(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
13 September 2001 (13.09.2001)

PCT

(10) International Publication Number
WO 01/66136 A2

(51) International Patent Classification[7]: A61K 39/00, 39/35, 35/66, A61P 37/08

(21) International Application Number: PCT/US00/33121

(22) International Filing Date: 6 December 2000 (06.12.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/195,035 6 March 2000 (06.03.2000) US
Not furnished 6 December 2000 (06.12.2000) US

(71) Applicant: PANACEA PHARMACEUTICALS, LLC. [US/US]; 640 Sasco Hill Road, Fairfield, CT 06430-2038 (US).

(72) Inventor: CAPLAN, Michael; 1217 Racebrook Road, Woodbridge, CT 06525 (US).

(74) Agent: MAH, Stanley, C.; Choate, Hall & Stewart, 53 State Street, Boston, MA 02109 (US).

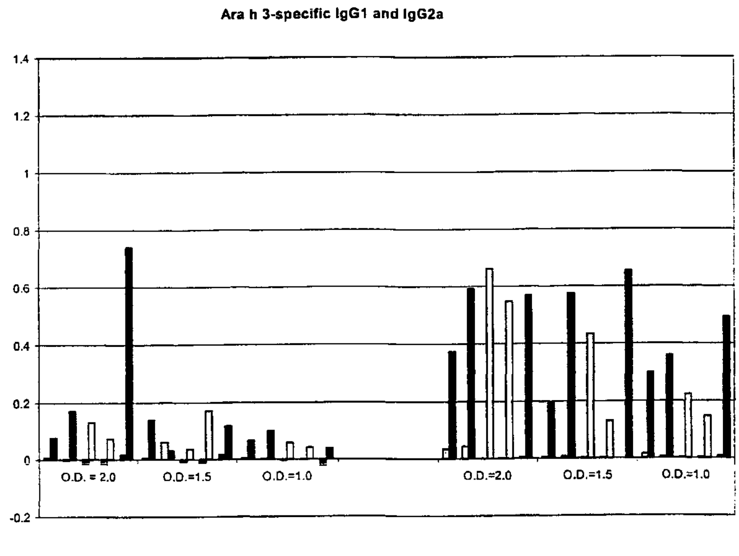
(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: MICROBIAL DELIVERY SYSTEM



(57) Abstract: The present invention provides methods and compositions for treating or preventing allergic responses, particularly anaphylactic allergic responses, in subjects who are allergic to allergens or susceptible to allergies. Methods of the present invention utilize administration of microorganisms to subjects, where the microorganisms produce allergens and protect the subjects from exposure to the allergens until phagocytosed by antigen-presenting cells. Particularly preferred microorganisms are gram-negative bacteria, gram-positive bacteria, and yeast. Particularly preferred allergens are proteins found in foods, venoms, drugs and latex that elicit allergic reactions and anaphylactic allergic reactions in individuals who are allergic to the proteins or are susceptible to allergies to the proteins. The proteins may also be modified to reduce the ability of the proteins to bind and crosslink IgE antibodies and thereby reduce the risk of eliciting anaphylaxis without affecting T-cell mediated Th1-type immunity.

WO 01/66136 A2

MICROBIAL DELIVERY SYSTEM

Priority Information

The present application claims priority under 35 U.S.C. 119(e) to the U.S. Provisional Patent Application Serial Number 60/195,035 entitled "Bacterial Polypeptide Delivery" filed March 6, 2000.

Related Applications

The present invention is generally in the area of controlled delivery of antigens for use in vaccination or induction of tolerance to allergens, and in particular relates to cellular delivery of proteins and polypeptides. This application is related to U.S.S.N. 60/169,330 entitled "Controlled Delivery of Antigens" filed Dec. 6, 1999; U.S.S.N. 09/141,220 entitled "Methods and Reagents for Decreasing Clinical Reaction to Allergy" filed Aug. 27, 1998; U.S.S.N. 09/455,294 entitled "Peptide Antigens" filed December 6, 1999; U.S.S.N. 09/494,096 filed January 28, 2000 entitled "Methods and Reagents for Decreasing Clinical Reaction to Allergy" by Bannon et al.; and U.S.S.N. 09/527,083 entitled "Immunostimulatory Nucleic Acids and Antigens" by Caplan filed March 16, 2000; the teachings of which are all incorporated herein by reference in their entirety.

Background of the Invention

Allergic reactions pose serious public health problems worldwide. Pollen allergy alone (allergic rhinitis or hay fever) affects about 10-15% of the population, and generates huge economic costs. For example, reports estimate that pollen allergy generated $1.8 billion of direct and indirect expenses in the United States in 1990 (*Fact Sheet*, National Institute of Allergy and Infectious Diseases; McMenamin, *Annals of Allergy* 73:35, 1994). Asthma, which can be triggered by exposure to antigens, is also a serious public health problem, and like anaphylactic allergic reactions, can lead to death in extreme cases. Asthma currently accounts for millions of visits yearly to hospitals and is increasing in frequency. The only treatment currently available is for alleviation of symptoms, for example, to relieve constriction of airways. More serious than the economic costs associated with pollen and other inhaled allergens (e.g., molds, dust mites, animal danders) is the risk of an anaphylactic allergic reaction observed with allergens such as food allergens, insect venoms, drugs, and latex.

Allergic reactions result when an individual's immune system overreacts, or reacts inappropriately, to an encountered antigen. Typically, there is no allergic reaction the first time an individual is exposed to a particular antigen. However, it is the initial response to an antigen that primes the system for subsequent allergic reactions. In particular, the antigen is taken up by antigen presenting cells (APC; e.g., macrophages and dendritic cells) that degrade the antigen and then display antigen fragments to T cells. T cells, in particular $CD4^+$ "helper" T-cells, respond by secreting a collection of cytokines that have effects on other immune system cells. The profile of cytokines secreted by responding $CD4^+$ T cells determines whether subsequent exposures to the antigen will induce allergic reactions. Two classes of $CD4^+$ T cells (Th1 and Th2) influence the type of immune response that is mounted against an antigen.

Th2 cells can secrete a variety of cytokines and interleukins including IL-4, IL-5, IL-6, IL-10 and IL-13. One effect of IL-4 is to stimulate the maturation of B cells that produce IgE antibodies specific for the antigen. Allergic responses to allergens are characterized by the production of antigen-specific IgE antibodies which are dependent on help from IL-4 secreting $CD4^+$ T cells. These antigen-specific IgE antibodies attach to receptors on the surface of mast cells, basophils and eosinophils, where they act as a trigger to initiate a rapid allergic reaction upon the next exposure to antigen. When the individual encounters the antigen a second time, the antigen is quickly bound by these surface-associated IgE molecules. Each antigen typically has more than one IgE binding site, so that the surface-bound IgE molecules quickly become crosslinked to one another through their simultaneous (direct or indirect) associations with antigen. Such cross-linking induces mast cell degranulation, resulting in the release of histamines and other substances that trigger allergic reactions. Individuals with high levels of IgE antibodies are known to be particularly prone to allergies.

Current treatments for allergies involve attempts to "vaccinate" a sensitive individual against a particular allergen by periodically injecting or treating the individual with a crude suspension of the raw allergen. The goal, through controlled administration of known amounts of antigen, is to modulate the IgE response mounted in the individual. If the therapy is successful, the individual's IgE response is diminished, or can even disappear. However, the therapy requires several rounds of vaccination, over an extended time period (3-5 years), and very often does not produce the desired results. Moreover, certain individuals suffer anaphylactic reactions to the vaccines, despite their intentional, controlled administration.

Clearly, there is a need for treatments and preventive methods for patients with allergies to allergens that elicit serious allergic responses including anaphylaxis.

Summary of the Invention

The present invention provides methods and compositions for modulating the immune response in a subject. It is an aspect of the present invention to provide a method of treating or preventing undesirable allergic reactions and anaphylactic allergic reactions to allergens in a subject. Methods of the present invention involve administering to subjects, microorganisms that express or produce allergens of interest. Without being limited to the proposed mechanism of action, after administration the microorganisms are taken up by antigen-presenting cells in the subject where the expressed antigens are released. After being processed inside the antigen-presenting cells and displayed on the cell surface, the processed antigens activate T-cell mediated immune responses. Use of genetically modified microorganisms to express and deliver allergens to a subject therefore reduces the exposure of the allergens to the subject's IgE antibodies, which lead to allergic reactions and possibly anaphylaxis. The present invention therefore reduces the risk of anaphylaxis during immunotherapy. Furthermore, the microorganisms may act as a natural adjuvant to enhance desirable Th1-type immune responses.

In a preferred embodiment, microorganisms are genetically modified to express selected polypeptides or proteins, and are used as delivery vehicles in accordance with the present invention. Such microorganisms include but are not limited to bacteria, viruses, fungi (including yeast), algae, and protozoa. Generally, preferred microorganisms for use in accordance with the present invention are single cell, single spore or single virion organisms. Additionally, included within the scope of the present invention are cells from multi-cellular organisms which have been modified to produce a polypeptide of interest.

In a particularly preferred embodiment, bacteria or yeast are used as microorganisms to express and deliver allergenic proteins to individuals to treat or prevent allergic responses, including anaphylactic allergic responses, to the allergens. Gram-positive and gram-negative bacteria may be used in the present invention has delivery vehicles. Antigens expressed by the bacteria may be secreted or non-secreted. Secretion of proteins may involve secretion into the cellular medium. For gram-negative bacteria and yeast, secretion may involve secretion into the periplasm. Secretion of polypeptides may be facilitated by secretion signal peptides. In certain preferred embodiments microorganisms expressing allergenic compounds may be administered to subjects in compositions as attenuated microorganisms, non-pathogenic microorganisms, non-infectious microorganisms, or as killed microorganisms. Preferably, the killed microorganisms are killed without degrading the antigenic properties of the polypeptides.

In another preferred embodiment, the allergens utilized are allergens found in foods, venom, drugs and a rubber-based products. Particularly preferred protein allergens are found in foods and venoms that elicit anaphylactic allergic responses in subjects who are allergic to the allergens. Included in the present invention are peptides and polypeptides whose amino acid sequences are found in the proteins allergens in nature. Also included in the present invention are allergens that have modifications that reduce the ability of the peptides, polypeptides and proteins to bind and crosslink IgE antibodies. Also included in the present invention are non-peptide allergens that are produced by microorganisms and include for example antibiotics such as penicillin.

In another aspect of the invention, compositions for use in treating or prevent allergic and anaphylactic allergic responses in a subject comprise microorganisms that have been engineered by the hand of man, and preferably by the introduction of one or more introduced nucleic acids, to produce allergens in accordance with the present invention. In certain preferred embodiments, the produced allergens are peptides, polypeptides, or proteins encoded by the introduced nucleic acids(s).

Brief Description of Figures

Figure 1. Experiments designed to determine the optimal temperature for heat-killing bacteria (*E. coli)* are depicted in graphic form. The number of surviving colonies in aliquots of samples are shown as a function of temperature (Celsius).

Figure 2. Determination of protein produce per cell. The optical density (O.D.) of the HIS-tagged Ara h 2 allergen was determined from an immunoblot where different concentrations of *E. coli* extract has been electrophoresed on SDS-PAGE gels. The allergen O.D. was used to estimate the amount of protein produced by that extract.

Figure 3. Results of ELISA analysis of Ara h 2-specific IgG antibodies produced in mice following injection of *E. coli* producing Ara h 2. IgG1 is on the left and IgG2a is on the right.

Figure 4. Results of ELISA analysis of Ara h 3-specific IgG antibodies produced in mice following injection of *E. coli* producing Ara h 3. IgG1 is on the left and IgG2a is on the right.

Definitions

"Allergen": An "allergen" is an antigen that (i) elicits an IgE response in an individual; and/or (ii) elicits an asthmatic reaction (e.g., chronic airway inflammation characterized by eosinophilia, airway hyperresponsiveness, and excess mucus production), whether or not such a reaction includes a detectable IgE response). Preferred allergens for the purpose of the present invention are peptide, polypeptide and protein allergens. An exemplary list of protein allergens is presented as an Appendix. This list was adapted from ftp://biobase.dk/pub/who-iuis/allergen.list (updated on March 1, 2000), which provides lists of known allergens. Other preferred allergens are chemical compounds such as small molecules that are produced by proteins.

"Allergic reaction": An allergic reaction is a clinical response by an individual to an antigen. Symptoms of allergic reactions can affect cutaneous (e.g., urticaria, angioedema, pruritus), respiratory (e.g., wheezing, coughing, laryngeal edema, rhinorrhea, watery/itching eyes) gastrointestinal (e.g., vomiting, abdominal pain, diarrhea), and/or cardiovascular (if a systemic reaction occurs) systems. For the purposes of the present invention, an asthmatic reaction is considered to be a form of allergic reaction.

"Anaphylactic antigen": An "anaphylactic antigen" according to the present invention is an antigen (or allergen) that is recognized to present a risk of anaphylactic reaction in allergic individuals when encountered in its natural state, under natural conditions. For example, for the purposes of the present invention, pollens and animal danders or excretions (e.g., saliva, urine) are not considered to be anaphylactic antigens. On the other hand, food antigens, insect antigens, drugs, and rubber (e.g., latex) antigens latex are generally considered to be anaphylactic antigens. Food antigens are particularly preferred anaphylactic antigens for use in the practice of the present invention. Particularly interesting anaphylactic antigens are those (e.g., nuts, seeds, and fish) to which reactions are commonly so severe as to create a risk of death.

"Anaphylaxis" or "anaphylactic reaction", as used herein, refers to an immune response characterized by mast cell degranulation secondary to antigen-induced cross-linking of the high-affinity IgE receptor on mast cells and basophils with subsequent mediator release and the production of pathological responses in target organs, e.g., airway, skin digestive tract and cardiovascular system. As is known in the art, the severity of an anaphylactic reaction may be monitored, for example, by assaying cutaneous reactions, puffiness around the eyes and mouth, and/or diarrhea, followed by respiratory reactions such as wheezing and labored respiration. The most severe anaphylactic reactions can result in loss of consciousness and/or death.

"Antigen": An "antigen" is (i) any compound or composition that elicits an immune response; and/or (ii) any compound that binds to a T cell receptor (e.g., when presented by an MHC molecule) or to an antibody produced by a B-cell. Those of ordinary skill in the art will appreciate that an antigen may be collection of different chemical compounds (e.g., a crude extract or preparation) or a single compound (e.g., a protein). Preferred antigens are peptide, polypeptide or protein antigens.

"Antigen presenting cells": "Antigen presenting cells" or APCs" include known APCs such as Langerhans cells, veiled cells of afferent lymphatics, dendritic cells and interdigitating cells of lymphoid organs. The term also includes mononuclear cells such as lymphocytes and macrophages which take up polypeptides and proteins according to the invention.

"Attenuation": "Attenuation" of microorganisms as used herein refers to the manipulation of the microorganisms so that the microorganisms do not induce significant toxic reactions in individuals or laboratory test animals. The manipulations include genetic methods and are well known in the art.

"IgE binding site": An IgE binding site is a region of an antigen that is recognized by an anti-antigen IgE molecule. Such a region is necessary and/or sufficient to result in (i) binding of the antigen to IgE; (ii) cross-linking of anti-antigen IgE; (iii) degranulation of mast cells containing surface-bound anti-antigen IgE; and/or (iv) development of allergic symptoms (e.g., histamine release). In general, IgE binding sites are defined for a particular antigen or antigen fragment by exposing that antigen or fragment to serum from allergic individuals (preferably of the species to whom inventive compositions are to be administered). It will be recognized that different individuals may generate IgE that recognize different epitopes on the same antigen. Thus, it is typically desirable to expose antigen or fragment to a representative pool of serum samples. For example, where it is desired that sites recognized by human IgE be identified in a given antigen or fragment, serum is preferably pooled from at least 5-10, preferably at least 15, individuals with demonstrated allergy to the antigen. Those of ordinary skill in the art will be well aware of useful pooling strategy in other contexts.

"Immunologic inducing agents": The term "immunological inducing agents" is used herein as agents that prompt the expression of Th1 stimulating cytokines by T-cells and include factors such as, CD40, CD40 ligand, oligonucleotides containing CpG motifs, TNF, and microbial extracts such as preparations of *Staphylococcus aureus,* heat killed *Listeria*, and modified cholera toxin.

"Inducible promoter": The term "inducible promoter", as used herein, means a promoter site which is activated directly by the presence or absence of a chemical agent or indirectly by an environmental stimulus such as temperature changes. A promoter is the region of DNA at which the enzyme RNA polymerase binds and initiates the process of gene transcription.

"Mast cell": As will be apparent from context, the term "mast cell" is often used herein to refer to one or more of mast cells, basophils, and other cells having IgE receptors, which when activated by crosslinking bound IgE molecules, releases histamines, vasodilators, and/or other mediators of allergic responses.

