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(54) **COLLAGEN V FOR USE IN THE TREATMENT OF ASTHMA**

COLLAGEN V FÜR DIE BEHANDLUNG VON ASTHMA

COLLAGEN V POUR LE TRAITEMENT DE L’ASTHME

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- **PALMANS E ET AL: "Repeated allergen exposure changes collagen composition in airways of sensitised Brown Norway rats." THE EUROPEAN RESPIRATORY JOURNAL : OFFICIAL JOURNAL OF THE EUROPEAN SOCIETY FOR CLINICAL RESPIRATORY PHYSIOLOGY AUG 2002 LNKD-PUBMED:12212956, vol. 20, no. 2, August 2002 (2002-08), pages 280-285, XP002602517 ISSN: 0903-1936**

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Description

BACKGROUND OF THE INVENTION

Field of the Invention

[0001] The present invention relates to compositions for use in methods for the treatment of asthma. In particular the invention relates to the treatment of asthma by administering type V Collagen (colV) or tolerogenic fragments thereof.

Description of the Related Art

[0002] Asthma is a heterogeneous disorder of the airways that afflicts millions of people. Airway inflammation, hyperresponsiveness, and obstruction characterize the condition. The disease often causes spasms of the bronchial smooth muscle system, and affects both the upper and lower respiratory tracts. There are several forms of asthma, characterized by varying degrees of severity. Mild asthma, for example, is defined as brief episodes of wheezing, with or without dyspnea or cough. Moderately severe asthma is defined as wheezing and dyspnea, and can be with or without cough and expectoration, but generally interferes with daily activities and/or sleeping. Severe asthma is characterized by incapacitation due to dyspnea, and the afflicted patient typically is unable to eat or sleep normally, is very anxious, and is often exhausted. A condition known as status asthmaticus is the most severe form of asthma, and generally requires intensive hospital care, and may even prove fatal. The disease may occur as a result of both allergic and nonallergic mechanisms.

[0003] While there are several treatments available for relieving the symptoms and discomfort associated with asthma, there are no cures. Moreover, the current treatments often cause side effects that exacerbate the discomfort and precipitate other debilitating conditions. Mild asthma generally is treated with beta-adrenergic drugs, as well as antihistamines, especially in the case of children, to prevent or abort sporadic episodes. Moderately severe and severe asthma are generally treated with adrenergic agents and bronchodilators, as well as corticosteroids. Other actions caused by antiasthmatic agents which limit their widespread use include headache, fatigue, dry mouth, nervousness, and in some cases addiction and substance abuse. Recent advances in the understanding of the pathogenesis and treatment of asthma is discussed more fully in Am J Respir Crit Care Med. 2008 May 15;177(10):1068-73.

[0004] Because asthma is so prevalent in both children and adults, there is an ongoing need for agents that can treat the disease, or at least relieve the symptoms that accompany the disease, without causing undesirable side effects. The present invention provides compositions and methods for the treatment of asthma and other advantages as described in the detailed description.

BRIEF SUMMARY OF THE INVENTION

[0005] One aspect of the present invention provides a compound for use in a method for treating asthma comprising administering to an asthma patient a therapeutically effective amount of type V collagen, or a tolerogenic fragment thereof. In one embodiment of the methods, the type V collagen or tolerogenic fragment thereof is administered orally, and may be administered at a dose of between 0.1 mg and 0.5 mg. In further embodiments, the type V collagen or tolerogenic fragment thereof is administered intravenously, by intrapulmonary instillation, by inhalation, or intramuscularly. In a yet a further embodiment, the method further comprises administering to the asthma patient a corticosteroid, a bronchodilator and/or a leukotriene modifier, or other known treatment for asthma.

[0006] A further aspect of the invention provides a compound for use in a method for preventing the development or worsening of asthma in a subject at risk for developing asthma comprising administering to the subject a therapeutically effective amount of type V collagen, or a tolerogenic fragment thereof. In one embodiment, the type V collagen or tolerogenic fragment thereof is administered orally and in certain embodiments, may be administered at a dose of between 0.1 mg and 0.5 mg. In a further embodiment, the type V collagen or tolerogenic fragment thereof is administered intravenously, by intrapulmonary instillation, by inhalation, intramuscularly, or by a combination of one or more of these routes.

[0007] One aspect of the present invention provides a method for identifying an asthma patient as a candidate for collagen V tolerance therapy comprising, contacting at least a portion of a sample of blood from the patient with collagen V or an antigenic fragment thereof; and measuring the level of antibodies that bind to the collagen V or antigenic fragment thereof *(i.e.,* measuring the level of Type V collagen-specific antibodies); wherein the presence of antibodies bound to the collagen V is indicative of asthma. In this regard, collagen V-specific antibody level may be used in conjuction with other clinical factors as described herein in diagnosing asthma. In one embodiment, the collagen V or antigenic fragment thereof is conjugated to a bead. In a further embodiment, the measuring comprises contacting the antibodies that bind to the collagen V or antigenic fragment thereof with a fluorescently labeled anti-IgG antibody; and detecting by flow

cytometry the amount of flurescently labeled anti-IgG antibody bound to the antibodies bound to the collagen V.

[0008] Another aspect of the present invention provides a method for identifying an individual at risk for developing asthma, comprising: contacting at least a portion of a sample of blood from the individual with collagen V or an antigenic fragment thereof; and measuring the level of antibodies that bind to the collagen V or an antigenic fragment thereof (i.e., measuring the level of Type V collagen-specific antibodies); wherein the presence of antibodies that bind to the collagen V is associated with a higher risk than would be expected in an individual with no antibodies that bind to the collagen V. In one embodiment, the collagen V or antigenic fragment thereof is conjugated to a bead. In another embodiment, the measuring comprises contacting the antibodies that bind to the collagen V or antigenic fragment thereof with a fluorescently labeled anti-IgG antibody; and detecting by flow cytometry the amount of fluorescently labeled anti-IgG antibody bound to the antibodies bound to the collagen V.

[0009] In certain embodiments of the methods for diagnosing or measuring risk for developing asthma, the anti-IgG antibody used in the methods detects all IgG subtypes. In further embodiments, the anti-IgG antibody specifically detects the IgG1 subtype, or the IgG2 subtype, or the IgG3 subtype, or the IgG4 subtype. In this regard, a switch from one subtype to another subtype may occur during the course of disease and may indicate worsening of disease. Therefore, an increase in one subtype over time may indicate worsening of disease.

[0010] A further aspect of the invention provides a method for monitoring the progression of asthma in an individual comprising, contacting at least a portion of a first sample of blood from the individual with collagen V or an antigenic fragment thereof; measuring the level of antibodies that bind to the collagen V or antigenic fragment thereof in the first sample of blood; contacting at least a portion of a second sample of blood from the individual taken at a later time point, with collagen V or an antigenic fragment thereof; measuring the level of antibodies that bind to the collagen V or antigenic fragment thereof in the second sample of blood; and comparing the level of antibodies that bind to the collagen V or antigenic fragment thereof in the second sample of blood to the level of antibodies that bind to the collagen V or antigenic fragment thereof in the first sample of blood; wherein an increase in the level of antibodies bound to the collagen V in the second sample as compared to the first sample is indicative of worsening of asthma and a decrease in the level of antibodies bound to the collagen V in the second sample as compared to the first sample is indicative of amelioration of asthma. Other clinical indicators of asthma may be used in conjuction with the methods provided herein. In certain embodiments, an increase in anti-collagen V antibodies of a particular IgG subtype (e.g., IgG1, IgG2, IgG3, or IgG4) is indicative of a progression of asthma. In certain embodiments, the collagen V or antigenic fragment thereof is conjugated to a bead. In another embodiment, the measuring comprises contacting the antibodies that bind to the collagen V or antigenic fragment thereof with a fluorescently labeled anti-IgG antibody; and detecting by flow cytometry the amount of flurescently labeled anti-IgG antibody bound to the antibodies bound to the collagen V. In certain embodiments of the methods for monitoring progression the anti-IgG antibody detects all IgG subtypes. In other embodiments, the anti-IgG antibody specifically detects the IgG1, IgG2, IgG3 or IgG4 subtype.

[0011] These and other aspects of the invention will be evident upon reference to the following detailed description and attached drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012]

Figure 1 is a bar graph showing elevated anti-type V collagen antibodies in COPD patients.

Figure 2 is a bar graph showing elevated anti-type V collagen antibodies in asthma patients.

Figure 3 shows prevention of ovalbumin-induced airway hyperresponsiveness in mice following intravenous administration of collagen V. n = 5 in all groups except col V where n = 2 - 5 for each data point.

Figures 4A-4E are bar graphs showing induction of IFN-γ transcripts in lung mononuclear cells by intravenous administration of col(v). Quantitative PCR was performed for the IL-4, IL-5, IL-13, IFN-γ and IL-10 cytokines shown. Only Col(V) IV induced IFN-γ transcripts in lung mononuclear cells. Data represent lung mononuclear cells of RNA pooled from 5 mice in each group.

DETAILED DESCRIPTION OF THE INVENTION

Type V Collagen

[0013] Collagen protein is made of polypeptide chains composed of a repeated sequence of amino acids primarily consisting of hydroxyproline (Hyp), glycine (Gly), and proline (Pro). Collagen is one of the most predominant proteins found in the human body, comprising about 80-85% of the extracellular matrix (ECM) in the dermal layer of normal (non-wounded) skin tissue.

[0014] Collagens are classified into several types based on sequence identity and function. Types I, II, & III collagen

molecules make up the main fibers of most animal extracellular structures. Type I forms about 90% of the body's collagen and is the primary component of bone, skin and tendons. Type II makes up the major fibers of cartilage. Collagen fibers are arranged in rigid plates in bones, in parallel bundles in tendons, and in a dense meshwork in cartilage. Type I and lesser amounts of type III make up tendons and skin. Type IV collagen molecules make up very fine, unstriated fibers present in basal laminae. Type V Collagen (colV) is a minor collagen present in the lung (Madri and Furthmayr, Human Pathology, 11:353-366, 1980) and is located in the peribronchiolar connective tissues (Madri and Furthmayr, Am. J. Pathol., 94:323-332, 1979), alveolar interstitium (Konomi *et al*., 1984), and capillary basement membranes (Madri and Furthmayr, 1979, Supra). Over a dozen other collagen types are known but are less well characterized.

[0015] Collagen polypeptide chains are characterized by a core helical domain made up of repeating glycine-X-Y triplets and globular N-terminal and C-terminal domains. Three such chains are wound around one another in a superhelix to generate an individual ropelike collagen molecule.

[0016] Previous work has demonstrated that autoimmunity to colV is associated with chronic allograft dysfunction (including obliterative bronchiolitis, bronchiolitis obliterans syndrome (BOS)), lung allograft rejection and with risk of developing IPF (see e.g., U.S. Patent 7,348,005 and WO 2007/120947). Furthermore, this work showed that administration of colV induced tolerance to alloantigens and to colV (see e.g., WO 2007/120947; Fig. 10). However, prior to the present invention, no association with autoimmunity to colV had been shown in asthma

[0017] Thus, the present invention relates to inducing tolerance to colV in asthma patients, or in subjects at risk for developing this disease.

[0018] ColV polynucleotide and polypeptide sequences are known to the skilled person and are available in public databases. Illustrative colV polynucleotides and polypeptides of the present invention include, but are not limited to Homo sapien collagen, type V, alpha 1 (COL5A1), mRNA NCBI Reference Sequence: NM_000093.3 version GI:89276750 (SEQ ID NO:1); alpha 1 type V collagen preproprotein [Homo sapiens]: Accession NP_000084, version GI:89276751 (SEQ ID NO:2); Homo sapien collagen, type V, alpha 2 (COL5A2), mRNA; accession NM_000393, version GI:89363016 (SEQ ID NO:3); alpha 2 type V collagen preproprotein [Homo sapiens]; accession NP_000384, version GI:89363017 (SEQ ID NO:4); Homo sapien collagen, type V, alpha 3 (COL5A3), mRNA; accession NM_015719, NM_015719.3, GI:110735434 (SEQ ID NO:5); collagen, type V, alpha 3 preproprotein [Homo sapien]; accession NP_056534, version NP_056534.2, GI:110735435 (SEQ ID NO:6).

[0019] As would be recognized by the skilled person, the preprocollagen is processed in a cell into procollagen which is exported from the cell and eventually formed into collagen fibrils and fibers. Thus, the present invention specifically contemplates procollagen and other processed or mature forms of collagen proteins described herein. In this regard, for example, amino acids 1-26 of SEQ ID NO:4 corresponds to the signal peptide which is cleaved during processing, amino acids 27-1229 is the collagen alpha-2(V) chain, and amino acids 1230-1499 correspond to the c-terminal propeptide. These positions within the sequences specifically disclosed herein would be recognized by the skilled person and are available through various public databases where annotation of sequences is provided. It should also be noted that certain amino acids of the collagen proteins are modified during processing (e.g., proline to hydroxyproline). Mature, modified forms of the type V collagen chains, particularly alpha-2 chains, are specifically contemplated herein. As noted elsewhere, the type V collagen and alpha chains thereof for use in the present invention may be purified from a variety of sources or produced recombinantly.

[0020] As used herein, the term "polypeptide" is used in its conventional meaning, *i.e*., as a sequence of amino acids. The polypeptides are not limited to a specific length of the product; thus, peptides, oligopeptides, and proteins are included within the definition of polypeptide, and such terms may be used interchangeably herein unless specifically indicated otherwise. This term also does not refer to or exclude post-expression modifications of the polypeptide, for example, glycosylations, acetylations, phosphorylations and the like, as well as other modifications known in the art, both naturally occurring and non-naturally occurring. A polypeptide may be an entire protein, or a subsequence thereof. Particular polypeptides of interest in the context of this invention are amino acid subsequences comprising tolerogenic fragments.

[0021] The present invention, in another aspect, provides polypeptide fragments comprising at least about 5, 10, 15, 20, 25, 50, or 100 contiguous amino acids, or more, including all intermediate lengths, of a collagen polypeptide compositions set forth herein, such as those set forth in SEQ ID NOs: 2, 4 or 6, or those encoded by a polynucleotide sequence set forth in a sequence of SEQ ID NOs: 1, 3 or 5.

[0022] In another aspect, the present invention provides variants of the polypeptide compositions described herein. Polypeptide variants generally encompassed by the present invention will typically exhibit at least about 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% or more identity (determined as described below), along its length, to a polypeptide sequences set forth herein.

[0023] In one embodiment, the polypeptide fragments and variants provided by the present invention are immunologically tolerogenic as described herein.

[0024] A polypeptide "variant," as the term is used herein, is a polypeptide that typically differs from a polypeptide specifically disclosed herein in one or more substitutions, deletions, additions and/or insertions. Such variants may be

naturally occurring or may be synthetically generated, for example, by modifying one or more of the above polypeptide sequences of the invention and evaluating their tolerogenic activity as described herein and/or using any of a number of techniques well known in the art.

[0025] In many instances, a variant will contain conservative substitutions. A "conservative substitution" is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydropathic nature of the polypeptide to be substantially unchanged. Modifications may be made in the structure of the polynucleotides and polypeptides of the present invention and still obtain a functional molecule that encodes a variant or derivative polypeptide with desirable characteristics, e.g., with tolerogenic characteristics. When it is desired to alter the amino acid sequence of a polypeptide to create an equivalent, or even an improved, tolerogenic variant or portion of a polypeptide of the invention, one skilled in the art will typically change one or more of the codons of the encoding DNA sequence according to Table 1.

[0026] For example, certain amino acids may be substituted for other amino acids in a protein structure without appreciable loss of interactive binding capacity with structures such as, for example, antigen-binding regions of antibodies or binding sites on substrate molecules. Since it is the interactive capacity and nature of a protein that defines that protein's biological functional activity, certain amino acid sequence substitutions can be made in a protein sequence, and, of course, its underlying DNA coding sequence, and nevertheless obtain a protein with like properties. It is thus contemplated that various changes may be made in the peptide sequences of the disclosed compositions, or corresponding DNA sequences which encode said peptides without appreciable loss of their tolerogenic utility or activity.

Table 1

Amino Acids			**Codons**					
Alanine	Ala	A	GCA	GCC	GCG	GCU		
Cysteine	Cys	C	UGC	UGU				
Aspartic acid	Asp	D	GAC	GAU				
Glutamic acid	Glu	E	GAA	GAG				
Phenylalanine	Phe	F	UUC	UUU				
Glycine	Gly	G	GGA	GGC	GGG	GGU		
Histidine	His	H	CAC	CAU				
Isoleucine	Ile	I	AUA	AUC	AUU			
Lysine	Lys	K	AAA	AAG				
Leucine	Leu	L	UUA	UUG	CUA	CUC	CUG	CUU
Methionine	Met	M	AUG					
Asparagine	Asn	N	AAC	AAU				
Proline	Pro	P	CCA	CCC	CCG	CCU		
Glutamine	Gin	Q	CAA	CAG				
Arginine	Arg	R	AGA	AGG	CGA	CGC	CGG	CGU
Serine	Ser	S	AGC	AGU	UCA	UCC	UCG	UCU
Threonine	Thr	T	ACA	ACC	ACG	ACU		
Valine	Val	V	GUA	GUC	GUG	GUU		
Tryptophan	Trp	W	UGG					
Tyrosine	Tyr	Y	UAC	UAU				

[0027] In making such changes, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a protein is generally understood in the art (Kyte and Doolittle, 1982). It is accepted that the relative hydropathic character of the amino acid contributes to the secondary structure of the resultant protein, which in turn defines the interaction of the protein with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like. Each amino acid has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics (Kyte and Doolittle, 1982). These values are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

[0028] It is known in the art that certain amino acids may be substituted by other amino acids having a similar hydropathic index or score and still result in a protein with similar biological activity, *i.e.*, still obtain a biological functionally equivalent protein. In making such changes, the substitution of amino acids whose hydropathic indices are within ± 2 is preferred,

those within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred. It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. U. S. Patent 4,554,101 states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the protein.

[0029] As detailed in U. S. Patent 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0 ± 1); glutamate (+3.0 ± 1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5 ± 1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4). It is understood that an amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent, and in particular, an immunologically equivalent protein. In such changes, the substitution of amino acids whose hydrophilicity values are within ±2 is preferred, those within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred.

[0030] As outlined above, amino acid substitutions are generally therefore based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions that take various of the foregoing characteristics into consideration are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

[0031] In addition, any polynucleotide may be further modified to increase stability *in vivo.* Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends; the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages in the backbone; and/or the inclusion of nontraditional bases such as inosine, queosine and wybutosine, as well as acetyl- methyl-, thio- and other modified forms of adenine, cytidine, guanine, thymine and uridine.

[0032] Amino acid substitutions may further be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the residues. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine and valine; glycine and alanine; asparagine and glutamine; and serine, threonine, phenylalanine and tyrosine. Other groups of amino acids that may represent conservative changes include: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his. A variant may also, or alternatively, contain nonconservative changes. In a preferred embodiment, variant polypeptides differ from a native sequence by substitution, deletion or addition of five amino acids or fewer. Variants may also (or alternatively) be modified by, for example, the deletion or addition of amino acids that have minimal influence on the tolerogenicity, secondary structure and hydropathic nature of the polypeptide.

[0033] As noted above, polypeptides may comprise a signal (or leader) sequence at the N-terminal end of the protein, which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification or identification of the polypeptide (*e.g.*, poly-His), or to enhance binding of the polypeptide to a solid support. For example, a polypeptide may be conjugated to an immunoglobulin Fc region.

[0034] When comparing polypeptide sequences, two sequences are said to be "identical" if the sequence of amino acids in the two sequences is the same when aligned for maximum correspondence, as described below. Comparisons between two sequences are typically performed by comparing the sequences over a comparison window to identify and compare local regions of sequence similarity. A "comparison window" as used herein, refers to a segment of at least about 20 contiguous positions, usually 30 to about 75, 40 to about 50, in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned.

[0035] Optimal alignment of sequences for comparison may be conducted using the Megalign program in the Lasergene suite of bioinformatics software (DNASTAR, Inc., Madison, WI), using default parameters. This program embodies several alignment schemes described in the following references: Dayhoff, M.O., (1978) A model of evolutionary change in proteins - Matrices for detecting distant relationships. In Dayhoff, M.O. (ed.) Atlas of Protein Sequence and Structure, National Biomedical Research Foundation, Washington DC Vol. 5, Suppl. 3, pp. 345-358; Hein J. (1990) Unified Approach to Alignment and Phylogenes, pp. 626-645 Methods in Enzymology vol. 183, Academic Press, Inc., San Diego, CA; Higgins, D.G. and Sharp, P.M., CABIOS 5:151-153 (1989); Myers, E.W. and Muller W., CABIOS 4:11-17 (1988); Robinson, E.D., Comb. Theor 11:105 (1971); Saitou, N. Nei, M., Mol. Biol. Evol. 4:406-425 (1987); Sneath, P.H.A. and Sokal, R.R., Numerical Taxonomy - the Principles and Practice of Numerical Taxonomy, Freeman Press, San Francisco, CA (1973); Wilbur, W.J. and Lipman, D.J., Proc. Natl. Acad., Sci. USA 80:726-730 (1983).

