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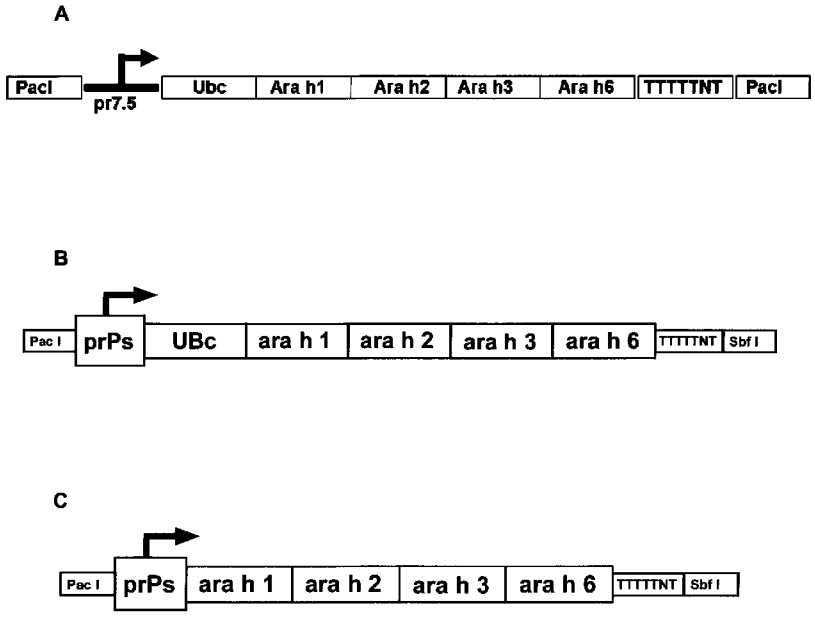
(54) **Title**: IMMUNE MODULATION

FIGURE 1



(57) **Abstract**: The present invention relates to a poxvirus vector comprising a nucleic acid sequence encoding a fusion protein comprising: (i) a peanut allergen selected from list consisting of at least two peanut allergens from ara h 1, ara h 2, ara h 3, ara h 4, ara h 5, ara h 6. ara h 7, ara h 8, ara h 9, ara h l O and ara h I I or a derivative or part thereof having at least 70% sequence identity thereto, and (ii) a profeasome degradation tag to enhance intracellular degradation of the fusion protein. Methods of desensitizing or inducing tolerance to a peanut allergen and/or suppressing an allergic response to a peanut allergen are also disclosed.

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IMMUNE MODULATION

TECHNICAL FIELD

[0001] The present specification relates generally to the field of prophylactic or therapeutic vaccines. In particular, the specification relates to a vaccine for the treatment of peanut allergies by suppressing the allergic response thereto.

BACKGROUND

[0002] In principle, allergic diseases are disorders of the immune system associated with a dysregulation of the T_H1 and T_H2 lymphocyte subsets [de Vries *et al.* 1999, Parronchi *et al.* 1999, Singh *et al.* 1999]. It has been postulated that with a declining incidence of infectious diseases due to vaccination, the use of antibiotics and other public health practices, a major source of T_H1 immune provocation has been lost, with a consequent increase in the T_H2 bias of immune responses towards environmental allergens [Holgate 1999, Shaheen *et al.* 1996].

[0003] Of the various allergic diseases that affect the general population, peanut-induced anaphylaxis is particularly severe and represents the most common contributor of emergency department admissions for treatment of anaphylactic reactions.

[0004] Allergies to peanut result from an aberrant immune response directed against an otherwise harmless environmental antigen. Peanut allergy and anaphylaxis are centred around a type 2 immune response, characterised by the generation of T_H2 T cells and IgE antibody secreting B cells. By contrast, a types 1 immune response can be characterised by antibodies predominately of IgG (IgG2a isotype in mice), activation of NK cells and phagocytic cells, and the development of cytotoxic T lymphocytes (CTL). Both type 1 and 2 responses are coordinated by helper T cells, which differentiate into several functionally different subsets including T_H1 and T_H2 lymphocytes. Theses subsets are characterised by their cytokine secretion profile [Mosmann *et al.* 1989], where T_H1 cells produce IFN-gamma and T_H2 cells typically secrete IL-4, IL-5 and IL-13.

[0005] Orally ingested peanut allergens first encounter the gut mucosal immune system. Microfold (M) cells are specialised follicle-associated cells that line the epithelium of the gastrointestinal tract and lie in close proximity to Peyer's patches. They are responsible for the induction of tolerising and/or protective gut-associated immune responses. Sensitization to food allergens occurs when exogenous food antigens are taken

up by M cells, and then presented to macrophages and dendritic cells (DCs) [DeLong *et al.* 2011]. Once internalised by macrophages and DCs, the antigens are endocytosed, then denatured and degraded into peptides of around 12-20 amino acids in length. A small fraction of these small peptide fragments are then transported intracellularly and presented on the cell-surface MHC class II molecules for specific interaction with $CD4^+$ T cells. These activated $CD4^+$ T cells subsequently expand in number and release T_H2 cytokines. The T_H2 cells, IL-4 and IL-5 promote the differentiation of B cells, which bear allergens bound to surface immunoglobulin (Ig) receptors, into cells that secrete allergen-specific IgE antibodies [Turcanu *et al.* 2010]. These IgE-producing B cells then expand in number and become plasma cells that continuously secrete allergen-specific IgE antibodies. Environmental exposure to peanuts results in binding of peanut allergens to specific IgE-coating on mast cells and basophils. Subsequently, Fc receptor cross-linking provides a potent activation stimulus that results in the degranulation of basophils and mast cells, which rapidly release a variety of preformed proinflammatory and vasoactive compounds such as prostaglandins, leukotrienes, serine proteases, histamine and cytokines into the extracellular fluid to produce an inflammatory response [Sicherer *et al.* 2010], all of which culminate in the clinical manifestation of an acute allergic reaction [Long 2002].

[0006] Local symptoms of peanut allergy include abdominal pain, vomiting, cramping and diarrhea, and are common even in cases of mild peanut allergy. This acute non-life threatening reaction causes a transient increase in intestinal permeability, which subsequently allows systemic distribution of macromolecules, such as whole peanut allergens, exacerbating the allergic response to subsequent exposure to peanut allergens, which can cause life-threatening anaphylactic reactions [Sanderson *et al.* 1993].

[0007] Unlike traditional immunotherapy for allergic reactions to grass pollens, dust mite and bee sting venom, subcutaneous desensitization injections of peanut extracts have unacceptable risk-benefits [Oppenheimer *et al.* 1992]. Therefore, at present, avoiding peanuts is the only available method for prevent further reactions. However, strict avoidance is often an unrealistic strategy for many individuals, particularly in light of accidental exposure to peanuts that often occurs through ingestion of processed foods or foods prepared in the same vicinity of those containing peanuts, *e.g.*, restaurants, schools, food courts and work canteens. Therefore, there remains a need for an effective therapeutic strategy for the treatment and prevention of the peanut allergy.

SUMMARY OF THE INVENTION

[0008] In an aspect of the present invention, there is provided a poxvirus vector comprising a nucleic acid sequence encoding a fusion protein comprising (i) at least two peanut allergens selected from list consisting of ara h 1, ara h 2, ara h 3, ara h 4, ara h 5, ara h 6and ara h 7 and a derivative or part thereof having at least 70% sequence identity thereto, and (ii) a proteasome degradation tag to enhance intracellular degradation of the fusion protein.

[0009] In an aspect of the present invention, there is provided a poxvirus vector comprising a nucleic acid sequence encoding a fusion protein comprising (i) at least two peanut allergens selected from list consisting of ara h 1, ara h 2, ara h 3, ara h 4, ara h 5, ara h 6, ara h 7, ara h 8, ara h 9, ara h10 and ara h 11 and a derivative or part thereof having at least 70% sequence identity thereto, and (ii) a proteasome degradation tag to enhance intracellular degradation of the fusion protein.

[0010] In another aspect of the present invention, there is provided a poxvirus vector comprising a nucleic acid sequence encoding a fusion protein comprising: (i) at least three peanut allergens selected from list consisting of ara h 1, ara h 2, ara h 3, ara h 4, ara h 5, ara h 6 and ara h 7 and a derivative or part thereof having at least 70% sequence identity thereto, and (ii) a proteasome degradation tag to enhance intracellular degradation of the fusion protein.

[0011] In another aspect of the present invention, there is provided a poxvirus vector comprising a nucleic acid sequence encoding a fusion protein comprising: (i) at least three peanut allergens selected from list consisting of ara h 1, ara h 2, ara h 3, ara h 4, ara h 5, ara h 6, ara h 7, ara h 8, ara h 9, ara h10 and ara h 11 and a derivative or part thereof having at least 70% sequence identity thereto, and (ii) a proteasome degradation tag to enhance intracellular degradation of the fusion protein.

[0012] In another aspect of the present invention, there is provided use of a poxvirus vector disclosed herein in, or in the manufacture of a medicament for, the treatment of peanut allergy.

[0013] In another aspect of the present invention, there is provided a method of inducing tolerance to or suppressing an allergic response in a subject or patient, the method comprising administering to the subject or patient an effective amount of the poxvirus

vector disclosed herein for a time and under conditions sufficient to elicit suppression/tolerance.

[0014] In another aspect of the present invention, there is provided a method of vaccinating a subject to induce tolerance to a peanut allergen comprising administering the poxvirus vector disclosed herein.

[0015] In another aspect of the present invention, there is provided a kit comprising the poxvirus vector disclosed herein.

[0016] The above summary is not and should not be seen in any way as an exhaustive of all embodiments of the present invention.

BRIEF DESCRIPTION OF THE FIGURES

[0017] **Figure 1** shows an arrangement of the PHAV Antigen according to an embodiment of the present invention including a proteasome degradation tag and multiple peanut allergens (Figures 1A and B) and the PHAV Antigen according to an embodiment of the present invention without a proteasome degradation tag (Figure 1C).

[0018] **Figure 2** shows the nucleic acid sequence of the UBc.PHAV expression cassette.

[0019] **Figure 3** shows the nucleic acid sequence of the PHAVag construct expression cassette, without the ubiquitin sequence.

[0020] **Figure 4** is a diagrammatic representation of the insertion of the PHAV expression cassettes into the A39R ORF of vaccinia virus Copenhagen strain by homologous recombination.

[0021] **Figure 5** lists the features of the homologous recombination cassette diagrammatically represented in Figure 4.

[0022] **Figure 6** shows the nucleic acid sequence of the UBc.PHAV homologous recombination cassette.

[0023] **Figure 7** shows the nucleic acid sequence of the PHAV homologous recombination cassette.

[0024] **Figure 8** is a diagrammatic representation of the pTC11 (UBc.PHAV) and

pTC12 (PHAV). The plasmids are shown in Figure 8.

[0025] **Figure 9** is a diagrammatic representation of the proteasomal degradation pathway in a cell.

[0026] **Figure 10** shows the levels of peanut protein-specific serum IgE (Figure 10A) and IgG2a (Figure 10B) antibodies before and after vaccination (17 day post vaccination) with the empty vector (SCV000) or the UBc.PHAV vector (SCV201C); *=$p<0.05$.

[0027] **Figure 11** shows the levels of IFN-gamma (IFN-g; a T_H1 cytokine; Figure 11A), IL4 (T_H2 cytokines; Figure 11B) and IL5 (T_H2 cytokines; Figures 11C) secreted by cultured lymphocytes obtained from the spleens of SCV000 and SCV201C vaccinate mice.

DETAILED DESCRIPTION

[0028] Reference to any prior art in this specification is not, and should not be taken as, an acknowledgment or any form of suggestion that this prior art forms part of the common general knowledge in any country.

[0029] Throughout this specification, unless the context requires otherwise, the words "comprise," "comprises" and "comprising" will be understood to imply the inclusion of a stated step or element or group of steps or elements but not the exclusion of any other step or element or group of steps or elements. Thus, use of the term "comprising" and the like indicates that the listed elements are required or mandatory, but that other elements are optional and may or may not be present. By "consisting of" is meant including, and limited to, whatever follows the phrase "consisting of". Thus, the phrase "consisting of" indicates that the listed elements are required or mandatory, and that no other elements may be present. By "consisting essentially of" is meant including any elements listed after the phrase, and limited to other elements that do not interfere with or contribute to the activity or action specified in the disclosure for the listed elements. Thus, the phrase "consisting essentially of" indicates that the listed elements are required or mandatory, but that other elements are optional and may or may not be present depending upon whether or not they affect the activity or action of the listed elements.

[0030] As used herein the singular forms "a", "an" and "the" include plural aspects unless the context clearly dictates otherwise. Thus, for example, reference to "a composition" includes a single composition, as well as two or more compositions;

reference to "an agent" includes one agent, as well as two or more agents; reference to "the invention" includes single and multiple aspects of the invention; and so forth.

[0031] Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. Any materials and methods similar or equivalent to those described herein can be used to practice or test the present invention.

[0032] The present specification enables a vaccine approach to the development of a therapeutic agent for treating or preventing peanut allergy. In particular, the specification enables an agent capable of providing therapy in the context of the major peanut allergens, *e.g.*, at least one, at least two, at least three, etc, of the most widespread or troublesome peanut allergens.

[0033] The present invention is predicated on the inventors' surprising finding that a DNA vaccine comprising a nucleic acid construct operatively encoding a fusion protein, the fusion protein comprising a peanut allergen (such as ara h 1) linked to a proteasome degradation tag (such as ubiquitin), is capable of inducing an immune response in a subject that is biased towards a T_H1 phenotype, thus resulting in the secretion of peanut allergen-specific IgG antibodies, as opposed to peanut allergen-specific IgE antibodies that would otherwise facilitate an allergic reaction upon exposure to the peanut allergen.

[0034] Accordingly, In an aspect of the present invention, there is provided a poxvirus vector comprising a nucleic acid sequence encoding a fusion protein comprising (i) at least two peanut allergens selected from list consisting of ara h 1, ara h 2, ara h 3, ara h 4, ara h 5, ara h 6 and ara h 7 and a derivative or part thereof having at least 70% sequence identity thereto, and (ii) a proteasome degradation tag to enhance intracellular degradation of the fusion protein.

[0035] In another aspect of the present invention, there is provided a poxvirus vector comprising a nucleic acid sequence encoding a fusion protein comprising (i) at least two peanut allergens selected from list consisting of ara h 1, ara h 2, ara h 3, ara h 4, ara h 5, ara h 6, ara h 7, ara h 8, ara h 9, ara h10 and ara h 11 and a derivative or part thereof having at least 70% sequence identity thereto, and (ii) a proteasome degradation tag to enhance intracellular degradation of the fusion protein.

[0036] In another aspect of the present invention, there is provided a poxvirus vector

comprising a nucleic acid sequence encoding a fusion protein comprising: (i) at least three peanut allergens selected from list consisting of ara h 1, ara h 2, ara h 3, ara h 4, ara h 5, ara h 6 and ara h 7 and a derivative or part thereof having at least 70% sequence identity thereto, and (ii) a proteasome degradation tag to enhance intracellular degradation of the fusion protein.

[0037] In another aspect of the present invention, there is provided a poxvirus vector comprising a nucleic acid sequence encoding a fusion protein comprising: (i) at least three peanut allergens selected from list consisting of ara h 1, ara h 2, ara h 3, ara h 4, ara h 5, ara h 6, ara h 7, ara h 8, ara h 9, ara h10 and ara h 11 and a derivative or part thereof having at least 70% sequence identity thereto, and (ii) a proteasome degradation tag to enhance intracellular degradation of the fusion protein.

[0038] In another aspect of the present invention, there is provided a poxvirus vector which expresses in the cell of a subject a fusion protein comprising: (i) a peanut allergen selected from list consisting of (a) at least two peanut allergens from ara h 1, ara h 2, ara h 3, ara h 4, ara h 5, ara h 6 and ara h 7 or a derivative or part thereof having at least 70% sequence identity thereto, or (b) at least three peanut allergens from ara h 1, ara h 2, ara h 3, ara h 4, ara h 5, ara h 6 and ara h 7, or a derivative or part thereof having at least 70% sequence identity thereto, and (ii) a proteasome degradation tag to enhance intracellular degradation of the fusion protein.

[0039] In another aspect of the present invention, there is provided a poxvirus vector which expresses in the cell of a subject a fusion protein comprising: (i) a peanut allergen selected from list consisting of (a) at least two peanut allergens from ara h 1, ara h 2, ara h 3, ara h 4, ara h 5, ara h 6 and ara h 7, ara h 8, ara h 9, ara h10 and ara h 11 or a derivative or part thereof having at least 70% sequence identity thereto, or (b) at least three peanut allergens from ara h 1, ara h 2, ara h 3, ara h 4, ara h 5, ara h 6, ara h 7, ara h 8, ara h 9, ara h10 and ara h 11 or a derivative or part thereof having at least 70% sequence identity thereto, and (ii) a proteasome degradation tag to enhance intracellular degradation of the fusion protein.

Peanut allergens

[0040] Peanut allergens would be known to persons skilled in the art and include any peptide of the *Arachis hypogaea* species to which a subject may be exposed to through, for

example, contact, inhalation, ingestion, injection, or the like. In an embodiment, the at least two peanut allergens are selected from the group consisting of ara h 1, ara h 2, ara h 3, ara h 4, ara h 5, ara h 6 and ara h 7. In another embodiment, the at least two peanut allergens are selected from the group consisting of ara h 1, ara h 2, ara h 3, ara h 4, ara h 5, ara h 6, ara h 7, ara h 8, ara h 9, ara h 10 and ara h 11.

[0041] The fusion protein can comprise any two or more peanut allergens ara h 1 to ara h 11. For example, the fusion protein may comprise the following peanut allergens:

(i) ara h 1, ara h 2, ara h 3, ara h 4, ara h 5, ara h 6, ara h 7, ara h 8, ara h 9, ara h 10 and ara h 11;

(ii) ara h 1, ara h 2, ara h 3, ara h 4, ara h 5, ara h 6, ara h 7, ara h 8, ara h 9 and ara h1 0;

(iii) ara h 1, ara h 2, ara h 3, ara h 4, ara h 5, ara h 6, ara h 7, ara h 8 and ara h 9;

(iv) ara h 1, ara h 2, ara h 3, ara h 4, ara h 5, ara h 6, ara h 7 and ara h 8;

(v) ara h 1, ara h 2, ara h 3, ara h 4, ara h 5, ara h 6 and ara h 7;

(vi) ara h 1, ara h 2, ara h 3, ara h 4, ara h 5 and ara h 6;

(vii) ara h 1, ara h 2, ara h 3, ara h 4 and ara h 5;

(viii) ara h 1, ara h 2, ara h 3 and ara h 4;

(ix) ara h 1, ara h 2, ara h 3 and ara h 6;

(x) ara h 1, ara h 2 and ara h 3;

(xi) ara h 1 and ara h 2;

(xii) ara h 1 and ara h 3;

(xiii) ara h 1 and ara h 4;

(xiv) ara h 1 and ara h 5;

(xv) ara h 1 and ara h 6;

(xvi) ara h 1 and ara h 7;

(xvii) ara h 1 and ara h 8;

(xviii) ara h 1 and ara h 9;

(xix) ara h 1 and ara h 10;

(xx) ara h 1 and ara h 11;

(xxi) ara h 2 and ara h 3;

(xxii) ara h 2 and ara h 4; and so on.

[0042] By employing a proteasome degradation tag (*e.g.*, ubiquitin) as a component of

the fusion protein, the synthesized fusion protein is targeted to proteasomal degradation, resulting in the generation of small peptide fragments, which enter the endoplasmic reticulum (ER) where they are complexed with MHC class I proteins and then transported to the cell surface to be presented to T lymphocytes. As a consequence, there is enhanced presentation of the protein fragments with MHC class I. Thus, it would be understood by persons skilled in the art that, where the nucleic acid sequence encodes a fusion protein comprising two or more peanut allergens, the two or more peanut allergens can appear in the fusion protein in any particular order, as the expressed fusion protein will be subjected to proteasomal degradation.

[0043] It would be understood by persons skilled in the art that the choice of peanut allergen or allergens is likely to depend on the particular therapeutic and/or prophylactic application. For example, where the vaccine is to be used to induce tolerance in a subject who is allergic to peanut allergen Ara h1, then the fusion protein would desirably comprise ara h 1; where the vaccine is to be used to induce tolerance in a subject who is allergic to peanut allergen ara h 2, then the fusion protein would desirably comprise ara h 2; where the vaccine is to be used to induce tolerance in a subject who is allergic to peanut allergens ara h 1 and ara h 2, then the fusion protein would desirably comprise ara h 1 and ara h 2; and so on.

[0044] In an embodiment, the peanut allergen is selected from the group including: arah 1, Clone P41B (GenBank Accession number L34402 or Swiss-Prot: P43238.1); ara h 1 Clone P17 (GenBank Accession number L38853); ara h 2 cDNA (GenBank Accession number L7797 or UniProtKB/TrEMBL: Q8GV20); ara h 3 cDNA (GenBank Accession number AF093541 or ACH91862); ara h 4 cDNA (GenBank Accession number AF086821); ara h 5 cDNA (GenBank Accession number AF059616); ara h 6 cDNA (GenBank Accession number AF092846 or UniProtKB/TrEMBL: Q647G9), ara h 7 cDNA (GenBank Accession number AF091737), ara h 8 (GenBank Accession number AY328088, EF436550), ara h 9 (GenBank Accession number EU159429, EU161278), ara h 10 (AY722694, AY722695) and ara h 11 (DQ097716).

[0045] In an embodiment, the fusion protein comprises at least four peanut allergens, more preferably at least four of the most common peanut allergens affecting individuals who are allergic to peanuts. In an embodiment, the fusion protein comprises peanut allergens ara h 1, ara h 2, ara h 3 and ara h 6.

[0046] As used herein, the term "peanut allergen", including specific examples such ara h 1, ara h 2, *etc*, is to be understood as also including a homologue or variant thereof. The term "homologue", as used herein with reference to homologs of nucleic acid sequences or polypeptides described herein (including, for example, any one of SEQ ID NOs: 1-12), should be understood to include, for example, orthologs, paralogs, mutants and variants of nucleic acids or polypeptides described herein. In some embodiments, the homologue comprises a nucleic acid or an amino acid sequence which comprises at least 70% sequence identity, at least 75% sequence identity, at least 80% sequence identity, at least 85% sequence identity, at least 90% sequence identity, at least 95% sequence identity, at least 96% sequence identity, at least 97% sequence identity, at least 98% sequence identity, or at least 99% sequence identity to the nucleic acid or amino acid sequence described herein.

[0047] Thus, in an embodiment, ara h 1 has an amino acid sequence of SEQ ID NO: 4 or an amino acid sequence having at least 70% identity thereto, ara h 2 comprises the amino acid sequence of SEQ ID NO:6 or an amino acid sequence having at least 70% identity thereto, ara h 3 comprises the amino acid sequence of SEQ ID NO:8 or an amino acid sequence having at least 70% identity thereto, and ara h 6 comprises the amino acid sequence of SEQ ID NO:10 or an amino acid sequence having at least 70% identity thereto.

[0048] In another embodiment, ara h 1 is encoded by the nucleic acid sequence of SEQ ID NO:3 or a nucleic acid sequence having at least 70% identity thereto, ara h 2 is encoded by the nucleic acid sequence of SEQ ID NO:5 or a nucleic acid sequence having at least 70% identity thereto, ara h 3 is encoded by the nucleic acid sequence of SEQ ID NO:7 or a nucleic acid sequence having at least 70% identity thereto and ara h 6 is encoded by the nucleic acid sequence of SEQ ID NO:9 or a nucleic acid sequence having at least 70% identity thereto.

