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(54) Title: NUCLEIC ACIDS FOR TREATMENT OF ALLERGIES

(57) Abstract: The present invention provides DNA vaccines for the treatment or prevention of an allergic response. The vaccines comprise the coding sequence for Allergen X or fragments thereof fused in-frame with the luminal domain of the lysosomal associated membrane protein (LAMP) and the targeting sequence of LAMP. The vaccines allow for presentation of properly configured three dimensional epitopes for production of an immune response when administered to a subject. The vaccines can be multivalent molecules, and/or can be provided as part of a multivalent vaccine containing two or more DNA constructs.



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NUCLEIC ACIDS FOR TREATMENT OF ALLERGIES

BACKGROUND OF THE INVENTION

Field of the Invention

[0001] The present invention relates to the fields of molecular biology and medicine. More specifically, the invention relates to nucleic acids for use as DNA vaccines, and methods of using them to treat subjects suffering from or susceptible to allergic reactions. Prime boost protocols utilizing the LAMP Constructs described herein are also described.

Discussion of the Related Art

[0002] Allergic reactions occur when the immune system reacts to harmless foreign substances, called allergens. For example, food allergies are an important public health issue due to the high risk of anaphylaxis, a potentially deadly systemic shock (Sampson et al. (1992) N. Engl. J. Med. 327:380-384; Bock et al. (2001) J. Allergy Clin. Immunol. 107:191-193). Young children are at greater risk of developing food allergies than the general public (Lack et al. (2003) N. Engl. J. Med 348:977-985; Zimmerman et al. (1989) J. Allergy Clin. Immunol. 83:764-770; Green et al. (2007) Pediatrics 120:1304-1310). During the first three years of life, 6-8% of children experience an allergic reaction caused by food (Bock (1987) Allergy 45:587-596; Burks and Sampson (1993) Curr. Prob. Pediatr. 23:230-252; Jansen et al. (1994) J. Allergy Clin. Immunol. 93:2:446-456; Sampson (1999) J. Allergy Clin. Immunol. 103:5:717-728). Nut allergies, e.g., peanut and nut allergies, affect up to 1-2% of the population, and the rate of occurrence of this food allergy is thought to be increasing in the general population, disproportionately affecting those of Asian ethnicity.

[0003] Anaphylaxis caused by exposure to an allergen, e.g., tree nuts or peanuts, results in a severe immune reaction characterized by overproduction of histamine and is responsible for half of U.S. anaphylaxis emergency room visits annually. For example, extreme reactions to nuts result in over 30,000 incidents of anaphylaxis and between 100 – 200 deaths in the U.S. each year. Nuts in trace amounts are commonly found in thousands of individually branded, but not labeled, packaged food items. More than one

and a half million Americans suffer symptoms from nut allergy and symptoms often persist throughout life. Many experience dangerous reactions on exposure to trace amounts.

[0004] There is no treatment for relieving nut allergy symptoms. Over the last ten years, the prevalence of nut allergies has doubled to affect 2% of adult Americans (Sampson (1999) *J. Allergy Clin. Immunol.* 103;5:717-728; Sicherer et al. (2003) *J. Allergy Clin. Immunol.* 112:1203-1207). While the symptoms for many other allergies like hay fever and short ragweed pollen are not life threatening, for a nut allergic individual, the ingestion of as little as 1/1000th of a nut can induce anaphylactic shock and death (Taylor et al. (2002) *J. Allergy Clin. Immunol.* 109 (1):24-30; Wensing et al. (2002) *J. Allergy Clin. Immunol.* 110(6):915-920). In the event that accidental ingestion triggers anaphylaxis, injections of epinephrine are used to open up airway passages (Stark and Sullivan (1986) *J. Allergy Clin. Immunol.* 78:76-83; Sampson (2003) *Pediatrics* 111(6):1601-1608).

[0005] Food allergies occur when an individual fails to develop oral tolerance and instead becomes sensitized to subsequent allergen exposure (Till et al. (2004) *J. Allergy Clin. Immunol.* 113(6):1025-1034). In allergic patients, allergens preferentially activate type 2 helper CD4⁺ T lymphocytes (Th₂), which produce the pro-allergic cytokines interleukin IL-4, IL-5, and IL-13 that help orchestrate inflammation underlying most allergic symptoms (Woodfolk (2007) *J. Allergy Clin. Immunol.* 118(2):260-294). IL-4 instructs antibody-producing B cells to secrete allergen-specific Immunoglobulin (Ig) E (Del Prete et al. (1988) *J. Immunol.* 140:4193-4198; Swain et al. (1990) *J. Immunol.* 145:3796-3806). Unlike neutralizing IgG, IgE binds to its high affinity receptor Fc-εR₁ expressed by mast cells and eosinophils (Blank et al. (1989) *Nature* 337:187-190; Benhamou et al. (1990) *J. Immunol.* 144:3071-3077), thus sensitizing these cells. Upon subsequent exposure, IgE binds the offending allergen, cross-links, and transduces a signal instructing mast cells to degranulate and release the volatile chemicals that trigger the allergic reaction.

[0006] Beside food allergies, other environmental agents can also generate an allergic response as described above in an individual. Examples of such environmental agents include, but are not limited to, pollen, dog dander, cat saliva, or dust mites.

[0007] Immunotherapy, the administration of increasing doses of an allergen to bring about tolerance, is a standard treatment for allergic diseases, but has not been approved for treating nut allergies due to frequent anaphylactic reactions (Nelson et al. (1997) *J. Allergy Clin. Immunol* 99;6:744-751; Oppenheimer et al. (1992) *J. Allergy Clin. Immunol* 90:256-262). In addition, the utility of immunotherapy is limited by the length of treatment, which requires up to 36 months of weekly or bi-weekly injections and results in varying degrees of success and compliance (Bousquet et al. (1998) *J. Allergy Clin. Immunol* 102:558-562; Rank and Li (2007) *Mayo Clin. Proc.* 82(9):1119-1123; Ciprandi et al. (2007) *Allergy Asthma Proc.* 28:40-43).

[0008] DNA vaccines have been proposed as a treatment of allergic disease (Raz et al., 1996; Hartl et al., 2004; Hsu et al., 1996; Cramer 2007; Weiss et al., 2006). The underlying rationale is that allergen protein encoded by a DNA vaccine will preferentially activate the allergen-specific Th1 cellular response with the production of interferons by APCs, natural killer (NK), and T cells, rather than the characteristic Th2-type response, such as secretion of IL-4, IL-5, and IL-13, and the formation of IgE by B lymphocytes and the maturation and recruitment of eosinophils in late-phase reactions. However, the mechanisms underlying the differential induction of the Th1 and Th2 T-cell phenotypes appear to involve a large number of factors, such as unique properties of the bacterial DNA of vaccine preparations, *e.g.*, unmethylated and CpG DNA residues, the cytokine milieu elicited by innate immunity, and the cellular trafficking properties of the allergens (Chen et al., 2001; Kaeche et al., 2002).

[0009] Generally speaking, DNA vaccines are engineered nucleic acids that include sequences encoding one or more epitopes. The nucleic acids are delivered to cells, typically antigen presenting cells (APCs), the nucleic acids are expressed, and the epitopes present on the expressed proteins are processed in the endosomal/lysosomal compartment, and ultimately presented on the surface of the cell. For example, U.S. Patent No. 5,633,234 to August et al. discloses and characterizes the endosomal/lysosomal targeting sequence of the lysosomal-associated membrane protein (LAMP), identifying critical residues in the C-terminal region of the protein necessary for targeting of the protein to the endosomal/lysosomal compartment. In addition, U.S. patent application publication number 2004/0157307 to Harris et al. discloses the use of the LAMP luminal domain as a "trafficking domain" to direct chimeric proteins expressed

from DNA vaccines through one or more cellular compartments/organelles, such as through the lysosomal vesicular pathway. The chimeric proteins include the luminal domain of a LAMP polypeptide, an antigenic domain comprising a peptide epitope sequence previously identified and selected from an antigen protein, a transmembrane domain, and an endosomal/lysosomal targeting sequence.

[0010] Although numerous LAMP chimeric vaccines have been proposed, their ability to function as effective vaccines has been inconsistent once tested *in vivo*. For example, in Godinho et al., PLoS ONE 9(6): 9(6): e99887. doi:10.1371/journal.pone.0099887, the authors reported that an intact luminal domain was necessary to target an antigen to the lysosomes and exosomes secretory pathway and that fragments of the luminal domain did not always work. *See, id.* at page 6. Moreover, the authors acknowledged that the ability to raise an immune response is dependent upon the particular antigen and the epitopes used. For example, on page 9, column 2, the authors state “accordingly, previous studies demonstrated that DNA vaccines that generate Gag secreted as VLP, or in a soluble form, induce different levels of T and B cell activation, which were also different from the response induced by cytoplasmic Gag.”

[0011] Thus, even with the presence of both LAMP domains, it has increasingly been found that the efficacy of a particular antigen to raise an immune response is highly dependent on the particular sequence used. In fact, different antigenic fragments of the same protein when inserted into the identical LAMP vaccine vectors have been found to not elicit the same immune response. Sometimes the antigen fragment generates an immune response and other times it does not. These observations make the ability to predict ahead of time which particular antigenic sequence from a protein of interest will raise an immune response difficult. Instead, in the case of LAMP vaccines, immune response activity needs to be determined by *in vivo* testing.

[0012] Therefore, there is a need to identify new and improved LAMP chimeric vaccines that can be used to effectively treat susceptibility to allergic reactions.

SUMMARY OF THE INVENTION

[0013] It is an object of this invention to provide vaccines comprising specific antigens(s) with enhanced immunogenicity, particularly, genetic vaccines such as DNA or RNA vaccines.

[0014] It is a further object of this invention to provide more effective methods of vaccination, through the use of the disclosed antigens which are directed to the lysosomal/endosomal compartment and related organelles (e.g., such as MIIC, CIIV, melanosomes, secretory granules, Birbeck granules, and the like) where they are processed and presented to major histocompatibility complex (MHC) class II molecules so that helper T cells are preferentially stimulated.

[0015] The nucleic acids, and thus the encoded proteins, polypeptides, and peptides of the invention can be used in methods of treating subjects, and in particular subjects suffering from or potentially developing allergies. In general, a method of treating according to the present invention comprises administering a nucleic acid of the invention to a subject in an amount sufficient to deliver the nucleic acid to one or more immune cells, and preferably to one or more antigen presenting cells (APC) of the immune system. Once delivered, the nucleic acid is expressed, the encoded protein processed inside the cell, and the epitope(s) displayed on the surface of the cell. The method of treating can be considered a method of using the nucleic acids and proteins to provide a therapeutic or prophylactic immune response in subjects suffering from or potentially developing allergies.

[0016] In one aspect, the invention provides a chimeric protein, comprising: a N-terminal domain comprising at least one Allergen X; and a trafficking domain; wherein the trafficking domain directs Allergen X to an endosomal compartment (e.g., a lysosome) in a cell. Preferably, the trafficking domain comprises the luminal domain of a LAMP polypeptide, such as a LAMP-1 or LAMP-2 polypeptide.

[0017] In another aspect, the chimeric protein comprises a targeting sequence that directs at least one Allergen X to an endosomal/lysosomal compartment or a related organelle for protein processing and peptide epitope binding to MHC II, such as the tetrapeptide sequence Tyr-Xaa-Xaa-Xbb, wherein Xaa is any amino acid and Xbb is a hydrophobic amino acid. In still another aspect, the targeting sequence comprises a dileucine sequence. In a further aspect, the targeting sequence comprises a cytosolic protein targeting domain from an endocytic receptor. Suitable domains, include, but are not limited to the targeting domain of a C-type lectin receptor, a DEC-205 polypeptide, gp200-MR6 protein, or homolog, ortholog, variant, or modified form thereof.

[0018] In one particularly preferred aspect, the protein comprises the luminal domain of a LAMP polypeptide and a cytoplasmic domain comprising the tetrapeptide sequence Tyr-Xaa-Xaa-Xbb, wherein Xaa is any amino acid and Xbb is a hydrophobic amino acid. The chimeric protein, preferably, further comprises a transmembrane protein. Still more preferably, the chimeric protein also comprises a signaling domain.

[0019] Allergen X can be used to generate chimeric proteins. In one aspect, preferred allergens comprise the amino acid sequence of Allergen X of any one of SEQ ID NO:Y as shown in Table 1. Representative examples of polynucleotides that can encode Allergen X (SEQ ID NO:Y) are shown as SEQ ID NO:Z in Table 1. These polynucleotides are inserted between a trafficking domain and a targeting domain. Insertion may be facilitated by the use of cloning sequences that flank the amino acid sequence of SEQ ID NO:Y, such as, for example, polynucleotides that encode “Leu-Glu” and “Glu-Phe” (e.g., “CTCGAG” and “GAATTC.”)

[0020] The invention further provides a nucleic acid molecule encoding any of the Allergen X of Table 1. The invention also provides a vector comprising the nucleic acid encoding Allergen X, such as, for example, SEQ ID NO:Z and SEQ ID NO:W, wherein the nucleic acid molecule is operably linked to an expression control sequence. In one preferred aspect, the vector is a vaccine vector, suitable for vaccinating a patient against Allergen X. In another aspect, the invention provides a delivery vehicle comprising the nucleic acid molecule for facilitating the introduction of the nucleic acid molecule into a cell. The delivery vehicle may be lipid-based (e.g., a liposome formulation), viral-based (e.g., comprising viral proteins encapsulating the nucleic acid molecule), or cell-based. In one preferred aspect, the vector is a vaccine vector.

[0021] The invention also provides a cell comprising any of the vectors described above. In one aspect, the cell is an antigen presenting cell. The antigen presenting cell may be a professional antigen presenting cell (e.g., a dendritic cell, macrophage, B cell, and the like) or an engineered antigen presenting cell (e.g., a non-professional antigen presenting cell engineered to express molecules required for antigen presentation, such as MHC class II molecules). The molecules required for antigen presentation may be derived from other cells, e.g., naturally occurring, or may themselves be engineered (e.g. mutated or modified to express desired properties, such as higher or lower affinity for an

antigenic epitope). In one aspect, the antigen presenting cell does not express any co-stimulatory signals and the antigen is an auto-antigen.

[0022] The invention additionally provides a kit comprising a plurality of cells comprising any of the vectors described above. At least two of the cells express different MHC class II molecules, and each cell comprises the same vector. In one aspect, a kit is provided comprising a vector and a cell for receiving the vector.

[0023] The invention also provides a transgenic animal comprising at least one of the cells described above.

[0024] The invention further provides a method for generating an immune response in an animal to Allergen X, comprising: administering to the animal a cell or a polynucleotide comprising a nucleic acid sequence encoding Allergen X (such as, for example, SEQ ID NO:Z or SEQ ID NO:W) as described above, wherein the cell expresses, or can be induced to express the chimeric protein in the animal. In one aspect, the cell comprises an MHC class II molecule compatible with MHC proteins of the animal, such that the animal does not generate an immune response against the MHC class II molecule. In one preferred aspect, the animal is a human.

[0025] In one aspect, the invention provides a method of treating subjects suffering from or potentially developing allergies to Allergen X, comprising administering to an animal, any of the vectors, host cells, polynucleotides, or polypeptides described above. In one embodiment, the vector is infectious for a cell of the animal. For example, the vector may be a viral vector, such as a vaccinia vector. Preferred chimeric vaccines administered to the animal include polynucleotides that encode any one of the amino acid sequences of SEQ ID NO:Y as shown in Table 1.

[0026] Prime boost protocols are also contemplated. For example, the invention further provides a method for generating an immune response in a subject to Allergen X, comprising priming the subject with a LAMP Construct as described herein followed by at least one boosting of the subject with Allergen X or a related antigen (e.g., a second Allergen derived from the same or highly similar protein sequence). Mixtures of allergens can be used in either or both the priming and the boosting step. Use of a LAMP Construct for the prime step followed by a boost step has been shown to significantly produce higher titers, indicating the power of LAMP in enhancing antibody response.

[0027] In a further aspect, a cell is obtained from a patient, the vector is introduced into the cell and the cell or progeny of the cell is reintroduced into the patient. In one aspect, the cell is a stem cell-capable of differentiating into an antigen presenting cell.

BRIEF DESCRIPTION OF THE FIGURES

[0028] The objects and features of the invention can be better understood with reference to the following detailed description and accompanying drawings.

[0029] **Figure 1** provides the different portions of Human LAMP-1 sequences used to generate constructs described herein.

[0030] **Figure 2** provides the known structural domains and amino acid sequence for Human Lamp-1 Protein (SEQ ID NO:4).

[0031] **Figure 3** provides the known structural domains and amino acid sequence of Mouse Lamp-1 Protein (SEQ ID NO:5)

[0032] **Figure 4** is a schematic representation of the different constructs described herein as provided as Table 1.

DETAILED DESCRIPTION

[0033] The invention provides chimeric proteins and nucleic acids encoding these proteins which can be used to generate vaccines against Allergen X. In one aspect, a chimeric protein comprises Allergen X and a domain for trafficking the protein to an endosomal/lysosomal compartment or related organelle. In one preferred aspect, the trafficking domain comprises a luminal domain of a LAMP polypeptide. Alternatively, or additionally, the chimeric protein comprises a trafficking domain of an endocytic receptor (e.g., such as C-type lectin, DEC-205 or gp200-MR6). The vaccines can be used to modulate an immune response. In one preferred aspect, the invention provides a method of treating or prevention an allergic response in a patient by providing a chimeric protein comprising Allergen X or a nucleic acid encoding Allergen X to the patient.

DEFINITIONS

[0034] The following definitions are provided for specific terms which are used in the following written description.

[0035] As used in the specification and claims, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a cell" includes a plurality of cells, including mixtures thereof. The term "a nucleic acid molecule" includes a plurality of nucleic acid molecules.

[0036] As used herein, the term "comprising" is intended to mean that the compositions and methods include the recited elements, but do not exclude other elements. "Consisting essentially of", when used to define compositions and methods, shall mean excluding other elements of any essential significance to the combination. Thus, a composition consisting essentially of the elements as defined herein would not exclude trace contaminants from the isolation and purification method and pharmaceutically acceptable carriers, such as phosphate buffered saline, preservatives, and the like. "Consisting of" shall mean excluding more than trace elements of other ingredients and substantial method steps for administering the compositions of this invention. Embodiments defined by each of these transition terms are within the scope of this invention.

[0037] As used herein, "the lysosomal/endosomal compartment" refers to membrane-bound acidic vacuoles containing LAMP molecules in the membrane, hydrolytic enzymes that function in antigen processing, and MHC class II molecules for antigen recognition and presentation. This compartment functions as a site for degradation of foreign materials internalized from the cell surface by any of a variety of mechanisms including endocytosis, phagocytosis and pinocytosis, and of intracellular material delivered to this compartment by specialized autolytic phenomena (de Duve, *Eur. J. Biochem.* 137: 391, 1983). The term "endosome" as used herein and in the claims encompasses a lysosome.

[0038] As used herein, a "lysosome-related organelle" refers to any organelle which comprises lysosomes and includes, but is not limited to, MIIC, CIIV, melanosomes, secretory granules, lytic granules, platelet-dense granules, basophil granules, Birbeck granules, phagolysosomes, secretory lysosomes, and the like. Preferably, such an organelle lacks mannose 6-phosphate receptors and comprises LAMP, but may or may not comprise an MHC class II molecule. For reviews, see, e.g., Blott and Griffiths, *Nature Reviews, Molecular Cell Biology*, 2002; Dell'Angelica, et al., *The FASEB Journal* 14: 1265-1278, 2000.

[0039] As used herein, the terms "polynucleotide" and "nucleic acid molecule" are used interchangeably to refer to polymeric forms of nucleotides of any length. The polynucleotides may contain deoxyribonucleotides, ribonucleotides, and/or their analogs. Nucleotides may have any three-dimensional structure, and may perform any function, known or unknown. The term "polynucleotide" includes, for example, single-stranded, double-stranded and triple helical molecules, a gene or gene fragment, exons, introns, mRNA, tRNA, rRNA, ribozymes, antisense molecules, cDNA, recombinant polynucleotides, branched polynucleotides, aptamers, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes, and primers. A nucleic acid molecule may also comprise modified nucleic acid molecules (e.g., comprising modified bases, sugars, and/or internucleotide linkers).

