N G D L L I

agctatgatgaacggccgaaggacaatggaaatggtggttccgatgcccctaacccgaat

S Y D E R P K D N G N G G S D A P N P N

catattgttcagcgtcgctccacagatggcggtaaaacttggagcgcgccaacctatatt

H I V Q R R S T D G G K T W S A P T Y I

catcagggtacggagactggcaagaaagtgggatattccgacccctcttatgtggtggat

H Q G T E T G K K V G Y S D P S Y V V D

catcaaaccggtacaatcttcaatttccatgtgaaatcatacgatcagggctggggaggt

H Q T G T I F N F H V K S Y D Q G W G G

agccgtgggggaacagacccggaaaaccgcgggattattcaggcagaggtgtctacgagc

S R G G T D P E N R G I I Q A E V S T S

acggataatggatggacgtggacacatcgcaccatcaccgcggatattacgaaagataaa

T D N G W T W T H R T I T A D I T K D K

ccgtggaccgcgcgttttgcggcgtccggccaaggcattcagatccagcatgggccgcat

P W T A R F A A S G Q G I Q I Q H G P H

gccggccgtctggtgcaacagtataccattcgtacggccggtggagcggtgcaggctgta

A G R L V Q Q Y T I R T A G G A V Q A V

tcggtttattccgatgatcatgggaaaacgtggcaggctggcaccccgattgggacgggt

S V Y S D D H G K T W Q A G T P I G T G

atggatgaaaacaaagttgtagagctgtctgacggctctctgatgctgaacagtcgtgcg

M D E N K V V E L S D G S L M L N S R A

tcggacgggagcggctttcgtaaggttgcgcatagcactgatggtgggcagacctggtcc

S D G S G F R K V A H S T D G G Q T W S

gaaccggtttcggacaaaaatttgccggattcggttgataatgcccagataattcgtgcg

E P V S D K N L P D S V D N A Q I I R A

tttcctaatgctgcccccgatgacccgcgcgcgaaagtacttcttctgagtcattcccca

F P N A A P D D P R A K V L L L S H S P

aatccacgtccgtggtcccgggatcgtggtacgataagcatgtcatgtgatgacggggcc

N P R P W S R D R G T I S M S C D D G A

tcatggaccacttccaaagtttttcacgaaccgtttgtgggctacacgactattgcagtt

S W T T S K V F H E P F V G Y T T I A V

cagagtgatggaagcatcggtctgctgtcggaggacgcgcacaatggcgctgattatggt

Q S D G S I G L L S E D A H N G A D Y G

ggcatctggtatcgtaattttacgatgaactggctgggagaacaatgtggacaaaaaccc

G I W Y R N F T M N W L G E Q C G Q K P

gcggaat**aagctt**

A E - A

FIG. 6

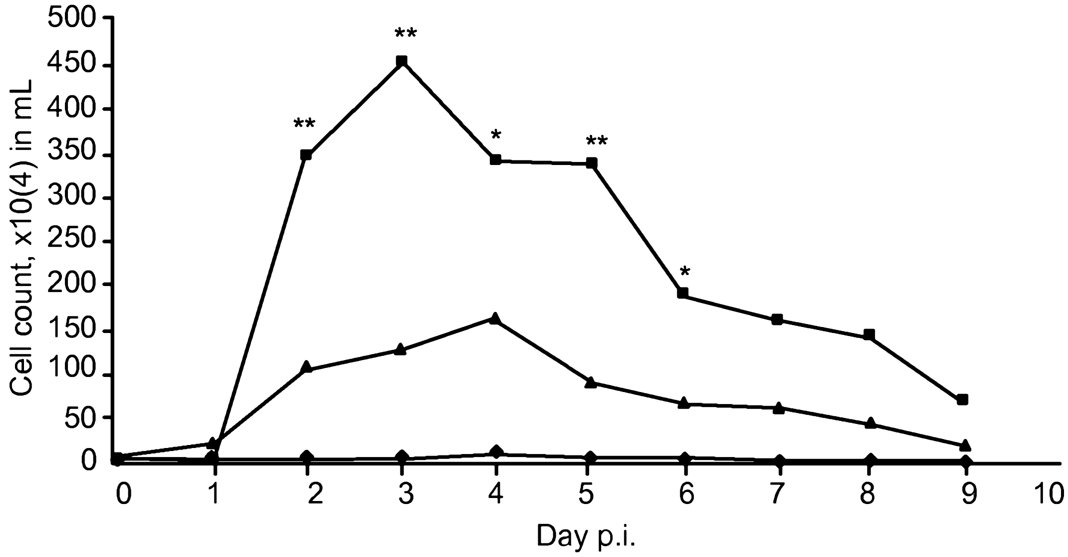

500
450
400
350
300
250
200
150
100
50
0
Cell count, x10(4) in mL
**
**
*
**
*
0 1 2 3 4 5 6 7 8 9 10
Day p.i.

FIG. 7A

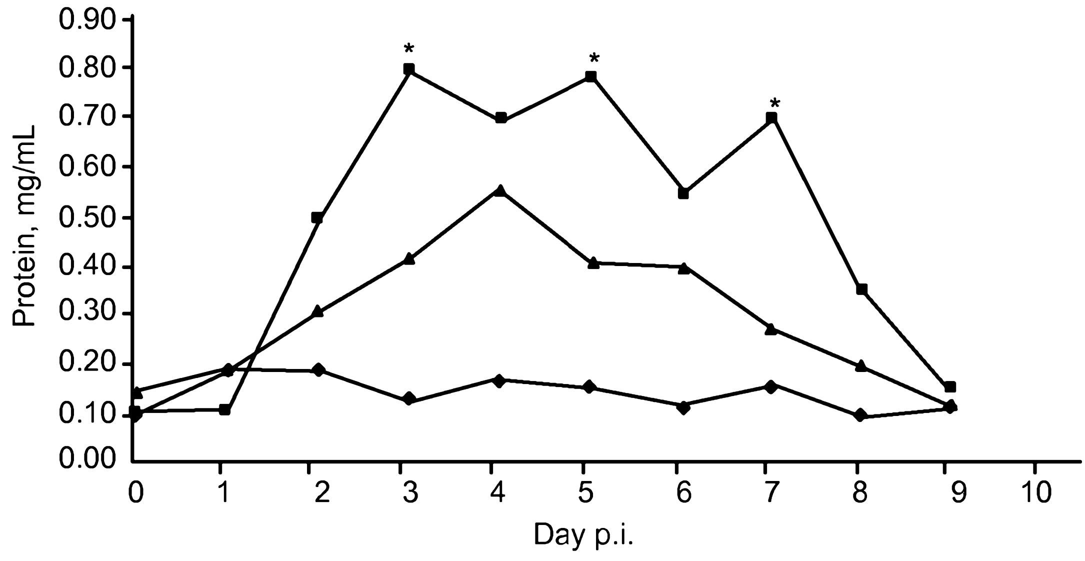

0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Protein, mg/mL
*
*
*
0 1 2 3 4 5 6 7 8 9 10
Day p.i.

FIG. 7B

PENH: Enhanced Pause = ((Te/RT)-1).(PEF/PIF)

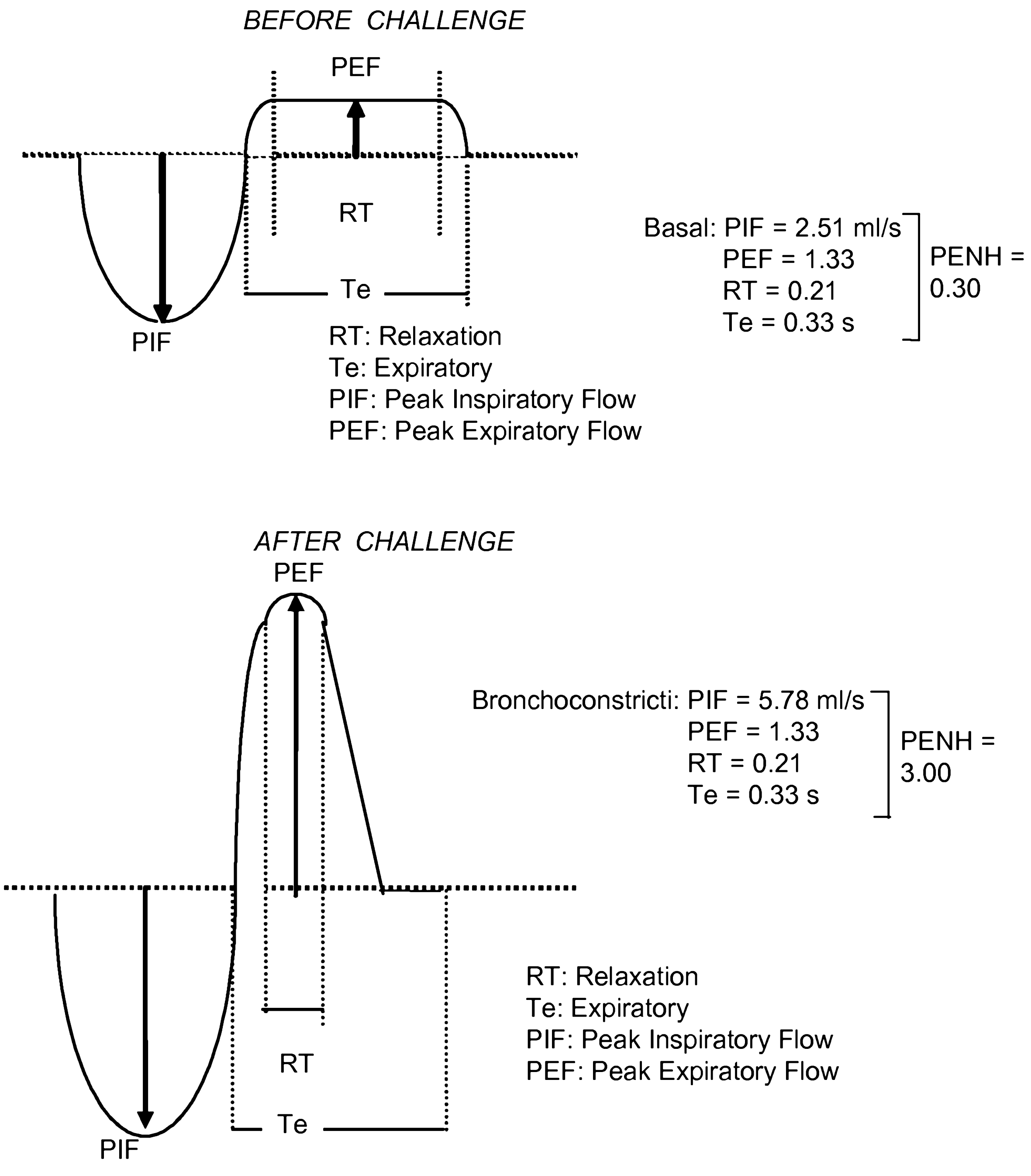


FIG. 8

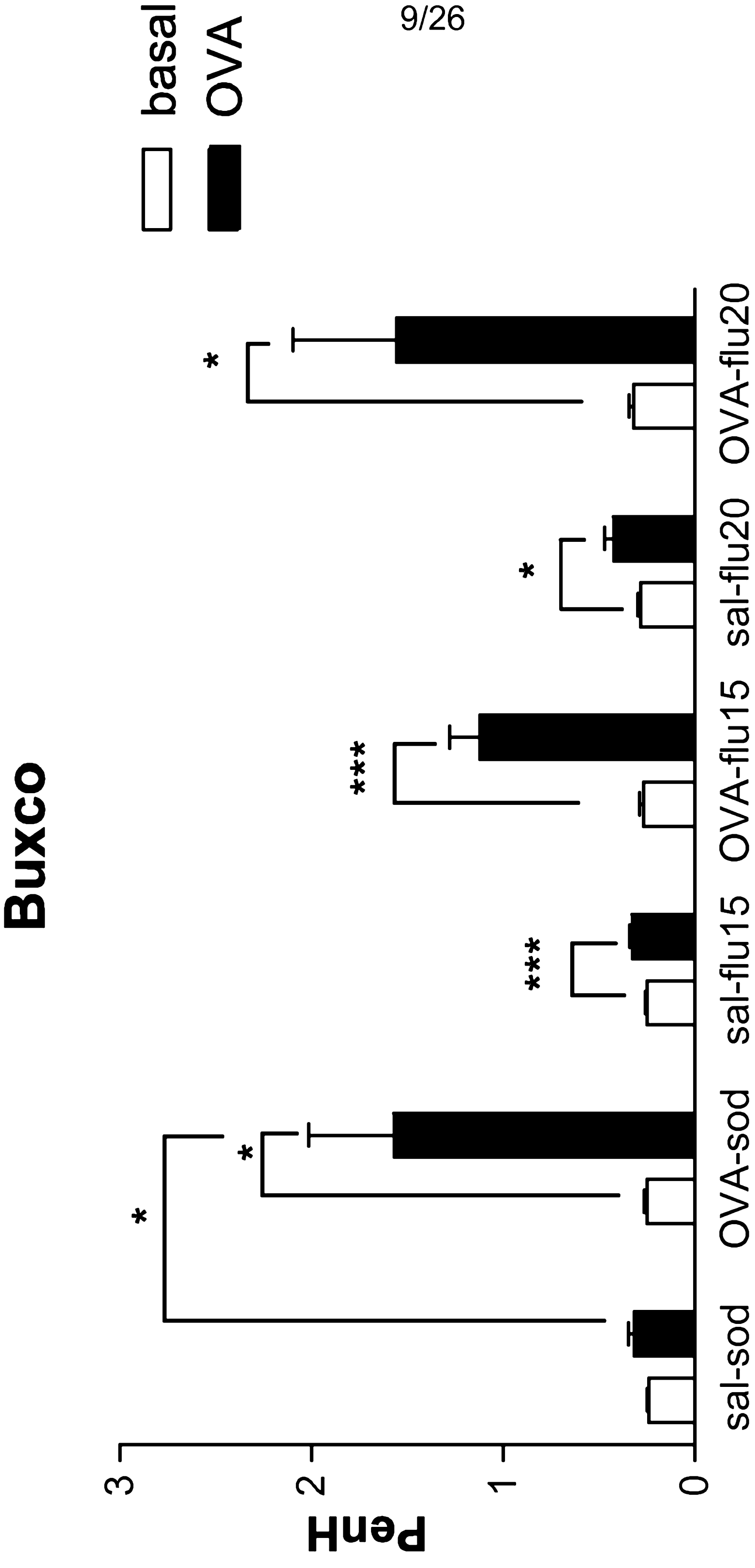

Buxco
basal
OVA
PenH
0
1
2
3
sal-sod
OVA-sod
sal-flu15
OVA-flu15
sal-flu20
OVA-flu20
*
*
***
***
*
*

FIG. 9

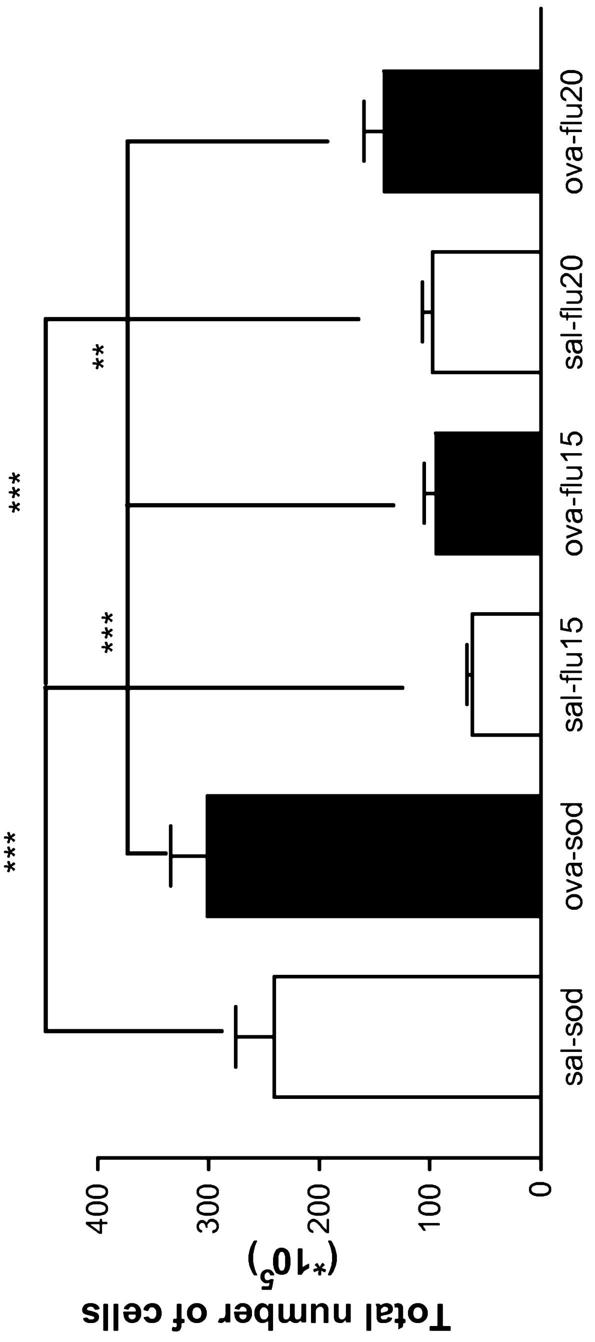

Total number of cells
(*10^5)
0
100
200
300
400
sal-sod
ova-sod
sal-flu15
ova-flu15
sal-flu20
ova-flu20
***
***
***
**

FIG. 10

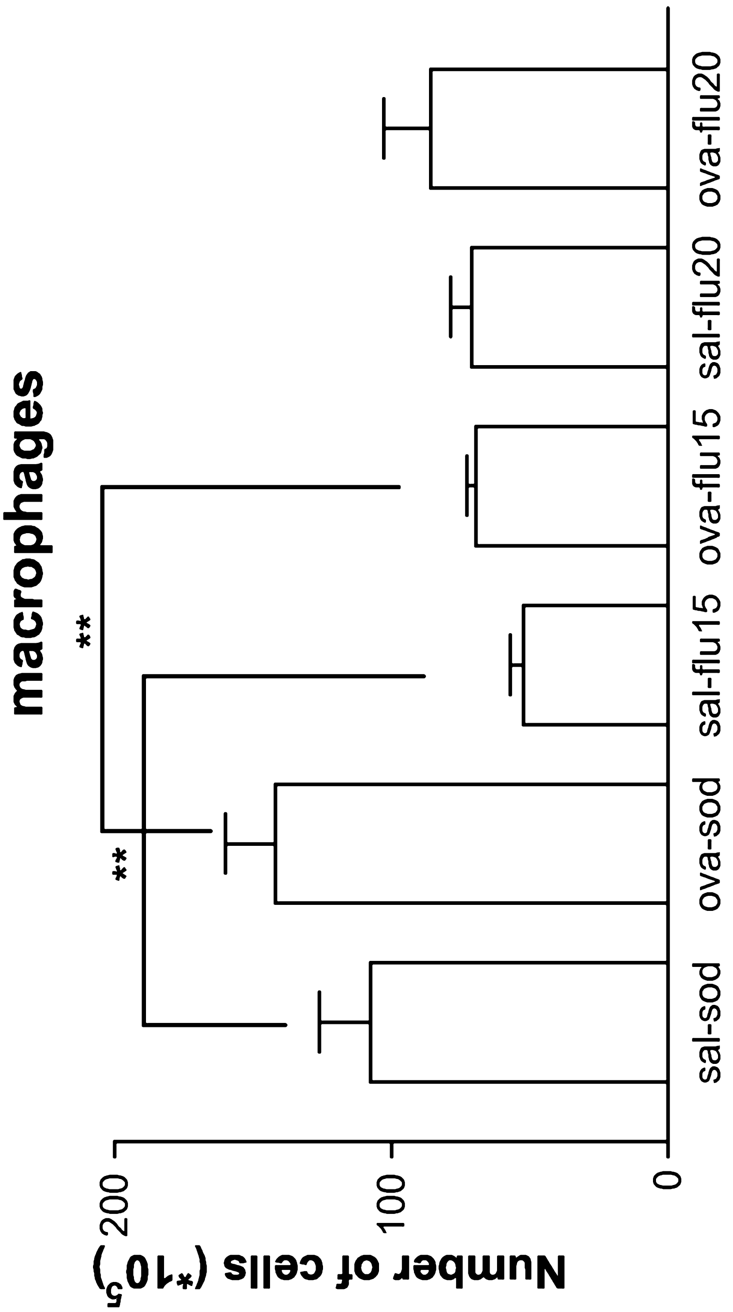

macrophages
Number of cells (*10^5)
200
100
0
sal-sod
ova-sod
sal-flu15
ova-flu15
sal-flu20
ova-flu20
**
**