"Microorganisms": "Microorganisms" as used herein are cells, bacteria, fungi, viruses, algae, and protozoa. Preferred microorganisms can be genetically manipulated to produce a desired polypeptide(s).

"Peptide": According to the present invention, a "peptide" comprises a string of at least three amino acids linked together by peptide bonds. Inventive peptides preferably contain only natural amino acids, although non-natural amino acids (i.e., compounds that do not occur in nature but that can be incorporated into a polypeptide chain; see, for example, http://www.cco.caltech.edu/~dadgrp/Unnatstruct.gif, which displays structures of non-natural amino acids that have been successfully incorporated into functional ion channels) and/or amino acid analogs as are known in the art may alternatively be employed. Also, one or more of the amino acids in an inventive peptide may be modified, for example, by the addition of a chemical entity such as a carbohydrate group, a phosphate group, a farnesyl group, an isofarnesyl group, a fatty acid group, a linker for conjugation, functionalization, or other modification, etc.

A peptide or polypeptide is derived from a protein if the amino acid sequence of the peptide or polypeptide is found within the amino acid sequence of the protein. The sequences are preferably identical but may have a sequence homology between approximately 80-100%. It is also recognized that amino acid residues may be replaced with other amino acids residues with similar physical properties such as hydrophobicity, hydrophilicity, charge, aromatic structures and polarity.

"Reduced IgE binding": An inventive composition or antigen is considered to have "reduced IgE binding" if it demonstrates a lower level of interaction with IgE when compared with unmodified antigen in any available assay. For example, a modified antigen is considered to have reduced IgE binding if (i) its affinity for anti-antigen IgE (assayed, for example, using direct binding studies or indirect competition studies) is reduced at least about 2-5 fold, preferably at least about 10, 20, 50, or 100 fold as compared with intact antigen ; (ii) ability of the modified antigen to support cross-linking of anti-antigen IgE is reduced at least about 2-fold, preferably at least about 5, 10, 20, 50, or 100 fold as compared with intact antigen; (iii) mast cells containing surface-bound anti-antigen IgE degranulate less (at least about 2 fold, preferably at least about 3, 5, 10, 20, 50, or 100 fold less) when contacted with modified as compared with unmodified antigen; and/or (iv) individuals contacted with modified antigen develop fewer (at least about 2 fold, preferably at least about 3, 5, 10, 20, 50, or 100 fold fewer) allergic symptoms, or developed symptoms are reduced in intensity when exposed to modified antigens as compared with unmodified antigens.

"Secretion signals": A secretion signal is any amino acid sequence which when conjugated to a peptide, polypeptide or protein facilitates the transport of the conjugate fusion proteins across cell membranes. For uses of secretion signals in microorganisms, transport of fusion proteins involves crossing an inner membrane into the periplasm. It is preferred that secretion signals also facilitate transport of fusion proteins across an outer membrane into an extracellular medium. Secretion of proteins into the extracellular medium is considered "excretion".

"Sensitized mast cell": A "sensitized" mast cell is a mast cell that has surface-bound antigen specific IgE molecules. The term is necessarily antigen specific. That is, at any given time, a particular mast cell will be "sensitized" to certain antigens (those that are recognized by the IgE on its surface) but will not be sensitized to other antigens.

"Small molecules": As used herein, the term "small molecule" refers to a compound either synthesized in the laboratory or found in nature. Typically, a small molecule is organic and is characterized in that it contains several carbon-carbon bonds, and has a molecular weight of less than 1500 daltons, although this characterization is not intended to be limiting for the purposes of the present invention. Examples of "small molecules" that are allergens include without limitation penicillin, alcohols, and aspirin. Non-organic small molecule allergens include sulfites present in wine, for example.

"Susceptible individual": According to the present invention, a person is susceptible to an allergic reaction if (i) that person has ever displayed symptoms of allergy after exposure to a given antigen; (ii) members of that person's genetic family have displayed symptoms of allergy against the allergen, particularly if the allergy is known to have a genetic component; and/or (iii) antigen-specific IgE are found in the individual, whether in serum or on mast cells.

"Th1 response" and "Th2 response": Certain preferred peptides, polypeptides, proteins and compositions of the present invention are characterized by their ability to suppress a Th2 response and/or to stimulate a Th1 response preferentially as compared with their ability to stimulate a Th2 response. Th1 and Th2 responses are well-established alternative immune system responses that are characterized by the production of different collections of cytokines and/or cofactors. For example, Th1 responses are generally associated with production of cytokines such as IL-1β, IL-2, IL-12, IL-18, IFNα, IFNγ, TNFβ, etc; Th2 responses are generally associated with the production of cytokines such as IL-4, IL-5, IL-10, etc. The extent of T cell subset suppression or stimulation may be determined by any available means including, for example, intra-cytoplasmic cytokine determination. In preferred embodiments of the invention, Th2 suppression is assayed, for example, by quantitation of IL-4, IL-5, and/or IL-13 in stimulated T cell culture supernatant or assessment of T cell intra-cytoplasmic (e.g., by protein staining or analysis of mRNA) IL-4, IL-5, and/or IL-13; Th1 stimulation is assayed, for example, by quantitation of IFNα, IFNγ, IL-2, IL-12, and/or IL-18 in activated T cell culture supernatant or assessment of intra-cytoplasmic levels of these cytokines.

Description of Certain Preferred Embodiments

The present invention provides compositions and methods for modulating the immune response in a subject. It is an aspect of the present invention that undesirable allergic immune responses to antigens in a subject are treated or prevented by administering modified cells, virions, or spores ("microorganisms") that express allergens of interest. By using genetically modified microorganisms to express and deliver allergens, exposure of the allergens to the subject's IgE-mediated allergic immune response is reduced or eliminated. Without limitation to the mechanisms proposed, it is expected that the modified microorganisms of the present invention are engulfed by antigen-presenting cells (APCs) such as macrophages and dendritic cells without exposing allergens to IgE antibodies. Once inside the APCs, the expressed allergens are released by lysis of the microorganisms or secretion of the antigen by the microorganisms. The allergens are then processed, for example through partial digestion by the APCs, and displayed on the cell surface.

Once the processed antigens are displayed on the cell surface, activation of the cytotoxic T cell response and helper T cell response promotes cellular immune response and Th1-mediated B cell response to protein allergens. In addition, the processed antigens have a reduced ability (or no ability) to bind and crosslink IgE antibodies located on the surface of mast cells and basophils leading to the release of histamines and other vasodilators responsible for allergic and sometimes fatal anaphylactic responses.

HOST MICROORGANISMS

Any microorganism capable of expressing (e.g., by expression of polypeptide or protein allergens, or by expression of polypeptide or protein enzymes involved in synthesis of small molecule allergens) allergens may be used as delivery vehicles in accordance with the present invention. Such microorganisms include but are not limited to bacteria, viruses, fungi (including yeast), algae, and protozoa. Generally, microorganisms are single cell, single spore or single virion organisms. Additionally, included within the scope of the present invention are cells from multi-cellular organisms which have been modified to produce a polypeptide of interest. Microorganisms that can be genetically manipulated to produce a desired polypeptide are preferred. (Ausubel et al. *Current Protocols in Molecular Biology*. Wiley and Sons, Inc. 1999, incorporated herein by reference) Genetic manipulation includes mutation of the host genome, insertion of genetic material into the host genome, deletion of genetic material of the host genome, transformation of the host with extrachromosomal genetic material, transformation with linear plasmids, transformation with circular plasmids, insertion of genetic material into the host (e.g., injection of mRNA), insertion of transposons, and chemical modification of genetic material. Methods for constructing nucleic acids (including an expressible gene), and introducing such nucleic acids into an expression system to express the encoded protein are well established in the art (see, for example, Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989, incorporated herein by reference).

Use of microorganisms such as bacteria and yeast for allergen delivery in accordance with the present invention offers many advantages over delivery of allergens that are not encapsulated inside microorganisms for immunotherapy. Generally, microorganisms, such as bacteria, are known to act as an adjuvant (for a review, see for example, Freytag et al. *Curr Top Microbiol Immunol* 236:215-36, 1999). Therefore, use of microorganisms to delivery allergens to subjects, and APCs of subjects, provides protection of the allergen from IgE-mediated allergic responses and also provides an adjuvant effect which elicits a Th1-type immune response from an individual susceptible to allergic responses. In addition, use of non-pathogenic, non-infectious, attenuated and/or killed microorganisms reduces or eliminates toxicity which may be associated with allergen delivery vehicles.

In a preferred embodiment, bacteria are used as protein delivery microorganisms. Generally, bacteria are classified as gram-negative or gram-positive depending on the structure of the cell walls. Those skilled in the art are capable of identifying gram-negative and gram-positive bacteria which may be used to express proteins in accordance with the present invention. Non-limiting examples of genera and species of gram-negative bacteria include *Escherichia coli, Vibro cholera, Salmonella, Listeria, Legionella, Shigella, Yersenia, Citrobacter, Enterobacter, Klebsiella, Morganella, Proteus, Providencia, Serratia, Plesiomonas, Aeromonas.* Non-limiting examples of genera and species of gram-positive bacteria which may be used in the present invention include *Bacillus subtilis, Sporolactobacillus, Clostridium, Arthrobacter, Micrococcus, Mycobacterium, Peptococcus, Peptostreptococcus, and Lactococcus.*

Gram-negative bacterial systems for use as delivery vehicles are known and may be used in the present invention. For example, *E. coli* is a well-studied bacteria, and methods of protein expression in *E. coli* are well-established. Most strains of *E. coli* have the advantage of being non-pathogenic since *E. coli* is found naturally in the gut. Therefore, *E. coli* is preferred as a delivery vehicle in the present invention. In addition, Calderwood *et al.* (US Patent 5,747,028) utilize *Vibrio cholerae* as a delivery vehicle for production of antigens for use as a live vaccine against infectious organisms. Miller and Mekalanos (US Patent 5,731,196) utilize *Salmonella* as delivery vehicle for production of antigens for use as a live vaccine against infectious organisms. Hess *et al.* (*Proc. Natl. Acad. Sci. USA* 93:1458-1463, 1996) utilize recombinant attenuated *Salmonella* which secretes antigenic determinants of *Listeria* as a live vaccine to protect against listeriosis. Donner *et al.* (WO 98/50067) utilize attenuated *Salmonella typhimurium* as a gram-negative host for secretion of polypeptides for controlling fertility and also teach that other attenuated gram-negative strains including *Yersinia* may be used to express and secrete such polypeptides.

Gram-positive bacteria have also been studied as delivery vehicles for proteins to modulate an immune response in a subject. WO 97/14806 describes the use of *Lactococcus* to deliver polypeptides into a body to enhance the immune response to the polypeptides. However, WO 97/14806 does not teach the use of *Lactococcus* to treat patients with food allergies and venom allergies which may result in anaphylaxis

In another preferred embodiment, yeast are used as protein delivery microorganisms. It is well known that yeast are amenable to genetic manipulation to express a protein or proteins of choice (Ausubel et al. supra). Furthermore, in general most yeast are non-pathogenic. Without limitation to these species, two well-characterized species of yeast are the budding yeast *Saccharomyces cerevisiae*, and the fission yeast, *Schizosaccharomyces pombe*. Moreover, the administration of yeast that express protein antigens to alter an immune response has been studied. Duke et al. (US Patent No. 5,830,463; "Duke") describe the use of yeast to express proteins after administration of the yeast to a mammal. However, Duke does not teach the use of yeast to treat patients with food allergies and venom allergies which may result in anaphylaxis.

Microorganisms of the present invention may be administered to a subject as live or dead microorganisms. Preferably if the microorganisms are administered as live microorganisms, they are non-pathogenic or attenuated pathogenic microorganisms. For applications of the invention where live microorganisms are administered to individuals, preferably the microorganisms are attenuated and/or are administered in suitable encapsulation materials and/or as pharmaceutical compositions as vaccines to decrease an individuals immune response to the microorganism and/or allergenic compounds. Generally, attenuation involves genetically modifying the infectious pathogenic microorganism to reduce or eliminate the infectious ability of the microorganism. Preferably, the microorganism is attenuated such that an individual inoculated with the microorganism does not suffer any cytotoxic effects from the presence of the microorganism. Particularly preferred attenuated microorganisms are infectious intracellular pathogens which are phagocytosed by antigen-presenting cells in individuals who are exposed to the microorganism. Examples of microorganisms which are intracellular pathogens include *Salmonella, Mycobacterium, Leishmania, Legionella, Listeria, and Shigella.*

Microorganisms of the present invention may be administered to subjects after killing the microorganisms. Any method of killing the microorganisms may be utilized that does not greatly alter the antigenicity of the expressed polypeptides. Methods of killing microorganism include but are not limited to using heat, antibiotics, chemicals such as iodine, bleach, ozone, and alcohols, radioactivity (i.e. irradiation), UV light, electricity, and pressure. Preferred methods of killing microorganisms are reproducible and kill at least 99% of the microorganisms. Particularly preferred is the use of heat above 50 degrees Celsius for a period of time that kills greater than 99% of the cells and preferably 100% of the cells.

INDUCIBLE SYSTEMS

In another preferred embodiment, the inventive expression of allergens by microorganisms is regulated so that synthesis occurs at a controlled time after the live microorganism is administered to an individual. Preferably the induction of protein synthesis is regulated so that activation occurs after the microorganism(s) is taken up by antigen-presenting cells (APCs) and phagocytosed into the endosome. A desirable result of this regulation is that production of the allergen of interest occurs inside the APCs and therefore reduces or eliminates the exposure of the allergen to IgE molecules bound to the surface of histamine-releasing mast cells and basophils. This reduces or eliminates the risk of anaphylaxis during administration of microorganisms that produce anaphylactic antigens.

Any method of controlling protein synthesis in the microorganism may be used in accordance with the present invention. Preferably, the method of controlling protein synthesis utilizes an inducible promoter operatively-linked to the gene of interest (e.g., a gene which encodes a signal peptide and protein antigen). Many systems for controlling transcription of a gene using an inducible promoter are known (Ausubel et al. *Current Protocols in Molecular Biology.* Wiley and Sons. New York. 1999). Generally, inducible systems either utilize activation of the gene or derepression of the gene. It is preferred that the present invention utilizes activation of a gene to induces transcription. However, inducible systems using derepression of a gene may also be used in the present invention. Systems using activation are preferred because these systems are able to tightly control inactivation (and hence basal level synthesis) since derepression may result in low levels of transcription if the derepression is not tight.

Methods of inducing transcription include but are not limited to induction by the presence or absence of a chemical agent, induction using a nutrient starvation inducible promoter, induction using a phosphate starvation inducible promoter and induction using a temperature sensitive inducible promoter. A particularly preferred system for regulating gene expression utilizes tetracycline controllable expression system. Systems which utilize the tetracycline controllable expression system are commercially available (see for example, Clontech, Palo Alto, CA).

Another particularly preferred system for regulating gene expression utilizes an ecdysone-inducible expression system which is also commercially available (Invitrogen, Carlsbad CA). The ecdysone-inducible expression system is based on the ability of ecdysone which is an insect hormone, to activate gene expression by binding to the ecdysone receptor. The expression system utilizes a modified heterologous protein containing the ecdysone receptor, a viral transactivation domain (from VP16) and the retinoid X receptor derived from mammalian cells to bind to a modified ecdysone response element in the presence of a ligand such as ecdysone or an analog (e.g. muristerone A, ponasterone A).

It is preferred that inducible systems for use in the present invention utilize inducing agents that are non-toxic to mammalians cells including humans. Furthermore, it is preferred that transcriptional inducing agents permeate cells membranes. More specifically for activation of protein synthesis in microorganisms after phagocytosis by APCs, transcriptional inducing agents must be able to pass through cells membranes of the APC and cell membranes of the microorganism to activate the expression of genes encoding protein allergens in accordance with the present invention. Since both tetracycline and ecdysone are able to pass through cell membranes and are non-toxic, tetracycline-inducible systems and ecdysone-inducible systems are ideally suited for use in the present invention. However, the use of inducible systems in the present invention is not limited to those systems.

It is also preferred that bacteria that have not been phagocytosed are killed before induction of genes expressing polypeptide allergens of interest. A preferred method of killing bacteria is to use antibiotics which are not permeable to mammalian cell membranes such that only bacteria that are not phagocytosed are killed. The use of antibiotics in accordance with the present embodiment reduces or eliminates the production of polypeptides by bacteria outside antigen presenting cells. It is important to reduce or eliminate exposure of allergen-producing bacteria to the immune system, especially bacteria that secrete polypeptides, which could elicit a potentially lethal anaphylactic reaction in an individual. Those having ordinary skill in the art are readily aware of antibiotics which may be used. Such antibiotics include but are not limited to penicillin, ampicillin, cephalosporin, griseofulvin, bacitracin, polymyxin b, amphotericin b, erythromycin, neomycin, streptomycin, tetracycline, vancomycin, gentamicin, and rifamycin

SECRETION SIGNALS

In another embodiment of the present invention, expressed allergens (and/or immunomodulatory molecules, such as cytokines; see below) are secreted by the microorganisms. Preferably, secretion of the allergens occurs inside a mammalian cell to reduce or eliminate exposure of allergens to a subject's allergic immune response. Secretion of polypeptides includes secretion into the extracellular medium and secretion of polypeptides into the periplasm of microorganisms such as gram-negative bacteria and yeast. Advantages of secreting allergens into the periplasm include reducing leakage of the allergens prior to phagocytosis of the microorganism. This advantage is most applicable in non-inducible systems. Advantages of secreting allergens into the extracellular medium in inducible systems include maximizing the amount of allergens available for processing by antigen-presenting cells after phagocytosis of the microorganisms of the present invention.