[0040] As used herein, the term "peptide" refers to a compound of two or more subunit amino acids, amino acid analogs, or peptidomimetics. The subunits may be linked by peptide bonds or by other bonds (e.g., as esters, ethers, and the like).

[0041] As used herein, the term "amino acid" refers to either natural and/or unnatural or synthetic amino acids, including glycine and both D or L optical isomers, and amino acid analogs and peptidomimetics. A peptide of three or more amino acids is commonly called an oligopeptide if the peptide chain is short. If the peptide chain is long (e.g., greater than about 10 amino acids), the peptide is commonly called a polypeptide or a protein. While the term "protein" encompasses the term "polypeptide", a "polypeptide" may be a less than full-length protein.

[0042] As used herein, the term "Allergen X" refers to the specific genes/proteins listed in the following Table 1, fragments thereof, or mixtures of the listed proteins that are known to induce allergies, i.e., IgE mediated reactions upon their repeated exposure to an individual. Generally, an allergen is any compound, substance, or material that is capable of evoking an allergic reaction. Allergens are usually understood as a subcategory of antigens, which are compounds, substances, or materials capable of evoking an immune response. For carrying out the invention, Allergen X may be selected, among other things, from natural or native allergens, modified natural allergens, synthetic allergens, recombinant allergens, allergoids, and mixtures or combinations thereof. Of particular interest is Allergen X that is capable of causing an IgE-mediated immediate type hypersensitivity.

Table 1

Allergen X	Amino Acid Sequence of Allergen X (SEQ ID NO: Y)	Representative Polynucleotide Sequences Encoding SEQ ID NO: Y (SEQ ID NO: Z)	Amino Acid Sequence of Full Length Construct (SEQ ID NO: V)	Representative Polynucleotide Sequence Encoding SEQ ID NO: V (SEQ ID NO: W)
Cor a 1	SEQ ID NO:7	SEQ ID NO:6		
Cor a 9	SEQ ID NO:9	SEQ ID NO:8		
Cor a 1 – Gly4 – Cor a 9	SEQ ID NO:11	SEQ ID NO:10		
Pru du 6	SEQ ID NO:13	SEQ ID NO:12		
Ana o 1	SEQ ID NO:15	SEQ ID NO:14		
Ana o 2	SEQ ID NO:17	SEQ ID NO: 16		
Ana o 3	SEQ ID NO:19	SEQ ID NO:18		
Ana o 2 – Gly4- Ana o 1 – Gly4- Ana o 3	SEQ ID NO:21	SEQ ID NO:20		
Jug n 1	SEQ ID NO:23	SEQ ID NO:22		
Jug r 2	SEQ ID NO:25	SEQ ID NO:24		
Jug n 1 – Gly4 – Jug r 2	SEQ ID NO:27	SEQ ID NO:26		
Amb a 1	SEQ ID NO:29	SEQ ID NO:28	SEQ ID NO:31	SEQ ID NO:30
Bet v 1-A	SEQ ID NO:33	SEQ ID NO:32	SEQ ID NO:35	SEQ ID NO:34
Can f 1	SEQ ID NO:37	SEQ ID NO:36	SEQ ID NO:39	SEQ ID NO:38
Cyn d 1	SEQ ID NO:41	SEQ ID NO:40	SEQ ID NO:43	SEQ ID NO:42
Der F 1 (19-321)	SEQ ID NO:45	SEQ ID NO:44	SEQ ID NO:47	SEQ ID NO:46
Der F 1 (99-321)	SEQ ID NO:49	SEQ ID NO:48	SEQ ID NO:51	SEQ ID NO:50

Der P2	SEQ ID NO:53	SEQ ID NO:52	SEQ ID NO:55	SEQ ID NO:54
DerF2	SEQ ID NO:57	SEQ ID NO:56	SEQ ID NO:59	SEQ ID NO:58
DerP1 (del)	SEQ ID NO:61	SEQ ID NO:60	SEQ ID NO:63	SEQ ID NO:62
Fel D 1	SEQ ID NO:65 SEQ ID NO:66	SEQ ID NO:64	SEQ ID NO:68	SEQ ID NO:67
Fel d 2	SEQ ID NO:70	SEQ ID NO:69	SEQ ID NO:72	SEQ ID NO:71
Fel d 4	SEQ ID NO:74	SEQ ID NO:73	SEQ ID NO:76	SEQ ID NO:75
Lit v 1	SEQ ID NO:78	SEQ ID NO:77	SEQ ID NO:80	SEQ ID NO:79
Lol p 5a	SEQ ID NO:82	SEQ ID NO:81	SEQ ID NO:84	SEQ ID NO:83
Phl p 1	SEQ ID NO:86	SEQ ID NO:85	SEQ ID NO:88	SEQ ID NO:87
Phl p 5	SEQ ID NO:90	SEQ ID NO:89	SEQ ID NO:92	SEQ ID NO:91
Der f 15	SEQ ID NO:93			
Der f 18	SEQ ID NO:94			
Zen-1	SEQ ID NO:95			
Cte f 1	SEQ ID NO:96			
Der F15-Der F18	SEQ ID NO:109			
Der F1-Der F2	SEQ ID NO:110			

[0043] As used herein, the amino acid sequence of Allergen X comprises any one of SEQ ID NO:Y. Preferred representative examples of polynucleotides that can encode Allergen X are shown as SEQ ID NO:Z in Table 1. Polynucleotides encoding Allergen X polypeptides are specifically contemplated.

[0044] As used herein, "Allergen X" also encompasses variants of Allergen X. For example, preferred embodiments include Allergen X polypeptide variants that have at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: Y. These variants retain either (a) the ability to raise an antibody that cross-reacts with the Allergen X from which it was derived and/or (b) Allergen X biological activity. Polynucleotides that encode these variant Allergen X polypeptides are specifically contemplated.

[0045] Additionally, more than one Allergen X can be combined and administered as a vaccine as described herein. When cloned, the order of the combination of Allergen X can vary within a construct. Moreover, it is specifically envisioned that the combinations of Allergen X can be cloned within a single vaccine construct, or can be delivered in a composition comprising multiple vaccine constructs or Allergen X. Specifically, Allergen X as described in Table 1 can be cloned into the LAMP Constructs described herein either individually, or in combination with one another. As used herein in this paragraph, the names Cora1, Cora9, Prudu6, Anao1, Anao2, Anao3, Jugn1, Jugr2, Amba1, Betv1-A, Canf1, Cynd1, DerF1(19-321), DerF1(99-321), DerP2, DerF2, DerP1(del), FelD1, FelD2, FelD4, Litv1, Lolp5a, Phlp1, Phlp5, Derf15, Derf18, Zen-1, and Ctef1 refer to the Allergen X described in Table 1. Specifically, the combinations of Allergen X can be cloned into the LAMP Constructs described herein as follows: (a) Cora1 and at least one of Cora9, Prudu6, Anao1, Anao2, Anao3, Jugn1, Jugr2, Amba1, Betv1-A, Canf1, Cynd1, DerF1(19-321), DerF1(99-321), DerP2, DerF2, DerP1(del), FelD1, FelD2, FelD4, Litv1, Lolp5a, Phlp1, Phlp5, Derf15, Derf18, Zen-1, and/or Ctef1; (b) Cora9 and at least one of Cora1, Prudu6, Anao1, Anao2, Anao3, Jugn1, Jugr2, Amba1, Betv1-A, Canf1, Cynd1, DerF1(19-321), DerF1(99-321), DerP2, DerF2, DerP1(del), FelD1, FelD2, FelD4, Litv1, Lolp5a, Phlp1, Phlp5, Derf15, Derf18, Zen-1, and/or Ctef1; (c) Prudu6 and at least one of Cora1, Cora9, Anao1, Anao2, Anao3, Jugn1, Jugr2, Amba1, Betv1-A, Canf1, Cynd1, DerF1(19-321), DerF1(99-321), DerP2, DerF2, DerP1(del), FelD1, FelD2, FelD4, Litv1,

Lolp5a, Phlp1, Phlp5, Derf15, Derf18, Zen-1, and/or Ctef1; (d) Anao1 and at least one of Cora1, Cora9, Prudu6, Anao2, Anao3, Jugn1, Jugr2, Amba1, Betv1-A, Canf1, Cynd1, DerF1(19-321), DerF1(99-321), DerP2, DerF2, DerP1(del), FelD1, FelD2, FelD4, Litv1, Lolp5a, Phlp1, Phlp5, Derf15, Derf18, Zen-1, and/or Ctef1; (e) Anao2 and at least one of Cora1, Cora9, Prudu6, Anao1, Anao3, Jugn1, Jugr2, Amba1, Betv1-A, Canf1, Cynd1, DerF1(19-321), DerF1(99-321), DerP2, DerF2, DerP1(del), FelD1, FelD2, FelD4, Litv1, Lolp5a, Phlp1, Phlp5, Derf15, Derf18, Zen-1, and/or Ctef1; (f) Anao3 and at least one of Cora1, Cora9, Prudu6, Anao1, Anao2, Jugn1, Jugr2, Amba1, Betv1-A, Canf1, Cynd1, DerF1(19-321), DerF1(99-321), DerP2, DerF2, DerP1(del), FelD1, FelD2, FelD4, Litv1, Lolp5a, Phlp1, Phlp5, Derf15, Derf18, Zen-1, and/or Ctef1; (g) Jugn1 and at least one of Cora1, Cora9, Prudu6, Anao1, Anao2, Anao3, Jugr2, Amba1, Betv1-A, Canf1, Cynd1, DerF1(19-321), DerF1(99-321), DerP2, DerF2, DerP1(del), FelD1, FelD2, FelD4, Litv1, Lolp5a, Phlp1, Phlp5, Derf15, Derf18, Zen-1, and/or Ctef1; (h) Jugr2 and at least one of Cora1, Cora9, Prudu6, Anao1, Anao2, Anao3, Jugn1, Amba1, Betv1-A, Canf1, Cynd1, DerF1(19-321), DerF1(99-321), DerP2, DerF2, DerP1(del), FelD1, FelD2, FelD4, Litv1, Lolp5a, Phlp1, Phlp5, Derf15, Derf18, Zen-1, and/or Ctef1; (i) Betv1-A and at least one of Cora1, Cora9, Prudu6, Anao1, Anao2, Anao3, Jugn1, Jugr2, Amba1, Canf1, Cynd1, DerF1(19-321), DerF1(99-321), DerP2, DerF2, DerP1(del), FelD1, FelD2, FelD4, Litv1, Lolp5a, Phlp1, Phlp5, Derf15, Derf18, Zen-1, and/or Ctef1; (j) Canf1 and at least one of Cora9, Prudu6, Anao1, Anao2, Anao3, Jugn1, Jugr2, Amba1, Betv1-A, Cynd1, DerF1(19-321), DerF1(99-321), DerP2, DerF2, DerP1(del), FelD1, FelD2, FelD4, Litv1, Lolp5a, Phlp1, Phlp5, Derf15, Derf18, Zen-1, and/or Ctef1; (k) Cynd1 and at least one of Cora9, Prudu6, Anao1, Anao2, Anao3, Jugn1, Jugr2, Amba1, Betv1-A, Canf1, DerF1(19-321), DerF1(99-321), DerP2, DerF2, DerP1(del), FelD1, FelD2, FelD4, Litv1, Lolp5a, Phlp1, Phlp5, Derf15, Derf18, Zen-1, and/or Ctef1; (l) DerF1(19-321) and at least one of Cora9, Prudu6, Anao1, Anao2, Anao3, Jugn1, Jugr2, Amba1, Betv1-A, Canf1, Cynd1, DerF1(99-321), DerP2, DerF2, DerP1(del), FelD1, FelD2, FelD4, Litv1, Lolp5a, Phlp1, Phlp5, Derf15, Derf18, Zen-1, and/or Ctef1; (m) DerF1(99-321) and at least one of Cora1, Cora9, Prudu6, Anao1, Anao2, Anao3, Jugn1, Jugr2, Amba1, Betv1-A, Canf1, Cynd1, DerF1(19-321), DerP2, DerF2, DerP1(del), FelD1, FelD2, FelD4, Litv1, Lolp5a, Phlp1, Phlp5, Derf15, Derf18, Zen-1, and/or Ctef1; (n) DerP2 and at least one of Cora1, Cora9, Prudu6, Anao1, Anao2, Anao3, Jugn1, Jugr2, Amba1, Betv1-A, Canf1, Cynd1, DerF1(19-321), DerF1(99-

321), DerF2, DerP1(del), FelD1, FelD2, FelD4, Litv1, Lolp5a, Phlp1, Phlp5, Derf15, Derf18, Zen-1, and/or Ctef1; (o) DerF2 and at least one of Cora1, Cora9, Prudu6, Anao1, Anao2, Anao3, Jugn1, Jugr2, Amba1, Betv1-A, Canf1, Cynd1, DerF1(19-321), DerF1(99-321), DerP2, DerP1(del), FelD1, FelD2, FelD4, Litv1, Lolp5a, Phlp1, Phlp5, Derf15, Derf18, Zen-1, and/or Ctef1; (p) DerP1(del) and at least one of Cora1, Cora9, Prudu6, Anao1, Anao2, Anao3, Jugn1, Jugr2, Amba1, Betv1-A, Canf1, Cynd1, DerF1(19-321), DerF1(99-321), DerP2, DerF2, FelD1, FelD2, FelD4, Litv1, Lolp5a, Phlp1, Phlp5, Derf15, Derf18, Zen-1, and/or Ctef1; (q) FelD1 and at least one of Cora1, Cora9, Prudu6, Anao1, Anao2, Anao3, Jugn1, Jugr2, Amba1, Betv1-A, Canf1, Cynd1, DerF1(19-321), DerF1(99-321), DerP2, DerF2, DerP1(del), FelD2, FelD4, Litv1, Lolp5a, Phlp1, Phlp5, Derf15, Derf18, Zen-1, and/or Ctef1; (r) FelD2 and at least one of Cora1, Cora9, Prudu6, Anao1, Anao2, Anao3, Jugn1, Jugr2, Amba1, Betv1-A, Canf1, Cynd1, DerF1(19-321), DerF1(99-321), DerP2, DerF2, DerP1(del), FelD1, FelD4, Litv1, Lolp5a, Phlp1, Phlp5, Derf15, Derf18, Zen-1, and/or Ctef1; (s) FelD4 and at least one of Cora1, Cora9, Prudu6, Anao1, Anao2, Anao3, Jugn1, Jugr2, Amba1, Betv1-A, Canf1, Cynd1, DerF1(19-321), DerF1(99-321), DerP2, DerF2, DerP1(del), FelD1, FelD2, Litv1, Lolp5a, Phlp1, Phlp5, Derf15, Derf18, Zen-1, and/or Ctef1; (t) Litv1 and at least one of Cora1, Cora9, Prudu6, Anao1, Anao2, Anao3, Jugn1, Jugr2, Amba1, Betv1-A, Canf1, Cynd1, DerF1(19-321), DerF1(99-321), DerP2, DerF2, DerP1(del), FelD1, FelD2, FelD4, Lolp5a, Phlp1, Phlp5, Derf15, Derf18, Zen-1, and/or Ctef1; (u) Lolp5a and at least one of Cora1, Cora9, Prudu6, Anao1, Anao2, Anao3, Jugn1, Jugr2, Amba1, Betv1-A, Canf1, Cynd1, DerF1(19-321), DerF1(99-321), DerP2, DerF2, DerP1(del), FelD1, FelD2, FelD4, Litv1, Phlp1, Phlp5, Derf15, Derf18, Zen-1, and/or Ctef1; (v) Phlp1 and at least one of Cora1, Cora9, Prudu6, Anao1, Anao2, Anao3, Jugn1, Jugr2, Amba1, Betv1-A, Canf1, Cynd1, DerF1(19-321), DerF1(99-321), DerP2, DerF2, DerP1(del), FelD1, FelD2, FelD4, Litv1, Lolp5a, Phlp5, Derf15, Derf18, Zen-1, and/or Ctef1; (w) Amba1 and at least one of Cora1, Cora9, Prudu6, Anao1, Anao2, Anao3, Jugn1, Jugr2, Betv1-A, Canf1, Cynd1, DerF1(19-321), DerF1(99-321), DerP2, DerF2, DerP1(del), FelD1, FelD2, FelD4, Litv1, Lolp5a, Phlp1, Phlp5, Derf15, Derf18, Zen-1, and/or Ctef1; (x) Phlp5 and at least one of Cora1, Cora9, Prudu6, Anao1, Anao2, Anao3, Jugn1, Jugr2, Amba1, Betv1-A, Canf1, Cynd1, DerF1(19-321), DerF1(99-321), DerP2, DerF2, DerP1(del), FelD1, FelD2, FelD4, Litv1, Lolp5a, Phlp1, Derf15, Derf18, Zen-1, and/or Ctef1; (y) Derf15 and at least one of Cora1, Cora9, Prudu6, Anao1, Anao2, Anao3,

Jugn1, Jugr2, Amba1, Betv1-A, Canf1, Cynd1, DerF1(19-321), DerF1(99-321), DerP2, DerF2, DerP1(del), FelD1, FelD2, FelD4, Litv1, Lolp5a, Phlp1, Phlp5, Derf18, Zen-1, and/or Ctef1; (z) Derf18 and at least one of Cora1, Cora9, Prudu6, Anao1, Anao2, Anao3, Jugn1, Jugr2, Amba1, Betv1-A, Canf1, Cynd1, DerF1(19-321), DerF1(99-321), DerP2, DerF2, DerP1(del), FelD1, FelD2, FelD4, Litv1, Lolp5a, Phlp1, Phlp5, Derf15, Zen-1, and/or Ctef1; (aa) Zen-1 and at least one of Cora1, Cora9, Prudu6, Anao1, Anao2, Anao3, Jugn1, Jugr2, Amba1, Betv1-A, Canf1, Cynd1, DerF1(19-321), DerF1(99-321), DerP2, DerF2, DerP1(del), FelD1, FelD2, FelD4, Litv1, Lolp5a, Phlp1, Phlp5, Derf15, Derf18, and/or Ctef1; and/or (bb) Ctef1 and at least one of Cora1, Cora9, Prudu6, Anao1, Anao2, Anao3, Jugn1, Jugr2, Amba1, Betv1-A, Canf1, Cynd1, DerF1(19-321), DerF1(99-321), DerP2, DerF2, DerP1(del), FelD1, FelD2, FelD4, Litv1, Lolp5a, Phlp1, Phlp5, Derf15, Derf18, and/or Zen-1.

[0046] The order of the combination of antigens as described above in a particular LAMP construct can vary as this list describes what a LAMP Construct comprises and not necessarily to describe the arrangement of the antigens within a particular construct. Moreover, it is specifically envisioned that these antigens can be combined within a single LAMP Construct, or can be delivered in a composition comprising multiple LAMP Constructs.

[0047] As used herein a "LAMP polypeptide" refers to LAMP-1, LAMP-2, CD63/LAMP-3, DC-LAMP, or any lysosomal associated membrane protein, or homologs, orthologs, variants (e.g., allelic variants) and modified forms (e.g., comprising one or more mutations, either naturally occurring or engineered). In one aspect, a LAMP polypeptide is a mammalian lysosomal associated membrane protein, e.g., such as a human or mouse lysosomal associated membrane protein. More generally, a "lysosomal associated membrane protein" refers to any protein comprising a domain found in the membrane of an endosomal/lysosomal compartment or lysosome-related organelle and which further comprises a luminal domain. Representative examples of LAMP-1 polypeptide include SEQ ID NO:4 (human LAMP-1), SEQ ID NO:5 (mouse LAMP-1), or canine LAMP (XP_534193) and any of these sequences can be used to generate DNA vaccines as described herein.