FIG. 11

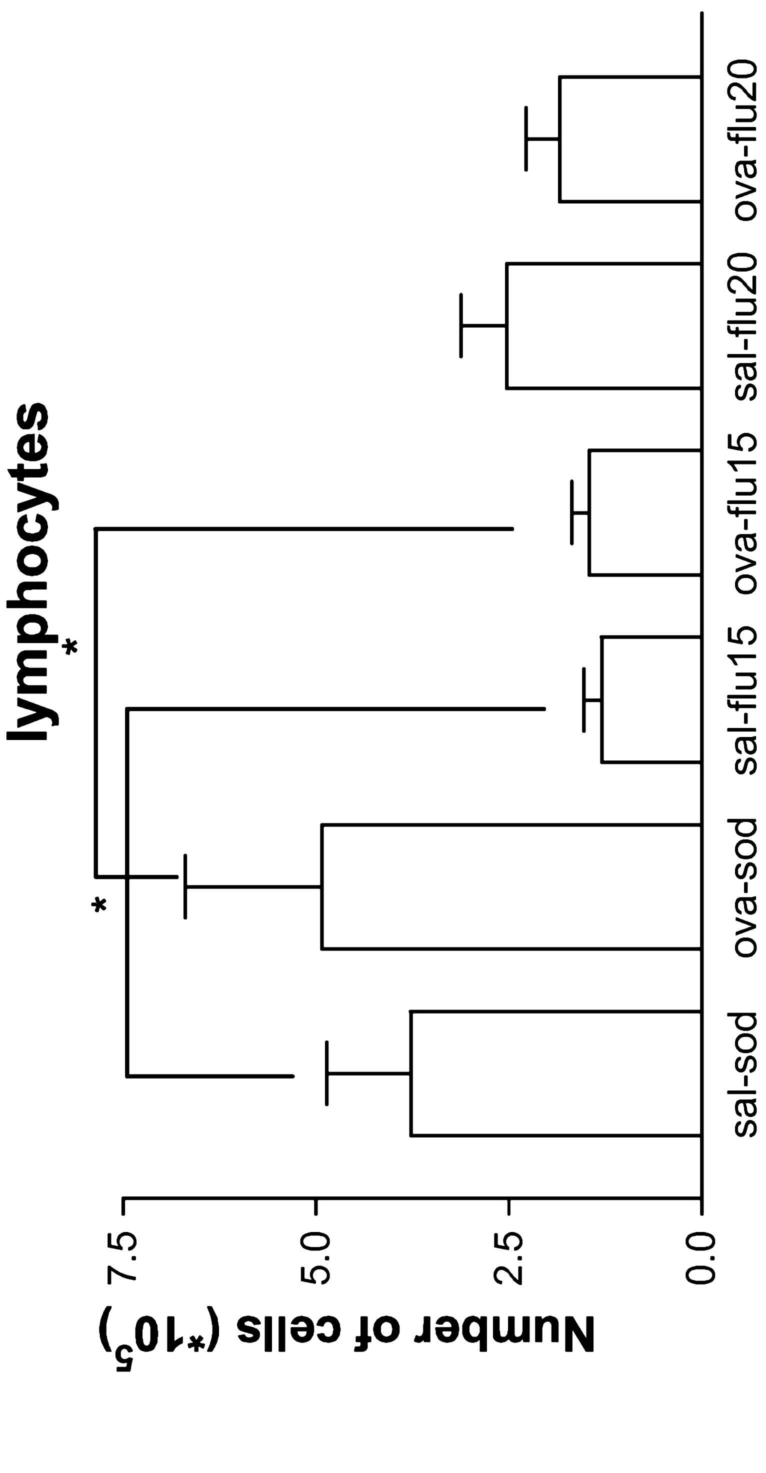

lymphocytes
Number of cells (*10^5)
7.5
5.0
2.5
0.0
sal-sod
ova-sod
sal-flu15
ova-flu15
sal-flu20
ova-flu20
*
*

FIG. 12

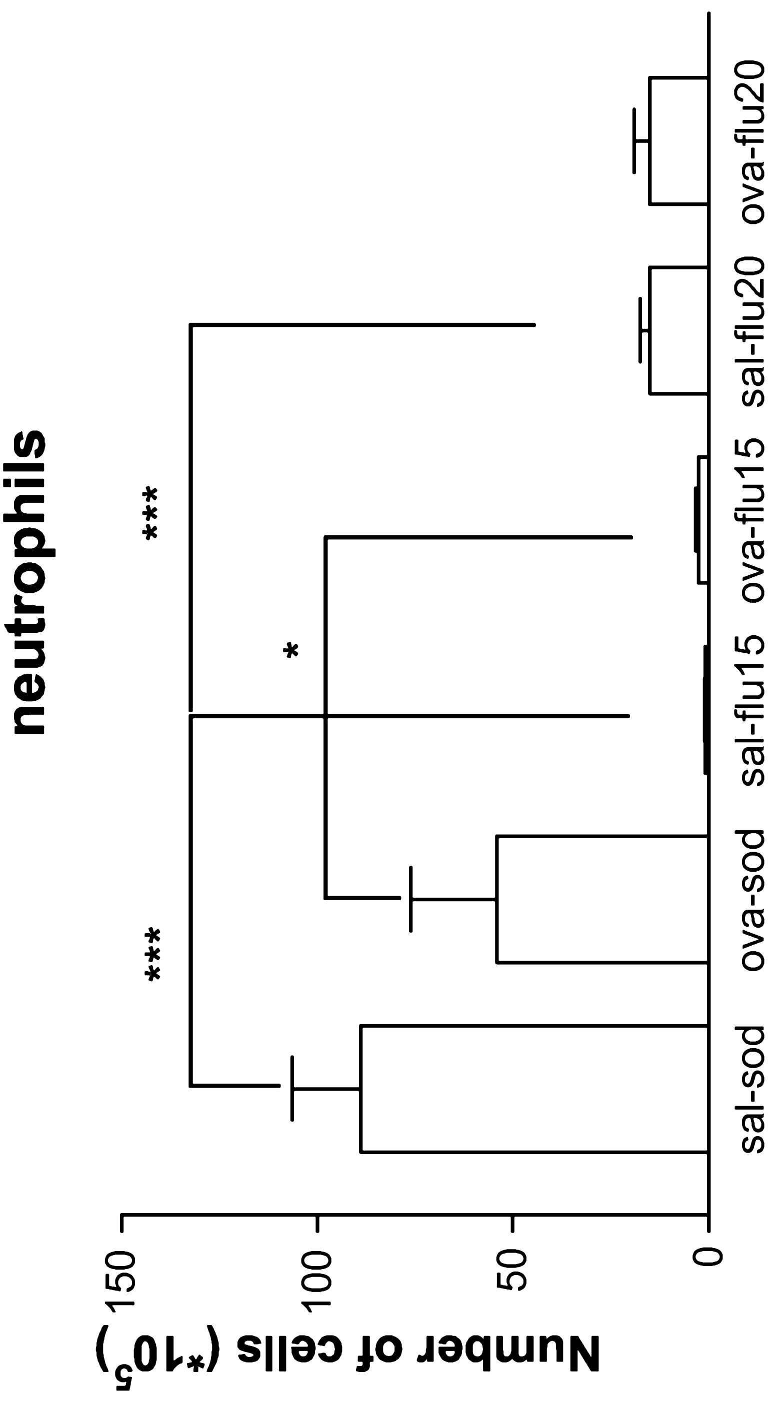

neutrophils
Number of cells (*10^5)
0
50
100
150
sal-sod
ova-sod
sal-flu15
ova-flu15
sal-flu20
ova-flu20
***
*
***

FIG. 13

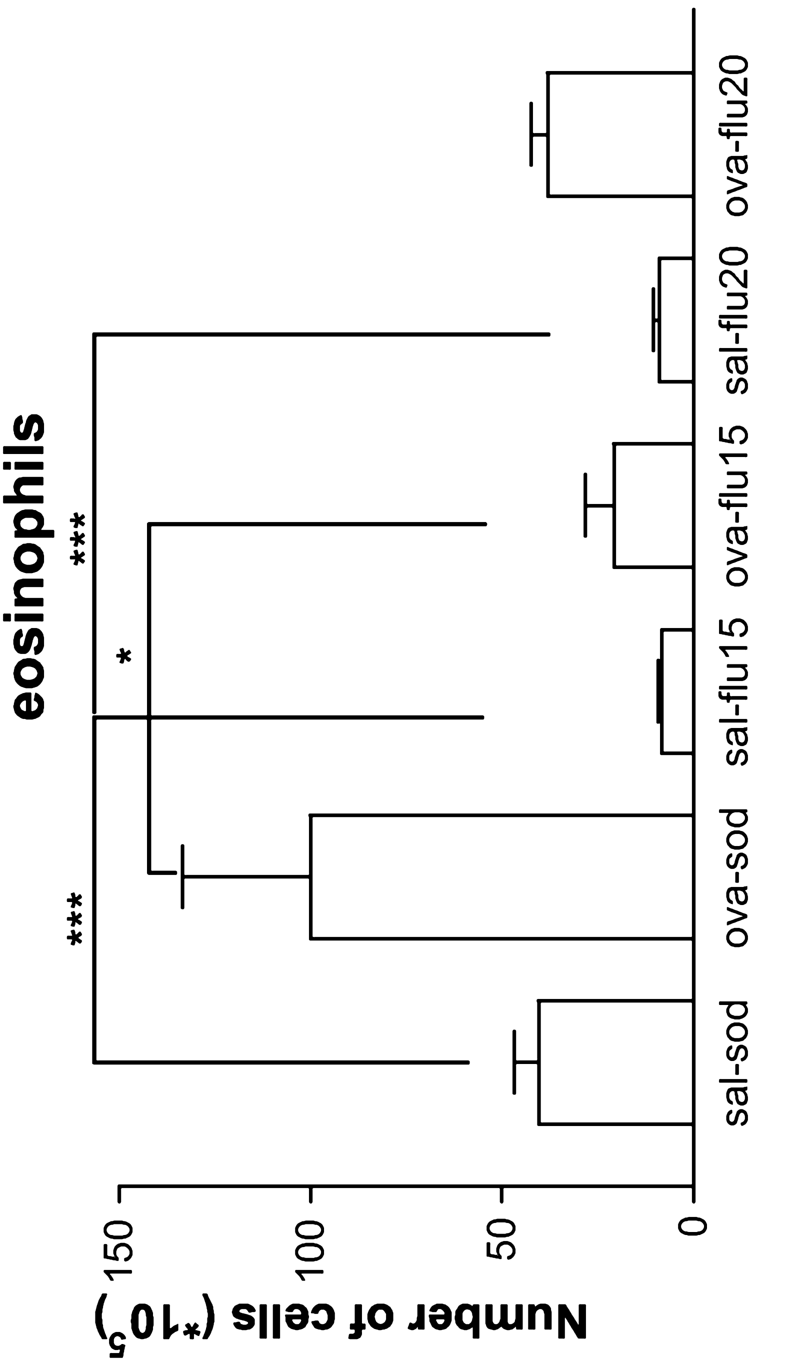

eosinophils
Number of cells (*10^5)
0
50
100
150
***
*
***
sal-sod
ova-sod
sal-flu15
ova-flu15
sal-flu20
ova-flu20

FIG. 14

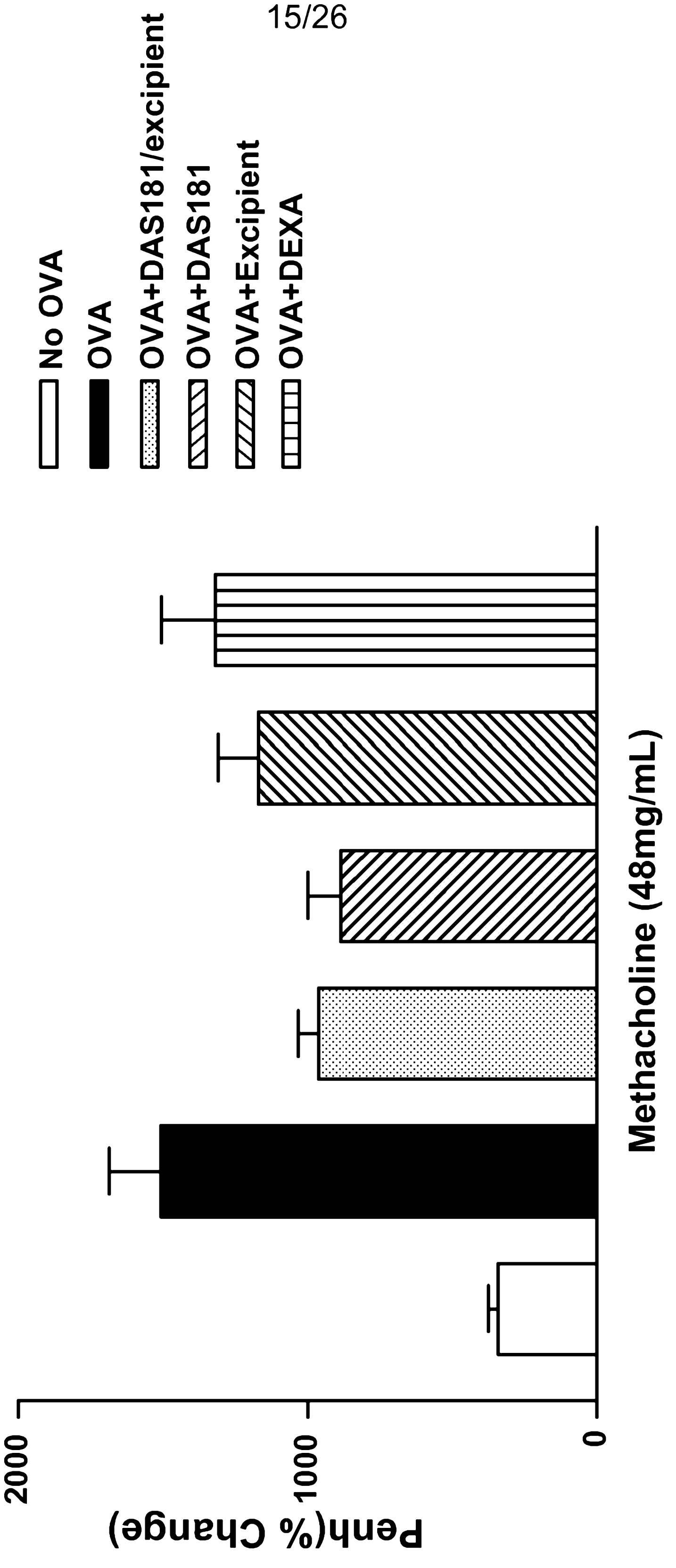

No OVA
OVA
OVA+DAS181/excipient
OVA+DAS181
OVA+Excipient
OVA+DEXA
Penh(% Change)
2000
1000
0
Methacholine (48mg/mL)

FIG. 15

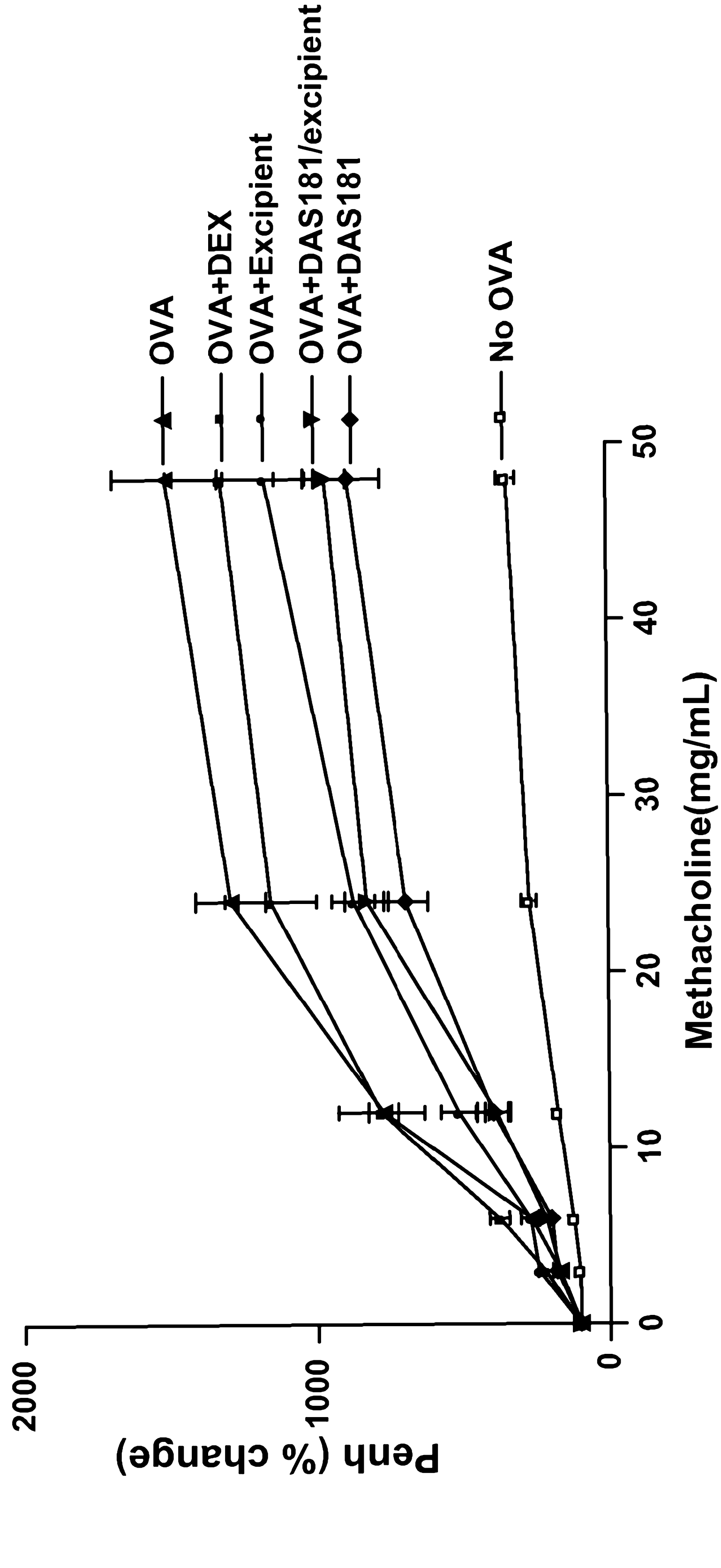
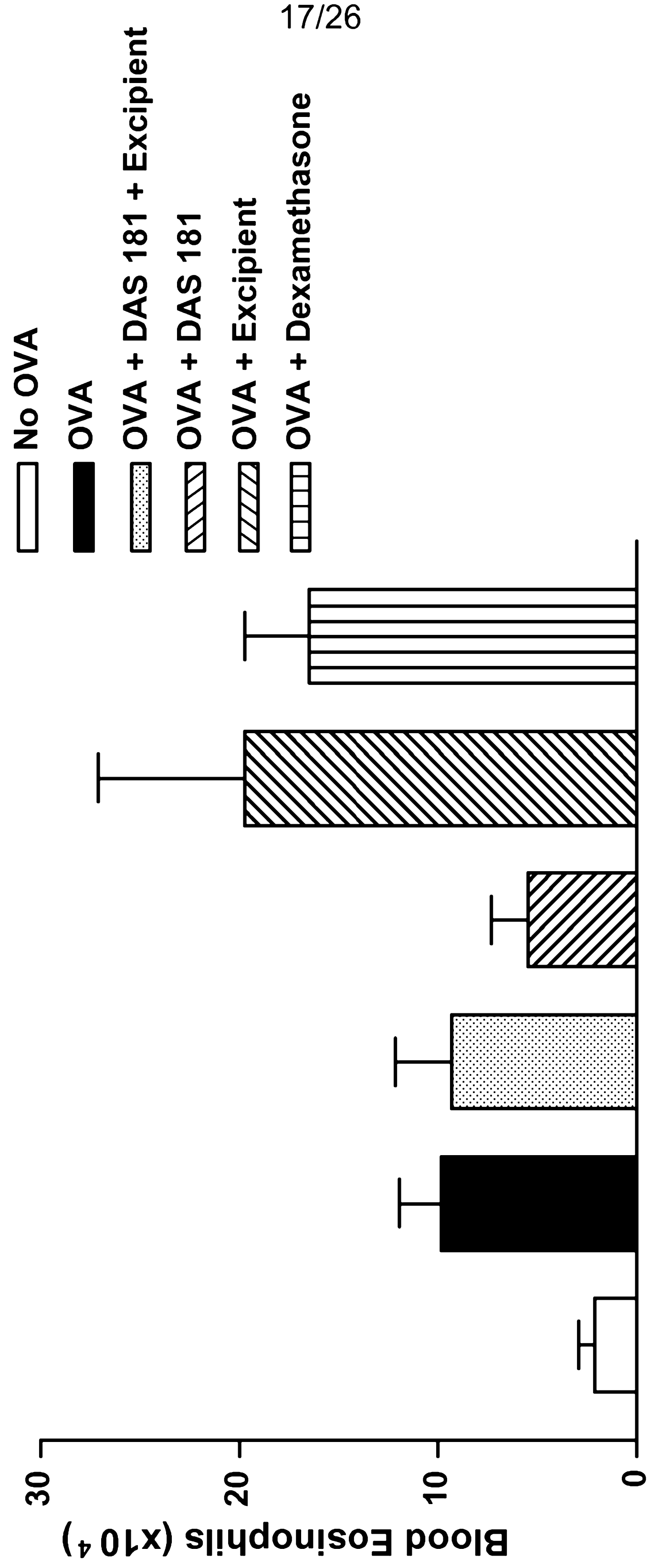

OVA
OVA+DEX
OVA+Excipient
OVA+DAS181/excipient
OVA+DAS181
No OVA
Penh (% change)
2000
1000
0
0
10
20
30
40
50
Methacholine(mg/mL)