To express secreted polypeptides in bacteria, a variety of bacterial secretion signals known in the art may be used. For example, the Sec-dependent process in *E. coli* is one which is well known (for a review see Driessen et al. *Curr. Opin. Microbiology* 1:216-22). In addition, the OmpA signal peptide in *E. coli* has been described by Wong and Sutherland (US Patent 5,223,407). Fusion proteins containing either of these secretion signal peptides are not fully secreted by the bacteria, but rather transported across the inner membrane of the gram-negative bacteria into the periplasm. These secretion signals may be used in the present invention to transport allergenic or immunomodulatory polypeptides into the periplasm of bacteria. After administration of the genetically engineered bacteria to an individual and subsequent phagocytosis by APCs, the allergenic or immunomodulatory polypeptides in the periplasm are released after degradation of the outer membrane by enzymes in the endosome of the APCs. Preferably, the bacteria synthesize and secrete the polypeptides into the periplasm and are killed, preferably heat-killed, before administration. However, it is recognized that attenuated bacteria may be used to secrete inventive allergens into the periplasm and administered to individuals.

In another preferred embodiment of secreted proteins or polypeptides, fusion proteins containing secretion signal sequences and allergenic or immunomodulatory sequences are fully secreted into the extracellular medium by a microorganism after synthesis of the protein. Such secretion signals include those found in hemolysin and listeriolysin. In a particularly preferred embodiment, the hemolysin complex of *E. coli* is used to transport allergenic or immunomodulatory polypeptides across the inner and outer membrane of a microorganism (e.g. *E. coli, Salmonella, Shigella, Vibrio, Yersinia, Citrobacter, Serratia, Pseudomonas*) into the extracellular medium (Spreng et al. *Mol. Microbiol.* 31:1589-1601, 1999, and references therein all of which are incorporated herein by reference). Fusion of HlyAs to proteins and polypeptides has been shown to result in secretion of these fusion proteins utilizing the hemolysin secretion system (Blight and Holland, *Trends Biotechnol.* 1994 Nov;12(11):450-5.; Gentschev et al., *Behring Inst Mitt.* 1994 Dec;(95):57-66)

The hemolysin protein (HlyA) contains a C-terminal transport signal (HlyAs) which is approximately 50-60 amino acids in length (Hess et al., *Mol Gen Genet.* 1990 Nov;224(2):201-8; Jarchau et al., *Mol Gen Genet.* 1994 Oct 17;245(1):53-60). The HlyA protein is secreted across the inner *and outer* cellular membranes by the hemolysin secretion system. This complex contains three membrane proteins. Two of these proteins, HlyB and HlyD, are located in the inner membrane, and the third TolC, is located at the outer membrane. Genes encoding these proteins are part of the hemolysin operon which consists of four genes *hlyC, hlyA, hlyB,* and *hlyD* (Wagner et al., *J Bacteriol.* 1983 Apr;154(1):200-10; Gentschev. *Gene.* 1996 Nov 7;179(1):133-40).

In a preferred embodiment for use of the Hly secretion system, DNA plasmids (vectors) are used to express fusion proteins containing the HlyAs signal peptide and allergenic or immunomodulatory polypeptides. The genes encoding the transport complex (hlyB, and hlyD) are encoded by the same vector. It is recognized that multiple vectors can be used to encode and express these genes, or that sequences encoding these genes can be inserted into the host genome for expression. Preferably, a single vector contains the complete hemolysin operon including the hly specific promoter and an enhancer-type regulator hlyR; the HlyA gene where only the minimal polypeptide sequence necessary to transport a fusion protein is present; and the antigen of interest. TolC protein is generally produced by the host *E. coli* system. However, in systems where tolC DNA is not encoded by a host organism, tolC can be encoded by a vector.

In a particularly preferred embodiment, the secretion plasmid pMOhly1 described in WO 98/50067 ("Donner") is used to express fusion proteins containing secretion signal sequences and polypeptides related to inducing anaphylaxis in individuals. The secretion vector pMOhly1 contains the complete hemolysin operon including the *hly* specific promoter and an enhancer-type regulator hlyR. A majority of the *hlyA* gene has been deleted so that HlyA encodes only the 34 amino terminal and 61 carboxyl terminal amino acids ($HlyA_s$). A unique Nsi restriction enzyme site between the amino terminal and carboxyl terminal residues of HlyA facilitates the insertion of heterologous genes or gene fragments into the reading frame of $HlyA_s$. The genetic information for antigens the size of 10-1000 amino acids can be inserted into this secretion vector pMOhly1, which facilitates the secretion of these antigens in attenuated *Salmonella* and other gram-negative attenuated inoculation strains (e.g. *E. coli, Vibrio cholera, Yersina enterocolitica*). In contrast to other secretion systems, the secretion of fusion proteins using a single plasmid is described by Donner. An advantage of the hemolysin secretion system in comparison to conventional transport systems is the larger size of the fusion proteins synthesized and secreted according to the methods taught in Donner. Conventional secretion systems for the presentation of antigens are only capable of secreting relatively short peptides to the outer part of the bacterial cell (Cardenas and Clements, *Clin Microbiol Rev.* 1992 Jul;5(3):328-42).

ANTIGENS AND ALLERGENS

In general, any allergen may be produced by microorganisms in accordance with the present invention. Preferred allergens are found in certain foods, venom, drugs or rubber and are capable of eliciting allergic responses, and in particular anaphylactic allergic responses in an individual. Particularly preferred allergens are protein or polypeptide allergens.

In a preferred embodiment, microorganisms of the present invention produce allergenic proteins that elicit allergies, possibly anaphylaxis, and are found in foods, venoms, drugs, and rubber-based products. Particularly preferred allergenic proteins that induce anaphylaxis, such as several protein allergens found in food (peanut, milk, egg, wheat), insect venom (i.e. bees, reptiles), drugs, and latex. Non-limiting examples of protein allergens found in food include proteins found in nuts (e.g., peanut walnut, almond, pecan, cashew, hazelnut, pistachio, pine nut, brazil nut), seafood (e.g. shrimp, crab, lobster, clams), fruit (e.g. plums, peaches, nectarines; *Ann Allergy Asthma Immunol* 7(6):504-8 (1996); cherries, *Allergy* 51(10):756-7 (1996)), seeds (sesame, poppy, mustard), and soy and dairy products (e.g., egg, milk).

Some protein allergens found in nuts are related to legume allergies and may be used instead of the legume proteins (e.g. peanuts, soybeans, lentils; *Ann Allergy Asthma Immunol* 77(6): 480-2 (1996). Also, protein antigens found in pollen-related food allergies may be used (e.g. birch pollen related to apple allergies). Other protein allergens found in foods include those found in young garlic (*Allergy* 54(6):626-9 (1999), and for children allergic to house dust mites, allergens found in snails (*Arch Pediatr* 4(8):767-9 (1997)). Protein allergens in wheat are known to cause exercise-induced allergies (*J Allergy Clin Immunol* 1999 May;103(5 Pt 1):912-7).

Stings from organisms that inject venoms, such as insect stings are known to cause anaphylaxis in individuals with allergies to the venom. In general, insect venom includes venom from Hymenoptera such as bees, hornets, wasps, yellow jackets, velvet ants, and fire ants. In particular for example, venom from honey bees of the genus *Apis* can cause anaphylaxis in stung victims who are allergic (Weber et al. *Allergy* 42:464-470). The venom from honey bees contains numerous compounds which have been extensively studied and characterized (see for a reference, Banks and Shipolini. *Chemistry and Pharmacology of Honey-bee Venom.* Chapter 7 of Venoms of the Hymenoptera. Ed. T. Piek. Academic Press. London. 1986). The two main components of bee venom are phospholipase A2 and melittin and are preferred protein allergens for use in the present invention for treating and preventing allergies to bee venom.

In certain uses of the present invention, it will be desirable to work in systems in which a single compound (e.g., a single protein) is responsible for most observed allergy. In other cases, the invention can be applied to more complex allergens. Therefore, collections of more than one antigen can be used so that immune responses to multiple antigens may be modulated simultaneously.

Appendix A presents a representative list of certain known protein antigens. As indicated, the amino acid sequence is known for many or all of these proteins, either through knowledge of the sequence of their cognate genes or through direct knowledge of protein sequence, or both. Of particular interest are anaphylactic antigens.

In another embodiment of allergenic antigens, microorganisms are genetically engineered to synthesize and secrete modified allergenic polypeptides that elicit anaphylaxis when exposed to individuals who are susceptible to anaphylactic shock. Preferably, the allergens are modified such that the ability to elicit anaphylaxis is reduced or eliminated. As previously discussed allergens elicit allergic responses which are sometimes severe enough to induce anaphylactic shock by crosslinking IgE antibodies bound to the surface of mast cells and basophils. The IgE crosslinking releases compounds such as histamines which causes symptoms related to allergies and anaphylactic shock. In accordance with the present invention, microorganisms are used to synthesize and secrete antigens which are modified to reduce or eliminate IgE binding sites while still maintaining antigenicity or immunomodulatory activity (U.S.S.N. 09/141,220 incorporated herein by reference). This reduces the risk of allergic or anaphylactic responses in individuals treated with vaccines containing these engineered microorganisms.

The amount of antigen to be employed in any particular composition or application will depend on the nature of the particular antigen and of the application for which it is being used, as will readily be appreciated by those of ordinary skill in the art. The experiments described in Examples 1-4 suggest that larger amounts of polypeptides are useful for inducing Th1 responses. The amount of antigen can be controlled by a variety of factors including but not limited to expression systems, inducible expression systems, levels of secretion and excretion, methods of killing bacteria before delivery. Those of ordinary skill in the art are capable of determining the desired levels of antigens to be produced by bacteria and delivered to individuals.

It is recognized that multiple antigenic molecules may be delivered by bacteria simultaneously in accordance with the methods of the present invention. Without limitation, different antigenic determinants for one antigenic protein may be delivered. Different antigenic determinants from different antigenic proteins may also be delivered. Further, multiple antigenic polypeptides and proteins may be delivered in accordance with the present invention. It is also recognized that single or multiple antigenic polypeptides and single or multiple cytokines may be delivered to individuals by bacteria in accordance with the present invention. For example but without limitation, allergenic antigens of the present invention and immunomodulatory molecules such as interleukins may be delivered by bacteria using secreted or non-secreted methods in accordance with the present invention.

ADJUVANTS AND IMMUNOSTIMULATORY AGENTS

Compositions and methods of the present invention include the use of adjuvants and immunomodulatory polypeptides or immunostimulatory factors to modulate an individual's immune response. Immunologic adjuvants are agents that enhance specific immune responses to vaccines. Formulation of vaccines with potent adjuvants is desirable for improving the performance of vaccines composed of antigens. Adjuvants may have diverse mechanisms of action and should be selected for use based on the route of administration and the type of immune response (antibody, cell-mediated, or mucosal immunity) that is desired for a particular vaccine

In general, immunomodulatory polypeptides include cytokines which are small proteins or biological factors (in the range of 5-20 kD) that are released by cells and have specific effects on cell-cell interaction, communication and behavior of other cells. As previously described, cytokines in accordance with the present invention are proteins that are secreted to T-cells to induce a Th1 or Th2 response. Preferably, the cytokine(s) to be administered is/are selected to reduce production of a Th2 response to antigens associated with anaphylaxis. One preferred method of reducing a Th2 response is through induction of the alternative response. Cytokines that, when expressed during antigen delivery into cells, induce a Th1 response in T cells (i.e., "Th1 stimulating cytokines") include IL-12, IL-2, I-18, IL-1 or fragments thereof, IFN , and/or IFNγ.

Other compounds that are immunomodulatory include immunological inducing agents. These inducing agents prompt the expression of Th1 stimulating cytokines by T-cells and include factors such as, CD40, CD40 ligand, oligonucleotides containing CpG motifs, TNF, and microbial extracts such as preparations of *Staphylococcus aureus,* heat killed *Listeria*, and modified cholera toxin, etc.

Those of ordinary skill in the art readily appreciate the preferred types of adjuvants for use with particular antigen compositions. In general, immunologic adjuvant include gel-type adjuvants (e.g. aluminum hydroxide/aluminum phosphate, calcium phosphate), microbial adjuvants (e.g. DNA such as CpG motifs; endotoxin such as monophosphoryl lipid A; exotoxins such as cholera toxin, *E. coli* heat labile toxin, and pertussis toxin; and muramyl dipeptide), oil-emulsion and emulsifier-based adjuvants (e.g. Freund's Incomplete Adjuvant, MF59, and SAF), particulate adjuvants (e.g. liposomes, biodegradable microspheres, and saponins), and synthetic adjuvants (e.g. nonionic block copolymers, muramyl peptide analogues, polyphosphazene, and synthetic polynucleotides).

Adjuvants that are known to stimulate Th2 responses are preferably avoided. Particularly preferred adjuvants include, for example, preparations (including heat-killed samples, extracts, partially purified isolates, or any other preparation of a microorganism or macroorganism component sufficient to display adjuvant activity) of microorganisms such as *Listeria monocytogenes* or others (e.g., Bacille Calmette-Guerin [BCG], Corynebacterium species, Mycobacterium species, Rhodococcus species, Eubacteria species, Bortadella species, and Nocardia species), and preparations of nucleic acids that include unmethylated CpG motifs (see, for example, U.S. Patent No. 5,830,877; and published PCT applications WO 96/02555, WO 98/18810, WO 98/16247, and WO 98/40100, each of which is incorporated herein by reference). Other preferred adjuvants reported to induce Th1-type responses and not Th2-type responses include, for example, Aviridine (N,N-dioctadecyl-N'N'-bis (2-hydroxyethyl) propanediamine) and CRL 1005. Particularly preferred are ones that induce IL-12 production, including microbial extracts such as fixed *Staphylococcus aureus*, *Streptococcal* preparations, *Mycobacterium tuberculosis*, lipopolysaccharide (LPS), monophosphoryl lipid A (MPLA) from gram negative bacterial lipopolysaccharides (Richards et al. *Infect Immun* 1998 Jun;66(6):2859-65), *listeria monocytogenes*, *toxoplasma gondii*, *leishmania major*. Some polymers are also adjuvants. For example, polyphosphazenes are described in U.S. Patent No. 5,500,161 to Andriavnov, et al. These can be used not only to encapsulate the microorganisms but also to enhance the immune response to the antigen.

If adjuvants are not synthesized by microorganisms in accordance with the present invention, adjuvants which are cytokines may be provided as impure preparations (e.g., isolates of cells expressing a cytokine gene, either endogenous or exogenous to the cell), but are preferably provided in purified form. Purified preparations are preferably at least about 90% pure, more preferably at least about 95% pure, and most preferably at least about 99% pure. Alternatively, genes encoding the cytokines or immunological inducing agents may be provided, so that gene expression results in cytokine or immunological inducing agent production either in the individual being treated or in another expression system (e.g., an *in vitro* transcription/translation system or a host cell) from which expressed cytokine or immunological inducing agent can be obtained for administration to the individual. It is recognized that microorganisms utilized to synthesize and deliver allergenic and/or immunomodulatory proteins according to the present invention can act as an adjuvant, and that preferred microorganisms are immunostimulatory adjuvants.

It will be appreciated by those of ordinary skill in the art that the inventive administration of microorganisms expressing cytokines and/or allergens may optionally be combined with the administration of any other desired immune system modulatory factor such as, for example, an adjuvant or other immunomodulatory compound.

METHODS OF ADMINISTRATION

Formulations can be delivered to a patient by any available route including for example enteral, parenteral, topical (including nasal, pulmonary or other mucosal route), oral or local administration. The compositions are preferably administered in an amount effective to elicit cellular immunity and production of Th1-related IgG while minimizing IgE mediated responses. Also preferred are compositions administered in an effective amount to active T-cell response, preferably Th1-type responses. For compositions of the present invention containing bacteria, administration is preferably delivered parenterally.