[0036] Alternatively, optimal alignment of sequences for comparison may be conducted by the local identity algorithm of Smith and Waterman, Add. APL. Math 2:482 (1981), by the identity alignment algorithm of Needleman and Wunsch, J. Mol. Biol. 48:443 (1970), by the search for similarity methods of Pearson and Lipman, Proc. Natl. Acad. Sci. USA 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, BLAST, FASTA, and TFASTA in

the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, WI), or by inspection.

[0037] One preferred example of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al., Nucl. Acids Res. 25:3389-3402 (1977), and Altschul et al., J. Mol. Biol. 215:403-410 (1990), respectively. BLAST and BLAST 2.0 can be used, for example with the parameters described herein, to determine percent sequence identity for the polynucleotides and polypeptides of the invention. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. For amino acid sequences, a scoring matrix can be used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment.

[0038] In one preferred approach, the "percentage of sequence identity" is determined by comparing two optimally aligned sequences over a window of comparison of at least 20 positions, wherein the portion of the polypeptide sequence in the comparison window may comprise additions or deletions *(i.e.,* gaps) of 20 percent or less, usually 5 to 15 percent, or 10 to 12 percent, as compared to the reference sequences (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the reference sequence *(i.e.,* the window size) and multiplying the results by 100 to yield the percentage of sequence identity.

[0039] Within other illustrative embodiments, a polypeptide may be a fusion polypeptide that comprises multiple polypeptides as described herein, or that comprises at least one polypeptide as described herein and an unrelated sequence, such as a His tag for purification, or a targeting peptide. A fusion partner may, for example, assist in expressing the protein (an expression enhancer) at higher yields than the native recombinant protein. Other fusion partners may be selected so as to increase the solubility of the polypeptide or to enable the polypeptide to be targeted to desired intracellular compartments. Still further fusion partners include affinity tags, which facilitate purification of the polypeptide. In certain embodiments, a fusion partner increases the tolerogenicity of the polypeptide or increases its uptake by cells. In further embodiments, a fusion partner comprises an immune response enhancer.

[0040] Fusion polypeptides may generally be prepared using standard techniques, including chemical conjugation. Preferably, a fusion polypeptide is expressed as a recombinant polypeptide, allowing the production of increased levels, relative to a non-fused polypeptide, in an expression system. Briefly, DNA sequences encoding the polypeptide components may be assembled separately, and ligated into an appropriate expression vector. The 3' end of the DNA sequence encoding one polypeptide component is ligated, with or without a peptide linker, to the 5' end of a DNA sequence encoding the second polypeptide component so that the reading frames of the sequences are in phase. This permits translation into a single fusion polypeptide that retains the biological activity of both component polypeptides.

[0041] A peptide linker sequence may be employed to separate the first and second polypeptide components by a distance sufficient to ensure that each polypeptide folds into its secondary and tertiary structures. Such a peptide linker sequence is incorporated into the fusion polypeptide using standard techniques well known in the art. Suitable peptide linker sequences may be chosen based on the following factors: (1) their ability to adopt a flexible extended conformation; (2) their inability to adopt a secondary structure that could interact with functional epitopes on the first and second polypeptides; and (3) the lack of hydrophobic or charged residues that might react with the polypeptide functional epitopes. Preferred peptide linker sequences contain Gly, Asn and Ser residues. Other near neutral amino acids, such as Thr and Ala may also be used in the linker sequence. Amino acid sequences which may be usefully employed as linkers include those disclosed in Maratea et al., Gene 40:39-46, 1985; Murphy et al., Proc. Natl. Acad. Sci. USA 83:8258-8262, 1986; U.S. Patent No. 4,935,233 and U.S. Patent No. 4,751,180. The linker sequence may generally be from 1 to about 50 amino acids in length. Linker sequences are not required when the first and second polypeptides have non-essential N-terminal amino acid regions that can be used to separate the functional domains and prevent steric interference.

[0042] The ligated DNA sequences are operably linked to suitable transcriptional or translational regulatory elements. The regulatory elements responsible for expression of DNA are located only 5' to the DNA sequence encoding the first polypeptides. Similarly, stop codons required to end translation and transcription termination signals are only present 3' to the DNA sequence encoding the second polypeptide.

[0043] One embodiment of the invention involves fusion polypeptides, and the polynucleotides encoding them, wherein the fusion partner comprises a targeting signal capable of directing a polypeptide to the endosomal/lysosomal compartment, as described in U.S. Patent No. 5,633,234. A tolerogenic polypeptide of the invention, when fused with this targeting signal, will associate more efficiently with MHC class II molecules and thereby provide enhanced *in vivo* stimulation of appropriate $CD4^+$ T cells specific for the polypeptide.

[0044] Polypeptides of the invention are prepared using any of a variety of well known synthetic and/or recombinant techniques, the latter of which are further described below. Polypeptides, portions and other variants generally less than

about 150 amino acids can be generated by synthetic means, using techniques well known to those of ordinary skill in the art. In one illustrative example, such polypeptides are synthesized using any of the commercially available solid-phase techniques, such as the Merrifield solid-phase synthesis method, where amino acids are sequentially added to a growing amino acid chain. *See* Merrifield, J. Am. Chem. Soc. 85:2149-2146, 1963. Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Perkin Elmer/Applied BioSystems Division (Foster City, CA), and may be operated according to the manufacturer's instructions.

[0045] In general, polypeptide compositions (including fusion polypeptides) of the invention are isolated. An "isolated" polypeptide is one that is removed from its original environment. For example, a naturally-occurring protein or polypeptide is isolated if it is separated from some or all of the coexisting materials in the natural system. Preferably, such polypeptides are also purified, *e.g.*, are at least about 90% pure, more preferably at least about 95% pure and most preferably at least about 99% pure.

[0046] ColV protein may be purified from a variety of sources or may be purchased from a commercial source (Collaborative Biomedical Products/ Becton, Dickinson and Company, Franklin Lakes, NJ USA). In order to practice some embodiments it may be necessary to obtain pure or partially pure collagen or a tolerogenic fragment, epitope or antigenic portion thereof. These materials for example type V collagen or fragments thereof can be readily obtained by a variety of means including but not limited to animal sources, human cadavers, or recombinant means. Additional methods include partial digests of collagen such as type V collagen. In this regard, Human type V collagen may be extracted from human placenta or other sources and purified by differential NaCl precipitation (Seyer and Kang, 1989). For example, placental tissues are minced, washed, and suspended in 0.5 M acetic acid containing 0.2 M NaCl, and digested by pepsin at 4°C. Supernatants are aspirated from centrifuged specimens, the pellet collected and the extraction procedure repeated. The supernatants are combined from the two digests, and col(V) was purified from the supernatants by differential NaCl precipitation from 0.5 M acetic acid (Smith *et al.,* 1985; Seyer and Kang, 1989). The type V collagen is generally soluble in 0.7 M NaCl and precipitated in 1.2 M NaCl.

[0047] For those embodiments where it is required to purify α(V) chains, the cycle of solubilization in acetic acid and NaCl precipitation may be repeated until a type V preparation with an α-chain ratio α 1 (V)/ α 2(V) of approximately 2 is obtained as determined by SDS-polyacrylamide gel electrophoresis (Smith *et al*., 1985), or other appropriate method known to the skilled artisan. Separation of α 1 (V) from α 2(V) may be achieved by chromatography on DEAE-cellulose (Seyer and Kang, 1989) or other methods known to the skilled person, such as those described in Protein Purification Protocols, Ed. Shawn Doonan, Humana Press, 1996). The α 1(V) and α 2(V) chains may be eluted from the column, and purity confirmed by SDS-polyacrylamide gel electrophoresis as previously reported (Smith, Jr. *et al*, 1985). Intact col(V), or α 1(V) and α 2(V) chains may be diluted in PBS (0.5 mg/ml) or other appropriate buffer until use.

[0048] The present invention, in certain embodiments, provides polynucleotides encoding the collagen proteins of the present invention. Illustrative polynucleotides are those set forth in SEQ ID NOs:1, 3 and 5, and fragments thereof that encode a tolerogenic fragment of a collagen protein as described herein.

[0049] The terms "DNA" and "polynucleotide" are used essentially interchangeably herein to refer to a DNA molecule that has been isolated free of total genomic DNA of a particular species. "Isolated", as used herein, means that a polynucleotide is substantially away from other coding sequences, and that the DNA molecule does not contain large portions of unrelated coding DNA, such as large chromosomal fragments or other functional genes or polypeptide coding regions. Of course, this refers to the DNA molecule as originally isolated, and does not exclude genes or coding regions later added to the segment by the hand of man.

[0050] As will be understood by those skilled in the art, the polynucleotide compositions of this invention can include genomic sequences, extra-genomic and plasmid-encoded sequences and smaller engineered gene segments that express, or may be adapted to express, proteins, polypeptides, peptides and the like. Such segments may be naturally isolated, or modified synthetically by the hand of man.

[0051] Polynucleotides compositions of the present invention may be identified, prepared and/or manipulated using any of a variety of well established techniques (see generally, Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1989; Ausubel et al. (2001 Current Protocols in Molecular Biology, Greene Publ. Assoc. Inc. & John Wiley & Sons, Inc., NY, NY) and other like references).

[0052] Many template dependent processes are available to amplify a target sequences of interest present in a sample. One of the best known amplification methods is the polymerase chain reaction (PCR™) which is described in detail in U.S. Patent Nos. 4,683,195, 4,683,202 and 4,800,159.

[0053] Any of a number of other template dependent processes, many of which are variations of the PCR ™ amplification technique, are readily known and available in the art. Illustratively, some such methods include the ligase chain reaction (referred to as LCR), described, for example, in Eur. Pat. Appl. Publ. No. 320,308 and U.S. Patent No. 4,883,750; Qbeta Replicase, described in PCT Intl. Pat. Appl. Publ. No. PCT/US87/00880; Strand Displacement Amplification (SDA) and Repair Chain Reaction (RCR). Still other amplification methods are described in Great Britain Pat. Appl. No. 2 202 328, and in PCT Intl. Pat. Appl. Publ. No. PCT/US89/01025. Other nucleic acid amplification procedures include transcription-based amplification systems (TAS) (PCT Intl. Pat. Appl. Publ. No. WO 88/10315), including nucleic acid sequence based

amplification (NASBA) and 3SR. Eur. Pat. Appl. Publ. No. 329,822 describes a nucleic acid amplification process involving cyclically synthesizing single-stranded RNA ("ssRNA"), ssDNA, and double-stranded DNA (dsDNA). PCT Intl. Pat. Appl. Publ. No. WO 89/06700 describes a nucleic acid sequence amplification scheme based on the hybridization of a promoter/primer sequence to a target single-stranded DNA ("ssDNA") followed by transcription of many RNA copies of the sequence. Other amplification methods such as "RACE" (Frohman, 1990), and "one-sided PCR" (Ohara, 1989) are also well-known to those of skill in the art.

[0054] An amplified portion of a polynucleotide of the present invention may be used to isolate a full length gene from a suitable library (e.g., a tumor cDNA library) using well known techniques.

[0055] As will be also recognized by the skilled artisan, polynucleotides of the invention may be single-stranded (coding or antisense) or double-stranded, and may be DNA (genomic, cDNA or synthetic) or RNA molecules. RNA molecules may include HnRNA molecules, which contain introns and correspond to a DNA molecule in a one-to-one manner, and mRNA molecules, which do not contain introns. Additional coding or non-coding sequences may, but need not, be present within a polynucleotide of the present invention, and a polynucleotide may, but need not, be linked to other molecules and/or support materials.

[0056] Polynucleotides may comprise a native sequence *(i.e.,* an endogenous sequence that encodes a polypeptide/protein of the invention or a portion thereof) or may comprise a sequence that encodes a variant or derivative, preferably a tolerogenic variant or derivative, of such a sequence.

[0057] In other related embodiments, the present invention provides polynucleotide variants having substantial identity to the sequences disclosed herein in SEQ ID NOs: 1, 3 and 5, for example those comprising at least 70% sequence identity, preferably at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% or higher, sequence identity compared to a polynucleotide sequence of this invention using the methods described herein, (e.g., BLAST analysis using standard parameters, as described below). One skilled in this art will recognize that these values can be appropriately adjusted to determine corresponding identity of proteins encoded by two nucleotide sequences by taking into account codon degeneracy, amino acid similarity, reading frame positioning and the like.

[0058] Typically, polynucleotide variants will contain one or more substitutions, additions, deletions and/or insertions, preferably such that the tolerogenic activity of the polypeptide encoded by the variant polynucleotide is not substantially diminished relative to a polypeptide encoded by a polynucleotide sequence specifically set forth herein. The term "variants" should also be understood to encompasses homologous genes of xenogenic origin.

[0059] In additional embodiments, the present invention provides polynucleotide fragments comprising or consisting of various lengths of contiguous stretches of sequence identical to or complementary to one or more of the sequences disclosed herein. For example, polynucleotides are provided by this invention that comprise or consist of at least about 10, 15, 20, 30, 40, 50, 75, 100, 150, 200, 300, 400, 500 or 1000 or more contiguous nucleotides of one or more of the sequences disclosed herein as well as all intermediate lengths there between. It will be readily understood that "intermediate lengths", in this context, means any length between the quoted values, such as 16, 17, 18, 19, *etc*.; 21, 22, 23, *etc*.; 30, 31, 32, *etc*.; 50, 51, 52, 53, *etc*.; 100, 101, 102, 103, *etc*.; 150, 151, 152, 153, *etc*.; including all integers through 200-500; 500-1,000, and the like. A polynucleotide sequence as described here may be extended at one or both ends by additional nucleotides not found in the native sequence. This additional sequence may consist of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides at either end of the disclosed sequence or at both ends of the disclosed sequence.

[0060] In another embodiment of the invention, polynucleotide compositions are provided that are capable of hybridizing under moderate to high stringency conditions to a polynucleotide sequence provided herein, or a fragment thereof, or a complementary sequence thereof. Hybridization techniques are well known in the art of molecular biology. For purposes of illustration, suitable moderately stringent conditions for testing the hybridization of a polynucleotide of this invention with other polynucleotides include prewashing in a solution of 5 X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0); hybridizing at 50°C-60°C, 5 X SSC, overnight; followed by washing twice at 65°C for 20 minutes with each of 2X, 0.5X and 0.2X SSC containing 0.1 % SDS. One skilled in the art will understand that the stringency of hybridization can be readily manipulated, such as by altering the salt content of the hybridization solution and/or the temperature at which the hybridization is performed. For example, in another embodiment, suitable highly stringent hybridization conditions include those described above, with the exception that the temperature of hybridization is increased, e.g., to 60-65°C or 65-70°C.

[0061] In one embodiment, such polynucleotide variants encode polypeptides that have a level of tolerogenic activity of at least about 50%, preferably at least about 70%, and more preferably at least about 90%, 95%, 96%, 97%, 98%, 99%, or more, of that for a polypeptide sequence specifically set forth herein.

[0062] The polynucleotides of the present invention, or fragments thereof, regardless of the length of the coding sequence itself, may be combined with other DNA sequences, such as promoters, polyadenylation signals, additional restriction enzyme sites, multiple cloning sites, other coding segments, and the like, such that their overall length may vary considerably. It is therefore contemplated that a nucleic acid fragment of almost any length may be employed, with the total length preferably being limited by the ease of preparation and use in the intended recombinant DNA protocol. For example, illustrative polynucleotide segments with total lengths of about 10,000, about 5000, about 3000, about

2,000, about 1,000, about 500, about 200, about 100, about 50 base pairs in length, and the like, (including all intermediate lengths) are contemplated to be useful in many implementations of this invention.

[0063] When comparing polynucleotide sequences, two sequences are said to be "identical" if the sequence of nucleotides in the two sequences is the same when aligned for maximum correspondence, as described below. Comparisons between two sequences are typically performed by comparing the sequences over a comparison window to identify and compare local regions of sequence similarity. A "comparison window" as used herein, refers to a segment of at least about 20 contiguous positions, usually 30 to about 75, 40 to about 50, in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned.

[0064] Optimal alignment of sequences for comparison may be conducted using the Megalign program in the Lasergene suite of bioinformatics software (DNASTAR, Inc., Madison, WI), using default parameters. This program embodies several alignment schemes described in the following references: Dayhoff, M.O. (1978) A model of evolutionary change in proteins - Matrices for detecting distant relationships. In Dayhoff, M.O. (ed.) Atlas of Protein Sequence and Structure, National Biomedical Research Foundation, Washington DC Vol. 5, Suppl. 3, pp. 345-358; Hein J., Unified Approach to Alignment and Phylogenes, pp. 626-645 (1990); Methods in Enzymology vol. 183, Academic Press, Inc., San Diego, CA; Higgins, D.G. and Sharp, P.M., CABIOS 5:151-153 (1989); Myers, E.W. and Muller W., CABIOS 4:11-17 (1988); Robinson, E.D., Comb. Theor 11:105 (1971); Saitou, N. Nei, M., Mol. Biol. Evol. 4:406-425 (1987); Sneath, P.H.A. and Sokal, R.R., Numerical Taxonomy - the Principles and Practice of Numerical Taxonomy, Freeman Press, San Francisco, CA (1973); Wilbur, W.J. and Lipman, D.J., Proc. Natl. Acad., Sci. USA 80:726-730 (1983).

[0065] Alternatively, optimal alignment of sequences for comparison may be conducted by the local identity algorithm of Smith and Waterman, Add. APL. Math 2:482 (1981), by the identity alignment algorithm of Needleman and Wunsch, J. Mol. Biol. 48:443 (1970), by the search for similarity methods of Pearson and Lipman, Proc. Natl. Acad. Sci. USA 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, BLAST, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, WI), or by inspection.

[0066] One preferred example of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al., Nucl. Acids Res. 25:3389-3402 (1977), and Altschul et al., J. Mol. Biol. 215:403-410 (1990), respectively. BLAST and BLAST 2.0 can be used, for example with the parameters described herein, to determine percent sequence identity for the polynucleotides of the invention. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. In one illustrative example, cumulative scores can be calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always >0) and N (penalty score for mismatching residues; always <0). Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, and expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff and Henikoff, Proc. Natl. Acad. Sci. USA 89:10915 (1989)) alignments, (B) of 50, expectation (E) of 10, M=5, N=-4 and a comparison of both strands.

[0067] Preferably, the "percentage of sequence identity" is determined by comparing two optimally aligned sequences over a window of comparison of at least 20 positions, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions *(i.e.,* gaps) of 20 percent or less, usually 5 to 15 percent, or 10 to 12 percent, as compared to the reference sequences (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid bases occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the reference sequence *(i.e.,* the window size) and multiplying the results by 100 to yield the percentage of sequence identity.

[0068] It will be appreciated by those of ordinary skill in the art that, as a result of the degeneracy of the genetic code, there are many nucleotide sequences that encode a polypeptide as described herein. Some of these polynucleotides bear minimal homology to the nucleotide sequence of any native gene. Nonetheless, polynucleotides that vary due to differences in codon usage are specifically contemplated by the present invention. Further, alleles of the genes comprising the polynucleotide sequences provided herein are within the scope of the present invention. Alleles are endogenous genes that are altered as a result of one or more mutations, such as deletions, additions and/or substitutions of nucleotides. The resulting mRNA and protein may, but need not, have an altered structure or function. Alleles may be identified using standard techniques (such as hybridization, amplification and/or database sequence comparison).

[0069] Therefore, in another embodiment of the invention, a mutagenesis approach, such as site-specific mutagenesis, is employed for the preparation of tolerogenic variants and/or derivatives of the polypeptides described herein. By this approach, specific modifications in a polypeptide sequence can be made through mutagenesis of the underlying polynucleotides that encode them. These techniques provides a straightforward approach to prepare and test sequence

variants, for example, incorporating one or more of the foregoing considerations, by introducing one or more nucleotide sequence changes into the polynucleotide.