[0049] The term "sequence identity" as used herein refers to the extent that sequences are identical on a nucleotide-by-nucleotide basis or an amino acid-by-amino acid basis over a window of comparison. Thus, a "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (*e.g.*, A, T, C, G, I) or the identical amino acid residue (*e.g.*, Ala, Pro, Ser, Thr, Gly, Val, Leu, Ile, Phe, Tyr, Trp, Lys,

Arg, His, Asp, Glu, Asn, Gln, Cys and Met) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (*i.e.*, the window size), and multiplying the result by 100 to yield the percentage of sequence identity. For the purposes of the present invention, "sequence identity" will be understood to mean the "match percentage" calculated by an appropriate method. For example, sequence identity analysis may be carried out using the DNASIS computer program (Version 2.5 for windows; available from Hitachi Software engineering Co., Ltd., South San Francisco, California, USA) using standard defaults as used in the reference manual accompanying the software. The sequence identity of the encompassed peanut allergen amino acid or nucleotide sequence is, in some embodiments, increased to at least 75%, or at least 80%, or at least 85%, or at least 90% or at least 95% or at least 98% sequence identity.

[0050] In some embodiments, the term "allergen" may also include a fragment of any one of the foregoing peptides. As such, the nucleic acid may comprise a nucleotide that encodes a fragment of one of the aforementioned peanut allergens.

[0051] In some embodiments, the peanut allergen includes a modified peanut allergen whereby repeat sequences of 8 or more bases are removed from a native peanut allergen sequence. In some embodiments, the fusion protein includes 2 or more peanut allergens. In some embodiments the fusion protein includes two or more peanut allergens, at least one of which is selected from the group consisting of ara h 1, ara h 2, ara h 3, ara h 4, ara h 5, ara h 6, ara h 7, ara h 8, ara h 9, ara h 10 and ara h 11. In some embodiments, the fusion protein includes ara h 1, ara h 2, ara h 3 and ara h 6, or homologues thereof.

[0052] In some embodiments, to facilitate expression of a single fusion protein, the nucleic acid is devoid of stop codons between two sequences encoding peanut allergens.

Proteasome degradation tag

[0053] The present inventors have surprisingly found that employing a proteasome degradation tag (such as ubiquitin) as a component of the fusion protein is able to overcome the apparent toxic and/or inhibitory effect that a non-ubiquitinated peanut allergen peptide construct has on recombinant expression. The use of a proteasome degradation tag targets the expressed fusion peptide to proteasomal degradation. As a result of ubiquitin-targeted proteasomal degradation, small peptide fragments of the fusion

peptide (*e.g.* peptides of about 8-12 amino acids in length) enter the endoplasmic reticulum (ER) where they are complexed with MHC class I proteins and subsequently transported to the cell surface to be presented to T lymphocytes. As a result, there is enhanced presentation of the fusion peptide fragments with MHC class I, resulting in a greater T_H1 immune response to peanut allergens. Thus, the proteasome degradation tag unexpectedly prevents the intact peanut allergen peptide construct from inhibiting recombinant expression in a host cell and biases the immune response towards a T_H1 phenotype.

[0054] The proteasome degradation tag may be any tag that targets the fusion protein for proteasomal degradation. In some embodiments, the proteasome degradation tag may include a ubiquitin molecule or a ubiquitin binding domain. In an embodiment, the proteasome degradation tag is a ubiquitin monomer, an illustrative example of which is ubiquitin C. In some embodiments, the ubiquitin monomer comprises the amino acid sequence of SEQ ID NO:2 or an amino acid sequence having at least 70% nucleotide sequence identity thereto.

[0055] In some embodiments, the C-terminal of the ubiquitin monomer is an alanine residue.

[0056] In another embodiment, the ubiquitin monomer is encoded by the nucleotide sequence of SEQ ID NO: 1 or a nucleotide sequence having at least 70% nucleotide sequence identity thereto.

[0057] The sequence encoding the proteasome degradation tag may be placed before or after the sequence encoding the at least one peanut allergen (*i.e.* the protein degradation tag may be C-terminal or N-terminal fusion protein).

[0058] Ubiquitin molecules may be derived from any suitable species. For a vaccine intended for human treatment, the ubiquitin molecule may be a human ubiquitin molecule or a ubiquitin molecule from another animal species that may have been codon optimised for expression in human cells. In some embodiments, the ubiquitin molecule may be a ubiquitin C monomer. Once expressed, the ubiquitin molecule may attract and bind to other ubiquitin molecules to form a polyubiquitin chain on the fusion protein. The ubiquitin molecule and/or the polyubiquitin chain may direct the fusion protein for proteasomal degradation.

[0059] In some embodiments, the nucleic acid construct operably encodes multiple ubiquitin molecules or one or more sequences encoding a truncated or modified ubiquitin molecule. If multiple ubiquitin molecules are encoded, one or more start and stop codons may be removed to allow translation of the entire fusion protein.

[0060] In some embodiments, a truncated ubiquitin molecule may involve exclusion of the lysine closest to the C-terminal of the native ubiquitin molecule. In some embodiments, a modified ubiquitin molecule may have one or more lysines of the native sequence removed or replaced (e.g. with arginine) from the sequence. In some embodiments, the ubiquitin molecule may only have a single lysine.

[0061] In some embodiments, the C-terminal of the ubiquitin molecule may be modified. For example, the C-terminal glycine of the native molecule may be replaced with alanine. Replacing the glycine with alanine or another amino acid, may prevent protease cleavage of the proteasome degradation tag from the allergen. Replacement of the glycine with alanine may also allow for the formation of a covalent bond between the proteasome degradation tag and the allergen. This covalent bond may be resistant to protease cleavage.

[0062] In some embodiments the proteasome degradation tag may include a ubiquitin binding domain. The protein degradation tag may be a member of the UbL (ubiquitin-like)-UBA (ubiquitin-associated) domain-containing protein family. In this regard, the expressed fusion protein may attract binding of ubiquitin molecules to the binding domain, leading to proteasomal degradation of the fusion protein.

Fusion protein

[0063] In some embodiments, the nucleic acid sequence encodes a fusion protein that has been optimized for expression in a subject. For example, the sequence for a peanut allergen fusion protein can be is optimized for expression in a human cell. Similarly, in some embodiments, the proteasome degradation tag is optimized for expression in a subject and/or may be a proteasome degradation tag cloned from the same species as the desired subject. In some embodiments, codon optimization involves replacing a codon with a different codon that encodes the same amino acid but is more efficiently or accurately translated in a target species (*e.g.* in humans).

[0064] In some embodiments, optimisation of a sequence for expression in a subject

also includes the removal of repeat sequences. For example, in some embodiments, repeat sequences of 8 or more bases are removed from the peanut allergen sequence. This may be particularly important if the sequence is constructed synthetically by back translation. Synthetic sequences generally lack the benefit of codon optimization through evolution. Therefore, disrupting randomly occurring destabilizing repeat sequences within the sequence by changing nucleotide bases without changing the amino acid sequence may improve expression of the sequence.

[0065] In some embodiments, the proteasome degradation tag is encoded by a nucleic acid sequence according to SEQ ID NO: 1 or a homologue thereof. In some embodiments, the peanut allergens of the fusion protein are encoded by a nucleic acid sequence according to SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, and/or SEQ ID NO: 9, or a homologue of any one of the foregoing.

[0066] In some embodiments, the proteasome degradation tag comprises an amino acid sequence according to SEQ ID NO: 2. In some embodiments, the peanut allergens of the fusion protein comprise an amino acid sequence according to SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, and/or SEQ ID NO: 10.

[0067] As conformational epitopes are not required for MHC-1 presentation and in some respects unwanted in order to prevent allergen-specific IgE antibody binding, the allergens expressed as part of the fusion protein are not required to be in their native structural form. This can allow for fusion proteins including multiple peanut allergens to be used and provides flexibility in the design of the fusion protein.

[0068] Accordingly, in some embodiments, the nucleic acid construct operably encodes 2 or more peanut allergens. For example, the fusion protein may encode 2, 3, 4, 5, 6, 7, 8, 9, 10 or more peanut allergens. For some nucleic acids, at least one of the allergens may be selected from the following peanut allergens or homologues thereof: ara h 1, ara h 2, ara h 3, ara h 4, ara h 5, ara h 6, ara h 7, ara h 8, ara h 9, ara h 10 or ara h 11. In an illustrative example, the nucleic acid construct operably encodes ara h 1, ara h 2, ara h 3 and ara h 6, or homologues thereof. For example, the nucleic acid construct may include a nucleic acid sequence according to SEQ ID NO: 11 or may encode a protein with an amino acid sequence according to SEQ ID NO: 12.

[0069] Each allergen may be fused to its own proteasome degradation tag and may be

operably connected to its own promoter (*e.g.* multiple fusion proteins may be expressed). Alternatively, the sequences for the proteasome degradation tag and the allergens may be arranged to allow for expression of a fusion protein including a proteasome degradation tag and the multiple allergens. This latter approach can prevent differential expression of the different allergens and/or prevent intramolecular recombination if multiple expression cassettes are used with identical promoters.

[0070] To allow translation of a fusion protein with 2 or more allergens, the nucleic acid may be devoid of stop codons between two sequences encoding peanut allergens. In some embodiments, the nucleic acid sequence may be devoid of stop codons between any of the sequences encoding peanut allergens and/or may be devoid of stop codons between the sequence encoding the proteasome degradation tag and a sequence encoding an allergen.

[0071] To drive translation, the sequence encoding the first part of the fusion protein may include a start codon at the 5' end of the sequence. Start codons may be absent from the sequence encoding the rest of the fusion protein. In this regard, expression of allergens that are not fused to the proteasome degradation tag may be minimized or prevented. This can minimize or prevent intact peanut allergens from being secreted from the cell or presented on the surface of the cell, which could otherwise stimulate a T_H2 immune response against the allergen.

[0072] In an embodiment, the fusion protein comprises the amino acid sequence of SEQ ID NO: 12 or an amino acid sequence having at least 70% identity thereto.

[0073] In another embodiment, the fusion protein is encoded by the nucleic acid sequence of SEQ ID NO: 11 or a nucleic acid sequence having at least 70% identity thereto.

[0074] In some embodiments, and in order to facilitate expression of the fusion protein as an intact protein and reduce differential expression of each allergen, the vector comprises a transcription control sequence (such as a promoter) and single start codon to facilitate expression of the intact fusion protein.

[0075] In some aspects, the present invention provides a nucleic acid cassette for desensitizing or inducing tolerance in a subject to a peanut allergen, the cassette including: i) the vaccine as described herein and ii) a terminal restriction enzyme linker at each end of

the sequence of the cassette. In some embodiments, at least one terminal restriction enzyme linker includes a Pac1 restriction enzyme recognition/cleavage sequence. In some embodiments the cassette is a viral vector cassette.

[0076] The nucleic acid construct may advantageously include a transcriptional control sequence operably connected to the nucleic acid sequence encoding the fusion protein.

[0077] The term "transcriptional control sequence" is to be understood to include any nucleic acid sequence which effects the transcription of an operably connected nucleic acid. Suitable transcriptional control sequences would be known to persons skilled in the art. Illustrative examples include a leader, polyadenylation sequence, promoter, enhancer or upstream activating sequence, and transcription terminator. Typically, a transcriptional control sequence at least includes a promoter. The term "promoter" as used herein, describes any nucleic acid which confers, activates or enhances expression of a nucleic acid molecule in a cell.

[0078] In some embodiments, at least one transcriptional control sequence is operably connected to the nucleic acid encoding the fusion protein. For the purposes of the present invention, a transcriptional control sequence is regarded as "operably connected" to a given gene or nucleotide sequence when the transcriptional control sequence is able to promote, inhibit or otherwise modulate the transcription of the gene or other nucleotide sequence.

[0079] A promoter may regulate the expression of an operably connected nucleotide sequence constitutively, or differentially, with respect to the cell, tissue, organ or developmental stage at which expression occurs, in response to external stimuli such as physiological stresses, pathogens, or metal ions, amongst others, or in response to one or more transcriptional activators. As such, the promoter used in accordance with the vaccine and/or methods of the present invention may include, for example, a constitutive promoter, an inducible promoter, a tissue-specific promoter or an activatable promoter. The present invention contemplates the use of any promoter which would be active in a cell of interest.

[0080] "Tissue specific promoters" include promoters which are preferentially or specifically expressed in one or more specific cells, tissues or organs in an organism and/or one or more developmental stages of the organism. It should be understood that a tissue specific promoter also be constitutive or inducible.

[0081] The promoter may also be a promoter that is activatable by one or more transcriptional activators, referred to herein as an "activatable promoter". For example, the activatable promoter may comprise a minimal promoter operably connected to an Upstream Activating Sequence (UAS), which comprises, *inter alia*, a DNA binding site for one or more transcriptional activators.

[0082] As referred to herein the term "minimal promoter" should be understood to include any promoter that incorporates at least a RNA polymerase binding site and, optionally a TATA box and transcription initiation site and/or one or more CAAT boxes.

[0083] As set out above, the activatable promoter may comprise a minimal promoter fused to an Upstream Activating Sequence (UAS). The UAS may be any sequence that can bind a transcriptional activator to activate the minimal promoter. Exemplary transcriptional activators include, for example: yeast derived transcription activators such as Gal4, Pdr1, Gcn4 and Ace1; the viral derived transcription activator, VP16; Hap1 (Hach *et al.*, *J Biol Chem* 278: 248-254, 2000); Gaf1 (Hoe *et al.*, *Gene* 215(2): 319-328, 1998); E2F (Albani *et al.*, *J Biol Chem* 275: 19258-19267, 2000); HAND2 (Dai and Cserjesi, *J Biol Chem* 277: 12604-12612, 2002); NRF-1 and EWG (Herzig *et al.*, *J Cell Sci* 113: 4263-4273, 2000); P/CAF (Itoh *et al.*, *Nucl Acids Res* 28: 4291 - 4298, 2000); MafA (Kataoka *et al.*, *J Biol Chem* 277: 49903-49910, 2002); human activating transcription factor 4 (Liang and Hai, *J Biol Chem* 272: 24088 - 24095, 1997); Bcl10 (Liu *et al.*, *Biochem Biophys Res Comm* 320(1): 1-6, 2004); CREB-H (Omori *et al.*, *Nucl Acids Res* 29: 2154 - 2162, 2001); ARR1 and ARR2 (Sakai *et al.*, *Plant J* 24(6): 703-711, 2000); Fos (Szuts and Bienz, *Proc Natl Acad Sci USA* 97: 5351-5356, 2000); HSF4 (Tanabe *et al.*, *J Biol Chem* 274: 27845 - 27856, 1999); MAML1 (Wu *et al.*, *Nat Genet* 26: 484-489, 2000).

[0084] The transcriptional control sequence may also include a terminator. The term "terminator" refers to a DNA sequence at the end of a transcriptional unit which signals termination of transcription. Terminators are 3'-non-translated DNA sequences generally containing a polyadenylation signal, which facilitate the addition of polyadenylate sequences to the 3'-end of a primary transcript. As with promoter sequences, the terminator may be any terminator sequence which is operable in the cells, tissues or organs in which it is intended to be used. In some embodiments, the nucleic acid sequence may include a viral early transcriptional stop sequence 3' of the sequence encoding the fusion protein.

Vectors

[0085] In an embodiment, the nucleic acid construct is operably incorporated in a vector.

[0086] In some embodiments, the vector may be an expression vector adapted for expression in a eukaryotic cell. As used herein, a "vector" may be any of a number of nucleic acids into which a desired sequence may be inserted. Vectors include, but are not limited to, plasmids, phagemids and virus genomes. In some embodiments, the expression vector is also able to be replicated in a host cell (*e.g.* a bacterial cell), and may also further comprise one or more endonuclease restriction sites at which the vector may be cut in a determinable fashion and into which a desired DNA sequence may be ligated such that the recombinant vector retains its ability to replicate in the host cell. In the case of plasmids, replication of the desired sequence may occur many times as the plasmid increases in copy number within the host bacterium or just a single time per host before the host reproduces by mitosis. In the case of phage, replication may occur actively during a lytic phase or passively during a lysogenic phase.

[0087] Expression vectors may contain transcriptional control sequences to drive expression of inserted nucleic acids in target cells (*e.g.* in a human cell). Transcriptional control sequences include those described above and include, for example, promoters.

[0088] Vectors may further contain one or more selectable marker sequences suitable for use in the identification of cells which have or have not been transformed or transfected with the vector. Markers include, for example, genes encoding proteins which increase or decrease either resistance or sensitivity to antibiotics or other compounds, genes which encode enzymes whose activities are detectable by standard assays known in the art (*e.g.*, β-galactosidase, luciferase), and genes which visibly affect the phenotype of transformed or transfected cells, hosts, colonies or plaques (*e.g.*, various fluorescent proteins such as green fluorescent protein, GFP). Some vectors may be capable of autonomous replication, also referred to as episomal vectors. Alternatively vectors may be adapted to insert into a chromosome, so called integrating vectors. The vector may be provided with transcription control sequences (promoter sequences) which mediate cell/tissue specific expression. These promoter sequences may be cell/tissue specific, inducible or constitutive.

[0089] In some embodiments, the vector may be a viral vector. Suitable viral vectors

would be known to persons skilled in the art. Illustrative examples of viral vectors include a retroviral vector, a lentiviral vector, an adenoviral vector, an adeno-associated viral vector, or a poxvirus viral vector. Poxviral vectors may include, for example, an avipox viral vector (*e.g.* fowlpox or canary pox). In some embodiments, the poxvirus viral vector may be a replication restricted viral vector including, for example, Modified Vaccinia Ankara (MVA) virus, an avipox virus or a crippled vaccinia virus. Use of viral vectors may be beneficial in further biasing a T_H1 response against cells expressing the degraded peanut allergen peptide fragments on MHC Class I molecules as the viral vector itself may promote IL-12 receptor expression on the cells. Furthermore, the activation of immune cells by viral vectors may initiate a complex network of cell-cell interactions and cytokine production cascades that result in the overall enhancement of T_H1 immune functions in an antigen-dependant manner.

[0090] In an embodiment, the viral vector is a poxvirus viral vector.

[0091] In some embodiments, the nucleic acid sequence includes a viral early transcriptional stop sequence 3' of the sequence encoding the fusion protein.

[0092] To facilitate cloning, the nucleic acid construct may be included in a nucleic acid cassette (*i.e.*, an expression cassette). Accordingly, in some embodiments, the present invention provides a nucleic acid cassette for desensitizing a subject to a peanut allergen, the cassette including: the nucleic acid construct operably encoding the fusion protein as described herein and a terminal restriction enzyme linker at each end of the sequence of the cassette.

[0093] The term "nucleic acid cassette" as used herein is intended to mean a nucleic acid sequence designed to introduce a nucleic acid molecule (*e.g.*, the nucleic acid construct as described herein) into a vector or genome.

[0094] The cassette will typically include a terminal restriction enzyme linker at each end of the sequence of the cassette. The terminal restriction enzyme linkers at each end may be the same or different terminal restriction enzyme linkers. In some embodiments, having the same terminal restriction enzyme linkers at each end can be advantageous if replication of the cassette in bacterial cells is desired (and the cassette includes an origin of replication) as the cassette may be circularized by digesting the cassette with the appropriate restriction enzyme and ligating the ends together. Similarly, a circular cassette

may be linearised by digesting the cassette with a single restriction enzyme.

[0095] In some embodiments, the terminal restriction enzyme linkers may include rare restriction enzyme recognition/cleavage sequences, such that unintended digestion of the nucleic acid or the vector or genome into which the cassette is to be introduced does not occur. In some embodiments, the terminal restriction enzyme linkers include a Pac1 restriction enzyme recognition/cleavage sequence.

[0096] The cassette may be cloned into a mammalian expression vector, a bacterial expression or cloning vector, an insect expression vector, a plant expression vector or a viral vector. Accordingly, the cassette may be a mammalian vector cassette, a bacterial vector cassette, an insect vector cassette, a plant vector cassette or a viral vector cassette.

Treatment and prevention of peanut allergy

[0097] The present inventors have surprisingly found that the vaccine of the present invention produces a biased anti-peanut protein T_H1 immune response, which will dominate over an existing allergen-specific T_H2 immune response and, in doing so, will desensitize an individual to subsequent exposure to the peanut allergen. Furthermore, expression of T_H1 cytokines (*e.g.* IFNγ, IL-12, TGF-β, IL2, *etc.*) can reduce the expression of T_H2 cytokines (*e.g.* IL-3, IL-4, IL-5, IL6, IL10, *etc.*), biasing the immune response against the allergen towards a T_H1 immune response, the result of which is the inhibition or amelioration of the activation and/or recruitment of IgE antibody producing B cells, mast cells and eosinophils, thereby reducing or preventing an allergic reaction to subsequent allergen exposure (*e.g.*, anaphylactic reactions). Accordingly, the vaccine of the present invention is suitable for use in the treatment of a peanut allergy in a subject.

[0098] The present inventors have also surprisingly found that the vaccine of the present invention produces a biased T_H1 immune response to peanut allergen that is independent of a pre-existing peanut allergy. Accordingly, the vaccine of the present invention is suitable for use in the prevention of a peanut allergy in a subject who may be at risk thereof.

[0099] Thus, in another aspect, there is provided use of the poxvirus vector disclosed herein in, or in the manufacture of a medicament for, inducing tolerance in a subject to a peanut allergen.

[0100] In an embodiment, the poxvirus vector disclosed herein is used as a

prophylactic to prevent or ameliorate peanut allergy in a subject at risk of developing a peanut allergy (*i.e.* tolerance may be induced in a subject at risk of developing allergy to a peanut allergen). Subjects at risk of developing a peanut allergy may include people already suffering from an allergy such as hayfever, asthma or other food allergies or people that have a family history of allergies.

[0101] In another aspect, there is provided a method of inducing tolerance in a subject to a peanut allergen, the method comprising administering to a subject in need thereof an effective amount of the poxvirus vector disclosed herein for a time and under conditions sufficient to elicit suppression and/or tolerance, for example, by inducing a peanut allergen-specific T_H1 response in the subject.

[0102] The terms "allergic reaction", "allergy", "allergic disorder" and the like, as used herein, are to be understood as meaning an immune disorder in which the immune system is hypersensitive to otherwise harmless environmental substances. These environmental substances that cause allergies are called "allergens." Common allergies include seasonal rhinoconjuctivitis (*e.g.*, allergies to grasses and pollen such as ragweed, timothy grass), allergies to pet dander such as cat dander or dog dander, food allergies such as peanut, dairy and wheat allergies, venom anaphylaxis, and asthma. An allergic disorder is typically characterised by the production of IgE.

[0103] Allergic diseases result from immune responses against otherwise harmless environmental antigens, characterised by the generation of T_H2 T cells, which produce IL-4 and IL-5 and promote the differentiation of B cells into IgE antibody secreting cells. IgE antibodies bind to high affinity receptors on basophils and mast cells. Allergen exposure leads to binding of allergen molecules by surface IgE and cross linking of the receptors thus causing activation and degranulation of basophils and mast cells. The latter release a variety of preformed proinflammatory and vasoactive compounds such as histamine, prostaglandins, leukotriens and cytokines, leading to inflammatory response. Binding of peanut allergen to the IgE antibodies that are bound to the surface of mast cells and basophils is the initiating event that eventually culminates in an allergic reaction. Preventing allergen binding to mast cell- and/or basophil-bound IgE will prevent the onset of an allergic reaction. The prevention of allergen specific IgE production upon exposure to peanut allergen will induce tolerance to peanut.

[0104] The term “tolerance”, as used herein, is taken to mean an inhibition (partial or

complete) of an allergic reaction to peanut allergen exposure. Inhibition may be prevention, retardation, reduction, abrogation or otherwise hindrance of an allergic reaction. Such inhibition may be in magnitude and/or be temporal in nature. In particular contexts, the terms "inhibit" and "prevent", and variations thereof may be used interchangeably. Tolerance can be assessed by any means known to persons skilled in the art. As an illustrative example, a skin-prick test can be used to measure the subject's response to an allergen or multiple allergens, before and/or after treatment with the poxvirus vector disclosed herein. For example, in a subject who is allergic to peanuts, a skin-prick test using one or more peanut allergens will typically produce an observable localised allergic response characterised by a localised rash, urticaria and/or swelling. Tolerance in the same individual following treatment with the poxvirus vector disclosed herein will typically manifest itself as a reduced localised allergic reaction to the skin-prick test. This reduction can be measured, for example, by the difference in size (*e.g.*, diameter) of the localised allergic reaction before and after treatment.