PHARMACEUTICAL COMPOSITIONS

Pharmaceutical compositions for use in accordance with the present invention may include a pharmaceutically acceptable excipient or carrier. As used herein, the term "pharmaceutically acceptable carrier" means a non-toxic, inert solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. Some examples of materials which can serve as pharmaceutically acceptable carriers are sugars such as lactose, glucose, and sucrose; starches such as corn starch and potato starch; cellulose and its derivatives such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients such as cocoa butter and suppository waxes; oils such as peanut oil, cottonseed oil; safflower oil; sesame oil; olive oil; corn oil and soybean oil; glycols; such as propylene glycol; esters such as ethyl oleate and ethyl laurate; agar; buffering agents such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol, and phosphate buffer solutions, as well as other non-toxic compatible lubricants such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, releasing agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the composition, according to the judgment of the formulator. The pharmaceutical compositions of this invention can be administered to humans and/or to other animals, orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, or drops), bucally, or as an oral or nasal spray.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active compounds, the liquid dosage forms may contain inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, the oral compositions can also include agents such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents.

Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution, suspension or emulsion in a nontoxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, U.S.P. and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid are used in the preparation of injectables.

In order to prolong the effect of an agent, it is often desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material with poor water solubility. The rate of absorption of the agent then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle. Injectable depot forms are made by forming microencapsule matrices of the drug in biodegradable polymers such as polylactide-polyglycolide. Depending upon the ratio of agent to polymer and the nature of the particular polymer employed, the rate of release of the agent can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides) Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissues.

Compositions for rectal or vaginal administration are preferably suppositories which can be prepared by mixing the compounds of this invention with suitable non-irritating excipients or carriers such as cocoa butter, polyethylene glycol or a suppository wax which are solid at ambient temperature but liquid at body temperature and therefore melt in the rectum or vaginal cavity and release the active compound.

Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound is mixed with at least one inert, pharmaceutically acceptable excipient or carrier such as sodium citrate or dicalcium phosphate and/or a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid, b) binders such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidinone, sucrose, and acacia, c) humectants such as glycerol, d) disintegrating agents such as agar -agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate, e) solution retarding agents such as paraffin, f) absorption accelerators such as quaternary ammonium compounds, g) wetting agents such as, for example, cetyl alcohol and glycerol monostearate, h) absorbents such as kaolin and bentonite clay, and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may also comprise buffering agents.

Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like.

The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes.

Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polethylene glycols and the like.

The compounds can also be in micro-encapsulated form with one or more excipients as noted above. The solid dosage forms of tablets, dragees, capsules, pills and granules can be prepared with coatings and shells such as enteric coatings, release controlling coatings and other coatings well known in the pharmaceutical formulating art. In such solid dosage forms the active compound may be admixed with at least one inert diluent such as sucrose, lactose or starch. Such dosage forms may also comprise, as is normal practice, additional substances other than inert diluents, e.g., tableting lubricants and other tableting aids such a magnesium stearate and microcrystalline cellulose. In the case of capsules, tablets and pills, the dosage forms may also comprise buffering agents. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes.

Dosage forms for topical or transdermal administration of an inventive pharmaceutical composition include ointments, pastes, creams, lotions, gels, powders, solutions, sprays, inhalants or patches. The active component is admixed under sterile conditions with a pharmaceutically acceptable carrier and any needed preservatives or buffers as may be required. Ophthalmic formulation, ear drops, eye drops are also contemplated as being within the scope of this invention.

The ointments, pastes, creams and gels may contain, in addition to an active compound of this invention, excipients such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

Powders and sprays can contain, in addition to the compounds of this invention, excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants such as chlorofluorohydrocarbons.

Transdermal patches have the added advantage of providing controlled delivery of a compound to the body. Such dosage forms can be made by dissolving or dispensing the compound in the proper medium. Absorption enhancers can also be used to increase the flux of the compound across the skin. The rate can be controlled by either providing a rate controlling membrane or by dispersing the compound in a polymer matrix or gel.

ENCAPSULATION

In a preferred embodiment, inventive compositions comprising live microorganisms are provided in association with an encapsulation device (see, for example, U.S.S.N. 60/169,330 entitled "Controlled Delivery of Antigens" filed Dec. 6, 1999, incorporated by reference herewith). Preferred encapsulation devices are biocompatible, are stable inside the body so that microorganisms are not released until after the encapsulation device reaches its intended destination (e.g. mucosal lining of the gut, endocytosis by antigen-presenting cells (APC)). For example, preferred systems of encapsulation are stable at physiological pH and degrade at acidic pH levels comparable to those found in the digestive tract or endosomes of APCs. Particularly preferred encapsulation compositions include but are not limited to ones containing liposomes, polylactide-co-glycolide (PLGA), chitosan, synthetic biodegradable polymers, environmentally responsive hydrogels, and gelatin PLGA nanoparticles. Inventive compositions may be encapsulated in combination with one or more adjuvants, targeting entities, or other agents including, for example, pharmaceutical carriers, diluents, excipients, oils, etc. Alternatively or additionally the encapsulation device itself may be associated with a targeting entity and/or an adjuvant.

Methods of encapsulating live cells are known and may also be used in accordance with the present invention for delivering antigen-secreting microorganisms to individuals. The following references are provided as examples of encapsulation of live cells. However, any method of encapsulating live cells may be used in the present invention. US Patent 5,084,350; US Patent 4,680,174; and US Patent 4,352,883 (all of which are incorporated herein by reference) describe the encapsulation of a prokaryotic or eukaryotic cell or cell culture in microcapsules. Briefly, US Patent 5,084,350; 4,680,174; and 4,352,883 disclose that a tissue sample, cell, or cell culture to be encapsulated is first prepared in finely divided form in accordance with well-known techniques and suspended in an aqueous medium suitable for maintenance and for supporting the ongoing metabolic processes of the particular cells involved. Media suitable for this purpose generally are available commercially. Thereafter, a water-soluble substance which is physiologically compatible with the cells and which can be rendered water-insoluble to form a shape-retaining coherent spheroidal mass or other shape is added to the medium. The solution is then formed into droplets containing cells together with their maintenance or growth medium and is immediately rendered water-insoluble and gelled to form shape-retaining, typically spheroidal coherent masses.

The material used to induce gelation of the culture medium may be any non-toxic water-soluble material which, by a change in the surrounding temperature, pH, ionic environment, or concentration, can be converted to shape-retaining masses. Preferably, the material also is one which comprises plural, easily ionized groups, e.g., carboxyl or amino groups, which can react by salt formation with polymers containing plural groups which ionize to form species of the opposite charge. Use of this type of material enables the deposition of a membrane of a selected porosity range without damage to the labile cells. The presently preferred materials for forming the gelled masses are water-soluble natural or synthetic polysaccharides. Many such commercially available materials are typically extracted from vegetable matter and are often used as additives in various foods. Sodium alginate is the presently preferred water-soluble polysaccharide. Other usable materials include acidic fractions of guar gum, gum arabic, carrageenan, pectin, tragacanth gum or xanthan gums. These materials may be gelled when multivalent ions are exchanged for the acidic hydrogen or alkali metal ion normally associated with the carboxyl groups.

USES

The compositions of the present invention may be employed to treat or prevent allergic reactions in a subject. Subjects are animal and human patients in need of treatment for allergies. Preferably, the animal is a domesticated mammal (e.g., a dog, a cat, a horse, a sheep, a pig, a goat, a cow, etc). Animals also include laboratory animals such as mice, rats, hamsters, monkeys, and rabbits. Any individual who suffers from allergy, or who is susceptible to allergy, may be treated. It will be appreciated that an individual can be considered susceptible to allergy without having suffered an allergic reaction to the particular antigen in question. For example, if the individual has suffered an allergic reaction to a related antigen (e.g., one from the same source or one for which shared allergies are common), that individual will be considered susceptible to allergy to the relevant antigen. Similarly, if members of an individual's family are allergic to a particular antigen, the individual may be considered to be susceptible to allergy to that antigen. More preferably, any individual who is susceptible to anaphylactic shock upon exposure to food allergens, venom allergens or rubber allergens may be treated according to the present invention.

The compositions of the present invention may be formulated for delivery by any route. Preferably, the compositions are formulated for injection, ingestion, or inhalation.

Modifications and variations of the methods and compositions described herein are intended to be within the scope of the following claims.

Other Embodiments

Those of ordinary skill in the art will readily appreciate that the foregoing represents merely certain preferred embodiments of the invention. Various changes and modifications to the procedures and compositions described above can be made without departing from the spirit or scope of the present invention, as set forth in the following claims.

Examples

Material and Methods

For general methods used to express proteins in microorganisms see Ausubel et al. (supra) and Sambrook et al. (supra) both of which are incorporated herein by reference. In addition, expression vectors for use in the present invention are widely available from commercial sources (see for example, Clontech, Palo Alto, CA; Invitrogen, Carlsbad, CA; Promega Corporation, Madison, WI; New England Biolabs, Beverly, MA).

The following experiments describe the encapsulation of allergens in bacteria for use as a delivery vehicle and/or adjuvant in immunotherapy in accordance with the teachings of the present invention. Recombinant peanut allergen proteins (Ara h 1, Ara h 2, and Ara h 3; Burks et al. *J Allergy Clin Immunol.* 88(2):172-9, 1991; Burks et al. *J Allergy Clin Immunol.* 90(6 Pt 1):962-9, 1992; Rabjohn et al. *J Clin Invest.* 103(4):535-42, 1999; incorporated herein by reference) were produced in *E. coli* BL21 cells by transforming the bacterial cells with cDNA clones encoding the proteins (see Appendix B; sequences cloned into pET24, Novagen, Madison, WI). The transformed cells were then injected into C3H/HEJ mice to determine if the allergen-expressing *E. coli* elicited an immune response.

Example 1. Methods of killing allergen-producing *E. coli*

Several methods of killing allergen-producing *E. coli* were tested. Preferably, the method of killing bacteria does not denature or proteolyze the recombinant allergen(s) produced by the bacteria. As non-limiting examples, *E. coli* were killed by heat (at temperatures ranging from 37 °C to 95 °C), by using ethanol (0.1% to 10%), and by using solutions containing iodine (0.1% to 10%). Survival was determined by plating 100 µl of cells onto the appropriate agar plates, and subsequently counting the resulting colonies. The most reproducible method was heat killing. Therefore, the preferred method of killing allergen-producing *E. coli* is to incubate the cells at 60 °C for 20 minutes which results in 100% death (i.e. no colonies formed; see Figure #).

Example 2. Growth of Bacteria.

The following protocol was developed for the preparation of allergen-producing *E. coli* cells for inoculation of mice.

DAY 1

Five milliliters (ml) of liquid cultures of LB (Luria-Bertani broth) containing kanamycin (30 micrograms/ml per each cell line used) were prepared in 50 ml sterile tubes or flasks. Cultures were inoculated with approximately 10 microliters from a frozen stock of the desired bacterial cell line containing the desired expression vectors. The inoculated cultures were incubated with shaking overnight at 37 °C.

DAY 2

The following morning, 100 ml of liquid LB (500 ml Erlenmeyer flask) containing kanamycin (30 micrograms/ml) were inoculated using a 1 ml aliquot from the 5 ml culture grown from the previous day. (The remaining 4 mls of culture were frozen. Optionally, the remaining 4 milliliters of culture can be stored at 4 °C for several weeks for inoculating subsequent cultures) The inoculated cultures were incubate with shaking at 37 °C until the optical density of the solution measured at 600 nM (OD_{600}) reached approximately 0.6 to 0.9.

DAY 3

To induce production of recombinant proteins, the cultures from the previous day were induced by adding isopropyl-beta-D-thiogalactopyranoside (IPTG; Sigma-Aldrich, St. Louis, MO) from a 1 M stock to a final concentration of 1 mM (100 microliters of 1 M IPTG per 100 mls of culture) when the OD_{600} of the culture reached approximately 0.6-0.9. The induced cultures were incubated overnight.

DAY 4

1.4 ml of culture from the previous day were aliquoted into each of five 1.5 ml microfuge tubes for each culture and heat killed at 60°C in a water bath for 20 minutes. The tubes were centrifuged at 16,000 x g for 5 minutes at room temperature and the supernatant discarded. The pellets were washed with 1X phosphate buffer saline (PBS) and centrifuged at 16,000 x g for 5 minutes at room temperature. Again, the supernatant was discarded and the pellets were resuspended in 250 microliters of 1X PBS. The resuspended pellets from the same original samples were combined. The OD_{600} were determined for each sample and diluted to the desired OD_{600} using 1X PBS.

Example 3. Production and release of allergen.

Release of allergen by heat-killed bacteria

In order to determine if the cells remained intact after heat-killing we measured the amount of allergen released into the media. A dot-blot assay was developed that utilized as controls, purified recombinant allergens applied to a filter at known concentrations and serum IgE from peanut sensitive patients. The assay detected and quantified the amount of allergen present in 100 microliters of supernatant after pelleting heat-killed bacteria. The level of allergen released varied and was dependent on the expression vector and protein tested. In general, more Ara h 2 was released than Ara h 1 and Ara h 3 (Ara h 2>>Ara h 1>Ara h 3).

Production of allergen.

In order to measure amounts of allergen in *E. coli,* we developed an immunoblot assay that utilizes a six histidine tag (HIS tag) that is present on all of our purified recombinant allergens and a HIS tag antibody to build a standard curve that could then be used to estimate amounts of allergen produced. The amount of allergen produced on a per cell basis varied depending on which clone was tested. In general, more Ara h 3 was produced than Ara h 2 and Ara h 1 (Ara h 3>Ara h 2>>Ara h1).

Our best estimates for amounts of allergen delivered in 100 μl of a 2.0 O.D. inoculum of *E. coli* varies from about 1 μg of Ara h 1 to about 20 μg of Ara h 3.

Figure 2 is an example of a standard curve generated for Ara h 2. The optical density (O.D.) of the HIS-tagged Ara h 2 allergen is then determined from an immunoblot where different concentrations of *E. coli* extract has been electrophoresed on SDS-PAGE gels. The allergen O.D. is then used to estimate the amount of protein produced by that extract.

Example 4. Immune response of mice.

The following protocol was utilized to determine the immune response of mice injected with allergen-producing bacteria. Blood was collected from the tail vein of each mouse used before the first injection. Enough blood was collected for antibody ELISA for each allergen and *E. coli* proteins. On Day Zero each mouse was injected with 100 microliters of the killed *E. coli* samples subcutaneously in the left hind flank. The mice were injected for the second time on Day 14 using the same procedure as Day Zero. On Day 21, a second blood sample was collected from each mouse. Blood samples at Day 0 and Day 21 were assayed for IgG1 and IgG2a antibodies to either Ara h 1, Ara h 2, or Ara h 3 by an ELISA assay.

Mice injected with *E. coli* producing Ara h 1 did not give detectable levels of any immunoglobulin to the Ara h 1 allergen and therefore, that data are not shown. Without limitation to theory, we speculate that this may be due to the relatively small amounts of Ara h 1 produced by these cells (see previous discussion). Mice injected with *E. coli* producing Ara h 2 contained relatively high levels of IgG1 and IgG2a. Again, without limitation to the cause, we speculated that this may be due to the amount of Ara h 2 released from these cells (see discussion above). Mice injected with *E. coli* producing Ara h 3 contained relatively high levels of IgG2a (indicative of a Th1-type response) and elicited relatively low levels of IgG1 (indicative of a Th2-type response.

Interpretation of results

The present data should be cautiously interpreted. The data in the Figures only represent O.D. levels and do not represent absolute amounts of immunoglobulin. Therefore comparisons between groups should take into consideration the data presented as O.D. However, the general trend suggests that for example, more mice exhibited an IgG2a response to Ara h 3 than mice that exhibit an IgG1 response to Ara h 3.