FIG. 16



No OVA
OVA
OVA + DAS 181 + Excipient
OVA + DAS 181
OVA + Excipient
OVA + Dexamethasone
Blood Eosinophils (x10 4)
30
20
10
0

FIG. 17

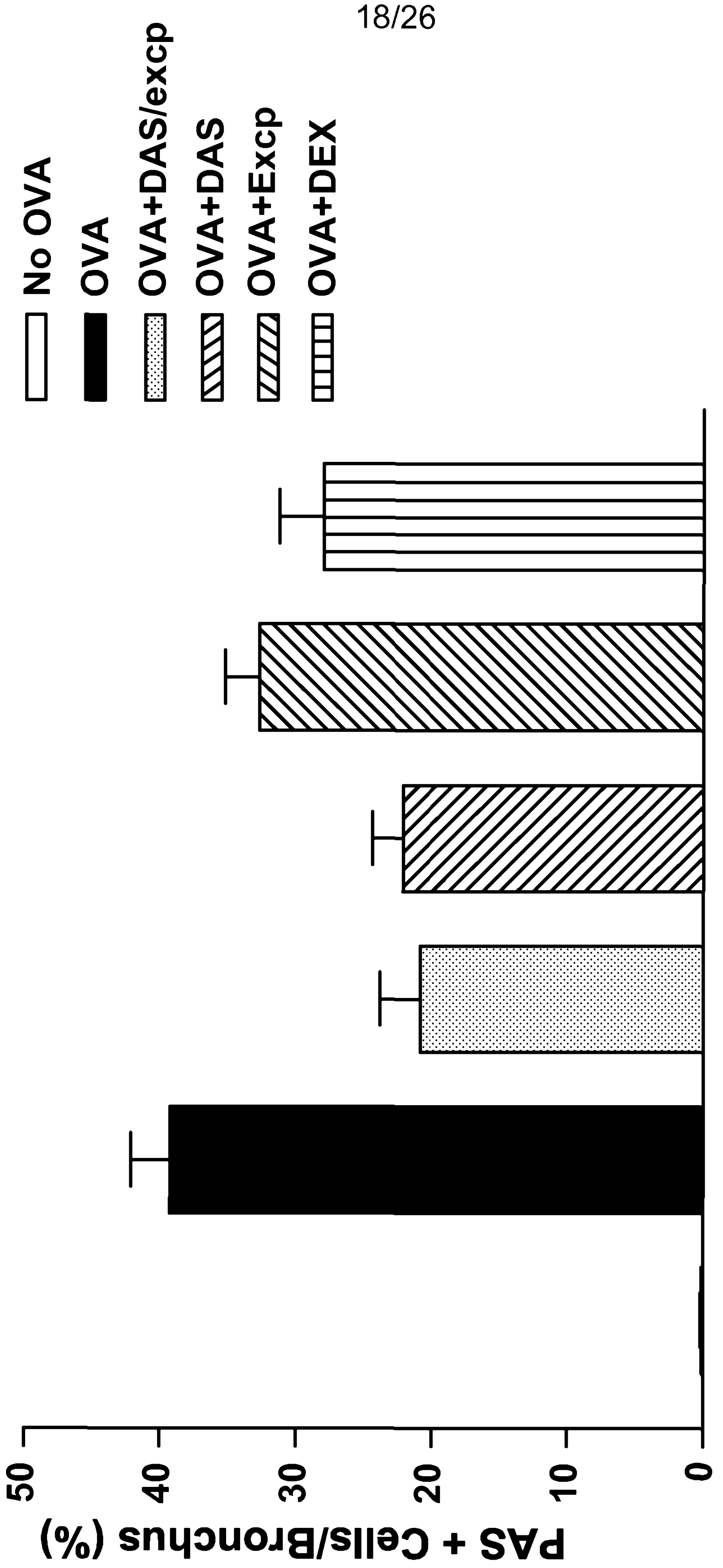
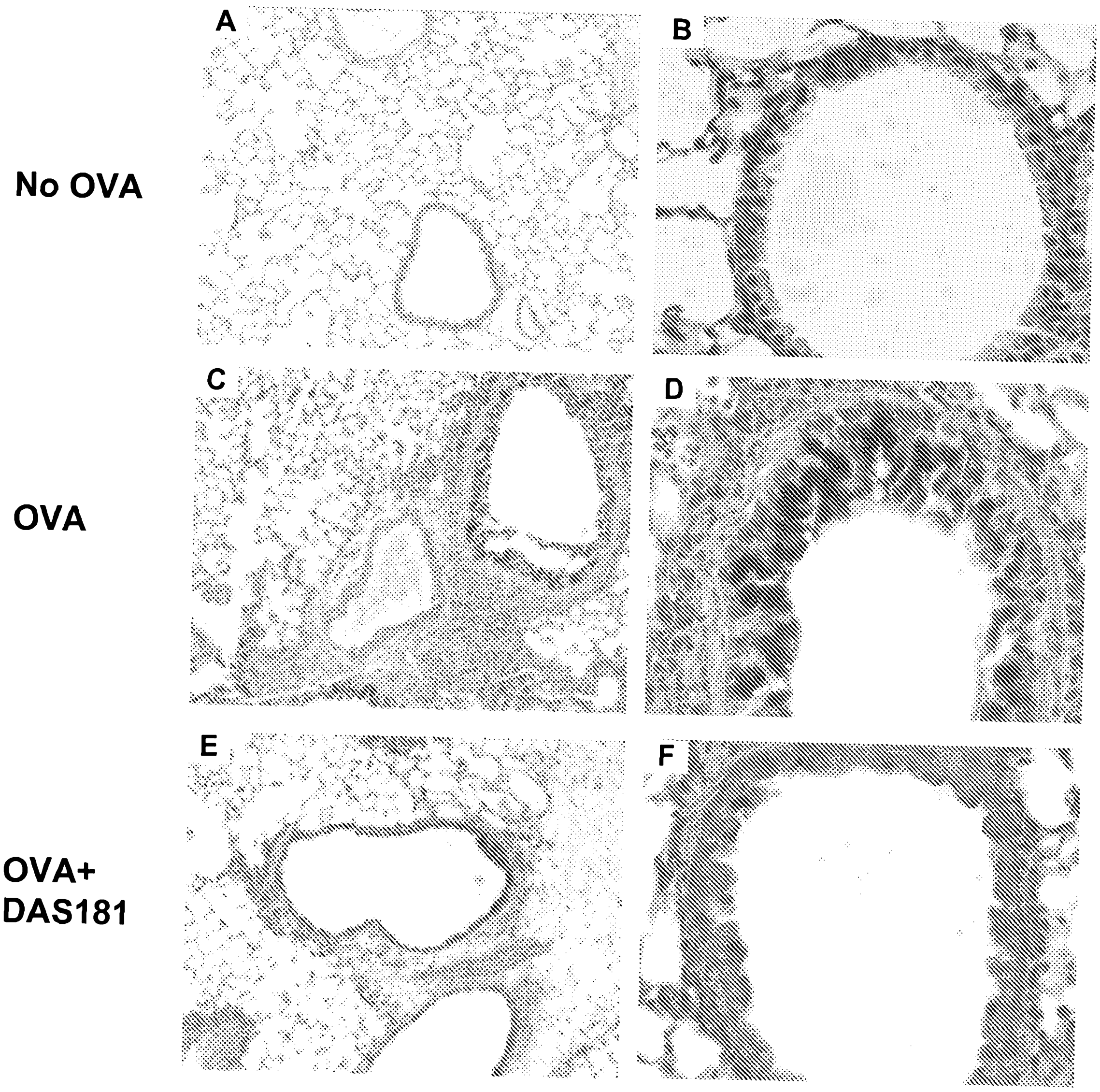

No OVA
OVA
OVA+DAS/excp
OVA+DAS
OVA+Excp
OVA+DEX
PAS + Cells/Bronchus (%)
50
40
30
20
10
0

FIG. 18

Lung Section PAS Staining



FIGS. 19A-F

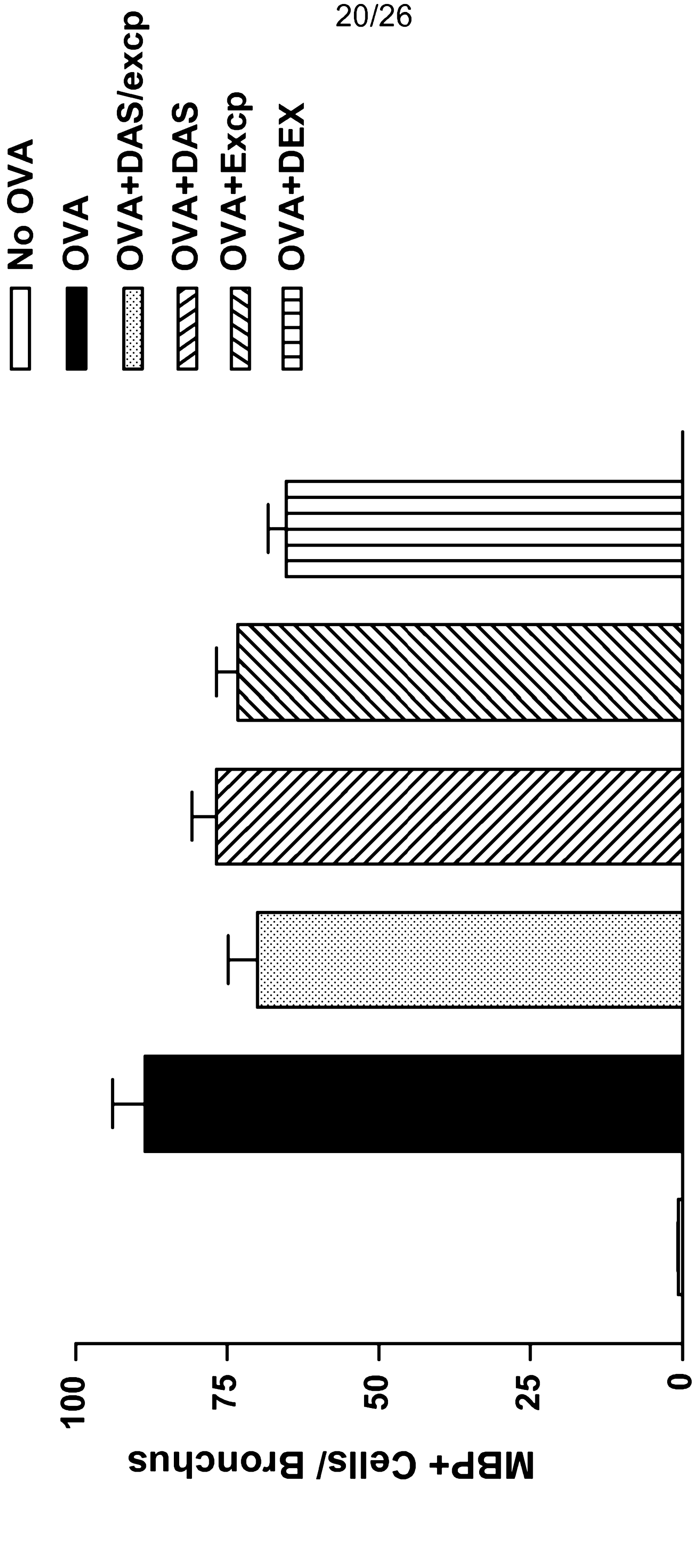

No OVA
OVA
OVA+DAS/excp
OVA+DAS
OVA+Excp
OVA+DEX
MBP+ Cells/ Bronchus
100
75
50
25
0

FIG. 20

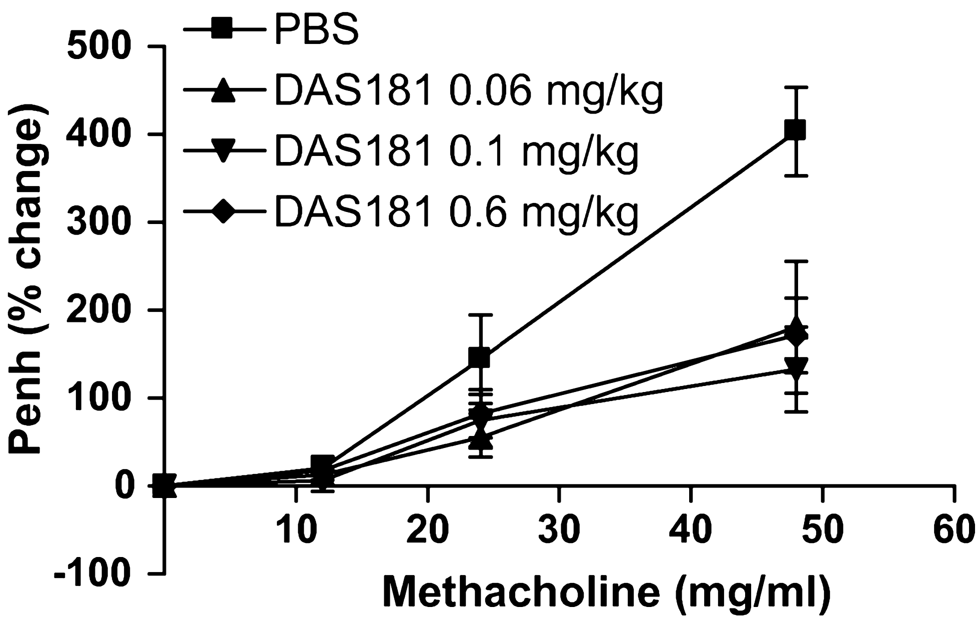


FIG. 21A

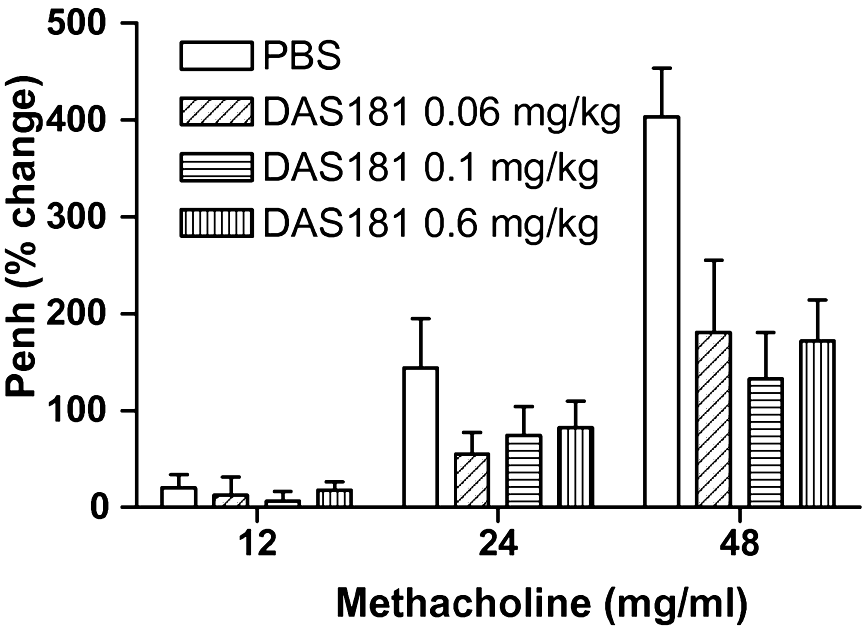
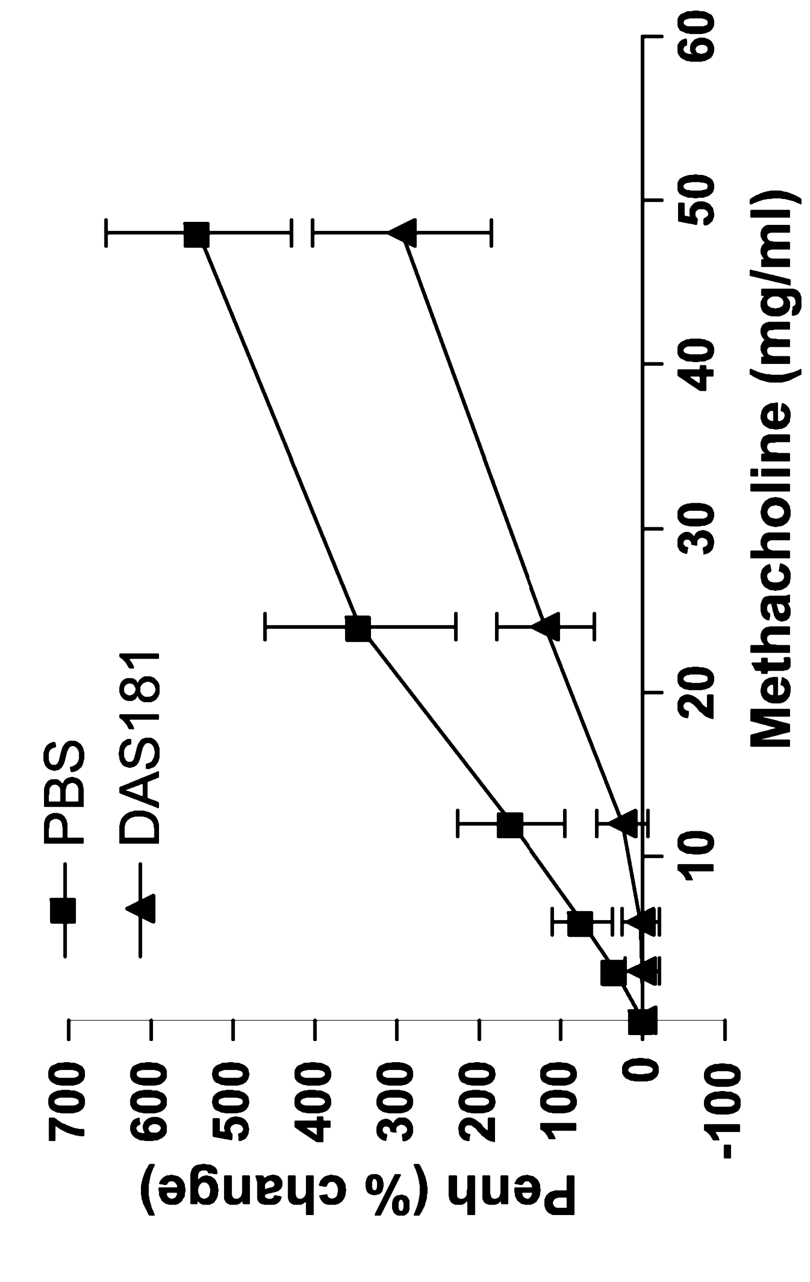


FIG. 21B



PBS
DAS181
Penh (% change)
700
600
500
400
300
200
100
0
-100
Methacholine (mg/ml)
10
20
30
40
50
60

FIG. 22

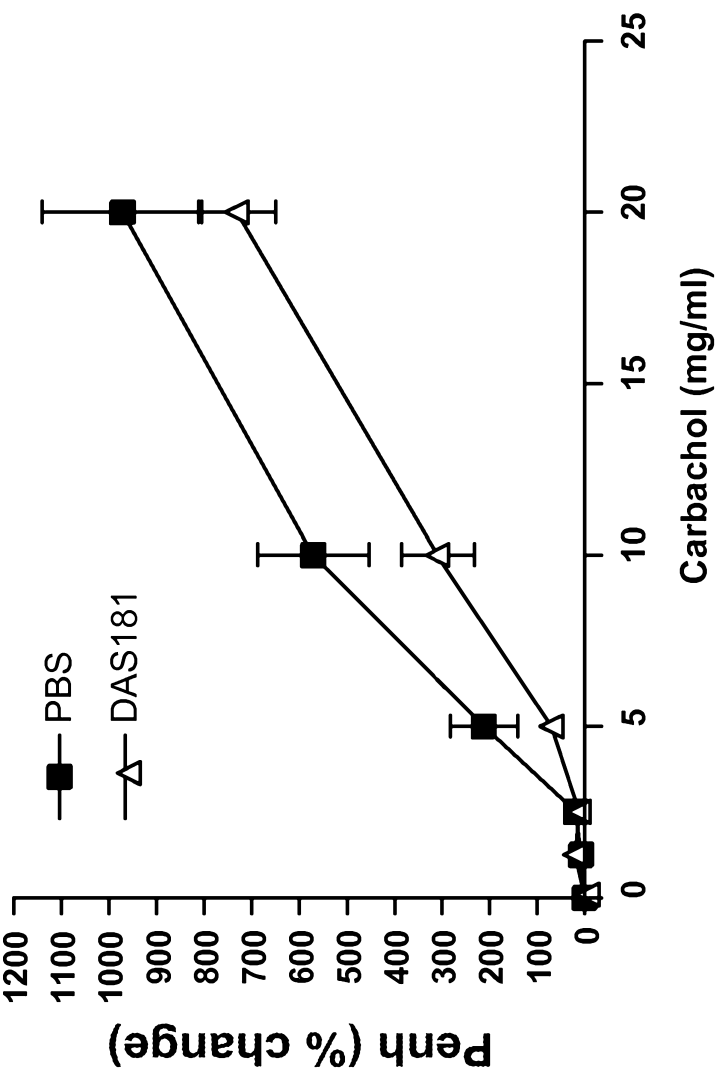

PBS
DAS181
Penh (% change)
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
0
5
10
15
20
25
Carbachol (mg/ml)