[0105] In another illustrative example, tolerance is assessed by the prevention, retardation, inhibition, reduction, abrogation or hindrance of the severity of allergic response following accidental exposure to a peanut allergen. For example, where a subject has a history of anaphylactic responses to peanut allergen exposure, tolerance as a result of treatment with the poxvirus vector in accordance with the present invention may be determined by the absence of an anaphylactic reaction following subsequent peanut allergen exposure, even though the subject may show other signs of an allergic reaction, such as a rash.

[0106] In another illustrative example, tolerance is assessed by determining the level of circulating peanut allergen-specific IgE antibodies in a subject. For instance, a subject who has a history of allergic reactions (including anaphylactic responses) to peanut allergen exposure will typically have a higher level of peanut allergen-specific IgE antibodies as compared, for example, to a subject who does not have a peanut allergy. In such individuals, tolerance may be determined by a reduction in the level of circulating peanut allergen-specific IgE antibodies following treatment with the vaccine of the present invention. Alternatively, or in addition, tolerance may be determined by a higher level of circulating peanut allergen-specific IgG antibodies following treatment with the poxvirus vector of the present invention, which is characteristic of a T_H1 immune response and

typically indicative of a tolerant state.

[0107] Alternatively, or in addition, tolerance may be determined by assessing the cytokine profile in a sample obtained from the subject (e.g., a blood sample, including a plasma or serum sample). For example, a higher level of IFN-gamma is indicative of a bias towards an allergen-specific T_H1 response, whereas a higher level of IL-4 and/or IL-5 is indicative of a bias towards an allergen-specific T_H2 response.

[0108] Alternatively, or in addition, tolerance may be determined by obtaining a sample of T lymphocytes from a subject who has been treated with the poxvirus vector in accordance with the present invention, as disclosed herein, and measuring the cytokine profile of the lymphocytes *ex vivo*. For example, a higher level of IFN-gamma production by the T lymphocytes is indicative of a bias towards an allergen-specific T_H1 response, whereas a higher level of IL-4 and/or IL-5 production by the T lymphocytes is indicative of a bias towards an allergen-specific T_H2 response. Methods of measuring the level of peanut allergen-specific IgE and/or IgG antibodies and cytokines that are capable of differentiating between a T_H1 and T_H2 response would be know to persons skilled in the art. Illustrative examples include radioimmunoassays (RIA) and enzyme linked immunosorbant assays (ELISA).

[0109] It would be understood by persons skilled in the art that the poxvirus vector disclosed herein is to be administered in either in a single dose or as part of a series of doses that provides the desired therapeutic or prophylactic effect in a subject in need thereof; namely, the induction of tolerance to a peanut allergen. Undesirable effects, *e.g.* side effects, may sometimes manifest along with the desired therapeutic and/or prophylactic effect; hence, a practitioner will generally balance the potential benefits against the potential risks in determining an appropriate effective amount. The exact amount of vaccine required will vary from subject to subject, depending on the species, age and general condition of the subject, mode of administration and the like. Thus, it may not be possible to specify an exact effective amount. However, an appropriate effective amount in any individual case may be determined by one of ordinary skill in the art using routine skills or experimentation. One of ordinary skill in the art would be able to determine the required amounts based on such factors as the subject's size and weight, the severity of a subject's symptoms, and the proposed route of administration.

[0110] The term "treatment" refers to any measurable or statistically significant

inhibition or amelioration in at least some subjects in one or more symptoms of peanut allergy.

[0111] In some embodiments, the poxvirus vector disclosed herein is exploited to desensitise a subject with a peanut allergy (*i.e.* a subject who is hypersensitive to one or more peanut allergens) to one or more peanut allergens. The term "desensitizing a subject" as used herein with reference to a peanut allergen is intended to mean that the sensitivity of the subject to the peanut allergen is reduced, ameliorated or eliminated. In this regard, symptoms of a peanut allergy in a subject are partially or completely reduced upon re-exposure to one or more peanut allergens.

[0112] In some embodiments, alternatively, or in addition, the nucleic acid sequence is exploited to induce tolerance in a subject to one or more peanut allergens. Induction of tolerance to the one or more peanut allergens is performed in a subject with a peanut allergy or in a subject who may be at risk of developing a peanut allergy (*i.e.* the nucleic acid may be exploited as part of a prophylactic treatment of peanut allergy).

[0113] While the poxvirus vector disclosed herein is exploited in different ways to desensitize or induce tolerance in a subject to a peanut allergen (as described herein), the general principle by which the poxvirus vector operates is the same. When the fusion peptide is expressed in a cell, it is targeted to proteasomal degradation by virtue of the proteasome degradation tag, which prevents the intact fusion protein from being secreted from the cell.

[0114] In an embodiment, there is provided a method of vaccinating a subject to induce tolerance to a peanut allergen comprising administering the poxvirus vector as disclosed herein. In a particular embodiment, the method is for inducing tolerance against at least two or at least three major peanut allergens.

[0115] The present invention extends to kits comprising the poxvirus vector, as disclosed herein.

[0116] The poxvirus vector of the present invention may be delivered to a cell *in vivo* or *ex vivo* (*e.g.* as naked DNA or in a vector) by methods known in the art. Illustrative examples include viral delivery, microinjection, gene gun, impalefection, hydrostatic pressure, electroporation, sonication, and/or lipofection. The poxvirus vector may also be delivered to a cell as a pharmaceutical composition.

[0117] Liposomes may serve as a carrier for the poxvirus vector. Liposomes are lipid-based vesicles which encapsulate a selected therapeutic agent (*e.g.* a vector) which is then introduced into a patient. The liposome may be manufactured either from pure phospholipid or a mixture of phospholipid and phosphoglyceride. Typically, liposomes can be manufactured with diameters of less than 200 nm, which enables them to be intravenously injected and able to pass through the pulmonary capillary bed. Furthermore, the biochemical nature of liposomes confers permeability across blood vessel membranes to gain access to selected tissues.

[0118] The poxvirus vector may be naked, that is, unassociated with any proteins or other agents which may affect the recipients' immune system. In this case, it is desirable for the poxvirus vector be in a physiologically acceptable solution, such as, but not limited to, sterile saline or sterile buffered saline. Alternatively, the vaccine may be associated with liposomes, such as lecithin liposomes or other liposomes known in the art. Agents which assist in the cellular uptake of nucleic acid molecules, such as, but not limited to, calcium ions, may also be used.

[0119] In the case of non-viral vectors, the amount of nucleic acid to be introduced into a recipient will have a very broad dosage range and may depend, for example, on the strength of the transcriptional and translational promoters used. In addition, the magnitude of the immune response may depend on the level of protein expression and on the immunogenicity of the expressed fusion protein product. An effective dose range may include about 1 ng to 5 mg, about 100 ng to 2.5 mg, about 1 μg to 750 μg, or about 10 μg to 300 μg of the nucleic acid (*e.g.* as part of a poxvirus vector).

[0120] The poxvirus vector may be administered or inoculated, subcutaneously, intramuscularly, intradermally, or by other modes such as intraperitoneal, intravenous, or inhalation, in the presence of adjuvants or other substances that have the capability of promoting DNA uptake or recruiting immune system cells to the site of inoculation. The chosen route of administration will depend on the composition and the disease status of patients. Relevant considerations include the types of immune cells to be activated, the time which the antigen is exposed to the immune system and the immunization schedule. It is also contemplated that booster treatments may be provided.

[0121] As described herein, the poxvirus vector is able to desensitize (*i.e.*, induce

tolerance in) a subject by expression of the fusion protein in a cell. The fusion protein is degraded within the cell and the degraded peanut allergen fragments are expressed on the cell surface in association with MHC Class I molecules. In some embodiments, no intact expressed peanut allergen is exposed to the subject's immune system during the methods of the present invention. This is as a result of the proteasome degradation tag, which drives the intracellular proteasomal degradation of the expressed fusion protein.

[0122] The method of desensitizing or inducing tolerance in a subject to a peanut allergen may involve administering the poxvirus vector, or a pharmaceutical composition including the poxvirus vector to the subject. Accordingly, the present invention provides a method of desensitizing a subject to a peanut allergen, wherein the method includes expressing the fusion protein in a cell of the subject, wherein the proteasome degradation tag of the expressed fusion protein targets the fusion protein for intracellular proteasomal degradation and association of the degraded peptides of the peanut allergen with MHC class I molecules to promote generation of a T_H1 response to the peanut allergen, thus desensitizing or inducing tolerance in the subject to the peanut allergen.

[0123] The present invention also provides a prophylactic treatment method for inducing tolerance to a peanut allergen in a subject, wherein the method includes expressing the fusion protein in a cell of the subject, wherein the proteasome degradation tag of the expressed fusion protein targets the fusion protein for intracellular proteasomal degradation and association of the degraded peptides of the peanut allergen with MHC class I molecules to promote generation of a T_H1 response to the peanut allergen, thus preventing sensitivity of the subject to the peanut allergen.

[0124] While these methods may involve expressing the fusion protein in a cell *in vivo*, other methods may include expressing the fusion protein in a cell *ex vivo*. As such, the present invention also provides a cell expressing the fusion protein. In this regard, the cell may be used for *in vitro* experiments, *in vivo* treatment and/or *ex vivo* treatments.

Subject

[0125] The terms "*subject*," "*individual*" and "*patient*" are used interchangeably herein to refer to any subject to which the present disclosure may be applicable, particularly a vertebrate subject, and even more particularly a mammalian subject. Suitable vertebrate animals that fall within the scope of the invention include, but are not restricted to, any

member of the subphylum Chordata including primates, rodents (*e.g.*, mice rats, guinea pigs), lagomorphs (*e.g.*, rabbits, hares), bovines (*e.g.*, cattle), ovines (*e.g.*, sheep), caprines (*e.g.*, goats), porcines (*e.g.*, pigs), equines (*e.g.*, horses), canines (*e.g.*, dogs), felines (*e.g.*, cats), avians (*e.g.*, chickens, turkeys, ducks, geese, companion birds such as canaries, budgerigars *etc*), marine mammals (*e.g.*, dolphins, whales), reptiles (snakes, frogs, lizards, *etc.*), and fish. In some embodiments, the subject is a primate (*e.g.*, a human, ape, monkey, chimpanzee).

[0126] In a preferred embodiment, the subject is a human. Accordingly, in some embodiments, the nucleic acid sequence encoding the fusion protein is codon optimized for expression in human cells.

Pharmaceutical compositions

[0127] The poxvirus vector according to the present invention may be provided in a form comprising a pharmaceutically or physiologically acceptable carrier and/or diluent.

[0128] Thus, in another aspect, there is provided a pharmaceutical composition for desensitizing or inducing tolerance in a subject to a peanut allergen, the composition comprising the poxvirus vector disclosed herein and a pharmaceutically acceptable carrier.

[0129] Pharmaceutical compositions are conveniently prepared according to conventional pharmaceutical compounding techniques. See, for example, Remington's Pharmaceutical Sciences, 18th Ed., Mack Publishing, Company, Easton, PA, U.S.A., 1990. These compositions may comprise, in addition to one of the active substances, a pharmaceutically acceptable excipient, carrier, buffer, stabilizer or other materials well known in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The carrier may take a wide variety of forms depending on the form of preparation desired for administration, *e.g.* intravenous, oral or parenteral.

[0130] In some embodiments, the present invention provides a method of desensitizing or inducing tolerance in a subject to a peanut allergen, the method including expressing the fusion peptide as described herein in a cell of the subject, wherein the proteasome degradation tag of the expressed fusion protein targets the fusion protein for intracellular proteasomal degradation and association of the degraded peptides of the peanut allergen with MHC class I molecules to promote generation of a T_H1 response to the peanut allergen, thus desensitizing or inducing tolerance in the subject to the peanut allergen.

[0131] In some embodiments, the present invention provides a prophylactic treatment method for inducing tolerance to a peanut allergen in a subject, the method including expressing the fusion peptide as described herein in a cell of the subject, wherein the proteasome degradation tag of the expressed fusion protein targets the fusion protein for intracellular proteasomal degradation and association of the degraded peptides of the peanut allergen with MHC class I molecules to promote generation of a T_H1 response to the peanut allergen, thus preventing sensitivity of the subject to the peanut allergen.

[0132] The present inventors have surprisingly found that a poxvirus vector comprising a nucleic acid sequence encoding a fusion protein comprising peanut allergens and a proteasome degradation tag, can, upon vaccination, produce a peanut-specific T_H1 immune response, as measured by the production of peanut allergen-specific IgG2a antibodies and peanut allergen-induced secretion of T_H1 cytokines from lymphocytes. As this poxvirus vector stimulated a peanut allergen-specific T_H1 immune response, it follows that the poxvirus vector disclosed herein can be used to desensitize (*i.e.*, induce tolerance in) subjects who are allergic to peanut allergens.

[0133] The present invention also provides a nucleic acid sequence for desensitizing or inducing tolerance in a subject to a peanut allergen, the nucleic acid including a sequence encoding a fusion protein, the fusion protein including a proteasome degradation tag and a peanut allergen. The nucleic acid may be used as a genetic vaccine.

[0134] As described herein, in some embodiments, the nucleic acid is included in an expression vector (*e.g.*, a viral vector) or pharmaceutical composition which can be administered to a subject to allow expression of the ubiquitinated fusion protein in a cell *in vivo*. Alternatively, the nucleic acid is expressed in an *ex vivo* cell (*e.g.*, an antigen presenting cell) that may then be administered to a subject. Alternatively, or in addition, the transfected cell can be used to stimulate and expand a T_H1 lymphocyte population *ex vivo*, which are then administered to the subject.

[0135] In some embodiments, establishment of T_H1 memory to the presented peptides of the peanut allergen can prevent or reduce T_H2 immune responses against the peanut allergen upon subsequent expose to a peanut allergen. In some embodiments, T_H1 memory against the peanut allergen is established by the activation and maintenance of peanut allergen specific $CD8^+$ T cells.

Cells

[0136] In another aspect of the present invention, there is provided a cell expressing the fusion protein as described herein, such as a host cell or an antigen presenting cell (*e.g.*, a dendritic cell). The transfected cell expressing the fusion protein can then be used to generate and/or expand a peanut allergen reactive T_H1 lymphocyte population *in vivo* or *ex vivo*. Thus, in an embodiment, the present disclosure enables a method of generating and/or expanding a peanut allergen reactive T_H1 lymphocyte population *ex vivo*, the method comprising culturing the cell (*i.e.*, a transfected cell expressing the fusion protein) as described herein with one or more T lymphocytes. In another embodiment, the present disclosure enables a method of generating and/or expanding a peanut allergen reactive T_H1 lymphocyte population *in vivo*, the method comprising administering a transfected cell as described herein in a subject in need thereof, wherein the administered transfected cell activates naïve T cells in the subject to become peanut allergen-specific T_H1 cells.

[0137] In some embodiments, the present invention provides a method of desensitizing or inducing tolerance in a subject to a peanut allergen, the method comprising: i) collecting lymphocytes from the subject; ii) co-culturing the lymphocytes with cells as described herein (*i.e.*, transfected cells expressing the fusion protein disclosed herein) to generate and/or expand a T_H1 lymphocyte population that recognizes the proteasomally degraded peanut allergen fusion protein associated with MHC Class I molecules on the cells; and iii) administering the T_H1 lymphocytes from (ii) to the subject.

[0138] In some embodiments, the cell may include a prokaryotic cell (*e.g.* a bacterial cell). The prokaryotic cell may be used to replicate the nucleic acid construct (*e.g.* in vector form) and/or in various cloning steps. In some embodiments, the cell may include a eukaryotic cell (*e.g.* a mammalian cell). In this regard, the present invention also includes a cell expressing the nucleic acid construct operably encoding the fusion protein.

[0139] The poxvirus vector as disclosed herein can also be used to activate naïve antigen presenting cells, which can then be reintroduced back into the subject to activate naïve T cells to become peanut allergen- specific T_H1 cells. Thus, in some embodiments, the present invention provides a method of desensitizing or inducing tolerance in a subject to a peanut allergen, the method comprising: i) collecting antigen presenting cells from the subject; ii) co-culturing the antigen presenting cells with the cells as described herein (*i.e.*,

transfected cells expressing the fusion protein disclosed herein) to generate and/or expand a population of activated T_H1 antigen presenting cell population; and iii) administering the activated T_H1 antigen presenting cell from (ii) to the subject to activate T lymphocytes towards an allergen-specific T_H1 phenotype. Suitable naïve antigen presenting cells would be known to persons skilled in the art. Illustrative examples include dendritic cells and fibroblasts.

[0140] The cell type expressing the fusion protein is only limited in that the cell should be a nucleated cell that expresses an MHC Class I molecule. In this regard, the cell may be a cell from a cell line (*e.g.* a CHO cell line, HEK cell line, fibroblast cell line, *etc.*) or a primary cell (*e.g.*, a fibroblast, a dendritic cell). In embodiments whereby the cell is intended as an *ex vivo* autologous treatment, the cell may be cell which may be readily removed from a subject (*e.g.* a cell in blood, lymph, bone marrow) and/or readily cultured from a tissue sample (*e.g.* fibroblast cells). In some embodiments, the cell may be a professional antigen presenting cell (*e.g.* a dendritic cell, macrophage, B-cell, epithelial cell, *etc.*) or may be a non-professional antigen presenting cell (*e.g.* a fibroblast, thymic epithelial cell, thyroid epithelial cell, glial cell, pancreatic beta cell, vascular endothelial cell, *etc.*).

[0141] Expressing the fusion protein in a cell *ex vivo* (*e.g.*, transfecting the cell with the poxvirus vector disclosed herein) can be advantageous in that the number of cells expressing the nucleic acid may be controlled. Furthermore, a wider range of nucleic acid delivery systems are available for cells *ex vivo*. The cells expressing the fusion protein (*i.e.*, the transfected cells) may then be administered to a subject to activate naïve T cells in the subject towards a peanut allergen-specific T_H1 phenotype, which can then desensitize or induce tolerance in the subject to one or more peanut allergens. Alternatively, the cells expressing the fusion protein may be cultured with lymphocytes *ex vivo* to generate peanut allergen reactive T_H1 lymphocytes, which may then be administered to the subject.

[0142] Accordingly, the present invention also provides a method of generating and/or expanding a peanut allergen reactive T_H1 lymphocyte *ex vivo*, wherein the method includes culturing a cell expressing the fusion protein with one or more T lymphocytes. The T lymphocytes may be included in a mixed lymphocyte population or may be isolated T lymphocytes. Mixed lymphocyte populations may be readily obtained from peripheral blood, lymph or bone marrow by methods known in the art. T cells may be isolated from

such mixed lymphocyte populations by methods known in the art including, for example, nylon wool isolation, FACS sorting, magnetic bead separation, *etc*. In some embodiments, particular T lymphocyte subsets may be isolated for culturing with the cell expressing the nucleic acid.

[0143] It would be understood by persons skilled in the art that, where cells are transfected *ex vivo* to express the fusion protein and/or where a population of T_H1 lymphocytes are generated and/or expanded *ex vivo*, as disclosed herein, it is often desirable to use autologous cells (*i.e.*, cells derived from the subject to be treated), thereby avoiding or minimising an immune response that may occur where allogeneic cells (*i.e.*, cells derived from a different subject) are used and administered to the subject.

[0144] *Ex vivo* expansion of peanut allergen reactive T_H1 lymphocyte may be used to generate large numbers of peanut allergen reactive T_H1 lymphocyte, which may then be administered to a subject as a prophylactic or therapeutic treatment of peanut allergy. In some instances, *ex vivo* expansion may accelerate the activation and expansion of peanut allergen reactive T_H1 lymphocytes compared with *in vivo* activation and expansion. Furthermore, *ex vivo* expansion allows control over the number and reactivity of peanut allergen reactive T_H1 lymphocytes that are expanded. In some embodiments, the peanut allergen reactive T_H1 lymphocytes may be autologous to the subject.

[0145] Accordingly, the present invention also provides a method of desensitizing a subject to a peanut allergen, the method including: (i) collecting lymphocytes from the subject; (ii) co-culturing the lymphocytes with cells expressing the fusion protein to generate and/or expand a T_H1 lymphocyte that recognizes proteasomally degraded fusion protein peptide fragments associated with MHC Class I molecules on the cells; and (iii) administering the T_H1 lymphocytes from (ii) to the subject. In some embodiments, the lymphocytes are collected from the subject before administration of the poxvirus vector as disclosed herein.

[0146] In some embodiments, the method may include isolating the lymphocytes from step (ii) prior to administration to the subject. Isolating the lymphocytes from step (ii) may include isolating all lymphocytes from the cells expressing the nucleic acid and/or may include isolating one or more lymphocyte types (*e.g.* all T cells lymphocytes, all T_H1 lymphocytes, *etc*.). Alternatively, the T_H1 lymphocytes from (ii) may be administered to the subject without isolating the lymphocytes from the cells expressing the fusion protein,

in which case the administered cells expressing the fusion protein may continue to activate further T_H1 lymphocytes *in vivo*. Methods for isolating lymphocytes from a subject, methods for isolating T cells and T cell subsets include those methods described above.

[0147] Also enabled herein are methods in which T lymphocytes are obtained, whether isolated or not, from the subject treated in accordance with the present invention, and determining whether the lymphocytes are biased towards a T_H1 phenotype, as disclosed herein (*e.g.*, determining the cytokine expression profile *ex vivo*). This approach has the added advantage of determining whether the administration of the poxvirus vector has induced a T_H1-biased allergen-specific immune response in the subject. Thus, in some embodiments, the method includes determining whether the lymphocytes isolated from step (ii) are biased towards a T_H1 phenotype prior to their administration to the subject.

[0148] In some embodiments, desensitization or tolerance induction of a subject to a peanut allergen may prevent or reduce hypersensitivity reactions against subsequent exposure of the subject to peanuts. As such, the methods described above may reduce the risk of anaphylactic reactions to peanuts in subjects previously allergic to peanuts upon subsequent exposure of the subject to peanuts and/or reduce the risk of anaphylactic reactions to peanuts in subjects at risk of developing a peanut allergy.

[0149] The present invention is further described by the following non-limiting examples. It is to be understood that the following description is for the purpose of describing particular embodiments only and is not intended to be limiting with respect to the above description.

EXAMPLES

Materials and Methods

[0150] ***Production of a PHAV Antigen:*** A nucleic acid sequence for a fusion protein (PHAV antigen) including a human Ubiquitin C monomer (Ubc) and four peanut allergens was designed as set out below and illustrated in Figure 1A.

[0151] The amino acid sequence for Ubc (NM_021009), ara h 1 (Swiss-Prot entry P43238), ara h 2 (TrEMBL entry Q8GV20), ara h 3 (Genbank Protein ACH91862) and ara h 6 (UniProtKB/TrEMBL entry Q647G9) were obtained from online protein sequence databases. The start codon amino acid Met (M) was removed from ara h 1, ara h 2, ara h 3 and ara h 6 protein sequences before joining the sequences to form one continuous protein

sequence in the order of: Ubc + ara h1+ ara h2+ ara h3 + ara h6. The DNA sequence coding this PHAVag protein was obtained by back translation using a *Homo Sapiens* codon preferred table.

[0152] The PHAVag amino acid sequence was back translated into a nucleotide sequence using Gene Designer (DNA2.0 Inc) and employed a *Homo Sapiens* codon optimisation set at a 10% threshold. Repeat sequences of 8 bases or more were also filtered out. The resulting sequence was further screened for secondary structure formation potential and destabilising elements by DNA2.0 Inc. The final nucleotide sequence of the PHAVag protein sequence was screened for the pox virus early transcriptional motif "TTTTTNT". However, none were found.