Appendix A

ALLERGEN SOURCE	SYSTEMATIC AND ORIGINAL NAMES	MW KDA	SEQ	ACCESSION NO. OR REFERENCES
WEED POLLENS				
Asterales				
Ambrosia artemisiifolia (short ragweed)	Amb a 1; antigen E	38	C	8,20
	Amb a 2; antigen K	38	C	8,21
	Amb a 3; Ra3	11	C	22
	Amb a 5; Ra5	5	C	11,23
	Amb a 6; Ra6	10	C	24,25
	Amb a 7; Ra7	12	P	26
	Amb a ?	11	C	27
Ambrosia trifida (giant ragweed)	Amb t 5; Ra5G	4.4	C	9,10,28
Artemisia vulgaris (mugwort)	Art v 1	27-29	C	28A
	Art v 2	35	P	29
Helianthus annuus (sunflower)	Hel a 1	34	-	29a
	Hel a 2; profilin	15.7	C	Y15210
Mercurialis annua	Mer a 1; profilin	14-15	C	Y13271
GRASS POLLENS				
Poales				
Cynodon dactylon (Bermuda grass)	Cyn d 1	32	C	30,S83343
	Cyn d 7		C	31,X91256
	Cyn d 12; profilin	14	C	31a,Y08390
Dactylis glomerata (orchard grass)	Dac g 1; AgDg1	32	P	32
	Dac g 2	11	C	33,S45354
	Dac g 3		C	33a,U25343
	Dac g 5	31	P	34
Holcus lanatus (velvet grass)	Hol l 1		C	Z27084,Z68893
Lolium perenne (rye grass)	Lol p 1; group I	27	C	35,36
	Lol p 2; group II	11	C	37,37a,X73363
	Lol p 3; group III	11	C	38
	Lol p 5; Lol p IX, Lol p Ib	31/35		34,39
	Lol p 11; trypsin inh. Related	16		39a
Phalaris aquatica (canary grass)	Pha a 1		C	40,S80654
Phleum pratense (timothy grass)	Phl p 1	27	C	X78813
	Phl p 2		C	41,X75925
	Phl p 4		P	41A
	Phl p 5; Ag25	32	C	42
	Phl p 6		C	43,Z27082
	Phl p 12; profilin		C	44,X77583
	Phl p 13; polygalacturonase	55-60	C	AJ238848
Poa pratensis (Kentucky blue grass)	Poa p 1; group I	33	P	46
	Poa p 5	31/34	C	34,47

Sorghum halepense (Johnson grass)	Sor h 1		C	48
TREE POLLENS				
Fagales				
Alnus glutinosa (alder)	Aln g 1	17	C	S50892
Betula verrucosa (birch)	Bet v 1	17	C	see list of isoallergens
				M65179
	Bet v 2; profilin	15	C	X79267
	Bet v 3	8	C	X87153/S54819
	Bet v 4		C	AF135127
	Bet v 5; isoflavone reductase homologue	33.5	C	
	Bet v 7; cyclophilin	18	C	P P81531
Carpinus betulus (hornbeam)	Car b 1	17	C	51
Castanea sativa (chestnut)	Cas s 1; Bet v 1 homologue	22	P	52
	Cas s5; chitinase			
Corylus avelana (hazel)	Cor a 1	17	C	53
Quercus alba (white oak)	Que a 1	17	P	54
Cryptomeria japonica (sugi)	Cry j 1	41-45	C	55,56
	Cry j 2		C	57,D29772
Juniperus ashei (mountain cedar)	Jun a 1	43	P	P81294
	Jun a 3	30	P	P81295
Juniperus oxycedrus (prickly juniper)	Jun o 2; calmodulin-like	29	C	AF031471
Juniperus sabinoides (mountain cedar)	Jun s 1	50	P	58
Juniperus virginiana (eastern red cedar)	Jun v 1	43	P	P81825
Oleales				
Fraxinus excelsior (ash)	Fra e 1	20	P	58A
Ligustrum vulgare (privet)	Lig v 1	20	P	58A
Olea europea (olive)	Ole e 1;	16	C	59,60
	Ole e 2; profilin	15-18	C	60A
	Ole e 3;	9.2		60B
	Ole e 4;	32	P	P80741
	Ole e 5; superoxide dismutase	16	P	P80740
	Ole e 6;	10	C	U86342
Syringa vulgaris (lilac)	Syr v 1	20	P	58A
MITES				
Acarus siro (mite)	Aca s 13; fatty acid-bind.prot.	14*	C	AJ006774

Blomia tropicalis (mite)	Blo t 5; Blo t 12; Bt11a Blo t 13; Bt6 fatty acid-binding prot		C C C	U59102 U27479 U58106
Dermatophagoides pteronyssinus (mite)	Der p 1; antigen P1 Der p 2; Der p 3; trypsin Der p 4; amylase Der p 5; Der p 6; chymotrypsin Der p 7; Der p 8; glutathione transferase Der p 9; collagenolytic serine prot. Der p 10; tropomyosin Der p 14; apolipophorin like p	25 14 28/30 60 14 25 22-28 36 	C C C C P C C P C C	61 62 63 64 65 66 67 67A 67B Y14906 Epton p.c.
Dermatophagoides microceras (mite)	Der m 1;	25	P	68
Dermatophagoides farinae (mite)	Der f 1; Der f 2; Der f 3; Der f 10; tropomyosin Der f 11; paramyosin Der f 14; Mag3, apolipophorin	25 14 30 98 	C C C C C C	69 70,71 63 72 72a D17686
Euroglyphus maynei (mite)	Eur m 14; apolipophorin	177	C	AF149827
Lepidoglyphus destructor (storage mite)	Lep d 2.0101; Lep d 2.0102;	15 15	C C	73,74,75 75
ANIMALS				
Bos domesticus (domestic cattle) (see also foods)	Bos d 2; Ag3,lipocalin Bos d 4; alpha-lactalbumin Bos d 5; beta-lactoglobulin Bos d 6; serum albumin Bos d 7; immunoglobulin Bos d 8; caseins	20 14.2 18.3 67 160 20-30	C C C C 	76,L42867 M18780 X14712 M73993 77 77
Canis familiaris (Canis domesticus (dog)	Can f 1; Can f 2; Can f ?; albumin	25 27 	C C C	78,79 78,79 S72946
Equus caballus (domestic horse)	Equ c 1; lipocalin Equ c 2; lipocali	25 18.5	C P	U70823 79A,79B
Felis domesticus (cat saliva)	Fel d 1; cat-1	38	C	15
Mus musculus (mouse urine)	Mus m 1; MUP	19	C	80,81
Rattus norvegius (rat urine)	Rat n 1	17	C	82,83
FUNGI				
Ascomycota				
Dothidiales				
Alternaria alternata	Alt a 1; Alt a 2; Alt a 3; heat shock protein	28 25 70	C C C	U82633 U87807,U87808 X78222,

	Alt a 6; ribosomal protein Alt a 7; YCP4 protein Alt a 10; aldehyde dehydrogenase Alt a 11; enolase Alt a 12;acid.ribosomal prot P1	11 22 53 45 11	C C C C C	U87806 X78225 X78227, P42041 U82437 X84216
Cladosporium herbarum	Cla h 1; Cla h 2; Cla h 3; aldehyde dehydrogenase Cla h 4; ribosomal protein Cla h 5; YCP4 protein Cla h 6; enolase Cla h 12;acid.ribosomal prot P1	13 23 53 11 22 46 11	 C C C C C	83a,83b 83a,83b X78228 X78223 X78224 X78226 X85180
Eurotiales				
	Asp fl 13; alkaline serine proteinase	34		84
Aspergillus Fumigatus	Asp f 1; Asp f 2; Asp f 3; peroxisomal protein Asp f 4; Asp f 5; metalloprotease Asp f 6; Mn superoxide dismutase Asp f 7; Asp f 8; ribosomal protein P2 Asp f 9; Asp f 10; aspartic protease Asp f 11; peptidyl-prolyl isom Asp f 12; heat shock prot. P70 Asp f 13; alkaline serine proteinase Asp f 15; Asp f 16; Asp f 17; Asp f 18; vacuolar serine Asp f ?; Asp f ?;	18 37 19 30 42 26.5 12 11 34 34 24 65 34 16 43 34 90 55 	C C C C C C C C C C C C C P P	83781,S39330 U56938 U20722 AJ001732 Z30424 U53561 AJ223315 AJ224333 AJ223327 X85092 84a U92465 84b AJ002026 g3643813 AJ224865 84c 85 86
Aspergillus niger	Asp n 14; beta-xylosidase Asp n 18; vacuolar serine proteinase Asp n ?;	105 34 85	C C C	AF108944 84b Z84377
Aspergillus oryzae	Asp o 2; TAKA-amylase A Asp o 13; alkaline serine proteinase	53 34	C C	D00434,M33218 X17561
Penicillium brevicompactum	Pen b 13; alkaline serine Proteinase	33		86a
Penicillium citrinum	Pen c 1; heat shock protein P70 Pen c 3; peroxisomal membrane protein Pen c 13; alkaline serine proteinase	70 33	C	U64207 86b 86a
Penicillium notatum	Pen n 1; N-acetyl glucosaminidase Pen n 13; alkaline serine proteinase Pen n 18; vacuolar serine proteinase	68 34 32		87 89 89
Penicillium oxalicum	Pen o 18; vacuolar serine proteinase	34		89

Onygenales				
Trichophyton rubrum	Tri r 2; Tri r 4; serine protease		C C	90 90
Trichophyton tonsurans	Tri t 1; Tri t 4; serine protease	30 83	P C	91 90
Saccharomycetales				
Candida albicans	Cand a 1	40	C	88
Candida boidinii	Cand b 2	20	C	J04984,J04985
Basidiomycota				
Basidiolelastomycetes				
Malassezia furfur	Mal f 1; Mal f 2; MF1 peroxisomal membrane protein Mal f 3; MF2 peroxisomal membrane protein Mal f 4, Mal f 5; Mal f 6; cyclophilin homologue	 21 20 35 18* 17*	 C C C C C	91a AB011804 AB011805 Takesako, p.c. AJ011955 AJ011956
Basidiomycetes				
Psilocybe cubensis	Psi c 1; Psi c 2; cyclophilin	16		91b
Coprinus comatus (shaggy cap)	Cop c 1; Cop c 2; Cop c 3; Cop c 5; Cop c 7;	11	C	AJ132235 Brander, p.c. Brander, p.c. Brander, p.c.
INSECTS				
Aedes aegyptii (mosquito)	Aed a 1; apyrase Aed a 2;	68 37	C C	L12389 M33157
Apis mellifera (honey bee)	Api m 1; phospholipase A2 Api m 2; hyaluronidase Api m 4; melittin Api m 6;	16 44 3 7-8	C C C P	92 93 94 Kettner,p.c.
Bombus pennsylvanicus (bumble bee)	Bom p 1; phospholipase Bom p 4; protease	16	P P	95 95
Blattella germanica (German cockroach)	Bla g 1; Bd90k Bla g 2; aspartic protease Bla g 4; calycin Bla g 5; glutathione transf. Bla g 6; troponin C	 36 21 22 27	C C C C C	96 97 98 98
Periplaneta americana (American cockroach)	Per a 1; Cr-PII Per a 3; Cr-PI Per a 7; tropomyosin	72-78 37	C C C	98A Y14854
Chironomus thummi thummi (midges)	Chi t 1-9; hemoglobin Chi t 1.01; component III Chi t 1.02; component IV Chi t 2.0101; component I Chi t 2.0102; component IA	16 16 16 16 16	C C C C C	99 P02229 P02230 P02221 P02221

	Chi t 3; component II-beta Chi t 4; component IIIA Chi t 5; component VI Chi t 6.01; component VIIA Chi t 6.02; component IX Chi t 7; component VIIB Chi t 8; component VIII Chi t 9; component X	16 16 16 16 16 16 16 16	C C C C C C C C	P02222 P02231 P02224 P02226 P02223 P02225 P02227 P02228
Dolichovespula maculata (white face hornet)	Dol m 1; phospholipase A1 Dol m 2; hyaluronidase Dol m 5; antigen 5	35 44 23	C C C	100 101 102,103
Dolichovespula arenaria (yellow hornet)	Dol a 5; antigen 5	23	C	104
Polistes annularies (wasp)	Pol a 1; phospholipase A1 Pol a 2; hyaluronidase Pol a 5; antigen 5	35 44 23	P P C	105 105 104
Polistes dominulus (Mediterranean paper wasp)	Pol d 1; Pol d 4; serine protease Pol d 5;	32-34	C	DR Hoffman DR Hoffman P81656
Polistes exclamans (wasp)	Pol e 1; phospholipase A1 Pol e 5; antigen 5	34 23	P C	107 104
Polistes fuscatus (wasp)	Pol f 5; antigen 5	23	C	106
Polistes metricus (wasp)	Pol m 5; antigen 5	23	P	106
Vespa crabo (European hornet)	Vesp c 1; phospholipase Vesp c 5.0101; antigen 5 Vesp c 5.0102; antigen 5	34 23 23	P C C	107 106 106
Vespa mandarina (giant asian hornet)	Vesp m 1.01; Vesp m 1.02; Vesp m 5;			DR Hoffman DR Hoffman P81657
Vespula flavopilosa (yellowjacket)	Ves f 5; antigen 5	23	C	106
Vespula germanica (yellowjacket)	Ves g 5; antigen 5	23	C	106
Vespula maculifrons (yellowjacket)	Ves m 1; phospholipase A1 Ves m 2; hyaluronidase Ves m 5; antigen 5	33.5 44 23	C P 23	108 109 104
Vespula pennsylvanica (yellowjacket)	Ves p 5; antigen 5	23	C	106
Vespula squamosa (yellowjacket)	Ves s 5; antigen 5	23	C	106
Vespula vidua (wasp)	Ves vi 5;	23	C	106
Vespula vulgaris (yellowjacket)	Ves v 1; phopholipase A1 Ves v 2; hyaluronidase Ves v 5; antigen 5	35 44 23	C P C	105A 105A 104
Myrmecia pilosula (Australian jumper	Myr p 1,		C	X70256

ant)	Myr p 2;		C	S81785
Solenopsis geminata (tropical fire ant)	Sol g 2; Sol g 4			DR Hoffman DR Hoffman
Solenopsis invicta (fire ant)	Sol i 2; Sol i 3; Soli 4;	13 24 13	C C C	110,111 110 110
Solenopsis saevissima (brazilian fire ant)	Sols 2;			DR Hoffman
FOODS				
Gadus callarias (cod)	Gad c 1; allergen M	12	C	112,113
Salmo salar (Atlantic salmon)	Sals 1; parvalbumin	12	C	X97824,X97825
Bos domesticus (domestic cattle)	Bos d 4; alpha-lactalbumin Bos d 5; beta-lactoglobulin Bos d 6; serum albumin Bos d 7; immunoglobulin Bos d 8; caseins	14.2 18.3 67 160 20-30	C C C	M18780 X14712 M73993 77 77
Gallus domesticus (chicken)	Gal d 1; ovomucoid Gald 2; ovalbumin Gald 3; conalbumin (Ag22) Gald 4; lysozyme Gal d 5; serum albumin	28 44 78 14 69	C C C C C	114,115 114,115 114,115 114,115 X60688
Metapenaeus ensis (shrimp)	Met e 1; tropomyosin		C	U08008
Penaeus aztecus (shrimp)	Pen a 1; tropomyosin	36	P	116
Penaeus indicus (shrimp)	Pen i 1; tropomyosin	34	C	117
Todarodes pacificus (squid)	Tod p 1; tropomyosin	38	P	117A
Haliotis Midae (abalone)	Hal m 1	49	-	117B
Apium graveolens (celery)	Api g 1; Bet v 1 homologue Api g 4; profilin Api g 5;	16* 55/58	C P	Z48967 AF129423 P81943
Brassica juncea (oriental mustard)	Bra j 1; 2S albumin	14	C	118
Brassica rapa (turnip)	Bra r 2; prohevein-like protein	25	?	P81729
Hordeum vulgare (barley)	Hor v 1; BMAI-1	15	C	119
Zea mays (maize, corn)	Zea m 14; lipid transfer prot.	9	P	P19656
Corylus avellana (hazelnut)	Cor a 1.0401; Bet v 1 homologue	17	C	AF136945
Malus domestica (apple)	Mal d 1; Bet v 1 homologue Mal d 3; lipid transfer protein	 9	C C	X83672 Pastorello
Pyrus communis	Pyr c 1; Bet v 1 homologue	18	C	AF05730

(pear)	Pyr c 4; profilin Pyr c 5; isoflavone reductase homologue	14 33.5	C C	AF129424 AF071477
Oryza sativa (rice)	Ory s 1;		C	U31771
Persea americana (avocado)	Pers a 1; endochitinase	32	C	Z78202
Prunus armeniaca (apricot)	Pru ar 1; Bet v 1 homologue Pru ar 3; lipid transfer protein	 9	C P	U93165
Prunus avium (sweet cherry)	Pru av 1; Bet v 1 homologue Pru av 2; thaumatin homologue Pru av 4; profilin	 15	C C C	U66076 U32440 AF129425
Prunus persica (peach)	Pru p 3;lipid transfer protein	10	P	P81402
Sinapis alba (yellow mustard)	Sin a 1; 2S albumin	14	C	120
Glycine max (soybean)	Gly m 1.0101; HPS Gly m 1.0102; HPS Gly m 2 Gly m 3; profilin	7.5 7 8 14	P P P C	121 121 A57106 AJ223982
Arachis hypogaea (peanut)	Ara h 1; vicilin Ara h 2; conglutin Ara h 3; glycinin Ara h 4; glycinin Ara h 5; profilin Ara h 6; conglutin homolog Ara h 7; conglutin homolog	63.5 17 14 37 15 15 15	C C C C C C C	L34402 L77197 AF093541 AF086821 AF059616 AF092846 AF091737
Actinidia chinensis (kiwi)	Act c 1; cysteine protease	30	P	P00785
Solanum tuberosum (potato)	Sol t 1; patatin	43	P	P15476
Bertholletia excelsa (Brazil nut)	Ber e 1; 2S albumin	9	C	P04403,M17146
Juglans regia (English walnut)	Jug r 1; 2S albumin Jug r 2; vicilin	44	C C	U66866 AF066055
Ricinus communis (Castor bean)	Ric c 1; 2S albumin		C	P01089
OTHERS				
Anisakis simplex (nematode)	Ani s 1 Ani s 2; paramyosin	24 97	P C	A59069 AF173004
Ascaris suum (worm)	Asc s 1;	10	P	122
Aedes aegyptii (mosquito)	Aed a 1; apyrase Aed a 2;	68 37	C C	L12389 M33157
Hevea brasiliensis (rubber)	Hev b 1; elongation factor Hev b 2; (1,3-glucanase Hev b 2; (1,3-glucanase Hev b 3 Hev b 4; component of microhelix protein complex	58 58 34/36 24 100/110/115	P P C P P	123,124 123,124 125 126,127 128