FIG. 23

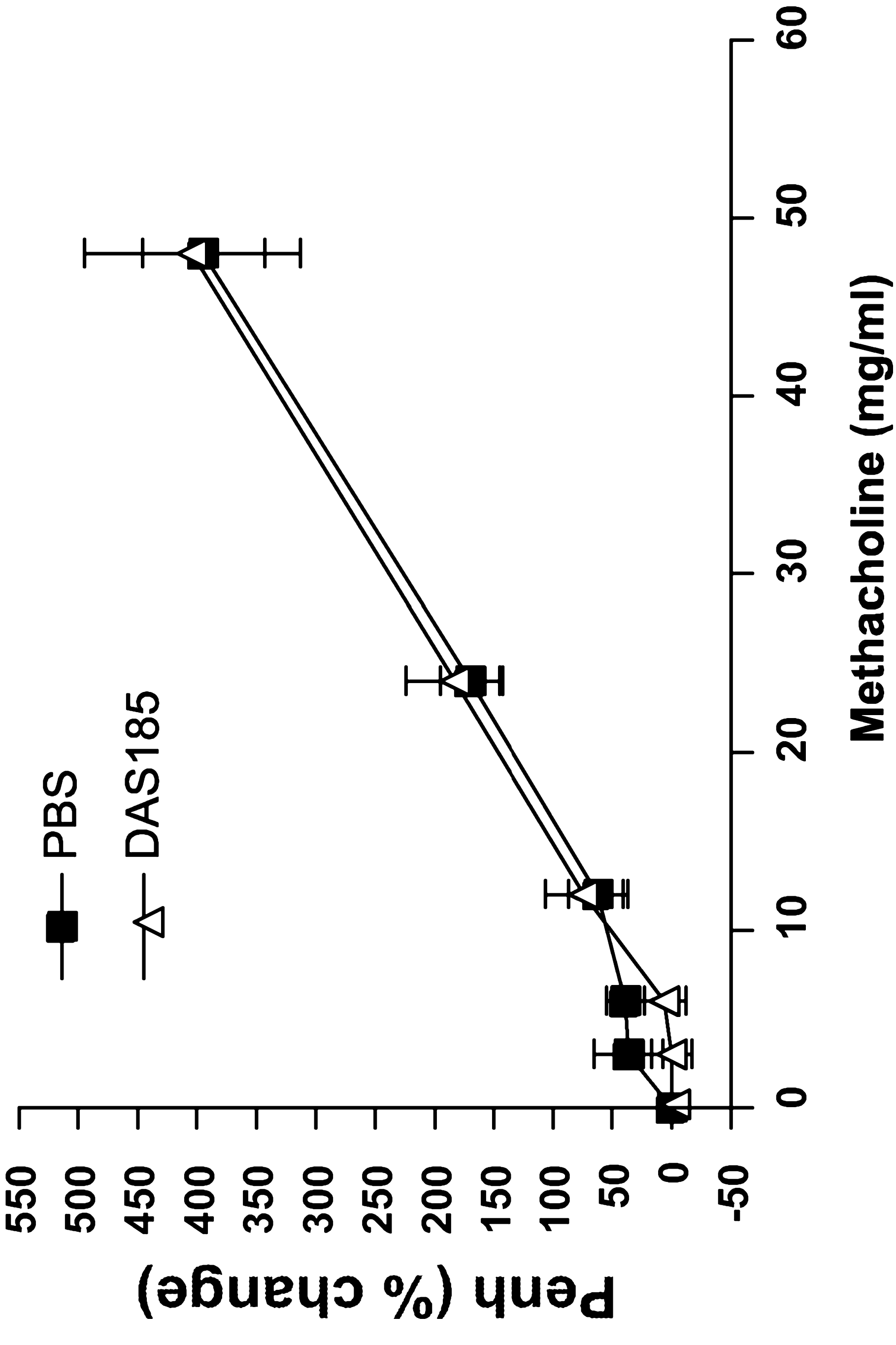

PBS
DAS185
Penh (% change)
550
500
450
400
350
300
250
200
150
100
50
0
-50
Methacholine (mg/ml)
0
10
20
30
40
50
60

FIG. 24

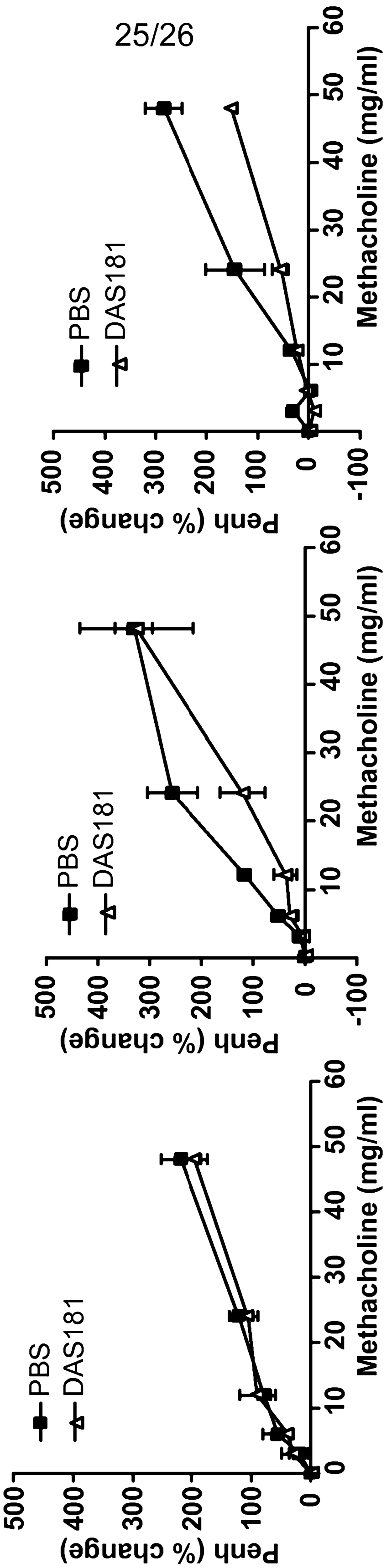

PBS
DAS181
Penh (% change)
Methacholine (mg/ml)
500
400
300
200
100
0
-100
0
10
20
30
40
50
60

FIG. 25

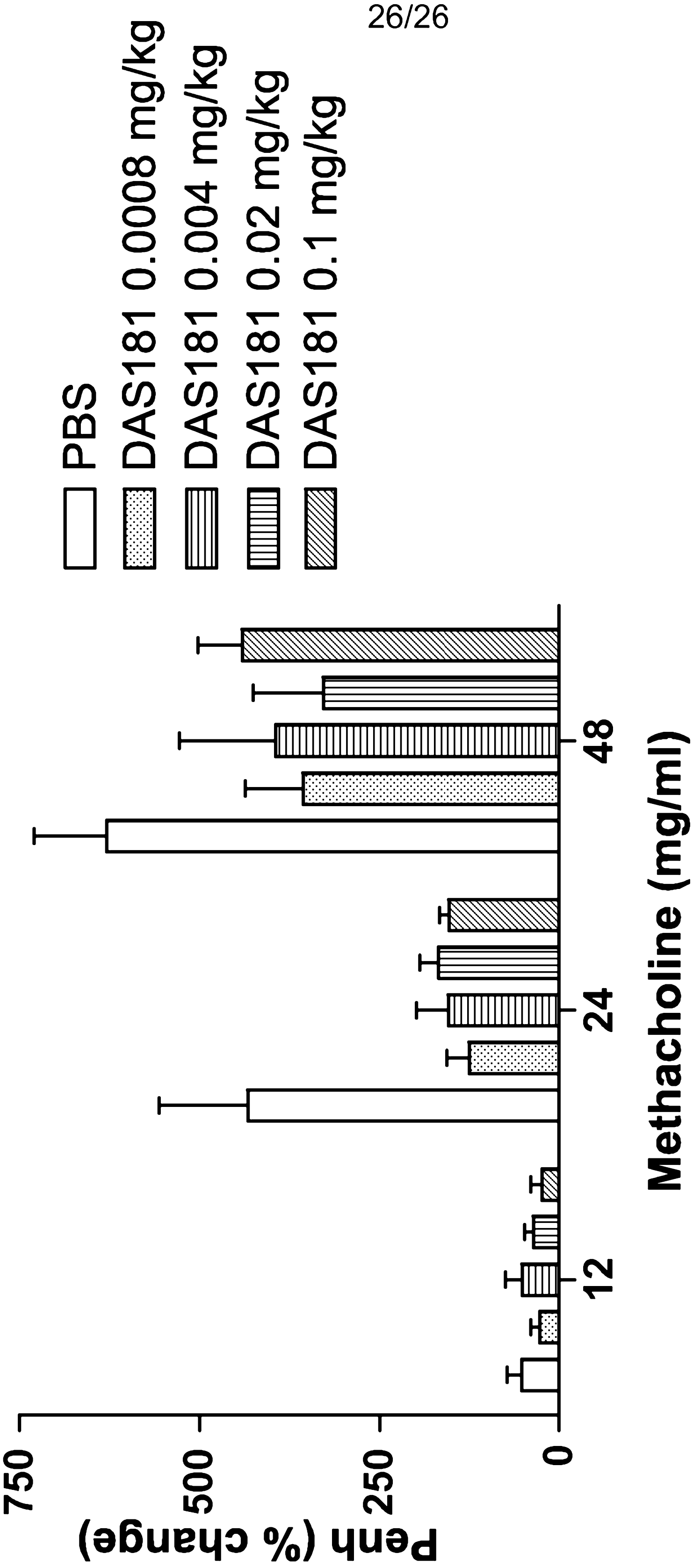

PBS
DAS181 0.0008 mg/kg
DAS181 0.004 mg/kg
DAS181 0.02 mg/kg
DAS181 0.1 mg/kg
Penh (% change)
750
500
250
0
12
24
48
Methacholine (mg/ml)

FIG. 26

SEQUENCE LISTING

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<110> NexBio, Inc.

<120> Methods, Compounds, and Compositions For
  Treatment and Prophylaxis in the Respiratory Tract


<130> 21865/0009WO1

<150> 61/322,813
<151> 2010-04-09

<150> 61/322,063
<151> 2010-05-06

<150> 61/381,420
<151> 2010-09-09

<150> 61/259,033
<151> 2009-11-06

<150> 61/259,055
<151> 2009-11-06

<160> 39

<170> FastSEQ for Windows Version 4.0

<210> 1
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<212> PRT
<213> Bos taurus

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 1               5                  10                  15
Arg Ile Ile Arg Tyr Phe Tyr Asn Ala Lys Ala Gly Leu Cys Gln Thr
            20                  25                  30
Phe Val Tyr Gly Gly Cys Arg Ala Lys Arg Asn Asn Phe Lys Ser Ala
        35                  40                  45
Glu Asp Cys Met Arg Thr Cys Gly Gly Ala
    50                  55


<210> 2
<211> 24
<212> PRT
<213> Homo sapiens

<400> 2
Asn Gly Arg Arg Ile Cys Leu Asp Leu Gln Ala Pro Leu Tyr Lys Lys
 1               5                  10                  15
Ile Ile Lys Lys Leu Leu Glu Ser
            20