[0070] Site-specific mutagenesis allows the production of mutants through the use of specific oligonucleotide sequences which encode the DNA sequence of the desired mutation, as well as a sufficient number of adjacent nucleotides, to provide a primer sequence of sufficient size and sequence complexity to form a stable duplex on both sides of the deletion junction being traversed. Mutations may be employed in a selected polynucleotide sequence to improve, alter, decrease, modify, or otherwise change the properties of the polynucleotide itself, and/or alter the properties, activity, composition, stability, or primary sequence of the encoded polypeptide.

[0071] In certain embodiments of the present invention, the inventors contemplate the mutagenesis of the disclosed polynucleotide sequences to alter one or more properties of the encoded polypeptide, such as the tolerogenicity of a polypeptide. The techniques of site-specific mutagenesis are well-known in the art, and are widely used to create variants of both polypeptides and polynucleotides. For example, site-specific mutagenesis is often used to alter a specific portion of a DNA molecule. In such embodiments, a primer comprising typically about 14 to about 25 nucleotides or so in length is employed, with about 5 to about 10 residues on both sides of the junction of the sequence being altered.

[0072] As will be appreciated by those of skill in the art, site-specific mutagenesis techniques have often employed a phage vector that exists in both a single stranded and double stranded form. Typical vectors useful in site-directed mutagenesis include vectors such as the M13 phage. These phage are readily commercially-available and their use is generally well-known to those skilled in the art. Double-stranded plasmids are also routinely employed in site directed mutagenesis that eliminates the step of transferring the gene of interest from a plasmid to a phage.

[0073] In general, site-directed mutagenesis in accordance herewith is performed by first obtaining a single-stranded vector or melting apart of two strands of a double-stranded vector that includes within its sequence a DNA sequence that encodes the desired peptide. An oligonucleotide primer bearing the desired mutated sequence is prepared, generally synthetically. This primer is then annealed with the single-stranded vector, and subjected to DNA polymerizing enzymes such as *E. coli* polymerase I Klenow fragment, in order to complete the synthesis of the mutation-bearing strand. Thus, a heteroduplex is formed wherein one strand encodes the original non-mutated sequence and the second strand bears the desired mutation. This heteroduplex vector is then used to transform appropriate cells, such as *E. coli* cells, and clones are selected which include recombinant vectors bearing the mutated sequence arrangement.

[0074] The preparation of sequence variants of the selected peptide-encoding DNA segments using site-directed mutagenesis provides a means of producing potentially useful species and is not meant to be limiting as there are other ways in which sequence variants of peptides and the DNA sequences encoding them may be obtained. For example, recombinant vectors encoding the desired peptide sequence may be treated with mutagenic agents, such as hydroxylamine, to obtain sequence variants. Specific details regarding these methods and protocols are found in the teachings of Maloy *et al*., 1994; Segal, 1976; Prokop and Bajpai, 1991; Kuby, 1994; and Maniatis *et al*., 1982.

[0075] As used herein, the term "oligonucleotide directed mutagenesis procedure" refers to template-dependent processes and vector-mediated propagation which result in an increase in the concentration of a specific nucleic acid molecule relative to its initial concentration, or in an increase in the concentration of a detectable signal, such as amplification. As used herein, the term "oligonucleotide directed mutagenesis procedure" is intended to refer to a process that involves the template-dependent extension of a primer molecule. The term template dependent process refers to nucleic acid synthesis of an RNA or a DNA molecule wherein the sequence of the newly synthesized strand of nucleic acid is dictated by the well-known rules of complementary base pairing (see, for example, Watson, 1987). Typically, vector mediated methodologies involve the introduction of the nucleic acid fragment into a DNA or RNA vector, the clonal amplification of the vector, and the recovery of the amplified nucleic acid fragment. Examples of such methodologies are provided by U. S. Patent No. 4,237,224.

[0076] In another approach for the production of polypeptide variants of the present invention, recursive sequence recombination, as described in U.S. Patent No. 5,837,458, may be employed. In this approach, iterative cycles of recombination and screening or selection are performed to "evolve" individual polynucleotide variants of the invention having, for example, enhanced tolerogenic activity.

[0077] In other embodiments of the invention, polynucleotide sequences or fragments thereof which encode polypeptides of the invention, or fusion proteins or functional equivalents thereof, may be used in recombinant DNA molecules to direct expression of a polypeptide in appropriate host cells. Due to the inherent degeneracy of the genetic code, other DNA sequences that encode substantially the same or a functionally equivalent amino acid sequence may be produced and these sequences may be used to clone and express a given polypeptide.

[0078] As will be understood by those of skill in the art, it may be advantageous in some instances to produce polypeptide-encoding nucleotide sequences possessing non-naturally occurring codons. For example, codons preferred by a particular prokaryotic or eukaryotic host can be selected to increase the rate of protein expression or to produce a recombinant RNA transcript having desirable properties, such as a half-life which is longer than that of a transcript generated from the naturally occurring sequence.

[0079] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally

known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

[0080] In another embodiment of the invention, natural, modified, or recombinant nucleic acid sequences may be ligated to a heterologous sequence to encode a fusion protein. For example, to screen peptide libraries for inhibitors of polypeptide activity, it may be useful to encode a chimeric protein that can be recognized by a commercially available antibody. A fusion protein may also be engineered to contain a cleavage site located between the polypeptide-encoding sequence and the heterologous protein sequence, so that the polypeptide may be cleaved and purified away from the heterologous moiety.

[0081] Sequences encoding a desired polypeptide may be synthesized, in whole or in part, using chemical methods well known in the art (see Caruthers, M. H. et al. (1980) Nucl. Acids Res. Symp. Ser. 215-223, Horn, T. et al. (1980) Nucl. Acids Res. Symp. Ser. 225-232). Alternatively, the protein itself may be produced using chemical methods to synthesize the amino acid sequence of a polypeptide, or a portion thereof. For example, peptide synthesis can be performed using various solid-phase techniques (Roberge, J. Y. et al. (1995) Science 269:202-204) and automated synthesis may be achieved, for example, using the ABI 431A Peptide Synthesizer (Perkin Elmer, Palo Alto, CA).

[0082] A newly synthesized peptide may be substantially purified by preparative high performance liquid chromatography (*e.g.*, Creighton, T. (1983) Proteins, Structures and Molecular Principles, WH Freeman and Co., New York, N.Y.) or other comparable techniques available in the art. The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing (*e.g.*, the Edman degradation procedure). Additionally, the amino acid sequence of a polypeptide, or any part thereof, may be altered during direct synthesis and/or combined using chemical methods with sequences from other proteins, or any part thereof, to produce a variant polypeptide.

[0083] In order to express a desired polypeptide, the nucleotide sequences encoding the polypeptide, or functional equivalents, may be inserted into appropriate expression vector, *i.e.*, a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence. Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding a polypeptide of interest and appropriate transcriptional and translational control elements. These methods include *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. Such techniques are described, for example, in Sambrook, J. et al. (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Plainview, N.Y., and Ausubel et al. (2001-2008 Current Protocols in Molecular Biology, Greene Publ. Assoc. Inc. & John Wiley & Sons, Inc., NY, NY).

[0084] A variety of expression vector/host systems may be utilized to contain and express polynucleotide sequences. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with virus expression vectors (*e.g.*, baculovirus); plant cell systems transformed with virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial expression vectors (*e.g.*, Ti or pBR322 plasmids); or animal cell systems.

[0085] The "control elements" or "regulatory sequences" present in an expression vector are those non-translated regions of the vector-enhancers, promoters, 5' and 3' untranslated regions-which interact with host cellular proteins to carry out transcription and translation. Such elements may vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the pBLUESCRIPT phagemid (Stratagene, La Jolla, Calif.) or pSPORT1 plasmid (Gibco BRL, Gaithersburg, MD) and the like may be used. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are generally preferred. If it is necessary to generate a cell line that contains multiple copies of the sequence encoding a polypeptide, vectors based on SV40 or EBV may be advantageously used with an appropriate selectable marker.

[0086] In bacterial systems, any of a number of expression vectors may be selected depending upon the use intended for the expressed polypeptide. For example, when large quantities are needed, for example for the induction of antibodies, vectors which direct high level expression of fusion proteins that are readily purified may be used. Such vectors include, but are not limited to, the multifunctional *E. coli* cloning and expression vectors such as pBLUESCRIPT (Stratagene), in which the sequence encoding the polypeptide of interest may be ligated into the vector in frame with sequences for the amino-terminal Met and the subsequent 7 residues of β-galactosidase so that a hybrid protein is produced; pIN vectors (Van Heeke, G. and S. M. Schuster (1989) J. Biol. Chem. 264:5503-5509); and the like. pGEX Vectors (Promega, Madison, Wis.) may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems may be designed to include heparin, thrombin, or factor XA protease cleavage sites so that the cloned polypeptide of interest can be

released from the GST moiety at will.

[0087] In the yeast, Saccharomyces cerevisiae, a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase, and PGH may be used. For reviews, see Ausubel *et al.* (supra) and Grant et al. (1987) Methods Enzymol. 153:516-544.

[0088] In cases where plant expression vectors are used, the expression of sequences encoding polypeptides may be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV may be used alone or in combination with the omega leader sequence from TMV (Takamatsu, N. (1987) EMBO J. 6:307-311. Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters may be used (Coruzzi, G. et al. (1984) EMBO J. 3:1671-1680; Broglie, R. et al. (1984) Science 224:838-843; and Winter, J. et al. (1991) Results Probl. Cell Differ. 17:85-105). These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. Such techniques are described in a number of generally available reviews (see, for example, Hobbs, S. or Murry, L. E. in McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York, N.Y.; pp. 191-196).

[0089] An insect system may also be used to express a polypeptide of interest. For example, in one such system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in Spodoptera frugiperda cells or in Trichoplusia larvae. The sequences encoding the polypeptide may be cloned into a non-essential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of the polypeptide-encoding sequence will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses may then be used to infect, for example, S. frugiperda cells or Trichoplusia larvae in which the polypeptide of interest may be expressed (Engelhard, E. K. et al. (1994) Proc. Natl. Acad. Sci. 91 :3224-3227).

[0090] In mammalian host cells, a number of viral-based expression systems are generally available. For example, in cases where an adenovirus is used as an expression vector, sequences encoding a polypeptide of interest may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain a viable virus which is capable of expressing the polypeptide in infected host cells (Logan, J. and Shenk, T. (1984) Proc. Natl. Acad. Sci. 81:3655-3659). In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells.

[0091] Specific initiation signals may also be used to achieve more efficient translation of sequences encoding a polypeptide of interest. Such signals include the ATG initiation codon and adjacent sequences. In cases where sequences encoding the polypeptide, its initiation codon, and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a portion thereof, is inserted, exogenous translational control signals including the ATG initiation codon should be provided. Furthermore, the initiation codon should be in the correct reading frame to ensure translation of the entire insert. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers which are appropriate for the particular cell system which is used, such as those described in the literature (Scharf, D. et al. (1994) Results Probl. Cell Differ. 20:125-162*).*

[0092] In addition, a host cell strain may be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation. glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" form of the protein may also be used to facilitate correct insertion, folding and/or function. Different host cells such as CHO, COS, HeLa, MDCK, HEK293, and WI38, which have specific cellular machinery and characteristic mechanisms for such post-translational activities, may be chosen to ensure the correct modification and processing of the foreign protein.

[0093] For long-term, high-yield production of recombinant proteins, stable expression is generally preferred. For example, cell lines which stably express a polynucleotide of interest may be transformed using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be proliferated using tissue culture techniques appropriate to the cell type.

[0094] Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler, M. et al. (1977) Cell 11:223-32) and adenine phosphoribosyltransferase (Lowy, I. et al. (1990) Cell 22:817-23) genes which can be employed in tk^- or $aprt^-$ cells, respectively. Also, antimetabolite, antibiotic or herbicide resistance can be used as the basis for selection; for example, dhfr which confers resistance to methotrexate (Wigler, M. et al. (1980) Proc. Natl. Acad. Sci. 77:3567-70); npt, which confers resistance to the aminoglycosides, neomycin and G-418 (Colbere-Garapin, F. et al (1981) J. Mol. Biol. 150:1-14); and als or pat, which confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively (Murry, *supra*).

Additional selectable genes have been described, for example, trpB, which allows cells to utilize indole in place of tryptophan, or hisD, which allows cells to utilize histinol in place of histidine (Hartman, S. C. and R. C. Mulligan (1988) Proc. Natl. Acad. Sci. 85:8047-51). The use of visible markers has gained popularity with such markers as anthocyanins, beta-glucuronidase and its substrate GUS, and luciferase and its substrate luciferin, being widely used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system (Rhodes, C. A. et al. (1995) Methods Mol. Biol. 55:121-131).

[0095] Although the presence/absence of marker gene expression suggests that the gene of interest is also present, its presence and expression may need to be confirmed. For example, if the sequence encoding a polypeptide is inserted within a marker gene sequence, recombinant cells containing sequences can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a polypeptide-encoding sequence under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

[0096] Alternatively, host cells that contain and express a desired polynucleotide sequence may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations and protein bioassay or immunoassay techniques which include, for example, membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein.

[0097] A variety of protocols for detecting and measuring the expression of polynucleotide-encoded products, using either polyclonal or monoclonal antibodies specific for the product are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on a given polypeptide may be preferred for some applications, but a competitive binding assay may also be employed. These and other assays are described, among other places, in Hampton, R. et al. (1990; Serological Methods, a Laboratory Manual, APS Press, St Paul. Minn.) and Maddox, D. E. et al. (1983; J. Exp. Med. 158:1211-1216).

[0098] A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides include oligolabeling, nick translation, end-labeling or PCR amplification using a labeled nucleotide. Alternatively, the sequences, or any portions thereof may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes *in vitro* by addition of an appropriate RNA polymerase such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits. Suitable reporter molecules or labels, which may be used include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

[0099] Host cells transformed with a polynucleotide sequence of interest may be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The protein produced by a recombinant cell may be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides of the invention may be designed to contain signal sequences which direct secretion of the encoded polypeptide through a prokaryotic or eukaryotic cell membrane. Other recombinant constructions may be used to join sequences encoding a polypeptide of interest to nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, Wash.). The inclusion of cleavable linker sequences such as those specific for Factor XA or enterokinase (Invitrogen. San Diego, Calif.) between the purification domain and the encoded polypeptide may be used to facilitate purification. One such expression vector provides for expression of a fusion protein containing a polypeptide of interest and a nucleic acid encoding 6 histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification on IMIAC (immobilized metal ion affinity chromatography) as described in Porath, J. et al. (1992, Prot. Exp. Purif. 3:263-281) while the enterokinase cleavage site provides a means for purifying the desired polypeptide from the fusion protein. A discussion of vectors which contain fusion proteins is provided in Kroll, D. J. et al. (1993; DNA Cell Biol. 12:441-453).

[0100] In addition to recombinant production methods, polypeptides of the invention, and fragments thereof, may be produced by direct peptide synthesis using solid-phase techniques (Merrifield J. (1963) J. Am. Chem. Soc. 85:2149-2154). Protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer). Alternatively, various fragments may be chemically synthesized separately and combined using chemical methods to produce the full length molecule.

Tolerance

[0101] Immunological tolerance is defined as immune unresponsiveness to an antigen, usually an antigen implicated

in causing disease. Although tolerance may be induced by administering antigens by different routes, oral tolerance refers to the oral administration of the antigen, which has resulted in suppression of disease activity in several animal models including experimental autoimmune encephalomyelitis-a rodent model of multiple sclerosis, myasthenia gravis, uveitis, insulin dependent diabetes, and collagen-induced arthritis (Faria and Weiner 1999). Early results from clinical trials in humans suggest that oral tolerance is effective in autoimmune uveitis, diabetes, nickel allergy, and possibly multiple sclerosis (Faria and Weiner 1999; Duda *et al.* 2000). There are few studies reporting oral tolerance induction in organ transplantation (Sayegh *et al.* 1996; Hancock *et al.* 1993; Ishido *et al.* 1999; Sayegh *et al.* 1992a; Sayegh *et al.* 1992b). In each report, tolerance was induced by feeding donor MHC-derived peptides or feeding allogeneic cells prior to transplantation (Sayegh *et al.* 1996; Hancock *et al.* 1993; Ishido *et al.* 1999; Sayegh *et al.* 1992a; Sayegh *et al.* 1992b). These techniques were effective in preventing rejection of cardiac and corneal allografts (Sayegh *et al.* 1996; Hancock *et al.* 1993; Ishido *et al.* 1999; Sayegh *et al.* 1992a; Sayegh *et al.* 1992b; Faria and Weiner 1999). In addition to diminished disease activity, immune suppression induced by oral tolerance in these studies was also quantitated by down regulation of delayed type hypersensitivity (DTH) responses to target antigens, as well as diminished cellular and humoral immunity (Faria and Weiner 1999; Mayer 2000; Garside and Mowat 1997).

[0102] There are three mechanisms by which oral (and other routes of administration) tolerance down regulates antigen-specific immune responses: 1. active suppression of antigen specific cells, 2. clonal anergy of antigen specific cells, and 3. clonal deletion of antigen specific cells (Faria and Weiner 1999, Miller *et al.* 1991; Chen *et al.* 1994; Chen *et al.* 1995). Although all three mechanisms can be operative simultaneously in response to oral tolerance, active suppression and clonal anergy are the key mechanisms of immune suppression induced by oral tolerance (Faria and Weiner 1999).

[0103] Active suppression describes the regulation of one lymphocyte subset by another in an antigen-specific manner. Depending on the antigen and disease state, the suppressor cells may be CD4+ and/or CD8+ T-lymphocytes which migrate from peripheral lymphoid tissues, such as spleen and peripheral lymph nodes, to sites of disease activity. Adoptive transfer of these cells to naive recipients has confirmed the role of these cells in active suppression in rodent models of ovalbumin-induced hypersensitivity, and multiple sclerosis. In vitro evidence of active suppression is demonstrated by data showing that tolerized lymphocytes from animals can suppress proliferation of other antigen-specific T-lymphocytes across a transwell cell culture system (Faria and Weiner 1999; Miller *et al.* 1991).

[0104] Clonal anergy refers to unresponsiveness of antigen-specific T-lymphocytes, which is characterized by diminished proliferation after exposure to an antigen, and is involved in oral tolerance in several animal models. Anergy could be the result of production of soluble suppressive factors by CD4+ or CD8+ T-lymphocytes themselves, other T-lymphocytes or cells in the local environment, or as result of decreased expression of appropriate costimulatory molecules (Faria and Weiner 1999). Clonal deletion refers to the elimination of antigen-specific T-lymphocytes, but has been reported rarely as a mechanism of oral tolerance to an antigen (Chen *et al.* 1995).

[0105] The soluble mediators that suppress the immune response during oral tolerance are derived mainly from regulatory or suppressor T-lymphocytes (Faria and Weiner 1999). There are five types of T-lymphocytes described by the cytokines they produce: Th1-type that produce interleukin-2 (IL-2) and gamma interferon (γIFN); Th2-type that produce IL-4 and IL-10; Th3-type that produce high levels or transforming growth factor beta (TGF- β), alone, or in conjunction with very low levels of IL-4, IL-10, or γIFN; Tr1 cells that produce high levels of IL-10 in conjunction with low levels of TGF- β (Faria and Weiner *et al.* 1999; Mayer 2000; Garside and Mowat 1997; Groux *et al.* 1997); and Th17 cells that produce IL-17 (see *e.g.*, Immunol Rev. 2008, 226:87-102; Nature. 2006 May 11;441(7090):235-8). Since Th3, Th2, and Tr1-T-lymphocytes have been shown to be the major mediators of active suppression induced by oral tolerance, then TGF- β, IL-4 and IL-10 are believed to be key cytokines in this process (Teng *et al.* 1998; Shi *et al.* 1999b). A report from Barone *et al.,* and others showing that oral tolerance induction occurred in the absence of these cytokines suggests that other mediators or cells could suppress the immune response (Barone *et al.* 1998; Shi *et al.* 1999a).

[0106] Although studies of oral tolerance have focused on T-lymphocyte-derived cytokines that suppress immune responses, nitric oxide, which is not produced by T-lymphocytes, is known to be a potent suppressor of alloimmmune responses (Garside and Mowat 1997). These data and others showing that nitric oxide modulates apoptosis, which is involved in the rejection response (Meyer *et al.* 1998; Kallio *et al.* 1997; Shiraishi *et al.* 1997; Shiraishi *et al.* 1995; Medot-Pirenne *et al.* 1999), suggests that nitric oxide could be a mediator of oral tolerance and prevent the rejection response. TGF- β, is a potent inducer of nitric oxide synthesis, and is a key mediator of active suppression in oral tolerance (Faria and Weiner 1999; Meyer *et al.* 1998; Vodovotz *et al.* 1998; Vodovitz *et al.* 1999). Therefore, immunosuppression induced by TGF- β in the tolerized host could be mediated, in part, by nitric oxide. However, production of nitric oxide in response to oral tolerance is unknown.