[0048] As used herein, "an endocytic receptor" refers to a transmembrane protein with either its C-terminal or N-terminal end facing the cytoplasm and which comprises a

trafficking domain (e.g., a luminal domain) for transporting a polypeptide or peptide conjugated to it (e.g., via a chemical bond) to an MHC class II molecule or to an intracellular compartment for subsequent association with an MHC class II molecule. Examples of endocytic receptors include, but are not limited to, Fc-receptors, complement receptors, scavenger receptors, integrins, lectins (e.g., C-type lectins), DEC-205 polypeptides, gp200-MR6 polypeptides, Toll-like receptors, heat shock protein receptors (e.g., CD 91), apoptotic body or necrotic body receptors (e.g., such as CD 14), or homologs, orthologs, variants (e.g., allelic variants) and modified forms thereof (e.g., comprising one or more mutations, either naturally occurring or engineered).

[0049] As used herein, "expression" refers to the process by which polynucleotides are transcribed into mRNA and/or translated into peptides, polypeptides, or proteins. If the polynucleotide is derived from genomic DNA, expression may include splicing of the mRNA transcribed from the genomic DNA.

[0050] As used herein, "under transcriptional control" or "operably linked" refers to expression (e.g., transcription or translation) of a polynucleotide sequence which is controlled by an appropriate juxtaposition of an expression control element and a coding sequence. In one aspect, a DNA sequence is "operatively linked" to an expression control sequence when the expression control sequence controls and regulates the transcription of that DNA sequence.

[0051] As used herein, "coding sequence" is a sequence which is transcribed and translated into a polypeptide when placed under the control of appropriate expression control sequences. The boundaries of a coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxyl) terminus. A coding sequence can include, but is not limited to, a prokaryotic sequence, cDNA from eukaryotic mRNA, a genomic DNA sequence from eukaryotic (e.g., mammalian) DNA, and even synthetic DNA sequences. A polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence.

[0052] As used herein, two coding sequences "correspond" to each other if the sequences or their complementary sequences encode the same amino acid sequences.

[0053] As used herein, "signal sequence" denotes the endoplasmic reticulum translocation sequence. This sequence encodes a signal peptide that communicates to a cell to direct a polypeptide to which it is linked (e.g., via a chemical bond) to an

endoplasmic reticulum vesicular compartment, to enter an exocytic/endocytic organelle, to be delivered either to a cellular vesicular compartment, the cell surface or to secrete the polypeptide. This signal sequence is sometimes clipped off by the cell in the maturation of a protein. Signal sequences can be found associated with a variety of proteins native to prokaryotes and eukaryotes.

[0054] As used herein, "trafficking" denotes movement or progression of the Allergen X polypeptide through all of the cellular organelles or compartments in the pathway from the rough endoplasmic reticulum to the endosomal/lysosomal compartment or related organelles where antigen processing and binding to MHC II occurs. "Transport" refers to delivery of a chimeric protein to one particular type of cellular compartment.

[0055] As used herein, a "trafficking sequence" which is substantially homologous to another trafficking sequence is one which shares substantial homology to the other trafficking sequence; however, the ultimate test for substantial homology is a functional assay in which a polypeptide comprising a sequence substantially homologous to a trafficking sequence is able to co-localize to the same endosomal compartment as the trafficking sequence.

[0056] As used herein, "a trafficking domain" refers to a series of continuous or discontinuous amino acids in a protein which are required for vesicular flow of the protein through one or more cellular compartments/organelles. A trafficking domain preferably comprises necessary sequences for proper protein folding to mediate this flow. In one aspect, a trafficking domain comprises a luminal sequence; preferably, such a sequence comprises one or more binding sites for interactions with a cellular folding protein such as a chaperone.

[0057] As used herein, "targeting" denotes the polypeptide sequence that directs the trafficking of Allergen X to the preferred site or cellular organelles or compartment where antigen processing and binding to MHC II occurs.

[0058] In contrast, as used herein, a "targeting domain" refers to a series of amino acids which are required for delivery to a cellular compartment/organelle. Preferably, a targeting domain is a sequence which binds to an adaptor or AP protein (e.g., such as an AP1, AP2, or AP3 protein). Exemplary targeting domain sequences are described in Dell'Angelica, 2000, *supra*.

[0059] A "chimeric DNA" is an identifiable segment of DNA within a larger DNA molecule that is not found in association with the larger molecule in nature. Thus, when the chimeric DNA encodes a protein segment, the segment coding sequence will be flanked by DNA that does not flank the coding sequence in any naturally occurring genome. Allelic variations or naturally occurring mutational events do not give rise to a chimeric DNA as defined herein.

[0060] Short stretches of polynucleotides that encode amino acids may be included at the ends of the polynucleotides that encode Allergen X to facilitate cloning into any of the vectors described herein. For example, use of cloning sequences that flank the amino acid sequence of SEQ ID NO:Y, such as, for example, polynucleotides that encode "Leu-Glu" and "Glu-Phe" (e.g., "CTCGAG" and "GAATTC") may be included in the construct design.

[0061] As used herein, a "nucleic acid delivery vector" is a nucleic acid molecule which can transport a polynucleotide of interest into a cell. Preferably, such a vector comprises a coding sequence operably linked to an expression control sequence. However, a polynucleotide sequence of interest may not necessarily comprise a coding sequence. For example, in one aspect a polynucleotide sequence of interest is an aptamer which binds to a target molecule. In another aspect, the sequence of interest is a complementary sequence of a regulatory sequence which binds to a regulatory sequence to inhibit regulation of the regulatory sequence. In still another aspect, the sequence of interest is itself a regulatory sequence (e.g., for titrating out regulatory factors in a cell).

[0062] As used herein, a "nucleic acid delivery vehicle" is defined as any molecule or group of molecules or macromolecules that can carry polynucleotides into a host cell (e.g., such as genes or gene fragments, antisense molecules, ribozymes, aptamers, and the like) and which occurs in association with a nucleic acid vector as described above.

[0063] As used herein, "nucleic acid delivery," or "nucleic acid transfer," refers to the introduction of an exogenous polynucleotide (e.g., such as a "transgene") into a host cell, irrespective of the method used for the introduction. The introduced polynucleotide may be stably or transiently maintained in the host cell. Stable maintenance typically requires that the introduced polynucleotide either contains an origin of replication compatible with the host cell or integrates into a replicon of the host cell such as an extrachromosomal replicon (e.g., a plasmid) or a nuclear or mitochondrial chromosome.

[0064] As used herein, a "viral vector" refers to a virus or viral particle that comprises a polynucleotide to be delivered into a host cell, either *in vivo*, *ex vivo* or *in vitro*. Examples of viral vectors include, but are not limited to, adenovirus vectors, adeno-associated virus vectors, retroviral vectors, and the like. In aspects where gene transfer is mediated by an adenoviral vector, a vector construct refers to the polynucleotide comprising the adenovirus genome or part thereof, and a selected, non-adenoviral gene, in association with adenoviral capsid proteins.

[0065] As used herein, "adenoviral-mediated gene transfer" or "adenoviral transduction" refers to the process by which a gene or nucleic acid sequences are transferred into a host cell by virtue of the adenovirus entering the cell. Preferably, the virus is able to replicate and/or integrate and be transcribed within the cell.

[0066] As used herein, "adenovirus particles" are individual adenovirus virions comprised of an external capsid and internal nucleic acid material, where the capsid is further comprised of adenovirus envelope proteins. The adenovirus envelope proteins may be modified to comprise a fusion polypeptide which contains a polypeptide ligand covalently attached to the viral protein, e.g., for targeting the adenoviral particle to a particular cell and/or tissue type.

[0067] As used herein, the term "administering a molecule to a cell" (e.g., an expression vector, nucleic acid, an accessory factor, a delivery vehicle, agent, and the like) refers to transducing, transfecting, microinjecting, electroporating, or shooting the cell with the molecule. In some aspects, molecules are introduced into a target cell by contacting the target cell with a delivery cell (e.g., by cell fusion or by lysing the delivery cell when it is in proximity to the target cell).

[0068] As used herein, the phrase "prime boost" describes the use of a LAMP Construct described herein used to prime a T-cell response followed by the use of a second LAMP Construct comprising an Allergen, a DNA vaccine comprising an Allergen or a recombinant Allergen to boost the response. These heterologous prime-boost immunizations elicit immune responses of greater height and breadth than can be achieved by priming and boosting with the same vector. The priming with a LAMP Construct comprising an Allergen X memory cells; the boost step expands the memory response. Preferably, use of the two different agents do not raise responses against each other and thus do not interfere with each other's activity. Mixtures of allergens are

specifically contemplated in the prime and/or boost step. Boosting can occur one or multiple times.

[0069] As used herein, "hybridization" refers to a reaction in which one or more polynucleotides react to form a complex that is stabilized via hydrogen bonding between the bases of the nucleotide residues. The hydrogen bonding may occur by Watson-Crick base pairing, Hoogsteen binding, or in any other sequence-specific manner. The complex may comprise two strands forming a duplex structure, three or more strands forming a multi-stranded complex, a single self-hybridizing strand, or any combination of these. A hybridization reaction may constitute a step in a more extensive process, such as the initiation of a PCR reaction, or the enzymatic cleavage of a polynucleotide by a ribozyme.

[0070] As used herein, a polynucleotide or polynucleotide region (or a polypeptide or polypeptide region) which has a certain percentage (for example, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%) of "sequence identity" to another sequence means that, when maximally aligned, using software programs routine in the art, that percentage of bases (or amino acids) are the same in comparing the two sequences.

[0071] Two sequences are "substantially homologous" or "substantially similar" when at least about 50%, at least about 60%, at least about 70%, at least about 75%, and preferably at least about 80%, and most preferably at least about 90 or 95% of the nucleotides match over the defined length of the DNA sequences. Similarly, two polypeptide sequences are "substantially homologous" or "substantially similar" when at least about 50%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, and preferably at least about 80%, and most preferably at least about 90 or 95% of the amino acid residues of the polypeptide match over a defined length of the polypeptide sequence. Sequences that are substantially homologous can be identified by comparing the sequences using standard software available in sequence data banks. Substantially homologous nucleic acid sequences also can be identified in a Southern hybridization experiment under, for example, stringent conditions as defined for that particular system. Defining appropriate hybridization conditions is within the skill of the art. For example, stringent conditions can be: hybridization at 5xSSC and 50% formamide at 42°C, and washing at 0.1xSSC and 0.1% sodium dodecyl sulfate at 60°C.

[0072] The terms "percent (%) sequence similarity", "percent (%) sequence identity", and the like, generally refer to the degree of identity or correspondence between different nucleotide sequences of nucleic acid molecules or amino acid sequences of polypeptides that may or may not share a common evolutionary origin (see Reeck et al., *supra*). Sequence identity can be determined using any of a number of publicly available sequence comparison algorithms, such as BLAST, FASTA, DNA Strider, GCG (Genetics Computer Group, Program Manual for the GCG Package, Version 7, Madison, Wisconsin), etc.

[0073] To determine the percent identity between two amino acid sequences or two nucleic acid molecules, the sequences are aligned for optimal comparison purposes. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (i.e., percent identity = number of identical positions/total number of positions (e.g., overlapping positions) x 100). In one embodiment, the two sequences are, or are about, of the same length. The percent identity between two sequences can be determined using techniques similar to those described below, with or without allowing gaps. In calculating percent sequence identity, typically exact matches are counted.

[0074] The determination of percent identity between two sequences can be accomplished using a mathematical algorithm. A non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul, *Proc. Natl. Acad. Sci. USA* 1990, 87:2264, modified as in Karlin and Altschul, *Proc. Natl. Acad. Sci. USA* 1993, 90:5873-5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul et al, *J. Mol. Biol.* 1990; 215: 403. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12, to obtain nucleotide sequences homologous to sequences of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3, to obtain amino acid sequences homologous to protein sequences of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al, *Nucleic Acids Res.* 1997, 25:3389. Alternatively, PSI-Blast can be used to perform an iterated search that detects distant relationship between molecules. See Altschul et al. (1997) *supra*. When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective

programs (e.g., XBLAST and NBLAST) can be used. See ncbi.nlm.nih.gov/BLAST/ on the WorldWideWeb.

[0075] Another non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, CABIOS 1988; 4: 1 1-17. Such an algorithm is incorporated into the ALIGN program (version 2.0), which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used.

[0076] In a preferred embodiment, the percent identity between two amino acid sequences is determined using the algorithm of Needleman and Wunsch (J. Mol. Biol. 1970, 48:444-453), which has been incorporated into the GAP program in the GCG software package (Accelrys, Burlington, MA; available at accelrys.com on the WorldWideWeb), using either a Blossum 62 matrix or a PAM250 matrix, a gap weight of 16, 14, 12, 10, 8, 6, or 4, and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package using a NWSgapdna.CMP matrix, a gap weight of 40, 50, 60, 70, or 80, and a length weight of 1, 2, 3, 4, 5, or 6. A particularly preferred set of parameters (and the one that can be used if the practitioner is uncertain about what parameters should be applied to determine if a molecule is a sequence identity or homology limitation of the invention) is using a Blossum 62 scoring matrix with a gap open penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5.

[0077] Statistical analysis of the properties described herein may be carried out by standard tests, for example, t-tests, ANOVA, or Chi squared tests. Typically, statistical significance will be measured to a level of $p=0.05$ (5%), more preferably $p=0.01$, $p=0.001$, $p=0.0001$, $p=0.000001$

[0078] "Conservatively modified variants" of domain sequences also can be provided. With respect to particular nucleic acid sequences, conservatively modified variants refer to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Specifically, degenerate codon substitutions can be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer, et al.,

1991, Nucleic Acid Res. 19: 5081; Ohtsuka, et al., 1985, J. Biol. Chem. 260: 2605-2608; Rossolini et al., 1994, Mol. Cell. Probes 8: 91-98).

[0079] The term "biologically active fragment", "biologically active form", "biologically active equivalent" of and "functional derivative" of a wild-type protein, possesses a biological activity that is at least substantially equal (e.g., not significantly different from) the biological activity of the wild type protein as measured using an assay suitable for detecting the activity. For example, a biologically active fragment comprising a trafficking domain is one which can co-localize to the same compartment as a full-length polypeptide comprising the trafficking domain.

[0080] As used herein, "*in vivo*" nucleic acid delivery, nucleic acid transfer, nucleic acid therapy" and the like, refer to the introduction of a vector comprising an exogenous polynucleotide directly into the body of an organism, such as a human or non-human mammal, whereby the exogenous polynucleotide is introduced into a cell of such organism *in vivo*.

[0081] As used herein, the term "*in situ*" refers to a type of *in vivo* nucleic acid delivery in which the nucleic acid is brought into proximity with a target cell (e.g., the nucleic acid is not administered systemically). For example, *in situ* delivery methods include, but are not limited to, injecting a nucleic acid directly at a site (e.g., into a tissue, such as a tumor or heart muscle), contacting the nucleic acid with cell(s) or tissue through an open surgical field, or delivering the nucleic acid to a site using a medical access device such as a catheter.

[0082] As used herein, the term "isolated" means separated from constituents, cellular and otherwise, in which the polynucleotide, peptide, polypeptide, protein, antibody, or fragments thereof, are normally associated with in nature. For example, with respect to a polynucleotide, an isolated polynucleotide is one that is separated from the 5' and 3' sequences with which it is normally associated in the chromosome. As is apparent to those of skill in the art, a non-naturally occurring polynucleotide, peptide, polypeptide, protein, antibody, or fragments thereof, does not require "isolation" to distinguish it from its naturally occurring counterpart.

[0083] As used herein, a "target cell" or "recipient cell" refers to an individual cell or cell which is desired to be, or has been, a recipient of exogenous nucleic acid molecules, polynucleotides and/or proteins. The term is also intended to include progeny of a single

cell, and the progeny may not necessarily be completely identical (in morphology or in genomic or total DNA complement) to the original parent cell due to natural, accidental, or deliberate mutation. A target cell may be in contact with other cells (e.g., as in a tissue) or may be found circulating within the body of an organism.

[0084] As used herein, a "subject" is a vertebrate, preferably a mammal, more preferably a human. Mammals include, but are not limited to, murines, simians, humans, farm animals, sport animals, and pets. In other preferred embodiments, the "subject" is a rodent (e.g., a guinea pig, a hamster, a rat, a mouse), murine (e.g., a mouse), canine (e.g., a dog), feline (e.g., a cat), equine (e.g., a horse), a primate, simian (e.g., a monkey or ape), a monkey (e.g., marmoset, baboon), or an ape (e.g., gorilla, chimpanzee, orangutan, gibbon). In other embodiments, non-human mammals, especially mammals that are conventionally used as models for demonstrating therapeutic efficacy in humans (e.g., murine, primate, porcine, canine, or rabbit animals) may be employed.

[0085] As used herein, the term "pharmaceutically acceptable carrier" encompasses any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, and emulsions, such as an oil/water or water/oil emulsion, and various types of wetting agents. The compositions also can include stabilizers and preservatives. For examples of carriers, stabilizers and adjuvants, see Martin Remington's Pharm. Sci., 15th Ed. (Mack Publ. Co., Easton (1975)).

[0086] A cell has been "transformed", "transduced", or "transfected" by exogenous or heterologous nucleic acids when such nucleic acids have been introduced inside the cell. Transforming DNA may or may not be integrated (covalently linked) with chromosomal DNA making up the genome of the cell. In prokaryotes, yeast, and mammalian cells for example, the transforming DNA may be maintained on an episomal element, such as a plasmid. In a eukaryotic cell, a stably transformed cell is one in which the transforming DNA has become integrated into a chromosome so that it is inherited by daughter cells through chromosome replication. This stability is demonstrated by the ability of the eukaryotic cell to establish cell lines or clones comprised of a population of daughter cells containing the transforming DNA. A "clone" is a population of cells derived from a single cell or common ancestor by mitosis. A "cell line" is a clone of a primary cell that is capable of stable growth in vitro for many generations (e.g., at least about 10).

[0087] As used herein, an "effective amount" is an amount sufficient to affect beneficial or desired results, e.g., such as an effective amount of nucleic acid transfer and/or expression, and/or the attainment of a desired therapeutic endpoint. An effective amount can be administered in one or more administrations, applications or dosages. In one aspect, an effective amount of a nucleic acid delivery vector is an amount sufficient to transform/transduce/transfect at least one cell in a population of cells comprising at least two cells.

[0088] As used herein, a "therapeutically effective amount" is used herein to mean an amount sufficient to prevent, correct and/or normalize an abnormal physiological response. In one aspect, a "therapeutically effective amount" is an amount sufficient to reduce by at least about 30 percent, more preferably by at least 50 percent, most preferably by at least 90 percent, a clinically significant feature of pathology, such as for example, a clinically significant feature of pathology, such as for example, antibody production, cytokine production, fever or white cell count, or level of histamine.

[0089] As used herein, the term "prevent" or "prevents" in the context of allergy immunotherapy, allergy treatment, or other terms that describe an intervention designed for an allergy patient, means the prevention of an IgE response in at least 20% of all patients. The term "prevent" does not mean total prevention from developing an IgE mediated disease in all patients, and such a definition is outside the scope of the present invention for treating allergy through a mechanism that reduces allergy symptoms, and is inconsistent with the use of the term in the art. It is well known to those skilled in the art of allergy immunotherapy that allergy treatments are not 100% effective in 100% of patients, and as such an absolute definition of "prevent" does not apply within the context of the present invention. The art-recognized concept of prevention is contemplated by the present invention.