[0153] At the end of the nucleotide sequence coding for the PHAV antigen, a "TAA" stop codon was added. The Pox virus early transcriptional stop sequence TTTTTAT was also added immediately after the stop codon. The expression cassette was flanked with Pac I linkers. As Pac I recognition sites were not present within the cassette, this cassette could be cloned into plasmids and excised whole from a plasmid by Pac I restriction endonuclease digestion.

[0154] As shown in Table 1, Ubc, ara h 1, ara h 2, ara h 3 and ara h 6 in the PHAV Antigen had around 75% nucleic acid sequence identity to the native sequences.

Table 1: Sequence comparison of PHAV Antigen components and native sequences

	Nucleotide sequence comparison		Amino acid sequence comparison	
	Number of differences to native sequence/ total length	% identity to native sequence	Number of differences to native sequence/ total length	% identity to native sequence
Ubc	53/228	76.8%	1/76	98.7%
Ara h 1	440/1875	76.5%	0/625	100%
Ara h 2	124/513	75.8%	0/171	100%
Ara h 3	405/1587	74.5%	22/529	95.8%
Ara h 6	103/435	76.3%	0/145	100%

[0155] A summary of the nucleic acid and amino sequences of the PHAV Antigen construct and components thereof is set out in Table 2.

Table 2: Sequence Summary

	Name	Sequence Type
1	Ubc	Nucleic acid
2	Ubc	Amino acid
3	ara h 1	Nucleic acid
4	ara h 1	Amino acid
5	ara h 2	Nucleic acid
6	ara h 2	Amino acid
7	ara h 3	Nucleic acid
8	ara h 3	Amino acid
9	ara h 6	Nucleic acid
10	ara h 6	Amino acid
11	PHAV Antigen	Nucleic acid
12	PHAV Antigen	Amino acid

[0156] ***Production of an alternative PHAV Antigen:*** A ubiquitinated peanut hypo-allergy vaccine antigen (UBc.PHAVag) was made comprising a PHAV antigen protein sequence made up of a fusion of the following protein coding sequences – ubiquitin C monomer, the peanut allergen ara h 1, peanut allergen ara h 2, peanut allergen ara h 3 and peanut allergen ara h 6.), pox virus early transcriptional stop sequence and finally another Pac 1 linker.

[0157] The ubiquitin C monomer was modified at the C-terminal to replace the terminal Gly (G) residue with Ala (A). The modified ubiquitin C targets the PHAV antigen upon synthesis to the proteasomal degradation pathway in the host cell (see Figure 9). This ensures that no intact protein is presented for antibody production and that the resulting peptide fragments are processed by the MHC class I pathway, triggering a T_H1 immune response to the PHAV antigen.

[0158] The configuration and features of the PHAV expression cassettes are shown diagrammatically in Figure 1B and include Pac I restriction endonuclease linkers at the 5' and 3' ends, as well as a vaccinia early/late promoter at the 5'end.

[0159] The amino acid sequence for UBc, ara h 1, ara h 2, ara h 3 and ara h 6 were obtained from either Swit-Prot or EMBL protein databases. The start codon encoding a Met (M) residue was removed from the ara h 1, ara h 2, ara h 3 and ara h 6 nucleic acid sequences before joining then up to form the continuous nucleic acid sequence encoding the protein sequence UBc+h1+h2+h3+h6, in that order.

[0160] The DNA sequence for coding this UBc.PHAVag was obtained by back translation using a Homo Sapiens codon preferred table. The UBc.PHAVag amino acid sequence was back translated into a nucleotide sequence using Gene Designer (DNA2.0 Inc) and employing Homo Sapiens codon optimisation set at 10% threshold and filtering out repeat sequences of 8 bases or more. The resulting sequence was further screened for secondary structure formation potential and destabilising elements by DNA2.0 Inc. The final nucleotide sequence encoding UBc.PHAVag was screened for pox virus early transcriptional motif "TTTTTNT" – none were found. At the end of the nucleotide sequence coding for UBc.PHAV, "TAA" stop codon was added. The Pox virus early transcriptional stop sequence TTTTTAT was also added immediately after the stop codon. The expression cassette was flanked with Pac I linkers and because Pac I recognition sites are not present within the cassette, this cassette can be cloned into plasmids and excised whole from plasmid by Pac I restriction endonuclease digestion. The DNA sequence of the UBc.PHAV expression cassette can be found in Figure 2.

[0161] A peanut hypollergen vaccine antigen was also constructed in which the ubiquitin monomer at the 5' end was omitted. This construct was identical to the UBc.PHAVag construct, as described above, but without the ubiquitin sequence. This construct was referred to as PHAVag and a diagrammatic representation of the configuration and features of PHAVag can be found in Figure 1C and the DNA sequence found in Figure 3.

[0162] Both the UBc.PHAVag and PHAVag expression cassettes were cloned into bacterial plasmids so that these expression cassette could be retrieved after cloning by PacI/Sbf I digestion and gel purification.

[0163] Additional ubiquitinated peanut hypo-allergy vaccine antigens could be made that include the following peanut allergens:

(i) ara h 1, ara h 2, ara h 3, ara h 4, ara h 5, ara h 6, ara h 7, ara h 8, ara h 9, ara h 10 and ara h 11;

(ii) ara h 1, ara h 2, ara h 3, ara h 4, ara h 5, ara h 6, ara h 7, ara h 8, ara h 9 and ara h 10;

(iii) ara h 1, ara h 2, ara h 3, ara h 4, ara h 5, ara h 6, ara h 7, ara h 8 and ara h 9;

(iv) ara h 1, ara h 2, ara h 3, ara h 4, ara h 5, ara h 6, ara h 7 and ara h 8;

(v) ara h 1, ara h 2, ara h 3, ara h 4, ara h 5, ara h 6 and ara h 7;

(vi) ara h 1, ara h 2, ara h 3, ara h 4, ara h 5 and ara h 6;

(vii) ara h 1, ara h 2, ara h 3, ara h 4 and ara h 5;

(viii) ara h 1, ara h 2, ara h 3 and ara h 4;

(ix) ara h 1, ara h 2 and ara h 3;

(x) ara h 1 and ara h 2;

(xi) ara h 1;

(xii) ara h 2;

(xiii) ara h 2, ara h 3, ara h 4, ara h 5, ara h 6, ara h 7, ara h 8, ara h 9, ara h 10, and ara h 11;

(xiv) ara h 2, ara h 3, ara h 4, ara h 5, ara h 6, ara h 7, ara h 8, ara h 9 and ara h 10;

(xv) ara h 2, ara h 3, ara h 4, ara h 5, ara h 6, ara h 7, ara h 8 and ara h 9;

(xvi) ara h 2, ara h 3, ara h 4, ara h 5, ara h 6, ara h 7 and ara h 8;

(xvii) ara h 2, ara h 3, ara h 4, ara h 5, ara h 6 and ara h 7;

(xviii) ara h 2, ara h 3, ara h 4, ara h 5 and ara h 6;

(xix) ara h 2, ara h 3, ara h 4 and ara h 5;

(xx) ara h 2, ara h 3 and ara h 4;

(xxi) ara h 2 and ara h 3;

(xxii) ara h 3;

(xxiii) ara h 1, ara h 3, ara h 4, ara h 5, ara h 6, ara h 7, ara h 8, ara h 9, ara h 10 and ara h 11;

(xxiv) ara h 1, ara h 3, ara h 4, ara h 5, ara h 6, ara h 7, ara h 8, ara h 9 and ara h 10;

(xxv) ara h 1, ara h 3, ara h 4, ara h 5, ara h 6, ara h 7, ara h 8 and ara h 9;

(xxvi) ara h 1, ara h 3, ara h 4, ara h 5, ara h 6, ara h 7 and ara h 8;

(xxvii) ara h 1, ara h 3, ara h 4, ara h 5, ara h 6 and ara h 7;

(xxviii) ara h 1, ara h 3, ara h 4, ara h 5 and ara h 6;

(xxix) ara h 1, ara h 3, ara h 4 and ara h 5;

(xxx) ara h 1, ara h 3 and ara h 4;

(xxxi) ara h 1 and ara h 3;

(xxxii) ara h 1, ara h 2, ara h 4, ara h 5, ara h 6, ara h 7, ara h 8, ara h 9, ara h 10 and ara h 11;

(xxxiii) ara h 1, ara h 2, ara h 4, ara h 5, ara h 6, ara h 7, ara h 8, ara h 9 and ara h 10;

(xxxiv) ara h 1, ara h 2, ara h 4, ara h 5, ara h 6, ara h 7, ara h 8 and ara h 9;

(xxxv) ara h 1, ara h 2, ara h 4, ara h 5, ara h 6, ara h 7 and ara h 8;

(xxxvi) ara h 1, ara h 2, ara h 4, ara h 5, ara h 6 and ara h 7;

(xxxvii) ara h 1, ara h 2, ara h 4, ara h 5 and ara h 6;

(xxxviii) ara h 1, ara h 3, ara h 4 and ara h 5;

(xxxix) ara h 1, ara h 2 and ara h 4;

(xl) ara h 1, ara h 4, ara h 5, ara h 6, ara h 7, ara h 8, ara h 9, ara h 10 and ara h 11;

(xli) ara h 1, ara h 4, ara h 5, ara h 6, ara h 7, ara h 8, ara h 9 and ara h 10;

(xlii) ara h 1, ara h 4, ara h 5, ara h 6, ara h 7, ara h 8 and ara h 9;

(xliii) ara h 1, ara h 4, ara h 5, ara h 6, ara h 7 and ara h 8;

(xliv) ara h 1, ara h 4, ara h 5, ara h 6 and ara h 7;

(xlv) ara h 1, ara h 4, ara h 5 and ara h 6;

(xlvi) ara h 1, ara h 4 and ara h 5;

(xlvii) ara h 1 and ara h 4;

(xlviii) ara h 4; and so on.

[0164] The amino acid sequences for ara h1, h2, h3, h4, h5, h6, h7, h8, h9, h10 and h11 are readily obtained from either Swit-Prot or EMBL protein databases. The start codon encoding a Met (M) residue and also the stop codon would be removed from the ara h nucleic acid sequences before joining them up to form a continuous nucleic acid sequence encoding a fusion protein of any two or more of the ara h proteins, and in any particular order. However, a start codon would be required at the start of the fusion protein coding sequence and stop codon to terminate expression of the encoded fusion protein.

[0165] ***Construction of vaccinia virus homologous recombination plasmid*:** The homologous recombination cassette consist of the following element, all of which were synthetically made by GeneArt GmbH of Life Technologies: (i) 500bp left homologous

recombination arm that flanks up-stream of the VACV-A39R ORF of the Copenhagen strain, (ii) EGFP expression cassette under the control of a vaccinia early/late promoter and terminating in the poxvirus early transcription stop sequence (TTTTTNT), (iii) Ecogpt expression cassette under the control of a vaccinia early/late promoter and terminating in the poxvirus early transcription stop sequence (TTTTTNT); (iv) the peanut hypoallergen vaccine antigen expression cassette (UBc.PHAVag or PHAVag) as described above, (v) 500bp right homologous recombination arm that flanks down-stream of the VACV-A39R ORF of the Copenhagen strain. A diagrammatic presentation of these cassettes can be found in Figure 4 and their DNA sequences can be found in Figures 6 and 7.

[0166] Both UBc.PHAV and PHAV homologous recombination cassettes were flanked with Not I restriction enzyme sites and cloned into plasmids to form clones pTC11 (UBc.PHAV) and pTC12 (PHAV). The plasmids are shown in Figure 8. As these cassettes were synthetically made, any TTTTTNT sequences occurring with the protein coding sequences of EGFP and Ecogpt where disrupted with silent mutations without affecting the encoded amino acid sequences.

[0167] ***Construction of Vaccinia Virus expressing the peanut hypoallergen vaccine antigens:*** The PHAV expression cassettes were inserted into the A39R ORF of vaccinia virus Copenhagen strain by homologous recombination. Figure 4 shows a map illustrating site of the insertion within the A39R ORF. Briefly, this was carried out by infecting BHK21 cells at a low multiplicity of infection (moi) of 0.01 pfu per cell for 45 minutes and then transfecting the cells with either of the Not I linearized pTC11 or pTC12 plasmid vectors. The infected/transfected cells were then harvested once the infection reach near completion. Harvested cells were then sonicated to make viral extracts and these virus extracts where subjected to one round of plaque purification under positive selection with Mycophenolic acid (MPA) in the presence of xanthine, hypoxanthine, aminopterin and thymidine. Plaque purified clones where then sequentially amplified under MPA positive selection to make a seed stock of virus. Recombinant vaccinia virus harbouring the UBc.PHAVag expression cassette was designated as SCV201C and the recombinant virus harbouring the PHAVag expression cassette was designated SCV202C.

[0168] Detailed protocols for making recombinant vaccinia virus using Ecogpt selection method can be found in *Smith 1993*. The method employed to make SCV201C and SCV202C is outlined below.

[0169] ***Homologous recombination:*** For each virus construction, three T25 flasks containing growth medium (RPMI-1640/10% FCS/2mM Glutamax/Pen-Strep) were seeded with BHK21 cells and culture until subconfluent at 37°C/5%CO_2. On the day of infection, two flasks were infected with VACV-COP at an moi 0.01 pfu/cell, where the other flask was not infected (uninfected control). After infecting flask 1 and 2 for 45 min at room temperature, the virus inoculums were removed and the monolayer of cells washed twice with PBS. After washing, 4 ml of Maintenance Medium (MM: RPMI-1640/2% FCS/2mM Glutamax/Pen-Strep) was added to each flask including Flask 3 that had also gone through the same washing step.

[0170] Transfection was carried out using Effectene Transfection reagent (Qiagen, Cat No 301425) and following the manufacturer's instructions. Briefly, 16µL of Enhancer was added to 2µg of linearized pTC11 or pTC12 in 150µuL of EC buffer and left to stand for 5 minutes at room temperature after thoroughly mixing. To this 25 µl of Effectene Transfection reagent was added, thoroughly mixed and left to stand at room temperature for 10 minutes. Finally, 1 ml of MM (RPMI-1640/2% FCS/2mM Glutamax/Pen-Strep) was added mixed thoroughly mixed gently together. This transfection mix was then added to flask 1 that had previously been infected with VACV-COP.

[0171] Flask 1 (homologous recombination), Flask 2 (infection only control) and Flask 3 (uninfected control) were incubated overnight at 37°C/5%CO_2 where the following day each flask had a media change with fresh MM containing 25µg/mL mycophenolic acid (MPA), 250µg/mL xanthine and 1^x HAT (Sigma Cat# H0262-10VL) – 5mL per flask and further incubated at 37°C/5%CO_2 until gross CPE can be seen in Flask 1 only. There was little or no sign of gross CPE in Flask 2 as the MPA treatment inhibited VACV-COP spread of infection, and the monolayer looked healthy in Flask 3.

[0172] Cells in Flask 1 were harvested by scraping the cells into the culture medium, then pelleted by low speed centrifugation (500g for 5 minutes at room temperature) followed by resuspending the cell pellet in 1mL of 10 mM Tris-HCl pH8. A viral extract was prepared by multiple freeze and thaw cycles and then stored at -80°C ready for plaque purification phase. The viral constructs were designated SCV201C (UBc.PHAV insertion) and SCV202C (PHAV insertion).

[0173] ***Plaque purification process:*** The homologous recombination extract was serially diluted and each dilution was used to infect one row of BHK21 cells cultured in a 48 well plate in the presence of MPA. The aim was to dilute the virus down to 1 pfu infection per well and look for wells that contain only 1 fluorescent plaque after approx. 30hr of infection before harvesting.

[0174] BHK21 cells were seeded into each well of a 48-well plate and culture to 100% in growth medium (RPMI-1640/10% FBS/2mM Glutamax/pen-strep) at 37°C/5% CO_2. Thereafter the medium was replaced with MM containing 25μg/mL MPA, 250μg/mL xanthine and 1^x HAT (Sigma Cat# H0262-10VL) and incubated further overnight.

[0175] For infection, the homologous recombination extracts (SCV201C and SCV202C) were thawed and briefly sonicated to break up lumps and aggregates. Tenfold serial dilution down to 10^{-5} of each viral extract was performed using MM (RPMI/2% FBS/Glutamax/PenStrep) in 1mL volumes. For each dilution, one row of the 48-well plate was seeded with 100uL of diluted virus after removing the growth medium from each well and washed once with PBS. The 48-well plate was left at room temperature for 45 minute for viral adsorption to occur. After viral adsorption, the virus inoculum was carefully removed from each well where residual inoculum was removed by a washing step consisting of 500μL of PBS per well. After washing, 500μL of MM (RPMI/2% FBS/Glutamax/PenStrep) containing 25μg/mL MPA, 250μg/mL xanthine and 1^x HAT (Sigma Cat# H0262-10VL) was added to each well and then incubated at 37°C/CO_2 until fluorescent green foci of infections could be clearly seen under a fluorescent microscope.

[0176] For harvesting, only wells containing a single fluorescent foci at the highest dilution possible was selected. The medium from selected wells were carefully removed and 100μL of 10mM TrisHCl pH8 was added. The plate was freeze-thawed three times and the contents of the selected wells were recovered.

[0177] One selected clone was then further amplified by infecting 1 well of a 6-well plate containing BHK21 cells at 100% confluency that had been pretreated overnight with 25μg/mL MPA, 250μg/mL xanthine and 1^x HAT (Sigma Cat# H0262-10VL), by removing the culture medium from the well and adding 10μL of viral extract diluted to 500μL in PBS. After 45 min at room temperature 2mL of MM containing 25μg/mL MPA, 250μg/mL xanthine and 1^x HAT (Sigma Cat# H0262-10VL) was added to the well and

incubated further at 37°C/5%CO_2 for 3 days until majority of the cells fluoresced green under a fluorescent microscope. The cells within the infected well were scraped into the culture medium and then pelleted at 500g for 5 minutes. The pelleted cells were resuspended in 500µL of 10mM TrisHCl pH8 and briefly sonicated to make a viral extract.

[0178] A portion of this extract was used for further amplification by infecting five T175 flask of BHK21 under MPA selection. The infected cells were recovered and then pelleted at 500g for 5 mins. The pelleted cells for all five flasks were resuspended in 5mL of 10mM TrisHCl pH8 and briefly sonicated to make a viral extract. Insoluble material was then remove by pelleting at 500g for 5 min. The supernatant (viral extract) was then titrated in BHK21 cells using the following procedure outlined below and the presence of the inserted UBc.PHAV within the A39R ORF was confirmed by PCR analysis.

[0179] ***Titration:*** Titration was carried out using 24-well plate format. Plaques were clearly distinguishable as Crystal violet counter-stained holes in the monolayer (plaques), as seen by the naked eye.

[0180] For each recombinant virus to be titrated, one 24 well plate was seeded with BHK21 cells and cultured to confluency in growth medium (RPMI/10% FBS/Glutamax/Pen Strep). On the day of titration, each viral stock was thawed and sonicated to break up lumps and clumps. Each virus was serially diluted in PBS down to 10^{-8}. The medium was removed from each well and starting from the 10^{-8} dilution, 500µL of each dilution was added to each well of a column in the 24 well plate (4 wells per dilution) and left to incubate at room temperature for 45mins for the virus to adsorb to the cells. After this, the virus inoculum was removed from each well, where each well was then washed once with PBS. After washing, 1mL of MM (RPMI/2% FBS/Glutamax/PenStrep) was added to each well and the plates were incubated at 37°C/5% CO_2 until plaques can been seen in the monolayers. For plaque counter staining, the medium from each well was removed and 500µL of Crystal Violet solution (0.4% $^{w}/_{v}$ in 20% ethanol) was added to each well. Staining was carried out at room temperature for 15-30 min where after the Crystal Violet stain was removed from each well and each well left to air dry before counting plaques. From the dilution that gave rise to 10-30 counts per well, the mean was calculated. This value was then multiplied by the reciprocal of the serial dilution and then further multiplied by 2 (*i.e.*, 2 x 500µL = 1mL) to produce the titre in pfu/mL.

[0181] ***Immunogenicity testing of SCV201C in C3H/HeJ mice:*** To test the immunogenicity of SCV201C and determine if the ubiquitinated PHAV antigen can induce a peanut protein specific T_H1 immune response 3 groups of C3H/HeJ mice (5 mice per group) where vaccinated with the following: (i) 10^6pfu of SCV201C administered intraperitoneally (IP), (ii) 10^6pfu of SCV000 administered intraperitoneally (IP), and (iii) PBS administered intraperitoneally (IP). Blood samples were taken just prior to vaccination (prebleed) and 17days after vaccination. Spleens for cytokine profiling was harvested 9 weeks are vaccination.

[0182] ***Preparation of soluble peanut protein extract:*** The method used to extract soluble peanut protein from roasted unsalted peanuts was derived from the procedures described by Sachs et al. (1981) and Burks et al. (1992). Roasted unsalted peanuts were purchased from a local grocery store. The nuts were then pulverized in a blender to a meal and then to a butter paste. Lipids/fats were removed from the peanut butter by the additions of de-fatting reagent hexane. To do this, n-hexane was added to the peanut butter and shaken vigorously to mix. The mixture was transferred to a glass beaker and left to settle into solvent and solid phases. The solvent phase (which contains the extracted lipids/fats) was removed from the solid phase. The solid phase was air dried into a cake. This cake was dissolved in 0.1M NH_4HCO_3 (2mL per gram) at 4°C for 36 hours with stirring to extract soluble proteins. The slurry was centrifuged for 15 min at 10,000g to remove solids. The supernatant was dialyzed against 5mM phosphate buffer (pH7 to pH8) or PBS using 3500 MWCO membrane/tubing. The dialyzed solution was centrifuge at 10,000g for 15min at 4°C to clarify the extract. Total protein concentration in the soluble extract was measured using standard techniques. The resulting soluble protein extract (10mg/ml) was kept at -20°C for storage, and thawed prior to use.

[0183] ***Quantification of peanut-specific serum IgE and IgG2a:*** Flat-bottom 96-well EIA/RIA ELISA plates (Costar) were coated with 2µg/well purified peanut extract in PBS and incubated at 37°C for 1hr then overnight at 4°C. Plates were washed with 200µl/well PBS three times before blocking with 5% skim milk with PBS and 0.05% Tween (SM+PBS+TW). Non-specific binding was blocked at 37°C for at least one hour before three 200µl/well washes with PBS with 0.05% Tween (PBS+TW). After washing, serum samples were first diluted 1:100 for IgE assays, or 1:500 for IgG1 and IgG2a assays, in SM+PBS+TW. Serum samples were serially diluted across three columns. Various wells

were left without serum as background controls. The plates were incubated at 37°C for one hour.

[0184] Plates were washed five times with 200µl of PBS and secondary antibody (1:500 Goat antimouse IgE HRP conjugate, Alpha Diagnostic; 1:1000 HRP rat anti-mouse IgG2a, BD Biosciences- BD Pharmingen) diluted in SM+PBS+TW was added (100µl/well). The plates were incubated for one hour and then washed five times as above, and 100µl/well of o-Phenylenediaminedihydrochloride (OPD) substrate solution prepared according to manufacturer's directions (SigmaFAST™ OPD, Sigma-Aldrich) was added. Reactions were stopped with 20µl/well 1MHCl when colour had begun to develop in 'blank' wells (ranging from five minutes in IgG1 and IgG2a assays to 45 minutes for IgE assay). Optical densities were measured at 450nm on a plate reader (EL808 Ultra Microplate Reader, Bio-tek Instruments Inc).

[0185] Optical densities for serial dilutions from each respective time point were plotted against dilution factor on a logarithmic scale using GraphPadPrisim V5.01 (GraphPad Software, San Diego, CA,USA). The endpoint titre for each time point was determined as the dilution value at which the curve intercepted the calculated cut-off optical density (minimum of three times standard errormean (SEM) of pre-bleed samples but greater than the highest optical density value measured for all pre-bleed samples).