	Hev b 5	16	C	U42640
	Hev b 6.01 hevein precursor	20	C	M36986/p02877
	Hev b 6.02 hevein	5	C	M36986/p02877
	Hev b 6.03 C-terminal fragment	14	C	M36986/p02877
	Hev b 7; patatin homologue	46	C	U80598
	Hev b 8; profilin	14	C	Y15042
	Hev b 9; enolase	51	C	AJ132580/AJ132581
	Hev b 10; Mn-superoxide dismut	26	C	AJ249148
Ctenocephalides felis felis (cat flea)	Cte f 1;	-	-	-
	Cte f 2; M1b	27	C	AF231352
Homo sapiens (human autoallergens)	Hom s 1;	73*	C	Y14314
	Hom s 2;	10.3*	C	X80909
	Hom s 3;	20.1*	C	X89985
	Hom s 4;	36*	C	Y17711
	Hom s 5;	42.6*	C	P02538

1. Marsh, D.G., and L.R. Freidhoff. 1992. ALBE, an allergen database. IUIS, Baltimore, MD, Edition 1.0.
2. Marsh, D. G., L. Goodfriend, T. P. King, H. Lowenstein, and T. A. E. Platts-Mills. 1986. Allergen nomenclature. Bull WHO 64:767-770.
3. King, T.P., P.S. Norman, and J.T. Cornell. 1964. Isolation and characterization of allergen from ragweed pollen. II. Biochemistry 3:458-468.
4. Lowenstein, H. 1980. Timothy pollen allergens. Allergy 35:188-191.
5. Aukrust, L. 1980. Purification of allergens in Cladosporium herbarum. Allergy 35:206-207.
6. Demerec, M., E. A. Adelberg, A. J. Clark, and P. E. Hartman. 1966. A proposal for a uniform nomenclature in bacterial genetics. Genetics 54:61-75.
7. Bodmer, J. G., E. D. Albert, W. F. Bodmer, B. Dupont, H. A. Erlich, B. Mach, S. G. E. Marsh, W. R. Mayr, P. Parham, T. Sasuki, G. M. Th. Schreuder, J. L. Strominger, A. Svejgaard, and P. I. Terasaki. 1991. Nomenclature for factors of the HLA system, 1990. Immunogenetics 33:301-309.
8. Griffith, I.J., J. Pollock, D.G. Klapper, B.L. Rogers, and A.K. Nault. 1991. Sequence polymorphism of Amb a I and Amb a II, the major allergens in Ambrosia artemisiifolia (short ragweed). Int. Arch. Allergy Appl. Immunol. 96:296-304.
9. Roebber, M., D. G. Klapper, L. Goodfriend, W. B. Bias, S. H. Hsu, and D. G. Marsh. 1985. Immunochemical and genetic studies of Amb t V (Ra5G), an Ra5 homologue from giant ragweed pollen. J. Immunol. 134:3062-3069.
10. Metzler, W. J., K. Valentine, M. Roebber, M. Friedrichs, D. G. Marsh, and L. Mueller. 1992. Solution structures of ragweed allergen Amb t V. Biochemistry 31:5117-5127.
11. Metzler, W. J., K. Valentine, M. Roebber, D. G. Marsh, and L. Mueller. 1992. Proton resonance assignments and three-dimensional solution structure of the ragweed allergen Amb a V by nuclear magnetic resonance spectroscopy. Biochemistry 31:8697-8705.
12. Goodfriend, L., A.M. Choudhury, J. Del Carpio, and T.P. King. 1979. Cytochromes C: New ragweed pollen allergens. Fed. Proc. 38:1415.
13. Ekramoddoullah, A. K. M., F. T. Kisil, and A. H. Sehon. 1982. Allergenic cross reactivity of cytochrome c from Kentucky bluegrass and perennial ryegrass pollens. Mol. Immunol. 19:1527-1534.
14. Ansari, A. A., E. A. Killoran, and D. G. Marsh. 1987. An investigation of human response to perennial ryegrass (Lolium perenne) pollen cytochrome c (Lol p X). J. Allergy Clin. Immunol. 80:229-235.
15. Morgenstern, J.P., I.J. Griffith, A.W. Brauer, B.L. Rogers, J.F. Bond, M.D. Chapman, and M. Kuo. 1991. Amino acid sequence of Fel d I, the major allergen of the domestic cat: protein sequence analysis and cDNA cloning. Proc. Natl. Acad. Sci. USA 88:9690-9694.
16. Griffith, I.J., S. Craig, J. Pollock, X. Yu, J.P. Morgenstern, and B.L.Rogers. 1992. Expression and genomic structure of the genes encoding FdI, the major allergen from the domestic cat. Gene 113:263-268.
17. Weber, A., L. Marz, and F. Altmann. 1986. Characteristics of the asparagine-linked oligosaccharide from honey-bee venom phospholipase A2. Comp. Biochem. Physiol. 83B:321-324.
18. Weber, A., H. Schroder, K. Thalberg, and L. Marz. 1987. Specific interaction of IgE antibodies with a carbohydrate epitope of honey bee venom phospholipase A2. Allergy 42:464-470.
19. Stanworth, D. R., K. J. Dorrington, T. E. Hugli, K. Reid, and M. W. Turner. 1990. Nomenclature for synthetic peptides representative of immunoglobulin chain sequences. Bulletin WHO 68:109-111.
20. Rafnar, T., I. J. Griffith, M. C. Kuo, J. F. Bond, B. L. Rogers, and D.G. Klapper. 1991. Cloning of Amb a I (Antigen E), the major allergen family of short ragweed pollen. J. Biol. Chem. 266: 1229-1236.
21. Rogers, B.L., J.P. Morgenstern, I.J. Griffith, X.B. Yu, C.M. Counsell, A.W. Brauer, T.P. King, R.D. Garman, and M.C. Kuo. 1991. Complete sequence of the allergen Amb a II: recombinant expression and reactivity with T cells from ragweed allergic patients. J. Immunol. 147:2547-2552.

22. Klapper, D.G., L. Goodfriend, and J.D. Capra. 1980. Amino acid sequence of ragweed allergen Ra3. Biochemistry 19:5729-5734.

23. Ghosh, B., M.P. Perry, T. Rafnar, and D.G. Marsh. 1993. Cloning and expression of immunologically active recombinant Amb a V allergen of short ragweed (Ambrosia artemisiifolia) pollen. J. Immunol. 150:5391-5399.

24. Roebber, M., R. Hussain, D. G. Klapper, and D. G. Marsh. 1983. Isolation and properties of a new short ragweed pollen allergen, Ra6. J. Immunol. 131:706-711.

25. Lubahn, B., and D.G. Klapper. 1993. Cloning and characterization of ragweed allergen Amb a VI (abst). J. Allergy Clin. Immunol. 91:338.

26. Roebber, M., and D.G. Marsh. 1991. Isolation and characterization of allergen Amb a VII from short ragweed pollen. J. Allergy Clin. Immunol. 87:324.

27. Rogers, B.L., J. Pollock, D.G. Klapper, and I.J. Griffith. 1993. Cloning, complete sequence, and recombinant expression of a novel allergen from short ragweed pollen (abst). J. Allergy Clin. Immunol. 91:339.

28. Goodfriend, L., A.M. Choudhury, D.G. Klapper, K.M. Coulter, G. Dorval, J. DelCarpio, and C.K. Osterland. 1985. Ra5G, a homologue of Ra5 in giant ragweed pollen: isolation, HLA-DR-associated activity and amino acid sequence. Mol. Immunol. 22:899-906.

28A. Breitenbach M, pers. comm.

29. Nilsen, B. M., K. Sletten, M. O'Neill, B. Smestead Paulsen, and H. van Halbeek. 1991. Structural analysis of the glycoprotein allergen Art v II from pollen of mugwort (Artemesia vulgaris). J. Biol. Chem. 266:2660-2668.

29A Jimenez A, Moreno C, Martinez J, Martinez A, Bartolome B, Guerra F, Palacios R 1994. Sensitization to sunflower pollen: only an occupational allergy? Int Arch Allergy Immunol 105:297-307.

30. Smith,P.M., Suphioglu,C., Griffith,I.J., Theriault,K., Knox,R.B. and Singh,M.B. 1996.
Cloning and expression in yeast Pichia pastoris of a biologically active form of Cyn d 1, the major allergen of Bermuda grass pollen. J. Allergy Clin. Immunol. 98:331-343.

31. Suphioglu,C., Ferreira,F. and Knox,R.B. 1997. Molecular cloning and immunological characterisation of Cyn d 7, a novel calcium-binding allergen from Bermuda grass pollen. FEBS Lett. 402:167-172.

31a. Asturias JA, Arilla MC, Gomez-Bayon N, Martinez J, Martinez A, and Palacios R. 1997. Cloning and high level expression of Cynodon dactylon (Bermuda grass) pollen profilin (Cyn d 12) in Escherichia coli: purification and characterization of the allergen. Clin Exp Allergy 27:1307-1313.

32. Mecheri, S., G. Peltre, and B. David. 1985. Purification and characterization of a major allergen from Dactylis glomerata pollen: The Ag Dg 1. Int. Arch. Allergy Appl. Immunol. 78:283-289.

33. Roberts, A.M., L.J. Bevan, P.S. Flora, I. Jepson, and M.R. Walker. 1993. Nucleotide sequence of cDNA encoding the Group II allergen of Cocksfoot/Orchard grass (Dactylis glomerata), Dac g II. Allergy 48:615-623.

33a. Guerin-Marchand,C., Senechal,H., Bouin,A.P., Leduc-Brodard,V., Taudou,G., Weyer,A., Peltre,G. and David,B. 1996. Cloning, sequencing and immunological characterization of Dac g 3, a major allergen from Dactylis glomerata pollen. Mol. Immunol. 33:797-806.

34. Klysner, S., K. Welinder, H. Lowenstein, and F. Matthiesen. 1992. Group V allergens in grass pollen IV. Similarities in amino acid compositions and amino terminal sequences of the group V allergens from Lolium perenne, Poa pratensis and Dactylis glomerata. Clin. Exp. Allergy 22: 491-497.

35. Perez, M., G. Y. Ishioka, L. E. Walker, and R. W. Chesnut. 1990. cDNA cloning and immunological characterization of the rye grass allergen Lol p I. J. Biol. Chem. 265:16210-16215.

36. Griffith, I. J., P. M. Smith, J. Pollock, P. Theerakulpisut, A. Avjioglu, S. Davies, T. Hough, M. B. Singh, R. J. Simpson, L. D. Ward, and R. B. Knox. 1991. Cloning and sequencing of Lol p I, the major allergenic protein of rye-grass pollen. FEBS Letters 279:210-215.

37. Ansari, A. A., P. Shenbagamurthi, and D.G. Marsh. 1989. Complete amino acid sequence of a Lolium perenne (perennial rye grass) pollen allergen, Lol p II. J. Biol. Chem. 264:11181-11185.

37a. Sidoli,A., Tamborini,E., Giuntini,I., Levi,S., Volonte,G., Paini,C., De Lalla,C., Siccardi,A.G., Baralle,F.E., Galliani,S. and Arosio,P. 1993. Cloning, expression, and immunological characterization of recombinant Lolium perenne allergen Lol p II. J. Biol. Chem. 268:21819-21825.

38. Ansari, A. A., P. Shenbagamurthi, and D. G. Marsh. 1989. Complete primary structure of a Lolium perenne (perennial rye grass) pollen allergen, Lol p III: Comparison with known Lol p I and II sequences. Biochemistry 28:8665-8670.

39. Singh, M. B., T. Hough, P. Theerakulpisut, A. Avjioglu, S. Davies, P. M. Smith, P. Taylor, R. J. Simpson, L. D. Ward, J. McCluskey, R. Puy, and R.B. Knox. 1991. Isolation of cDNA encoding a newly identified major allergenic protein of rye-grass pollen: Intracellular targeting to the amyloplost. Proc. Natl. Acad. Sci. 88:1384-1388.

39a. van Ree R, Hoffman DR, van Dijk W, Brodard V, Mahieu K, Koeleman CA, Grande M, van Leeuwen WA, Aalberse RC. 1995. Lol p XI, a new major grass pollen allergen, is a member of a family of soybean trypsin inhibitor-related proteins. J Allergy Clin Immunol 95:970-978.

40. Suphioglu,C. and Singh,M.B. 1995. Cloning, sequencing and expression in Escherichia coli of Pha a 1 and four isoforms of Pha a 5, the major allergens of canary grass pollen. Clin. Exp. Allergy 25:853-865.

41. Dolecek,C., Vrtala,S., Laffer,S., Steinberger,P., Kraft,D., Scheiner,O. and Valenta,R. 1993. Molecular characterization of Phl p II, a major timothy grass (Phleum pratense) pollen allergen. FEBS Lett. 335:299-304.

41A. Fischer S, Grote M, Fahlbusch B, Muller WD, Kraft D, Valenta R. 1996. Characterization of Phl p 4, a major timothy grass (Phleum pratense) pollen allergen. J Allergy Clin Immunol 98:189-198.

42. Matthiesen, F., and H. Lowenstein. 1991. Group V allergens in grass pollens. I. Purification and characterization of the group V allergen from Phleum pratense pollen, Phl p V. Clin. Exp. Allergy 21:297-307.

43. Petersen,A., Bufe,A., Schramm,G., Schlaak,M. and Becker,W.M. 1995. Characterization of the allergen group VI in timothy grass pollen (Phl p 6). II. cDNA cloning of Phl p 6 and structural comparison to grass group V. Int. Arch. Allergy Immunol. 108:55-59.

44. Valenta,R., Ball,T., Vrtala,S., Duchene,M., Kraft,D. and Scheiner,O. 1994. cDNA cloning and expression of timothy grass (Phleum pratense) pollen profilin in Escherichia coli: comparison with birch pollen profilin. Biochem. Biophys. Res. Commun. 199:106-118.