[0090] The term "oromucosal administration" refers to a route of administration where the dosage form is placed under the tongue or anywhere else in the oral cavity to allow the active ingredient to come in contact with the mucosa of the oral cavity or the pharynx of the patient in order to obtain a local or systemic effect of the active ingredient. An example of an oromucosal administration route is sublingual administration. The term "sublingual administration" refers to a route of administration where a dosage form is placed underneath the tongue in order to obtain a local or systemic effect of the active

ingredient. As used herein, the term "intradermal delivery" means delivery of the vaccine to the dermis in the skin. However, the vaccine will not necessarily be located exclusively in the dermis. The dermis is the layer in the skin located between about 1.0 and about 2.0 mm from the surface in human skin, but there is a certain amount of variation between individuals and in different parts of the body. In general, it can be expected to reach the dermis by going 1.5 mm below the surface of the skin. The dermis is located between the stratum corneum and the epidermis at the surface and the subcutaneous layer below. Depending on the mode of delivery, the vaccine may ultimately be located solely or primarily within the dermis, or it may ultimately be distributed within the epidermis and the dermis.

[0091] An "antibody" is any immunoglobulin, including antibodies and fragments thereof, that binds a specific epitope. The term encompasses polyclonal, monoclonal, and chimeric antibodies (e.g., bispecific antibodies). An "antibody combining site" is that structural portion of an antibody molecule comprised of heavy and light chain variable and hypervariable regions that specifically binds antigen. Exemplary antibody molecules are intact immunoglobulin molecules, substantially intact immunoglobulin molecules, and those portions of an immunoglobulin molecule that contains the paratope, including Fab, Fab', F(ab')₂ and F(v) portions, which portions are preferred for use in the therapeutic methods described herein.

[0092] An "epitope" is a structure, usually made up of a short peptide sequence or oligosaccharide, that is specifically recognized or specifically bound by a component of the immune system. T-cell epitopes have generally been shown to be linear oligopeptides. Two epitopes correspond to each other if they can be specifically bound by the same antibody. Two epitopes correspond to each other if both are capable of binding to the same B cell receptor or to the same T cell receptor, and binding of one antibody to its epitope substantially prevents binding by the other epitope (e.g., less than about 30%, preferably, less than about 20%, and more preferably, less than about 10%, 5%, 1%, or about 0.1% of the other epitope binds). In the present invention, multiple Allergen X can be combined to make up an Allergen X antigen (see, for example, SEQ ID NOs: 11, 21, 27, 109 and 110).

[0093] The term "antigenic material" as used herein covers any substance that will elicit an innate or adaptive immune response.

[0094] The term "antigen presenting cell" as used herein includes any cell which presents on its surface an antigen in association with a major histocompatibility complex molecule, or portion thereof, or, alternatively, one or more non-classical MHC molecules, or a portion thereof. Examples of suitable APCs are discussed in detail below and include, but are not limited to, whole cells such as macrophages, dendritic cells, B cells, hybrid APCs, and foster antigen presenting cells.

[0095] As used herein an "engineered antigen-presenting cell" refers to an antigen-presenting cell that has a non-natural molecular moiety on its surface. For example, such a cell may not naturally have a costimulator on its surface or may have an additional artificial costimulator in addition to a natural costimulator on its surface, or may express a non-natural class II molecule on its surface. In preferred embodiments, the engineered antigen-presenting cells have one or more Allergen X on its surface.

[0096] As used herein, "immune effector cells" refers to cells capable of binding an antigen and which mediate an immune response. These cells include, but are not limited to, T cells, B cells, monocytes, macrophages, NK cells and cytotoxic T lymphocytes (CTLs), for example CTL lines, CTL clones, and CTLs from tumor, inflammatory, or other infiltrates.

[0097] An "isolated" or "purified" population of cells is substantially free of cells and materials with which it is associated in nature. By substantially free or substantially purified APCs it is meant at least 50% of the population are APCs, preferably at least 70%, more preferably at least 80%, and even more preferably at least 90%, are free of non-APCs cells with which they are associated in nature.

[0098] As used herein, a "genetic modification" refers to any addition, deletion or disruption to a cell's normal nucleotides. Any method which can achieve the genetic modification of APCs are within the spirit and scope of this invention. Art recognized methods include viral mediated gene transfer, liposome mediated transfer, transformation, transfection and transduction, e.g., viral-mediated gene transfer such as the use of vectors based on DNA viruses such as adenovirus, adeno-associated virus and herpes virus, as well as retroviral based vectors.

[0099] The practice of the present invention employs, unless otherwise indicated, conventional molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Maniatis,

Fritsch & Sambrook, In Molecular Cloning: A Laboratory Manual (1982); DNA Cloning: A Practical Approach, Volumes I and II (D. N. Glover, ed., 1985); Oligonucleotide Synthesis (M. J. Gait, ed., 1984); Nucleic Acid Hybridization (B. D. Hames & S. J. Higgins, eds., 1985); Transcription and Translation (B. D. Hames & S. I. Higgins, eds., 1984); Animal Cell Culture (R. I. Freshney, ed., 1986); Immobilized Cells and Enzymes (IRL Press, 1986); B. Perbal, A Practical Guide to Molecular Cloning (1984).

Allergen X Antigen Sequences

[0100] The present invention provides polynucleic acids, polyaminoacids, host cells, vectors directed to Allergen X and methods of treating subjects in need of such polynucleic acids, polyaminoacids, host cells, or vectors. Broadly speaking, the polynucleic acids can be thought of as nucleic acid (e.g., DNA, RNA) vaccines for the intracellular production of allergenic sequences (polyaminoacids) that elicit a protective immune response within the body of the subject to whom the polynucleic acid is administered. The polynucleic acids, when administered, preferentially evoke a cell-mediated immune response via the MHC-II pathway and production of IgG antibodies by activating an allergen-specific T-helper type 1 (Th1) cellular response with the production of interferons by APCs, NK cells, and T cells rather than a Th2-type response, which involves production of IgE antibodies, granulocytes (e.g., eosinophils), and other substances. To an extent, both an MHC-II and an MHC-I response can be generated; however, the invention provides a response that is primarily or substantially an MHC-II response. Preferably, the nucleic acids do not encode an antibiotic resistance gene.

[0101] The invention is based, at least in part, on the recognition that a combination of certain structural, and thus functional, elements provides advantageous properties to the nucleic acid vaccines and the encoded allergens, and allows for allergy treatment methods that satisfy unmet needs in the art. In the various embodiments of the invention, which are intended to be understood as standing alone as independent embodiments and as embodiments that combine two or more features of the independent embodiments, the combinations include the use of a lysosomal trafficking domain to direct Allergen X to lysosomes with MHC II proteins. Doing so allows for predominantly an IgG response as opposed to an IgE response to the Allergen X. Yet further, independent embodiments or combinations of embodiments provide constructs

containing a sufficient length of a nucleic acid sequence (e.g., the full length nucleic acid sequence or a fragment thereof, a variant of the full length nucleic acid sequence or a fragment thereof, etc.) to encode an amino acid sequence that provides a naturally-occurring three-dimensional structure of an epitope. In preferred embodiments, the nucleic acid sequence encodes Allergen X (SEQ ID NO:Y). In other embodiments, the nucleic acid sequence encodes at least one Allergen X. Although it is recognized in the art that an immune response can be generated against the primary sequence of an epitope, the present invention recognizes that nucleic acid vaccines for the production of an MHC-II immune response to encoded epitopes preferably uses nucleic acid constructs that encode enough sequence data to produce a correct three-dimensional peptide structure in the region comprising Allergen X, at least at the time when Allergen X is delivered to a lysosome for processing. While not being limited to any particular molecular theory, it is believed that delivery of a properly three-dimensionally folded protein, polypeptide, or peptide to an endosome improves processing and presentation of allergenic epitopes for an immune response.

[0102] Thus, the invention provides chimeras which comprise Allergen X and luminal sequences of a polypeptide that result in trafficking of the chimera to the endosomal/lysosomal compartment for antigen processing and antigen epitope association with MHC II. In one aspect, the chimeric protein additionally comprises cytoplasmic targeting sequences that direct the chimera to endosomal/lysosomal compartments. Additionally, the chimeric protein also may comprise a signal sequence and/or a transmembrane sequence. Suitable trafficking domains are provided by LAMP-1, LAMP-2, DC-LAMP, Trp-1, DEC-205, gp200-MR6, and other polypeptides, as discussed below. The signal sequence and transmembrane sequence may, but do not have to be, from these polypeptides. However, in one aspect, an antigen/LAMP chimera comprises a full-length LAMP polypeptide.

[0103] Allergen X can be used to generate chimeric proteins. In one aspect, preferred compositions comprise the amino acid sequence of Allergen X of any one of SEQ ID NO:Y as shown in Table 1 or polynucleotides that encode any one of the SEQ ID NO:Y. Preferred representative examples of polynucleotides that can encode Allergen X are shown as SEQ ID NO:Z in Table 1. Further preferred representative embodiments are constructs comprising Allergen X along with either human, murine, or canine LAMP

sequences (see, for example, SEQ ID NO:W and SEQ ID NO:V). As described in Table 3 in the Examples, it was surprisingly found that the chimeric LAMP vaccines comprising the specific Allergen X amino acid sequences corresponding to SEQ ID NOs: Y generated an unexpectedly robust immune response as compared to other constructs tested.

[0104] Allergen X can also include Allergen X variants. For example, preferred embodiments include Allergen X polypeptide variants that have at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO:Y. These variants retain either (a) the ability to raise an antibody that cross-reacts with the Allergen X from which it was derived and/or (b) Allergen X biological activity. Polynucleotides that encode these polypeptides are specifically contemplated.

[0105] Synthetic Allergen X antigens and altered Allergen X antigens also can be used in the methods described herein. Synthetic antigenic Allergen X peptide epitopes have modified amino acid sequences relative to the natural Allergen X sequence. Further encompassed by the term "synthetic Allergen X antigenic peptides" are multimers (concatemers) of synthetic antigenic Allergen X peptides, optionally including intervening amino acid sequences. For example, synthetic Allergen X antigenic peptide epitopes of the present invention can be designed based on known amino acid sequences of antigenic Allergen X peptide epitopes.

[0106] Also included within the scope of the invention are antigenic Allergen X peptides that are differentially modified during or after translation, e.g., by phosphorylation, glycosylation, crosslinking, acylation, proteolytic cleavage, linkage to an antibody molecule, membrane molecule or other ligand (Ferguson et al., *Ann. Rev. Biochem.* 57: 285-320, 1998).

[0107] Isolated Allergen X peptides can be synthesized using an appropriate solid state synthetic procedure (Steward and Young, *Solid Phase Peptide Synthesis*, Freeman, San Francisco, Calif. 1968). A preferred method is the Merrifield process (Merrifield, *Recent Progress in Hormone Res.* 23: 451, 1967). The Allergen X antigenic activity of these peptides may conveniently be tested using, for example, the assays as described herein.

[0108] Once an isolated Allergen X peptide is obtained, it may be purified by standard methods including chromatography (e.g., ion exchange, affinity, and sizing

column chromatography), centrifugation, differential solubility, or by any other standard technique for protein purification. For immunoaffinity chromatography, an Allergen X epitope may be isolated by binding it to an affinity column comprising antibodies that were raised against that peptide, or a related peptide, and were affixed to a stationary support. Alternatively, affinity tags such as hexa-His (Invitrogen), Maltose binding domain (New England Biolabs), influenza coat sequence (Kolodziej, et al., *Methods Enzymol.* 194: 508-509, 1991), and glutathione-S-transferase can be attached to the peptides to allow easy purification by passage over an appropriate affinity column. Isolated Allergen X peptides also can be physically characterized using such techniques as proteolysis, nuclear magnetic resonance, and x-ray crystallography.

[0109] Having isolated and identified the Allergen X peptide sequence of a desired epitope, nucleic acids comprising sequences encoding these epitopes can be sequenced readily.

Endocytic Trafficking Sequences

[0110] The available data suggest the following sequence of events in the intracellular transport of MHC class II molecules: MHC class II molecules with the invariant chain are assembled in the endoplasmic reticulum and transported through the Golgi with other membrane proteins including MHC class I. The molecules are then targeted to specific endosomal/lysosomal organelles by an unknown mechanism, segregating from the MHC class I molecules which follow a constitutive route to the cell surface. In the endocytotic/lysosomal route, the invariant chain is removed from MHC class II by proteases acting in an acidic environment. At the same time, antigenic fragments of proteins that have entered the endocytic/lysosomal pathway are generated by these proteases and the resulting peptides bind to the class II molecules and are carried to the cell surface.

[0111] The biosynthesis and vacuolar targeting mechanisms of the hydrolytic enzymes present in the lysosomal/endosomal compartment have been extensively studied (Kornfeld and Mellman, *Ann. Rev. Cell Biol.* 5: 483, 1989). Newly synthesized hydrolases in the Golgi apparatus acquire mannose 6-phosphate groups that serve as specific recognition markers for the binding of these enzymes to mannose 6-phosphate receptors which are then targeted in some unknown manner to a prelysosomal vacuole.

There the receptor-enzyme complex is dissociated by low pH, and the receptors recycle to the Golgi apparatus, while the enzyme-containing vacuole matures into a lysosome.

[0112] The localization of the lysosomal membrane glycoproteins is controlled by a targeting mechanism independent of the well-defined mannose 6-phosphate receptor (MPR) pathway for hydrolytic lysosomal enzymes (Kornfeld and Mellman, 1989, *supra*). Recent studies describe a distinct vesicular compartment with lysosomal properties and characterized by high concentration of lysosomal-associated membrane protein (LAMP-1) and MHC class II molecules (Peters, et al., *EMBO J.* 9: 3497, 1990). Lysosomal/Endosomal Compartment Kinetic analysis of intracellular transport and targeting of newly synthesized LAMP-1 and other similar proteins indicate that the molecule is synthesized in the endoplasmic reticulum, processed in the Golgi cisternae and transported to lysosomes within one hour of its biosynthesis, without detectable accumulation in the plasma membrane (Barriocanal, et al., *J Biol. Chem.* 15: 261(35): 16755-63, 1986; D'Sousa, et al., *Arch. Biochem. Biophys.* 249: 522, 1986; Green, et al., *J. Cell Biol.*, 105: 1227, 1987).

[0113] Studies of the structure and function of the lysosomal membrane were initiated in 1981 by August and colleagues with the discovery of major cellular glycoproteins that were subsequently termed lysosomal-associated membrane proteins one and two (LAMP-1 and LAMP-2) due to their predominant localization in the lysosomal membrane (Hughes, et al., *J. Biol. Chem.* 256: 664, 1981; Chen, et al., *J. Cell Biol.* 101:85, 1985). Analogous proteins were subsequently identified in rat, chicken and human cells (Barriocanal, et al., 1986, *supra*; Lewis, et al., *J. Cell Biol.* 100:1839, 1985; Fambourgh, et al., *J. Cell Biol.* 106: 61, 1988; Mane, et al., *Arch. Biochem. Biophys.* 268: 360, 1989).

[0114] Typically, LAMP-1, as deduced from a cDNA clone (Chen, et al., *J. Biol. Chem.* 263: 8754, 1988) consists of a polypeptide core of about 382 amino acids with a large (346-residue) luminal amino-terminal domain followed by a 24-residue hydrophobic transmembrane region and short (12-residue) carboxyl-terminal cytoplasmic tail. See, Figures 2 and 3. The luminal domain is highly glycosylated, being substituted with about 20 asparagine linked complex-type oligosaccharides and consists of two to about 160-residue homology units that are separated by a proline/serine-rich region. Each of these homologous domains contains 4 uniformly spaced cysteine residues,

disulfide bonded to form four 36-38-residue loops symmetrically placed within the two halves of the luminal domain (Arterburn, et al., J. Biol. Chem. 265: 7419, 1990; see, also Chen, et al., J. Biol. Chem. 25: 263(18): 8754-8, 1988). The LAMP-2 molecule is highly similar to LAMP-1 in overall amino acid sequence (Cha, et al., J. Biol. Chem. 265: 5008, 1990).

[0115] LAMP-1 and LAMP-2 are not specifically found in antigen presenting cells (dendritic cells). Their precise function is unknown, but they presumably are involved in some manner with lysosome function. Their colocalization with MHC II in the multilaminar MIIC vesicular compartment of APCs has no known functional association to antigen processing or presentation; however, chimeric antigens comprising LAMP cytoplasmic domains, as discussed above, show enhanced immunogenicity (see, U.S. Pat. No. 5,633,234).

[0116] The invention provides chimeric Allergen X proteins comprising the luminal domain of a lysosomal associated membrane protein, such as a LAMP polypeptide, or a bioactive fragment or modified form thereof (collectively referred to as "a LAMP-luminal domain"). In one aspect, the LAMP luminal domain comprises at least two homology units. Preferably, each homology unit is separated by a proline/serine-rich region. More preferably, each homology domain comprises 4 cysteine residues capable of forming four 36-38-residue loops symmetrically placed within the two halves of the luminal domain when disulfide bonded together. Most preferably, the luminal domain comprises sequences necessary to target and traffic a polypeptide to which it is linked (e.g., via a chemical bond) to an endosomal/lysosomal compartment or lysosome-related organelle for binding to an MHC class II molecule or for delivery to another compartment/organelle where it will bind to an MHC class II molecule.

[0117] In a preferred embodiment, the portion of the luminal domain of LAMP-1 that can be used in the present invention, includes, but is not limited to SEQ ID NOs:2 or 3 or amino acids 29-381 of SEQ ID NO:4 (or the corresponding sequence of SEQ ID NO:5). In further preferred embodiments, the transmembrane domain/cytoplasmic tail of LAMP-1 can be used in the present invention. For example, SEQ ID NO:1 or amino acids 382-417 of SEQ ID NO:4 (or the corresponding sequence of SEQ ID NO:5) can be used in the chimeric vaccines as described herein. Moreover, any combination of the

luminal domain fragments with the transmembrane domain/cytoplasmic tail fragments can be used.

[0118] In another aspect, the chimeric protein additionally, or alternatively, comprises a dileucine-based signal comprising at least one leucine-leucine pair or at least one leucine/isoleucine pair. Preferably, the protein further comprises an acidic residue 4-5 residues upstream of the pair. More preferably, this signal domain binds to an AP complex polypeptide (see, e.g., Bonafacino and Dell'Angelica, *J. Cell Biol.* 145: 923-926, 1999). Suitable dileucine-based domains can be found in tyrosinase (TM-X₁₀-EKQPLL-X₅-YHSL-X₅) (SEQ ID NO:97); TRP-2 (TM-X₇-EANQPLL-X₁₂) (SEQ ID NO:98); and Pmel7 (TM-X₃₄-ENSPLL-X₅) (SEQ ID NO:99) and P-protein (see, e.g., Dell'Angelica, 2000, *supra*), for example.

[0119] In a preferred aspect, the chimeric protein also comprises a cytoplasmic domain for targeting and/or trafficking a chimeric Allergen X protein to an endosomal/lysosomal compartment or lysosome-related organelle. In one aspect, the cytoplasmic domain comprises the tail of a LAMP polypeptide. The eleven amino-acid sequence of the cytoplasmic tail of LAMP-1 and other similar lysosomal membrane glycoproteins has the following sequence: Arg-Lys-Arg-Ser-His-Ala-Gly-Tyr-Gln-Thr-Ile-COOH (Chen, et al., 1988, *supra*)(Residues 25-35 of SEQ ID NO:1). In LAMP-1, these sequences are from amino acids 372-382 of the full-length polypeptide.

[0120] The known cytoplasmic tail sequences of lysosomal membrane proteins, LAMP-1 (Chen, et al., 1988, *supra*), LAMP-2 (Cha, et al., 1990, *supra*) and CD63 (Hotta, et al., *Cancer Res.* 48: 2955, 1988), have been aligned with the Tyr-containing internalization signal in the cytoplasmic tail of LAP (Pohlman, et al., *EMBO J.* 7: 2343, 1988) in Table 2. The Tyr residue is known to be required for endosomal/lysosomal targeting, and it was demonstrated in U.S. Pat. No. 5,633,234 that the complete sequence required to target other molecules to lysosomes requires the Tyr-X-X-hyd sequence (i.e., a "Tyr motif"), a Tyr followed by two amino acids followed by a hydrophobic residue.