[0107] Antigen-specific T-lymphocyte activation induced by APCs requires bi-directional interaction between the T-lymphocyte and APC. Initially, APCs present MHC molecules that bind to the T-cell-receptor which stimulates upregulated expression of CD40-ligand (CD40L) on T-lymphocytes. CD40L, in turn, binds to its receptor, CD40, on the APC. Signaling through CD40 induces the expression of CD80 and CD86 on the APC which, upon binding to their receptor, CD28, on the T-lymphocyte, results in co-stimulation and subsequent T-lymphocyte activation (Liu *et al.* 1999; Li *et al.* 1999;

Lederman and Siciu-Foca 1999). Although studies of oral tolerance induction have focused on T-lymphocyte function, a recent study from Taams *et al* (1998), reported that tolerance induction may affect function of APCs, with similar data from other investigators (Wu *et al.* 1998; Finkelman *et al.* 1996; Viney *et al.* 1998). For example, a report from Wu. *et al* (1998), showing that expression of CD80 is decreased on APCs from the lymph nodes and spleens of orally tolerized mice suggests that ineffective APCs could contribute to impaired T-lymphocyte activation in tolerized recipients. Furthermore, studies in vitro showing that suppressor T-lymphocytes inhibit expression of CD86 in APCs highlights another mechanism of how tolerance induces impaired APC function (Liu *et al.* 1999; Li *et al.* 1999; Lederman and Siciu-Foca 1999).

[0108] Administration of col(V) prevents proliferative responses to alloantigens, in addition to preventing proliferative responses to itself, and prevents the development of acute rejection pathology in recipient lungs (see e.g., U.S. Patent 7,348,005; WO 2007/120947). Thus, col(V) may induce anergy to donor alloantigens and to itself; or alternatively, the lack of proliferative responses to donor antigens and to colV may be due to clonal deletion of alloantigen-specific lung lymphocytes; or yet alternatively may result from suppressor cell activity.

[0109] Without being bound by theory, tolerance induction by colV may be induced through linked suppression (see, *e.g.*, Hum Immunol. 2008 Nov;69(11):715-20). Also, it is thought that differential binding of colV to the collagen receptors on lymphocytes, leading to differential activation signals may be involved (see e.g., Cell Signal. 2006 Aug;18(8):1108-16). For example, only collagen V induced IL-17 signaling in T cells.

[0110] Oral administration of antigens is an effective method of inducing peripheral T-cell tolerance. This phenomenon, often referred to as oral tolerance, has been well studied in various models of autoimmune diseases in animals including encephalomyelitis, uveitis, diabetes, myasthenia gravis, and arthritis. However, the mechanisms for inducing tolerance are not completely understood. All of the known mechanisms for tolerance induction, including clonal anergy, clonal deletion, and regulation by IL-4, IL-10, or TGF-beta-mediated active suppression may have a role in oral tolerance (Faria and Weiner, 1999). Generally, higher doses of antigen are reported to induce anergy or clonal deletion (Chen *et al.,* 1995; Whitacre *et al.,* 1991), whereas low doses induce cytokine regulation and active suppression (Faria and Weiner, 1999; Chen *et al.,* 1994). In the animal model of cardiac transplantation, oral administration of allogeneic splenocytes has been shown to be effective in tolerance induction by bypassing Th1 activation and selectively stimulating induction of Th-2 derived inhibitory cytokines such as IL-4 (Hancock *et al*., 1993; Ishido *et al.,* 1999).

[0111] Thus, oral tolerance is a method of downregulating an immune response in a subject by orally administering an antigen *(i.e.* by feeding) to the subject. Oral tolerance is characterized by decreased levels of systemic antibody production, as well as decreased delayed type hypersensitivity responses (DTH), T cell proliferation, cytotoxic responses and graft rejection (Alpan et al. 2001. J. Immunol. 166:4843-52; Chen et al. 1995. Nature 376:177-80; Weiner. 1997. Imm. Today. 7:335-44; Sayegh et al. 1992. Transplantation. 53:163-6).

[0112] Other routes for inducing tolerance are also contemplated herein, in particular by intramuscular, subcutaneous, intradermal and intravenous injection. Intradermal injections are contemplated herein where low doses tend to induce tolerance and high doses induce an immune response.

[0113] Studies of tolerance have focused primarily on the effect of the tolerizing antigen on T-lymphocyte function, and the role of T-lymphocytes in suppressing immune activation (Faria and Weiner 1999; Mayer 2000; Garside and Mowat 1997). However, immune responses to any antigen require interactions between APCs and T-lymphocytes, and the T-lymphocyte may affect APC function (Liu *et al.* 1999; Li *et al.* 1999; Lederman and Siciu-Foca 1999). Therefore, down-regulated antigen presentation by APCs from tolerized hosts could contribute to tolerance induction either indirectly as a result of interactions with suppressor T-lymphocytes, or possibly as a result of direct effects of the tolerizing antigen on the APC.

[0114] Thus, in certain aspects of the invention, the present invention provides methods for restoring or reinforcing self-tolerance to colV in asthma patients. One embodiment is a method of treating asthma by administering colV either by oral therapy (Yasufuku *et al,* 2001 ; Yasufuku, *et al,* 2002) interstitially into the lung or by other desensitization strategies on a dosage regimen designed to increase the patient tolerance for collagens including, but not necessarily limited to colV and antigenic components and variants thereof.

[0115] By "tolerogenic fragment" is meant a fragment that can induce tolerance to the full-length protein of which it is a fragment (e.g., full-length type V collagen, and tolerogenic fragments thereof). In certain embodiments, a tolerogenic fragment can induce tolerance to the full-length type V collagen at least as well as the full-length type V collagen protein can and in certain embodiments may be more effective than the full-length collagen protein at inducing tolerance. However, in certain embodiments, a tolerogenic fragment induces tolerance to the full-length type V collagen but may not induce tolerance as effectively as the full-length type V collagen protein. Such tolerogenic fragments may still be useful in the present invention particularly where said tolerogenic fragments have other advantageous properties, such as ease of preparation or purification as compared to the full-length protein. As would be recognized by the skilled person, a variety of known assays can be used to assess induction of tolerance, including measuring delayed-type hypersensitivity (DTH) responses, measuring cytokine productions by ELISA or other methods, T cell proliferation or cytotoxicity assays, B cell proliferation assays, antibody production, and the like. Such assays are known in the art and

are described, for example, in Current Protocols in Immunology, Edited by: John E. Coligan, Ada M. Kruisbeek, David H. Margulies, Ethan M. Shevach, Warren Strober (2001 John Wiley & Sons, NY, NY); Ausubel et al. (2001 Current Protocols in Molecular Biology, Greene Publ. Assoc. Inc. & John Wiley & Sons, Inc., NY, NY); US Patent No. 7,348,005; and elsewhere.

[0116] Thus, a tolerogenic fragment is a fragment of a tolerogenic polypeptide such as type V collagen, or any one or more of the alpha chains thereof, that itself is immunologically tolerogenic *(i.e.,* induces tolerance) with regard to the specific B-cells and/or T-cells that recognize the polypeptide via their surface receptors (*e.g*., B cell antibody receptor or T cell receptor). Tolerogenic fragments may generally be identified using well known techniques, such as those summarized in Paul, Fundamental Immunology, 3rd ed., (Raven Press, 1993) and references cited therein. Such techniques include screening polypeptides for the ability to reduce T cell and/or B cell reactivity. As used herein, antisera and antibodies are "antigen-specific" if they specifically bind to an antigen *(i.e.,* they react specifically with the protein in an ELISA or other immunoassay, and do not react detectably with unrelated proteins). Such antisera and antibodies may be prepared as described herein, and using well-known techniques.

[0117] In one embodiment, a tolerogenic fragment of a polypeptide of the present invention is a portion that induces B cell and/or T cell tolerance at a level that is not substantially less than the tolerogenic activity of the full-length polypeptide (*e.g*., in an appropriate assay such as antibody production, which may be measured by ELISA, and/or T cell reactivity assay (T cell proliferation or cytokine production assay). Preferably, the level of tolerogenic activity of the tolerogenic portion is at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or greater than about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of the tolerogenic activity of the full-length polypeptide. In some instances, tolerogenic fragments will be identified that have a level of tolerogenic activity greater than that of the corresponding full-length polypeptide, e.g., having greater than about 100%, 110%, 120%, 130%, 140% or 150% or more tolerogenic activity.

[0118] In certain embodiments, tolerogenic fragments may be identified using computer analysis, such as the Tsites program (see Rothbard and Taylor, EMBO J. 7:93-100, 1988; Deavin et al., Mol. Immunol. 33:145-155, 1996), which searches for peptide motifs that have the potential to elicit Th responses. CTL peptides with motifs appropriate for binding to murine and human class I or class II MHC may be identified according to BIMAS (Parker et al., J. Immunol. 152:163, 1994) and other HLA peptide binding prediction analyses. Alternatively, portions that bind to a particular MHC molecule can be identified by using defined peptide binding motifs such as those described in Rammensee et al., Immunogenetics 41:178-228, 1995. To confirm peptide binding to murine and human class I or class II MHC molecules, peptide binding assays known in the art may be used. To confirm immunogenicity or tolerogenicity, a peptide may be tested using an HLA A2 or other transgenic mouse model and/or an *in vitro* stimulation assay using dendritic cells, fibroblasts or peripheral blood cells.

[0119] It should be noted that in certain embodiments, a tolerogenic fragment of the invention is also an immunogenic fragment. In this regard, as would be recognized by the skilled artisan, highly immunogenic fragments, such as immunodominant epitopes of proteins like colV, may be tolerogenic when administered correctly, *e.g*., generally in low doses over extended periods of time. Thus, the present invention also contemplates the identification and use of immunogenic fragments of colV where such immunogenic fragments may be used to induce tolerance. In this regard, the level of immunogenic activity of an immunogenic portion is at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or greater than about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of the immunogenic activity of the full-length polypeptide. In some instances, immunogenic fragments will be identified that have a level of immunogenic activity greater than that of the corresponding full-length polypeptide, e.g., having greater than about 100%, 110%, 120%, 130%, 140% or 150% or more immunogenic activity.

[0120] The same analyses may be used to identify immunogenic fragments that are used to identify tolerogenic fragments, including using computer analysis, such as the Tsites program (see Rothbard and Taylor, EMBO J. 7:93-100, 1988; Deavin et al., Mol. Immunol. 33:145-155, 1996), which searches for peptide motifs that have the potential to elicit Th responses. CTL peptides with motifs appropriate for binding to murine and human class I or class II MHC may be identified according to BIMAS (Parker et al., J. Immunol. 152:163, 1994) and other HLA peptide binding prediction analyses. Alternatively, portions that bind to a particular MHC molecule can be identified by using defined peptide binding motifs such as those described in Rammensee et al., Immunogenetics 41:178-228, 1995. To confirm peptide binding to murine and human class I or class II MHC molecules, peptide binding assays known in the art may be used. To confirm immunogenicity or tolerogenicity, a peptide may be tested using an HLA A2 or other transgenic mouse model and/or an *in vitro* stimulation assay using dendritic cells, fibroblasts or peripheral blood cells.

[0121] Intact type V collagen or any one or more of its component alpha chains that have tolerogenic or immunogenic activity, are contemplated for use in the methods of the present invention. As such, a tolerogenic fragment or immunogenic fragment of collagen V may refer to a fragment of intact type V collagen or may refer to a tolerogenic or immunogenic fragment of any one of the component alpha chains. In certain embodiments, the colV as used herein may comprise the collagen molecule composed of the three alpha chains. In a further embodiment, colV may comprise any one or more of the alpha chains, such as those set forth in SEQ ID NOs:2, 4 or 6, encoded by the polynucleotides set forth in SEQ ID NOs:1, 3, or 5, or a tolerogenic fragment or an immunogenic fragment thereof.

[0122] In certain embodiments, two or more tolerogenic or immunogenic fragments may be used concurrently, either administered separately, mixed in a composition, or as a fusion protein. In this regard, any number of tolerogenic fragments or immunogenic fragments may be used to induce tolerance to colV, such as 2, 3, 4, 5, 6, 7, 8, 9, 10 or more tolerogenic or immunogenic fragments, either in a composition as separate fragments or as a fusion protein, with or without linkers. In certain embodiments, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more fragments may be used in the methods of the present invention.

[0123] Detecting the presence of antibodies to collagen in accordance with some embodiments may be accomplished using any of a number of immunoassay procedures, such as by ELISA procedures. A wide range of immunoassay techniques is available as can be seen by reference to standard immunoassay textbooks these include, but are not limited to singlesite and two-site or "sandwich" assays of the non-competitive types, as well as the traditional competitive binding assays.

[0124] Sandwich assays are among the most useful and commonly used antibody based assay methods and may be used to practice various embodiments. A number of variations of the sandwich assay technique exist, and all are intended to be encompassed by various embodiments. Briefly, in a typical assay to detect antibodies in a sample, an unlabelled antigen is immobilized on a solid substrate and the sample to be tested is contacted with the bound antigen molecule. After a suitable period of incubation, *i.e.* for a period of time sufficient to allow formation of an antibody-antigen complex, a second antibody such as anti-human IgG, labeled with a reporter molecule capable of producing a detectable signal, is then added and incubated, allowing time sufficient for the formation of an antibody-antigen-labeled antibody. Any unreacted material is washed away, and the presence of the antibody to be detected in the sample is determined by observation of a signal produced by the reporter molecule. The results may either be qualitative, e.g., by simple observation of the visible signal, or may be quantitated by comparing the signal generated by a sample of interest with a control sample containing known amounts of antibody to be detected. Variations on this assay include a simultaneous assay, in which both the sample and labeled antibody are added simultaneously to the bound antigen. These techniques are well known, including any minor variations as will be readily apparent to those in the art. In the typical sandwich assay, antigen is immobilized, for example by being either covalently or passively bound to a solid surface. In some embodiments the solid surface is typically glass or a polymer, the most commonly used polymers being cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene. The solid supports may be in the form of tubes, beads, discs, or microplates, or any other surface suitable for conducting an immunoassay. Various binding processes are well-known in the art and generally consist of crosslinking, covalent binding or physical adsorption of the antigen to a given surface. The immobilized antigen is then washed in preparation for the addition of the test sample. An aliquot of the sample to be tested is then contacted with the immobilized antigen and incubated for a period of time sufficient (*e.g.* 2-40 minutes) and under suitable conditions (*e.g.* 25°C) to allow binding of any antibody to collagen present in the sample. The actual length of contact time, buffer conditions, temperatures and the like are readily adjustable parameters and are typically readily arrived at for a given test. Following the incubation period, the immobilized antigen including any bound antibody is washed and dried, and incubated with a second antibody specific for the bound antibody, for example anti-human IgG. The second antibody is linked to a reporter molecule which is used to indicate the binding of the second antibody to the antibody-immobilized antigen complex.

[0125] One particular method for measuring antibodies to colV is described in WO 2007/120947. Specifically, this bead assay detects antibodies to type V collagen as may be present in serum and/or lung lavage fluid from patients that have an autoimmune response type V collagen. Type V collagen-coated beads along with other necessary reagents are provided for this assay. Briefly, a typical assay is as follows: 1) Streptavidin-coated beads (e.g., such as those from Polyscience, Warrington, PA) are washed with sterile PBS. Beads are suspended in an appropriate volume of PBS with human type V collagen. 2) A positive control is generated by following the same procedures in 1 above, using rabbit antibody to human collagen V antibody (Bioten) (Abeam, Cambridge, MA). 3) For each assay, conjugated beads are washed in PBS, and incubated in PBS plus serum of lung lavage fluid. The beads are then washed with PBS containing 10% FCS. 4) The beads are then suspended in sterile PBS+10% FBS and incubated at room temperature with secondary antibody. Typically, anti-human IgG antibody conjugated with R-PE is used (Sigma, Saint Louis) although as would be understood by the skilled artisan, other suitable antibodies are available. In this regard, anti-human IgG1-, IgG2-, IgG3-, or IgG4-specific antibodies may be used in certain embodiments in order to detect switching from one subtype to another during the course of disease. The beads are washed in PBS containing 10% FCS, suspended in PBS/FCS solution and analyzed using a flow cytometer. For the positive control, known amounts of anti-colV antisera or antibody may be added to the bead assay.

[0126] Typically, antibodies to colV found in COPD and asthma patients are IgG but other classes of antibodies may also be present, such as IgM. Further, in certain embodiments of the present invention, the subtype of IgG antibodies to type V collagen as may be present in serum and/or lung lavage fluid from patients changes through the course of the disease. In this regard, the IgG subtype switching may occur during the course of a disease and certain subtypes may be indicative of worsening disease. Thus, any one or more of the IgG subtypes may be present during the course of disease, e.g. IgG1, IgG2, IgG3, or IgG4, or any combination thereof. The present invention provides for methods for

detecting Type V collagen-specific IgG1, IgG2, IgG3, IgG4 subtype antibodies using the bead assay as described herein. As would be recognized by the skilled person, IgG subtype-specific antibodies are commercially available and may be used in the methods described herein. In certain embodiments, an increase in IgG1 indicates worsening of disease. In another embodiment, a switch to IgG2 subtype indicates worsening of disease. In further embodiment, a switch to IgG3 subtype indicates worsening of disease. In yet an additional embodiment, a switch to IgG4 subtype indicates worsening of disease.

Compositions, Pharmaceutical Compositions and Methods of Use

[0127] Administration of the tolerogenic compounds or compositions of the invention, or their pharmaceutically acceptable salts, in pure form or in an appropriate pharmaceutical composition, can be carried out via any of the accepted modes of administration of agents for serving similar utilities. As noted elsewhere herein, one route contemplated herein for inducing tolerance is oral administration. However, any of a variety of other routes may also be used, in particular including intravenous, intramuscular, intradermal, subcutaneous injection, and other routes. The pharmaceutical compositions of the invention can be prepared by combining a compound of the invention with an appropriate pharmaceutically acceptable carrier, diluent or excipient, and may be formulated into preparations in solid, semi-solid, liquid or gaseous forms, such as tablets, capsules, powders, granules, ointments, solutions, suppositories, injections, inhalants, gels, microspheres, and aerosols. In addition, other pharmaceutically active ingredients (including other immunosuppressive agents) and/or suitable excipients such as salts, buffers and stabilizers may, but need not, be present within the composition.

[0128] Administration may be achieved by a variety of different routes, including oral, parenteral, nasal, intravenous, intradermal, subcutaneous or topical. Preferred modes of administration depend upon the nature of the condition to be treated or prevented. An amount that, following administration, reduces, inhibits, prevents or delays the onset of an anti-colV immune response or clinical indication of such a response is considered effective.

[0129] In certain embodiments, the amount administered is sufficient to result in reduced immune activity as described elsewhere herein (*e.g.*, T cell response, B cell response, anti-colV antibody level, and the like). The precise dosage and duration of treatment is a function of the disease being treated and may be determined empirically using known testing protocols or by testing the compositions in model systems known in the art and extrapolating therefrom. Controlled clinical trials may also be performed. Dosages may also vary with the severity of the condition to be alleviated. A pharmaceutical composition is generally formulated and administered to exert a therapeutically useful effect while minimizing undesirable side effects. The composition may be administered one time, or may be divided into a number of smaller doses to be administered at intervals of time. For any particular subject, specific dosage regimens may be adjusted over time according to the individual need.

[0130] The compositions of the present invention may be administered alone or in combination with other known treatments, such as immunosuppressive regimens, radiation therapy, chemotherapy, transplantation, oral collagen therapy, immunotherapy, hormone therapy, photodynamic therapy, *etc.*

[0131] Typical routes of administering these and related pharmaceutical compositions thus include, without limitation, oral, topical, transdermal, inhalation, intrapulmonary instillation, parenteral, sublingual, buccal, rectal, vaginal, and intranasal. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intrasternal injection or infusion techniques. Compositions of the invention are formulated so as to allow the active ingredients contained therein to be bioavailable upon administration of the composition to a patient. Compositions that will be administered to a subject or patient take the form of one or more dosage units, where for example, a tablet may be a single dosage unit, and a container of a compound of the invention in aerosol form may hold a plurality of dosage units. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in this art; for example, see Remington: The Science and Practice of Pharmacy, 20th Edition (Philadelphia College of Pharmacy and Science, 2000). The composition to be administered will, in any event, contain a therapeutically effective amount of a compound of the invention, or a pharmaceutically acceptable salt thereof, for treatment of a disease or condition of interest in accordance with the teachings of this invention.