<210> 3
<211> 27
<212> PRT
<213> Homo sapiens

<400> 3
Gly Arg Glu Leu Cys Leu Asp Pro Lys Glu Asn Trp Val Gln Arg Val
 1               5                  10                  15
Val Glu Lys Phe Leu Lys Arg Ala Glu Asn Ser
            20                  25
```

<210> 4
<211> 34
<212> PRT
<213> Homo sapiens

<400> 4
Gln Ile His Phe Phe Phe Ala Lys Leu Asn Cys Arg Leu Tyr Arg Lys
 1   5   10   15
Ala Asn Lys Ser Ser Lys Leu Val Ser Ala Asn Arg Leu Phe Gly Asp
            20   25   30
Lys Ser

<210> 5
<211> 34
<212> PRT
<213> Homo sapiens

<400> 5
Glu Leu Arg Val Arg Leu Ala Ser His Leu Arg Lys Leu Arg Lys Arg
 1   5   10   15
Leu Leu Arg Asp Ala Asp Asp Leu Gln Lys Arg Leu Ala Val Tyr Gln
            20   25   30
Ala Gly

<210> 6
<211> 12
<212> PRT
<213> Homo sapiens

<400> 6
Arg Arg Leu Arg Arg Met Glu Ser Glu Ser Glu Ser
 1   5   10

<210> 7
<211> 21
<212> PRT
<213> Homo sapiens

<400> 7
Lys Arg Lys Lys Lys Gly Gly Lys Asn Gly Lys Asn Arg Arg Asn Arg
 1   5   10   15
Lys Lys Lys Asn Pro
            20

<210> 8
<211> 379
<212> PRT
<213> Homo sapiens

<400> 8
Met Ala Ser Leu Pro Val Leu Gln Lys Glu Ser Val Phe Gln Ser Gly
 1   5   10   15
Ala His Ala Tyr Arg Ile Pro Ala Leu Leu Tyr Leu Pro Gly Gln Gln
            20   25   30
Ser Leu Leu Ala Phe Ala Glu Gln Arg Ala Ser Lys Lys Asp Glu His
        35   40   45
Ala Glu Leu Ile Val Leu Arg Arg Gly Asp Tyr Asp Ala Pro Thr His
    50   55   60
Gln Val Gln Trp Gln Ala Gln Glu Val Val Ala Gln Ala Arg Leu Asp
65   70   75   80

Gly His Arg Ser Met Asn Pro Cys Pro Leu Tyr Asp Ala Gln Thr Gly
85 90 95
Thr Leu Phe Leu Phe Phe Ile Ala Ile Pro Gly Gln Val Thr Glu Gln
100 105 110
Gln Gln Leu Gln Thr Arg Ala Asn Val Thr Arg Leu Cys Gln Val Thr
115 120 125
Ser Thr Asp His Gly Arg Thr Trp Ser Ser Pro Arg Asp Leu Thr Asp
130 135 140
Ala Ala Ile Gly Pro Ala Tyr Arg Glu Trp Ser Thr Phe Ala Val Gly
145 150 155 160
Pro Gly His Cys Leu Gln Leu Asn Asp Arg Ala Arg Ser Leu Val Val
165 170 175
Pro Ala Tyr Ala Tyr Arg Lys Leu His Pro Ile Gln Arg Pro Ile Pro
180 185 190
Ser Ala Phe Cys Phe Leu Ser His Asp His Gly Arg Thr Trp Ala Arg
195 200 205
Gly His Phe Val Ala Gln Asp Thr Leu Glu Cys Gln Val Ala Glu Val
210 215 220
Glu Thr Gly Glu Gln Arg Val Val Thr Leu Asn Ala Arg Ser His Leu
225 230 235 240
Arg Ala Arg Val Gln Ala Gln Ser Thr Asn Asp Gly Leu Asp Phe Gln
245 250 255
Glu Ser Gln Leu Val Lys Lys Leu Val Glu Pro Pro Pro Gln Gly Cys
260 265 270
Gln Gly Ser Val Ile Ser Phe Pro Ser Pro Arg Ser Gly Pro Gly Ser
275 280 285
Pro Gln Trp Leu Leu Tyr Thr His Pro Thr His Ser Trp Gln Arg Ala
290 295 300
Asp Leu Gly Ala Tyr Leu Asn Pro Arg Pro Pro Ala Pro Glu Ala Trp
305 310 315 320
Ser Glu Pro Val Leu Leu Ala Lys Gly Ser Cys Ala Tyr Ser Asp Leu
325 330 335
Gln Ser Met Gly Thr Gly Pro Asp Gly Ser Pro Leu Phe Gly Cys Leu
340 345 350
Tyr Glu Ala Asn Asp Tyr Glu Glu Ile Val Phe Leu Met Phe Thr Leu
355 360 365
Lys Gln Ala Phe Pro Ala Glu Tyr Leu Pro Gln
370 375

<210> 9
<211> 424
<212> PRT
<213> Homo sapiens

<400> 9
Leu Ala Gly Gly Ser Val Arg Trp Gly Ala Leu His Val Leu Gly Thr
1 5 10 15
Ala Ala Leu Ala Glu His Arg Ser Met Asn Pro Cys Pro Val His Asp
20 25 30
Ala Gly Thr Gly Thr Val Phe Leu Phe Phe Ile Ala Val Leu Gly His
35 40 45
Thr Pro Glu Ala Val Gln Ile Ala Thr Gly Arg Asn Ala Ala Arg Leu
50 55 60
Cys Cys Val Ala Ser Arg Asp Ala Gly Leu Ser Trp Gly Ser Ala Arg
65 70 75 80
Asp Leu Thr Glu Glu Ala Ile Gly Gly Ala Val Gln Asp Trp Ala Thr
85 90 95
Phe Ala Val Gly Pro Gly His Gly Val Gln Leu Pro Ser Gly Arg Leu
100 105 110
Leu Val Pro Ala Tyr Thr Tyr Arg Val Asp Arg Leu Glu Cys Phe Gly
115 120 125
Lys Ile Cys Arg Thr Ser Pro His Ser Phe Ala Phe Tyr Ser Asp Asp
130 135 140
His Gly Arg Thr Trp Arg Cys Gly Gly Leu Val Pro Asn Leu Arg Ser
145 150 155 160
Gly Glu Cys Gln Leu Ala Ala Val Asp Gly Gly Gln Ala Gly Ser Phe
165 170 175

Leu Tyr Cys Asn Ala Arg Ser Pro Leu Gly Ser Arg Val Gln Ala Leu
180 185 190
Ser Thr Asp Glu Gly Thr Ser Phe Leu Pro Ala Glu Arg Val Ala Ser
195 200 205
Leu Pro Glu Thr Ala Trp Gly Cys Gln Gly Ser Ile Val Gly Phe Pro
210 215 220
Ala Pro Ala Pro Asn Arg Pro Arg Asp Asp Ser Trp Ser Val Gly Pro
225 230 235 240
Arg Ser Pro Leu Gln Pro Pro Leu Leu Gly Pro Gly Val His Glu Pro
245 250 255
Pro Glu Glu Ala Ala Val Asp Pro Arg Gly Gly Gln Val Pro Gly Gly
260 265 270
Pro Phe Ser Arg Leu Gln Pro Arg Gly Asp Gly Pro Arg Gln Pro Gly
275 280 285
Pro Arg Pro Gly Val Ser Gly Asp Val Gly Ser Trp Thr Leu Ala Leu
290 295 300
Pro Met Pro Phe Ala Ala Pro Pro Gln Ser Pro Thr Trp Leu Leu Tyr
305 310 315 320
Ser His Pro Val Gly Arg Arg Ala Arg Leu His Met Gly Ile Arg Leu
325 330 335
Ser Gln Ser Pro Leu Asp Pro Arg Ser Trp Thr Glu Pro Trp Val Ile
340 345 350
Tyr Glu Gly Pro Ser Gly Tyr Ser Asp Leu Ala Ser Ile Gly Pro Ala
355 360 365
Pro Glu Gly Gly Leu Val Phe Ala Cys Leu Tyr Glu Ser Gly Ala Arg
370 375 380
Thr Ser Tyr Asp Glu Ile Ser Phe Cys Thr Phe Ser Leu Arg Glu Val
385 390 395 400
Leu Glu Asn Val Pro Ala Ser Pro Lys Pro Pro Asn Leu Gly Asp Lys
405 410 415
Pro Arg Gly Cys Cys Trp Pro Ser
420

<210> 10
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 10
Gly Gly Gly Gly Ser
1 5

<210> 11
<211> 2706
<212> DNA
<213> Actinomyces viscosus

<400> 11
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ctggccgcca ccggcctgat cgccgccgca cccccggcgc acgccgtccc cacgtctgac 120
ggcctggccg acgtcaccat cacgcaggtg aacgcgcccg cggacggcct ctactccgtc 180
ggcgatgtca tgaccttcaa catcaccctg accaacacca gcggcgaggc ccactcctac 240
gccccggcct cgacgaacct gtccgggaac gtctccaagt gccggtggcg caacgtcccg 300
gccgggacga ccaagaccga ctgcaccggc ctggccacgc acacggtgac cgccgaggac 360
ctcaaggccg gtggcttcac ccgcagatc gcctacgagg tcaaggccgt ggagtacgcc 420
gggaaggccc tgagcacccc ggagacgatc aagggcgcga cgagcccagt caaggccaac 480
tcgctgcggg tcgagtcgat cacgccgtcg tcgagccagg agaactacaa gctgggcgac 540
accgtcagct acacggtgcg cgtgcgctcg gtgtcggaca agacgatcaa cgtcgccgcc 600
accgaatcct ccttcgacga cctgggccgc cagtgccact ggggcggcct caagccgggc 660
aagggcgccg tctacaactg caagccgctc acccacacga tcacgcaagc cgacgtcgac 720
gccggccgct ggacgccatc gatcaccctg acggccaccg gaaccgacgg cgccaccctc 780
cagacgctca ccgccaccgg caacccgatc aacgtcgtcg gcgaccaccc gcaggccacg 840
cccgcaccgg cgcccgacgc gagcacggag ctgccggcct caatgagcca ggcccagcac 900

ctggccgcca acacggccac cgacaactac cgcatcccgg cgatcaccac cgccccccaat 960
ggggacctgc tcatctccta cgacgagcgc ccgaaggaca acggcaacgg cggcagcgac 1020
gcccccaacc cgaaccacat cgtccagcgc cgctccaccg acggcggcaa gacctggtcg 1080
gcgcccacct acatccacca gggcacggag accggcaaga aggtcggcta ctccgacccg 1140
agctacgtcg tcgatcacca gacgggcacg atcttcaact tccacgtcaa gtcctacgac 1200
cagggctggg gcggctcgcg cggcggcacc gacccggaga accggggcat catccaggcc 1260
gaggtgtcga cctccacgga caacggctgg acctggacgc accgcacgat caccgcggac 1320
atcacgaagg acaagccgtg gaccgcgcgt ttcgcggcct cgggccaggg catccagatt 1380
cagcacgggc cccacgccgg gcgcctggtg cagcagtaca cgatcaggac cgccggcggc 1440
gcggtgcagg ccgtctcggt ctactccgac gaccacggga agacgtggca ggccggcacg 1500
ccgatcggga ccggcatgga tgagaacaag gtcgttgagc tctccgacgg ctccctcatg 1560
ctcaactcgc gcgcctcgga tggctccggc ttccgcaagg tggcccactc caccgacggt 1620
gggcagacct ggagcgagcc ggtgtccgac aagaacctgc ccgactcggt ggacaacgcc 1680
cagatcatcc gagccttccc gaacgccgcg ccggacgacc cgcgcgccaa ggtgctgctg 1740
ctgagccact caccgaaccc gcggccgtgg tcgcgtgacc gcggcaccat ctcgatgtcc 1800
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ggcgccgact acggcggcat ctggtaccgc aacttcacga tgaactggct cggcgagcag 1980
tgcggccaga agccggcgga gccgagcccg gcgccgtcgc cgacggcggc accctcagcg 2040
gcaccgacgg agaagccggc cccgtcggcc gcgccgagcg ctgagcccac gcaggcaccg 2100
gcaccatcct ccgcgcccga gccgagcgct gcgcccgagc cgagcagcgc cccggcgccg 2160
gagcccacga ccgctccgag cacggagccc acaccggctc ctgcgcccag ctccgcacct 2220
gagcagaccg atgggccgac cgctgcgccc gcaccggaga cgtcctctgc accggccgcc 2280
gaaccgacgc aggccccgac ggtggcgcct tctgttgagc ccacgcaggc tccgggtgcg 2340
cagccgagct cagcacccaa gccgggggcg acgggtcggg ccccgtcggt ggtgaacccg 2400
aaggcgaccg gggcggcgac ggagcctggg acgccgtcat cgagcgcgag cccggcaccg 2460
agccggaacg cggcgccgac gccgaagccg ggcatggagc ccgatgagat tgatcggccg 2520
tctgacggca ccatggcgca gccgaccggt ggcgccagcg cgccgagtgc cgcgccgacg 2580
caggcggcga aggccggcag caggctgtct cgcacgggga ccaacgcgct gctgatcctg 2640
ggccttgcgg gtgtcgcggt tgtcggcggg tacctgctgc tgcgggctcg ccgttcgaag 2700
aactga 2706

<210> 12
<211> 901
<212> PRT
<213> Actinomyces viscosus

<400> 12
Met Thr Ser His Ser Pro Phe Ser Arg Arg Arg Leu Pro Ala Leu Leu
1 5 10 15
Gly Ser Leu Pro Leu Ala Ala Thr Gly Leu Ile Ala Ala Ala Pro Pro
20 25 30
Ala His Ala Val Pro Thr Ser Asp Gly Leu Ala Asp Val Thr Ile Thr
35 40 45
Gln Val Asn Ala Pro Ala Asp Gly Leu Tyr Ser Val Gly Asp Val Met
50 55 60
Thr Phe Asn Ile Thr Leu Thr Asn Thr Ser Gly Glu Ala His Ser Tyr
65 70 75 80
Ala Pro Ala Ser Thr Asn Leu Ser Gly Asn Val Ser Lys Cys Arg Trp
85 90 95
Arg Asn Val Pro Ala Gly Thr Thr Lys Thr Asp Cys Thr Gly Leu Ala
100 105 110
Thr His Thr Val Thr Ala Glu Asp Leu Lys Ala Gly Gly Phe Thr Pro
115 120 125
Gln Ile Ala Tyr Glu Val Lys Ala Val Glu Tyr Ala Gly Lys Ala Leu
130 135 140
Ser Thr Pro Glu Thr Ile Lys Gly Ala Thr Ser Pro Val Lys Ala Asn
145 150 155 160
Ser Leu Arg Val Glu Ser Ile Thr Pro Ser Ser Ser Gln Glu Asn Tyr
165 170 175
Lys Leu Gly Asp Thr Val Ser Tyr Thr Val Arg Val Arg Ser Val Ser
180 185 190
Asp Lys Thr Ile Asn Val Ala Ala Thr Glu Ser Ser Phe Asp Asp Leu
195 200 205
Gly Arg Gln Cys His Trp Gly Gly Leu Lys Pro Gly Lys Gly Ala Val
210 215 220
Tyr Asn Cys Lys Pro Leu Thr His Thr Ile Thr Gln Ala Asp Val Asp
225 230 235 240

Ala Gly Arg Trp Thr Pro Ser Ile Thr Leu Thr Ala Thr Gly Thr Asp
245 250 255
Gly Ala Thr Leu Gln Thr Leu Thr Ala Thr Gly Asn Pro Ile Asn Val
260 265 270
Val Gly Asp His Pro Gln Ala Thr Pro Ala Pro Ala Pro Asp Ala Ser
275 280 285
Thr Glu Leu Pro Ala Ser Met Ser Gln Ala Gln His Leu Ala Ala Asn
290 295 300
Thr Ala Thr Asp Asn Tyr Arg Ile Pro Ala Ile Thr Thr Ala Pro Asn
305 310 315 320
Gly Asp Leu Leu Ile Ser Tyr Asp Glu Arg Pro Lys Asp Asn Gly Asn
325 330 335
Gly Gly Ser Asp Ala Pro Asn Pro Asn His Ile Val Gln Arg Arg Ser
340 345 350
Thr Asp Gly Gly Lys Thr Trp Ser Ala Pro Thr Tyr Ile His Gln Gly
355 360 365
Thr Glu Thr Gly Lys Lys Val Gly Tyr Ser Asp Pro Ser Tyr Val Val
370 375 380
Asp His Gln Thr Gly Thr Ile Phe Asn Phe His Val Lys Ser Tyr Asp
385 390 395 400
Gln Gly Trp Gly Gly Ser Arg Gly Gly Thr Asp Pro Glu Asn Arg Gly
405 410 415
Ile Ile Gln Ala Glu Val Ser Thr Ser Thr Asp Asn Gly Trp Thr Trp
420 425 430
Thr His Arg Thr Ile Thr Ala Asp Ile Thr Lys Asp Lys Pro Trp Thr
435 440 445
Ala Arg Phe Ala Ala Ser Gly Gln Gly Ile Gln Ile Gln His Gly Pro
450 455 460
His Ala Gly Arg Leu Val Gln Gln Tyr Thr Ile Arg Thr Ala Gly Gly
465 470 475 480
Ala Val Gln Ala Val Ser Val Tyr Ser Asp Asp His Gly Lys Thr Trp
485 490 495
Gln Ala Gly Thr Pro Ile Gly Thr Gly Met Asp Glu Asn Lys Val Val
500 505 510
Glu Leu Ser Asp Gly Ser Leu Met Leu Asn Ser Arg Ala Ser Asp Gly
515 520 525
Ser Gly Phe Arg Lys Val Ala His Ser Thr Asp Gly Gly Gln Thr Trp
530 535 540
Ser Glu Pro Val Ser Asp Lys Asn Leu Pro Asp Ser Val Asp Asn Ala
545 550 555 560
Gln Ile Ile Arg Ala Phe Pro Asn Ala Ala Pro Asp Asp Pro Arg Ala
565 570 575
Lys Val Leu Leu Leu Ser His Ser Pro Asn Pro Arg Pro Trp Ser Arg
580 585 590
Asp Arg Gly Thr Ile Ser Met Ser Cys Asp Asp Gly Ala Ser Trp Thr
595 600 605
Thr Ser Lys Val Phe His Glu Pro Phe Val Gly Tyr Thr Thr Ile Ala
610 615 620
Val Gln Ser Asp Gly Ser Ile Gly Leu Leu Ser Glu Asp Ala His Asn
625 630 635 640
Gly Ala Asp Tyr Gly Gly Ile Trp Tyr Arg Asn Phe Thr Met Asn Trp
645 650 655
Leu Gly Glu Gln Cys Gly Gln Lys Pro Ala Glu Pro Ser Pro Ala Pro
660 665 670
Ser Pro Thr Ala Ala Pro Ser Ala Ala Pro Thr Glu Lys Pro Ala Pro
675 680 685
Ser Ala Ala Pro Ser Ala Glu Pro Thr Gln Ala Pro Ala Pro Ser Ser
690 695 700
Ala Pro Glu Pro Ser Ala Ala Pro Glu Pro Ser Ser Ala Pro Ala Pro
705 710 715 720
Glu Pro Thr Thr Ala Pro Ser Thr Glu Pro Thr Pro Ala Pro Ala Pro
725 730 735
Ser Ser Ala Pro Glu Gln Thr Asp Gly Pro Thr Ala Ala Pro Ala Pro
740 745 750
Glu Thr Ser Ser Ala Pro Ala Ala Glu Pro Thr Gln Ala Pro Thr Val
755 760 765
Ala Pro Ser Val Glu Pro Thr Gln Ala Pro Gly Ala Gln Pro Ser Ser
770 775 780

Ala Pro Lys Pro Gly Ala Thr Gly Arg Ala Pro Ser Val Val Asn Pro
785 790 795 800
Lys Ala Thr Gly Ala Ala Thr Glu Pro Gly Thr Pro Ser Ser Ser Ala
805 810 815
Ser Pro Ala Pro Ser Arg Asn Ala Ala Pro Thr Pro Lys Pro Gly Met
820 825 830
Glu Pro Asp Glu Ile Asp Arg Pro Ser Asp Gly Thr Met Ala Gln Pro
835 840 845
Thr Gly Gly Ala Ser Ala Pro Ser Ala Ala Pro Thr Gln Ala Ala Lys
850 855 860
Ala Gly Ser Arg Leu Ser Arg Thr Gly Thr Asn Ala Leu Leu Ile Leu
865 870 875 880
Gly Leu Ala Gly Val Ala Val Val Gly Gly Tyr Leu Leu Leu Arg Ala
885 890 895
Arg Arg Ser Lys Asn
900

<210> 13
<211> 1887
<212> DNA
<213> Actinomyces viscosus

<400> 13
ggcgaccacc cgcaggccac gcccgcaccg gcgcccgacg cgagcacgga gctgccggcc 60
tcaatgagcc aggcccagca cctggccgcc aacacggcca ccgacaacta ccgcatcccg 120
gcgatcacca ccgcccccaa tggggacctg ctcatctcct acgacgagcg cccgaaggac 180
aacggcaacg gcggcagcga cgcccccaac ccgaaccaca tcgtccagcg ccgctccacc 240
gacggcggca agacctggtc ggcgcccacc tacatccacc agggcacgga gaccggcaag 300
aaggtcggct actccgaccc gagctacgtc gtcgatcacc agacgggcac gatcttcaac 360
ttccacgtca agtcctacga ccagggctgg ggcggctcgc gcggcggcac cgacccggag 420
aaccggggca tcatccaggc cgaggtgtcg acctccacgg acaacggctg gacctggacg 480
caccgcacga tcaccgcgga catcacgaag gacaagccgt ggaccgcgcg tttcgcggcc 540
tcgggccagg gcatccagat tcagcacggg ccccacgccg ggcgcctggt gcagcagtac 600
acgatcagga ccgccggcgg cgcggtgcag gccgtctcgg tctactccga cgaccacggg 660
aagacgtggc aggccggcac gccgatcggg accggcatgg atgagaacaa ggtcgttgag 720
ctctccgacg gctccctcat gctcaactcg cgcgcctcgg atggctccgg cttccgcaag 780
gtggcccact ccaccgacgg tgggcagacc tggagcgagc cggtgtccga caagaacctg 840
cccgactcgg tggacaacgc ccagatcatc cgagccttcc cgaacgccgc gccggacgac 900
ccgcgcgcca aggtgctgct gctgagccac tcaccgaacc cgcggccgtg gtcgcgtgac 960
cgcggcacca tctcgatgtc ctgcgacgac ggcgcctcct ggacgaccag caaggtcttc 1020
cacgagccct tcgtcggata cacgacgatc gcggtgcagt ccgacggcag catcgggctg 1080
ctcagcgagg acgcccacaa cggcgccgac tacggcggca tctggtaccg caacttcacg 1140
atgaactggc tcggcgagca gtgcggccag aagccggcgg agccgagccc ggcgccgtcg 1200
ccgacggcgg caccctcagc ggcaccgacg gagaagccgg ccccgtcggc cgcgccgagc 1260
gctgagccca cgcaggcacc ggcaccatcc tccgcgcccg agccgagcgc tgcgcccgag 1320
ccgagcagcg ccccggcgcc ggagcccacg accgctccga gcacggagcc cacaccggct 1380
cctgcgccca gctccgcacc tgagcagacc gatgggccga ccgctgcgcc cgcaccggag 1440
acgtcctctg caccggccgc cgaaccgacg caggccccga cggtggcgcc ttctgttgag 1500
cccacgcagg ctccgggtgc gcagccgagc tcagcaccca agccgggggc gacgggtcgg 1560
gccccgtcgg tggtgaaccc gaaggcgacc ggggcggcga cggagcctgg gacgccgtca 1620
tcgagcgcga gcccggcacc gagccggaac gcggcgccga cgccgaagcc gggcatggag 1680
cccgatgaga ttgatcggcc gtctgacggc accatggcgc agccgaccgg tggcgccagc 1740
gcgccgagtg ccgcgccgac gcaggcggcg aaggccggca gcaggctgtc tcgcacgggg 1800
accaacgcgc tgctgatcct gggccttgcg ggtgtcgcgg ttgtcggcgg gtacctgctg 1860
ctgcgggctc gccgttcgaa gaactga 1887

<210> 14
<211> 628
<212> PRT
<213> Actinomyces viscosus

<400> 14
Gly Asp His Pro Gln Ala Thr Pro Ala Pro Ala Pro Asp Ala Ser Thr
1 5 10 15
Glu Leu Pro Ala Ser Met Ser Gln Ala Gln His Leu Ala Ala Asn Thr
20 25 30
Ala Thr Asp Asn Tyr Arg Ile Pro Ala Ile Thr Thr Ala Pro Asn Gly

35 40 45
Asp Leu Leu Ile Ser Tyr Asp Glu Arg Pro Lys Asp Asn Gly Asn Gly
50 55 60
Gly Ser Asp Ala Pro Asn Pro Asn His Ile Val Gln Arg Arg Ser Thr
65 70 75 80
Asp Gly Gly Lys Thr Trp Ser Ala Pro Thr Tyr Ile His Gln Gly Thr
85 90 95
Glu Thr Gly Lys Lys Val Gly Tyr Ser Asp Pro Ser Tyr Val Val Asp
100 105 110
His Gln Thr Gly Thr Ile Phe Asn Phe His Val Lys Ser Tyr Asp Gln
115 120 125
Gly Trp Gly Gly Ser Arg Gly Gly Thr Asp Pro Glu Asn Arg Gly Ile
130 135 140
Ile Gln Ala Glu Val Ser Thr Ser Thr Asp Asn Gly Trp Thr Trp Thr
145 150 155 160
His Arg Thr Ile Thr Ala Asp Ile Thr Lys Asp Lys Pro Trp Thr Ala
165 170 175
Arg Phe Ala Ala Ser Gly Gln Gly Ile Gln Ile Gln His Gly Pro His
180 185 190
Ala Gly Arg Leu Val Gln Gln Tyr Thr Ile Arg Thr Ala Gly Gly Ala
195 200 205
Val Gln Ala Val Ser Val Tyr Ser Asp Asp His Gly Lys Thr Trp Gln
210 215 220
Ala Gly Thr Pro Ile Gly Thr Gly Met Asp Glu Asn Lys Val Val Glu
225 230 235 240
Leu Ser Asp Gly Ser Leu Met Leu Asn Ser Arg Ala Ser Asp Gly Ser
245 250 255
Gly Phe Arg Lys Val Ala His Ser Thr Asp Gly Gly Gln Thr Trp Ser
260 265 270
Glu Pro Val Ser Asp Lys Asn Leu Pro Asp Ser Val Asp Asn Ala Gln
275 280 285
Ile Ile Arg Ala Phe Pro Asn Ala Ala Pro Asp Asp Pro Arg Ala Lys
290 295 300
Val Leu Leu Leu Ser His Ser Pro Asn Pro Arg Pro Trp Ser Arg Asp
305 310 315 320
Arg Gly Thr Ile Ser Met Ser Cys Asp Asp Gly Ala Ser Trp Thr Thr
325 330 335
Ser Lys Val Phe His Glu Pro Phe Val Gly Tyr Thr Thr Ile Ala Val
340 345 350
Gln Ser Asp Gly Ser Ile Gly Leu Leu Ser Glu Asp Ala His Asn Gly
355 360 365
Ala Asp Tyr Gly Gly Ile Trp Tyr Arg Asn Phe Thr Met Asn Trp Leu
370 375 380
Gly Glu Gln Cys Gly Gln Lys Pro Ala Glu Pro Ser Pro Ala Pro Ser
385 390 395 400
Pro Thr Ala Ala Pro Ser Ala Ala Pro Thr Glu Lys Pro Ala Pro Ser
405 410 415
Ala Ala Pro Ser Ala Glu Pro Thr Gln Ala Pro Ala Pro Ser Ser Ala
420 425 430
Pro Glu Pro Ser Ala Ala Pro Glu Pro Ser Ser Ala Pro Ala Pro Glu
435 440 445
Pro Thr Thr Ala Pro Ser Thr Glu Pro Thr Pro Ala Pro Ala Pro Ser
450 455 460
Ser Ala Pro Glu Gln Thr Asp Gly Pro Thr Ala Ala Pro Ala Pro Glu
465 470 475 480
Thr Ser Ser Ala Pro Ala Ala Glu Pro Thr Gln Ala Pro Thr Val Ala
485 490 495
Pro Ser Val Glu Pro Thr Gln Ala Pro Gly Ala Gln Pro Ser Ser Ala
500 505 510
Pro Lys Pro Gly Ala Thr Gly Arg Ala Pro Ser Val Val Asn Pro Lys
515 520 525
Ala Thr Gly Ala Ala Thr Glu Pro Gly Thr Pro Ser Ser Ser Ala Ser
530 535 540
Pro Ala Pro Ser Arg Asn Ala Ala Pro Thr Pro Lys Pro Gly Met Glu
545 550 555 560
Pro Asp Glu Ile Asp Arg Pro Ser Asp Gly Thr Met Ala Gln Pro Thr
565 570 575
Gly Gly Ala Ser Ala Pro Ser Ala Ala Pro Thr Gln Ala Ala Lys Ala

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            580                 585                 590
Gly Ser Arg Leu Ser Arg Thr Gly Thr Asn Ala Leu Leu Ile Leu Gly
        595                 600                 605
Leu Ala Gly Val Ala Val Val Gly Gly Tyr Leu Leu Leu Arg Ala Arg
    610                 615                 620
Arg Ser Lys Asn
625
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<210> 15
<211> 1182
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 15

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agcatgtctc aggctcagca tcttgcagca aatacggcta ctgataatta tcgcattcca 120
gcgattacaa ccgctccgaa tggtgattta ctgattagct atgatgaacg gccgaaggac 180
aatggaaatg gtggttccga tgcccctaac ccgaatcata ttgttcagcg tcgctccaca 240
gatggcggta aaacttggag cgcgccaacc tatattcatc agggtacgga gactggcaag 300
aaagtgggat attccgaccc ctcttatgtg gtggatcatc aaaccggtac aatcttcaat 360
tttcatgtga aatcatacga tcagggctgg ggaggtagcc gtgggggaac agacccggaa 420
aaccgcggga ttattcaggc agaggtgtct acgagcacgg ataatggatg gacgtggaca 480
catcgcacca tcaccgcgga tattacgaaa gataaaccgt ggaccgcgcg ttttgcggcg 540
tccggccaag gcattcagat ccagcatggg ccgcatgccg gccgtctggt gcaacagtat 600
accattcgta cggccggtgg agcggtgcag gctgtatcgg tttattccga tgatcatggg 660
aaaacgtggc aggctggcac ccccgattggg acgggtatgg atgaaaacaa agttgtagag 720
ctgtctgacg gctctctgat gctgaacagt cgtgcgtcgg acgggagcgg ctttcgtaag 780
gttgcgcata gcactgatgg tgggcagacc tggtccgaac cggtttcgga caaaaatttg 840
ccggattcgg ttgataatgc ccagataatt cgtgcgtttc ctaatgctgc ccccgatgac 900
ccgcgcgcga aagtacttct tctgagtcat tccccaaatc cacgtccgtg gtcccgggat 960
cgtggtacga taagcatgtc atgtgatgac ggggcctcat ggaccacttc caaagttttt 1020
cacgaaccgt ttgtgggcta cacgactatt gcagttcaga gtgatggaag catcggtctg 1080
ctgtcggagg acgcgcacaa tggcgctgat tatggtggca tctggtatcg taattttacg 1140
atgaactggc tgggagaaca atgtggacaa aaacccgcgg aa                   1182
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<210> 16
<211> 394
<212> PRT
<213> Actinomyces viscosus

<400> 16

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Gly Asp His Pro Gln Ala Thr Pro Ala Pro Ala Pro Asp Ala Ser Thr
1               5                   10                  15
Glu Leu Pro Ala Ser Met Ser Gln Ala Gln His Leu Ala Ala Asn Thr
            20                  25                  30
Ala Thr Asp Asn Tyr Arg Ile Pro Ala Ile Thr Thr Ala Pro Asn Gly
        35                  40                  45
Asp Leu Leu Ile Ser Tyr Asp Glu Arg Pro Lys Asp Asn Gly Asn Gly
    50                  55                  60
Gly Ser Asp Ala Pro Asn Pro Asn His Ile Val Gln Arg Arg Ser Thr
65                  70                  75                  80
Asp Gly Gly Lys Thr Trp Ser Ala Pro Thr Tyr Ile His Gln Gly Thr
                85                  90                  95
Glu Thr Gly Lys Lys Val Gly Tyr Ser Asp Pro Ser Tyr Val Val Asp
            100                 105                 110
His Gln Thr Gly Thr Ile Phe Asn Phe His Val Lys Ser Tyr Asp Gln
        115                 120                 125
Gly Trp Gly Gly Ser Arg Gly Gly Thr Asp Pro Glu Asn Arg Gly Ile
    130                 135                 140
Ile Gln Ala Glu Val Ser Thr Ser Thr Asp Asn Gly Trp Thr Trp Thr
145                 150                 155                 160
His Arg Thr Ile Thr Ala Asp Ile Thr Lys Asp Lys Pro Trp Thr Ala
                165                 170                 175
Arg Phe Ala Ala Ser Gly Gln Gly Ile Gln Ile Gln His Gly Pro His
```

```
                180                     185                     190
Ala Gly Arg Leu Val Gln Gln Tyr Thr Ile Arg Thr Ala Gly Gly Ala
        195                     200                     205
Val Gln Ala Val Ser Val Tyr Ser Asp Asp His Gly Lys Thr Trp Gln
    210                     215                     220
Ala Gly Thr Pro Ile Gly Thr Gly Met Asp Glu Asn Lys Val Val Glu
225                     230                     235                     240
Leu Ser Asp Gly Ser Leu Met Leu Asn Ser Arg Ala Ser Asp Gly Ser
                245                     250                     255
Gly Phe Arg Lys Val Ala His Ser Thr Asp Gly Gly Gln Thr Trp Ser
            260                     265                     270
Glu Pro Val Ser Asp Lys Asn Leu Pro Asp Ser Val Asp Asn Ala Gln
        275                     280                     285
Ile Ile Arg Ala Phe Pro Asn Ala Ala Pro Asp Asp Pro Arg Ala Lys
    290                     295                     300
Val Leu Leu Leu Ser His Ser Pro Asn Pro Arg Pro Trp Ser Arg Asp
305                     310                     315                     320
Arg Gly Thr Ile Ser Met Ser Cys Asp Asp Gly Ala Ser Trp Thr Thr
                325                     330                     335
Ser Lys Val Phe His Glu Pro Phe Val Gly Tyr Thr Thr Ile Ala Val
            340                     345                     350
Gln Ser Asp Gly Ser Ile Gly Leu Leu Ser Glu Asp Ala His Asn Gly
        355                     360                     365
Ala Asp Tyr Gly Gly Ile Trp Tyr Arg Asn Phe Thr Met Asn Trp Leu
    370                     375                     380
Gly Glu Gln Cys Gly Gln Lys Pro Ala Glu
385                     390
```