[0186] ***Statistical Analyses***: Statistical comparisons were performed using GraphPad Prism V5.01 (GraphPad Software, San Diego, CA, USA). Two-way analysis of variance (ANOVA) with Bonferroni post-testing was used to deduce significant differences among the ELISA results.

Example 1: Ubiquitination of PHAV antigen enabled the successful expression of SCV201C but not SCV202C

[0187] The expression of SCV201C following insertion of the UBc.PHAVag expression cassette into the A39R of vaccinia virus was successful. After homologous recombination and during the plaque purification step, MPA resistant plaques could be clearly identify and amplified in the presence of MPA to produce a seed stock that gave sufficient titres to proceed to the next step of immunogenicity testing in mice.

[0188] By contrast, the expression of SCV202C was difficult to progress beyond the plaque purification step, as no clearly discernable plaques could be found at the high dilution range. Fluorescent infected cells could be detected at the low dilution range and at these dilution only 100% CPE was seen in the infected wells as opposed to discernable plaques. When these wells were harvested and subjected to further amplification in the presence of MPA, very little virus titre was obtained most of which consisted of parental virus as determined by PCR analysis and plaque assays showing the lack of fluorescent plaque in the absence of MPA.

[0189] The expression of PHAVag following infection had an inhibitory or toxic effect on virus propagation, which was overcome with the SCV201C construct. Without being bound by theory or by a particular mode of application, it is postulated that the inhibitory or toxic effect of the synthesized PHAVag was overcome by the use of a proteasome degradation tag such as ubiquitin to target the expressed PHAVag to proteasomal degradation.

[0190] This inhibitory effect of viral propagation by expressing the intact PHAVag was further confirmed because the construction a recombinant vaccinia containing only the Ecogpt and EFGP expression cassettes inserted into the A39R ORF was easily achievable (designated as SCV000).

Example 2: Antigen-specific antibody responses following vaccination

[0191] The results are present in Figure 10 for both peanut protein-specific serum IgE (Figure 10A) and IgG2a (Figure 10B) antibody level before and after vaccination (17 day post vaccination). It can be clearly seen that vaccination with SCV201C produced significant levels of peanut protein-specific IgG2a after 17 day post vaccination. These level where significantly higher than the vector only control (SCV000) and PBS control, demonstrating SCV201C produces a specific anti-peanut protein antibody response. It is to be noted that SCV201C produced a much smaller IgE response as compared to an IgG2a response; that is, an endpoint dilution of 1:2,500 for IgE as compared to an endpoint dilution approaching 1: 1,000,000 for IgG2a. Moreover, the IgE response was not much more above the responses induced by the empty vector (SCV000) or PBS controls.

[0192] These results show that SCV201C produces an IgG2a response to peanut proteins, but very little IgE response, indicating that SCV201C had initiated a peanut-specific T_H1 biased immune response in response to PHAVag.

Example 3: Lymphocyte cytokine profile following SCV201C vaccination in mice

[0193] Spleens were harvested from mice and stored in complete RPMI before being transferred to a 60mm tissue culture dish. Spleens were then cut into three sections and disaggregated into single-cell suspension. The cells were then filtered and washed with 5% RPMI (300g x 5 minutes). Red blood cells were then lysed in 5ml of alkaline lysis buffer for 5 minutes, then diluted to 20ml with 5% RPMI and centrifuged at 200g for 5 minutes. Cells were then resuspended and counted. Meanwhile, 96-well plates with control RPMI, soluble peanut-antigen (100μg/ml), and ConA (5ug/ml) wells were prepared. Lymphocytes were then add at 400,000 cells/well and incubated at 37C for 96 hours.

[0194] After the 96 hour incubation period, 100μl of supernatant from each was collected and frozen at -80°C. Th1/Th2 cytokines were then quantified by flow cytometry according to the manufacturer's instructions (BD Biosciences # 551287). The samples were then run on a BD FACSCanto II flow cytometer. Cytokine concentrations were determined using Soft Flow FCAP Array software. All further analysis was done in Graph Pad 6.0.

[0195] The results presented in Figure 11 clearly show that vaccination with SCV201C produces a biased T_H1 immune response to peanut protein exposure. This is illustrated by the significantly higher level of IFN-gamma (IFN-g; a T_H1 cytokine; Figure 11A) as compared to levels of IL4 and IL5 (T_H2 cytokines; Figures 11B and 11C) secreted by cultured lymphocytes obtained from the spleens of the SCV201C vaccinate mice.

Conclusion

[0196] Vaccination of mice with SCV201C produced a biased anti-peanut protein T_H1 immune response. An allergen-specific T_H1 immune response will dominate over an existing allergen-specific T_H2 immune response and, in doing so, will desensitize an individual to subsequent exposure to the allergen. The studies disclosed herein show that ubiquitinated peanut hypoallergen vaccine antigen (UBc.PHAVag) stimulates an anti-peanut protein-specific T_H1 immune response. Thus, vaccines containing the ubiquitinated

hypoallergen vaccine antigen as herein described can be used to desensitize individuals to peanut allergens and can therefore be used to treat and/or prevent allergic reactions in individuals that are triggered by exposure to peanut allergens.

[0197] As noted above, the expression of the SCV201C construct was successful following infection, whereas the expression of the non-ubiquitinated SCV202C construct was difficult to progress beyond the plaque purification step. The expression of PHAVag following infection therefore appears to have an inhibitory or toxic effect on virus propagation, which was overcome with the ubiquitinated SCV201C construct. Without being bound by theory or by a particular mode of application, it is postulated that the inhibitory or toxic effect of the synthesized PHAVag was overcome by the use of ubiquitin, targeting the expressed PHAVag to proteasomal degradation. As a result of ubiquitin-targeted proteasomal degradation of PHAVag, the small peptide fragments of PHAVag enter the endoplasmic reticulum (ER) where they are complexed with MHC class I proteins and then transported to the cell surface to be presented to T lymphocytes (see, for example, Figure 9). The consequence of this is that there is enhanced presentation of the PHAVag fragments with MHC class I, resulting in a greater T_H1 immune response to peanut allergens. Thus, the proteasome degradation tag (*e.g.*, ubiquitin) unexpectedly prevent the artificial, intact PHAVag fusion protein from inhibiting virus replication.

[0198] Ara h 1, ara h 2, ara h 3 are the three major peanut allergens that have been shown to cause peanut-specific allergic reactions in susceptible individuals. Ara h 6 has been implicated in childhood susceptibility to peanut allergy (Flinterman *et al.* 2007). Ara h 7 is recognised in 43% peanut allergic individuals, ara h 8 is recognised in 85% peanut allergic individuals, ara h 4 is recognised in 54% peanut allergic individuals and ara h 5 is recognised in 13% peanut allergic individuals.

[0199] The disclosure of every patent, patent application, and publication cited herein is hereby incorporated herein by reference in its entirety.

[0200] Many modifications will be apparent to those skilled in the art without departing from the scope of the present invention.

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CLAIMS:

1. A poxvirus vector comprising a nucleic acid sequence encoding a fusion protein comprising (i) at least two peanut allergens selected from the group consisting of ara h 1, ara h 2, ara h 3, ara h 4, ara 5, ara h 6, ara h 7, ara h 8, ara h 9, ara h 10 and ara h 11 and a derivative or part thereof having at least 70% sequence identity thereto, and (ii) a proteasome degradation tag to enhance intracellular degradation of the fusion protein.

2. The poxvirus vector of claim 1, wherein the nucleic acid sequence encodes a fusion protein comprising: (i) at least two peanut allergens selected from list consisting of ara h 1, ara h 2, ara h 3, ara h 4, ara h 5, ara h 6 and ara h 7, and a derivative or part thereof having at least 70% sequence identity thereto, and (ii) a proteasome degradation tag to enhance intracellular degradation of the fusion protein.

3. The poxvirus vector of claim 1, wherein the nucleic acid sequence encodes a fusion protein comprising: (i) at least three peanut allergens selected from the group consisting of ara h 1, ara h 2, ara h 3, ara h 4, ara h 5, ara h 6, ara h 7, ara h 8, ara h 9, ara h 10 and ara h 11 and a derivative or part thereof having at least 70% sequence identity thereto, and (ii) a proteasome degradation tag to enhance intracellular degradation of the fusion protein.

4. The poxvirus vector of claim 3, wherein the nucleic acid sequence encodes a fusion protein comprising: (i) at least three peanut allergens selected from the group consisting of ara h 1, ara h 2, ara h 3, ara h 4, ara h 5, ara h 6 and ara h 7, and a derivative or part thereof having at least 70% sequence identity thereto, and (ii) a proteasome degradation tag to enhance intracellular degradation of the fusion protein.

5. The poxvirus vector of any one of claims 1 to 4, wherein the vector comprises a promoter and single start codon to facilitate expression of the intact fusion protein.

6. The poxvirus vector of any one of claims 1 to 5, wherein the proteasome degradation tag comprises a ubiquitin monomer.

7. The poxvirus vector of any one of claim 1 to 6, wherein the nucleic acid sequence encodes a fusion protein comprising four peanut allergens which are ara h 1, ara h 2, ara h 3, and ara h 6, or a derivative or part thereof having at least 70% sequence identity thereto.

8. The poxvirus vector of any one of claims 1 to 7, wherein the fusion protein comprises an amino acid sequence set out in SEQ ID NO: 12 or a derivative or part thereof

having at least 70% sequence identity thereto.

9. The poxvirus vector of any one of claims 1 to 7, wherein the poxvirus vector comprises the nucleic acid sequence set out in one of SEQ ID NO: 11 or a derivative or part thereof having at least 70% sequence identity thereto.

10. The poxvirus vector of any one of claims 1 to 7, wherein the ubiquitin monomer comprises a nucleotide sequence set out in one of SEQ ID NO: 1 or a derivative thereof having at least 70% nucleotide sequence identity thereto.

11. The poxvirus vector of any one of claims 1 to 7, wherein the proteasome degradation tag comprises ubiquitin C.

12. The poxvirus vector of any one of claims 1 to 12, wherein the nucleic acid sequence encodes a fusion protein comprising peanut allergens ara h 1, ara h 2, ara h 3 and ara h 6.

13. The poxvirus vector of any one of claims 1 to 12, wherein the poxvirus vector is a vaccinia vector.

14. The poxvirus vector of claim 13, wherein the poxvirus vector is a modified vaccinia vector or an avipox vector.

15. The poxvirus vector of any one of claims 1 to 14, comprising a pharmaceutically or physiologically acceptable carrier and/or diluent.

16. The poxvirus vector of any one of claims 1 to 15, for use in the treatment of a peanut allergy in a subject.

17. Use of a poxvirus vector of any one of claims 1 to 14 in, or in the manufacture of a medicament for, the treatment of peanut allergy.

18. A method of inducing tolerance to or suppressing an allergic response in a subject or patient, the method comprising administering to the subject or patient an effective amount of the poxvirus vector of any one of claims 1 to 15 for a time and under conditions sufficient to elicit suppression/tolerance.

19. A method of vaccinating a subject to induce tolerance to a peanut allergen comprising administering the poxvirus vector of any one of claims 1 to 15.

20. The use of claim 15 or the method of claim 18 or claim 19, for inducing tolerance

against at least two or at least three major peanut allergens.

21. A kit comprising the poxvirus vector of any one of claim 1 to 15.

22. The poxvirus vector of any one of claims 1 to 15, for use in a human subject.

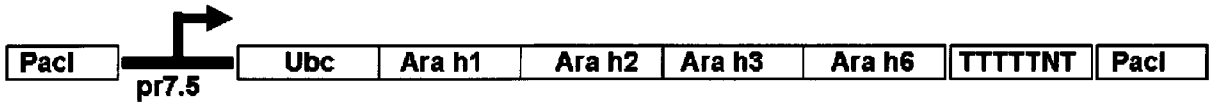
23. The poxvirus vector of claim 22, wherein the nucleic acid sequence encoding the fusion protein is codon optimized for expression in human cells.

24. A method of desensitizing or inducing tolerance in a subject to a peanut allergen, the method comprising: i) collecting lymphocytes from the subject; ii) co-culturing the lymphocytes with a cell transfected with the poxvirus vector of any one of claims 1 to 15 *ex vivo* to generate and/or expand a T_H1 lymphocyte population that recognizes the proteasomally degraded peanut allergen fusion protein associated with MHC Class I molecules on the cell; and iii) administering the T_H1 lymphocyte population from (ii) to the subject.

25. A method of desensitizing or inducing tolerance in a subject to a peanut allergen, the method comprising: i) collecting naïve antigen presenting cells from the subject; ii) co-culturing the antigen presenting cells with a cell transfected with the poxvirus vector of any one of claims 1 to 15 *ex vivo* to generate a population of activated antigen presenting cells that recognize the proteasomally degraded peanut allergen fusion protein associated with MHC Class I molecules on the cell; and iii) administering the activated antigen presenting cell population from (ii) to the subject.

FIGURE 1

A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pr7.5
Ubc
Ara h1
Ara h2
Ara h3
Ara h6
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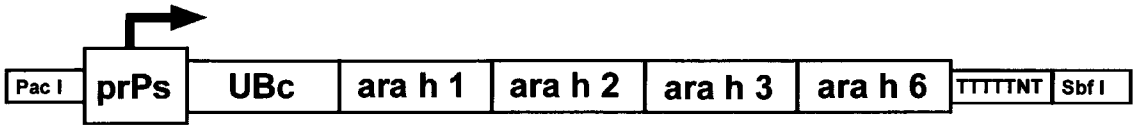
PacI

B


Pac I
prPs
UBc
ara h 1
ara h 2
ara h 3
ara h 6
TTTTTNT
Sbf I

C

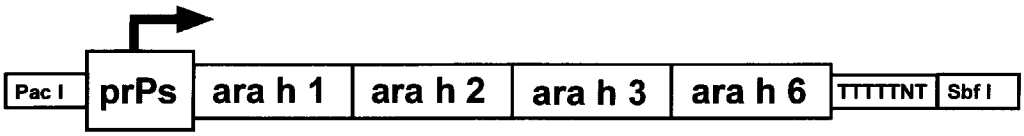

Pac I
prPs
ara h 1
ara h 2
ara h 3
ara h 6
TTTTTNT
Sbf I

FIGURE 2

UBc.PHAVag Expression Cassette

```
LOCUS       UBc.PHAVag expression cassette        4705 bp    DNA     linear   SYN
10-MAR-2014
DEFINITION  SCV-UBcPHAVag expression cassette
ORGANISM  Vaccinia Virus
COMMENT     Textco/File created by Gene Construction Kit (TextcoBioSoftware)
FEATURES             Location/Qualifiers
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                     /label="prPs"
                     /note="Vaccinia early/late promoter"
     CDS             53..4690
                     /label="UBc.PHAV"
                     /note="Protein coding sequence of the ubiquitinated peanut
hypoallergy vaccine antigen"
     CDS             53..280
                     /label="Ubc"
                     /note="Ubiquitin C monomer.  Terminal amino acid changed to
""A"""
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                     /note="Protein coding sequence of ara h 3"
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misc_feature    4691..4697
                     /label="T5NT"
                     /note="Pox virus early transcriptional stop sequence"
ORIGIN
        1 TTAattaaca AAAAATTGAA ATTTTATTTT TTTTTTTTGG AATATAAATA AtATGCAGAT
       61 CTTTGTGAAA ACACTCACGG GAAAAACTAT AACTCTTGAG GTGGAGCCCT CTGACACAAT
      121 CGAAAATGTG AAAGCCAAGA TCCAAGATAA GGAAGGCATC CCTCCAGACC AGCAACGGCT
      181 CATCTTTGCG GGCAAACAAC TGGAGGATGG GCGCACTCTC AGTGATTACA ATATTCAAAA
      241 GGAATCTACA CTGCACCTGG TTCTTAGGCT GCGGGGAGCC CGAGGCAGAG TCAGCCCTCT
      301 GATGCTGCTG TTGGGGATCT TGGTTCTTGC ATCCGTTTCA GCCACACATG CCAAGTCCAG
      361 CCCATACCAG AAAAAGACCG AGAACCCATG TGCTCAGCGG TGCCTCCAGT CATGTCAGCA
      421 GGAACCCGAT GACCTCAAGC AAAAGGCGTG TGAAAGCAGA TGTACGAAAC TGGAATACGA
      481 CCCCAGATGC GTGTACGATC CACGAGGCCA TACTGGCACC ACCAATCAAA GATCACCACC
      541 TGGCGAGCGA ACCAGGGGAC GACAGCCAGG GGATTACGAT GACGATAGAC GACAGCCTCG
      601 CCGGGAAGAG GGTGGGCGCT GGGGTCCAGC CGGTCCCAGA GAGCGAGAAC GGGAAGAGGA
      661 CTGGCGCCAA CCCCGGGAGG ACTGGCGCAG ACCTTCACAC CAGCAGCCCC GGAAAATACG
      721 CCCGGAGGGC AGAGAGGGTG AACAGGAATG GGGAACCCCT GGCAGTCACG TCAGGGAAGA
      781 AACCAGCCGG AACAACCCCT TCTATTTCCC CTCCCGGAGG TTTAGCACTC GGTACGGGAA
      841 CCAAAACGGA CGCATTCGCG TACTTCAGAG ATTTGATCAA CGCTCACGGC AGTTCCAGAA
      901 CCTTCAAAAC CATCGGATTG TTCAGATCGA GGCCAAACCA AACACTTTGG TTCTGCCAAA
      961 ACATGCTGAT GCAGACAACA TACTGGTGAT ACAGCAGGGT CAAGCCACAG TGACAGTAGC
     1021 CAATGGGAAC AATCGCAAAT CATTCAATCT CGATGAGGGA CACGCACTGA GGATCCCTTC
     1081 TGGCTTTATC TCCTATATAC TGAATCGGCA CGACAATCAA AATCTCAGGG TTGCTAAGAT
     1141 CTCTATGCCA GTCAACACTC CGGGTCAGTT CGAGGATTTC TTTCCCGCGT CCTCACGGGA
     1201 CCAGTCTAGT TATCTTCAAG GATTCAGCAG AAACACCTTG GAAGCGGCCT TTAACGCCGA
     1261 GTTTAACGAG ATCAGGCGGG TGCTTCTCGA GGAGAACGCT GGCGGGGAAC AGGAGGAGAG
     1321 AGGCCAACGG CGGTGGTCTA CCAGGTCCAG TGAGAACAAT GAGGGCGTGA TCGTCAAGGT
```

FIGURE 2 (CONTINUED)

```
1381 ATCTAAGGAG CATGTCGAGG AACTGACCAA ACATGCAAAG TCCGTTTCCA AGAAAGGCTC
1441 CGAGGAGGAA GGGGACATTA CGAATCCGAT CAACCTTCGG GAGGGCGAGC CGGATCTGTC
1501 AAATAACTTT GGAAAACTCT TCGAAGTCAA GCCCGACAAA AAGAATCCGC AGTTGCAAGA
1561 TCTGGACATG ATGCTCACGT GTGTCGAGAT TAAGGAAGGA GCACTGATGT TGCCTCACTT
1621 TAACTCCAAA GCCATGGTGA TAGTCGTAGT AAACAAAGGA ACCGGCAATC TGGAGTTGGT
1681 GGCTGTCCGA AAGGAACAAC AGCAAAGAGG GCGGAGGGAA GAAGAGGAAG ATGAGGACGA
1741 GGAGGAGGAG GGATCAAACC GGGAGGTACG CCGATACACA GCGAGGCTGA AAGAGGGAGA
1801 CGTGTTTATC ATGCCGGCAG CACATCCTGT CGCTATCAAC GCCTCTAGCG AGCTCCATTT
1861 GCTGGGGTTC GGGATCAATG CGGAGAACAA TCATCGCATT TTCCTGGCAG GCGACAAGGA
1921 CAACGTTATT GACCAAATTG AGAAGCAAGC CAAGGACCTG GCCTTCCCTG GATCAGGTGA
1981 ACAGGTCGAG AAGCTCATCA AAAACCAGAA GGAATCCCAC TTTGTATCTG CCAGACCACA
2041 GTCACAGTCC CAGAGCCCCT CTAGTCCCGA GAAGGAGAGC CCCGAAAAGG AAGATCAAGA
2101 GGAGGAGAAC CAGGGTGGAA AGGGCCCACT GCTTTCCATT CTCAAAGCCT TCAATGCTAA
2161 GCTGACAATA TTGGTGGCAC TGGCACTGTT CCTTCTTGCT GCACACGCGT CAGCCCGGCA
2221 GCAGTGGGAA TTGCAGGGCG ATCGAAGGTG TCAGTCACAG CTGGAGAGGG CGAACCTCCG
2281 GCCTTGTGAA CAGCACCTGA TGCAGAAGAT TCAGCGGGAC GAGGATTCTT ACGGGCGAGA
2341 TCCTTACAGT CCCTCCCAAG ATCCATATAG CCCGTCTCAA GACCCAGATC GCAGGGACCC
2401 ATATAGCCCC AGCCCCTATG ATCGAAGAGG TGCCGGAAGC AGCCAGCATC AGGAAAGGTG
2461 CTGCAATGAG CTGAACGAGT TCGAGAACAA CCAGAGATGT ATGTGCGAGG CTCTGCAGCA
2521 GATTATGGAA AATCAATCTG ACCGGCTGCA GGGACGGCAG CAGGAGCAGC AGTTCAAAAG
2581 GGAGCTCCGC AACCTTCCAC AGCAGTGCGG TTTGCGCGCA CCTCAGCGCT GCGACTTGGA
2641 GGTGGAAAGC GGAGGTAGAG ACAGATACGC GAAGCTGCTG GAACTCAGCT TCTGTTTCTG
2701 TTTCCTGGTA CTCGGCGCTT CATCAATATC TTTTAGGCAG CAGCCAGAGG AAAATGCCTG
2761 CCAGTTCCAA CGGCTGAACG CTCAGCGACC AGACAATAGG ATCGAATCAG AAGGTGGATA
2821 CATCGAGACT TGGAACCCGA ATAACCAGGA GTTCGAATGT GCAGGCGTGG CACTGTCTCG
2881 CCTTGTTCTC CGACGCAATG CGCTCAGGCG CCCATTCTAT TCCAATGCAC CCCAAGAAAT
2941 CTTTATCCAA CAGGGCAGAG GGTACTTCGG GCTGATCTTT CCCGGCTGTC CCCGGCACTA
3001 TGAGGAACCC CACACACAGG GCAGAAGGAG CCAGAGCCAG CGGCCTCCCC GGAGATTGCA
3061 AGGGGAGGAT CAGAGCCAGC AGCAGAGAGA TTCTCATCAG AAAGTACATA GGTTCGATGA
3121 GGGTGACCTG ATAGCTGTGC CAACCGGTGT TGCCTTTTGG TTGTATAATG ACCACGACAC
3181 AGACGTGGTG GCTGTGTCTC TGACCGATAC AAACAACAAT GACAATCAGC TTGATCAGTT
3241 CCCTAGGCGC TTTAACCTGG CTGGCAACAC CGAACAGGAG TTCTTGAGAT ATCAGCAGCA
3301 GTCTAGGCAG TCTAGGAGGA GGTCCCTGCC ATACTCCCCT TACAGCCCTC AGAGTCAGCC
3361 TAGGCAGGAA GAGAGAGAAT TCAGTCCCAG AGGCCAGCAC TCTAGGCGGG AGCGGGCTGG
3421 GCAGGAGGAG GAAAACGAAG GTGGCAATAT CTTTAGCGGC TTCACTCCAG AGTTTCTGGA
3481 ACAGGCATTC CAAGTAGATG ACAGACAGAT CGTCCAGAAC CTTAGGGGCG AGACTGAATC
3541 AGAAGAGGAA GGGGCAATCG TGACGGTGCG CGGAGGCTTG CGCATCCTGT CCCCTGACCG
3601 CAAACGCAGG GCCGACGAGG AAGAAGAGTA TGACGAGGAT GAATATGAAT ATGATGAGGA
3661 GGATCGAAGG CGCGGAAGGG GCAGTAGGGG ACGAGGGAAC GGCATAGAAG AAACTATTTG
3721 TACCGCGTCC GCCAAGAAGA ATATTGGGCG AAACCGCAGT CCCGACATAT ACAATCCTCA
3781 AGCCGGCAGC CTTAAAACCG CCAACGATCT GAACCTGCTG ATCCTCCGCT GGCTGGGGCC
3841 AAGCGCCGAA TATGGGAATC TGTACCGAAA TGCTCTGTTT GTGGCCCACT ACAATACAAA
3901 TGCCCACTCT ATTATCTACC GCCTCAGAGG GAGGGCTCAT GTGCAAGTGG TCGACAGCAA
3961 TGGGAATCGC GTGTACGATG AGGAGCTCCA AGAAGGGCAT GTCCTTGTTG TGCCTCAGAA
4021 TTTCGCAGTT GCGGGCAAAT CACAGAGTGA GAACTTCGAG TACGTTGCCT TTAAGACCGA
4081 TTCCAGACCC TCCATTGCAA ACCTGGCCGG AGAGAACAGT GTTATTGACA ATCTGCCGGA
4141 GGAAGTGGTT GCTAACAGTT ATGGGCTTCA GCGCGAACAG GCTCGGCAGC TGAAGAACAA
4201 CAATCCGTTC AAGTTTTTCG TCCCTCCATC CCAGCAGTCA CCCAGAGCTG TGGCCGCCAA
4261 ATCCACTATT CTTGTGGCCC TCTTGGCACT CGTGCTGGTC GCCCATGCTT CTGCTATGCG
4321 AAGGGAGAGA GGGCGCCAAG GTGACTCAAG CAGTTGCGAA CGACAAGTGG ACAGAGTGAA
4381 CCTCAAACCT TGCGAACAGC ACATTATGCA GAGAATTATG GGAGAGCAAG AGCAGTATGA
4441 TAGTTATGAT ATCAGATCAA CACGCTCTTC CGATCAGCAA CAGCGGTGTT GCGATGAACT
4501 CAACGAAATG GAGAATACGC AGCGGTGCAT GTGTGAGGCT CTTCAGCAAA TCATGGAAAA
4561 CCAATGCGAT CGGCTCCAAG ATCGACAGAT GGTGCAGCAG TTTAAGCGCG AGCTGATGAA
4621 TTTGCCACAA CAGTGCAACT TTCGGGCTCC CCAGAGATGC GACCTCGATG TCAGCGGAGG
4681 GAGATGCTAA TTTTTATCCT GCAGG
```