[0132] A pharmaceutical composition of the invention may be in the form of a solid or liquid. In one aspect, the carrier(s) are particulate, so that the compositions are, for example, in tablet or powder form. The carrier(s) may be liquid, with the compositions being, for example, an oral oil, injectable liquid or an aerosol, which is useful in, for example, inhalatory administration.

[0133] In certain embodiments, the therapeutic compound(s) are directly administered as a pressurized aerosol or nebulized formulation to the patient's lungs via inhalation. Such formulations may contain any of a variety of known aerosol propellants useful for endopulmonary and/or intranasal inhalation administration. In addition, water may be present, with or without any of a variety of cosolvents, surfactants, stabilizers (e.g., antioxidants, chelating agents, inert gases and buffers). For compositions to be administered from multiple dose containers, antimicrobial agents are typically added. Such compositions are also generally filtered and sterilized, and may be lyophilized to provide enhanced stability

and to improve solubility.

[0134] When intended for oral administration, the pharmaceutical composition is preferably in either solid or liquid form, where semi-solid, semi-liquid, suspension and gel forms are included within the forms considered herein as either solid or liquid.

[0135] As a solid composition for oral administration, the pharmaceutical composition may be formulated into a powder, granule, compressed tablet, pill, capsule, chewing gum, wafer or the like form. Such a solid composition will typically contain one or more inert diluents or edible carriers. In addition, one or more of the following may be present: binders such as carboxymethylcellulose, ethyl cellulose, microcrystalline cellulose, gum tragacanth or gelatin; excipients such as starch, lactose or dextrins, disintegrating agents such as alginic acid, sodium alginate, Primogel, corn starch and the like; lubricants such as magnesium stearate or Sterotex; glidants such as colloidal silicon dioxide; sweetening agents such as sucrose or saccharin; a flavoring agent such as peppermint, methyl salicylate or orange flavoring; and a coloring agent.

[0136] When the pharmaceutical composition is in the form of a capsule, for example, a gelatin capsule, it may contain, in addition to materials of the above type, a liquid carrier such as polyethylene glycol or oil.

[0137] The pharmaceutical composition may be in the form of a liquid, for example, an elixir, syrup, solution, emulsion or suspension. The liquid may be for oral administration or for delivery by injection, as two examples. When intended for oral administration, preferred composition contain, in addition to the present compounds, one or more of a sweetening agent, preservatives, dye/colorant and flavor enhancer. In a composition intended to be administered by injection, one or more of a surfactant, preservative, wetting agent, dispersing agent, suspending agent, buffer, stabilizer and isotonic agent may be included.

[0138] The liquid pharmaceutical compositions of the invention, whether they be solutions, suspensions or other like form, may include one or more of the following adjuvants: sterile diluents such as water for injection, saline solution, preferably physiological saline, Ringer's solution, isotonic sodium chloride, fixed oils such as synthetic mono or diglycerides which may serve as the solvent or suspending medium, polyethylene glycols, glycerin, propylene glycol or other solvents; antibacterial agents such as benzyl alcohol or methyl paraben; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic. Physiological saline is a preferred adjuvant. An injectable pharmaceutical composition is preferably sterile.

[0139] A liquid pharmaceutical composition of the invention intended for either parenteral or oral administration should contain an amount of a compound of the invention such that a suitable dosage will be obtained. Typically, this amount is at least 0.01% of a compound of the invention in the composition. When intended for oral administration, this amount may be varied to be between 0.1 and about 70% of the weight of the composition. Certain oral pharmaceutical compositions contain between about 4% and about 75% of the compound of the invention. Certain pharmaceutical compositions and preparations according to the present invention are prepared so that a parenteral dosage unit contains between 0.01 to 10% by weight of the compound prior to dilution of the invention.

[0140] The pharmaceutical composition of the invention may be intended for topical administration, in which case the carrier may suitably comprise a solution, emulsion, ointment or gel base. The base, for example, may comprise one or more of the following: petrolatum, lanolin, polyethylene glycols, bee wax, mineral oil, diluents such as water and alcohol, and emulsifiers and stabilizers. Thickening agents may be present in a pharmaceutical composition for topical administration. If intended for transdermal administration, the composition may include a transdermal patch or iontophoresis device. Topical formulations may contain a concentration of the compound of the invention from about 0.1 to about 10% w/v (weight per unit volume).

[0141] The pharmaceutical composition of the invention may be intended for rectal administration, in the form, for example, of a suppository, which will melt in the rectum and release the drug. The composition for rectal administration may contain an oleaginous base as a suitable nonirritating excipient. Such bases include, without limitation, lanolin, cocoa butter and polyethylene glycol.

[0142] The pharmaceutical composition of the invention may include various materials, which modify the physical form of a solid or liquid dosage unit. For example, the composition may include materials that form a coating shell around the active ingredients. The materials that form the coating shell are typically inert, and may be selected from, for example, sugar, shellac, and other enteric coating agents. Alternatively, the active ingredients may be encased in a gelatin capsule.

[0143] The pharmaceutical composition of the invention in solid or liquid form may include an agent that binds to the compound of the invention and thereby assists in the delivery of the compound. Suitable agents that may act in this capacity include a monoclonal or polyclonal antibody, a protein or a liposome.

[0144] The pharmaceutical composition of the invention may consist of dosage units that can be administered as an aerosol. The term aerosol is used to denote a variety of systems ranging from those of colloidal nature to systems consisting of pressurized packages. Delivery may be by a liquefied or compressed gas or by a suitable pump system that dispenses the active ingredients. Aerosols of compounds of the invention may be delivered in single phase, bi-

phasic, or tri-phasic systems in order to deliver the active ingredient(s). Delivery of the aerosol includes the necessary container, activators, valves, subcontainers, and the like, which together may form a kit. One of ordinary skill in the art, without undue experimentation may determine preferred aerosols.

[0145] The pharmaceutical compositions of the invention may be prepared by methodology well known in the pharmaceutical art. For example, a pharmaceutical composition intended to be administered by injection can be prepared by combining a compound of the invention with sterile, distilled water so as to form a solution. A surfactant may be added to facilitate the formation of a homogeneous solution or suspension. Surfactants are compounds that non-covalently interact with the compound of the invention so as to facilitate dissolution or homogeneous suspension of the compound in the aqueous delivery system.

[0146] The compounds of the invention, or their pharmaceutically acceptable salts, are administered in a therapeutically effective amount, which will vary depending upon a variety of factors including the activity of the specific compound employed; the metabolic stability and length of action of the compound; the age, body weight, general health, sex, and diet of the patient; the mode and time of administration; the rate of excretion; the drug combination; the severity of the particular disorder or condition; and the subject undergoing therapy. Generally, a therapeutically effective daily dose is (for a 70 kg mammal) from about 0.001 mg/kg (*i.e.*, 0.07 mg) to about 100 mg/kg (*i.e.*, 7.0 g); preferably a therapeutically effective dose is (for a 70 kg mammal) from about 0.01 mg/kg (*i.e.*, 0.7 mg) to about 50 mg/kg (*i.e.*, 3.5 g); more preferably a therapeutically effective dose is (for a 70 kg mammal) from about 1 mg/kg (*i.e.*, 70 mg) to about 25 mg/kg (*i.e.*, 1.75 g).

[0147] In certain embodiments, the dose of colV administered orally is from .001 mg to 500mg per day. In one particular embodiment, the oral dose of colV as described herein is from 0.01 mg to 50mg per day. In a further embodiment, the oral dose of colV as described herein is from 0.1 mg to 0.5mg per day. In one embodiment, the oral dose of colV is 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, or 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, or 5.0 mg per day. In another embodiment, the oral dose of colV may be 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, or 8.0 mg per day. In certain embodiments, the dose may given in a single dose, or may be given in multiple doses over the course of the day, for example in 2, 3 or 4 doses per day for a total of a particular mg/day dose.

[0148] As described elsewhere herein, in certain embodiments, a therapeutically effective dose of colV as used herein is a dose sufficient to induce tolerance to colV measured using any of a variety of methods as described herein. In certain embodiments, induction of tolerance to colV results in a decrease in serum anti-colV antibodies as measured using the methods described herein, such as an ELISA. In a further embodiment, a therapeutically effective dose of colV as used herein is a dose sufficient to induce T cell tolerance to colV as measured using any of a variety of methods as described herein, such as cytokine release assays, intracellular cytokine staining and flow cytometry, ELISPOT, and the like. Functional T cell assays, such as proliferation of cytotoxicity assays may also be used.

[0149] Compounds of the invention, or pharmaceutically acceptable salts thereof, may also be administered simultaneously with, prior to, or after administration of one or more other therapeutic agents. Such combination therapy includes administration of a single pharmaceutical dosage formulation which contains a compound of the invention and one or more additional active agents, as well as administration of the compound of the invention and each active agent in its own separate pharmaceutical dosage formulation. For example, a compound of the invention and the other active agent can be administered to the patient together in a single oral dosage composition such as a tablet or capsule, or each agent administered in separate oral dosage formulations. Where separate dosage formulations are used, the compounds of the invention and one or more additional active agents can be administered at essentially the same time, *i.e.*, concurrently, or at separately staggered times, *i.e.*, sequentially; combination therapy is understood to include all these regimens.

[0150] The compounds of the present invention may be administered to an individual afflicted with a disease or disorder as described herein, such as severe and persistent asthma. For *in vivo* use for the treatment of human disease, the compounds described herein are generally incorporated into a pharmaceutical composition prior to administration. A pharmaceutical composition comprises one or more of the compounds described herein in combination with a physiologically acceptable carrier or excipient as described elsewhere herein. To prepare a pharmaceutical composition, an effective amount of one or more of the compounds is mixed with any pharmaceutical carrier(s) or excipient known to those skilled in the art to be suitable for the particular mode of administration. A pharmaceutical carrier may be liquid, semi-liquid or solid. Solutions or suspensions used for parenteral, intradermal, subcutaneous or topical application may include, for example, a sterile diluent (such as water), saline solution, fixed oil, polyethylene glycol, glycerin, propylene glycol or other synthetic solvent; antimicrobial agents (such as benzyl alcohol and methyl parabens); antioxidants (such as ascorbic acid and sodium bisulfite) and chelating agents (such as ethylenediaminetetraacetic acid (EDTA)); buffers (such as acetates, citrates and phosphates). If administered intravenously, suitable carriers include physiological saline or phosphate buffered saline (PBS), and solutions containing thickening and solubilizing agents, such as glucose, polyethylene glycol, polypropylene glycol and mixtures thereof.

[0151] The compounds described herein may be prepared with carriers that protect it against rapid elimination from

the body, such as time release formulations or coatings. Such carriers include controlled release formulations, such as, but not limited to, implants and microencapsulated delivery systems, and biodegradable, biocompatible polymers, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, polyorthoesters, polylactic acid and others known to those of ordinary skill in the art.

[0152] In certain embodiments, adjuvants which assist in inducing tolerance include dexamethasone (see *e.g.*, Y. Kang, et al., J. Immunol. 2008, 180: 5172-5176), lipopolysaccharides (LPS) and cholera toxin β-subunit and may be added to the formulations. Certain other tolerogenic carriers are also contemplated for use with the colV compositions of the present invention. Such carriers include mineral oil carriers such as incomplete, Freund's adjuvant (IFA) or complete Freund's adjuvant (CFA). IFA is an emulsion of mineral oil. CFA is a preparation of mineral oil containing various amounts of killed organisms of Mycobacterium. However, IFA and CFA are not allowed for human use because the mineral oil is not metabolizable and cannot be degraded by the body.

[0153] In certain embodiments, fat emulsions, which have been in use for many years for intravenous nutrition of human patients, can also act as a vehicle for tolerogenic polypeptide therapy using the polypeptides of the present invention. Two examples of such emulsions are the available commercial fat emulsions known as Intralipid and Lipofundin. "Intralipid" is a registered trademark of Kabi Pharmacia, Sweden, for a fat emulsion for intravenous nutrition, described in U.S. Pat. No. 3,169,094. "Lipofundin" is a registered trademark of B. Braun Melsungen, Germany. Both contain soybean oil as fat (100 or 200 g in 1,000 ml distilled water: 10% or 20%, respectively). Egg-yolk phospholipids are used as emulsifiers in Intralipid (12 g/l distilled water) and egg-yolk lecithin in Lipofundin (12 g/l distilled water). Isotonicity results from the addition of glycerol (25 g/l) both in Intralipid and Lipofundin. It is believed that these vehicles are actually biologically active carriers which when complexed with the suspected auto-antigen, promote a TH1 to TH2 shift of the autoimmune T cells. In certain embodiments, such a vehicle is a fat emulsion comprising 10-20% triglycerides of plant and/or animal origin, 1.2-2.4% phospholipids of plant and/or animal origin, 2.25-4.5% osmo-regulator, 0-0.05% anti-oxidant, and sterile water to 100%.

[0154] In certain embodiments, colV or toleragenic fragments thereof may be linked to the diphtheria toxin receptor to enhance GI uptake.

[0155] The tolerogenic compositions of the present invention may be used to treat asthma.

[0156] In one embodiment the invention provides a method for treating asthma comprising administering to an asthma patient a therapeutically effective amount of type V collagen, or a tolerogenic fragment thereof, or an immunogenic fragment thereof administered in a dose effective to induce tolerance. In this regard, the asthma patient may have severe, persistent asthma. In another embodiment, the present invention provides methods for reducing the severity of asthma in an asthma patient.

[0157] As noted elsewhere herein, intact colV or any one or more of its component α chains may be administered, or a tolerogenic fragment of any of the aforementioned molecules. Further, also as noted elsewhere herein, an immunogenic fragment of intact colV or any one or more of its component α chains may be administered in any of the methods described herein, using a dose effective to induce tolerance to colV. Such a dose will vary depending upon a variety of factors including the activity of the specific proteins or fragments employed; the metabolic stability and length of action of these compounds; the age, body weight, general health, sex, and diet of the patient; the mode and time of administration; the rate of excretion; the drug combination; the severity of the particular disorder or condition; and the subject undergoing therapy. Thus, such tolerogenic doses may be determined empirically using known testing protocols or by testing the compositions in model systems known in the art and extrapolating therefrom. Controlled clinical trials may also be performed. Dosages may also vary with the severity of the condition to be alleviated.

[0158] As would be readily appreciated by the skilled artisan, a variety of factors can be assessed to determine the effectiveness of the compounds and methods of the invention for treating, preventing, or reducing the severity of asthma. Such factors include typical clinical symptoms of asthma which can be assessed by the skilled clinician. These symptoms may include, but are not limited to: for asthma: wheezing; chest tightness or pain; rapid heart rate; sweating; peak flow rates as measured by a peak flow meter; frequent cough, especially at night; loss of breath easily or shortness of breath; feeling very tired or weak when exercising; wheezing or coughing after exercise; feeling tired, easily upset, grouchy, or moody; decreases or changes in lung function as measured on a peak flow meter; signs of a cold, or allergies (sneezing, runny nose, cough, nasal congestion, sore throat, and headache); trouble sleeping. The compositions and methods of the present invention can be used in conjunction with other known treatments for asthma, such as, but not limited to corticosteroids (e.g., prednisone, fluticasone, methylprednisolone), bronchodilators (e.g., short- and long-acting β2-agonists, theophylline, pirbuterol, ephedrine, albuterol, salmeterol, levalbuterol, clenbuterol ipratropium bromide), and leukotriene modifiers (e.g., montelukast, zafirlukast, zileuton).

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EXAMPLES

REFERENCE EXAMPLE 1

ANTI-COLLAGEN V ANTIBODIES ARE INCREASED IN COPD PATIENTS

[0160] Plasma was obtained from normal volunteers (non smoking adults, age 18-55), and volunteers with documented emphysema. Levels of anti-colV antibodies in COPD patients and control healthy subjects were detected by the flow cytometry bead assay as described in WO 2007/120947.

[0161] Briefly, 1) Streptavidin-coated beads (5 μm, binding capacity 10-20 μg/1 x 10^7 beads (Polyscience, Warrington, PA)) were washed two times with sterile PBS. Beads (1 x 10^7) were suspended in 100 μl of PBS with 40 μg of human Type V collagen and incubated for 60 minutes at 4°C. 2) A positive control was generated by following the same procedures in 1 above, using 20 μm of rabbit antibody to human collagen V antibody (bioten) (Abeam, Cambridge, MA). 3) For each assay, 1 x 10^6 conjugated beads were washed two times in PBS, and incubated in 100 μl PBS plus 50 μl serum. After incubating for 30-minutes at room temperature, the beads were washed three times with PBS containing 10% FCS. 4) The beads were suspended in 100 μl of sterile PBS+10% FBS and incubated for about 30 minutes at room temperature with secondary antibody. Typically, 5 μl of anti-human IgG antibody conjugated with R-PE was used (Sigma, Saint Louis). The beads were washed three times in PBS containing 10% FCS, suspended in 300 μl of PBS/FCS solution and analyzed using a flow cytometer.

[0162] As shown in Figure 1, anti-type V collagen antibodies are significantly elevated in COPD patients (N=16) as compared to controls (N=42).

EXAMPLE 2

ANTI-COLLAGEN V ANTIBODIES ARE INCREASED IN ASTHMA PATIENTS

[0163] Plasma was obtained from normal volunteers (non smoking adults, age 18 -55), and volunteers with documented chronic asthma. Levels of anti-colV antibodies in the asthma patients and control volunteer subjects were measured using the bead assay as outlined in Example 1.

[0164] As shown in Figure 2, elevated levels of anti-type V collagen antibodies were found in 8 of 20 asthmatics.

EXAMPLE 3

INTRAVENOUS COLLAGEN V PREVENTS OVALBUMIN-INDUCED AIRWAY HYPER-RESPONSIVENESS IN MICE

[0165] This Example demonstrates that intravenous administration of collagen V prevents ovalbumin-induced airway hyper-responsiveness in this well-established murine asthma model.

[0166] Balb/c mice were injected via tail vein with 100 μg col (V), alone, or col(V) mixed in complete Freund's adjuvant, (CFA), or PBS, or CFA, alone. Seven days later mice received an IP injection of ovalbumin in alum and this was repeated seven days later. Seven days after the last ova/alum injection, mice in each group were challenged with increasing doses of aerosolized ova followed by measurements of airways resistance (PenH).

[0167] The results showed that col(V), alone, abrogated ova-induced airway hyperresponsiveness (see Figure 3). Further experiments were conducted which confirm these results. These results are summarized below in Table 2.

Table 2: Col(V) alone abrogates ova-induced airway hyperresponsiveness

	PBS			Col(V)			2-WAY ANOVA
	PenH Units			PenH Units			
Mg/ml methacholine	Mean	SEM	N	Mean	SEM	N	P value
Baseline	0.419913	0.021817	6	0.564856	0.092752	6	>0.05
Saline	0.466943	0.034769	6	0.519667	0.073273	6	>0.05
10	1.413495	0.189206	6	1.615039	0.172871	6	>0.05
25	2.448044	0.438264	6	2.914945	0.510278	6	>0.05
50	4.463914	0.319439	6	3.147791	0.531683	6	>0.05
100	5.121152	0.327822	6	3.661426	0.544324	6	>0.05
200	6.064105	0.368829	6	3.387585	0.46153	6	<0.001
300	6.825607	0.499452	6	3.700204	0.657257	6	<0.001

EXAMPLE 4

INTRAVENOUS COL(V) INDUCES IFN-γ TRANSCRIPTS IN LUNG MONONUCLEAR CELLS

[0168] This example shows intravenous injection of col(V) alone induced a TH1 response in lung mononuclear cells, characterized by induction of IFN-γ transcripts. Balb/c mice were injected via tail vein with 100 ug col(V) alone, CFA alone at the base of the tail, colV plus CFA the base of the tale or PBS i.v. alone. Seven days later mice received an IP injection of ovalbumin in alum and this was repeated seven days later. Seven days after the last ova/alum injection, mice in each group were challenged with increasing doses of aerosolized methacholine followed by measurements of airways resistance in response to methacholine challenge (PenH). RNA was extracted from mononuclear cells isolated from the lung parenchyma of mice in each treatment group and quantitative PCR performed for determining expression levels of IL-4, IL-5, IL-13, IFN-γ and IL-10 (see Figure 4; data represent lung mononuclear cells of RNA pooled from 5 mice in each group).

[0169] The results of the experiment show that Col(V) alone administered intravenously induced IFN-γ transcripts in lung mononuclear cells. IFN-γ is antagonistic to IL-13 and IL4, two cytokines thought to play a key role in asthma pathogenesis. Therefore, without being bound by theory, induction of this TH1 response which counteracts the effects of IL4/IL13 may play a part in the colV-mediated protective effect in ova-induced asthma.