46. Esch, R. E., and D. G. Klapper. 1989. Isolation and characterization of a major cross-reactive grass group I allergenic determinant. Mol. Immunol. 26:557-561.
47. Olsen, E., L. Zhang, R. D. Hill, F. T. Kisil, A. H. Sehon, and S. Mohapatra. 1991. Identification and characterization of the Poa p IX group of basic allergens of Kentucky bluegrass pollen. J. Immunol. 147:205-211.
48. Avjioglu, A., M. Singh, and R.B. Knox. 1993. Sequence analysis of Sor h I, the group I allergen of Johnson grass pollen and it comparison to rye-grass Lol p I (abst). J. Allergy Clin. Immunol. 91:340.
51. Larsen, J.N., P. Str°man, and H. Ipsen. 1992. PCR based cloning and sequencing of isogenes encoding the tree pollen major allergen Car b I from Carpinus betulus, hornbeam. Mol. Immunol. 29:703-711.
52. Kos T, Hoffmann-Sommergruber K, Ferreira F, Hirschwehr R, Ahorn H, Horak F, Jager S, Sperr W, Kraft D, Scheiner O. 1993. Purification, characterization and N-terminal amino acid sequence of a new major allergen from European chestnut pollen--Cas s 1. Biochem Biophys Res Commun 196:1086-92.
53. Breiteneder, H., F. Ferreira, K. Hoffman-Sommergruber, C. Ebner, M. Breitenbach, H. Rumpold, D. Kraft, and O. Scheiner. 1993. Four recombinant isoforms of Cor a I, the major allergen of hazel pollen. Europ. J. Biochem. 212:355-362.
54. Ipsen, H., and B.C. Hansen. 1991. The NH2-terminal amino acid sequence of the immunochemically partial identical major allergens of alder (Alnus glutinosa) Aln g I, birch (Betula verrucosa) Bet v I, hornbeam (Carpinus betulus) Car b I and oak (Quercus alba) Que a I pollens. Mol. Immunol. 28:1279-1288.
55. Taniai, M., S. Ando, M. Usui, M. Kurimoto, M. Sakaguchi, S. Inouye, and T. Matuhasi. 1988. N-terminal amino acid sequence of a major allergen of Japanese cedar pollen (Cry j I). FEBS Lett. 239:329-332.
56. Griffith, I.J., A. Lussier, R. Garman, R. Koury, H. Yeung, and J. Pollock. 1993. The cDNA cloning of Cry j I, the major allergen of Cryptomeria japonica (Japanese cedar) (abst). J. Allergy Clin. Immunol. 91:339.
57. Sakaguchi, M., S. Inouye, M. Taniai, S. Ando, M. Usui, and T. Matuhasi. 1990. Identification of the second major allergen of Japanese cedar pollen. Allergy 45:309-312.
58 Gross GN, Zimburean JM, Capra JD 1978. Isolation and partial characterization of the allergen in mountain cedar pollen. Scand J Immunol 8:437-41
58A Obispo TM, Melero JA, Carpizo JA, Carreira J, Lombardero M 1993. The main allergen of Olea europaea (Ole e I) is also present in other species of the oleaceae family. Clin Exp Allergy 23:311-316.
59. Cardaba, B., D. Hernandez, E. Martin, B. de Andres, V. del Pozo, S. Gallardo, J.C. Fernandez, R. Rodriguez, M. Villalba, P. Palomino, A. Basomba, and C. Lahoz. 1993. Antibody response to olive pollen antigens: association between HLA class II genes and IgE response to Ole e I (abst). J. Allergy Clin. Immunol. 91:338.
60. Villalba, M., E. Batanero, C. Lopez-Otin, L.M. Sanchez, R.I. Monsalve, M.A. Gonzalez de la Pena, C. Lahoz, and R. Rodriguez. 1993. Amino acid sequence of Ole e I, the major allergen from olive tree pollen (Olea europaea). Europ.J. Biochem. 216:863-869.
60A. Asturias JA, Arilla MC, Gomez-Bayon N, Martinez J, Martinez A, Palacios R 1997. Cloning and expression of the panallergen profilin and the major allergen (Ole e 1) from olive tree pollen. J Allergy Clin Immunol 100:365-372.
60B. Batanero E, Villalba M, Ledesma A Puente XS, Rodriguez R. 1996. Ole e 3, an olive-tree allergen, belongs to a widespread family of pollen proteins. Eur J Biochem 241: 772-778.
61. Chua, K. Y., G. A. Stewart, and W. R. Thomas. 1988. Sequence analysisof cDNA encoding for a major house dust mite allergen, Der p I. J. Exp. Med. 167:175-182.
62. Chua, K. Y., C. R. Doyle, R. J. Simpson, K. J. Turner, G. A. Stewart, and W. R. Thomas. 1990. Isolation of cDNA coding for the major mite allergen Der p II by IgE plaque immunoassay. Int. Arch. Allergy Appl. Immunol. 91:118-123.
63. Smith WA, Thomas WR. 1996. Comparative analysis of the genes encoding group 3 allergens from Dermatophagoides pteronyssinus and Dermatophagoides farinae. Int Arch Allergy Immunol 109: 133-40.
64. Lake, F.R., L.D. Ward, R.J. Simpson, P.J. Thompson, and G.A. Stewart. 1991. House dust mite-derived amylase: Allergenicity and physicochemical characterisation. J. Allergy Clin. Immunol. 87:1035-1042.
65. Tovey, E. R., M. C. Johnson, A. L. Roche, G. S. Cobon, and B. A. Baldo. 1989. Cloning and sequencing of a cDNA expressing a recombinant house dust mite protein that binds human IgE and corresponds to an important low molecular weight allergen. J. Exp. Med. 170:1457-1462.
66. Yasueda, H., T. Shida, T. Ando, S. Sugiyama, and H. Yamakawa. 1991. Allergenic and proteolytic properties of fourth allergens from Dermatophagoides mites. In: "Dust Mite Allergens and Asthma. Report of the 2nd international workshop" A. Todt, Ed., UCB Institute of Allergy, Brussels, Belgium, pp. 63-64.
67. Shen, H.-D., K.-Y. Chua, K.-L. Lin, K.-H. Hsieh, and W.R. Thomas. 1993. Molecular cloning of a house dust mite allergen with common antibody binding specificities with multiple components in mite extracts. Clin. Exp. Allergy 23:934-40.
67A. O'Neil GM, Donovan GR, Baldo BA. 1994. Cloning and charaterisation of a major allergen of the house dust mite Dermatophagoides pteronyssinus, homologous with glutathione S-transferase. Biochim Biophys Acta,1219:521-528.
67B. King C, Simpson RJ, Moritz RL, Reed GE, Thompson PJ, Stewart GA. 1996. The isolation and characterization of a novel collagenolytic serine protease allergen (Der p 9) from the dust mite Dermatophagoides pteronyssinus. J Allergy Clin Immunol 98:739-47.
68. Lind P, Hansen OC, Horn N. 1988. The binding of mouse hybridoma and human IgE antibodies to the major fecal allergen, Der p I of D. pteronyssinus. J. Immunol. 140:4256-4262.
69. Dilworth, R. J., K. Y. Chua, and W. R. Thomas. 1991. Sequence analysis of cDNA coding for a mojor house dust allergn Der f I. Clin. Exp. Allergy 21:25-32.
70. Nishiyama, C., T. Yunki, T. Takai, Y. Okumura, and H. Okudaira. 1993. Determination of three disulfide bonds in a major house dust mite allergen, Der f II. Int. Arch. Allergy Immunol. 101:159-166.
71. Trudinger, M., K. Y. Chua, and W. R. Thomas. 1991. cDNA encoding the major dust mite allergen Der f II. Clin. Exp. Allergy 21:33-38.
72. Aki T, Kodama T, Fujikawa A, Miura K, Shigeta S, Wada T, Jyo T, Murooka Y, Oka S, Ono K. 1995. Immunochemical characteristion of recombinant and native tropomyosins as a new allergen from the house dust mite Dermatophagoides farinae. J Allergy Clin Immunol 96:74-83.
73. van Hage-Hamsten, M., T. Bergman, E. Johansson, B. Persson, H. Jornvall, B. Harfast, and S.G.O. Johansson. 1993. N-terminal amino acid sequence of major allergen of the mite lepidoglyphus destructor (abst). J. Allergy Clin. Immunol. 91:353.

74. Varela J, Ventas P, Carreira J, Barbas JA, Gimenez-Gallego G, Polo F. Primary structure of Lep d I, the main Lepidoglyphus destructor allergen. Eur J Biochem 225:93-98, 1994.
75. Schmidt M, van der Ploeg I, Olsson S, van Hage Hamsten M. The complete cDNA encoding the Lepidoglyphus destructor major allergen Lep d 1. FEBS Lett 370:11-14, 1995.
76. Rautiainen J, Rytkonen M, Pelkonen J, Pentikainen J, Perola O, Virtanen T, Zeiler T, Mantyjarvi R. BDA20, a major bovine dander allergen characterized at the sequence level is Bos d 2. Submitted.
77. Gjesing B, Lowenstein H. Immunochemistry of food antigens. Ann Allergy 53:602, 1984.
78. de Groot, H., K.G.H. Goei, P. van Swieten, and R.C. Aalberse. 1991. Affinity purification of a major and a minor allergen from dog extract: Serologic activity of affiity-purified Can f I and Can f I-depleted extract. J. Allergy Clin. Immunol. 87:1056-1065.
79. Konieczny, A. Personal communication; Immunologic Pharmaceutical Corp.
79A. Bulone, V. 1998. Separation of horse dander allergen proteins by two-dimensional electrophoresis. Molecular characterisation and identification of Equ c 2.0101 and Equ c 2.0102 as lipocalin proteins. Eur J Biochem 253:202-211.
79B. Swiss-Prot acc. P81216, P81217.
80. McDonald, B., M. C. Kuo, J. L. Ohman, and L. J. Rosenwasser. 1988. A 29 amino acid peptide derived from rat alpha 2 euglobulin triggers murine allergen specific human T cells (abst). J. Allergy Clin. Immunol. 83:251.
81. Clarke, A. J., P. M. Cissold, R. A. Shawi, P. Beattie, and J. Bishop. 1984. Structure of mouse urinary protein genes: differential splicing configurations in the 3'-non-coding region. EMBO J 3:1045-1052.
82. Longbottom, J. L. 1983. Chracterization of allergens from the urines of experimental animals. McMillan Press, London, pp. 525-529.
83. Laperche, Y., K. R. Lynch, K. P. Dolans, and P. Feigelsen. 1983. Tissue-specific control of alpha 2u globulin gene expression: constitutive synthesis in submaxillary gland. Cell 32:453-460.
83A. Aukrust L, Borch SM. 1979. Partial purification and characterization of two Cladosporium herbarum allergens. Int Arch Allergy Appl Immunol 60:68-79.
83B. Sward-Nordmo M, Paulsen BS, Wold JK. 1988. The glycoprotein allergen Ag-54 (Cla h II) from Cladosporium herbarum. Structural studies of the carbohydrate moiety. Int Arch Allergy Appl Immunol 85:288-294.
84. Shen, et al. J. Allergy Clin. Immunol. 103:S157, 1999.
84A. Crameri R. Epidemiology and molecular basis of the involvement of Aspergillus fumigatus in allergic diseases. Contrib. Microbiol. Vol. 2, Karger, Basel (in press).
84B. Shen, et al. (manuscript submitted), 1999
84C. Shen HD, Ling WL, Tan MF, Wang SR, Chou H, Han SIH. Vacuolar serine proteinase: A major allergen of Aspergillus fumigatus. 10th International Congress of Immunology, Abstract, 1998.
85. Kumar, A., L.V. Reddy, A. Sochanik, and V.P. Kurup. 1993. Isolation and characterization of a recombinant heat shock protein of Aspergillus fumigatus. J. Allergy Clin. Immunol. 91:1024-1030.
86. Teshima, R., H. Ikebuchi, J. Sawada, S. Miyachi, S. Kitani, M. Iwama, M. Irie, M. Ichinoe, and T. Terao. 1993. Isolation and characterization of a major allergenic component (gp55) of Aspergillus fumigatus. J. Allergy Clin. Immunol. 92:698-706.
86A. Shen HD, Lin WL, Tsai JJ, Liaw SF, Han SH. 1996. Allergenic components in three different species of Penicillium: crossreactivity among major allergens. Clin Exp Allergy 26:444-451.
86B. Shen, et al. Abstract; The XVIII Congress of the European Academy of Allergology and Clinical Immunology, Brussels, Belgium, 3-7 July 1999.
87. Shen HD, Liaw SF, Lin WL, Ro LH, Yang HL, Han SH. 1995. Molecular cloning of cDNA coding for the 68 kDa allergen of Penicillium notatum using MoAbs. Clin Exp Allergy 25:350-356.
88. Shen, H.D., K.B. Choo, H.H. Lee, J.C. Hsieh, and S.H. Han. 1991. The 40 kd allergen of Candida albicans is an alcohol dehydrogenease: molecular cloning and immunological analysis using monoclonal antibodies. Clin. Exp. Allergy 21:675-681.
89. Shen, et al. Clin. Exp. Allergy (in press), 1999.
90. Woodfolk JA, Wheatley LM, Piyasena RV, Benjamin DC, Platts-Mills TA.1998. Trichophyton antigens associated with IgE antibodies and delayed type hypersensitivity. Sequence homology to two families of serine proteinases. J Biol Chem 273:29489-96.
91. Deuell, B., L.K. Arruda, M.L. Hayden, M.D. Chapman and T.A.E. Platts-Mills. 1991. Trichophyton tonsurans Allergen I. J. Immunol. 147:96-101.
91A. Schmidt M, Zargari A, Holt P, Lindbom L, Hellman U, Whitley P, van der Ploeg I, Harfast B, Scheynius A. 1997. The complete cDNA sequence and expression of the first major allergenic protein of Malassezia furfur, Mal f 1. Eur J Biochem 246:181-185.
91B. Horner WE, Reese G, Lehrer SB. 1995. Identification of the allergen Psi c 2 from the basidiomycete Psilocybe cubensis as a fungal cyclophilin. Int Arch Allergy Immunol 107:298-300.
92. Kuchler, K., M. Gmachl, M. J. Sippl, and G. Kreil. 1989. Analysis of the cDNA for phospholipase A2 from honey bee venom glands: The deduced amino acid sequence reveals homology to the corresponding vertebrate enzymes. Eur. J. Biochem. 184:249-254.
93. Gmachl, M., and G. Kreil. 1993. Bee venom hyaluronidase is homologous to a membrane protein of mammalian sperm. Proc. Natl. Acad. Sci. USA 90:3569-3573.
94. Habermann, E. 1972. Bee and wasp venoms. Science 177:314-322.
95. Jacobson, R.S., and D.R. Hoffman. 1993. Characterization of bumblebee venom allergens (abst). J. Allergy Clin. Immunol. 91:187.
96. Arruda LK, Vailes LD, Mann BJ, Shannon J, Fox JW, Vedvick TS, Hayden ML, Chapman MD. Molecular cloning of a major cockroach (Blattella germanica) allergen, Bla g 2. Sequence homology to the aspartic proteases. J Biol Chem 270:19563-19568, 1995.
97. Arruda LK, Vailes LD, Hayden ML, Benjamin DC, Chapman MD. Cloning of cockroach allergen, Bla g 4, identifies ligand binding proteins (or calycins) as a cause of IgE antibody responses. J Biol Chem 270:31196-31201, 1995.
98. Arruda LK, Vailes LD, Benjamin DC, Chapman MD. Molecular cloning of German Cockroach (Blattella germanica) allergens. Int Arch Allergy Immunol 107:295-297, 1995.