FIGURE 3

PHAVag Expression Cassette

```
LOCUS       PHAVag expression cassette        4480 bp    DNA     linear   SYN 10-
MAR-2014
DEFINITION  PHAVag expression cassette
ORGANISM  Vaccinia virus
          Unclassified.
COMMENT     Textco/File created by Gene Construction Kit (TextcoBioSoftware)
FEATURES             Location/Qualifiers
misc_feature    9..52
                     /label="prPs"
                     /note="Vaccinia early/late promoter"
                     /note="Pox virus early transcriptional stop"
     CDS             53..1930
                     /label="ara h1"
                     /note="Protein coding sequence of ara h 1"
     CDS             1931..2443
                     /label="ara h2"
                     /note="Protein coding sequence of ara h 2"
     CDS             2444..4030
                     /label="ara h3"
                     /note="Protein coding sequence of ara h 3"
     CDS             4031..4465
                     /label="ara h6"
                     /note="Protein coding sequence of ara h 6"
     CDS             53..4465
                     /label="PHAVag"
                     /note="Protein coding sequence of the peanut hypoallergen
vaccine antigen"
misc_feature    4466..4472
                     /label="Transcription stop"
ORIGIN
        1 TTAattaaca AAAAATTGAA ATTTTATTTT TTTTTTTTGG AATATAAATA AtatgCGAGG
       61 CAGAGTCAGC CCTCTGATGC TGCTGTTGGG GATCTTGGTT CTTGCATCCG TTTCAGCCAC
      121 ACATGCCAAG TCCAGCCCAT ACCAGAAAAA GACCGAGAAC CCATGTGCTC AGCGGTGCCT
      181 CCAGTCATGT CAGCAGGAAC CCGATGACCT CAAGCAAAAG GCGTGTGAAA GCAGATGTAC
      241 GAAACTGGAA TACGACCCCA GATGCGTGTA CGATCCACGA GGCCATACTG GCACCACCAA
      301 TCAAAGATCA CCACCTGGCG AGCGAACCAG GGGACGACAG CCAGGGGATT ACGATGACGA
      361 TAGACGACAG CCTCGCCGGG AAGAGGGTGG GCGCTGGGGT CCAGCCGGTC CCAGAGAGCG
      421 AGAACGGGAA GAGGACTGGC GCCAACCCCG GGAGGACTGG CGCAGACCTT CACACCAGCA
      481 GCCCCGGAAA ATACGCCCGG AGGGCAGAGA GGGTGAACAG GAATGGGGAA CCCCTGGCAG
      541 TCACGTCAGG GAAGAAACCA GCCGGAACAA CCCCTTCTAT TTCCCCTCCC GGAGGTTTAG
      601 CACTCGGTAC GGGAACCAAA ACGGACGCAT TCGCGTACTT CAGAGATTTG ATCAACGCTC
      661 ACGGCAGTTC CAGAACCTTC AAAACCATCG GATTGTTCAG ATCGAGGCCA AACCAAACAC
      721 TTTGGTTCTG CCAAAACATG CTGATGCAGA CAACATACTG GTGATACAGC AGGGTCAAGC
      781 CACAGTGACA GTAGCCAATG GGAACAATCG CAAATCATTC AATCTCGATG AGGGACACGC
      841 ACTGAGGATC CCTTCTGGCT TTATCTCCTA TATACTGAAT CGGCACGACA ATCAAAATCT
      901 CAGGGTTGCT AAGATCTCTA TGCCAGTCAA CACTCCGGGT CAGTTCGAGG ATTTCTTTCC
      961 CGCGTCCTCA CGGGACCAGT CTAGTTATCT TCAAGGATTC AGCAGAAACA CCTTGGAAGC
     1021 GGCCTTTAAC GCCGAGTTTA ACGAGATCAG GCGGGTGCTT CTCGAGGAGA ACGCTGGCGG
     1081 GGAACAGGAG GAGAGAGGCC AACGGCGGTG GTCTACCAGG TCCAGTGAGA ACAATGAGGG
     1141 CGTGATCGTC AAGGTATCTA AGGAGCATGT CGAGGAACTG ACCAAACATG CAAAGTCCGT
     1201 TTCCAAGAAA GGCTCCGAGG AGGAAGGGGA CATTACGAAT CCGATCAACC TTCGGGAGGG
     1261 CGAGCCGGAT CTGTCAAATA ACTTTGGAAA ACTCTTCGAA GTCAAGCCCG ACAAAAAGAA
     1321 TCCGCAGTTG CAAGATCTGG ACATGATGCT CACGTGTGTC GAGATTAAGG AAGGAGCACT
     1381 GATGTTGCCT CACTTTAACT CCAAAGCCAT GGTGATAGTC GTAGTAAACA AAGGAACCGG
     1441 CAATCTGGAG TTGGTGGCTG TCCGAAAGGA ACAACAGCAA AGAGGGCGGA GGGAAGAAGA
     1501 GGAAGATGAG GACGAGGAGG AGGAGGGATC AAACCGGGAG GTACGCCGAT ACACAGCGAG
```

FIGURE 3 (CONTINUED)

```
1561 GCTGAAAGAG GGAGACGTGT TTATCATGCC GGCAGCACAT CCTGTCGCTA TCAACGCCTC
1621 TAGCGAGCTC CATTTGCTGG GGTTCGGGAT CAATGCGGAG AACAATCATC GCATTTTCCT
1681 GGCAGGCGAC AAGGACAACG TTATTGACCA AATTGAGAAG CAAGCCAAGG ACCTGGCCTT
1741 CCCTGGATCA GGTGAACAGG TCGAGAAGCT CATCAAAAAC CAGAAGGAAT CCCACTTTGT
1801 ATCTGCCAGA CCACAGTCAC AGTCCCAGAG CCCCTCTAGT CCCGAGAAGG AGAGCCCCGA
1861 AAAGGAAGAT CAAGAGGAGG AGAACCAGGG TGGAAAGGGC CCACTGCTTT CCATTCTCAA
1921 AGCCTTCAAT GCTAAGCTGA CAATATTGGT GGCACTGGCA CTGTTCCTTC TTGCTGCACA
1981 CGCGTCAGCC CGGCAGCAGT GGGAATTGCA GGGCGATCGA AGGTGTCAGT CACAGCTGGA
2041 GAGGGCGAAC CTCCGGCCTT GTGAACAGCA CCTGATGCAG AAGATTCAGC GGGACGAGGA
2101 TTCTTACGGG CGAGATCCTT ACAGTCCCTC CCAAGATCCA TATAGCCCGT CTCAAGACCC
2161 AGATCGCAGG GACCCATATA GCCCCAGCCC CTATGATCGA AGAGGTGCCG GAAGCAGCCA
2221 GCATCAGGAA AGGTGCTGCA ATGAGCTGAA CGAGTTCGAG AACAACCAGA GATGTATGTG
2281 CGAGGCTCTG CAGCAGATTA TGGAAAATCA ATCTGACCGG CTGCAGGGAC GGCAGCAGGA
2341 GCAGCAGTTC AAAAGGGAGC TCCGCAACCT TCCACAGCAG TGCGGTTTGC GCGCACCTCA
2401 GCGCTGCGAC TTGGAGGTGG AAAGCGGAGG TAGAGACAGA TACGCGAAGC TGCTGGAACT
2461 CAGCTTCTGT TTCTGTTTCC TGGTACTCGG CGCTTCATCA ATATCTTTTA GGCAGCAGCC
2521 AGAGGAAAAT GCCTGCCAGT TCCAACGGCT GAACGCTCAG CGACCAGACA ATAGGATCGA
2581 ATCAGAAGGT GGATACATCG AGACTTGGAA CCCGAATAAC CAGGAGTTCG AATGTGCAGG
2641 CGTGGCACTG TCTCGCCTTG TTCTCCGACG CAATGCGCTC AGGCGCCCAT TCTATTCCAA
2701 TGCACCCCAA GAAATCTTTA TCCAACAGGG CAGAGGGTAC TTCGGGCTGA TCTTTCCCGG
2761 CTGTCCCCGG CACTATGAGG AACCCCACAC ACAGGGCAGA AGGAGCCAGA GCCAGCGGCC
2821 TCCCCGGAGA TTGCAAGGGG AGGATCAGAG CCAGCAGCAG AGAGATTCTC ATCAGAAAGT
2881 ACATAGGTTC GATGAGGGTG ACCTGATAGC TGTGCCAACC GGTGTTGCCT TTTGGTTGTA
2941 TAATGACCAC GACACAGACG TGGTGGCTGT GTCTCTGACC GATACAAACA ACAATGACAA
3001 TCAGCTTGAT CAGTTCCCTA GGCGCTTTAA CCTGGCTGGC AACACCGAAC AGGAGTTCTT
3061 GAGATATCAG CAGCAGTCTA GGCAGTCTAG GAGGAGGTCC CTGCCATACT CCCCTTACAG
3121 CCCTCAGAGT CAGCCTAGGC AGGAAGAGAG AGAATTCAGT CCCAGAGGCC AGCACTCTAG
3181 GCGGGAGCGG GCTGGGCAGG AGGAGGAAAA CGAAGGTGGC AATATCTTTA GCGGCTTCAC
3241 TCCAGAGTTT CTGGAACAGG CATTCCAAGT AGATGACAGA CAGATCGTCC AGAACCTTAG
3301 GGGCGAGACT GAATCAGAAG AGGAAGGGGC AATCGTGACG GTGCGCGGAG GCTTGCGCAT
3361 CCTGTCCCCT GACCGCAAAC GCAGGGCCGA CGAGGAAGAA GAGTATGACG AGGATGAATA
3421 TGAATATGAT GAGGAGGATC GAAGGCGCGG AAGGGGCAGT AGGGGACGAG GGAACGGCAT
3481 AGAAGAAACT ATTTGTACCG CGTCCGCCAA GAAGAATATT GGGCGAAACC GCAGTCCCGA
3541 CATATACAAT CCTCAAGCCG GCAGCCTTAA AACCGCCAAC GATCTGAACC TGCTGATCCT
3601 CCGCTGGCTG GGGCCAAGCG CCGAATATGG GAATCTGTAC CGAAATGCTC TGTTTGTGGC
3661 CCACTACAAT ACAAATGCCC ACTCTATTAT CTACCGCCTC AGAGGGAGGG CTCATGTGCA
3721 AGTGGTCGAC AGCAATGGGA ATCGCGTGTA CGATGAGGAG CTCCAAGAAG GGCATGTCCT
3781 TGTTGTGCCT CAGAATTTCG CAGTTGCGGG CAAATCACAG AGTGAGAACT TCGAGTACGT
3841 TGCCTTTAAG ACCGATTCCA GACCCTCCAT TGCAAACCTG GCCGGAGAGA ACAGTGTTAT
3901 TGACAATCTG CCGGAGGAAG TGGTTGCTAA CAGTTATGGG CTTCAGCGCG AACAGGCTCG
3961 GCAGCTGAAG AACAACAATC CGTTCAAGTT TTTCGTCCCT CCATCCCAGC AGTCACCCAG
4021 AGCTGTGGCC GCCAAATCCA CTATTCTTGT GGCCCTCTTG GCACTCGTGC TGGTCGCCCA
4081 TGCTTCTGCT ATGCGAAGGG AGAGAGGGCG CCAAGGTGAC TCAAGCAGTT GCGAACGACA
4141 AGTGGACAGA GTGAACCTCA AACCTTGCGA ACAGCACATT ATGCAGAGAA TTATGGGAGA
4201 GCAAGAGCAG TATGATAGTT ATGATATCAG ATCAACACGC TCTTCCGATC AGCAACAGCG
4261 GTGTTGCGAT GAACTCAACG AAATGGAGAA TACGCAGCGG TGCATGTGTG AGGCTCTTCA
4321 GCAAATCATG GAAAACCAAT GCGATCGGCT CCAAGATCGA CAGATGGTGC AGCAGTTTAA
4381 GCGCGAGCTG ATGAATTTGC CACAACAGTG CAACTTTCGG GCTCCCCAGA GATGCGACCT
4441 CGATGTCAGC GGAGGGAGAT GCTAATTTTT ATCCTGCAGG
```

FIGURE 4

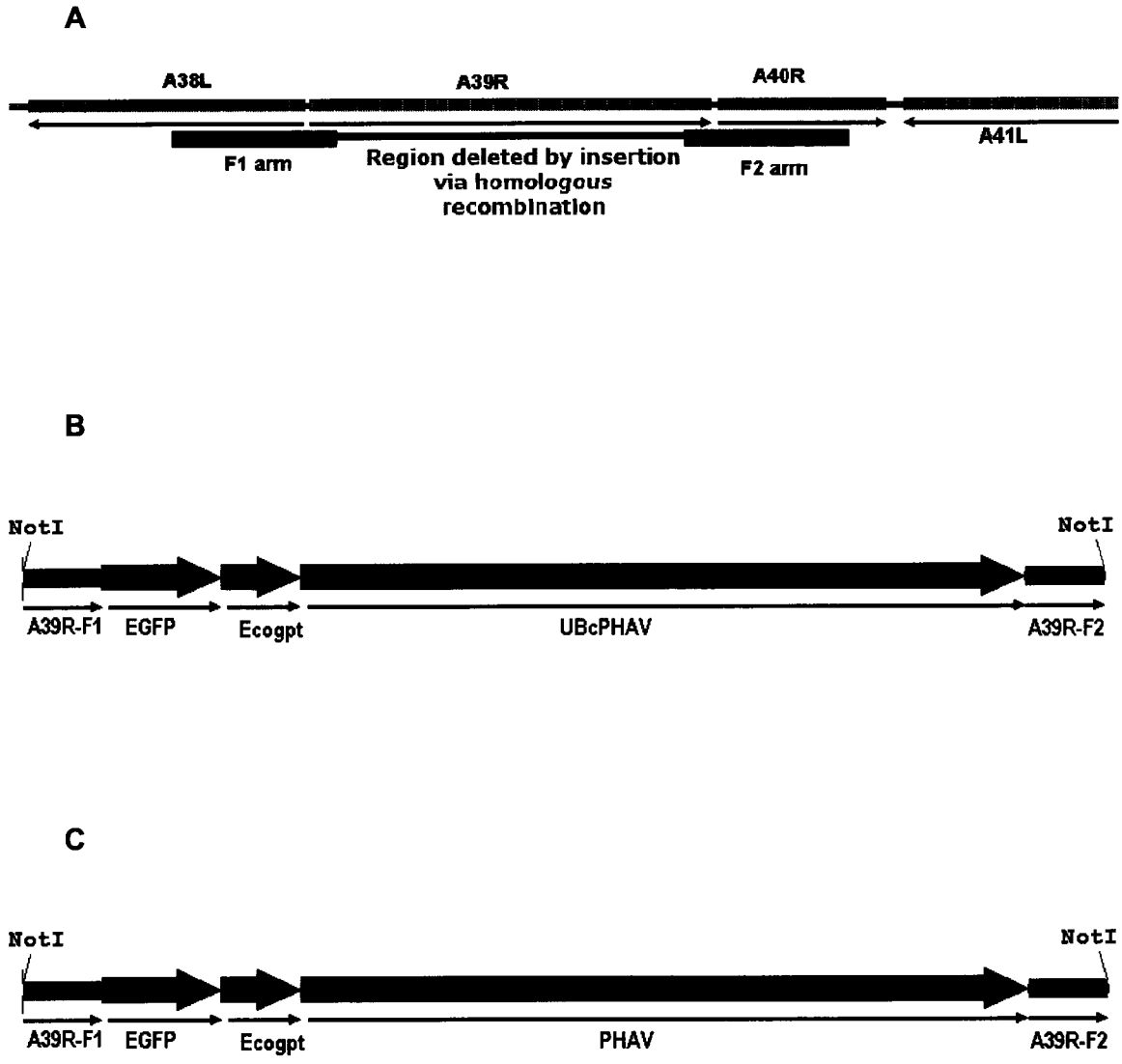

A
A38L
A39R
A40R
A41L
F1 arm
Region deleted by insertion via homologous recombination
F2 arm
B
NotI
NotI
A39R-F1
EGFP
Ecogpt
UBcPHAV
A39R-F2
C
NotI
NotI
A39R-F1
EGFP
Ecogpt
PHAV
A39R-F2

FIGURE 5

Homologous recombination cassette feature table

Element	Description	Size
Not 1	RE site flanking homologous recombination cassette	8 bp
A39R-F1	Homologous recombination arm 1	501 bp
EGFP	Enhance Fluorescent Green Protein expression cassette consisting of a vaccinia early/late promoter followed by the protein coding sequence of EGFP and terminated with the poxvirus early transcriptional stop sequence (TTTTTNT)	771 bp
Ecogpt	E. coli guanine phosphoribosyltransferase expression cassette consisting of a vaccinia early/late promoter followed by the protein coding sequence of Ecogpt and terminated with the poxvirus early transcriptional stop sequence (TTTTTNT)	510 bp
prPs	Vaccinia strong synthetic early/late promoter	44 bp
UbcPHAVag Or PHAV	Peanut hypoallergen vaccine antigen expression cassette UBc.PHAV PHAV	 4705bp 4480 bp
A39R-F2	Homologous recombination arm 2	501 bp
Not I	RE site flanking homologous recombination cassette	8 bp

FIGURE 6

Ubiquitinated Peanut HypoAllergen Vaccine (UBc.PHAV) antigen homologous recombination cassette

```
LOCUS       UBcPHAV HR Cassette        7010 bp    DNA     linear   SYN 10-MAR-
2014
DEFINITION  UBcPHAV Homologous Recombination Cassette
COMMENT     Textco/File created by Gene Construction Kit (TextcoBioSoftware)
ORGANISM  Vaccinia virus Copenhagen strain
FEATURES             Location/Qualifiers
     CDS             1849..6486
                     /label="UBcPHAV"
                     /note="Ubiquitinated PHAV antigen protein coding sequence"
misc_feature    9..509
                     /label="A39R-F1"
                     /note=" VACV-COP A39R homologous recombination arm F1"
misc_feature    517..560
                     /label="prPS"
                     /note=" Vaccinia virus early/late promoter"
     CDS             561..1280
                     /label="EGFP"
                     /note="Enhanced Green Fluorescent Protein coding sequence"
misc_feature    1281..1287
                     /label="T5NT"
                     /note=" Poxvirus early transcriptional stop sequence"
misc_feature    1288..1331
                     /label="prPs"
                     /note="Vaccinia virus early/late promoter"
     CDS             1332..1790
                     /label="Ecogpt"
                     /note="Ecogpt protein coding sequence: E. coli guanine
phosphoribosyltransferase"
misc_feature    1791..1797
                     /label="T5NT"
                     /note=" Poxvirus early transcriptional stop sequence"
misc_feature    1805..1848
                     /label="prPs"
                     /note="Vaccinia virus early/late promoter"
     CDS             1849..2076
                     /label="UBc"
                     /note="  Human Ubiquitin C monomer.  Terminal amino acid
changed [A]"
     CDS             2077..3951
                     /label="arah1"
                     /note=" Peanut allergen ara h 1 protein coding sequence"
     CDS             3952..4464
                     /label="arah2"
                     /note=" Peanut allergen ara h 2 protein coding sequence"
     CDS             4465..6051
                     /label="arah3"
                     /note="Peanut allergen ara h 3 protein coding sequence"
     CDS             6052..6486
                     /label="arah6"
                     /note=" Peanut allergen ara h 6 protein coding sequence"
misc_feature    6487..6493
                     /label="T5NT"
                     /note=" Pox virus early transcriptional stop"
misc_feature    6502..7002
                     /label="A39R-F2"
                    /note=" VACV-COP A39R homologous recombination arm F2"
```