SEQUENCE LISTING

[0170]

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<120> COMPOSITIONS AND METHODS FOR THE TREATMENT OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE AND ASTHMA

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<213> Homo sapiens

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Pro Ser Arg Ala Ala Gln Pro Ala Asp Leu Leu Lys Val Leu Asp Phe
        35                  40                  45
His Asn Leu Pro Asp Gly Ile Thr Lys Thr Thr Gly Phe Cys Ala Thr
    50                  55                  60
Arg Arg Ser Ser Lys Gly Pro Asp Val Ala Tyr Arg Val Thr Lys Asp
65                  70                  75                  80
Ala Gln Leu Ser Ala Pro Thr Lys Gln Leu Tyr Pro Ala Ser Ala Phe
                85                  90                  95
Pro Glu Asp Phe Ser Ile Leu Thr Thr Val Lys Ala Lys Lys Gly Ser
            100                 105                 110
Gln Ala Phe Leu Val Ser Ile Tyr Asn Glu Gln Gly Ile Gln Gln Ile
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Gly Leu Glu Leu Gly Arg Ser Pro Val Phe Leu Tyr Glu Asp His Thr
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Ser Asp Gly Lys Trp His Arg Ile Ala Leu Ser Val His Lys Lys Asn
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Val Thr Leu Ile Leu Asp Cys Lys Lys Lys Thr Thr Lys Phe Leu Asp
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Arg Ser Asp His Pro Met Ile Asp Ile Asn Gly Ile Ile Val Phe Gly
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Pro Asn Pro Asp Glu Tyr Tyr Thr Glu Gly Asp Gly Glu Gly Glu Thr
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Tyr Tyr Tyr Glu Tyr Pro Tyr Tyr Glu Asp Pro Glu Asp Leu Gly Lys
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Glu Pro Thr Pro Ser Lys Lys Pro Val Glu Ala Ala Lys Glu Thr Thr
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Pro Glu Thr Ser Glu Gly Ala Gly Lys Glu Glu Asp Val Gly Ile Gly
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Asp Tyr Asp Tyr Val Pro Ser Glu Asp Tyr Tyr Thr Pro Ser Pro Tyr
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Asp Asp Leu Thr Tyr Gly Glu Gly Glu Glu Asn Pro Asp Gln Pro Thr
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Asp Pro Gly Ala Gly Ala Glu Ile Pro Thr Ser Thr Ala Asp Thr Ser
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Glu Gly Glu Phe Thr Glu Glu Thr Ile Arg Asn Leu Asp Glu Asn Tyr
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Ala Gly Pro Met Gly Leu Thr Gly Arg Pro Gly Pro Val Gly Pro Pro
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Gly Ser Gly Gly Leu Lys Gly Glu Pro Gly Asp Val Gly Pro Gln Gly
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Pro Arg Gly Val Gln Gly Pro Pro Gly Pro Ala Gly Lys Pro Gly Arg
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Arg Gly Arg Ala Gly Ser Asp Gly Ala Arg Gly Met Pro Gly Gln Thr
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Gly Leu Pro Gly Glu Pro Gly Pro Arg Gly Leu Leu Gly Pro Lys Gly
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690 695 700
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Ala Ile Gly Pro Pro Gly Glu Lys Gly Pro Leu Gly Lys Pro Gly Leu
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Pro Gly Met Pro Gly Ala Asp Gly Pro Pro Gly His Pro Gly Lys Glu
755 760 765
Gly Pro Pro Gly Glu Lys Gly Gly Gln Gly Pro Pro Gly Pro Gln Gly
770 775 780
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Pro Lys Gly Ser Ile Gly Phe Pro Gly Phe Pro Gly Ala Asn Gly Glu
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Gly Pro Thr Gly Pro Arg Gly Glu Arg Gly Pro Arg Gly Ile Thr Gly
915 920 925
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Pro Gly Glu Arg Gly Pro Asn Gly Pro Gln Gly Pro Thr Gly Phe Pro
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Thr Phe Leu Arg Leu Leu Ser Ala Ser Ala His Gln Asn Val Thr Tyr
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His Cys Tyr Gln Ser Val Ala Trp Gln Asp Ala Ala Thr Gly Ser Tyr
1745                1750                1755                1760
Asp Lys Ala Leu Arg Phe Leu Gly Ser Asn Asp Glu Glu Met Ser Tyr
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Asp Asn Asn Pro Tyr Ile Arg Ala Leu Val Asp Gly Cys Ala Thr Lys
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Lys Gly Tyr Gln Lys Thr Val Leu Glu Ile Asp Thr Pro Lys Val Glu
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Gln Val Pro Ile Val Asp Ile Met Phe Asn Asp Phe Gly Glu Ala Ser
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<210> 4
<211> 1499
<212> PRT
<213> Homo sapiens

<400> 4

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            20                  25                  30
Glu Gly Tyr Gly Glu Glu Ile Ala Cys Thr Gln Asn Gly Gln Met Tyr
        35                  40                  45
Leu Asn Arg Asp Ile Trp Lys Pro Ala Pro Cys Gln Ile Cys Val Cys
    50                  55                  60
Asp Asn Gly Ala Ile Leu Cys Asp Lys Ile Glu Cys Gln Asp Val Leu
65                  70                  75                  80
Asp Cys Ala Asp Pro Val Thr Pro Pro Gly Glu Cys Cys Pro Val Cys
                85                  90                  95
Ser Gln Thr Pro Gly Gly Gly Asn Thr Asn Phe Gly Arg Gly Arg Lys
            100                 105                 110
Gly Gln Lys Gly Glu Pro Gly Leu Val Pro Val Val Thr Gly Ile Arg
        115                 120                 125
Gly Arg Pro Gly Pro Ala Gly Pro Pro Gly Ser Gln Gly Pro Arg Gly
    130                 135                 140
Glu Arg Gly Pro Lys Gly Arg Pro Gly Pro Arg Gly Pro Gln Gly Ile
145                 150                 155                 160
Asp Gly Glu Pro Gly Val Pro Gly Gln Pro Gly Ala Pro Gly Pro Pro
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Gly His Pro Ser His Pro Gly Pro Asp Gly Leu Ser Arg Pro Phe Ser
            180                 185                 190
Ala Gln Met Ala Gly Leu Asp Glu Lys Ser Gly Leu Gly Ser Gln Val
        195                 200                 205
Gly Leu Met Pro Gly Ser Val Gly Pro Val Gly Pro Arg Gly Pro Gln
    210                 215                 220
Gly Leu Gln Gly Gln Gln Gly Gly Ala Gly Pro Thr Gly Pro Pro Gly
225                 230                 235                 240
Glu Pro Gly Asp Pro Gly Pro Met Gly Pro Ile Gly Ser Arg Gly Pro
                245                 250                 255
Glu Gly Pro Pro Gly Lys Pro Gly Glu Asp Gly Glu Pro Gly Arg Asn
            260                 265                 270
Gly Asn Pro Gly Glu Val Gly Phe Ala Gly Ser Pro Gly Ala Arg Gly
        275                 280                 285
Phe Pro Gly Ala Pro Gly Leu Pro Gly Leu Lys Gly His Arg Gly His
    290                 295                 300
Lys Gly Leu Glu Gly Pro Lys Gly Glu Val Gly Ala Pro Gly Ser Lys
305                 310                 315                 320
Gly Glu Ala Gly Pro Thr Gly Pro Met Gly Ala Met Gly Pro Leu Gly
                325                 330                 335
Pro Arg Gly Met Pro Gly Glu Arg Gly Arg Leu Gly Pro Gln Gly Ala
            340                 345                 350
Pro Gly Gln Arg Gly Ala His Gly Met Pro Gly Lys Pro Gly Pro Met
        355                 360                 365
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Gly Pro Leu Gly Ile Pro Gly Ser Ser Gly Phe Pro Gly Asn Pro Gly
    370                 375                 380
Met Lys Gly Glu Ala Gly Pro Thr Gly Ala Arg Gly Pro Glu Gly Pro
385                 390                 395                 400
Gln Gly Gln Arg Gly Glu Thr Gly Pro Pro Gly Pro Val Gly Ser Pro
                405                 410                 415
Gly Leu Pro Gly Ala Ile Gly Thr Asp Gly Thr Pro Gly Ala Lys Gly
            420                 425                 430
Pro Thr Gly Ser Pro Gly Thr Ser Gly Pro Pro Gly Ser Ala Gly Pro
        435                 440                 445
Pro Gly Ser Pro Gly Pro Gln Gly Ser Thr Gly Pro Gln Gly Ile Arg
    450                 455                 460
Gly Gln Pro Gly Asp Pro Gly Val Pro Gly Phe Lys Gly Glu Ala Gly
465                 470                 475                 480
Pro Lys Gly Glu Pro Gly Pro His Gly Ile Gln Gly Pro Ile Gly Pro
                485                 490                 495
Pro Gly Glu Glu Gly Lys Arg Gly Pro Arg Gly Asp Pro Gly Thr Val
            500                 505                 510
Gly Pro Pro Gly Pro Val Gly Glu Arg Gly Ala Pro Gly Asn Arg Gly
        515                 520                 525
Phe Pro Gly Ser Asp Gly Leu Pro Gly Pro Lys Gly Ala Gln Gly Glu
    530                 535                 540
Arg Gly Pro Val Gly Ser Ser Gly Pro Lys Gly Ser Gln Gly Asp Pro
545                 550                 555                 560
Gly Arg Pro Gly Glu Pro Gly Leu Pro Gly Ala Arg Gly Leu Thr Gly
                565                 570                 575
Asn Pro Gly Val Gln Gly Pro Glu Gly Lys Leu Gly Pro Leu Gly Ala
            580                 585                 590
Pro Gly Glu Asp Gly Arg Pro Gly Pro Pro Gly Ser Ile Gly Ile Arg
        595                 600                 605
Gly Gln Pro Gly Ser Met Gly Leu Pro Gly Pro Lys Gly Ser Ser Gly
    610                 615                 620
Asp Pro Gly Lys Pro Gly Glu Ala Gly Asn Ala Gly Val Pro Gly Gln
625                 630                 635                 640
Arg Gly Ala Pro Gly Lys Asp Gly Glu Val Gly Pro Ser Gly Pro Val
                645                 650                 655
Gly Pro Pro Gly Leu Ala Gly Glu Arg Gly Glu Gln Gly Pro Pro Gly
            660                 665                 670
Pro Thr Gly Phe Gln Gly Leu Pro Gly Pro Pro Gly Pro Pro Gly Glu
        675                 680                 685
Gly Gly Lys Pro Gly Asp Gln Gly Val Pro Gly Asp Pro Gly Ala Val
    690                 695                 700
Gly Pro Leu Gly Pro Arg Gly Glu Arg Gly Asn Pro Gly Glu Arg Gly
705                 710                 715                 720
Glu Pro Gly Ile Thr Gly Leu Pro Gly Glu Lys Gly Met Ala Gly Gly
                725                 730                 735
His Gly Pro Asp Gly Pro Lys Gly Ser Pro Gly Pro Ser Gly Thr Pro
            740                 745                 750
Gly Asp Thr Gly Pro Pro Gly Leu Gln Gly Met Pro Gly Glu Arg Gly
        755                 760                 765
Ile Ala Gly Thr Pro Gly Pro Lys Gly Asp Arg Gly Gly Ile Gly Glu
    770                 775                 780
Lys Gly Ala Glu Gly Thr Ala Gly Asn Asp Gly Ala Arg Gly Leu Pro
785                 790                 795                 800
Gly Pro Leu Gly Pro Pro Gly Pro Ala Gly Pro Thr Gly Glu Lys Gly
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Glu Pro Gly Pro Arg Gly Leu Val Gly Pro Pro Gly Ser Arg Gly Asn
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Pro Gly Ser Arg Gly Glu Asn Gly Pro Thr Gly Ala Val Gly Phe Ala
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Gly Pro Gln Gly Pro Asp Gly Gln Pro Gly Val Lys Gly Glu Pro Gly
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Glu Pro Gly Gln Lys Gly Asp Ala Gly Ser Pro Gly Pro Gln Gly Leu
865                 870                 875                 880
Ala Gly Ser Pro Gly Pro His Gly Pro Asn Gly Val Pro Gly Leu Lys
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Ser Ala Gly Arg Val Gly Pro Pro Gly Pro Ala Gly Ala Pro Gly Pro
915 920 925
Ala Gly Pro Leu Gly Glu Pro Gly Lys Glu Gly Pro Pro Gly Leu Arg
930 935 940
Gly Asp Pro Gly Ser His Gly Arg Val Gly Asp Arg Gly Pro Ala Gly
945 950 955 960
Pro Pro Gly Gly Pro Gly Asp Lys Gly Asp Pro Gly Glu Asp Gly Gln
965 970 975
Pro Gly Pro Asp Gly Pro Pro Gly Pro Ala Gly Thr Thr Gly Gln Arg
980 985 990
Gly Ile Val Gly Met Pro Gly Gln Arg Gly Glu Arg Gly Met Pro Gly
995 1000 1005
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1125 1130 1135
Gly Asp Arg Gly Gln Lys Gly His Arg Gly Phe Thr Gly Leu Gln Gly
1140 1145 1150
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1155 1160 1165
Pro Gly Pro Phe Gly Pro Arg Gly Pro Pro Gly Pro Val Gly Pro Ser
1170 1175 1180
Gly Lys Glu Gly Asn Pro Gly Pro Leu Gly Pro Ile Gly Pro Pro Gly
1185 1190 1195 1200
Val Arg Gly Ser Val Gly Glu Ala Gly Pro Glu Gly Pro Pro Gly Glu
1205 1210 1215
Pro Gly Pro Pro Gly Pro Pro Gly Pro Pro Gly His Leu Thr Ala Ala
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Leu Gly Asp Ile Met Gly His Tyr Asp Glu Ser Met Pro Asp Pro Leu
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Pro Glu Phe Thr Glu Asp Gln Ala Ala Pro Asp Asp Lys Asn Lys Thr
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Asp Pro Gly Val His Ala Thr Leu Lys Ser Leu Ser Ser Gln Ile Glu
1265 1270 1275 1280
Thr Met Arg Ser Pro Asp Gly Ser Lys Lys His Pro Ala Arg Thr Cys
1285 1290 1295
Asp Asp Leu Lys Leu Cys His Ser Ala Lys Gln Ser Gly Glu Tyr Trp
1300 1305 1310
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1315 1320 1325
Asn Met Glu Thr Gly Glu Thr Cys Ile Ser Ala Asn Pro Ser Ser Val
1330 1335 1340
Pro Arg Lys Thr Trp Trp Ala Ser Lys Ser Pro Asp Asn Lys Pro Val
1345 1350 1355 1360
Trp Tyr Gly Leu Asp Met Asn Arg Gly Ser Gln Phe Ala Tyr Gly Asp
1365 1370 1375
His Gln Ser Pro Asn Thr Ala Ile Thr Gln Met Thr Phe Leu Arg Leu
1380 1385 1390
Leu Ser Lys Glu Ala Ser Gln Asn Ile Thr Tyr Ile Cys Lys Asn Ser
1395 1400 1405

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Val Gly Tyr Met Asp Asp Gln Ala Lys Asn Leu Lys Lys Ala Val Val
    1410                1415                1420
Leu Lys Gly Ala Asn Asp Leu Asp Ile Lys Ala Glu Gly Asn Ile Arg
1425                1430                1435                1440
Phe Arg Tyr Ile Val Leu Gln Asp Thr Cys Ser Lys Arg Asn Gly Asn
                1445                1450                1455
Val Gly Lys Thr Val Phe Glu Tyr Arg Thr Gln Asn Val Ala Arg Leu
            1460                1465                1470
Pro Ile Ile Asp Leu Ala Pro Val Asp Val Gly Gly Thr Asp Gln Glu
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<210> 5
<211> 6192
<212> DNA
<213> Homo sapiens