<210> 17
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 17

```
Met Val Lys Arg Lys Lys
 1               5
```

<210> 18
<211> 1281
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 18

```
ccatggttaa gcgcaaaaaa aaaggcggca aaaacggtaa aaatcgtcgt aaccgtaaga 60
aaaaaaatcc tggagatcat ccacaagcta caccagcacc tgcaccagat gctagcactg 120
agctgccagc aagcatgtct caggctcagc atcttgcagc aaatacggct actgataatt 180
atcgcattcc agcgattaca accgctccga atggtgattt actgattagc tatgatgaac 240
ggccgaagga caatggaaat ggtggttccg atgcccctaa cccgaatcat attgttcagc 300
gtcgctccac agatggcggt aaaacttgga gcgcgccaac ctatattcat cagggtacgg 360
agactggcaa gaaagtggga tattccgacc cctcttatgt ggtggatcat caaaccggta 420
caatcttcaa ttttcatgtg aaatcatacg atcagggctg gggaggtagc cgtgggggaa 480
cagacccgga aaccgcgggg attattcagg cagaggtgtc tacgagcacg gataatggat 540
ggacgtggac acatcgcacc atcaccgcgg atattacgaa agataaaccg tggaccgcgc 600
gttttgcggc gtccggccaa ggcattcaga tccagcatgg gccgcatgcc ggccgtctgg 660
tgcaacagta taccattcgt acggccggtg gagcggtgca ggctgtatcg gtttattccg 720
atgatcatgg gaaaacgtgg caggctggca ccccgattgg gacgggtatg gatgaaaaca 780
aagttgtaga gctgtctgac ggctctctga tgctgaacag tcgtgcgtcg gacgggagcg 840
gctttcgtaa ggttgcgcat agcactgatg gtgggcagac ctggtccgaa ccggtttcgg 900
acaaaaattt gccggattcg gttgataatg cccagataat tcgtgcgttt cctaatgctg 960
cccccgatga cccgcgcgcg aaagtacttc ttctgagtca ttccccaaat ccacgtccgt 1020
```

ggtcccggga tcgtggtacg ataagcatgt catgtgatga cggggcctca tggaccactt 1080
ccaaagtttt tcacgaaccg tttgtgggct acacgactat tgcagttcag agtgatggaa 1140
gcatcggtct gctgtcggag gacgcgcaca atggcgctga ttatggtggc atctggtatc 1200
gtaattttac gatgaactgg ctgggagaac aatgtggaca aaaacccgcg gaataagctt 1260
aaaaacccgc ggaataagct t 1281

<210> 19
<211> 444
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 19
Met Val Lys Arg Lys Lys Lys Gly Gly Lys Asn Gly Lys Asn Arg Arg
1 5 10 15
Asn Arg Lys Lys Lys Asn Pro Gly Asp His Pro Gln Ala Thr Pro Ala
20 25 30
Pro Ala Pro Asp Ala Ser Thr Glu Leu Pro Ala Ser Met Ser Gln Ala
35 40 45
Gln His Leu Ala Ala Asn Thr Ala Thr Asp Asn Tyr Arg Ile Pro Ala
50 55 60
Ile Thr Thr Ala Pro Asn Gly Asp Leu Leu Ile Ser Tyr Asp Glu Arg
65 70 75 80
Pro Lys Asp Asn Gly Asn Gly Gly Ser Asp Ala Pro Asn Pro Asn His
85 90 95
Ile Val Gln Arg Arg Ser Thr Asp Gly Gly Lys Thr Trp Ser Ala Pro
100 105 110
Thr Tyr Ile His Gln Gly Thr Glu Thr Gly Lys Lys Val Gly Tyr Ser
115 120 125
Asp Pro Ser Tyr Val Val Asp His Gln Thr Gly Thr Ile Phe Asn Phe
130 135 140
His Val Lys Ser Tyr Asp Gln Gly Trp Gly Gly Ser Arg Gly Gly Thr
145 150 155 160
Asp Pro Glu Asn Arg Gly Ile Ile Gln Ala Glu Val Ser Thr Ser Thr
165 170 175
Asp Asn Gly Trp Thr Trp Thr His Arg Thr Ile Thr Ala Asp Ile Thr
180 185 190
Lys Asp Lys Pro Trp Thr Ala Arg Phe Ala Ala Ser Gly Gln Gly Ile
195 200 205
Gln Ile Gln His Gly Pro His Ala Gly Arg Leu Val Gln Gln Tyr Thr
210 215 220
Ile Arg Thr Ala Gly Gly Ala Val Gln Ala Val Ser Val Tyr Ser Asp
225 230 235 240
Asp His Gly Lys Thr Trp Gln Ala Gly Thr Pro Ile Gly Thr Gly Met
245 250 255
Asp Glu Asn Lys Val Val Glu Leu Ser Asp Gly Ser Leu Met Leu Asn
260 265 270
Ser Arg Ala Ser Asp Gly Ser Gly Phe Arg Lys Val Ala His Ser Thr
275 280 285
Asp Gly Gly Gln Thr Trp Ser Glu Pro Val Ser Asp Lys Asn Leu Pro
290 295 300
Asp Ser Val Asp Asn Ala Gln Ile Ile Arg Ala Phe Pro Asn Ala Ala
305 310 315 320
Pro Asp Asp Pro Arg Ala Lys Val Leu Leu Leu Ser His Ser Pro Asn
325 330 335
Pro Arg Pro Trp Ser Arg Asp Arg Gly Thr Ile Ser Met Ser Cys Asp
340 345 350
Asp Gly Ala Ser Trp Thr Thr Ser Lys Val Phe His Glu Pro Phe Val
355 360 365
Gly Tyr Thr Thr Ile Ala Val Gln Ser Asp Gly Ser Ile Gly Leu Leu
370 375 380
Ser Glu Asp Ala His Asn Gly Ala Asp Tyr Gly Gly Ile Trp Tyr Arg
385 390 395 400
Asn Phe Thr Met Asn Trp Leu Gly Glu Gln Cys Gly Gln Lys Pro Ala
405 410 415
Glu Gly Ala Asp Tyr Gly Gly Ile Trp Tyr Arg Asn Phe Thr Met Asn

420 425 430
Trp Leu Gly Glu Gln Cys Gly Gln Lys Pro Ala Glu
435 440

<210> 20
<211> 1248
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 20
atgggagatc atccacaagc tacaccagca cctgcaccag atgctagcac tgagctgcca 60
gcaagcatgt ctcaggctca gcatcttgca gcaaatacgg ctactgataa ttatcgcatt 120
ccagcgatta caaccgctcc gaatggtgat ttactgatta gctatgatga acggccgaag 180
gacaatggaa atggtggttc cgatgcccct aacccgaatc atattgttca gcgtcgctcc 240
acagatggcg gtaaaacttg gagcgcgcca acctatattc atcagggtac ggagactggc 300
aagaaagtgg gatattccga cccctcttat gtggtggatc atcaaaccgg tacaatcttc 360
aattttcatg tgaaatcata cgatcagggc tggggaggta gccgtggggg aacagacccg 420
gaaaaccgcg ggattattca ggcagaggtg tctacgagca cggataatgg atggacgtgg 480
acacatcgca ccatcaccgc ggatattacg aaagataaac cgtggaccgc gcgttttgcg 540
gcgtccggcc aaggcattca gatccagcat gggccgcatg ccggccgtct ggtgcaacag 600
tataccattc gtacggccgg tggagcggtg caggctgtat cggtttattc cgatgatcat 660
gggaaaacgt ggcaggctgg caccccgatt gggacgggta tggatgaaaa caaagttgta 720
gagctgtctg acggctctct gatgctgaac agtcgtgcgt cggacgggag cggctttcgt 780
aaggttgcgc atagcactga tggtgggcag acctggtccg aaccggtttc ggacaaaaat 840
ttgccggatt cggttgataa tgcccagata attcgtgcgt ttcctaatgc tgcccccgat 900
gacccgcgcg cgaaagtact tcttctgagt cattccccaa atccacgtcc gtggtcccgg 960
gatcgtggta cgataagcat gtcatgtgat gacggggcct catggaccac ttccaaagtt 1020
tttcacgaac cgtttgtggg ctacacgact attgcagttc agagtgatgg aagcatcggt 1080
ctgctgtcgg aggacgcgca caatggcgct gattatggtg gcatctggta tcgtaatttt 1140
acgatgaact ggctgggaga acaatgtgga caaaaacccg cgaagcgcaa aaaaaaaggc 1200
ggcaaaaacg gtaaaaatcg tcgtaaccgt aagaaaaaaa atccttga 1248

<210> 21
<211> 415
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 21
Met Gly Asp His Pro Gln Ala Thr Pro Ala Pro Ala Pro Asp Ala Ser
1 5 10 15
Thr Glu Leu Pro Ala Ser Met Ser Gln Ala Gln His Leu Ala Ala Asn
20 25 30
Thr Ala Thr Asp Asn Tyr Arg Ile Pro Ala Ile Thr Thr Ala Pro Asn
35 40 45
Gly Asp Leu Leu Ile Ser Tyr Asp Glu Arg Pro Lys Asp Asn Gly Asn
50 55 60
Gly Gly Ser Asp Ala Pro Asn Pro Asn His Ile Val Gln Arg Arg Ser
65 70 75 80
Thr Asp Gly Gly Lys Thr Trp Ser Ala Pro Thr Tyr Ile His Gln Gly
85 90 95
Thr Glu Thr Gly Lys Lys Val Gly Tyr Ser Asp Pro Ser Tyr Val Val
100 105 110
Asp His Gln Thr Gly Thr Ile Phe Asn Phe His Val Lys Ser Tyr Asp
115 120 125
Gln Gly Trp Gly Gly Ser Arg Gly Gly Thr Asp Pro Glu Asn Arg Gly
130 135 140
Ile Ile Gln Ala Glu Val Ser Thr Ser Thr Asp Asn Gly Trp Thr Trp
145 150 155 160
Thr His Arg Thr Ile Thr Ala Asp Ile Thr Lys Asp Lys Pro Trp Thr
165 170 175
Ala Arg Phe Ala Ala Ser Gly Gln Gly Ile Gln Ile Gln His Gly Pro

```
            180                     185                     190
His Ala Gly Arg Leu Val Gln Gln Tyr Thr Ile Arg Thr Ala Gly Gly
        195                     200                     205
Ala Val Gln Ala Val Ser Val Tyr Ser Asp Asp His Gly Lys Thr Trp
    210                     215                     220
Gln Ala Gly Thr Pro Ile Gly Thr Gly Met Asp Glu Asn Lys Val Val
225                     230                     235                     240
Glu Leu Ser Asp Gly Ser Leu Met Leu Asn Ser Arg Ala Ser Asp Gly
                245                     250                     255
Ser Gly Phe Arg Lys Val Ala His Ser Thr Asp Gly Gly Gln Thr Trp
            260                     265                     270
Ser Glu Pro Val Ser Asp Lys Asn Leu Pro Asp Ser Val Asp Asn Ala
        275                     280                     285
Gln Ile Ile Arg Ala Phe Pro Asn Ala Ala Pro Asp Asp Pro Arg Ala
    290                     295                     300
Lys Val Leu Leu Leu Ser His Ser Pro Asn Pro Arg Pro Trp Ser Arg
305                     310                     315                     320
Asp Arg Gly Thr Ile Ser Met Ser Cys Asp Asp Gly Ala Ser Trp Thr
                325                     330                     335
Thr Ser Lys Val Phe His Glu Pro Phe Val Gly Tyr Thr Thr Ile Ala
            340                     345                     350
Val Gln Ser Asp Gly Ser Ile Gly Leu Leu Ser Glu Asp Ala His Asn
        355                     360                     365
Gly Ala Asp Tyr Gly Gly Ile Trp Tyr Arg Asn Phe Thr Met Asn Trp
    370                     375                     380
Leu Gly Glu Gln Cys Gly Gln Lys Pro Ala Lys Arg Lys Lys Lys Gly
385                     390                     395                     400
Gly Lys Asn Gly Lys Asn Arg Arg Asn Arg Lys Lys Lys Asn Pro
                405                     410                     415
```

<210> 22
<211> 1293
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 22

```
atgggagatc atccacaagc tacaccagca cctgcaccag atgctagcac tgagctgcca 60
gcaagcatgt ctcaggctca gcatcttgca gcaaatacgg ctactgataa ttatcgcatt 120
ccagcgatta caaccgctcc gaatggtgat ttactgatta gctatgatga acggccgaag 180
gacaatggaa atggtggttc cgatgcccct aacccgaatc atattgttca gcgtcgctcc 240
acagatggcg gtaaaacttg gagcgcgcca acctatattc atcagggtac ggagactggc 300
aagaaagtgg gatattccga cccctcttat gtggtggatc atcaaaccgg tacaatcttc 360
aattttcatg tgaaatcata cgatcagggc tggggaggta gccgtggggg aacagacccg 420
gaaaaccgcg ggattattca ggcagaggtg tctacgagca cggataatgg atggacgtgg 480
acacatcgca ccatcaccgc ggatattacg aaagataaac cgtggaccgc gcgttttgcg 540
gcgtccggcc aaggcattca gatccagcat gggccgcatg ccggccgtct ggtgcaacag 600
tataccattc gtacggccgg tggagcggtg caggctgtat cggtttattc cgatgatcat 660
gggaaaacgt ggcaggctgg caccccgatt gggacgggta tggatgaaaa caaagttgta 720
gagctgtctg acggctctct gatgctgaac agtcgtgcgt cggacgggag cggctttcgt 780
aaggttgcgc atagcactga tggtggcag acctggtccg aaccggtttc ggacaaaaat 840
ttgccggatt cggttgataa tgcccagata attcgtgcgt ttcctaatgc tgcccccgat 900
gacccgcgcg cgaaagtact tcttctgagt cattccccaa atccacgtcc gtggtcccgg 960
gatcgtggta cgataagcat gtcatgtgat gacggggcct catggaccac ttccaaagtt 1020
tttcacgaac cgtttgtggg ctacacgact attgcagttc agagtgatgg aagcatcggt 1080
ctgctgtcgg aggacgcgca caatggcgct gattatggtg gcatctggta tcgtaatttt 1140
acgatgaact ggctgggaga acaatgtgga caaaaacccg cggaaccgag cccagcccct 1200
agccctactg cagcaccgtc cgctgcaaag cgcaaaaaaa aaggcggcaa aaacggtaaa 1260
aatcgtcgta accgtaagaa aaaaaatcct tga                              1293
```

<210> 23
<211> 430
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 23
```
Met Gly Asp His Pro Gln Ala Thr Pro Ala Pro Ala Pro Asp Ala Ser
1               5                   10                  15
Thr Glu Leu Pro Ala Ser Met Ser Gln Ala Gln His Leu Ala Ala Asn
            20                  25                  30
Thr Ala Thr Asp Asn Tyr Arg Ile Pro Ala Ile Thr Thr Ala Pro Asn
        35                  40                  45
Gly Asp Leu Leu Ile Ser Tyr Asp Glu Arg Pro Lys Asp Asn Gly Asn
    50                  55                  60
Gly Gly Ser Asp Ala Pro Asn Pro Asn His Ile Val Gln Arg Arg Ser
65                  70                  75                  80
Thr Asp Gly Gly Lys Thr Trp Ser Ala Pro Thr Tyr Ile His Gln Gly
                85                  90                  95
Thr Glu Thr Gly Lys Lys Val Gly Tyr Ser Asp Pro Ser Tyr Val Val
            100                 105                 110
Asp His Gln Thr Gly Thr Ile Phe Asn Phe His Val Lys Ser Tyr Asp
        115                 120                 125
Gln Gly Trp Gly Gly Ser Arg Gly Gly Thr Asp Pro Glu Asn Arg Gly
    130                 135                 140
Ile Ile Gln Ala Glu Val Ser Thr Ser Thr Asp Asn Gly Trp Thr Trp
145                 150                 155                 160
Thr His Arg Thr Ile Thr Ala Asp Ile Thr Lys Asp Lys Pro Trp Thr
                165                 170                 175
Ala Arg Phe Ala Ala Ser Gly Gln Gly Ile Gln Ile Gln His Gly Pro
            180                 185                 190
His Ala Gly Arg Leu Val Gln Gln Tyr Thr Ile Arg Thr Ala Gly Gly
        195                 200                 205
Ala Val Gln Ala Val Ser Val Tyr Ser Asp Asp His Gly Lys Thr Trp
    210                 215                 220
Gln Ala Gly Thr Pro Ile Gly Thr Gly Met Asp Glu Asn Lys Val Val
225                 230                 235                 240
Glu Leu Ser Asp Gly Ser Leu Met Leu Asn Ser Arg Ala Ser Asp Gly
                245                 250                 255
Ser Gly Phe Arg Lys Val Ala His Ser Thr Asp Gly Gly Gln Thr Trp
            260                 265                 270
Ser Glu Pro Val Ser Asp Lys Asn Leu Pro Asp Ser Val Asp Asn Ala
        275                 280                 285
Gln Ile Ile Arg Ala Phe Pro Asn Ala Ala Pro Asp Asp Pro Arg Ala
    290                 295                 300
Lys Val Leu Leu Leu Ser His Ser Pro Asn Pro Arg Pro Trp Ser Arg
305                 310                 315                 320
Asp Arg Gly Thr Ile Ser Met Ser Cys Asp Asp Gly Ala Ser Trp Thr
                325                 330                 335
Thr Ser Lys Val Phe His Glu Pro Phe Val Gly Tyr Thr Thr Ile Ala
            340                 345                 350
Val Gln Ser Asp Gly Ser Ile Gly Leu Leu Ser Glu Asp Ala His Asn
        355                 360                 365
Gly Ala Asp Tyr Gly Gly Ile Trp Tyr Arg Asn Phe Thr Met Asn Trp
    370                 375                 380
Leu Gly Glu Gln Cys Gly Gln Lys Pro Ala Glu Pro Ser Pro Ala Pro
385                 390                 395                 400
Ser Pro Thr Ala Ala Pro Ser Ala Ala Lys Arg Lys Lys Lys Gly Gly
                405                 410                 415
Lys Asn Gly Lys Asn Arg Arg Asn Arg Lys Lys Lys Asn Pro
            420                 425                 430
```