FIGURE 6 (CONTINUED)

```
ORIGIN
        1 GCGGCCGCaatgcccgtaaagataaacatcaacattgtttggtaatcattaaaccaatta
       61 gtatgaagttgaactaatttcacagtagattttattccagtgttatcctcgcatgtataa
      121 gtacctggtaagatatctttatattctataatcaatgagacatcactatccgataacgaa
      181 tgaagtctagcactagtatgccatttacttaatattgtcgtcttggaagttttattataa
      241 gttaaaatatcatggttatccaatttccatctaatatactttgtcggattatctatagta
      301 cacggaataatgatggtatcattacatgctgtatactctatggtctttgtagttgttata
      361 acaaccaacgtatagaggtatatcaacgatattctaactcttgacatttttttatttattt
      421 aaaatgataccttttgttatttatttttattctattttgctaacggtattgaatggcataag
      481 tttgaaacgagtgaagaaataatttctacGCGCGCCcaAA AAATTGAAAT TTTATTTTTT
      541 TTTTTTGGAA TATAAATAAt ATGGTGAGCA AGGGCGAGGA GCTGTTCACC GGGGTGGTGC
      601 CCATCCTGGT CGAGCTGGAC GGCGACGTAA ACGGCCACAA GTTCAGCGTG TCCGGCGAGG
      661 GCGAGGGCGA TGCCACCTAC GGCAAGCTGA CCCTGAAGTT CATCTGCACC ACCGGCAAGC
      721 TGCCCGTGCC CTGGCCCACC CTCGTGACCA CCCTGACCTA CGGCGTGCAG TGCTTCAGCC
      781 GCTACCCCGA CCACATGAAG CAGCACGACT TCTTCAAGTC CGCCATGCCC GAAGGCTACG
      841 TCCAGGAGCG CACCATCTTC TTCAAGGACG ACGGCAACTA CAAGACCCGC GCCGAGGTGA
      901 AGTTCGAGGG CGACACCCTG GTGAACCGCA TCGAGCTGAA GGGCATCGAC TTCAAGGAGG
      961 ACGGCAACAT CCTGGGGCAC AAGCTGGAGT ACAACTACAA CAGCCACAAC GTCTATATCA
     1021 TGGCCGACAA GCAGAAGAAC GGCATCAAGG TGAACTTCAA GATCCGCCAC AACATCGAGG
     1081 ACGGCAGCGT GCAGCTCGCC GACCACTACC AGCAGAACAC CCCCATCGGC GACGGCCCCG
     1141 TGCTGCTGCC CGACAACCAC TACCTGAGCA CCCAGTCCGC CCTGAGCAAA GACCCCAACG
     1201 AGAAGCGCGA TCACATGGTC CTGCTGGAGT TCGTGACCGC CGCCGGGATC ACTCTCGGCA
     1261 TGGACGAGCT GTACAAGTAA tttttatcaA AAAATTGAAA TTTTATTTTT TTTTTTGGA
     1321 ATATAAATAA tATGAGCGAA AAATACATCG TCACCTGGGA CATGTTGCAG ATCCATGCAC
     1381 GTAAACTCGC AAGCCGACTG ATGCCTTCTG AACAATGGAA AGGCATTATT GCCGTAAGCC
     1441 GTGGCGGTCT GGTACCGGGT GCGTTACTGG CGCGTGAACT GGGTATTCGT CATGTCGATA
     1501 CCGTTTGTAT TTCCAGCTAC GATCACGACA ACCAGCGCGA GCTTAAAGTG CTGAAACGCG
     1561 CAGAAGGCGA TGGCGAAGGC TTCATCGTTA TTGATGACCT GGTGGATACC GGTGGTACTG
     1621 CGGTTGCGAT TCGTGAAATG TATCCAAAAG CGCACTTTGT CACCATCTTC GCAAAACCGG
     1681 CTGGTCGTCC GCTGGTTGAT GACTATGTTG TTGATATCCC GCAAGATACC TGGATTGAAC
     1741 AGCCGTGGGA TATGGGCGTC GTATTCGTCC CGCCAATCTC CGGTCGCTAA tttttatATT
     1801 TTAAcaAAAA ATTGAAATTT TATTTTTTTT TTTTGGAATA TAAATAAtAT GCAGATCTTT
     1861 GTGAAAACAC TCACGGGAAA AACTATAACT CTTGAGGTGG AGCCCTCTGA CACAATCGAA
     1921 AATGTGAAAG CCAAGATCCA AGATAAGGAA GGCATCCCTC CAGACCAGCA ACGGCTCATC
     1981 TTTGCGGGCA AACAACTGGA GGATGGGCGC ACTCTCAGTG ATTACAATAT TCAAAAGGAA
     2041 TCTACACTGC ACCTGGTTCT TAGGCTGCGG GGAGCCCGAG GCAGAGTCAG CCCTCTGATG
     2101 CTGCTGTTGG GGATCTTGGT TCTTGCATCC GTTTCAGCCA CACATGCCAA GTCCAGCCCA
     2161 TACCAGAAAA AGACCGAGAA CCCATGTGCT CAGCGGTGCC TCCAGTCATG TCAGCAGGAA
     2221 CCCGATGACC TCAAGCAAAA GGCGTGTGAA AGCAGATGTA CGAAACTGGA ATACGACCCC
     2281 AGATGCGTGT ACGATCCACG AGGCCATACT GGCACCACCA ATCAAAGATC ACCACCTGGC
     2341 GAGCGAACCA GGGGACGACA GCCAGGGGAT TACGATGACG ATAGACGACA GCCTCGCCGG
     2401 GAAGAGGGTG GGCGCTGGGG TCCAGCCGGT CCCAGAGAGC GAGAACGGGA AGAGGACTGG
     2461 CGCCAACCCC GGGAGGACTG GCGCAGACCT TCACACCAGC AGCCCCGGAA AATACGCCCG
     2521 GAGGGCAGAG AGGGTGAACA GGAATGGGGA ACCCCTGGCA GTCACGTCAG GGAAGAAACC
     2581 AGCCGGAACA ACCCCTTCTA TTTCCCCTCC CGGAGGTTTA GCACTCGGTA CGGGAACCAA
     2641 AACGGACGCA TTCGCGTACT TCAGAGATTT GATCAACGCT CACGGCAGTT CCAGAACCTT
     2701 CAAAACCATC GGATTGTTCA GATCGAGGCC AAACCAAACA CTTTGGTTCT GCCAAAACAT
     2761 GCTGATGCAG ACAACATACT GGTGATACAG CAGGGTCAAG CCACAGTGAC AGTAGCCAAT
     2821 GGGAACAATC GCAAATCATT CAATCTCGAT GAGGGACACG CACTGAGGAT CCCTTCTGGC
     2881 TTTATCTCCT ATATACTGAA TCGGCACGAC AATCAAAATC TCAGGGTTGC TAAGATCTCT
     2941 ATGCCAGTCA ACACTCCGGG TCAGTTCGAG GATTTCTTTC CCGCGTCCTC ACGGGACCAG
     3001 TCTAGTTATC TTCAAGGATT CAGCAGAAAC ACCTTGGAAG CGGCCTTTAA CGCCGAGTTT
     3061 AACGAGATCA GGCGGGTGCT TCTCGAGGAG AACGCTGGCG GGGAACAGGA GGAGAGAGGC
     3121 CAACGGCGGT GGTCTACCAG GTCCAGTGAG AACAATGAGG GCGTGATCGT CAAGGTATCT
     3181 AAGGAGCATG TCGAGGAACT GACCAAACAT GCAAAGTCCG TTTCCAAGAA AGGCTCCGAG
     3241 GAGGAAGGGG ACATTACGAA TCCGATCAAC CTTCGGGAGG GCGAGCCGGA TCTGTCAAAT
     3301 AACTTTGGAA AACTCTTCGA AGTCAAGCCC GACAAAAAGA ATCCGCAGTT GCAAGATCTG
     3361 GACATGATGC TCACGTGTGT CGAGATTAAG GAAGGAGCAC TGATGTTGCC TCACTTTAAC
     3421 TCCAAAGCCA TGGTGATAGT CGTAGTAAAC AAAGGAACCG GCAATCTGGA GTTGGTGGCT
     3481 GTCCGAAAGG AACAACAGCA AAGAGGGCGG AGGGAAGAAG AGGAAGATGA GGACGAGGAG
```

FIGURE 6 (CONTINUED)

```
3541 GAGGAGGGAT CAAACCGGGA GGTACGCCGA TACACAGCGA GGCTGAAAGA GGGAGACGTG

3601 TTTATCATGC CGGCAGCACA TCCTGTCGCT ATCAACGCCT CTAGCGAGCT CCATTTGCTG
3661 GGGTTCGGGA TCAATGCGGA GAACAATCAT CGCATTTTCC TGGCAGGCGA CAAGGACAAC
3721 GTTATTGACC AAATTGAGAA GCAAGCCAAG GACCTGGCCT TCCCTGGATC AGGTGAACAG
3781 GTCGAGAAGC TCATCAAAAA CCAGAAGGAA TCCCACTTTG TATCTGCCAG ACCACAGTCA
3841 CAGTCCCAGA GCCCCTCTAG TCCCGAGAAG GAGAGCCCCG AAAAGGAAGA TCAAGAGGAG
3901 GAGAACCAGG GTGGAAAGGG CCCACTGCTT TCCATTCTCA AAGCCTTCAA TGCTAAGCTG
3961 ACAATATTGG TGGCACTGGC ACTGTTCCTT CTTGCTGCAC ACGCGTCAGC CCGGCAGCAG
4021 TGGGAATTGC AGGGCGATCG AAGGTGTCAG TCACAGCTGG AGAGGGCGAA CCTCCGGCCT
4081 TGTGAACAGC ACCTGATGCA GAAGATTCAG CGGGACGAGG ATTCTTACGG GCGAGATCCT
4141 TACAGTCCCT CCCAAGATCC ATATAGCCCG TCTCAAGACC CAGATCGCAG GGACCCATAT
4201 AGCCCCAGCC CCTATGATCG AAGAGGTGCC GGAAGCAGCC AGCATCAGGA AAGGTGCTGC
4261 AATGAGCTGA ACGAGTTCGA GAACAACCAG AGATGTATGT GCGAGGCTCT GCAGCAGATT
4321 ATGGAAAATC AATCTGACCG GCTGCAGGGA CGGCAGCAGG AGCAGCAGTT CAAAAGGGAG
4381 CTCCGCAACC TTCCACAGCA GTGCGGTTTG CGCGCACCTC AGCGCTGCGA CTTGGAGGTG
4441 GAAAGCGGAG GTAGAGACAG ATACGCGAAG CTGCTGGAAC TCAGCTTCTG TTTCTGTTTC
4501 CTGGTACTCG GCGCTTCATC AATATCTTTT AGGCAGCAGC CAGAGGAAAA TGCCTGCCAG
4561 TTCCAACGGC TGAACGCTCA GCGACCAGAC AATAGGATCG AATCAGAAGG TGGATACATC
4621 GAGACTTGGA ACCCGAATAA CCAGGAGTTC GAATGTGCAG GCGTGGCACT GTCTCGCCTT
4681 GTTCTCCGAC GCAATGCGCT CAGGCGCCCA TTCTATTCCA ATGCACCCCA AGAAATCTTT
4741 ATCCAACAGG GCAGAGGGTA CTTCGGGCTG ATCTTTCCCG GCTGTCCCCG GCACTATGAG
4801 GAACCCCACA CACAGGGCAG AAGGAGCCAG AGCCAGCGGC CTCCCCGGAG ATTGCAAGGG
4861 GAGGATCAGA GCCAGCAGCA GAGAGATTCT CATCAGAAAG TACATAGGTT CGATGAGGGT
4921 GACCTGATAG CTGTGCCAAC CGGTGTTGCC TTTTGGTTGT ATAATGACCA CGACACAGAC
4981 GTGGTGGCTG TGTCTCTGAC CGATACAAAC AACAATGACA ATCAGCTTGA TCAGTTCCCT
5041 AGGCGCTTTA ACCTGGCTGG CAACACCGAA CAGGAGTTCT TGAGATATCA GCAGCAGTCT
5101 AGGCAGTCTA GGAGGAGGTC CCTGCCATAC TCCCCTTACA GCCCTCAGAG TCAGCCTAGG
5161 CAGGAAGAGA GAGAATTCAG TCCCAGAGGC CAGCACTCTA GGCGGGAGCG GGCTGGGCAG
5221 GAGGAGGAAA ACGAAGGTGG CAATATCTTT AGCGGCTTCA CTCCAGAGTT TCTGGAACAG
5281 GCATTCCAAG TAGATGACAG ACAGATCGTC CAGAACCTTA GGGGCGAGAC TGAATCAGAA
5341 GAGGAAGGGG CAATCGTGAC GGTGCGCGGA GGCTTGCGCA TCCTGTCCCC TGACCGCAAA
5401 CGCAGGGCCG ACGAGGAAGA AGAGTATGAC GAGGATGAAT ATGAATATGA TGAGGAGGAT
5461 CGAAGGCGCG GAAGGGGCAG TAGGGGACGA GGGAACGGCA TAGAAGAAAC TATTTGTACC
5521 GCGTCCGCCA AGAAGAATAT TGGGCGAAAC CGCAGTCCCG ACATATACAA TCCTCAAGCC
5581 GGCAGCCTTA AAACCGCCAA CGATCTGAAC CTGCTGATCC TCCGCTGGCT GGGGCCAAGC
5641 GCCGAATATG GGAATCTGTA CCGAAATGCT CTGTTTGTGG CCCACTACAA TACAAATGCC
5701 CACTCTATTA TCTACCGCCT CAGAGGGAGG GCTCATGTGC AAGTGGTCGA CAGCAATGGG
5761 AATCGCGTGT ACGATGAGGA GCTCCAAGAA GGGCATGTCC TTGTTGTGCC TCAGAATTTC
5821 GCAGTTGCGG GCAAATCACA GAGTGAGAAC TTCGAGTACG TTGCCTTTAA GACCGATTCC
5881 AGACCCTCCA TTGCAAACCT GGCCGGAGAG AACAGTGTTA TTGACAATCT GCCGGAGGAA
5941 GTGGTTGCTA ACAGTTATGG GCTTCAGCGC GAACAGGCTC GGCAGCTGAA GAACAACAAT
6001 CCGTTCAAGT TTTTCGTCCC TCCATCCCAG CAGTCACCCA GAGCTGTGGC CGCCAAATCC
6061 ACTATTCTTG TGGCCCTCTT GGCACTCGTG CTGGTCGCCC ATGCTTCTGC TATGCGAAGG
6121 GAGAGAGGGC GCCAAGGTGA CTCAAGCAGT TGCGAACGAC AAGTGGACAG AGTGAACCTC
6181 AAACCTTGCG AACAGCACAT TATGCAGAGA ATTATGGGAG AGCAAGAGCA GTATGATAGT
6241 TATGATATCA GATCAACACG CTCTTCCGAT CAGCAACAGC GGTGTTGCGA TGAACTCAAC
6301 GAAATGGAGA ATACGCAGCG GTGCATGTGT GAGGCTCTTC AGCAAATCAT GGAAAACCAA
6361 TGCGATCGGC TCCAAGATCG ACAGATGGTG CAGCAGTTTA AGCGCGAGCT GATGAATTTG
6421 CCACAACAGT GCAACTTTCG GGCTCCCCAG AGATGCGACC TCGATGTCAG CGGAGGGAGA
6481 TGCTAATTTT TATCCTGCAG Gacttcgaaaccgttatttatagcagatatagggatagga
6541 gtaggaatgccacaaatgaaaaaaatacttaaaatgtaatcttaatcgagtacaccacac
6601 gacaatgaacaaacctaagacagattatgctggttatgcttgctgcgtaatatgcggtct
6661 aattgtcggaattatttttacagcgacactattaaaagttgtagaacgtaaattagttca
6721 tacaccatcaatagataaaacgataaaagatgcatatattagagaagattgtcctactga
6781 ctggataagctataataataaatgtatccatttatctactgatcgaaaaacctgggagga
6841 aggacgtaatacatgcaaagctctaaatccaaattcggatctaattaagatagagactcc
6901 aaacgagttaagtttttaagaagccttagacgaggctattgggtaggagaatccgaaat
6961 attaaaccagacaaccccatataatttatagctaaaaatgcGCGGCCGC
```

FIGURE 7

Peanut HypoAllergen Vaccine (PHAV) antigen homologous recombination cassette

```
LOCUS  PHAV HR cassette         6785 bp    DNA     linear   SYN 10-MAR-2014
DEFINITION  PHAV homologous recombination cassette
ORGANISM  Vaccinia virus Copenhagen strain
COMMENT     Textco/File created by Gene Construction Kit (TextcoBioSoftware)
FEATURES             Location/Qualifiers
     CDS             1849..6261
                     /label="PHAV"
                     /note=" PHAV antigen protein coding sequence"
misc_feature    9..509
                     /label="A39R-F1"
                     /note=" VACV-COP A39R homologous recombination arm 1"
misc_feature    517..560
                     /label="prPs"
                     /note="Vaccinia virus early/late promoter"
     CDS             561..1280
                     /label="EGFP"
                     /note=" Enhanced Green Fluorescent Protein coding sequence"
misc_feature    1281..1287
                     /label="T5NT"
                     /note=" Poxvirus early transcriptional stop sequence"
misc_feature    1288..1331
                     /label="prPs"
                     /note=" Vaccinia virus early/late promoter"
     CDS             1332..1790
                     /label="Ecogpt"
                     /note="Ecogpt protein coding sequence: E. coli guanine
phosphoribosyltransferase"
misc_feature    1791..1797
                     /label="T5NT"
                     /note="  Poxvirus early transcriptional stop sequence"
misc_feature    1805..1848
                     /label="prPs"
                     /note=" Vaccinia virus early/late promoter"
     CDS             1849..3726
                     /label="arah1"
                     /note="Peanut allergen ara h 1 protein coding sequence"
     CDS             3727..4239
                     /label="arah2"
                     /note=" Peanut  allergenara h 2 protein coding sequence"
     CDS             4240..5826
                     /label="arah3"
                     /note="Peanut allergen ara h 3 protein coding sequence"
     CDS             5827..6261
                     /label="arah6"
                     /note=" Peanut allergen ara h 6 protein coding sequence"
misc_feature    6262..6268
                     /label="T5NT"
                     /note=" Pox virus early transcriptional stop"
misc_feature    6277..6777
                     /label="A39R-F2"
                     /note=" VACV-COP A39R homologous recombination arm 2"
ORIGIN
        1 GCGGCCGCaatgcccgtaaagataaacatcaacattgtttggtaatcattaaaccaatta
       61 gtatgaagttgaactaatttcacagtagattttattccagtgttatcctcgcatgtataa
      121 gtacctggtaagatatctttatattctataatcaatgagacatcactatccgataacgaa
      181 tgaagtctagcactagtatgccatttacttaatattgtcgtcttggaagttttattataa
```

FIGURE 7 (CONTINUED)

```
 241 gttaaaatatcatggttatccaatttccatctaatatactttgtcggattatctatagta
 301 cacggaataatgatggtatcattacatgctgtatactctatggtctttgtagttgttata
 361 acaaccaacgtatagaggtatatcaacgatattctaactcttgacatttttttatttattt
 421 aaaatgataccttttgttatttatttttattctattttgctaacggtattgaatggcataag
 481 tttgaaacgagtgaagaaataatttctacGCGCGCCcaAA AAATTGAAAT TTTATTTTTT
 541 TTTTTTGGAA TATAAATAAt ATGGTGAGCA AGGGCGAGGA GCTGTTCACC GGGGTGGTGC
 601 CCATCCTGGT CGAGCTGGAC GGCGACGTAA ACGGCCACAA GTTCAGCGTG TCCGGCGAGG
 661 GCGAGGGCGA TGCCACCTAC GGCAAGCTGA CCCTGAAGTT CATCTGCACC ACCGGCAAGC
 721 TGCCCGTGCC CTGGCCCACC CTCGTGACCA CCCTGACCTA CGGCGTGCAG TGCTTCAGCC
 781 GCTACCCCGA CCACATGAAG CAGCACGACT TCTTCAAGTC CGCCATGCCC GAAGGCTACG
 841 TCCAGGAGCG CACCATCTTC TTCAAGGACG ACGGCAACTA CAAGACCCGC GCCGAGGTGA
 901 AGTTCGAGGG CGACACCCTG GTGAACCGCA TCGAGCTGAA GGGCATCGAC TTCAAGGAGG
 961 ACGGCAACAT CCTGGGGCAC AAGCTGGAGT ACAACTACAA CAGCCACAAC GTCTATATCA
1021 TGGCCGACAA GCAGAAGAAC GGCATCAAGG TGAACTTCAA GATCCGCCAC AACATCGAGG
1081 ACGGCAGCGT GCAGCTCGCC GACCACTACC AGCAGAACAC CCCCATCGGC GACGGCCCCG
1141 TGCTGCTGCC CGACAACCAC TACCTGAGCA CCCAGTCCGC CCTGAGCAAA GACCCCAACG
1201 AGAAGCGCGA TCACATGGTC CTGCTGGAGT TCGTGACCGC CGCCGGGATC ACTCTCGGCA
1261 TGGACGAGCT GTACAAGTAA tttttatcaA AAAATTGAAA TTTTATTTTT TTTTTTTGGA
1321 ATATAAATAA tATGAGCGAA AAATACATCG TCACCTGGGA CATGTTGCAG ATCCATGCAC
1381 GTAAACTCGC AAGCCGACTG ATGCCTTCTG AACAATGGAA AGGCATTATT GCCGTAAGCC
1441 GTGGCGGTCT GGTACCGGGT GCGTTACTGG CGCGTGAACT GGGTATTCGT CATGTCGATA
1501 CCGTTTGTAT TTCCAGCTAC GATCACGACA ACCAGCGCGA GCTTAAAGTG CTGAAACGCG
1561 CAGAAGGCGA TGGCGAAGGC TTCATCGTTA TTGATGACCT GGTGGATACC GGTGGTACTG
1621 CGGTTGCGAT TCGTGAAATG TATCCAAAAG CGCACTTTGT CACCATCTTC GCAAAACCGG
1681 CTGGTCGTCC GCTGGTTGAT GACTATGTTG TTGATATCCC GCAAGATACC TGGATTGAAC
1741 AGCCGTGGGA TATGGGCGTC GTATTCGTCC CGCCAATCTC CGGTCGCTAA tttttatATT
1801 TTAAcaAAAA ATTGAAATTT TATTTTTTTT TTTTGGAATA TAAATAAtatgCGAGGCAGA
1861 GTCAGCCCTC TGATGCTGCT GTTGGGGATC TTGGTTCTTG CATCCGTTTC AGCCACACAT
1921 GCCAAGTCCA GCCCATACCA GAAAAAGACC GAGAACCCAT GTGCTCAGCG GTGCCTCCAG
1981 TCATGTCAGC AGGAACCCGA TGACCTCAAG CAAAAGGCGT GTGAAAGCAG ATGTACGAAA
2041 CTGGAATACG ACCCCAGATG CGTGTACGAT CCACGAGGCC ATACTGGCAC CACCAATCAA
2101 AGATCACCAC CTGGCGAGCG AACCAGGGGA CGACAGCCAG GGGATTACGA TGACGATAGA
2161 CGACAGCCTC GCCGGGAAGA GGGTGGGCGC TGGGGTCCAG CCGGTCCCAG AGAGCGAGAA
2221 CGGGAAGAGG ACTGGCGCCA ACCCCGGGAG GACTGGCGCA GACCTTCACA CCAGCAGCCC
2281 CGGAAAATAC GCCCGGAGGG CAGAGAGGGT GAACAGGAAT GGGGAACCCC TGGCAGTCAC
2341 GTCAGGGAAG AAACCAGCCG GAACAACCCC TTCTATTTCC CCTCCCGGAG GTTTAGCACT
2401 CGGTACGGGA ACCAAAACGG ACGCATTCGC GTACTTCAGA GATTTGATCA ACGCTCACGG
2461 CAGTTCCAGA ACCTTCAAAA CCATCGGATT GTTCAGATCG AGGCCAAACC AAACACTTTG
2521 GTTCTGCCAA AACATGCTGA TGCAGACAAC ATACTGGTGA TACAGCAGGG TCAAGCCACA
2581 GTGACAGTAG CCAATGGGAA CAATCGCAAA TCATTCAATC TCGATGAGGG ACACGCACTG
2641 AGGATCCCTT CTGGCTTTAT CTCCTATATA CTGAATCGGC ACGACAATCA AAATCTCAGG
2701 GTTGCTAAGA TCTCTATGCC AGTCAACACT CCGGGTCAGT TCGAGGATTT CTTTCCCGCG
2761 TCCTCACGGG ACCAGTCTAG TTATCTTCAA GGATTCAGCA GAAACACCTT GGAAGCGGCC
2821 TTTAACGCCG AGTTTAACGA GATCAGGCGG GTGCTTCTCG AGGAGAACGC TGGCGGGGAA
2881 CAGGAGGAGA GAGGCCAACG GCGGTGGTCT ACCAGGTCCA GTGAGAACAA TGAGGGCGTG
2941 ATCGTCAAGG TATCTAAGGA GCATGTCGAG GAACTGACCA AACATGCAAA GTCCGTTTCC
3001 AAGAAAGGCT CCGAGGAGGA AGGGGACATT ACGAATCCGA TCAACCTTCG GGAGGGCGAG
3061 CCGGATCTGT CAAATAACTT TGGAAAACTC TTCGAAGTCA AGCCCGACAA AAAGAATCCG
3121 CAGTTGCAAG ATCTGGACAT GATGCTCACG TGTGTCGAGA TTAAGGAAGG AGCACTGATG
3181 TTGCCTCACT TTAACTCCAA AGCCATGGTG ATAGTCGTAG TAAACAAAGG AACCGGCAAT
3241 CTGGAGTTGG TGGCTGTCCG AAAGGAACAA CAGCAAAGAG GGCGGAGGGA AGAAGAGGAA
3301 GATGAGGACG AGGAGGAGGA GGGATCAAAC CGGGAGGTAC GCCGATACAC AGCGAGGCTG
3361 AAAGAGGGAG ACGTGTTTAT CATGCCGGCA GCACATCCTG TCGCTATCAA CGCCTCTAGC
3421 GAGCTCCATT TGCTGGGGTT CGGGATCAAT GCGGAGAACA ATCATCGCAT TTTCCTGGCA
3481 GGCGACAAGG ACAACGTTAT TGACCAAATT GAGAAGCAAG CCAAGGACCT GGCCTTCCCT
3541 GGATCAGGTG AACAGGTCGA GAAGCTCATC AAAAACCAGA AGGAATCCCA CTTTGTATCT
3601 GCCAGACCAC AGTCACAGTC CCAGAGCCCC TCTAGTCCCG AGAAGGAGAG CCCCGAAAAG
3661 GAAGATCAAG AGGAGGAGAA CCAGGGTGGA AAGGGCCCAC TGCTTTCCAT TCTCAAAGCC
3721 TTCAATGCTA AGCTGACAAT ATTGGTGGCA CTGGCACTGT TCCTTCTTGC TGCACACGCG
3781 TCAGCCCGGC AGCAGTGGGA ATTGCAGGGC GATCGAAGGT GTCAGTCACA GCTGGAGAGG
```