<400> 5

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<210> 6

<211> 1745
<212> PRT
<213> Homo sapiens

5

10

15

20

25

30

35

40

45

50

55

<400> 6

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

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Pro Arg Gly Leu Gln Gly Pro His Gly Pro Pro Gly Arg Val Gly Lys
        515                 520                 525
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    530                 535                 540
Gly Pro Lys Gly Asp Arg Gly Phe Asp Gly Leu Pro Gly Leu Pro Gly
545                 550                 555                 560
Glu Lys Gly Gln Arg Gly Asp Phe Gly His Val Gly Gln Pro Gly Pro
                565                 570                 575
Pro Gly Glu Asp Gly Glu Arg Gly Ala Glu Gly Pro Pro Gly Pro Thr
            580                 585                 590
Gly Gln Ala Gly Glu Pro Gly Pro Arg Gly Leu Leu Gly Pro Arg Gly
        595                 600                 605
Ser Pro Gly Pro Thr Gly Arg Pro Gly Val Thr Gly Ile Asp Gly Ala
    610                 615                 620
Pro Gly Ala Lys Gly Asn Val Gly Pro Pro Gly Glu Pro Gly Pro Pro
625                 630                 635                 640
Gly Gln Gln Gly Asn His Gly Ser Gln Gly Leu Pro Gly Pro Gln Gly
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Leu Ile Gly Thr Pro Gly Glu Lys Gly Pro Pro Gly Asn Pro Gly Ile
            660                 665                 670
Pro Gly Leu Pro Gly Ser Asp Gly Pro Leu Gly His Pro Gly His Glu
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Gly Pro Thr Gly Glu Lys Gly Ala Gln Gly Pro Pro Gly Ser Ala Gly
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Pro Pro Gly Tyr Pro Gly Pro Arg Gly Val Lys Gly Thr Ser Gly Asn
705                 710                 715                 720
Arg Gly Leu Gln Gly Glu Lys Gly Glu Lys Gly Glu Asp Gly Phe Pro
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Gly Phe Lys Gly Asp Val Gly Leu Lys Gly Asp Gln Gly Lys Pro Gly
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Ala Pro Gly Pro Arg Gly Glu Asp Gly Pro Glu Gly Pro Lys Gly Gln
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Ala Gly Gln Ala Gly Glu Glu Gly Pro Pro Gly Ser Ala Gly Glu Lys
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Gly Lys Leu Gly Val Pro Gly Leu Pro Gly Tyr Pro Gly Arg Pro Gly
785                 790                 795                 800
Pro Lys Gly Ser Ile Gly Phe Pro Gly Pro Leu Gly Pro Ile Gly Glu
                805                 810                 815
Lys Gly Lys Ser Gly Lys Thr Gly Gln Pro Gly Leu Glu Gly Glu Arg
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Gly Pro Pro Gly Ser Arg Gly Glu Arg Gly Gln Pro Gly Ala Thr Gly
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Pro Gly Glu Lys Gly Leu Pro Gly Leu Gln Gly Pro Pro Gly Phe Pro
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Pro Gly Pro Ala Gly Val Leu Gly Pro Gln Gly Lys Thr Gly Glu Val
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Gly Pro Leu Gly Glu Arg Gly Pro Pro Gly Pro Pro Gly Pro Pro Gly
    930                 935                 940
Glu Gln Gly Leu Pro Gly Leu Glu Gly Arg Glu Gly Ala Lys Gly Glu
945                 950                 955                 960
Leu Gly Pro Pro Gly Pro Leu Gly Lys Glu Gly Pro Ala Gly Leu Arg
                965                 970                 975
Gly Phe Pro Gly Pro Lys Gly Gly Pro Gly Asp Pro Gly Pro Thr Gly
            980                 985                 990
Leu Lys Gly Asp Lys Gly Pro Pro Gly Pro Val Gly Ala Asn Gly Ser
        995                 1000                1005
Pro Gly Glu Arg Gly Pro Leu Gly Pro Ala Gly Gly Ile Gly Leu Pro
    1010                1015                1020
Gly Gln Ser Gly Ser Glu Gly Pro Val Gly Pro Ala Gly Lys Lys Gly
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1025                1030                1035                1040
Ser Arg Gly Glu Arg Gly Pro Pro Gly Pro Thr Gly Lys Asp Gly Ile
                1045                1050                1055
Pro Gly Pro Leu Gly Pro Leu Gly Pro Pro Gly Ala Ala Gly Pro Ser
            1060                1065                1070
Gly Glu Glu Gly Asp Lys Gly Asp Val Gly Ala Pro Gly His Lys Gly
        1075                1080                1085
Ser Lys Gly Asp Lys Gly Asp Ala Gly Pro Pro Gly Gln Pro Gly Ile
    1090                1095                1100
Arg Gly Pro Ala Gly His Pro Gly Pro Pro Gly Ala Asp Gly Ala Gln
1105                1110                1115                1120
Gly Arg Arg Gly Pro Pro Gly Leu Phe Gly Gln Lys Gly Asp Asp Gly
                1125                1130                1135
Val Arg Gly Phe Val Gly Val Ile Gly Pro Pro Gly Leu Gln Gly Leu
            1140                1145                1150
Pro Gly Pro Pro Gly Glu Lys Gly Glu Val Gly Asp Val Gly Ser Met
        1155                1160                1165
Gly Pro His Gly Ala Pro Gly Pro Arg Gly Pro Gln Gly Pro Thr Gly
    1170                1175                1180
Ser Glu Gly Thr Pro Gly Leu Pro Gly Gly Val Gly Gln Pro Gly Ala
1185                1190                1195                1200
Val Gly Glu Lys Gly Glu Arg Gly Asp Ala Gly Asp Pro Gly Pro Pro
                1205                1210                1215
Gly Ala Pro Gly Ile Pro Gly Pro Lys Gly Asp Ile Gly Glu Lys Gly
            1220                1225                1230
Asp Ser Gly Pro Ser Gly Ala Ala Gly Pro Pro Gly Lys Lys Gly Pro
        1235                1240                1245
Pro Gly Glu Asp Gly Ala Lys Gly Ser Val Gly Pro Thr Gly Leu Pro
    1250                1255                1260
Gly Asp Leu Gly Pro Pro Gly Asp Pro Gly Val Ser Gly Ile Asp Gly
1265                1270                1275                1280
Ser Pro Gly Glu Lys Gly Asp Pro Gly Asp Val Gly Gly Pro Gly Pro
                1285                1290                1295
Pro Gly Ala Ser Gly Glu Pro Gly Ala Pro Gly Pro Pro Gly Lys Arg
            1300                1305                1310
Gly Pro Ser Gly His Met Gly Arg Glu Gly Arg Glu Gly Glu Lys Gly
        1315                1320                1325
Ala Lys Gly Glu Pro Gly Pro Asp Gly Pro Pro Gly Arg Thr Gly Pro
    1330                1335                1340
Met Gly Ala Arg Gly Pro Pro Gly Arg Val Gly Pro Glu Gly Leu Arg
1345                1350                1355                1360
Gly Ile Pro Gly Pro Val Gly Glu Pro Gly Leu Leu Gly Ala Pro Gly
                1365                1370                1375
Gln Met Gly Pro Pro Gly Pro Leu Gly Pro Ser Gly Leu Pro Gly Leu
            1380                1385                1390
Lys Gly Asp Thr Gly Pro Lys Gly Glu Lys Gly His Ile Gly Leu Ile
        1395                1400                1405
Gly Leu Ile Gly Pro Pro Gly Glu Ala Gly Glu Lys Gly Asp Gln Gly
    1410                1415                1420
Leu Pro Gly Val Gln Gly Pro Pro Gly Pro Lys Gly Asp Pro Gly Pro
1425                1430                1435                1440
Pro Gly Pro Ile Gly Ser Leu Gly His Pro Gly Pro Pro Gly Val Ala
                1445                1450                1455
Gly Pro Leu Gly Gln Lys Gly Ser Lys Gly Ser Pro Gly Ser Met Gly
            1460                1465                1470
Pro Arg Gly Asp Thr Gly Pro Ala Gly Pro Pro Gly Pro Pro Gly Ala
        1475                1480                1485
Pro Ala Glu Leu His Gly Leu Arg Arg Arg Arg Arg Phe Val Pro Val
    1490                1495                1500
Pro Leu Pro Val Val Glu Gly Gly Leu Glu Glu Val Leu Ala Ser Leu
1505                1510                1515                1520
Thr Ser Leu Ser Leu Glu Leu Glu Gln Leu Arg Arg Pro Pro Gly Thr
                1525                1530                1535
Ala Glu Arg Pro Gly Leu Val Cys His Glu Leu His Arg Asn His Pro
            1540                1545                1550
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His Leu Pro Asp Gly Glu Tyr Trp Ile Asp Pro Asn Gln Gly Cys Ala
            1555                1560                1565
Arg Asp Ser Phe Arg Val Phe Cys Asn Phe Thr Ala Gly Gly Glu Thr
        1570                1575                1580
Cys Leu Tyr Pro Asp Lys Lys Phe Glu Ile Val Lys Leu Ala Ser Trp
1585                1590                1595                1600
Ser Lys Glu Lys Pro Gly Gly Trp Tyr Ser Thr Phe Arg Arg Gly Lys
                1605                1610                1615
Lys Phe Ser Tyr Val Asp Ala Asp Gly Ser Pro Val Asn Val Val Gln
            1620                1625                1630
Leu Asn Phe Leu Lys Leu Leu Ser Ala Thr Ala Arg Gln Asn Phe Thr
        1635                1640                1645
Tyr Ser Cys Gln Asn Ala Ala Ala Trp Leu Asp Glu Ala Thr Gly Asp
    1650                1655                1660
Tyr Ser His Ser Ala Arg Phe Leu Gly Thr Asn Gly Glu Glu Leu Ser
1665                1670                1675                1680
Phe Asn Gln Thr Thr Ala Ala Thr Val Ser Val Pro Gln Asp Gly Cys
                1685                1690                1695
Arg Leu Arg Lys Gly Gln Thr Lys Thr Leu Phe Glu Phe Ser Ser Ser
            1700                1705                1710
Arg Ala Gly Phe Leu Pro Leu Trp Asp Val Ala Ala Thr Asp Phe Gly
        1715                1720                1725
Gln Thr Asn Gln Lys Phe Gly Phe Glu Leu Gly Pro Val Cys Phe Ser
    1730                1735                1740
Ser
1745
```

Claims

1. A composition for use in the treatment of asthma in a patient or for use in the prevention of the development or worsening of asthma in a subject at risk for developing asthma, comprising a therapeutically effective amount of type V collagen, or a tolerogenic fragment thereof.

2. The composition of claim 1 wherein the patient has emphysema or chronic obstructive bronchitis.

3. The composition of claim 1 wherein the type V collagen or tolerogenic fragment thereof is prepared for oral administration.

4. The composition of claim 3 comprising between 0.1 mg and 0.5 mg of type V collagen.

5. The composition of claim 1 wherein the type V collagen or tolerogenic fragment thereof is prepared for intravenous administration, intrapulmonary instillation, administration by inhalation, or for intramuscular administration.

6. The composition of claim 1, further comprising a bronchodilator or for use in conjunction with a bronchodilator.

7. The composition of claim 1 further comprising a corticosteroid or for use in conjunction with a corticosteroid.

8. The composition of claim 1 further comprising a leukotriene modifier or for use in conjunction with a leukotriene modifier.

9. A method for identifying an individual at risk for developing asthma or identifying an asthma patient as a candidate for collagen V tolerance therapy comprising,
contacting at least a portion of a sample of blood from the patient with collagen V or an antigenic fragment thereof; and measuring the level of antibodies that bind to the collagen V or antigenic fragment thereof;
wherein the presence of antibodies specifically bound to the collagen V is indicative that the asthma patient would benefit from collagen V tolerance therapy.

10. The method of claim 9 wherein the collagen V or antigenic fragment thereof is conjugated to a bead.

11. The method of claim 9 wherein the measuring comprises
contacting the antibodies that specifically bind to the collagen V or antigenic fragment thereof with a fluorescently labeled anti-IgG antibody; and
detecting by flow cytometry the amount of fluorescently labeled anti-IgG antibody bound to the antibodies bound to the collagen V.

12. A method for monitoring the progression of asthma in an individual comprising,
contacting at least a portion of a first sample of blood from the individual with collagen V or an antigenic fragment thereof;
measuring the level of antibodies that specifically bind to the collagen V or antigenic fragment thereof in the first sample of blood;