98A. Wu CH, Lee MF, Liao SC. 1995. Isolation and preliminary characterization of cDNA encoding American cockroach allergens. J Allergy Clin Immunol 96: 352-9.
99. Mazur, G., X. Baur, and V. Liebers. 1990. Hypersensitivity to hemoglobins of the Diptera family Chironomidae: Structural and functional studies of their immunogenic/allergenic sites. Monog. Allergy 28:121-137.
100. Soldatova, L., L. Kochoumian, and T.P. King. 1993. Sequence similarity of a hornet (D. maculata) venom allergen phospholipase A1 with mammalian lipases. FEBS Letters 320:145-149.
101. Lu, G., L. Kochoumian and T.P. King. Whiteface hornet venom allergen hyaluronidase: cloning and its sequence similarity with other proteins (abst.). 1994. J. Allergy Clin. Immunol. 93:224.
102. Fang, K. S. F., M. Vitale, P. Fehlner, and T. P. King. 1988. cDNA cloning and primary structure of a white-faced hornet venom allergen, antigen 5. Proc. Natl. Acad. Sci., USA 85:895-899.
103. King, T. P., D. C. Moran, D. F. Wang, L. Kochoumian, and B.T. Chait. 1990. Structural studies of a hornet venom allergen antigen 5, Dol m V and its sequence similarity with other proteins. Prot. Seq. Data Anal. 3:263-266.
104. Lu, G., M. Villalba, M.R. Coscia, D.R. Hoffman, and T.P. King. 1993. Sequence analysis and antigen cross reactivity of a venom allergen antigen 5 from hornets, wasps and yellowjackets. J. Immunol. 150: 2823-2830.
105. King, T. P. and Lu, G. 1997. Unpublished data.
105A. King TP, Lu G, Gonzalez M, Qian N and Soldatova L. 1996. Yellow jacket venom allergens, hyaluronidase and phospholipase: sequence similarity and antigenic cross-reactivity with their hornet and wasp homologs and possible implications for clinical allergy. J. Allergy Clin. Immunol. 98:588-600.
106. Hoffman, D.R. 1993. Allergens in hymenoptera venom XXV: The amino acid sequences of antigen 5 molecules and the structural basis of antigenic cross-reactivity. J. Allergy Clin. Immunol. 92:707-716.
107. Hoffman, D.R. 1992. Unpublished data.
108. Hoffman, D. R. 1993. The complete amino acid sequence of a yellowjacket venom phospholipase (abst). J. Allergy Clin. Immunol. 91:187.
109. Jacobson, R.S., D.R. Hoffman, and D.M. Kemeny. 1992. The cross-reactivity between bee and vespid hyaluronidases has a structural basis (abst). J. Allergy Clin. Immunol. 89:292.
110. Hoffman, D.R. 1993. Allergens in Hymenoptera venom XXIV: The amino acid sequences of imported fire ant venom allergens Sol i II, Sol i III, and Sol i IV. J. Allergy Clin. Immunol. 91:71-78.
111. Schmidt, M., R.B. Walker, D.R. Hoffman, and T.J. McConnell. 1993. Nucleotide sequence of cDNA encoding the fire ant venom protein Sol i II. FEBS Letters 319:138-140.
112. Elsayed S, Bennich H. The primary structure of Allergen M from cod. Scand J Immunol 3:683-686, 1974.
113. Elsayed S, Aas K, Sletten K, Johansson SGO. Tryptic cleavage of a homogeneous cod fish allergen and isolation of two active polypeptide fragments. Immunochemistry 9:647-661, 1972.
114. Hoffman, D. R. 1983. Immunochemical identification of the allergens in egg white. J. Allergy Clin. Immunol. 71:481-486.
115. Langeland, T. 1983. A clinical and immunological study of allergy to hen's egg white. IV. specific IgE antibodies to individual allergens in hen's egg white related to clinical and immunolgical parameters in egg-allergic patients. Allergy 38:493-500.
116. Daul, C.B., M. Slattery, J.E. Morgan, and S.B. Lehrer. 1993. Common crustacea allergens: identification of B cell epitopes with the shrimp specific monoclonal antibodies. In: "Molecular Biology and Immunology of Allergens" (D. Kraft and A. Sehon, eds.). CRC Press, Boca Raton. pp. 291-293.
117. K.N. Shanti, B.M. Martin, S. Nagpal, D.D. Metcalfe, P.V. Subba Rao. 1993. Identification of tropomyosin as the major shrimp allergen and characterization of its IgE-binding epitopes. J. Immunol. 151:5354-5363.
117A. M. Miyazawa, H. Fukamachi, Y. Inagaki, G. Reese, C.B. Daul, S.B. Lehrer, S. Inouye, M. Sakaguchi. 1996. Identification of the first major allergen of a squid (Todarodes pacificus). J. Allergy Clin. Immunol. 98:948-953.
117B A. Lopata et al. 1997. Characteristics of hypersensitivity reactions and identification of a uniques 49 kDa IgE binding protein (Hal-m-1) in Abalone (Haliotis midae). J.Allergy Clin. Immunol. Submitted
118. Monsalve, R.I., M.A. Gonzalez de la Pena, L. Menendez-Arias, C. Lopez-Otin, M. Villalba, and R. Rodriguez. 1993. Characterization of a new mustard allergen, Bra j IE. Detection of an allergenic epitope. Biochem. J. 293:625-632.
119. Mena, M., R. Sanchez-Monge, L. Gomez, G. Salcedo, and P. Carbonero. 1992. A major barley allergen associated with baker's asthma disease is a glycosylated monomeric inhibitor of insect alpha-amylase: cDNA cloning and chromosomal location of the gene. Plant Molec. Biol. 20:451-458.
120. Menendez-Arias, L., I. Moneo, J. Dominguez, and R. Rodriguez. 1988. Primary structure of the major allergen of yellow mustard (Sinapis alba L.) seed, Sin a I. Eur. J. Biochem. 177:159-166.
121. Gonzalez R, Varela J, Carreira J, Polo F. Soybean hydrophobic protein and soybean hull allergy. Lancet 346:48-49, 1995.
122. Christie, J. F., B. Dunbar, I. Davidson, and M. W. Kennedy. 1990. N-terminal amino acid sequence identity between a major allergen of Ascaris lumbricoides and Ascaris suum and MHC-restricted IgE responses to it. Immunology 69:596-602.
123. Czuppon AB, Chen Z, Rennert S, Engelke T, Meyer HE, Heber M, Baur X. The rubber elongation factor of rubber trees (Hevea brasiliensis) is the major allergen in latex. J Allergy Clin Immunol 92:690-697, 1993.
124. Attanayaka DPSTG, Kekwick RGO, Franklin FCH. 1991. Molecular cloning and nucleotide sequencing of the rubber elongation factor gene from hevea brasiliensis. Plant Mol Biol 16:1079-1081.
125. Chye ML, Cheung KY. 1995. (1,3-glucanase is highly expressed in Laticifers of Hevea brasiliensis. Plant Mol Biol 26:397-402.
126. Alenius H, Palosuo T, Kelly K, Kurup V, Reunala T, Makinen-Kiljunen S, Turjanmaa K Fink J. 1993. IgE reactivity to 14-kD and 27-kD natural rubber proteins in Latex-allergic children with Spina bifida and other congenital anomalies. Int Arch Allergy Immunol 102:61-66.
127. Yeang HY, Cheong KF, Sunderasan E, Hamzah S, Chew NP, Hamid S, Hamilton RG, Cardosa MJ. 1996. The 14.6 kD (REF, Hev b 1) and 24 kD (Hev b 3) rubber particle proteins are recognized by IgE from Spina Bifida patients with Latex allergy. J Allerg Clin Immunol in press.
128. Sunderasan E, Hamzah S, Hamid S, Ward MA, Yeang HY, Cardosa MJ. 1995. Latex B-serum (-1,3-glucanase (Hev b 2) and a component of the microhelix (Hev b 4) are major Latex allergens. J nat Rubb Res 10:82-99.

Claims

We claim:

1. A method of treating an allergy in a subject susceptible to an anaphylactic allergic response to an allergen, the method comprising steps of:

providing a composition comprising microorganisms that produce the allergen; and

administering the composition to the subject at an effective and non-toxic dose.

2. The method of claim 1 , wherein in the step of providing, the microorganism is selected from the group consisting of: bacteria, fungi, viruses, algae, and protozoa.

3. The method of claim 1 , wherein in the step of providing, the microorganism is selected from the group consisting of: gram-negative bacteria, gram-positive bacteria, and yeast.

4. The method of claim 1 , wherein in the step of providing, the microorganism is selected from the group consisting of: *E. coli, Lactococcus, Listeria, Vibrio, Salmonella* and *S. cerevisiae.*

5. The method of claim 1 , wherein in the step of providing, the allergen is found in foods, venoms, or latex.

6. The method of claim 1 , wherein in the step of providing, the allergen is a protein found in peanuts, milk, eggs, seafood, nuts, dairy products and fruit.

7. The method of claim 1, wherein in the step of providing, the allergen is a protein found in bee venom.

8. The method of claim 1 , wherein in the step of providing, the allergen is Ara h 1, Ara h 2, Ara h 3, or a polypeptide portion thereof.

9. The method of claim 1 , wherein in the step of providing, the allergen is protein modified to have a reduced ability to bind and crosslink IgE antibodies.

10. The method of claim 1, wherein in the step of providing, the microorganisms produce a portion of the allergen.

11. The method of claim 10, wherein in the step of providing, the portion of the allergen produced has a reduced number of IgE binding sites as compared to the allergen.

12. The method of claim 1 , wherein in the step of providing, the allergen is a polypeptide and production of the allergen is inducible; and wherein after the step of administering, the method further comprises the step of inducing expression of the polypeptide.

13. The method of claim 12, wherein in the step of inducing, the polypeptide is secreted into a periplasm or secreted outside the cell.

14. The method of claim 1 , wherein the step of providing comprises providing a composition comprising gram-negative bacteria or yeast that secretes the allergen into a periplasm.

15. The method of claim 1, wherein in the step of providing, the allergen is a small molecule.

16. A composition comprising a microorganism that produces an allergen that elicits an anaphylactic allergic reaction in a subject allergic to the allergen.

17. The composition of claim 16, wherein the allergen is a polypeptide or small molecule.

18. The composition of claim 16, wherein the microorganism is selected from the group consisting of: bacteria, fungi, viruses, algae, and protozoa.

19. The composition of claim 16, wherein the microorganism is selected from the group consisting of: gram-negative bacteria, gram-positive bacteria, and yeast.

20. The composition of claim 16, wherein the microorganism is selected from the group consisting of: *E. coli, Lactococcus, Listeria, Vibrio, Salmonella* and *S. cerevisiae*

21. The composition of claim 16, wherein the allergen found in foods, venoms, or latex.

22. The composition of claim 16, wherein the allergen is an allergen found in peanuts, milk, eggs, seafood, nuts, dairy products and fruit.

23. The composition of claim 16, wherein the allergen found in bee venom.

24. The composition of claim 16, wherein the protein is Ara h 1, Ara h 2, Ara h 3, or a polypeptide portion thereof.

25. The composition of claim 16, wherein the allergen is modified to have a reduced ability to bind and crosslink IgE antibodies.

26. The composition of claim 16, wherein the microorganism produces a portion of the allergen.

27. The composition of claim 16, wherein the portion of the allergen produced has a reduced number of IgE binding sites as compared to the allergen.

28. The composition of claim 16, wherein production of the allergen is inducible.

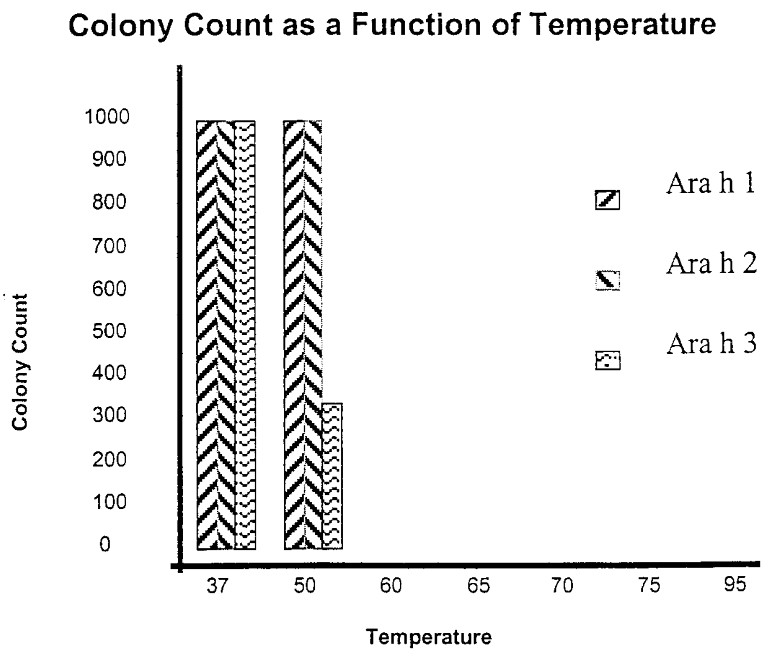
29. The composition of claim 16, wherein the allergen is a polypeptide which is secreted into a periplasm or secreted outside the cell.

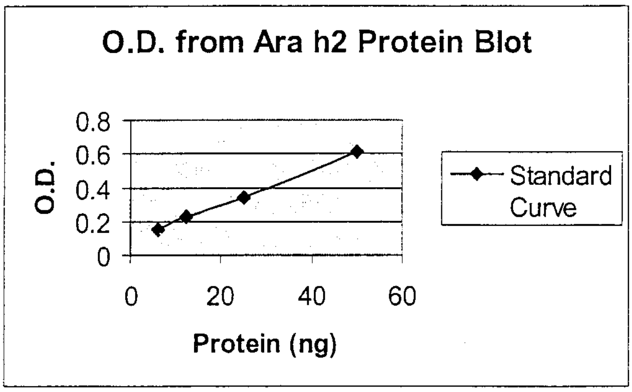
30. The composition of claim 16, wherein the microorganism is a gram-negative bacteria or yeast that secretes the allergen into a periplasm.

31. A pharmaceutical composition comprising microorganisms that produce an allergen that elicits an anaphylactic allergic response in a subject susceptible to the anaphylactic allergic response, and further comprises an pharmaceutically acceptible carrier.

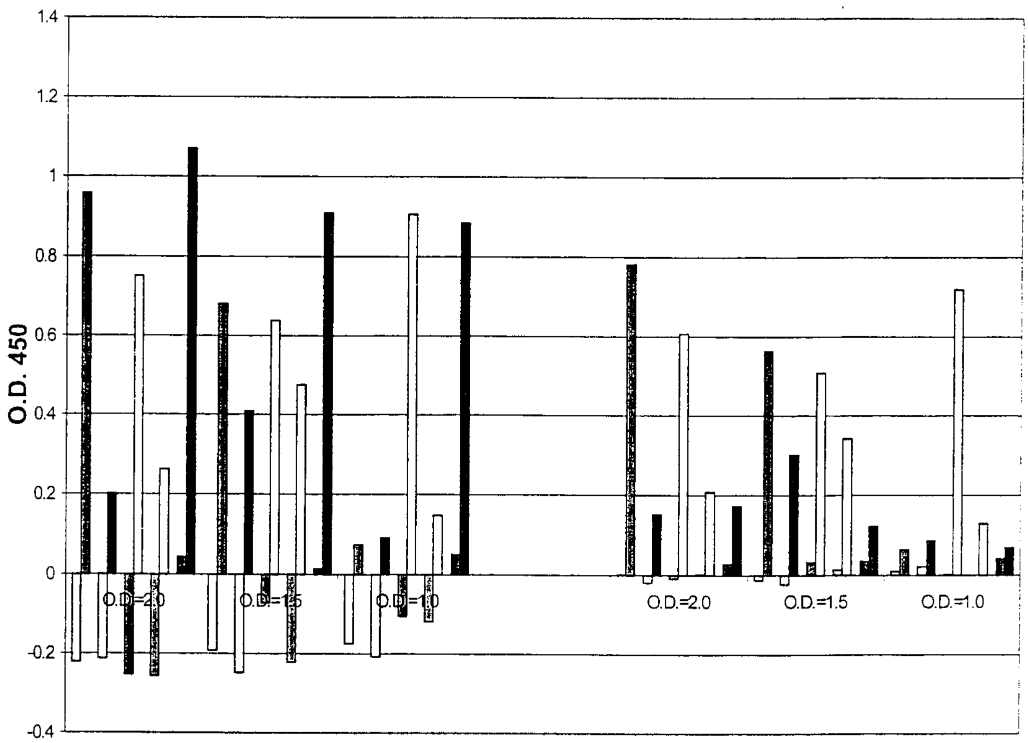
32. The pharmaceutical composition of claim 31, wherein the allergen is a polypeptide or a small molecule.

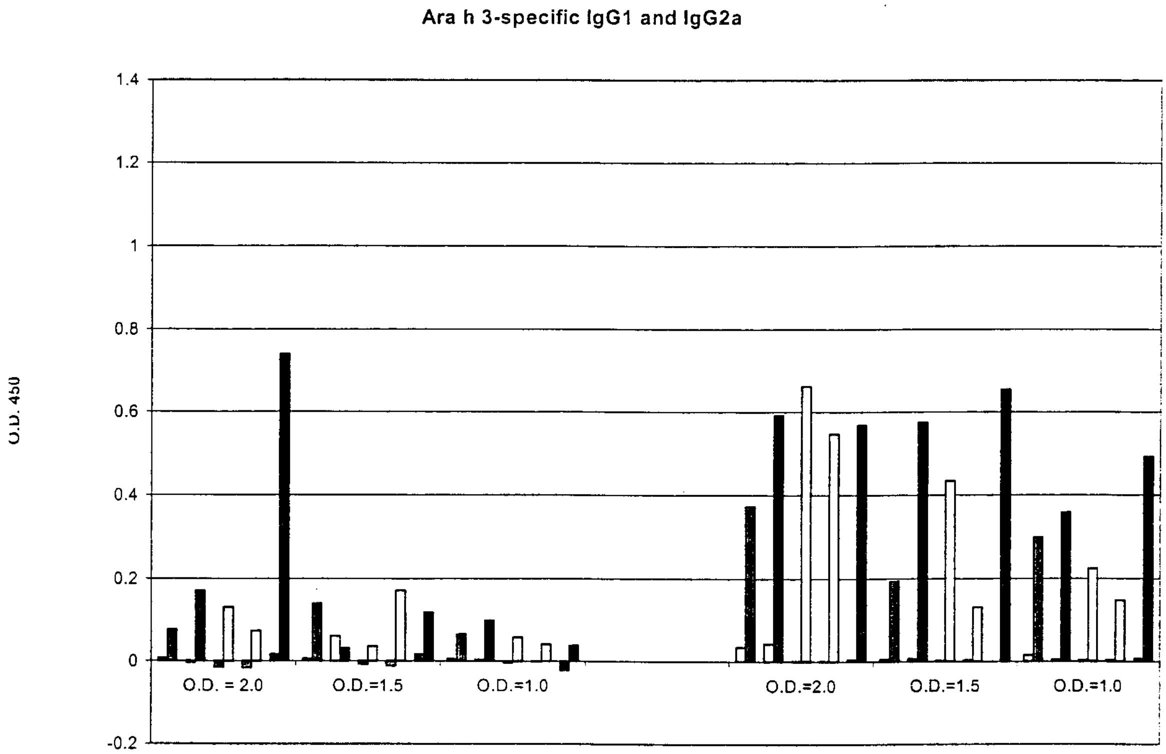
33. The pharmaceutical composition of claim 31, wherein the microorganisms produce a portion of an allergen that elicits an anaphylactic allergic response in a subject susceptible to the anaphylactic allergic response.


Colony Count as a Function of Temperature
1000
900
800
700
600
500
400
300
200
100
0
Colony Count
37
50
60
65
70
75
95
Temperature
Ara h 1
Ara h 2
Ara h 3


O.D. from Ara h2 Protein Blot
O.D.
0.8
0.6
0.4
0.2
0
0
20
40
60
Protein (ng)
Standard Curve

Ara h 2 IgG1 and IgG2a




Ara h 3-specific IgG1 and IgG2a
O.D. 450
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
O.D. = 2.0
O.D.=1.5
O.D.=1.0
O.D.=2.0
O.D.=1.5
O.D.=1.0