FIGURE 7 (CONTINUED)

```
3841 GCGAACCTCC GGCCTTGTGA ACAGCACCTG ATGCAGAAGA TTCAGCGGGA CGAGGATTCT
3901 TACGGGCGAG ATCCTTACAG TCCCTCCCAA GATCCATATA GCCCGTCTCA AGACCCAGAT
3961 CGCAGGGACC CATATAGCCC CAGCCCCTAT GATCGAAGAG GTGCCGGAAG CAGCCAGCAT
4021 CAGGAAAGGT GCTGCAATGA GCTGAACGAG TTCGAGAACA ACCAGAGATG TATGTGCGAG
4081 GCTCTGCAGC AGATTATGGA AAATCAATCT GACCGGCTGC AGGGACGGCA GCAGGAGCAG
4141 CAGTTCAAAA GGGAGCTCCG CAACCTTCCA CAGCAGTGCG GTTTGCGCGC ACCTCAGCGC
4201 TGCGACTTGG AGGTGGAAAG CGGAGGTAGA GACAGATACG CGAAGCTGCT GGAACTCAGC
4261 TTCTGTTTCT GTTTCCTGGT ACTCGGCGCT TCATCAATAT CTTTTAGGCA GCAGCCAGAG
4321 GAAAATGCCT GCCAGTTCCA ACGGCTGAAC GCTCAGCGAC CAGACAATAG GATCGAATCA
4381 GAAGGTGGAT ACATCGAGAC TTGGAACCCG AATAACCAGG AGTTCGAATG TGCAGGCGTG
4441 GCACTGTCTC GCCTTGTTCT CCGACGCAAT GCGCTCAGGC GCCCATTCTA TTCCAATGCA
4501 CCCCAAGAAA TCTTTATCCA ACAGGGCAGA GGGTACTTCG GGCTGATCTT TCCCGGCTGT
4561 CCCCGGCACT ATGAGGAACC CCACACACAG GGCAGAAGGA GCCAGAGCCA GCGGCCTCCC
4621 CGGAGATTGC AAGGGGAGGA TCAGAGCCAG CAGCAGAGAG ATTCTCATCA GAAAGTACAT
4681 AGGTTCGATG AGGGTGACCT GATAGCTGTG CCAACCGGTG TTGCCTTTTG GTTGTATAAT
4741 GACCACGACA CAGACGTGGT GGCTGTGTCT CTGACCGATA CAAACAACAA TGACAATCAG
4801 CTTGATCAGT TCCCTAGGCG CTTTAACCTG GCTGGCAACA CCGAACAGGA GTTCTTGAGA
4861 TATCAGCAGC AGTCTAGGCA GTCTAGGAGG AGGTCCCTGC CATACTCCCC TTACAGCCCT
4921 CAGAGTCAGC CTAGGCAGGA AGAGAGAGAA TTCAGTCCCA GAGGCCAGCA CTCTAGGCGG
4981 GAGCGGGCTG GGCAGGAGGA GGAAAACGAA GGTGGCAATA TCTTTAGCGG CTTCACTCCA
5041 GAGTTTCTGG AACAGGCATT CCAAGTAGAT GACAGACAGA TCGTCCAGAA CCTTAGGGGC
5101 GAGACTGAAT CAGAAGAGGA AGGGGCAATC GTGACGGTGC GCGGAGGCTT GCGCATCCTG
5161 TCCCCTGACC GCAAACGCAG GGCCGACGAG GAAGAAGAGT ATGACGAGGA TGAATATGAA
5221 TATGATGAGG AGGATCGAAG GCGCGGAAGG GGCAGTAGGG GACGAGGGAA CGGCATAGAA
5281 GAAACTATTT GTACCGCGTC CGCCAAGAAG AATATTGGGC GAAACCGCAG TCCCGACATA
5341 TACAATCCTC AAGCCGGCAG CCTTAAAACC GCCAACGATC TGAACCTGCT GATCCTCCGC
5401 TGGCTGGGGC CAAGCGCCGA ATATGGGAAT CTGTACCGAA ATGCTCTGTT TGTGGCCCAC
5461 TACAATACAA ATGCCCACTC TATTATCTAC CGCCTCAGAG GGAGGGCTCA TGTGCAAGTG
5521 GTCGACAGCA ATGGGAATCG CGTGTACGAT GAGGAGCTCC AAGAAGGGCA TGTCCTTGTT
5581 GTGCCTCAGA ATTTCGCAGT TGCGGGCAAA TCACAGAGTG AGAACTTCGA GTACGTTGCC
5641 TTTAAGACCG ATTCCAGACC CTCCATTGCA AACCTGGCCG GAGAGAACAG TGTTATTGAC
5701 AATCTGCCGG AGGAAGTGGT TGCTAACAGT TATGGGCTTC AGCGCGAACA GGCTCGGCAG
5761 CTGAAGAACA ACAATCCGTT CAAGTTTTTC GTCCCTCCAT CCCAGCAGTC ACCCAGAGCT
5821 GTGGCCGCCA AATCCACTAT TCTTGTGGCC CTCTTGGCAC TCGTGCTGGT CGCCCATGCT
5881 TCTGCTATGC GAAGGGAGAG AGGGCGCCAA GGTGACTCAA GCAGTTGCGA ACGACAAGTG
5941 GACAGAGTGA ACCTCAAACC TTGCGAACAG CACATTATGC AGAGAATTAT GGGAGAGCAA
6001 GAGCAGTATG ATAGTTATGA TATCAGATCA ACACGCTCTT CCGATCAGCA ACAGCGGTGT
6061 TGCGATGAAC TCAACGAAAT GGAGAATACG CAGCGGTGCA TGTGTGAGGC TCTTCAGCAA
6121 ATCATGGAAA ACCAATGCGA TCGGCTCCAA GATCGACAGA TGGTGCAGCA GTTTAAGCGC
6181 GAGCTGATGA ATTTGCCACA ACAGTGCAAC TTTCGGGCTC CCCAGAGATG CGACCTCGAT
6241 GTCAGCGGAG GGAGATGCTA ATTTTTATCC TGCAGGacttcgaaaccgttatttatagca
6301 gatatagggataggagtaggaatgccacaaatgaaaaaaatacttaaaatgtaatcttaa
6361 tcgagtacaccacacgacaatgaacaaacctaagacagattatgctggttatgcttgctg
6421 cgtaatatgcggtctaattgtcggaattatttttacagcgacactattaaaagttgtaga
6481 acgtaaattagttcatacaccatcaatagataaaacgataaaagatgcatatattagaga
6541 agattgtcctactgactggataagctataataataaatgtatccatttatctactgatcg
6601 aaaaacctgggaggaaggacgtaatacatgcaaagctctaaatccaaattcggatctaat
6661 taagatagagactccaaacgagttaagtttttaagaagccttagacgaggctattgggt
6721 aggagaatccgaaatattaaaccagacaaccccatataattttatagctaaaaatgcGCG
6781 GCCGC
```

FIGURE 8

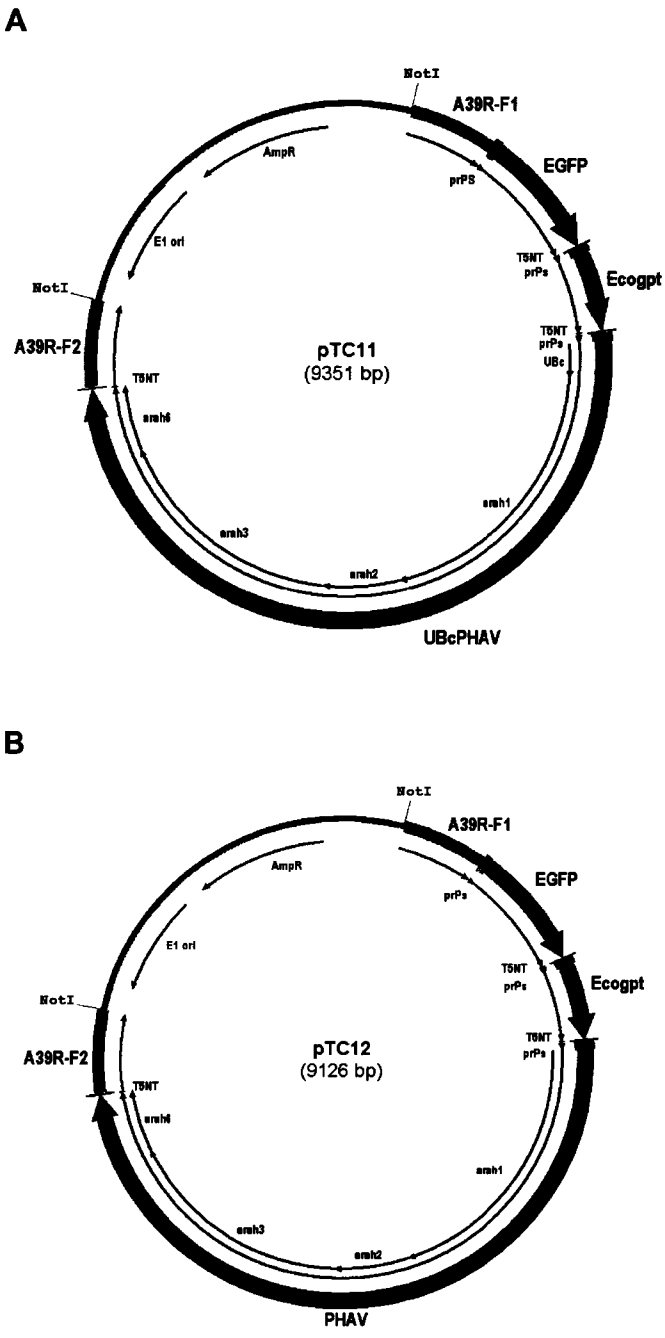

A
NotI
A39R-F1
EGFP
AmpR
prPS
E1 ori
T5NT prPs
Ecogpt
NotI
A39R-F2
T5NT prPs UBc
pTC11
(9351 bp)
T5NT
arah6
arah1
arah3
arah2
UBcPHAV
B
NotI
A39R-F1
EGFP
AmpR
prPs
E1 ori
T5NT prPs
Ecogpt
NotI
A39R-F2
T5NT prPs
pTC12
(9126 bp)
T5NT
arah6
arah1
arah3
arah2
PHAV

FIGURE 9

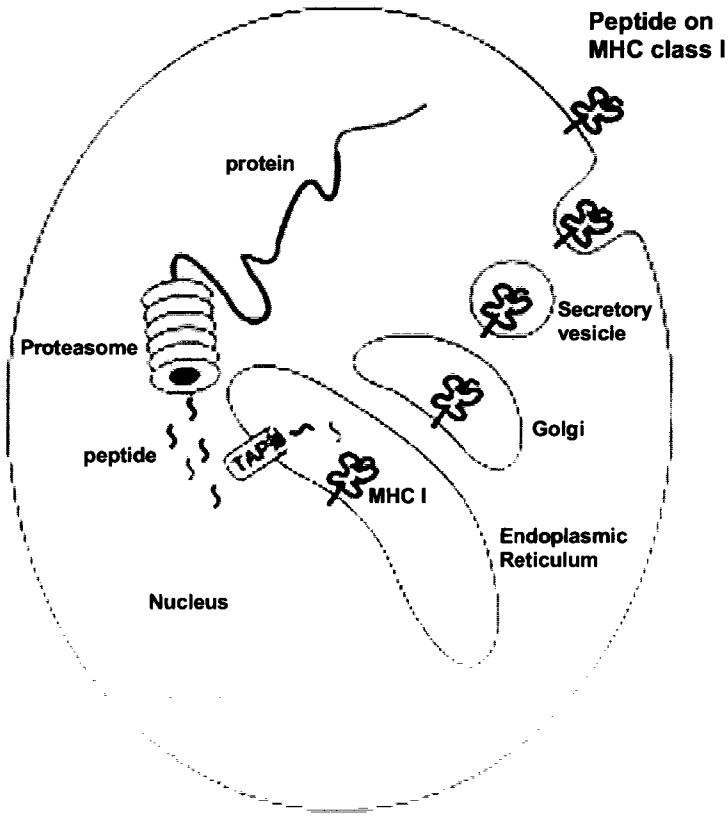
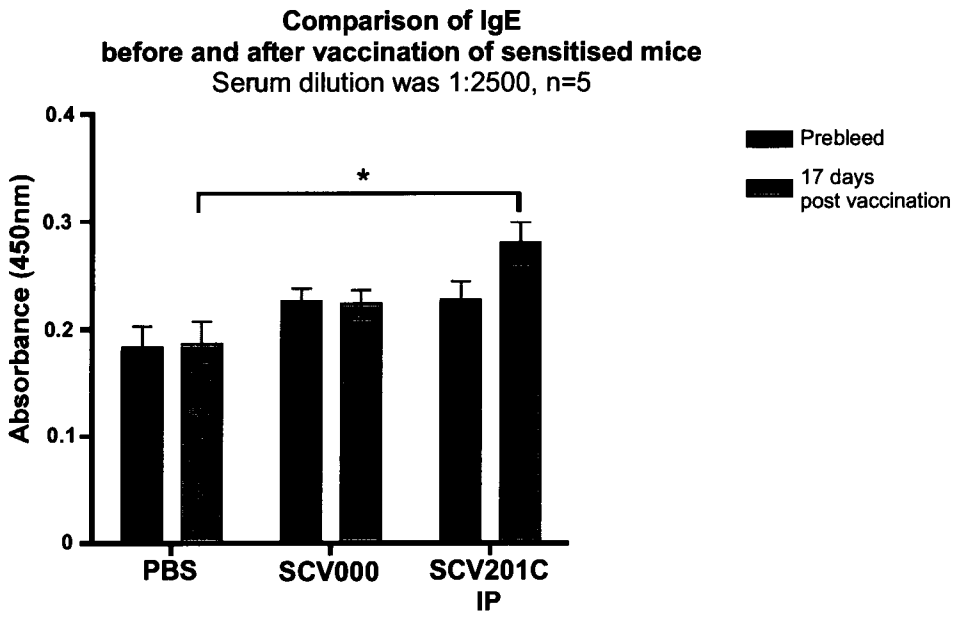

Peptide on
MHC class I
protein
Secretory
vesicle
Proteasome
Golgi
peptide
TAP
MHC I
Endoplasmic
Reticulum
Nucleus

FIGURE 10

A


Comparison of IgE
before and after vaccination of sensitised mice
Serum dilution was 1:2500, n=5
Absorbance (450nm)
0.4
0.3
0.2
0.1
0
*
PBS
SCV000
SCV201C
IP
Prebleed
17 days
post vaccination

B

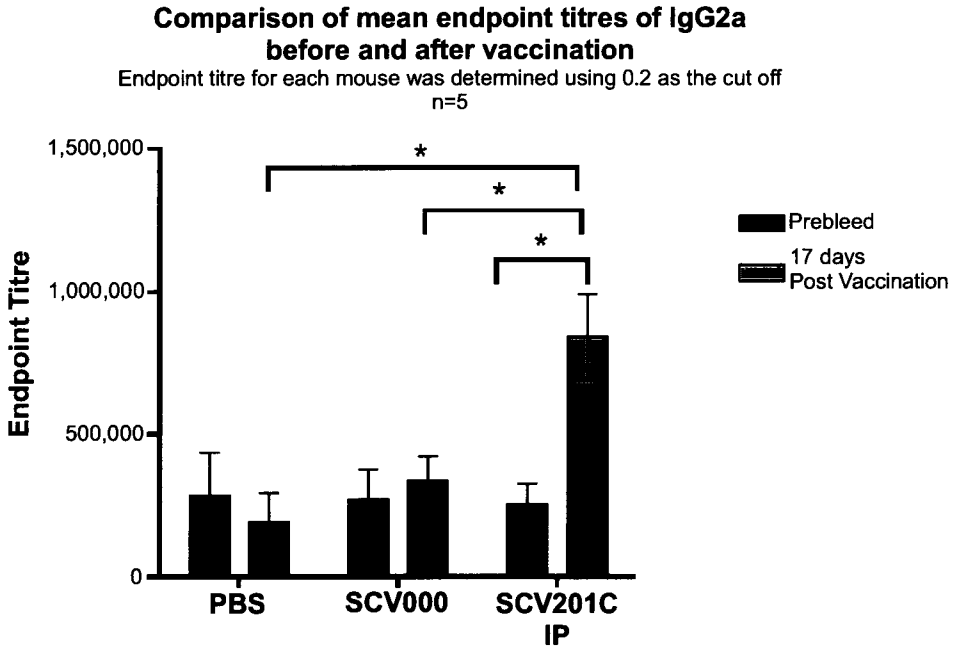
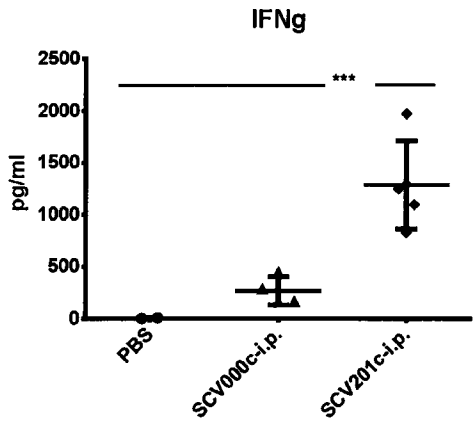

Comparison of mean endpoint titres of IgG2a
before and after vaccination
Endpoint titre for each mouse was determined using 0.2 as the cut off
n=5
Endpoint Titre
1,500,000
1,000,000
500,000
0
*
*
*
PBS
SCV000
SCV201C
IP
Prebleed
17 days
Post Vaccination

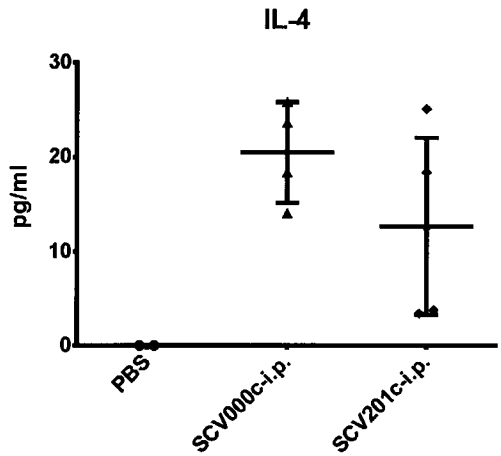
FIGURE 11

A

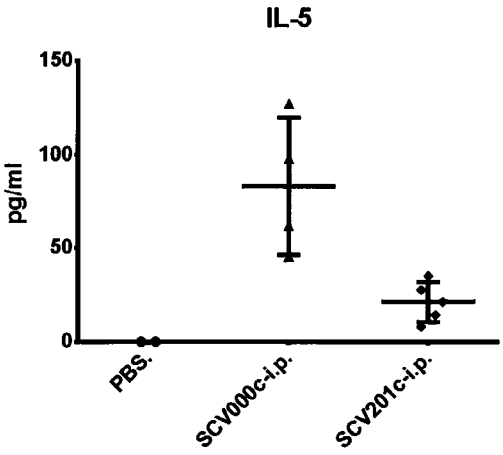


Group B is significantly greater than group A ($p < 0.005$)

B



C



INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2014/000286

A. CLASSIFICATION OF SUBJECT MATTER

A61K 39/35 (2006.01) ***C12N 15/62 (2006.01)*** ***C12N 7/01 (2006.01)*** ***C12N 15/29 (2006.01)*** ***A61K 31/711 (2006.01)***
A61K 31/713 (2006.01) ***A61P 37/08 (2006.01)***

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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

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

Databases used: MEDLINE, AGRICOLA, BIOSIS, HCAPLUS, EMBASE, WPI, EPODOC, GENOMEQUEST, ESPACENET
Keywords used: PEANUT ALLERGEN, ARA H1-H11, PROTEASOME DEGRADATION TAG, UBIQUITIN, CHIMERIC, FUSED, FUSION, POXVIRUS, VACCINIA, VACCINE, TOLERIZE, DESENSITIZE, SUPPRESS ALLERGIC, SEQ ID NO: 11, SEQ ID NO: 12, HOWLEY, PAUL MICHAEL, SEMENTIS LIMITED, and like terms

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

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

Date of the actual completion of the international search	Date of mailing of the international search report
4 April 2014	04 April 2014
Name and mailing address of the ISA/AU	**Authorised officer**
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustralia.gov.au Facsimile No.: +61 2 6283 7999	Alison Knight AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. +61 2 6283 2847

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2014/000286

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

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

   a. (means)

   [X] on paper

   [ ] in electronic form

   b. (time)

   [X] in the international application as filed

   [ ] together with the international application in electronic form

   [ ] subsequently to this Authority for the purposes of search

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

3. Additional comments:
   SEQ ID NOs: 11-12 were searched

INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation).	DOCUMENTS CONSIDERED TO BE RELEVANT	**PCT/AU2014/000286**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2007/104581 A1 (PAUL-EHRLICH-INSTITUT BUNDESAMT FUR SERA UND IMPFSTOFFE) 20 September 2007 p4 line 25-p5 line 11; p10, lines 19-26; Examples, p17-18	1-25
A	Liu Y, et. al. Blockade of peanut allergy with a novel Ara h 2-Fcγ fusion protein in mice. J Allergy Clin Immunol. 2013, 131(1):213-21.e1-5.Epub 2012 Nov 27. Abstract; Figure 1; Figure 4	1-25
A	Li XM, et. al. Engineered recombinant peanut protein and heat-killed *Listeria monocytogenes* coadministration protects against peanut-induced anaphylaxis in a murine model. J Immunol. 2003, 170(6):3289-95. Abstract; Figure 2	1-25
A	Li XM, et. al. Persistent protective effect of heat-killed *Escherichia coli* producing "engineered," recombinant peanut proteins in a murine model of peanut allergy. J Allergy Clin Immunol. 2003, 112(1):159-67. Abstract; Figures 3-4	1-25

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/AU2014/000286**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		**Patent Family Member/s**	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
WO 2007/104581 A1	20 Sep 2007	EP 1994159 A1	26 Nov 2008
		US 2009191157 A1	30 Jul 2009
		WO 2007104581 A1	20 Sep 2007

End of Annex

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.