contacting at least a portion of a second sample of blood from the individual taken at a later time point, with collagen V or an antigenic fragment thereof;
measuring the level of antibodies that specifically bind to the collagen V or antigenic fragment thereof in the second sample of blood; and
comparing the level of antibodies that specifically bind to the collagen V or antigenic fragment thereof in the second sample of blood to the level of antibodies that specifically bind to the collagen V or antigenic fragment thereof in the first sample of blood;
wherein an increase in the level of antibodies bound to the collagen V in the second sample as compared to the first sample is indicative of worsening of asthma and a decrease in the level of antibodies bound to the collagen V in the second sample as compared to the first sample is indicative of amelioration of asthma.

13. The method of claim 12 wherein the collagen V or antigenic fragment thereof is conjugated to a bead.

14. The method of claim 12 wherein the measuring comprises
contacting the antibodies that bind to the collagen V or antigenic fragment thereof with a fluorescently labeled anti-IgG antibody; and
detecting by flow cytometry the amount of fluorescently labeled anti-IgG antibody bound to the antibodies bound to the collagen V.

15. The method of claim 14 or 11 wherein the anti-IgG antibody detects one or more of the IgG subtypes selected from IgG1, IgG2, IgG3 and IgG4.

Patentansprüche

1. Eine Zusammensetzung zur Verwendung bei der Behandlung von Asthma bei einem Patienten oder zur Verwendung bei der Vorbeugung der Entwicklung oder Verschlimmerung von Asthma bei einem Individuum mit dem Risiko für die Entwicklung von Asthma, umfassend eine therapeutisch wirksame Menge von Typ-V-Kollagen oder einem tolerogenen Fragment davon.

2. Die Zusammensetzung nach Anspruch 1, wobei der Patient Emphysem oder chronisch-obstruktive Bronchitis hat.

3. Die Zusammensetzung nach Anspruch 1, wobei das Typ-V-Kollagen oder tolerogene Fragment davon zur oralen Verabreichung hergestellt ist.

4. Die Zusammensetzung nach Anspruch 3, umfassend zwischen 0,1 mg und 0,5 mg Typ-V-Kollagen.

5. Die Zusammensetzung nach Anspruch 1, wobei das Typ-V-Kollagen oder tolerogene Fragment davon zur intravenösen Verabreichung, intrapulmonalen Instillation, Verabreichung durch Inhalation oder zur intramuskulären Verabreichung hergestellt ist.

6. Die Zusammensetzung nach Anspruch 1, weiterhin umfassend einen Bronchodilator oder zur Verwendung in Verbindung mit einem Bronchodilator.

7. Die Zusammensetzung nach Anspruch 1, weiterhin umfassend ein Corticosteroid oder zur Verwendung in Verbindung mit einem Corticosteroid.

8. Die Zusammensetzung nach Anspruch 1, weiterhin umfassend einen Leukotrien-Modifikator oder zur Verwendung in Verbindung mit einem Leukotrien-Modifikator.

9. Ein Verfahren zur Identifikation eines Individuums mit dem Risiko für die Entwicklung von Asthma oder zur Identifikation eines Asthmapatienten als Kandidat für eine Kollagen-V-Toleranz-Therapie, umfassend
Kontaktieren mindestens eines Teils einer Blutprobe von dem Patienten mit Kollagen V oder einem antigenen Fragment davon; und
Messen der Menge der Antikörper, die an das Kollagen V oder das antigene Fragment davon binden;
wobei das Vorhandensein von spezifisch an das Kollagen V gebundenen Antikörpern indikativ dafür ist, dass der Asthmapatient von einer Kollagen-V-Toleranz-Therapie profitieren würde.

10. Das Verfahren nach Anspruch 9, wobei das Kollagen V oder das antigene Fragment davon an ein Kügelchen konjugiert wird.

11. Das Verfahren nach Anspruch 9, wobei das Messen umfasst:

Kontaktieren der Antikörper, die an das Kollagen V oder das antigene Fragment davon spezifisch binden, mit einem fluoreszenzmarkierten Anti-IgG-Antikörper; und
Feststellen der Menge des fluoreszenzmarkierten Anti-IgG-Antikörpers, der gebunden ist an die Antikörper, die an das Kollagen V gebunden sind, mittels Durchflusszytometrie.

12. Ein Verfahren zur Kontrolle des Verlaufs des Asthmas bei einem Individuum, umfassend
Kontaktieren mindestens eines Teils einer ersten Blutprobe von dem Individuum mit Kollagen V oder einem antigenen Fragment davon;
Messen der Menge der Antikörper, die an das Kollagen V oder das antigene Fragment davon in der ersten Blutprobe spezifisch binden;
Kontaktieren mindestens eines Teils einer zu einem späteren Zeitpunkt entnommenen zweiten Blutprobe von dem Individuum mit Kollagen V oder einem antigenen Fragment davon;
Messen der Menge der Antikörper, die an das Kollagen V oder das antigene Fragment davon in der zweiten Blutprobe spezifisch binden; und
Vergleichen der Menge der Antikörper, die an das Kollagen V oder das antigene Fragment davon in der zweiten Blutprobe spezifisch binden, mit der Menge der Antikörper, die an das Kollagen V oder das antigene Fragment davon in der ersten Blutprobe spezifisch binden;
wobei eine Erhöhung der Menge der an das Kollagen V gebundenen Antikörper in der zweiten Probe im Vergleich zu der ersten Probe indikativ ist für eine Verschlimmerung des Asthmas und eine Verringerung der Menge der an das Kollagen V gebundenen Antikörper in der zweiten Probe im Vergleich zu der ersten Probe indikativ ist für eine Besserung des Asthmas.

13. Das Verfahren nach Anspruch 12, wobei das Kollagen V oder das antigene Fragment davon an ein Kügelchen konjugiert wird.

14. Das Verfahren nach Anspruch 12, wobei das Messen umfasst:

Kontaktieren der Antikörper, die an das Kollagen V oder das antigene Fragment davon spezifisch binden, mit einem fluoreszenzmarkierten Anti-IgG-Antikörper; und
Feststellen der Menge des fluoreszenzmarkierten Anti-IgG-Antikörpers, der gebunden ist an die Antikörper, die an das Kollagen V gebunden sind, mittels Durchflusszytometrie.

15. Das Verfahren nach Anspruch 14 oder 11, wobei der Anti-IgG-Antikörper einen oder mehrere der IgG-Subtypen, ausgewählt aus IgG1, IgG2, IgG3 und IgG4, nachweist.

Revendications

1. Une composition pour utilisation dans le traitement de l’asthme chez un patient ou pour utilisation dans la prévention du développement ou de l’aggravation de l’asthme chez un sujet risquant de développer de l’asthme, comprenant une quantité thérapeutiquement efficace de collagène de type V, ou un fragment tolérogénique de celui-ci.

2. La composition selon la revendication 1 dans laquelle le patient a un emphysème ou une bronchite chronique obstructive.

3. La composition selon la revendication 1 dans laquelle le collagène de type V ou le fragment tolérogénique de celui-ci est préparé pour une administration orale.

4. La composition selon la revendication 3 comprenant entre 0,1 mg et 0,5 mg de collagène de type V.

5. La composition selon la revendication 1 dans laquelle le collagène de type V ou le fragment tolérogénique de celui-ci est préparé pour une administration par voie intraveineuse, une instillation intra-pulmonaire, une administration par inhalation ou pour une administration par voie intramusculaire.

6. La composition selon la revendication 1, comprenant en outre un bronchodilatateur, ou pour une utilisation conjointement avec un bronchodilatateur.

7. La composition selon la revendication 1, comprenant en outre un corticostéroïde, ou pour une utilisation conjointement avec un corticostéroïde.

8. La composition selon la revendication 1, comprenant en outre un modificateur de leucotriène, ou pour une utilisation conjointement avec un modificateur de leucotriène.

9. Un procédé pour identifier un individu risquant de développer de l'asthme ou pour identifier un patient atteint d'asthme comme étant un candidat pour une thérapie tolérant le collagène V, consistant à
mettre en contact au moins une partie d'un échantillon de sang du patient avec du collagène V ou un fragment antigénique de celui-ci ; et
mesurer le niveau d'anticorps qui se lient au collagène V ou au fragment antigénique de celui-ci ;
dans lequel la présence d'anticorps spécifiquement liés au collagène V indique que le patient atteint d'asthme pourrait bénéficier d'une thérapie tolérant le collagène V.

10. Le procédé selon la revendication 9 dans lequel le collagène V ou un fragment antigénique de celui-ci est conjugué à une bille.

11. Le procédé selon la revendication 9, dans lequel la mesure consiste à:

mettre en contact les anticorps qui se lient spécifiquement au collagène V ou au fragment antigénique de celui-ci avec un anticorps anti-IgG marqué par fluorescence ; et
détecter par cytométrie en flux la quantité d'anticorps anti-IgG marqués par fluorescence liés aux anticorps liés au collagène V.

12. Un procédé pour surveiller la progression de l'asthme chez un individu, consistant à :

mettre en contact au moins une partie d'un échantillon de sang de l'individu avec du collagène V ou un fragment antigénique de celui-ci ; et
mesurer le niveau d'anticorps qui se lient spécifiquement au collagène V ou au fragment antigénique de celui-ci dans le premier échantillon de sang ;
mettre en contact au moins une partie d'un deuxième échantillon de sang de l'individu, prélevé à un moment ultérieur, avec du collagène V ou un fragment antigénique de celui-ci ;
mesurer le niveau d'anticorps qui se lient spécifiquement au collagène V ou au fragment antigénique de celui-ci dans le deuxième échantillon de sang ; et
comparer le niveau d'anticorps qui se lient spécifiquement au collagène V ou au fragment antigénique de celui-ci dans le deuxième échantillon de sang au niveau d'anticorps qui se lient spécifiquement au collagène V ou au fragment antigénique de celui-ci dans le premier échantillon de sang ;
dans lequel une augmentation du niveau des anticorps liés au collagène V dans le deuxième échantillon en comparaison avec le premier échantillon indique une aggravation de l'asthme, et une diminution du niveau des anticorps liés au collagène V dans le deuxième échantillon en comparaison avec le premier échantillon indique une amélioration de l'asthme.

13. Le procédé selon la revendication 12 dans lequel le collagène V ou un fragment antigénique de celui-ci est conjugué

à une bille.

14. Le procédé selon la revendication 12 dans lequel la mesure consiste à :

mettre en contact les anticorps qui se lient au collagène ou au fragment antigénique de celui-ci avec un anticorps anti-IgG marqué par fluorescence ; et
détecter par cytométrie en flux la quantité d'anticorps anti-IgG marqués par fluorescence liés aux anticorps liés au collagène V.

15. Le procédé selon la revendication 14 ou 11 dans lequel l'anticorps anti-IgG détecte un ou plusieurs sous-types IgG choisis parmi IgG1, IgG2, IgG3 et IgG4.

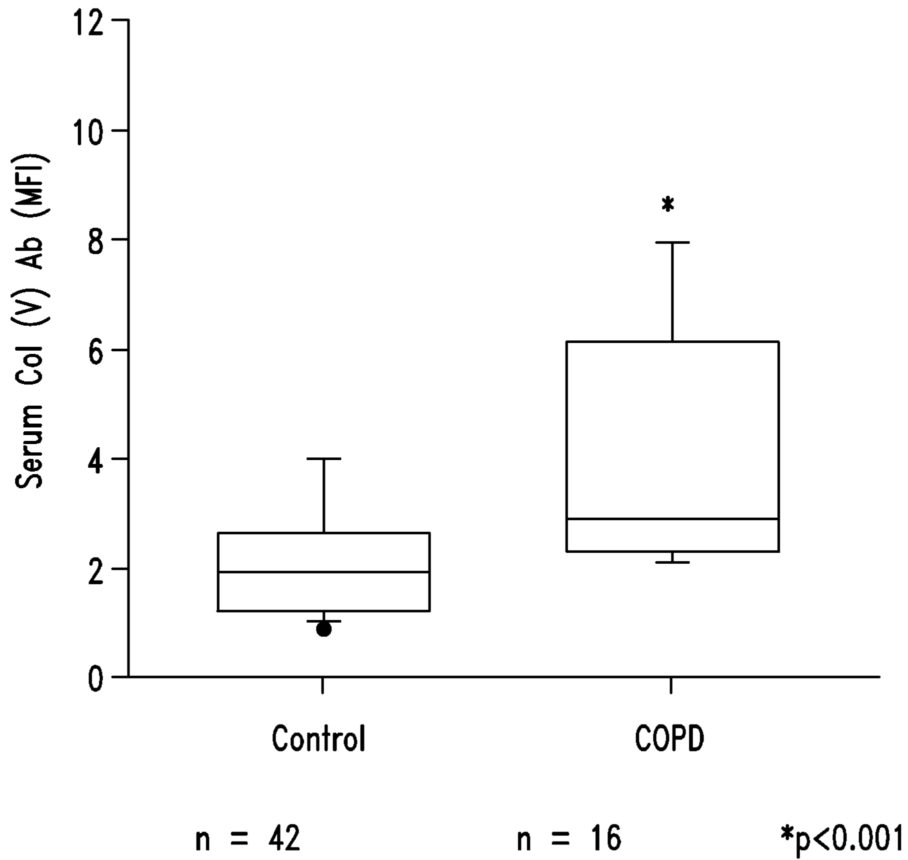

FIG. 1

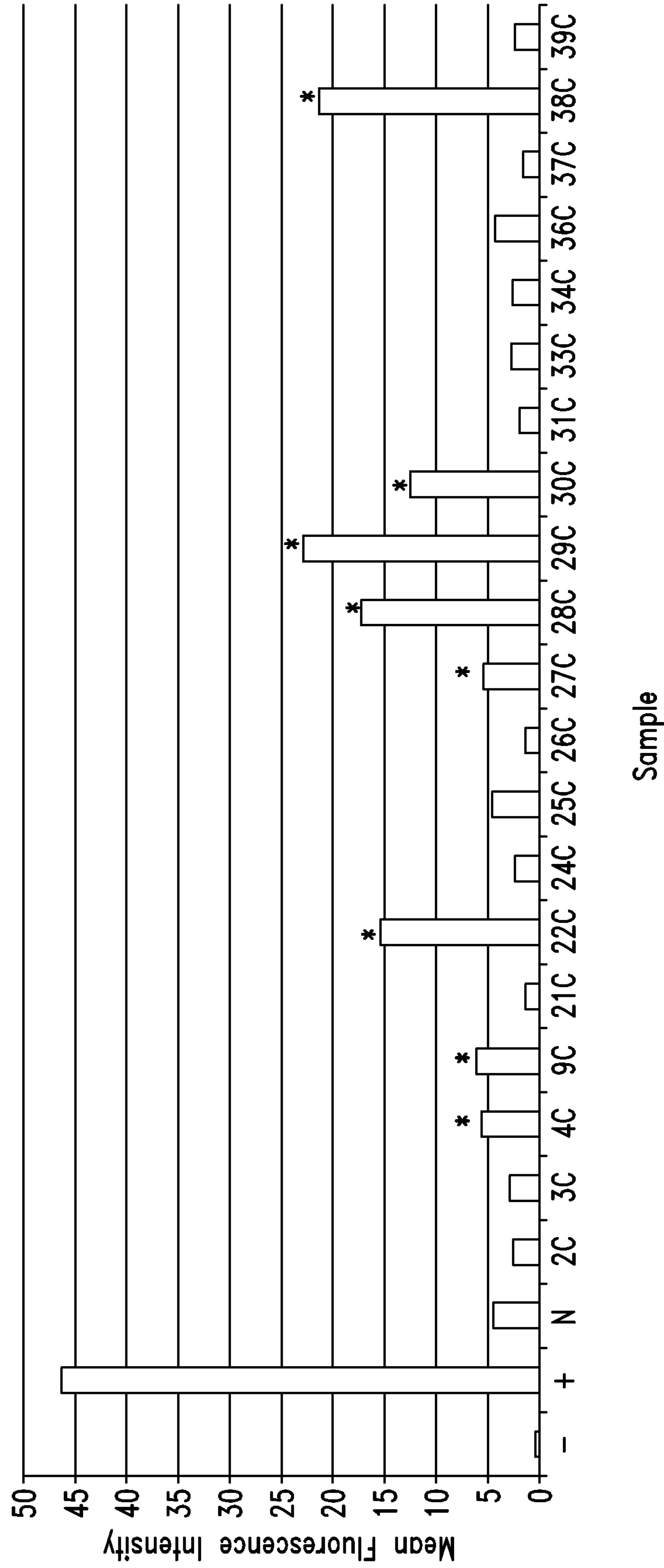

FIG. 2

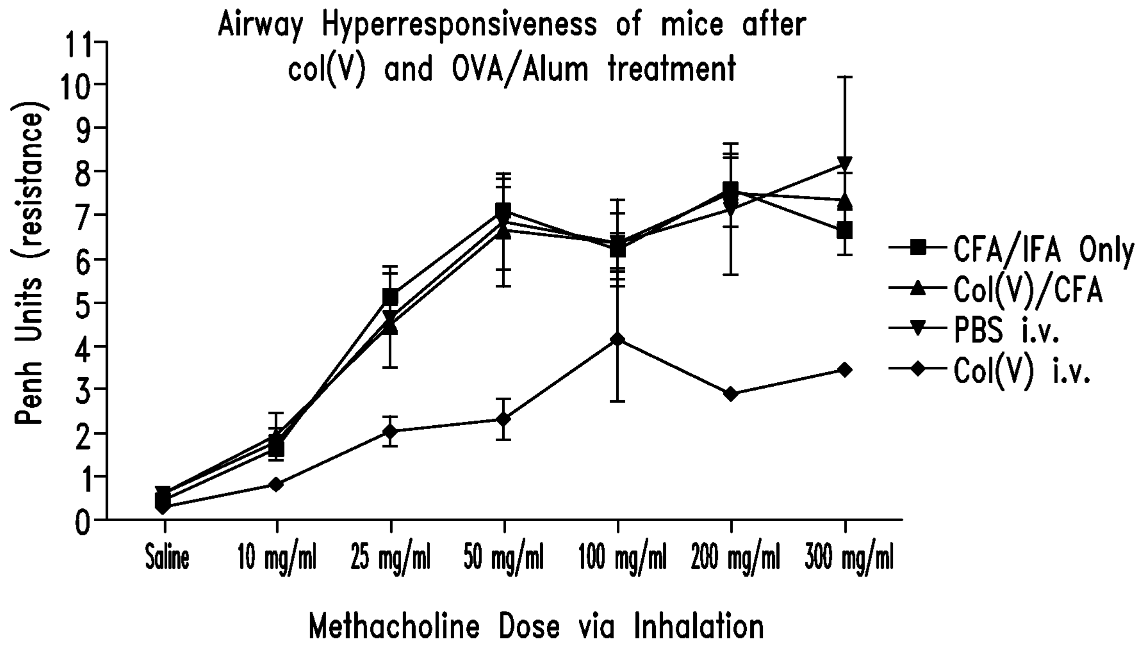

FIG. 3

Lung Mononuclear Cell Transcript Levels

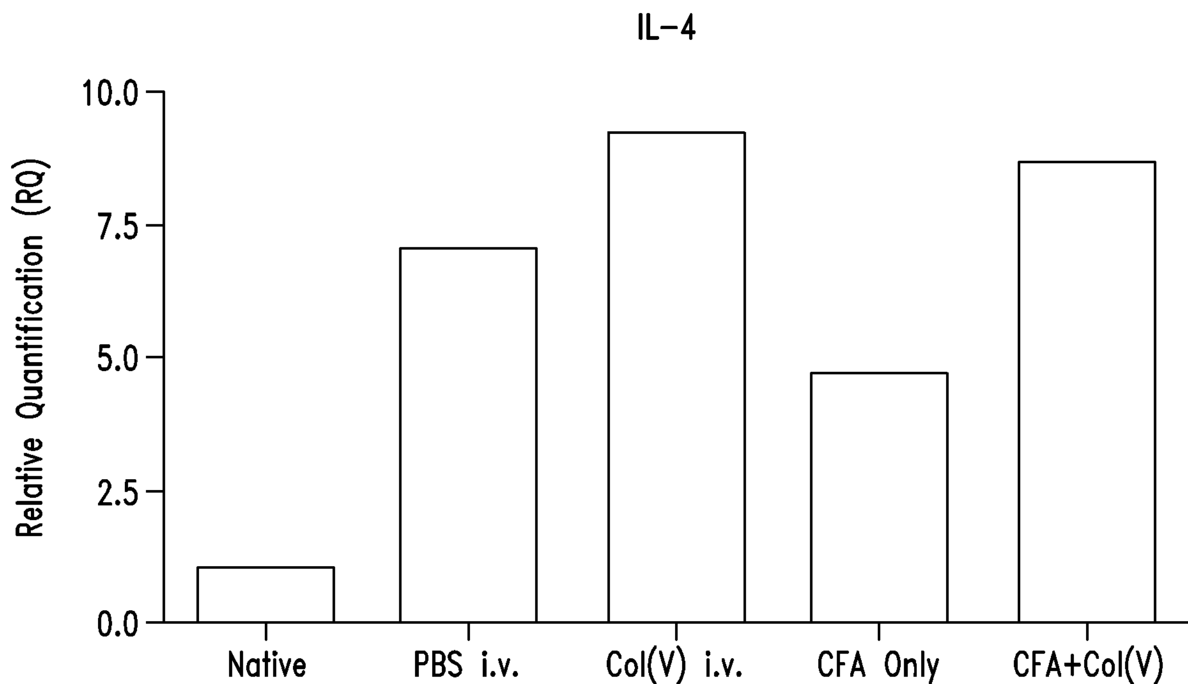

FIG. 4A

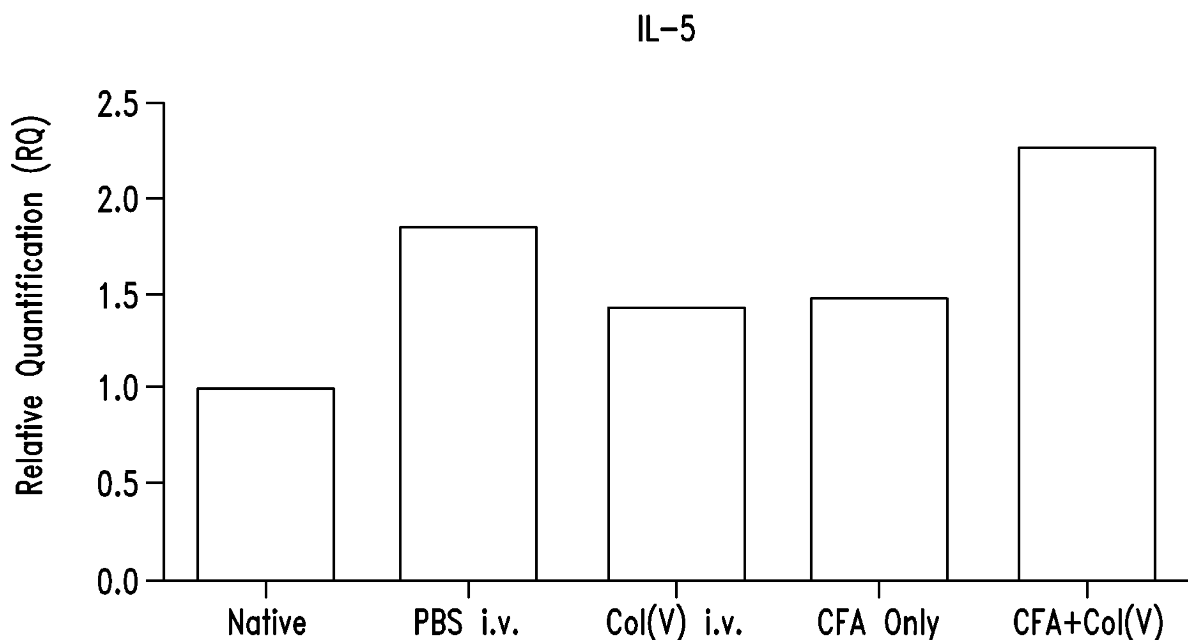

FIG. 4B

Lung Mononuclear Cell Transcript Levels

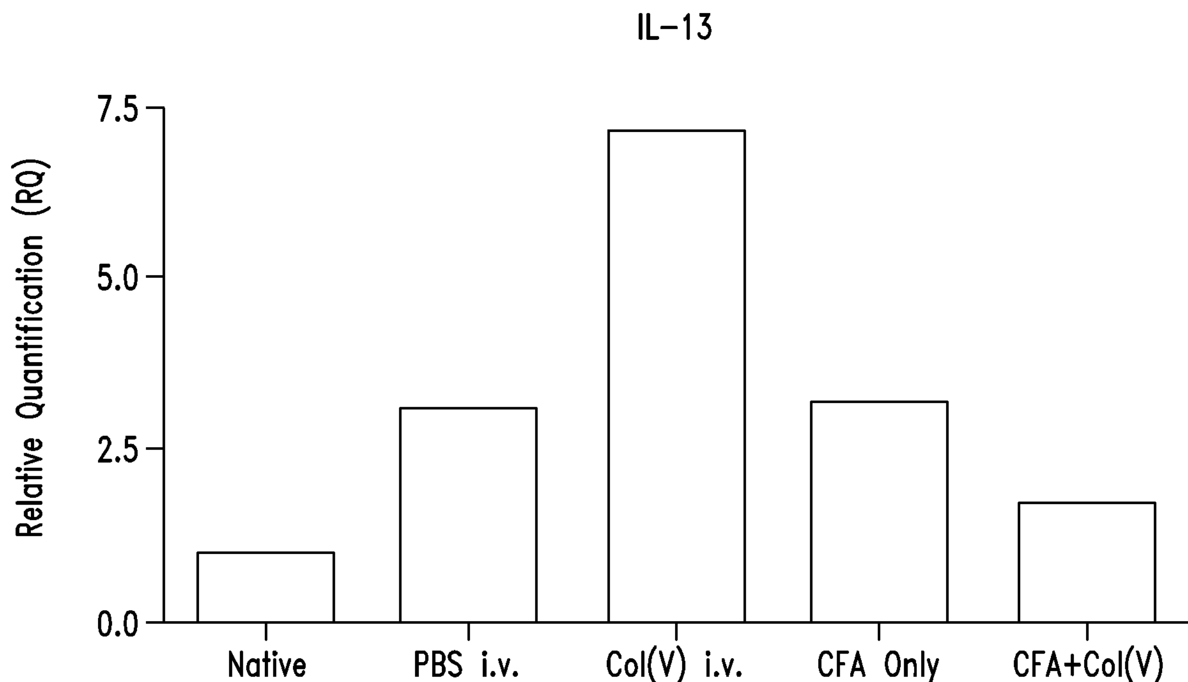

FIG. 4C

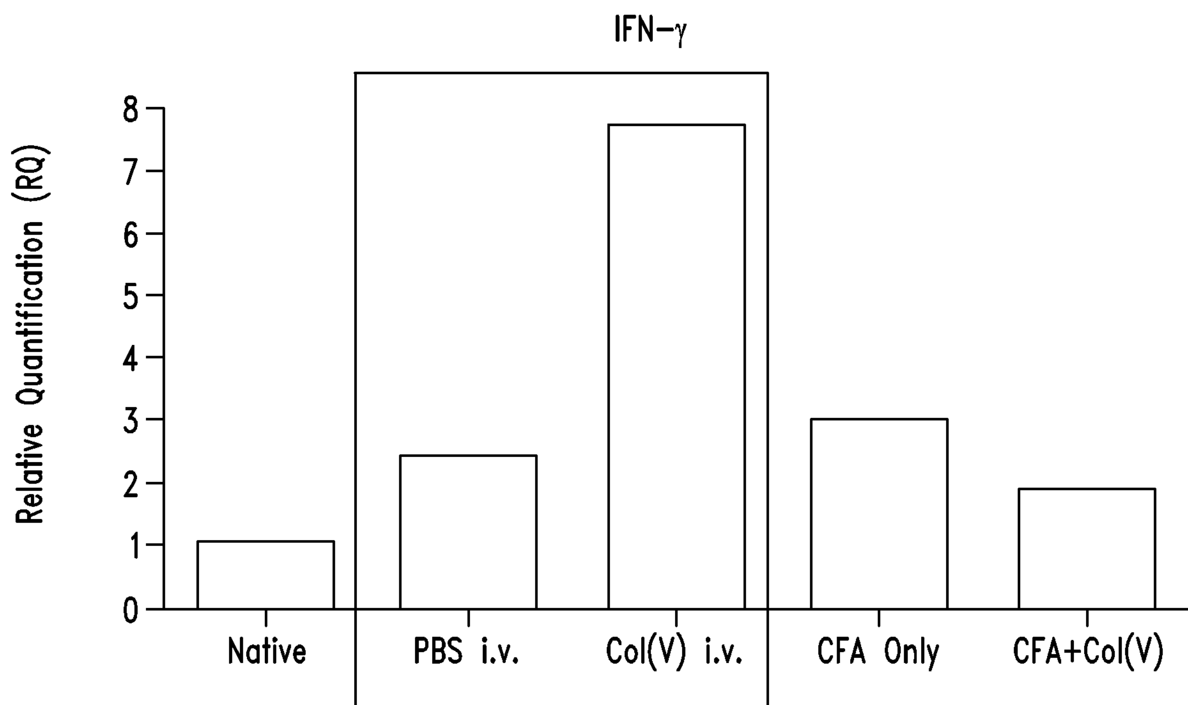

FIG. 4D

Lung Mononuclear Cell Transcript Levels

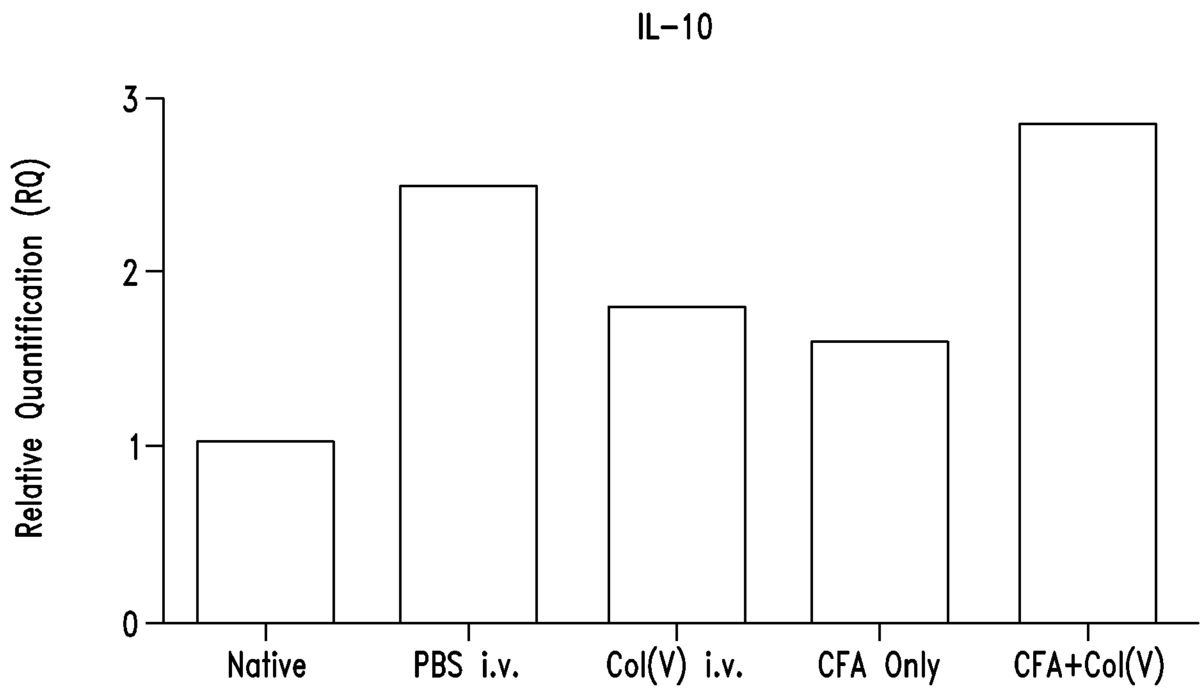

FIG. 4E

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专利名称(译)	胶原蛋白v用于治疗哮喘		
公开(公告)号	EP2421552B1	公开(公告)日	2016-12-14
申请号	EP2010716439	申请日	2010-04-22
[标]申请(专利权)人(译)	印第安纳UNIV RES TECH		
申请(专利权)人(译)	印第安纳大学研究与科技股份有限公司		
当前申请(专利权)人(译)	印第安纳大学研究与科技股份有限公司		
[标]发明人	WILKES DAVID S		
发明人	WILKES, DAVID, S.		
IPC分类号	A61K38/39 G01N33/567 G01N33/53 A61P11/06 A61P37/02		
CPC分类号	A61K38/39 G01N33/6854 G01N2333/78 G01N2800/122 G01N2800/50 G01N2800/52 G01N2800/56 A61P11/00 A61P11/06 A61P11/08 A61K9/0019 A61K9/0053 A61K9/0073 A61K39/0008		
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摘要(译)

本发明提供了用于治疗或预防包括COPD和哮喘的肺部疾病的化合物和方法。特别地，本发明提供了包含V型胶原蛋白或其耐受性片段的化合物，用于治疗COPD和哮喘。


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