Table 2		
Cytoplasmic Tail Sequences of the Major Lysosomal Membrane Proteins*		
LAMP-1	RKRSHA <u>GYQTI</u>	(Residues 25-35 of SEQ ID NO:1)

LAMP-2	KHHHA <u>G</u> YEQF	(SEQ ID NO:100)
CD63	KSIRS <u>G</u> YEV	(SEQ ID NO:101)
LAP	RMEAPP <u>G</u> YRHVADGEDHA	(SEQ ID NO:102)

*The conserved Gly-Tyr-X-X-hydrophobic residue motif in the cytoplasmic domain of the described lysosomal membrane proteins is underlined, where X is any amino acid. The complete cytoplasmic tail sequence of the listed proteins is shown from the transmembrane region to the carboxyl terminus.

[0121] The importance of a hydrophobic residue at or near the carboxyl-terminal position is shown by results obtained from modification of the Tyr-Gln-Thr-Ile sequence of LAMP-1 (Residues 32-35 of SEQ ID NO:1). Mutant cDNA molecules in which Ile was substituted with two other hydrophobic residues, Leu or Phe, and a polar residue, Thr. Substituting Leu (Tyr-Gln-Thr-Leu) (SEQ ID NO:103) and Phe (Tyr-Gln-Thr-Phe) (SEQ ID NO:104) does not affect lysosomal targeting, whereas the Thr-containing mutant protein (Tyr-Gln-Thr-Thr) (SEQ ID NO:105) accumulates at the cell surface. Mutants containing Ala substituted for Gln (Tyr-Ala-Thr-Ile) (SEQ ID NO:106), Thr (Tyr-Gln-Ala-Ile) (SEQ ID NO:107), and both residues (Tyr-Ala-Ala-Ile) (SEQ ID NO:108) have no effect on targeting to the lysosomal membrane, indicating that these positions may be occupied by charged, polar, or nonpolar residues.

[0122] The preferred targeting signal to the lysosomal/endosomal compartment, therefore, includes a tetrapeptide sequence located in the cytoplasmic domain, near the transmembrane domain and also near the C-terminus. The cytoplasmic domain is preferably a short amino acid sequence (less than 70 amino acids, preferably less than 30 amino acids, most preferably less than 20 amino acids) ending in a free carboxyl group. In a more preferred embodiment, the tetrapeptide is at the C-terminal end of a short cytoplasmic tail that contains the targeting signal, or is in a context similar to LAMP-1.

[0123] A suitable four amino acid sequence for the tetrapeptide may be obtained by amino acid substitutions, so long as the motif consists of Tyr-X-X-Hyd (where X may be any amino acid and Hyd denotes a hydrophobic amino acid), and the ability to confer lysosomal/endosomal targeting is conserved. A particularly preferred tetrapeptide has the sequence Tyr-Gln-Thr-Ile (Residues 32-35 of SEQ ID NO:1). In the most preferred embodiment, the entire LAMP cytoplasmic tail in conjunction with its transmembrane domain, and most preferably, its luminal domain is coupled to the primary sequence of

the antigenic domain for highly efficient MHC class II processing and presentation. However, the cytoplasmic domain is not necessary to facilitate trafficking so long as a luminal domain of a LAMP polypeptide is provided.

[0124] In another aspect, the endosomal targeting domain comprises a transmembrane sequence. Many proteins that will serve as the source of the antigenic domain for particular immune stimulatory constructs will be surface antigens that include a transmembrane domain in their primary sequence. Such a transmembrane domain can be retained, and the cytoplasmic domain replaced with a lysosomal/endosomal targeting domain as taught herein (e.g., a domain comprising a LAMP luminal domain).

[0125] In one preferred aspect, the transmembrane domain of LAMP (see, Chen, et al., J. Biol. Chem. 263: 8754, 1988) is coupled to the primary sequence of the Allergen X derived polypeptide and the sequence of the luminal domain. The structure of a transmembrane domain in a polypeptide is well known in the art (see, e.g., Bangham, Anal. Biochem. 174: 142, 1988; Klein, et al., Biochem. Biophys. Acta 815: 468, 1985; Kyle & Doolittle, J. Mol. Biol. 157: 105, 1982). Usually the transmembrane region appears in the primary sequence as a sequence of 20-25 hydrophobic amino acid residues flanked by hydrophilic regions. Such sequences can be found, for example, in most cell surface antigen sequences listed by Genbank as well as many other membrane proteins. The particular transmembrane sequence is not critical, so long as it serves to connect the antigenic domain to the luminal domain and cytoplasmic tail and anchors the construct in the membranous compartment.

[0126] Additional, or alternative sorting motifs, can include, but are not limited to, one or more of: a targeting domain, a tyrosine motif domain as described above; a dileucine and tyrosine-based domain; a proline rich domain; and S-V-V domain (see, e.g., Blott and Grifitts, Nature 3: 122-131, 2002).

Endocytic Receptor Sequences

[0127] Antigen access to the MHC II vesicular compartment of antigen presenting cells, such as dendritic cells, is normally by endocytosis of foreign antigens. The trafficking domains of endocytic receptors can be used to generate chimeric polypeptides to carry Allergen X to endosomal/lysosomal compartments or to lysosome-related organelles for association with class II MHC molecules and subsequent processing.

[0128] In one aspect, therefore, the invention provides at least one Allergen X linked to a trafficking domain of an endocytic receptor (e.g., via in-frame fusion of nucleic acid sequences encoding the trafficking domain and antigen). The trafficking domain localizes Allergen X to an endosomal/lysosomal compartment or to a lysosome-related organelle for association with an MHC class II molecule in the compartment/organelle or in a subsequent compartment to which Allergen X is delivered.

[0129] Endocytic receptors according to the invention, include, but are not limited to receptors for microorganisms, Fc receptors (e.g., CD64, CD32, CD16, CD23, and CD89); complement receptors (e.g., CR1 or CD35, CR3, CR4); scavenger receptors or receptors which bind to acetylated or modified lipoproteins, polyribonucleotides, lipopolysaccharides and silica particles (e.g., such as SRA, MARCO), integrins (CD49e/CD29; CD49d/CD29; CD51/CD61); lectins (e.g., such as dectin-1, C-type lectins, and the like), and Toll-like receptors (e.g., TLRs). For a review of such receptors, see Underhill and Ozinsky, *Annu. Rev. Immunol.* 20: 825-52, 2002, for example.

[0130] In one aspect, the endocytic receptor is obtained from a professional antigen presenting cell such as a dendritic cell. A number of endocytic receptors of dendritic cells have been identified, including the macrophage mannose receptor (MMR), phospholipaseA₂-receptor, Endo 180, and DEC-205 and its human homologue, gp200-MR6 (McKay, et al., 1998). DEC-205 is reported to differ from the MMR, at least, in that it targets antigenic material to an endosomal/lysosomal compartment co-localized with LAMP and MHC II, whereas MMR is found in peripheral endosomes lacking LAMP and MHC II (Mahnke, et al., *J. Cell Biol.* 151(3): 673-684, 2000).

[0131] DEC-205 also demonstrates a greatly enhanced presentation of endocytosed antigen to CD4⁺ T-cells, as compared to that by the MMR. This difference in trafficking and antigen delivery to MHC II between the two molecules is reported to result from the presence in the cytosolic tail of DEC-205, in addition to the coated pit uptake sequence, of an EDE triad that is lacking in the MMR. The distal portion of the cytosolic tail containing the EDE sequence was shown to be required for the targeting to the deeper endosome/lysosome compartment containing LAMP and MHC II, and EDE was not replaced by an AAA sequence. Mahnke et al., 2000, *supra*, have also shown that these cytoplasmic tail trafficking signals are sufficient to traffic and recycle a CD 16

chimera to the MHC II/LAMP site and to mediate a 100-fold increase in antigen presentation.

[0132] The sequence similarity between DEC-205 and gp200-MR6, particularly, in the cytoplasmic domain, makes this sequence a suitable trafficking sequence as well. Further, gp200-MR6 has been shown to have the further important property of IL-4 regulation. McKay et al., *Eur J Immunol.* 28(12): 4071-83, 1998, have shown that ligation of gp200-MR6 can mimic IL-4 and have an antiproliferative, pro-maturational influence within the immune system, causing up-regulation of costimulatory molecules on B lymphocytes.

[0133] However, DEC-205 fusions with LAMP do not traffic to the endosomal compartment but rather localize to the cell surface. Chimeric proteins which combine LAMP domains and at least one Allergen X, and endocytic receptor domains such as DEC-205 domains, however, are able to traffic to endosomal compartments, co-localizing with endogenous LAMP.

[0134] Therefore, in a preferred aspect, a chimeric Allergen X protein of the invention comprises a luminal domain of a lysosomal membrane polypeptide (e.g., such as a LAMP luminal domain) and the targeting domain of an endocytic receptor (e.g., such as DEC-205 or gp 200-MR6 polypeptide). Such constructs may not only show correct targeting but improved antigenicity as well. In a further aspect, both the targeting and trafficking domain of an endocytic receptor is provided along with at least one Allergen X. Chimeric Allergen X proteins may additionally, or alternatively, comprise the luminal domain of an endocytic receptor. In still a further aspect, a chimeric protein may comprise a full-length endocytic receptor polypeptide along with at least one Allergen X.

[0135] In one aspect, the targeting domain of the endocytic receptor comprises the 31 amino acid cytoplasmic domain of a DEC polypeptide (see, e.g., Manhke, et al., 2000, *supra*). In another aspect, the targeting domain comprises residues 7-9 of the DEC-205 cytoplasmic tail. Preferably, the domain comprises a Tyr motif. More preferably, the targeting domain also comprises residues 18-27 of the DEC-205 cytoplasmic tail. In a further aspect, the targeting domain comprises an EDE domain. The sequence of DEC-205 is provided in Kato, et al., *Immunogenetics* 47(6): 442-50, 1998, while that of gp200-MR6 is provided in McKay, et al., 1998, *supra*, for example.

[0136] The chimeric Allergen X protein may additionally comprise a cytoplasmic targeting domain for targeting at least one Allergen X to an endosomal/lysosomal compartment or a lysosome-related organelle (e.g., such as a cytoplasmic LAMP domain) as well as one or more of the other domains described above (e.g., signal sequence, transmembrane sequence, etc.). As above, additional, or alternative sorting motifs, can include, but are not limited to, one or more of the M6P domain; a tyrosine motif domain; a di-leucine and tyrosine-based domain; a proline rich domain; and S-V-V domain (see, e.g., Blott and Grifitts, Nature 3: 122-131, 2002).

Vaccine Compositions

[0137] Although a number of antigens have been hypothesized to be capable of combination with LAMP to produce novel chimeric vaccines; in actuality when tested, these vaccines do not always work.

[0138] Thus, this invention provides a vaccine composition for eliciting an immune response in a mammal to Allergen X. The composition comprises a vaccine vector which comprises a chimeric DNA segment comprising a sequence encoding at least one Allergen X (SEQ ID NO:Y). Preferably, the DNA segment further includes a sequence encoding a luminal domain of a lysosome associated membrane polypeptide (e.g., such as a LAMP polypeptide (e.g., SEQ ID NOs:2, 3, 4 or 5), homolog, ortholog, variant, or modified form thereof) or the trafficking domain of an endocytic receptor. Preferably, the luminal domain or trafficking domain traffics the antigen to an endosomal/lysosomal compartment or to a lysosome-related organelle of a cell, where it binds to an MHC class II molecule or is processed for delivery to another compartment/organelle where it will subsequently bind to an MHC class II molecule. More preferably, Allergen X is processed within the compartment/organelle (or subsequent compartment to which it is delivered) to generate an Allergen X epitope which is presented on the surface of the cell and which is bound to the MHC class II molecule.

[0139] The vector also may encode one or more of a transmembrane domain, a cytoplasmic domain containing an endosomal/lysosomal targeting signal directing the protein to an endosomal/lysosomal compartment or lysosome-related organelle, and a dileucine domain, a Tyr motif, a proline rich domain, and/or S-V-V domain.

[0140] The domains may be provided in sequence or separated by nucleic acids encoding linker polypeptides or which encode other amino acid sequences with desired functionalities (e.g., protein stabilizing sequences, and the like). Generally, where linker sequences are included, these encode linker polypeptides which range from about one to about 50 amino acids. The minimal requirement of the vector is that it encode a chimeric Allergen X protein with the desired trafficking properties. Such properties can be readily tested using assays routine in the art.

[0141] For example, immunofluorescence microscopy can be used to confirm the trafficking of an Allergen X chimeric protein to an appropriate compartment/organelle. ³⁵S methionine pulse-chase labeling analysis can be used to monitor the synthesis and degradation of the Allergen X chimeric protein to demonstrate that the rates of synthesis of the Allergen X chimeric protein vs. the endogenous Allergen X protein are essentially equal and/or that the processing of the Allergen X chimeric protein occurs properly.

[0142] In particular embodiments, the protein encoded by the chimeric DNA segment contains an intraluminal domain comprising at least one Allergen X epitope which is a peptide that complexes with major histocompatibility complex (MHC) class II molecules, an endosomal/lysosomal trafficking sequence as described above, and a cytoplasmic domain which contains an endosomal/lysosomal targeting sequence. Preferably, the targeting sequence comprises the tetrapeptide sequence Tyr-Xaa-Xaa-Xbb, wherein Xbb is a hydrophobic amino acid.

[0143] In another aspect, the protein encoded by the chimeric DNA segment comprises a full length lysosomal membrane associated polypeptide, such as a LAMP polypeptide, homolog, ortholog, variant or modified form thereof, which comprises sequences for targeting and trafficking both membrane-bound and non-membrane bound antigenic material to an endosomal/lysosomal compartment.

[0144] In preferred embodiments, the chimeric vaccines comprise (a) any one of the polynucleotides of SEQ ID NO:Z or SEQ ID NO:W as shown in Table 1; (b) a polynucleotide encoding a polypeptide encoded by any one of the polynucleotide identified as SEQ ID NO:Z or SEQ ID NO:W as shown in Table 1; (c) a polynucleotide that encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: Y or SEQ ID NO:V as shown in Table 1; (d) a polynucleotide encoding a luminal domain (such as, for example, SEQ ID NOs:2 or 3 or as shown in SEQ ID Nos: 4 or 5), one or more of SEQ ID

NO:Y as shown in Table 1, and a trafficking domain (such as SEQ ID NO:1, or as shown in SEQ ID Nos: 4 or 5); (e) a polynucleotide encoding a lumenal domain (such as, for example, SEQ ID NOs:2 or 3 or as shown in SEQ ID Nos: 4 or 5) and one or more of SEQ ID NO:Y as shown in Table 1; (f) a polynucleotide encoding one or more of SEQ ID NO:Y as shown in Table 1 and a trafficking domain (such as SEQ ID NO:1, or as shown in SEQ ID Nos: 4 or 5) or (g) a polynucleotide at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, 96%, 97%, 98% or 99% identity to any one of the polynucleotides of (a)-(f).

Assembly of Sequences Encoding Allergen X Chimeric Proteins

[0145] Procedures for construction of chimeric proteins are well known in the art (see e.g., Williams, et al., J. Cell Biol. 111: 955, 1990). DNA sequences encoding the desired segments can be obtained from readily available recombinant DNA materials such as those available from the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Md. 20852, U.S.A., or from DNA libraries that contain the desired DNA.

[0146] Such DNA segments minimally include: sequences encoding an antigenic domain from the Allergen X protein and a lumenal domain of a lysosomal membrane associated polypeptide for trafficking a polypeptide linked to the lumenal domain to an endosomal/lysosomal compartment or lysosome-related organelle and/or a trafficking domain of an endocytic receptor for trafficking to an endosomal/lysosomal compartment and or lysosome-related organelle. Additional DNA segments may include, but are not limited to, sequences encoding: cytoplasmic targeting sequences for targeting the chimeric protein to an endosomal/lysosomal compartment or lysosome-related organelle, transmembrane sequences, signal sequences, di-leucine sequences, Tyr motifs, proline rich domains, M6P sequences, Ser-Val-Val sequences and as well as cloning sequences and the like.

[0147] The DNA segments corresponding to the desired domain sequences are then assembled with appropriate control and signal sequences using routine procedures of recombinant DNA methodology. See, e.g., as described in U.S. Pat. No. 4,593,002, and Langford, et al., Molec. Cell. Biol. 6: 3191, 1986.

[0148] A DNA sequence encoding a protein or polypeptide can be synthesized chemically or isolated by one of several approaches. The DNA sequence to be synthesized

can be designed with the appropriate codons for the desired amino acid sequence. In general, one will select preferred codons for the intended host in which the sequence will be used for expression. The complete sequence may be assembled from overlapping oligonucleotides prepared by standard methods and assembled into a complete coding sequence. See, e.g., Edge, *Nature* 292: 756, 1981; Nambair, et al. *Science* 223: 1299, 1984; Jay, et al., *J. Biol. Chem.* 259: 6311, 1984.

[0149] In one aspect, one or more of the nucleic acids encoding the domain sequences of the chimeric protein are isolated individually using the polymerase chain reaction (M. A. Innis, et al., In *PCR Protocols: A Guide to Methods and Applications*, Academic Press, 1990). The domains are preferably isolated from publicly available clones known to contain them, but they may also be isolated from genomic DNA or cDNA libraries. Preferably, isolated fragments are bordered by compatible restriction endonuclease sites which allow a chimeric DNA encoding the immunogenic protein sequence to be constructed. This technique is well known to those of skill in the art. Domain sequences may be fused directly to each other (e.g., with no intervening sequences), or inserted into one another (e.g., where domain sequences are discontinuous), or may be separated by intervening sequences (e.g., such as linker sequences).

[0150] The basic strategies for preparing oligonucleotide primers, probes and DNA libraries, as well as their screening by nucleic acid hybridization, are well known to those of ordinary skill in the art. See, e.g., Sambrook, et al., 1989, *supra*; Perbal, 1984, *supra*. The construction of an appropriate genomic DNA or cDNA library is within the skill of the art. See, e.g., Perbal, 1984, *supra*. Alternatively, suitable DNA libraries or publicly available clones are available from suppliers of biological research materials, such as Clontech and Stratagene, as well as from public depositories such as the American Type Culture Collection.

[0151] Selection may be accomplished by expressing sequences from an expression library of DNA and detecting the expressed peptides immunologically. Clones which express peptides that bind to MHC II molecules and to the desired antibodies/T cell receptors are selected. These selection procedures are well known to those of ordinary skill in the art (see, e.g., Sambrook, et al., 1989, *supra*).

[0152] Once a clone containing the coding sequence for the desired polypeptide sequence has been prepared or isolated, the sequence can be cloned into any suitable vector, preferably comprising an origin of replication for maintaining the sequence in a host cell.

[0153] In preferred embodiments, the chimeric proteins are encoded by (a) any one of the polynucleotides of SEQ ID NO:Z or SEQ ID NO:W as shown in Table 1; (b) a polynucleotide encoding a polypeptide encoded by any one of the polynucleotides of SEQ ID NO:Z or SEQ ID NO:W as shown in Table 1; (c) a polynucleotide that encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: Y or SEQ ID NO:V as shown in Table 1; (d) a polynucleotide encoding a luminal domain (such as, for example, SEQ ID NOs: 2 or 3 or as shown in SEQ ID Nos: 4 or 5), one or more of SEQ ID NO:Y as shown in Table 1, and a trafficking domain (such as SEQ ID NO:1 or as shown in SEQ ID Nos: 4 or 5); (e) a polynucleotide encoding a luminal domain (such as, for example, SEQ ID NOs:2 or 3 or as shown in SEQ ID Nos: 4 or 5) and one or more of SEQ ID NO:Y as shown in Table 1; (f) a polynucleotide encoding one or more of SEQ ID NO:Y as shown in Table 1 and a trafficking domain (such as SEQ ID NO:1, or as shown in SEQ ID Nos: 4 or 5); or (g) polynucleotides with at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, 96%, 97%, 98% or 99% identity to any one of the polynucleotides of (a)-(f). Polypeptides encoded by these polynucleotides are additional preferred embodiments.