<210> 24
<211> 1203
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 24
atgggagagc tgccagcaag catgtctcag gctcagcatc ttgcagcaaa tacggctact 60
gataattatc gcattccagc gattacaacc gctccgaatg gtgatttact gattagctat 120
gatgaacggc cgaaggacaa tggaaatggt ggttccgatg cccctaaccc gaatcatatt 180
gttcagcgtc gctccacaga tggcggtaaa acttggagcg cgccaaccta tattcatcag 240
ggtacggaga ctggcaagaa agtgggatat tccgacccct cttatgtggt ggatcatcaa 300
accggtacaa tcttcaattt tcatgtgaaa tcatacgatc agggctgggg aggtagccgt 360
gggggaacag acccggaaaa ccgcgggatt attcaggcag aggtgtctac gagcacggat 420
aatggatgga cgtggacaca tcgcaccatc accgcggata ttacgaaaga taaaccgtgg 480
accgcgcgtt ttgcggcgtc cggccaaggc attcagatcc agcatgggcc gcatgccggc 540
cgtctggtgc aacagtatac cattcgtacg gccggtggag cggtgcaggc tgtatcggtt 600
tattccgatg atcatgggaa aacgtggcag gctggcaccc cgattgggac gggtatggat 660
gaaaacaaag ttgtagagct gtctgacggc tctctgatgc tgaacagtcg tgcgtcggac 720
gggagcggct ttcgtaaggt tgcgcatagc actgatggtg ggcagacctg gtccgaaccg 780
gtttcggaca aaaatttgcc ggattcggtt gataatgccc agataattcg tgcgtttcct 840
aatgctgccc ccgatgaccc gcgcgcgaaa gtacttcttc tgagtcattc ccccaaatcca 900
cgtccgtggt cccgggatcg tggtacgata agcatgtcat gtgatgacgg ggcctcatgg 960
accacttcca aagtttttca cgaaccgttt gtgggctaca cgactattgc agttcagagt 1020
gatggaagca tcggtctgct gtcggaggac gcgcacaatg gcgctgatta tggtggcatc 1080
tggtatcgta attttacgat gaactggctg ggagaacaat gtggacaaaa acccgcgaag 1140
cgcaaaaaaa aaggcggcaa aaacggtaaa aatcgtcgta accgtaagaa aaaaaatcct 1200
tga 1203

<210> 25
<211> 400
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 25
Met Gly Glu Leu Pro Ala Ser Met Ser Gln Ala Gln His Leu Ala Ala
1 5 10 15
Asn Thr Ala Thr Asp Asn Tyr Arg Ile Pro Ala Ile Thr Thr Ala Pro
20 25 30
Asn Gly Asp Leu Leu Ile Ser Tyr Asp Glu Arg Pro Lys Asp Asn Gly
35 40 45
Asn Gly Gly Ser Asp Ala Pro Asn Pro Asn His Ile Val Gln Arg Arg
50 55 60
Ser Thr Asp Gly Gly Lys Thr Trp Ser Ala Pro Thr Tyr Ile His Gln
65 70 75 80
Gly Thr Glu Thr Gly Lys Lys Val Gly Tyr Ser Asp Pro Ser Tyr Val
85 90 95
Val Asp His Gln Thr Gly Thr Ile Phe Asn Phe His Val Lys Ser Tyr
100 105 110
Asp Gln Gly Trp Gly Gly Ser Arg Gly Gly Thr Asp Pro Glu Asn Arg
115 120 125
Gly Ile Ile Gln Ala Glu Val Ser Thr Ser Thr Asp Asn Gly Trp Thr
130 135 140
Trp Thr His Arg Thr Ile Thr Ala Asp Ile Thr Lys Asp Lys Pro Trp
145 150 155 160
Thr Ala Arg Phe Ala Ala Ser Gly Gln Gly Ile Gln Ile Gln His Gly
165 170 175
Pro His Ala Gly Arg Leu Val Gln Gln Tyr Thr Ile Arg Thr Ala Gly
180 185 190
Gly Ala Val Gln Ala Val Ser Val Tyr Ser Asp Asp His Gly Lys Thr
195 200 205
Trp Gln Ala Gly Thr Pro Ile Gly Thr Gly Met Asp Glu Asn Lys Val
210 215 220
Val Glu Leu Ser Asp Gly Ser Leu Met Leu Asn Ser Arg Ala Ser Asp
225 230 235 240
Gly Ser Gly Phe Arg Lys Val Ala His Ser Thr Asp Gly Gly Gln Thr
245 250 255
Trp Ser Glu Pro Val Ser Asp Lys Asn Leu Pro Asp Ser Val Asp Asn
260 265 270
Ala Gln Ile Ile Arg Ala Phe Pro Asn Ala Ala Pro Asp Asp Pro Arg
275 280 285

Ala Lys Val Leu Leu Leu Ser His Ser Pro Asn Pro Arg Pro Trp Ser
    290   295   300
Arg Asp Arg Gly Thr Ile Ser Met Ser Cys Asp Asp Gly Ala Ser Trp
305   310   315   320
Thr Thr Ser Lys Val Phe His Glu Pro Phe Val Gly Tyr Thr Thr Ile
                325   330   335
Ala Val Gln Ser Asp Gly Ser Ile Gly Leu Leu Ser Glu Asp Ala His
            340   345   350
Asn Gly Ala Asp Tyr Gly Gly Ile Trp Tyr Arg Asn Phe Thr Met Asn
        355   360   365
Trp Leu Gly Glu Gln Cys Gly Gln Lys Pro Ala Lys Arg Lys Lys Lys
    370   375   380
Gly Gly Lys Asn Gly Lys Asn Arg Arg Asn Arg Lys Lys Lys Asn Pro
385   390   395   400

<210> 26
<211> 1248
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 26
atgggagagc tgccagcaag catgtctcag gctcagcatc ttgcagcaaa tacggctact 60
gataattatc gcattccagc gattacaacc gctccgaatg gtgatttact gattagctat 120
gatgaacggc cgaaggacaa tggaaatggt ggttccgatg cccctaaccc gaatcatatt 180
gttcagcgtc gctccacaga tggcggtaaa acttggagcg cgccaaccta tattcatcag 240
ggtacggaga ctggcaagaa agtgggatat tccgacccct cttatgtggt ggatcatcaa 300
accggtacaa tcttcaattt tcatgtgaaa tcatacgatc agggctgggg aggtagccgt 360
gggggaacag acccggaaaa ccgcgggatt attcaggcag aggtgtctac gagcacggat 420
aatggatgga cgtggacaca tcgcaccatc accgcggata ttacgaaaga taaaccgtgg 480
accgcgcgtt ttgcggcgtc cggccaaggc attcagatcc agcatgggcc gcatgccggc 540
cgtctggtgc aacagtatac cattcgtacg gccggtggag cggtgcaggc tgtatcggtt 600
tattccgatg atcatgggaa aacgtggcag gctggcaccc cgattgggac gggtatggat 660
gaaaacaaag ttgtagagct gtctgacggc tctctgatgc tgaacagtcg tgcgtcggac 720
gggagcggct ttcgtaaggt tgcgcatagc actgatggtg ggcagacctg gtccgaaccg 780
gtttcggaca aaaatttgcc ggattcggtt gataatgccc agataattcg tgcgtttcct 840
aatgctgccc ccgatgaccc gcgcgcgaaa gtacttcttc tgagtcattc cccaaatcca 900
cgtccgtggt cccgggatcg tggtacgata agcatgtcat gtgatgacgg ggcctcatgg 960
accacttcca aagtttttca cgaaccgttt gtgggctaca cgactattgc agttcagagt 1020
gatggaagca tcggtctgct gtcggaggac gcgcacaatg gcgctgatta tggtggcatc 1080
tggtatcgta attttacgat gaactggctg ggagaacaat gtggacaaaa acccgcggaa 1140
ccgagcccag cccctagccc tactgcagca ccgtccgctg caaagcgcaa aaaaaaaggc 1200
ggcaaaaacg gtaaaaatcg tcgtaaccgt aagaaaaaaa atccttga   1248

<210> 27
<211> 415
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 27
Met Gly Glu Leu Pro Ala Ser Met Ser Gln Ala Gln His Leu Ala Ala
1   5   10   15
Asn Thr Ala Thr Asp Asn Tyr Arg Ile Pro Ala Ile Thr Thr Ala Pro
            20   25   30
Asn Gly Asp Leu Leu Ile Ser Tyr Asp Glu Arg Pro Lys Asp Asn Gly
        35   40   45
Asn Gly Gly Ser Asp Ala Pro Asn Pro Asn His Ile Val Gln Arg Arg
    50   55   60
Ser Thr Asp Gly Gly Lys Thr Trp Ser Ala Pro Thr Tyr Ile His Gln
65   70   75   80
Gly Thr Glu Thr Gly Lys Lys Val Gly Tyr Ser Asp Pro Ser Tyr Val
                85   90   95

Val Asp His Gln Thr Gly Thr Ile Phe Asn Phe His Val Lys Ser Tyr
100 105 110
Asp Gln Gly Trp Gly Gly Ser Arg Gly Gly Thr Asp Pro Glu Asn Arg
115 120 125
Gly Ile Ile Gln Ala Glu Val Ser Thr Ser Thr Asp Asn Gly Trp Thr
130 135 140
Trp Thr His Arg Thr Ile Thr Ala Asp Ile Thr Lys Asp Lys Pro Trp
145 150 155 160
Thr Ala Arg Phe Ala Ala Ser Gly Gln Gly Ile Gln Ile Gln His Gly
165 170 175
Pro His Ala Gly Arg Leu Val Gln Gln Tyr Thr Ile Arg Thr Ala Gly
180 185 190
Gly Ala Val Gln Ala Val Ser Val Tyr Ser Asp Asp His Gly Lys Thr
195 200 205
Trp Gln Ala Gly Thr Pro Ile Gly Thr Gly Met Asp Glu Asn Lys Val
210 215 220
Val Glu Leu Ser Asp Gly Ser Leu Met Leu Asn Ser Arg Ala Ser Asp
225 230 235 240
Gly Ser Gly Phe Arg Lys Val Ala His Ser Thr Asp Gly Gly Gln Thr
245 250 255
Trp Ser Glu Pro Val Ser Asp Lys Asn Leu Pro Asp Ser Val Asp Asn
260 265 270
Ala Gln Ile Ile Arg Ala Phe Pro Asn Ala Ala Pro Asp Asp Pro Arg
275 280 285
Ala Lys Val Leu Leu Leu Ser His Ser Pro Asn Pro Arg Pro Trp Ser
290 295 300
Arg Asp Arg Gly Thr Ile Ser Met Ser Cys Asp Asp Gly Ala Ser Trp
305 310 315 320
Thr Thr Ser Lys Val Phe His Glu Pro Phe Val Gly Tyr Thr Thr Ile
325 330 335
Ala Val Gln Ser Asp Gly Ser Ile Gly Leu Leu Ser Glu Asp Ala His
340 345 350
Asn Gly Ala Asp Tyr Gly Gly Ile Trp Tyr Arg Asn Phe Thr Met Asn
355 360 365
Trp Leu Gly Glu Gln Cys Gly Gln Lys Pro Ala Glu Pro Ser Pro Ala
370 375 380
Pro Ser Pro Thr Ala Ala Pro Ser Ala Ala Lys Arg Lys Lys Lys Gly
385 390 395 400
Gly Lys Asn Gly Lys Asn Arg Arg Asn Arg Lys Lys Lys Asn Pro
405 410 415

<210> 28
<211> 1221
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 28
ccatggggca tcaccatcac catcatctag agggagatca tccacaagct acaccagcac 60
ctgcaccaga tgctagcact gagctgccag caagcatgtc tcaggctcag catcttgcag 120
caaatacggc tactgataat tatcgcattc cagcgattac aaccgctccg aatggtgatt 180
tactgattag ctatgatgaa cggccgaagg acaatggaaa tggtggttcc gatgccccta 240
acccgaatca tattgttcag cgtcgctcca cagatggcgg taaaacttgg agcgcgccaa 300
cctatattca tcagggtacg gagactggca agaaagtggg atattccgac cctcttatg 360
tggtggatca tcaaaccggt acaatcttca attttcatgt gaaatcatac gatcagggct 420
ggggaggtag ccgtggggga acagacccgg aaaaccgcgg gattattcag gcagaggtgt 480
ctacgagcac ggataatgga tggacgtgga cacatcgcac catcaccgcg gatattacga 540
aagataaacc gtggaccgcg cgttttgcgg cgtccggcca aggcattcag atccagcatg 600
ggccgcatgc cggccgtctg gtgcaacagt ataccattcg tacggccggt ggagcggtgc 660
aggctgtatc ggtttattcc gatgatcatg ggaaaacgtg gcaggctggc accccgattg 720
ggacgggtat ggatgaaaac aaagttgtag agctgtctga cggctctctg atgctgaaca 780
gtcgtgcgtc ggacgggagc ggctttcgta aggttgcgca tagcactgat ggtgggcaga 840
cctggtccga accggtttcg gacaaaaatt tgccggattc ggttgataat gcccagataa 900
ttcgtgcgtt tcctaatgct gcccccgatg acccgcgcgc gaaagtactt cttctgagtc 960
attccccaaa tccacgtccg tggtcccggg atcgtggtac gataagcatg tcatgtgatg 1020

acggggcctc atggaccact tccaaagttt ttcacgaacc gtttgtgggc tacacgacta 1080
ttgcagttca gagtgatgga agcatcggtc tgctgtcgga ggacgcgcac aatggcgctg 1140
attatggtgg catctggtat cgtaatttta cgatgaactg gctgggagaa caatgtggac 1200
aaaaacccgc ggaataagct t 1221

<210> 29
<211> 404
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 29
Met Gly His His His His His His Leu Glu Gly Asp His Pro Gln Ala
1   5   10   15
Thr Pro Ala Pro Ala Pro Asp Ala Ser Thr Glu Leu Pro Ala Ser Met
            20   25   30
Ser Gln Ala Gln His Leu Ala Ala Asn Thr Ala Thr Asp Asn Tyr Arg
        35   40   45
Ile Pro Ala Ile Thr Thr Ala Pro Asn Gly Asp Leu Leu Ile Ser Tyr
    50   55   60
Asp Glu Arg Pro Lys Asp Asn Gly Asn Gly Gly Ser Asp Ala Pro Asn
65   70   75   80
Pro Asn His Ile Val Gln Arg Arg Ser Thr Asp Gly Gly Lys Thr Trp
                85   90   95
Ser Ala Pro Thr Tyr Ile His Gln Gly Thr Glu Thr Gly Lys Lys Val
            100   105   110
Gly Tyr Ser Asp Pro Ser Tyr Val Val Asp His Gln Thr Gly Thr Ile
        115   120   125
Phe Asn Phe His Val Lys Ser Tyr Asp Gln Gly Trp Gly Gly Ser Arg
    130   135   140
Gly Gly Thr Asp Pro Glu Asn Arg Gly Ile Ile Gln Ala Glu Val Ser
145   150   155   160
Thr Ser Thr Asp Asn Gly Trp Thr Trp Thr His Arg Thr Ile Thr Ala
                165   170   175
Asp Ile Thr Lys Asp Lys Pro Trp Thr Ala Arg Phe Ala Ala Ser Gly
            180   185   190
Gln Gly Ile Gln Ile Gln His Gly Pro His Ala Gly Arg Leu Val Gln
        195   200   205
Gln Tyr Thr Ile Arg Thr Ala Gly Gly Ala Val Gln Ala Val Ser Val
    210   215   220
Tyr Ser Asp Asp His Gly Lys Thr Trp Gln Ala Gly Thr Pro Ile Gly
225   230   235   240
Thr Gly Met Asp Glu Asn Lys Val Val Glu Leu Ser Asp Gly Ser Leu
                245   250   255
Met Leu Asn Ser Arg Ala Ser Asp Gly Ser Gly Phe Arg Lys Val Ala
            260   265   270
His Ser Thr Asp Gly Gly Gln Thr Trp Ser Glu Pro Val Ser Asp Lys
        275   280   285
Asn Leu Pro Asp Ser Val Asp Asn Ala Gln Ile Ile Arg Ala Phe Pro
    290   295   300
Asn Ala Ala Pro Asp Asp Pro Arg Ala Lys Val Leu Leu Leu Ser His
305   310   315   320
Ser Pro Asn Pro Arg Pro Trp Ser Arg Asp Arg Gly Thr Ile Ser Met
                325   330   335
Ser Cys Asp Asp Gly Ala Ser Trp Thr Thr Ser Lys Val Phe His Glu
            340   345   350
Pro Phe Val Gly Tyr Thr Thr Ile Ala Val Gln Ser Asp Gly Ser Ile
        355   360   365
Gly Leu Leu Ser Glu Asp Ala His Asn Gly Ala Asp Tyr Gly Gly Ile
    370   375   380
Trp Tyr Arg Asn Phe Thr Met Asn Trp Leu Gly Glu Gln Cys Gly Gln
385   390   395   400
Lys Pro Ala Glu

<210> 30
<211> 1257
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 30
ccatgaagcg caaaaaaaaa ggcggcaaaa acggtaaaaa tcgtcgtaac cgtaagaaaa 60
aaaatcctgg agatcatcca caagctacac cagcacctgc accagatgct agcactgagc 120
tgccagcaag catgtctcag gctcagcatc ttgcagcaaa tacggctact gataattatc 180
gcattccagc gattacaacc gctccgaatg gtgatttact gattagctat gatgaacggc 240
cgaaggacaa tggaaatggt ggttccgatg cccctaaccc gaatcatatt gttcagcgtc 300
gctccacaga tggcggtaaa acttggagcg cgccaaccta tattcatcag ggtacggaga 360
ctggcaagaa agtgggatat tccgacccct cttatgtggt ggatcatcaa accggtacaa 420
tcttcaattt tcatgtgaaa tcatacgatc agggctgggg aggtagccgt gggggaacag 480
acccggaaaa ccgcgggatt attcaggcag aggtgtctac gagcacggat aatggatgga 540
cgtggacaca tcgcaccatc accgcggata ttacgaaaga taaaccgtgg accgcgcgtt 600
ttgcggcgtc cggccaaggc attcagatcc agcatgggcc gcatgccggc cgtctggtgc 660
aacagtatac cattcgtacg gccggtggag cggtgcaggc tgtatcggtt tattccgatg 720
atcatgggaa aacgtggcag gctggcaccc cgattgggac gggtatggat gaaaacaaag 780
ttgtagagct gtctgacggc tctctgatgc tgaacagtcg tgcgtcggac gggagcggct 840
ttcgtaaggt tgcgcatagc actgatggtg ggcagacctg gtccgaaccg gtttcggaca 900
aaaatttgcc ggattcggtt gataatgccc agataattcg tgcgtttcct aatgctgccc 960
ccgatgaccc gcgcgcgaaa gtacttcttc tgagtcattc ccccaaatcca cgtccgtggt 1020
cccgggatcg tggtacgata agcatgtcat gtgatgacgg ggcctcatgg accacttcca 1080
aagtttttca cgaaccgttt gtgggctaca cgactattgc agttcagagt gatggaagca 1140
tcggtctgct gtcggaggac gcgcacaatg gcgctgatta tggtggcatc tggtatcgta 1200
attttacgat gaactggctg ggagaacaat gtggacaaaa acccgcggaa taagctt 1257

<210> 31
<211> 416
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 31
Met Lys Arg Lys Lys Lys Gly Gly Lys Asn Gly Lys Asn Arg Arg Asn
1 5 10 15
Arg Lys Lys Lys Asn Pro Gly Asp His Pro Gln Ala Thr Pro Ala Pro
20 25 30
Ala Pro Asp Ala Ser Thr Glu Leu Pro Ala Ser Met Ser Gln Ala Gln
35 40 45
His Leu Ala Ala Asn Thr Ala Thr Asp Asn Tyr Arg Ile Pro Ala Ile
50 55 60
Thr Thr Ala Pro Asn Gly Asp Leu Leu Ile Ser Tyr Asp Glu Arg Pro
65 70 75 80
Lys Asp Asn Gly Asn Gly Gly Ser Asp Ala Pro Asn Pro Asn His Ile
85 90 95
Val Gln Arg Arg Ser Thr Asp Gly Gly Lys Thr Trp Ser Ala Pro Thr
100 105 110
Tyr Ile His Gln Gly Thr Glu Thr Gly Lys Lys Val Gly Tyr Ser Asp
115 120 125
Pro Ser Tyr Val Val Asp His Gln Thr Gly Thr Ile Phe Asn Phe His
130 135 140
Val Lys Ser Tyr Asp Gln Gly Trp Gly Gly Ser Arg Gly Gly Thr Asp
145 150 155 160
Pro Glu Asn Arg Gly Ile Ile Gln Ala Glu Val Ser Thr Ser Thr Asp
165 170 175
Asn Gly Trp Thr Trp Thr His Arg Thr Ile Thr Ala Asp Ile Thr Lys
180 185 190
Asp Lys Pro Trp Thr Ala Arg Phe Ala Ala Ser Gly Gln Gly Ile Gln
195 200 205
Ile Gln His Gly Pro His Ala Gly Arg Leu Val Gln Gln Tyr Thr Ile
210 215 220

Arg Thr Ala Gly Gly Ala Val Gln Ala Val Ser Val Tyr Ser Asp Asp
225 230 235 240
His Gly Lys Thr Trp Gln Ala Gly Thr Pro Ile Gly Thr Gly Met Asp
245 250 255
Glu Asn Lys Val Val Glu Leu Ser Asp Gly Ser Leu Met Leu Asn Ser
260 265 270
Arg Ala Ser Asp Gly Ser Gly Phe Arg Lys Val Ala His Ser Thr Asp
275 280 285
Gly Gly Gln Thr Trp Ser Glu Pro Val Ser Asp Lys Asn Leu Pro Asp
290 295 300
Ser Val Asp Asn Ala Gln Ile Ile Arg Ala Phe Pro Asn Ala Ala Pro
305 310 315 320
Asp Asp Pro Arg Ala Lys Val Leu Leu Leu Ser His Ser Pro Asn Pro
325 330 335
Arg Pro Trp Ser Arg Asp Arg Gly Thr Ile Ser Met Ser Cys Asp Asp
340 345 350
Gly Ala Ser Trp Thr Thr Ser Lys Val Phe His Glu Pro Phe Val Gly
355 360 365
Tyr Thr Thr Ile Ala Val Gln Ser Asp Gly Ser Ile Gly Leu Leu Ser
370 375 380
Glu Asp Ala His Asn Gly Ala Asp Tyr Gly Gly Ile Trp Tyr Arg Asn
385 390 395 400
Phe Thr Met Asn Trp Leu Gly Glu Gln Cys Gly Gln Lys Pro Ala Glu
405 410 415

<210> 32
<211> 43
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<220>
<221> misc_feature
<222> 17, 18, 20, 21
<223> n = A,T,C or G

<400> 32
ttttcgtctc ccatgvnnvn naagcgcaaa aaaaaaggcg gca 43

<210> 33
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<220>
<221> VARIANT
<222> 2, 3
<223> Xaa = Any Amino Acid

<400> 33
Met Xaa Xaa Lys Arg Lys Lys Lys Gly Gly
1 5 10

<210> 34
<211> 1272
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 34
ccatgaagcg caaaaaaaaa ggcggcaaaa acggtaaaaa tcgtcgtaac cgtaagaaaa 60
aaaatcctgg tggtggtggt tctggagatc atccacaagc tacaccagca cctgcaccag 120
atgctagcac tgagctgcca gcaagcatgt ctcaggctca gcatcttgca gcaaatacgg 180
ctactgataa ttatcgcatt ccagcgatta caaccgctcc gaatggtgat ttactgatta 240
gctatgatga acggccgaag gacaatggaa atggtggttc cgatgcccct aacccgaatc 300
atattgttca gcgtcgctcc acagatggcg gtaaaacttg gagcgcgcca acctatattc 360
atcagggtac ggagactggc aagaaagtgg gatattccga cccctcttat gtggtggatc 420
atcaaaccgg tacaatcttc aattttcatg tgaaatcata cgatcagggc tggggaggta 480
gccgtggggg aacagacccg gaaaaccgcg ggattattca ggcagaggtg tctacgagca 540
cggataatgg atggacgtgg acacatcgca ccatcaccgc ggatattacg aaagataaac 600
cgtggaccgc gcgttttgcg gcgtccggcc aaggcattca gatccagcat gggccgcatg 660
ccggccgtct ggtgcaacag tataccattc gtacggccgg tggagcggtg caggctgtat 720
cggtttattc cgatgatcat gggaaaacgt ggcaggctgg caccccgatt gggacgggta 780
tggatgaaaa caaagttgta gagctgtctg acggctctct gatgctgaac agtcgtgcgt 840
cggacgggag cggctttcgt aaggttgcgc atagcactga tggtggcag acctggtccg 900
aaccggtttc ggacaaaaat ttgccggatt cggttgataa tgcccagata attcgtgcgt 960
ttcctaatgc tgcccccgat gacccgcgcg cgaaagtact tcttctgagt cattccccaa 1020
atccacgtcc gtggtcccgg gatcgtggta cgataagcat gtcatgtgat gacggggcct 1080
catggaccac ttccaaagtt tttcacgaac cgtttgtggg ctacacgact attgcagttc 1140
agagtgatgg aagcatcggt ctgctgtcgg aggacgcgca caatggcgct gattatggtg 1200
gcatctggta tcgtaatttt acgatgaact ggctgggaga acaatgtgga caaaaacccg 1260
cggaataagc tt 1272

<210> 35
<211> 421
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 35
Met Lys Arg Lys Lys Lys Gly Gly Lys Asn Gly Lys Asn Arg Arg Asn
1 5 10 15
Arg Lys Lys Lys Asn Pro Gly Gly Gly Gly Ser Gly Asp His Pro Gln
20 25 30
Ala Thr Pro Ala Pro Ala Pro Asp Ala Ser Thr Glu Leu Pro Ala Ser
35 40 45
Met Ser Gln Ala Gln His Leu Ala Ala Asn Thr Ala Thr Asp Asn Tyr
50 55 60
Arg Ile Pro Ala Ile Thr Thr Ala Pro Asn Gly Asp Leu Leu Ile Ser
65 70 75 80
Tyr Asp Glu Arg Pro Lys Asp Asn Gly Asn Gly Gly Ser Asp Ala Pro
85 90 95
Asn Pro Asn His Ile Val Gln Arg Arg Ser Thr Asp Gly Gly Lys Thr
100 105 110
Trp Ser Ala Pro Thr Tyr Ile His Gln Gly Thr Glu Thr Gly Lys Lys
115 120 125
Val Gly Tyr Ser Asp Pro Ser Tyr Val Val Asp His Gln Thr Gly Thr
130 135 140
Ile Phe Asn Phe His Val Lys Ser Tyr Asp Gln Gly Trp Gly Gly Ser
145 150 155 160
Arg Gly Gly Thr Asp Pro Glu Asn Arg Gly Ile Ile Gln Ala Glu Val
165 170 175
Ser Thr Ser Thr Asp Asn Gly Trp Thr Trp Thr His Arg Thr Ile Thr
180 185 190
Ala Asp Ile Thr Lys Asp Lys Pro Trp Thr Ala Arg Phe Ala Ala Ser
195 200 205
Gly Gln Gly Ile Gln Ile Gln His Gly Pro His Ala Gly Arg Leu Val
210 215 220
Gln Gln Tyr Thr Ile Arg Thr Ala Gly Gly Ala Val Gln Ala Val Ser
225 230 235 240
Val Tyr Ser Asp Asp His Gly Lys Thr Trp Gln Ala Gly Thr Pro Ile
245 250 255
Gly Thr Gly Met Asp Glu Asn Lys Val Val Glu Leu Ser Asp Gly Ser
260 265 270
Leu Met Leu Asn Ser Arg Ala Ser Asp Gly Ser Gly Phe Arg Lys Val

```
        275                 280                 285
Ala His Ser Thr Asp Gly Gly Gln Thr Trp Ser Glu Pro Val Ser Asp
    290                 295                 300
Lys Asn Leu Pro Asp Ser Val Asp Asn Ala Gln Ile Ile Arg Ala Phe
305                 310                 315                 320
Pro Asn Ala Ala Pro Asp Asp Pro Arg Ala Lys Val Leu Leu Leu Ser
                325                 330                 335
His Ser Pro Asn Pro Arg Pro Trp Ser Arg Asp Arg Gly Thr Ile Ser
            340                 345                 350
Met Ser Cys Asp Asp Gly Ala Ser Trp Thr Thr Ser Lys Val Phe His
        355                 360                 365
Glu Pro Phe Val Gly Tyr Thr Thr Ile Ala Val Gln Ser Asp Gly Ser
    370                 375                 380
Ile Gly Leu Leu Ser Glu Asp Ala His Asn Gly Ala Asp Tyr Gly Gly
385                 390                 395                 400
Ile Trp Tyr Arg Asn Phe Thr Met Asn Trp Leu Gly Glu Gln Cys Gly
                405                 410                 415
Gln Lys Pro Ala Glu
            420
```

<210> 36
<211> 1275
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 36

```
ccatggttaa gcgcaaaaaa aaaggcggca aaaacggtaa aaatcgtcgt aaccgtaaga 60
aaaaaaatcc tggtggtggt ggttctggag atcatccaca agctacacca gcacctgcac 120
cagatgctag cactgagctg ccagcaagca tgtctcaggc tcagcatctt gcagcaaata 180
cggctactga taattatcgc attccagcga ttacaaccgc tccgaatggt gatttactga 240
ttagctatga tgaacggccg aaggacaatg gaaatggtgg ttccgatgcc cctaacccga 300
atcatattgt tcagcgtcgc tccacagatg gcggtaaaac ttggagcgcg ccaacctata 360
ttcatcaggg tacggagact ggcaagaaag tgggatattc cgacccctct tatgtggtgg 420
atcatcaaac cggtacaatc ttcaattttc atgtgaaatc atacgatcag ggctggggag 480
gtagccgtgg gggaacagac ccggaaaacc gcgggattat tcaggcagag gtgtctacga 540
gcacggataa tggatggacg tggacacatc gcaccatcac cgcggatatt acgaaagata 600
aaccgtggac cgcgcgtttt gcggcgtccg gccaaggcat tcagatccag catgggccgc 660
atgccggccg tctggtgcaa cagtatacca ttcgtacggc cggtggagcg gtgcaggctg 720
tatcggttta ttccgatgat catgggaaaa cgtggcaggc tggcaccccg attgggacgg 780
gtatggatga aaacaaagtt gtagagctgt ctgacggctc tctgatgctg aacagtcgtg 840
cgtcggacgg gagcggcttt cgtaaggttg cgcatagcac tgatggtggg cagacctggt 900
ccgaaccggt ttcggacaaa aatttgccgg attcggttga taatgcccag ataattcgtg 960
cgtttcctaa tgctgccccc gatgacccgc gcgcgaaagt acttcttctg agtcattccc 1020
caaatccacg tccgtggtcc cgggatcgtg gtacgataag catgtcatgt gatgacgggg 1080
cctcatggac cacttccaaa gtttttcacg aaccgtttgt gggctacacg actattgcag 1140
ttcagagtga tggaagcatc ggtctgctgt cggaggacgc gcacaatggc gctgattatg 1200
gtggcatctg gtatcgtaat tttacgatga actggctggg agaacaatgt ggacaaaaac 1260
ccgcggaata agctt                                                  1275
```

<210> 37
<211> 422
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 37

```
Met Val Lys Arg Lys Lys Lys Gly Gly Lys Asn Gly Lys Asn Arg Arg
 1               5                  10                  15
Asn Arg Lys Lys Lys Asn Pro Gly Gly Gly Gly Ser Gly Asp His Pro
            20                  25                  30
Gln Ala Thr Pro Ala Pro Ala Pro Asp Ala Ser Thr Glu Leu Pro Ala
        35                  40                  45
```

Ser Met Ser Gln Ala Gln His Leu Ala Ala Asn Thr Ala Thr Asp Asn
    50   55   60
Tyr Arg Ile Pro Ala Ile Thr Thr Ala Pro Asn Gly Asp Leu Leu Ile
65   70   75   80
Ser Tyr Asp Glu Arg Pro Lys Asp Asn Gly Asn Gly Gly Ser Asp Ala
                85   90   95
Pro Asn Pro Asn His Ile Val Gln Arg Arg Ser Thr Asp Gly Gly Lys
            100   105   110
Thr Trp Ser Ala Pro Thr Tyr Ile His Gln Gly Thr Glu Thr Gly Lys
        115   120   125
Lys Val Gly Tyr Ser Asp Pro Ser Tyr Val Val Asp His Gln Thr Gly
    130   135   140
Thr Ile Phe Asn Phe His Val Lys Ser Tyr Asp Gln Gly Trp Gly Gly
145   150   155   160
Ser Arg Gly Gly Thr Asp Pro Glu Asn Arg Gly Ile Ile Gln Ala Glu
                165   170   175
Val Ser Thr Ser Thr Asp Asn Gly Trp Thr Trp Thr His Arg Thr Ile
            180   185   190
Thr Ala Asp Ile Thr Lys Asp Lys Pro Trp Thr Ala Arg Phe Ala Ala
        195   200   205
Ser Gly Gln Gly Ile Gln Ile Gln His Gly Pro His Ala Gly Arg Leu
    210   215   220
Val Gln Gln Tyr Thr Ile Arg Thr Ala Gly Gly Ala Val Gln Ala Val
225   230   235   240
Ser Val Tyr Ser Asp Asp His Gly Lys Thr Trp Gln Ala Gly Thr Pro
                245   250   255
Ile Gly Thr Gly Met Asp Glu Asn Lys Val Val Glu Leu Ser Asp Gly
            260   265   270
Ser Leu Met Leu Asn Ser Arg Ala Ser Asp Gly Ser Gly Phe Arg Lys
        275   280   285
Val Ala His Ser Thr Asp Gly Gly Gln Thr Trp Ser Glu Pro Val Ser
    290   295   300
Asp Lys Asn Leu Pro Asp Ser Val Asp Asn Ala Gln Ile Ile Arg Ala
305   310   315   320
Phe Pro Asn Ala Ala Pro Asp Asp Pro Arg Ala Lys Val Leu Leu Leu
                325   330   335
Ser His Ser Pro Asn Pro Arg Pro Trp Ser Arg Asp Arg Gly Thr Ile
            340   345   350
Ser Met Ser Cys Asp Asp Gly Ala Ser Trp Thr Thr Ser Lys Val Phe
        355   360   365
His Glu Pro Phe Val Gly Tyr Thr Thr Ile Ala Val Gln Ser Asp Gly
    370   375   380
Ser Ile Gly Leu Leu Ser Glu Asp Ala His Asn Gly Ala Asp Tyr Gly
385   390   395   400
Gly Ile Trp Tyr Arg Asn Phe Thr Met Asn Trp Leu Gly Glu Gln Cys
                405   410   415
Gly Gln Lys Pro Ala Glu
            420

<210> 38
<211> 416
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 38
Met Lys Arg Lys Lys Lys Gly Gly Lys Asn Gly Lys Asn Arg Arg Asn
 1   5   10   15
Arg Lys Lys Lys Asn Pro Gly Asp His Pro Gln Ala Thr Pro Ala Pro
            20   25   30
Ala Pro Asp Ala Ser Thr Glu Leu Pro Ala Ser Met Ser Gln Ala Gln
        35   40   45
His Leu Ala Ala Asn Thr Ala Thr Asp Asn Tyr Arg Ile Pro Ala Ile
    50   55   60
Thr Thr Ala Pro Asn Gly Asp Leu Leu Ile Ser Tyr Asp Glu Arg Pro

```
65                  70                  75                  80
Lys Asp Asn Gly Asn Gly Gly Ser Asp Ala Pro Asn Pro Asn His Ile
                85                  90                  95
Val Gln Arg Arg Ser Thr Asp Gly Gly Lys Thr Trp Ser Ala Pro Thr
            100                 105                 110
Tyr Ile His Gln Gly Thr Glu Thr Gly Lys Lys Val Gly Tyr Ser Asp
        115                 120                 125
Pro Ser Tyr Val Val Asp His Gln Thr Gly Thr Ile Phe Asn Phe His
    130                 135                 140
Val Lys Ser Tyr Asp Gln Gly Trp Gly Gly Ser Arg Gly Gly Thr Asp
145                 150                 155                 160
Pro Glu Asn Arg Gly Ile Ile Gln Ala Glu Val Ser Thr Ser Thr Asp
                165                 170                 175
Asn Gly Trp Thr Trp Thr His Arg Thr Ile Thr Ala Asp Ile Thr Lys
            180                 185                 190
Asp Lys Pro Trp Thr Ala Arg Phe Ala Ala Ser Gly Gln Gly Ile Gln
        195                 200                 205
Ile Gln His Gly Pro His Ala Gly Arg Leu Val Gln Gln Tyr Thr Ile
    210                 215                 220
Arg Thr Ala Gly Gly Ala Val Gln Ala Val Ser Val Tyr Ser Asp Asp
225                 230                 235                 240
His Gly Lys Thr Trp Gln Ala Gly Thr Pro Ile Gly Thr Gly Met Asp
                245                 250                 255
Glu Asn Lys Val Val Glu Leu Ser Asp Gly Ser Leu Met Leu Asn Ser
            260                 265                 270
Arg Ala Ser Asp Gly Ser Gly Phe Arg Lys Val Ala His Ser Thr Asp
        275                 280                 285
Gly Gly Gln Thr Trp Ser Glu Pro Val Ser Asp Lys Asn Leu Pro Asp
    290                 295                 300
Ser Val Asp Asn Ala Gln Ile Ile Arg Ala Phe Pro Asn Ala Ala Pro
305                 310                 315                 320
Asp Asp Pro Arg Ala Lys Val Leu Leu Leu Ser His Ser Pro Asn Pro
                325                 330                 335
Arg Pro Trp Ser Arg Asp Arg Gly Thr Ile Ser Met Ser Cys Asp Asp
            340                 345                 350
Gly Ala Ser Trp Thr Thr Ser Lys Val Phe His Glu Pro Phe Val Gly
        355                 360                 365
Tyr Thr Thr Ile Ala Val Gln Ser Asp Gly Ser Ile Gly Leu Leu Ser
    370                 375                 380
Glu Asp Ala His Asn Gly Ala Asp Tyr Gly Gly Ile Trp Tyr Arg Asn
385                 390                 395                 400
Phe Thr Met Asn Trp Leu Gly Glu Gln Cys Gly Gln Lys Pro Ala Glu
                405                 410                 415
```

<210> 39
<211> 421
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 39

```
Val Lys Arg Lys Lys Lys Gly Gly Lys Asn Gly Lys Asn Arg Arg Asn
 1               5                  10                  15
Arg Lys Lys Lys Asn Pro Gly Gly Gly Gly Ser Gly Asp His Pro Gln
            20                  25                  30
Ala Thr Pro Ala Pro Ala Pro Asp Ala Ser Thr Glu Leu Pro Ala Ser
        35                  40                  45
Met Ser Gln Ala Gln His Leu Ala Ala Asn Thr Ala Thr Asp Asn Tyr
    50                  55                  60
Arg Ile Pro Ala Ile Thr Thr Ala Pro Asn Gly Asp Leu Leu Ile Ser
65                  70                  75                  80
Tyr Asp Glu Arg Pro Lys Asp Asn Gly Asn Gly Gly Ser Asp Ala Pro
                85                  90                  95
Asn Pro Asn His Ile Val Gln Arg Arg Ser Thr Asp Gly Gly Lys Thr
            100                 105                 110
```

```
Trp Ser Ala Pro Thr Tyr Ile His Gln Gly Thr Glu Thr Gly Lys Lys
        115                 120                 125
Val Gly Tyr Ser Asp Pro Ser Tyr Val Val Asp His Gln Thr Gly Thr
    130                 135                 140
Ile Phe Asn Phe His Val Lys Ser Tyr Asp Gln Gly Trp Gly Gly Ser
145                 150                 155                 160
Arg Gly Gly Thr Asp Pro Glu Asn Arg Gly Ile Ile Gln Ala Glu Val
                165                 170                 175
Ser Thr Ser Thr Asp Asn Gly Trp Thr Trp Thr His Arg Thr Ile Thr
            180                 185                 190
Ala Asp Ile Thr Lys Asp Lys Pro Trp Thr Ala Arg Phe Ala Ala Ser
        195                 200                 205
Gly Gln Gly Ile Gln Ile Gln His Gly Pro His Ala Gly Arg Leu Val
    210                 215                 220
Gln Gln Tyr Thr Ile Arg Thr Ala Gly Gly Ala Val Gln Ala Val Ser
225                 230                 235                 240
Val Tyr Ser Asp Asp His Gly Lys Thr Trp Gln Ala Gly Thr Pro Ile
                245                 250                 255
Gly Thr Gly Met Asp Glu Asn Lys Val Val Glu Leu Ser Asp Gly Ser
            260                 265                 270
Leu Met Leu Asn Ser Arg Ala Ser Asp Gly Ser Gly Phe Arg Lys Val
        275                 280                 285
Ala His Ser Thr Asp Gly Gly Gln Thr Trp Ser Glu Pro Val Ser Asp
    290                 295                 300
Lys Asn Leu Pro Asp Ser Val Asp Asn Ala Gln Ile Ile Arg Ala Phe
305                 310                 315                 320
Pro Asn Ala Ala Pro Asp Asp Pro Arg Ala Lys Val Leu Leu Leu Ser
                325                 330                 335
His Ser Pro Asn Pro Arg Pro Trp Ser Arg Asp Arg Gly Thr Ile Ser
            340                 345                 350
Met Ser Cys Asp Asp Gly Ala Ser Trp Thr Thr Ser Lys Val Phe His
        355                 360                 365
Glu Pro Phe Val Gly Tyr Thr Thr Ile Ala Val Gln Ser Asp Gly Ser
    370                 375                 380
Ile Gly Leu Leu Ser Glu Asp Ala His Asn Gly Ala Asp Tyr Gly Gly
385                 390                 395                 400
Ile Trp Tyr Arg Asn Phe Thr Met Asn Trp Leu Gly Glu Gln Cys Gly
                405                 410                 415
Gln Lys Pro Ala Glu
            420
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