Nucleic Acid Delivery Vehicles

[0154] In one aspect, a nucleic acid vector encoding a chimeric vaccine is introduced into a cell. The cell may be a host cell for replicating the nucleic acid or for expressing the chimeric vaccine. Preferably, the host cell for expressing the chimeric vaccine is an antigen presenting cell (described further below).

[0155] The nucleic acid vector minimally comprises a polynucleotide sequence for insertion into a target cell and an expression control sequence operably linked thereto to control expression of the polynucleotide sequence (e.g., transcription and/or translation) in the cell. Examples include plasmids, phages, autonomously replicating sequences (ARS), centromeres, and other sequences which are able to replicate or be replicated *in vitro* or in a host cell (e.g., such as a bacterial, yeast, or insect cell) and/or target cell (e.g.,

such as a mammalian cell, preferably an antigen presenting cell) and/or to convey the sequences encoding the chimeric vaccine to a desired location within the target cell.

[0156] In preferred embodiments, the vectors/vehicles described herein comprise (a) any one of the polynucleotides of SEQ ID NO:Z or SEQ ID NO:W as shown in Table 1; (b) a polynucleotide encoding a polypeptide encoded by any one of the polynucleotides of SEQ ID NO:Z or SEQ ID NO:W as shown in Table 1; (c) a polynucleotide that encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: Y or SEQ ID NO:V as shown in Table 1; (d) a polynucleotide encoding a luminal domain (such as, for example, SEQ ID NOs:2 or 3 or as shown in SEQ ID Nos: 4 or 5), one or more of SEQ ID NO:Y as shown in Table 1, and a trafficking domain (such as SEQ ID NO:1, or as shown in SEQ ID Nos: 4 or 5); (e) a polynucleotide encoding a luminal domain (such as, for example, SEQ ID NOs:2 or 3 or as shown in SEQ ID Nos: 4 or 5) and one or more of SEQ ID NO:Y as shown in Table 1; (f) a polynucleotide encoding one or more of SEQ ID NO:Y as shown in Table 1 and a trafficking domain (such as SEQ ID NO:1, or as shown in SEQ ID Nos: 4 or 5); or (g) polynucleotides with at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, 96%, 97%, 98% or 99% identity to any one of the polynucleotides of (a)-(f).

[0157] Recombinant expression vectors may be derived from micro-organisms which readily infect animals, including man, horses, cows, pigs, llamas, giraffes, dogs, cats or chickens. Preferred vectors include those which have already been used as live vaccines, such as vaccinia. These recombinants can be directly inoculated into a host, conferring immunity not only to the microbial vector, but also to express foreign antigens. Preferred vectors contemplated herein as live recombinant vaccines include RNA viruses, adenovirus, herpesviruses, poliovirus, and vaccinia and other pox viruses, as taught in Flexner, *Adv. Pharmacol.* 21: 51, 1990, for example.

[0158] Expression control sequences include, but are not limited to, promoter sequences to bind RNA polymerase, enhancer sequences or negative regulatory elements to bind to transcriptional activators and repressors, respectively, and/or translation initiation sequences for ribosome binding. For example, a bacterial expression vector can include a promoter such as the lac promoter and for transcription initiation, the Shine-Dalgarno sequence and the start codon AUG (Sambrook, et al., 1989, *supra*). Similarly, a eukaryotic expression vector preferably includes a heterologous, homologous, or chimeric

promoter for RNA polymerase II, a downstream polyadenylation signal, the start codon AUG, and a termination codon for detachment of a ribosome.

[0159] Expression control sequences may be obtained from naturally occurring genes or may be designed. Designed expression control sequences include, but are not limited to, mutated and/or chimeric expression control sequences or synthetic or cloned consensus sequences. Vectors that contain both a promoter and a cloning site into which a polynucleotide can be operatively linked are well known in the art. Such vectors are capable of transcribing RNA *in vitro* or *in vivo*, and are commercially available from sources such as Stratagene (La Jolla, Calif.) and Promega Biotech (Madison, Wis.).

[0160] In order to optimize expression and/or transcription, it may be necessary to remove, add or alter 5' and/or 3' untranslated portions of the vectors to eliminate extra, or alternative translation initiation codons or other sequences that may interfere with, or reduce, expression, either at the level of transcription or translation. Alternatively, consensus ribosome binding sites can be inserted immediately 5' of the start codon to enhance expression. A wide variety of expression control sequences--sequences that control the expression of a DNA sequence operatively linked to it--may be used in these vectors to express the DNA sequences of this invention. Such useful expression control sequences include, for example, the early or late promoters of SV40, CMV, vaccinia, polyoma, adenovirus, herpes virus and other sequences known to control the expression of genes of mammalian cells, and various combinations thereof.

[0161] In one aspect, the nucleic acid delivery vector comprises an origin of replication for replicating the vector. Preferably, the origin functions in at least one type of host cell which can be used to generate sufficient numbers of copies of the sequence for use in delivery to a target cell. Suitable origins therefore include, but are not limited to, those which function in bacterial cells (e.g., such as *Escherichia* sp., *Salmonella* sp., *Proteus* sp., *Clostridium* sp., *Klebsiella* sp., *Bacillus* sp., *Streptomyces* sp., and *Pseudomonas* sp.), yeast (e.g., such as *Saccharomyces* sp. or *Pichia* sp.), insect cells, and mammalian cells. In one preferred aspect, an origin of replication is provided which functions in the target cell into which the nucleic acid delivery vehicle is introduced (e.g., a mammalian cell, such as a human cell). In another aspect, at least two origins of replication are provided, one that functions in a host cell and one that functions in a target cell.

[0162] The nucleic acid delivery vector may alternatively, or additionally, comprise sequences to facilitate integration of at least a portion of the nucleic acid delivery vector into a target cell chromosome. For example, the nucleic acid delivery vector may comprise regions of homology to target cell chromosomal DNA. In one aspect, the delivery vector comprises two or more recombination sites which flank a nucleic acid sequence encoding the chimeric vaccine.

[0163] The vector may additionally comprise a detectable and/or selectable marker to verify that the vector has been successfully introduced in a target cell and/or can be expressed by the target cell. These markers can encode an activity, such as, but not limited to, production of RNA, peptide, or protein, or can provide a binding site for RNA, peptides, proteins, inorganic and organic compounds or compositions and the like.

[0164] Examples of detectable/selectable markers genes include, but are not limited to: DNA segments that encode products which provide resistance against otherwise toxic compounds (e.g., antibiotics); DNA segments that encode products which are otherwise lacking in the recipient cell (e.g., tRNA genes, auxotrophic markers); DNA segments that encode products which suppress the activity of a gene product; DNA segments that encode products which can be readily identified (e.g., phenotypic markers such as beta-galactosidase, a fluorescent protein (GFP, CFP, YFG, BFP, RFP, EGFP, EYFP, EBFP, dsRed, mutated, modified, or enhanced forms thereof, and the like), and cell surface proteins); DNA segments that bind products which are otherwise detrimental to cell survival and/or function; DNA segments that otherwise inhibit the activity of other nucleic acid segments (e.g., antisense oligonucleotides); DNA segments that bind products that modify a substrate (e.g., restriction endonucleases); DNA segments that can be used to isolate or identify a desired molecule (e.g., segments encoding specific protein binding sites); primer sequences; DNA segments, which when absent, directly or indirectly confer resistance or sensitivity to particular compounds; and/or DNA segments that encode products which are toxic in recipient cells.

[0165] The marker gene can be used as a marker for conformation of successful gene transfer and/or to isolate cells expressing transferred genes and/or to recover transferred genes from a cell. For example, in one aspect, the marker gene is used to isolate and purify antigen presenting cells expressing the chimeric vaccines.

[0166] As discussed above, homologs, variants, and modified forms of any of the domain sequences can be used so long as they retain the ability to function with their respective domain function. For example, a modified luminal sequence must retain the ability to traffic both membrane and non-membrane antigenic materials to an endosomal compartment with at least about 50%, at least about 60%, at least 70%, at least about 80%, at least about 90%, or at least about 100% efficacy as compared to the original domain sequence, i.e., an efficacy that results in sufficient antigen presentation by a cell comprising the chimeric sequence for it to mount an immune response. In one aspect, sequences containing a suitable trafficking signal may be identified by constructing a chimeric DNA containing the well-characterized antigenic domain of ovalbumin, a transmembrane domain, and the cytoplasmic domain of a protein containing a putative lysosomal/endosomal targeting signal. Efficiency of targeting can be measured by determining the ability of antigen presenting cells, expressing the chimeric protein, to stimulate HA epitope-specific, MHC class II restricted T-cells (see, e.g., Example 5 of U.S. Pat. No. 5,633,234).

[0167] Substantially similar genes may be provided, e.g., genes with greater than about 50%, greater than about 60%, greater than about 70%, greater than 80%, greater than about 90%, and preferably, greater than about 95% identity to a known gene. Percent identity can be determined using software programs known in the art, for example those described in Current Protocols In Molecular Biology (F. M. Ausubel et al., eds., 1987) Supplement 30, section 7.7.18, Table 7.7.1. Preferably, default parameters are used for alignment. A preferred alignment program is BLAST, using default parameters. In particular, preferred programs are BLASTN and BLASTP, using the following default parameters: Genetic code=standard; filter=none; strand=both; cutoff=60; expect=10; Matrix=BLOSUM62; Descriptions=50 sequences; sort by=HIGH SCORE; Databases=non-redundant, GenBank+EMBL+DDBJ+PDB+GenBank CDS translations+SwissProtein+SPupdate+PIR. Details of these programs can be found at the following Internet address: <http://www.ncbi.nlm.nih.gov/cgi-bin/BLAST>.

[0168] "Conservatively modified variants" of genes also can be provided. With respect to particular nucleic acid sequences, conservatively modified variants refer to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical

sequences. Specifically, degenerate codon substitutions can be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzner, et al., 1991, *Nucleic Acid Res.* 19: 5081; Ohtsuka, et al., 1985, *J. Biol. Chem.* 260: 2605-2608; Rossolini et al., 1994, *Mol. Cell. Probes* 8: 91-98).

[0169] Substantially similar domain sequences may initially be identified by selecting a sequence which specifically hybridizes to a domain sequence of interest under stringent hybridization conditions. Examples of stringent hybridization conditions include: incubation temperatures of about 25 degrees C to about 37 degrees C; hybridization buffer concentrations of about 6xSSC to about 10xSSC; formamide concentrations of about 0% to about 25%; and wash solutions of about 6xSSC. Examples of moderate hybridization conditions include: incubation temperatures of about 40 degrees C to about 50 degrees C.; buffer concentrations of about 9xSSC to about 2xSSC; formamide concentrations of about 30% to about 50%; and wash solutions of about 5xSSC to about 2xSSC. Examples of high stringency conditions include: incubation temperatures of about 55 degrees C to about 68 degrees C.; buffer concentrations of about 1xSSC to about 0.1xSSC; formamide concentrations of about 55% to about 75%; and wash solutions of about 1xSSC, 0.1xSSC, or deionized water. In general, hybridization incubation times are from 5 minutes to 24 hours, with 1, 2, or more washing steps, and wash incubation times are about 1, 2, or 15 minutes. SSC is 0.15 M NaCl and 15 mM citrate buffer. It is understood that equivalents of SSC using other buffer systems can be employed. Similarity can be verified by sequencing, but preferably, is also or alternatively, verified by function (e.g., ability to traffic to an endosomal compartment, and the like), using assays suitable for the particular domain in question.

[0170] Performing assays to determine the suitability of homologous, variant, or modified domain sequences is merely a matter of screening for sequences which express the appropriate activity. Such screening is routine in the art.

[0171] The nucleic acid delivery vector may be provided as naked nucleic acids or in a delivery vehicle associated with one or more molecules for facilitating entry of a nucleic acid into a cell. Suitable delivery vehicles include, but are not limited to: liposomal formulations, polypeptides, polysaccharides, lipopolysaccharides, viral formulations

(e.g., including viruses, viral particles, artificial viral envelopes and the like), cell delivery vehicles, and the like.

Lipid-Based Formulations

[0172] Delivery vehicles designed to facilitate intracellular delivery of biologically active molecules must interact with both non-polar and polar environments (in or on, for example, the plasma membrane, tissue fluids, compartments within the cell, and the like). Therefore, preferably, delivery vehicles are designed to contain both polar and non-polar domains or a translocating sequence for translocating a nucleic acid into a cell.

[0173] Compounds having polar and non-polar domains are termed amphiphiles. Cationic amphiphiles have polar groups that are capable of being positively charged at, or around, physiological pH for interacting with negatively charged polynucleotides such as DNA.

[0174] The nucleic acid vectors described above can be provided in formulations comprising lipid monolayers or bilayers to facilitate transfer of the vectors across a cell membrane. Liposomes or any form of lipid membrane, such as planar lipid membranes or the cell membrane of an intact cell, e.g., a red blood cell, can be used. Liposomal formulations can be administered by any means, including administration intravenously or orally.

[0175] Liposomes and liposomal formulations can be prepared according to standard methods and are well known in the art, see, e.g., Remington's; Akimaru, 1995, Cytokines Mol. Ther. 1: 197-210; Alving, 1995, Immunol. Rev. 145: 5-31; Szoka, 1980, Ann. Rev. Biophys. Bioeng. 9: 467; U.S. Pat. No. 4,235,871; U.S. Pat. No. 4,501,728; and U.S. Pat. No. 4,837,028. In one aspect, the liposome comprises a targeting molecule for targeting a liposome:nucleic acid vector complex to a particular cell type. In a particularly preferred aspect, a targeting molecule comprises a binding partner (e.g., a ligand or receptor) for a biomolecule (e.g., a receptor or ligand) on the surface of a blood vessel or a cell found in a target tissue.

[0176] Liposome charge is an important determinant in liposome clearance from the blood, with negatively charged liposomes being taken up more rapidly by the reticuloendothelial system (Juliano, 1975, Biochem. Biophys. Res. Commun. 63: 651) and thus having shorter half-lives in the bloodstream. Incorporating

phosphatidylethanolamine derivatives enhances the circulation time by preventing liposomal aggregation. For example, incorporation of N-(omega-carboxy)acylamidophosphatidylethanolamines into large unilamellar vesicles of L-alpha-distearoylphosphatidylcholine dramatically increases the in vivo liposomal circulation lifetime (see, e.g., Ahl, 1997, *Biochim. Biophys. Acta* 1329: 370-382). Liposomes with prolonged circulation half-lives are typically desirable for therapeutic and diagnostic uses. For a general discussion of pharmacokinetics, see, e.g., Remington's, Chapters 37-39, Lee, et al., In *Pharmacokinetic Analysis: A Practical Approach* (Technomic Publishing AG, Basel, Switzerland 1996).

[0177] Typically, liposomes are prepared with about 5 to 15 mole percent negatively charged phospholipids, such as phosphatidylglycerol, phosphatidylserine or phosphatidyl-inositol. Added negatively charged phospholipids, such as phosphatidylglycerol, also serve to prevent spontaneous liposome aggregation, and thus minimize the risk of undersized liposomal aggregate formation. Membrane-rigidifying agents, such as sphingomyelin or a saturated neutral phospholipid, at a concentration of at least about 50 mole percent, and 5 to 15 mole percent of monosialylganglioside can also impart desirably liposome properties, such as rigidity (see, e.g., U.S. Pat. No. 4,837,028).

[0178] Additionally, the liposome suspension can include lipid-protective agents which protect lipids against free-radical and lipid-peroxidative damages on storage. Lipophilic free-radical quenchers, such as alpha-tocopherol and water-soluble iron-specific chelators, such as ferrioxianine, are preferred.

[0179] The nucleic acid delivery vehicles of the invention can include multilamellar vesicles of heterogeneous sizes. For example, vesicle-forming lipids can be dissolved in a suitable organic solvent or solvent system and dried under vacuum or an inert gas to form a thin lipid film. If desired, the film can be redissolved in a suitable solvent, such as tertiary butanol, and then lyophilized to form a more homogeneous lipid mixture which is in a more easily hydrated powderlike form. This film is covered with an aqueous solution of the peptide or polypeptide complex and allowed to hydrate, typically over a 15 to 60 minute period with agitation. The size distribution of the resulting multilamellar vesicles can be shifted toward smaller sizes by hydrating the lipids under more vigorous agitation conditions or by adding solubilizing detergents such as deoxycholate. The

hydration medium preferably comprises the nucleic acid at a concentration which is desired in the interior volume of the liposomes in the final liposome suspension.

[0180] Following liposome preparation, the liposomes can be sized to achieve a desired size range and relatively narrow distribution of liposome sizes. One preferred size range is about 0.2 to 0.4 microns, which allows the liposome suspension to be sterilized by filtration through a conventional filter, typically a 0.22 micron filter. Filter sterilization can be carried out on a high throughput basis if the liposomes have been sized down to about 0.2 to 0.4 microns. Several techniques are available for sizing liposome to a desired size (see, e.g., U.S. Pat. No. 4,737,323).

[0181] Suitable lipids include, but are not limited to, DOTMA (Felgner, et al., 1987, Proc. Natl. Acad. Sci. USA 84: 7413-7417), DOGS or Transfectain.TM. (Behr, et al., 1989, Proc. Natl. Acad. Sci. USA 86: 6982-6986), DNERIE or DORIE (Felgner, et al., Methods 5: 67-75), DC-CHOL (Gao and Huang, 1991, BBRC 179: 280-285), DOTAPTM (McLachlan, et al., 1995, Gene Therapy 2: 674-622), Lipofectamine.RTM. and glycerolipid compounds (see, e.g., EP901463 and WO98/37916).

[0182] Other molecules suitable for complexing with nucleic acid delivery vectors include cationic molecules, such as, polyamidoamine (Haensler and Szoka, 1993, Bioconjugate Chem. 4: 372-379), dendritic polysine (WO 95/24221), polyethylene irinine or polypropylene h-nine (WO 96/02655), polylysine (U.S. Pat. No. 5,595,897; FR 2 719 316), chitosan (U.S. Pat. No. 5,744,166), DNA-gelatin coarcervates (see, e.g., U.S. Pat. No. 6,207,195; U.S. Pat. No. 6,025,337; U.S. Pat. No. 5,972,707) or DEAE dextran (Lopata, et al., 1984, Nucleic Acid Res. 12: 5707-5717).

Viral-Based Gene Delivery Vehicles

[0183] In one aspect, the nucleic acid delivery vehicle comprises a virus or viral particle. In this aspect, preferably, the nucleic acid vector comprises a viral vector. Viral vectors, such as retroviruses, adenoviruses, adeno-associated viruses and herpes viruses, are often made up of two components, a modified viral genome and a coat structure surrounding it (see, e.g., Smith et al., 1995, Ann. Rev. Microbiol. 49: 807-838), although sometimes viral vectors are introduced in naked form or coated with proteins other than viral proteins. Most current vectors have coat structures similar to a wild-type virus. This

structure packages and protects the viral nucleic acid and provides the means to bind and enter target cells.

[0184] Preferably, viral vectors are modified from wild-type viral genomes to disable the growth of the virus in a target cell while enabling the virus to grow in a host cell (e.g., such as a packaging or helper cell) used to prepare infectious particles. Vector nucleic acids generally essential cis-acting viral sequences for replication and packaging in a helper line and expression control sequences for regulating the expression of a polynucleotide being delivered to a target cell. Other viral functions are expressed in trans in specific packaging or helper cell lines as are known in the art.

[0185] Preferred vectors are viral vectors derived from a virus selected from the group consisting of herpes viruses, cytomegaloviruses, foamy viruses, lentiviruses, Semliki forrest virus, AAV (adeno-associated virus), poxviruses, adenoviruses and retroviruses. Such viral vectors are well known in the art.

[0186] In one preferred aspect, a viral vector used is an adenoviral vector. The adenoviral genome consists of a linear double-stranded DNA molecule of approximately 36 kb carrying more than about thirty genes necessary to complete the viral replication cycle. The early genes are divided into 4 regions (E1 to E4) that are essential for viral replication with the exception of the E3 region, which is believed to modulate the anti-viral host immune response. The E1 region (E1A and E1B) encodes proteins responsible for the regulation of transcription of the viral genome. Expression of the E2 region genes (E2A and E2B) leads to the synthesis of the polypeptides needed for viral replication. The proteins encoded by the E3 region prevent cytolysis by cytotoxic T cells and tumor necrosis factor (Wold and Gooding, 1991, *Virology* 184: 1-8). The proteins encoded by the E4 region are involved in DNA replication, late gene expression and splicing and host cell shut off (Halbert, et al., 1985, *J. Virol.* 56: 250-257). The late genes generally encode structural proteins contributing to the viral capsid. In addition, the adenoviral genome carries at cis-acting 5' and 3' ITRs (Inverted Terminal Repeat) and packaging sequences essential for DNA replication. The ITRs harbor origins of DNA replication while the encapsidation region is required for the packaging of adenoviral DNA into infectious particles.

[0187] Adenoviral vectors can be engineered to be conditionally replicative (CRAd vectors) in order to replicate selectively in specific cells (e.g., such as proliferative cells)

as described in Heise and Kim (2000, J. Clin. Invest. 105: 847-851). In another aspect, an adenoviral vector is replication-defective for the E1 function (e.g., by total or partial deletion or mutagenesis of E1). The adenoviral backbone of the vector may comprise additional modifications (deletions, insertions or mutations in one or more viral genes). An example of an E2 modification is illustrated by the thermosensitive mutation localized on the DBP (DNA Binding Protein) encoding gene (Ensinger et al., 1972, J. Virol. 10: 328-339). The adenoviral sequence may also be deleted of all or part of the E4 region (see, e.g., EP 974 668; Christ, et al., 2000, Human Gene Ther. 11: 415-427; Lusky, et al., 1999, J. Virol. 73: 8308-8319). Additional deletions within the non-essential E3 region may allow the size of the polynucleotide being delivered to be increased (Yeh, et al., 1997, FASEB Journal 11: 615-623). However, it may be advantageous to retain all or part of the E3 sequences coding for polypeptides (e.g., such as gp19k) allowing the virus to escape the immune system (Gooding, et al., 1990, Critical Review of Immunology 10: 53-71) or inflammatory reactions (EP 00440267.3).

[0188] Second generation vectors retaining the ITRs and packaging sequences and comprising substantial genetic modifications to abolish the residual synthesis of the viral antigens also may be used in order to improve long-term expression of the expressed gene in the transduced cells (see, e.g., WO 94/28152; Lusky, et al., 1998, J. Virol 72: 2022-2032).

[0189] The polynucleotide being introduced into the cell may be inserted in any location of the viral genome, with the exception of the cis-acting sequences. Preferably, it is inserted in replacement of a deleted region (E1, E3 and/or E4), preferably, within a deleted E1 region.

[0190] Adenoviruses can be derived from any human or animal source, in particular canine (e.g. CAV-1 or CAV-2 Genbank ref. CAVIGENOM and CAV77082, respectively), avian (Genbank ref. AAVEDSDNA), bovine (such as BAV3; Reddy, et al., 1998, J. Virol. 72: 1394-1402), murine (Genbank ref. ADRMUSMAVI), ovine, feline, porcine or simian sources or alternatively, may be a hybrid virus. Any serotype can be employed. However, the human adenoviruses of the C sub-group are preferred, especially adenoviruses 2 (Ad2) and 5 (Ad5). Such viruses are available, for example, from the ATCC.

[0191] Adenoviral particles or empty adenoviral capsids also can be used to transfer nucleic acid delivery vectors by a virus-mediated cointernalization process as

described in U.S. Pat. No. 5,928,944. This process can be accomplished in the presence of cationic agent(s) such as polycarbenes or lipid vesicles comprising one or more lipid layers.

[0192] Adenoviral particles may be prepared and propagated according to any conventional technique in the field of the art (e.g., WO 96/17070) using a complementation cell line or a helper virus, which supplies in trans the missing viral genes necessary for viral replication. The cell lines 293 (Graham et al., 1977, *J. Gen. Virol.* 36: 59-72) and PERC6 (Fallaux et al., 1998, *Human Gene Therapy* 9: 1909-1917) are commonly used to complement E1 deletions. Other cell lines have been engineered to complement defective vectors (Yeh, et al., 1996, *J. Virol.* 70: 559-565; Kroughak and Graham, 1995, *Human Gene Ther.* 6: 1575-1586; Wang, et al., 1995, *Gene Ther.* 2: 775-783; Lusky, et al., 1998, *J. Virol.* 72: 2022-203; EP 919627 and WO 97/04119). The adenoviral particles can be recovered from the culture supernatant but also from the cells after lysis and optionally further purified according to standard techniques (e.g., chromatography, ultracentrifugation, as described in WO 96/27677, WO 98/00524 WO 98/26048 and WO 00/50573).

[0193] Cell-type specific targeting may be achieved with vectors derived from adenoviruses having a broad host range by the modification of viral surface proteins. For example, the specificity of infection of adenoviruses is determined by the attachment to cellular receptors present at the surface of permissive cells. In this regard, the fiber and penton present at the surface of the adenoviral capsid play a critical role in cellular attachment (Defer, et al., 1990, *J. Virol.* 64: 3661-3673). Thus, cell targeting of adenoviruses can be carried out by genetic modification of the viral gene encoding fiber and/or penton, to generate modified fiber and/or penton capable of specific interaction with unique cell surface receptors. Examples of such modifications are described in Wickarn, et al., 1997, *J. Virol.* 71: 8221-8229; Arriberg, et al., 1997, *Virol. Chem* 268: 6866-6869; Roux, et al., 1989, *Proc. Natl. Acad. Sci. USA* 86: 9079-9083; Miller and Vile, 1995, *FASEB J.* 9: 190-199; WO 93/09221, and in WO 95/28494.

[0194] In a particularly preferred aspect, adeno-associated viral sequences are used as vectors. Vectors derived from the human parvovirus AAV-2 (adeno-associated virus type 2) are among the most promising gene delivery vehicles currently being developed. Several of the features of this system for packaging a single-stranded DNA

suggest it as a possible alternative to naked DNA for delivery of genetic vaccines. A primary attractive feature, in contrast to other viral vectors such as vaccinia or adenovirus, is that AAV vectors do not express any viral genes. The only viral DNA sequences included in the vaccine construct are the 145 bp inverted terminal repeats (ITR). Thus, as in immunization with naked DNA, the only gene expressed is that of the antigen, or antigen chimera. Additionally, AAV vectors are known to transduce both dividing and non-dividing cells, such as human peripheral blood monocyte-derived dendritic cells, with persistent transgene expression, and with the possibility of oral and intranasal delivery for generation of mucosal immunity. Moreover, the amount of DNA required appears to be much less by several orders of magnitude, with maximum responses at doses of 10^{10} to 10^{11} particles or copies of DNA in contrast to naked DNA doses of 50 ug or about 10^{15} copies.

[0195] In one aspect, AAV vectors are packaged by co-transfection of a suitable cell line (e.g., human 293 cells) with the DNA contained in the AAV ITR chimeric protein encoding constructs and an AAV helper plasmid ACG2 containing the AAV coding region (AAV rep and cap genes) without the ITRs. The cells are subsequently infected with the adenovirus Ad5. Vectors can be purified from cell lysates using methods known in the art (e.g., such as cesium chloride density gradient ultracentrifugation) and are validated to ensure that they are free of detectable replication-competent AAV or adenovirus (e.g., by a cytopathic effect bioassay). AAV titer may be determined by quantitative PCR with virus DNA samples prepared after digestion with proteinase K. Preferably, vector titers produced by such a method are approximately 5×10^{12} to 1×10^{13} DNase resistant particles per ml.

[0196] In other aspects, retroviral vectors are used. Retroviruses are a class of integrative viruses which replicate using a virus-encoded reverse transcriptase, to replicate the viral RNA genome into double stranded DNA which is integrated into chromosomal DNA of the infected cells (e.g., target cells). Such vectors include those derived from murine leukemia viruses, especially Moloney (Gilboa, et al., 1988, Adv. Exp. Med. Biol. 241: 29) or Friend's FB29 strains (WO 95/01447). Generally, a retroviral vector is deleted of all or part of the viral genes gag, pol and env and retains 5' and 3' LTRs and an encapsidation sequence. These elements may be modified to increase expression level or stability of the retroviral vector. Such modifications include the replacement of the

retroviral encapsidation sequence by one of a retrotransposon such as VL30 (see, e.g., U.S. Pat. No. 5,747,323). Preferably, the polynucleotide of interest is inserted downstream of the encapsidation sequence, preferably in opposite direction relative to the retroviral genome. Cell specific targeting may be achieved by the conjugation of antibodies or antibody fragments to the retroviral envelope protein as is known in the art.

[0197] Retroviral particles are prepared in the presence of a helper virus or in an appropriate complementation (packaging) cell line which contains integrated into its genome the retroviral genes for which the retroviral vector is defective (e.g. gag/pol and env). Such cell lines are described in the prior art (Miller and Rosman, 1989, *BioTechniques* 7: 980; Danos and Mulligan, 1988, *Proc. Natl. Acad. Sci. USA* 85: 6460; Markowitz, et al., 1988, *Virol.* 167: 400). The product of the env gene is responsible for the binding of the viral particle to the viral receptors present on the surface of the target cell and, therefore determines the host range of the retroviral particle. In the context of the invention, it is advantageous to use a packaging cell line, such as the PA317 cells (ATCC CRL 9078) or 293EI6 (WO97/35996) containing an amphotropic envelope protein, to allow infection of human and other species' target cells. The retroviral particles are preferably recovered from the culture supernatant and may optionally be further purified according to standard techniques (e.g. chromatography, ultracentrifugation).

[0198] Other suitable viruses include poxviruses. The genome of several members of poxyiridae has been mapped and sequenced. A poxyiral vector may be obtained from any member of the poxyiridae, in particular canarypox, fowlpox and vaccinia virus. Suitable vaccinia viruses include, but are not limited to, the Copenhagen strain (Goebel, et al., 1990, *Virol.* 179: 247-266; Johnson, et al., 1993, *Virol.* 196: 381-401), the Wyeth strain and the modified Ankara (MVA) strain (Antoine, et al., 1998, *Virol.* 244: 365-396). The general conditions for constructing a vaccinia virus vector are known in the art (see, e.g., EP 83 286 and EP 206 920; Mayr et al., 1975, *Infection* 3: 6-14; Sutter and Moss, 1992, *Proc. Natl. Acad. Sci. USA* 89: 10847-10851). Preferably, the polynucleotide of interest is inserted within a non-essential locus such as the nOD7coding intergenic regions or any gene for which inactivation or deletion does not significantly impair viral growth and replication.

[0199] Poxyriral particles are prepared as described in the art (Piccini, et al., 1987, *Methods of Enzymology* 153: 545-563; U.S. Pat. No. 4,769,330; U.S. Pat. No. 4,772,848;

U.S. Pat. No. 4,603,112; U.S. Pat. No. 5,100,587 and U.S. Pat. No. 5,179,993). Generally, a donor plasmid is constructed, amplified by growth in *E. coli* and isolated by conventional procedures. Then, it is introduced into a suitable cell culture (e.g. chicken embryo fibroblasts) together with a poxvirus genome, to produce, by homologous recombination, poxyviral particles. These can be recovered from the culture supernatant or from the cultured cells after a lysis step (e.g., chemical lysis, freezing/thawing, osmotic shock, sonication and the like). Consecutive rounds of plaque purification can be used to remove contaminating wild type virus. Viral particles can then be purified using the techniques known in the art (e.g., chromatographic methods or ultracentrifugation on cesium chloride or sucrose gradients).

[0200] The use of vaccinia as a live virus vaccine in the global campaign to eradicate smallpox made vaccinia an obvious choice for development as a live recombinant vaccine vector. Live recombinant vaccinia viruses expressing close to 100 different foreign proteins have been reported, and a number of these are effective experimental vaccines (reviewed by Moss and Flexner, 1987). Vaccinia is particularly versatile as an expression vector because of its large genomic size, capability of accepting at least 25,000 base pairs of foreign DNA, and its ability to infect most eukaryotic cell types, including insect cells (*ibid.*). Unlike other DNA viruses, poxviruses replicate exclusively in the cytoplasm of infected cells, reducing the possibility of genetic exchange of recombinant viral DNA with the host chromosome. Recombinant vaccinia vectors have been shown to properly process and express proteins from a variety of sources including man, other mammals, parasites, RNA and DNA viruses, bacteria and bacteriophage.

[0201] The virus is capable of infecting most mammals, making it a useful vector for studying a broad range of human and animal diseases. The expression of DNA encoding a foreign protein is controlled by host virus regulatory elements, including upstream promoter sequences and, where necessary, RNA processing signals. Insertion of foreign DNA into nonessential regions of the vaccinia virus genome has been carried out by homologous recombination (Panicali, et al., *Proc. Nat'l. Acad. Sci. USA*, 79: 4927, 1982; Mackett, et al., *Proc. Nat'l. Acad. Sci. USA*, 79: 7415, 1982).

[0202] Expression of foreign genes by the DNA may occur because of transcriptional regulatory elements at or near the site of insertion or by more precise genetic engineering. Plasmid vectors that greatly facilitate insertion and expression of

foreign genes have been constructed (Mackett, et al., J. Virol, 49: 857, 1982). These vectors contain an expression site, composed of a vaccinia transcriptional promoter and one or more unique restriction endonuclease sites for insertion of the foreign coding sequence flanked by DNA from a nonessential region of the vaccinia genome. The choice of promoter determines both the time (e.g., early or late) and level of expression, whereas the flanking DNA sequence determines the site of homologous recombination.

[0203] Only about one in a thousand virus particles produced by this procedure is a recombinant. Although recombinant virus plaques can be identified by DNA hybridization, efficient selection procedures have been developed. By using segments of nonessential vaccinia virus thymidine kinase (TK) gene as flanking sequences, the foreign gene recombines into the TK locus and by insertion inactivates the TK gene. Selection of TK virus is achieved by carrying out the virus plaque assay in TK cells in the presents of 5-bromodeoxyuridine. Phosphorylation of the nucleoside analogue and consequent lethal incorporation into viral DNA occurs only in cells infected with TK+ parental virus. Depending on the efficiency of the transfection and recombination, up to 80 of the plaques are desired recombinants, and the rest are spontaneous TK mutants.

[0204] Plasmid vectors that contain the *E. coli* beta-galactosidase gene, as well as an expression site for a second gene, permit an alternative method of distinguishing recombinant from parental virus (Chakrabarti, et al., Mol. Cell. Biol., 5: 3403, 1985). Plaques formed by such recombinants can be positively identified by the blue color that forms upon addition of an appropriate indicator. By combining both TK selection and beta-galactosidase expression, recombinant virus is readily and quickly isolated. The recombinants are then amplified by propagation in suitable cell lines and expression of the inserted gene is checked by appropriate enzymological, immunological or physical procedures.

[0205] An upper limit to the amount of genetic information that can be added to the vaccinia virus genome is not yet known. However, the addition of nearly 25,000 base pairs of foreign DNA had no apparent deleterious effect on virus yield (Smith, et al., Gene, 25:21, 1983). Were it necessary, large segments of the vaccinia virus genome could be deleted to provide additional capacity (Moss, et al., J. Virol. 40: 387, 1981).

[0206] Viral capsid molecules may include targeting moieties to facilitate targeting and/or entry into cells. Suitable targeting molecules, include, but are not limited to:

chemical conjugates, lipids, glycolipids, hormones, sugars, polymers (e.g. PEG, polylysine, PEI and the like), peptides, polypeptides (see, e.g., WO 94/40958), vitamins, antigens, lectins, antibodies and fragments thereof. Preferably, such targeting molecules recognize and bind to cell-specific markers, tissue-specific markers, cellular receptors, viral antigens, antigenic epitopes or tumor-associated markers.

[0207] A composition based on viral particles may be formulated in the form of doses of between 10 and 10^{14} i.u. (infectious units), and preferably, between 10 and 10^{11} i.u. The titer may be determined by conventional techniques. The doses of nucleic acid delivery vector are preferably comprised between 0.01 and 10 mg/kg, more especially between 0.1 and 2 mg/kg.

Cell-Based Delivery Vehicles

[0208] The nucleic acid vectors according to the invention can be delivered to target cells by means of other cells ("delivery cells") which comprise the vectors. Methods for introducing vectors into cells are known in the art and include microinjection of DNA into the nucleus of a cell (Capechi, et al., 1980, Cell 22: 479-488); transfection with CaPO_4 (Chen and Okayama, 1987, Mol. Cell Biol. 7: 2745-2752), electroporation (Chu, et al., 1987, Nucleic Acid Res. 15: 1311-1326); lipofection/liposome fusion (Feigner, et al., 1987, Proc. Natl. Acad. Sci. USA 84: 7413-7417) and particle bombardment (Yang, et al., 1990, Proc. Natl. Acad. Sci. USA 87: 9568-9572). Suitable cells include autologous and non-autologous cells, and may include xenogenic cells. Delivery cells may be induced to deliver their contents to the target cells by inducing their death (e.g., by providing inducible suicide genes to these cells).

Accessory Molecules

[0209] The compositions according to the invention may comprise one or more accessory molecules for facilitating the introduction of a nucleic acid delivery vector into a cell and/or for enhancing a particular therapeutic effect.

[0210] In addition, the composition according to the present invention may include one or more stabilizing substance(s), such as lipids, nuclease inhibitors, hydrogels, hyaluronidase (WO 98/53853), collagenase, polymers, chelating agents (EP 890362), in order to inhibit degradation within the animal/human body and/or improve

transfection/infection of the vector into a target cell. Such substances may be used alone or in combination (e.g., cationic and neutral lipids).

[0211] It has also been shown that adenovirus proteins are capable of destabilizing endosomes and enhancing the uptake of DNA into cells. The mixture of adenoviruses to solutions containing a lipid-complexed DNA vector or the binding of DNA to polylysine covalently attached to adenoviruses using protein cross-linking agents may substantially improve the uptake and expression of a nucleic acid delivery vector (see, e.g., Curiel, et al., 1992, Am. J. Respir. Cell. Mol. Biol. 6: 247-252).

Host Cells

[0212] Nucleic acid vectors according to the invention can be expressed in a variety of host cell, including, but not limited to: prokaryotic cells (e.g., *E. coli*, *Staphylococcus* sp., *Bacillus* sp.); yeast cells (e.g., *Saccharomyces* sp.); insect cells; nematode cells; plant cells; amphibian cells (e.g., *Xenopus*); avian cells; and mammalian cells (e.g., human cells, mouse cells, mammalian cell lines, primary cultured mammalian cells, such as from dissected tissues).

[0213] The molecules can be expressed in host cells isolated from an organism, host cells which are part of an organism, or host cells which are introduced into an organism. In one aspect, fusion molecules are expressed in host cells *in vitro*, e.g., in culture. In another aspect, fusion molecules are expressed in a transgenic organism (e.g., a transgenic mouse, rat, rabbit, pig, primate, etc.) that comprises somatic and/or germline cells comprising nucleic acids encoding the fusion molecules. Methods for constructing transgenic animals are well known in the art and are routine.

[0214] Nucleic acid vectors also can be introduced into cells *in vitro*, and the cells (e.g., such as stem cells, hematopoietic cells, lymphocytes, and the like) can be introduced into the host organism. The cells may be heterologous or autologous with respect to the host organism. For example, cells can be obtained from the host organism, nucleic acid vectors introduced into the cells *in vitro*, and then reintroduced into the host organism.

Antigen Presenting Cells

[0215] In a preferred aspect of the invention, a nucleic acid delivery vehicle such as described above is introduced into a natural or engineered antigen presenting cell.

[0216] The term "antigen presenting cell" (APC) as used herein includes any cell which presents on its surface an antigen in association with a major histocompatibility complex molecule, preferably a class II molecule, or portion thereof. Examples of suitable APCs are discussed in detail below and include, but are not limited to, whole cells such as macrophages, dendritic cells, B cells, hybrid APCs, and foster antigen presenting cells. Methods of making hybrid APCs are described and known in the art.

[0217] Dendritic cells (DCs) are potent antigen-presenting cells. It has been shown that DCs provide all the signals required for T cell activation and proliferation. These signals can be categorized into two types. The first type, which gives specificity to the immune response, is mediated through interaction between the T-cell receptor/CD3 ("TCR/CD3") complex and an antigenic peptide presented by a major histocompatibility complex ("MHC" defined above) class I or II protein on the surface of APCs. This interaction is necessary, but not sufficient, for T cell activation to occur. In fact, without the second type of signals, the first type of signals can result in T cell anergy. The second type of signals, called co-stimulatory signals, is neither antigen-specific nor MHC-restricted, and can lead to a full proliferation response of T cells and induction of T cell effector functions in the presence of the first type of signals.

[0218] Several molecules have been shown to enhance co-stimulatory activity. These include, but are not limited to, heat stable antigen (HSA), chondroitin sulfate-modified MHC invariant chain (Ii-CS), intracellular adhesion molecule I (ICAM-1), and B7 co-stimulatory molecule on the surface of APCs and its counter-receptor CD28 or CTLA-4 on T cells.

[0219] Other important co-stimulatory molecules are CD40, CD54, CD80, CD86. As used herein, the term "co-stimulatory molecule" encompasses any single molecule or combination of molecules which, when acting together with a peptide/MHC complex bound by a TCR on the surface of a T cell, provides a co-stimulatory effect which achieves activation of the T cell that binds the peptide. The term thus encompasses B7, or other co-stimulatory molecule(s) on an APC, fragments thereof (alone, complexed with another molecule(s), or as part of a fusion protein) which, together with peptide/MHC complex, binds to a cognate ligand and results in activation of the T cell when the TCR on the surface of the T cell specifically binds the peptide. Co-stimulatory molecules are

commercially available from a variety of sources, including, for example, Beckman Coulter.

[0220] In one aspect of the invention, the method described in Romani et al., *J. Immunol. Methods* 196: 135-151, 1996, and Bender et al, *J. Immunol. Methods* 196: 121-135, 1996, are used to generate both immature and mature dendritic cells from the peripheral blood mononuclear cells (PBMCs) of a mammal, such as a murine, simian or human. Briefly, isolated PBMCs are pre-treated to deplete T- and B-cells by means of an immunomagnetic technique. Lymphocyte-depleted PBMC are then cultured for in RPMI medium 9 e.g., about 7 days), supplemented with human plasma (preferably autologous plasma) and GM-CSF/IL-4, to generate dendritic cells. Dendritic cells are nonadherent when compared to their monocyte progenitors. Thus, on approximately day 7, non-adherent cells are harvested for further processing.

[0221] The dendritic cells derived from PBMC in the presence of GM-CSF and IL-4 are immature, in that they can lose their nonadherence property and revert back to macrophage cell fate if the cytokine stimuli are removed from the culture. The dendritic cells in an immature state are very effective in processing native protein antigens for the MHC class II restricted pathway (Romani, et al., *J. Exp. Med.* 169:1169, 1989). Further maturation of cultured dendritic cells is accomplished by culturing for 3 days in a macrophage-conditioned medium (CM), which contains the necessary maturation factors. Mature dendritic cells are less able to capture new proteins for presentation but are much better at stimulating resting T cells (both CD4 and CD8) to grow and differentiate.

[0222] Mature dendritic cells can be identified by their change in morphology, such as by the formation of more motile cytoplasmic processes; by their nonadherence; by the presence of at least one of the following markers: CD83, CD68, HLA-DR or CD86; or by the loss of Fc receptors such as CD 115 (reviewed in Steinman, *Annu. Rev. Immunol.* 9: 271, 1991). Mature dendritic cells can be collected and analyzed using typical cytofluorography and cell sorting techniques and devices, such as FACScan and FACStar. Primary antibodies used for flow cytometry are those specific to cell surface antigens of mature dendritic cells and are commercially available. Secondary antibodies can be biotinylated Igs followed by FITC- or PE-conjugated streptavidin.

[0223] Alternatively, others have reported that a method for upregulating (activating) dendritic cells and converting monocytes to an activated dendritic cell phenotype. This method involves the addition of calcium ionophore to the culture media convert monocytes into activated dendritic cells. Adding the calcium ionophore A23187, for example, at the beginning of a 24-48 hour culture period resulted in uniform activation and dendritic cell phenotypic conversion of the pooled "monocyte plus DC" fractions: characteristically, the activated population becomes uniformly CD 14 (Leu M3) negative, and upregulates HLA-DR, HLA-DQ, ICAM-1, 137.1, and 137.2. Furthermore, this activated bulk population functions as well on a small numbers basis as a further purified. Specific combination(s) of cytokines have been used successfully to amplify (or partially substitute) for the activation/conversion achieved with calcium ionophore: these cytokines include but are not limited to G-CSF, GM-CSF, IL-2, and IL-4. Each cytokine when given alone is inadequate for optimal upregulation.

[0224] The second approach for isolating APCs is to collect the relatively large numbers of precommitted APCs already circulating in the blood. Previous techniques for isolating committed APCs from human peripheral blood have involved combinations of physical procedures such as metrizamide gradients and adherence/nonadherence steps (Freudenthal et al. PNAS 87: 7698-7702, 1990); Percoll gradient separations (Mehta-Damani, et al., J. Immunol. 153: 996-1003, 1994); and fluorescence activated cell sorting techniques (Thomas et al., J. Immunol. 151: 6840-52, 1993).

[0225] It should be obvious to those of skill in the art that there are many methods routine in the art for isolating professional antigen presenting cells (or their precursors) and that such methods and others which may be developed are not limiting and are encompassed within the scope of the invention.

[0226] In one embodiment, the APCs and therefore the cells presenting one or more antigens are autologous. In another embodiment, the APCs presenting the antigen are allogeneic, i.e., derived from a different subject.

[0227] As discussed above, nucleic acids encoding chimeric molecules can be introduced into APCs using the methods described above or others known in the art, including, but not limited to, transfection, electroporation, fusion, microinjection, viral-based delivery, or cell based delivery. Arthur et al., Cancer Gene Therapy 4(1): 17-25, 1997, reports a comparison of gene transfer methods in human dendritic cells.

[0228] Known, partial and putative human leukocyte antigen (HLA), the genetic designation for the human MHC, amino acid and nucleotide sequences, including the consensus sequence, are published (see, e.g., Zemmour and Parham, *Immunogenetics* 33: 310-320, 1991), and cell lines expressing HLA variants are known and generally available as well, many from the American Type Culture Collection ("ATCC"). Therefore, using PCR, MHC class II-encoding nucleotide sequences are readily operatively linked to an expression vector of this invention that is then used to transform an appropriate cell for expression therein.

[0229] Professional APCs can be used, such as macrophages, B cells, monocytes, dendritic cells, and Langerhans cells. These are collected from the blood or tissue of 1) an autologous donor; 2) a heterologous donor having a different HLA specificity than the host to be treated; or 3) from a xenogeneic donor of a different species using standard procedures (Coligan, et. al., *Current Protocols in Immunology*, sections 3 and 14, 1994). The cells may be isolated from a normal host or a patient having an infectious disease, cancer, autoimmune disease, or allergy.

[0230] Professional APCs may be obtained from the peripheral blood using leukopheresis and "FICOLL/HYPAQUE" density gradient centrifugation (stepwise centrifugation through Ficoll and discontinuous Percoll density gradients). Procedures are utilized which avoid the exposure of the APCs to antigens which could be internalized by the APCs, leading to activation of T cells not specific for the antigens of interest.

[0231] Cells which are not naturally antigen presenting can be engineered to be antigen presenting by introducing sequences encoding appropriate molecules. For example, nucleic acid sequences encoding MHC class II molecules, accessory molecules, co-stimulatory molecules and antigen processing assisting molecules can be introduced after direct synthesis, cloning, purification of DNA from cells containing such genes, and the like. One expedient means to obtain genes for encoding the molecules used in the compositions and methods described herein is by polymerase chain reaction (PCR) amplification on selected nucleic acid templates with selected oligonucleotide primer pairs. For example, epithelial cells, endothelial cells, tumor cells, fibroblasts, activated T cells, eosinophils, keratinocytes, astrocytes, microglial cells, thymic cortical epithelial cells, Schwann cells, retinal pigment epithelial cells, myoblasts, vascular smooth muscle cells, chondrocytes, enterocytes, thyrocytes and kidney tubule cells can be used. These

may be primary cells recently explanted from a host and not extensively passaged in cell culture to form an established cell line, or established cell lines that are relatively homogeneous and capable of proliferating for many generations or indefinitely.

[0232] Cells that are not professional APCs are isolated from any tissue of an autologous donor; a heterologous donor or a xenogeneic donor, where they reside using a variety of known separation methods (Darling, *Animal Cells: Culture and Media*. J. Wiley, New York, 1994; Freshney, *Culture of Animal Cells*. Alan R. Liss, Inc., New York, 1987). Non-autologous cells, e.g., heterologous or xenogeneic cells, can be engineered *ex vivo* to express HLA class I and class II molecules that match known human HLA specificities. These cells can then be introduced into a human subject matching the HLA specificity of the engineered cells. The cells are further engineered *ex vivo* to express one or more chimeric vaccines according to the invention.

[0233] The engineered cells are maintained in cell culture by standard cell culture methods (Darling, *Animal Cells: Culture and Media*". J. Wiley, New York, 1994; Freshney, *Culture of Animal Cells*". Alan R. Liss, Inc., New York, 1987). Cell lines for use in the present invention are obtained from a variety of sources (e.g., ATCC Catalogue of Cell Lines & Hybridomas, American Type Culture Collection, 8th edition, 1995), or are produced using standard methods (Freshney, *Culture of Immortalized Cells*, Wiley-Liss, New York, 1996). Non-transformed cell lines are preferred for use in human subjects.

[0234] In one aspect, CD34+ precursors that are differentiating under the influence of GM-CSF into dendritic cells are obtained from the body of a subject and nucleic acids encoding chimeric vaccines according to the invention are introduced into the cells, which are then re-injected into the subject. Utilizing the construct containing antigenic sequences linked to an endosomal/lysosomal targeting signal (and preferably comprising a LAMP-like luminal polypeptide) will enhance the association of peptides derived from a particular antigen with MHC class II molecules on the transduced antigen presenting cells, resulting in significantly more potent systemic T cell dependent immune responses. While the antigen presenting cells transfected in this strategy are preferably autologous cells, any MHC class II cells that effectively present antigen in the host may be used as described above.

Peptide Vaccines

[0235] Also within the scope of this invention are vaccines containing cell-free peptide immunogens, where the immunogen contains at least one Allergen X fused to sequences of a lysosomal membrane polypeptide (e.g., such as a LAMP polypeptide or a homolog, ortholog, variant, or modified version thereof) or sequences of an endocytic receptor for targeting and trafficking Allergen X to an endosomal/lysosomal compartment or lysosome-related organelle for binding to an MHC class II molecule or for delivery to another compartment/organelle for binding to an MHC class II molecule. Preferably, Allergen X is processed within the compartment/organelle (or subsequent compartment/organelle to which it is delivered) to generate an Allergen X epitope bound to an MHC class II molecule capable of modulating an immune response.

[0236] The chimeric vaccine may also comprise a transmembrane region and/or cytoplasmic tail with lysosomal targeting region (preferably from a LAMP polypeptide), and/or di-leucine domain, Tyr motif, MR6 domain, proline rich domain, and/or Ser-Val-Val domain. The chimeric vaccine also may be bound in a membranous structure to facilitate its administration to the body of an organism. For example, the chimeric vaccine may be incorporated into liposomes, as described in U.S. Pat. No. 4,448,765.

[0237] In preferred embodiments, a peptide vaccine of the present invention comprises: (a) a polypeptide encoded by any one of the polynucleotides of SEQ ID NO:Z or SEQ ID NO:W as shown in Table 1; (b) a polypeptide comprising the amino acid sequence of SEQ ID NO: Y or SEQ ID NO:V as shown in Table 1; (c) a polypeptide comprising a luminal domain (such as, for example, SEQ ID NOs:2 or 3 or as shown in SEQ ID Nos: 4 or 5), one or more of SEQ ID NO:Y as shown in Table 1, and a trafficking domain (such as SEQ ID NO:1, or as shown in SEQ ID Nos: 4 or 5); (d) a polypeptide comprising a luminal domain (such as, for example, SEQ ID NOs:2 or 3 or as shown in SEQ ID Nos: 4 or 5) and one or more of SEQ ID NO:Y as shown in Table 1; (e) a polypeptide comprising one or more of SEQ ID NO:Y as shown in Table 1 and a trafficking domain (such as SEQ ID NO:1, or as shown in SEQ ID Nos: 4 or 5); or (f) polypeptides with at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, 96%, 97%, 98% or 99% identity to any one of the polynucleotides of (a)-(e).

[0238] When a protein or polypeptide is to be used as an immunogen, it may be produced by expression of any one or more of the DNA constructs described above in a

recombinant cell or it may be prepared by chemical synthesis. For example, the Merrifield technique (Journal of American Chemical Society, vol. 85, pp. 2149-2154, 1968), can be used.

[0239] Priming with the LAMP Constructs as polynucleotides, the encoded proteins of the LAMP Constructs, and/or cells (such as antigen presenting cells which express the LAMP Constructs described herein) followed by boosting with Allergen X is a preferred embodiment of the invention. In further preferred embodiments, priming with a LAMP Construct as described herein followed by boosting with an allergen, and preferably an allergen derived from the protein by which Allergen X is derived, is specifically contemplated and can be used to generate an even more robust immune response, especially in view of antibody repertoire diversity and titer.

Administration

[0240] Vaccine material according to this invention may contain the immune stimulatory constructs described above or may be recombinant microorganisms, or antigen presenting cells which express the immune stimulatory constructs. Preparation of compositions containing vaccine material according to this invention and administration of such compositions for immunization of individuals are accomplished according to principles of immunization that are well known to those skilled in the art.

[0241] Large quantities of these materials may be obtained by culturing recombinant or transformed cells containing replicons that express the chimeric Allergen X proteins described herein. Culturing methods are well-known to those skilled in the art and are taught in one or more of the documents cited above. The vaccine material is generally produced by culture of recombinant or transformed cells and formulated in a pharmacologically acceptable solution or suspension, which is usually a physiologically-compatible aqueous solution, or in coated tablets, tablets, capsules, suppositories or ampules, as described in the art, for example in U.S. Pat. No. 4,446,128, incorporated herein by reference. Administration may be any suitable route, including oral, rectal, intranasal or by injection where injection may be, for example, transdermal, subcutaneous, intramuscular or intravenous.

[0242] The vaccine composition is administered to a mammal in an amount sufficient to induce an immune response in the mammal. A minimum preferred amount

for administration is the amount required to elicit antibody formation to a concentration at least 4 times that which existed prior to administration. A typical initial dose for administration would be 10-5000 micrograms when administered intravenously, intramuscularly or subcutaneously, or 10^5 to 10^{11} plaque forming units of a recombinant vector, although this amount may be adjusted by a clinician doing the administration as commonly occurs in the administration of vaccines and other agents which induce immune responses. A single administration may usually be sufficient to induce immunity, but multiple administrations may be carried out to assure or boost the response (such as with a recombinantly produced derived from the same protein from which Allergen X is derived).

[0243] Vaccines may be tested initially in a non-human mammal (e.g., a mouse or primate). For example, assays of the immune responses of inoculated mice can be used to demonstrate greater antibody, T cell proliferation, and cytotoxic T cell responses to the lysosome-targeted chimeric proteins than to wild type antigen. Chimeric proteins can be evaluated in Rhesus monkeys to determine whether a DNA vaccine formulation that is highly effective in mice will also elicit an appropriate monkey immune response. In one aspect, each monkey receives a total of 5 mg DNA per immunization, delivered IM and divided between 2 sites, with immunizations at day 0 and at weeks 4, 8, and 20, with additional doses optional. Antibody responses, ADCC, CD4+ and CD8+ T-cell cytokine production, CD4+ and CD8+ T-cell antigen-specific cytokine staining can be measured to monitor immune responses to the vaccine.

[0244] Further description of suitable methods of formulation and administration according to this invention may be found in U.S. Pat. No. 4,454,116 (constructs), U.S. Pat. No. 4,681,762 (recombinant bacteria), and U.S. Pat. Nos. 4,592,002 and 4,920,209 (recombinant viruses).

Treatment of Allergies

[0245] This invention provides a formulation useful for the treatment of pollinosis correlated with Allergen X. It has previously been determined that delivering a DNA plasmid encoding the protein coding sequence of an allergen to an animal can increase IFN-gamma production and lower IL-4 production, which is useful in treating animals allergic to the specific allergen. The present invention provides an improved vaccine

composition for treating patients with an allergy correlated to Allergen X. The fusion protein of the invention has a specific intracellular trafficking pattern that intersects with MHC class II vesicles, and results in enhanced presentation of Allergen X to the immune system, specifically resulting in an enhanced antibody response. Nucleic acids and compositions provided by the present invention are useful for conducting allergy immunotherapy.

[0246] The present invention provides a formulation that when administered to a cell results in an increased specific antibody response. The increased antibody response to Allergen X is useful for treating an IgE-mediated allergic disease. IgE has certain properties related to its cellular restriction and the resulting intracellular signaling upon binding cognate allergen. IgE is generated against an allergen when B cells receive IL-4 secreted by Th2 cells. This helps instruct B cells to produce IgE class antibodies. Upon secretion by B cells, IgE binds to Fc-εRI, its high affinity receptor expressed by mast cells and eosinophils, resulting in these cells and the animal becoming sensitized to future allergen exposure. Consequently, the symptoms of allergy can be triggered upon the ingestion, inhalation, or mucosal contact with an allergen. Due to the binding properties of antibodies, it has been proposed that one way of reducing allergy symptoms is to chelate free allergen available for binding by IgE through competition with other antibody classes. In particular, an allergy formulation that increases IgG has been proposed to be a pathway for reducing allergic disease. The invention described herein induces enhanced IgG production, thus causing a decrease in the ratio of IgE to IgG in a clinically significant manner.

[0247] In a particularly preferred embodiment, the invention provides a method of treatment or prevention of an allergy to Allergen X. In one preferred method, polynucleotides encoding Allergen X described herein and a luminal trafficking domain of a lysosome associated membrane polypeptide (e.g., LAMP polypeptide, homolog, ortholog, variant, or modified form thereof) or the luminal trafficking domain of an endocytic receptor, and a cytoplasmic targeting domain of a LAMP polypeptide, homolog, ortholog, variant, or modified form thereof, or of an endocytic receptor for targeting the Allergen X antigen to an endosomal/lysosomal compartment or lysosome-related organelle and for association with an MHC class II molecule either within the compartment/organelle or within another compartment/organelle to which the antigen

is delivered. Such chimeric DNA molecules can encode additional domain sequences as described above (e.g., sequences encoding transmembrane domains, signal sequences, cytoplasmic domains for targeting to an endosomal/lysosomal compartment or lysosome-related organelles, di-leucine domains, Tyr motif domains, proline rich domains, Ser-Val-Val domains, and the like).

[0248] More generally, in one embodiment, this invention provides a vaccine composition for modulating an immune response in a mammal to Allergen X (i.e., stimulating, enhancing, or reducing such a response). Preferably, the composition comprises a vaccine vector, wherein the vector contains a chimeric DNA segment which encodes a protein containing Allergen X and a luminal trafficking domain of a lysosome associated membrane polypeptide (e.g., LAMP polypeptide, homolog, ortholog, variant, or modified form thereof) or the luminal trafficking domain of an endocytic receptor, and a targeting domain, such as the cytoplasmic targeting domain of a LAMP polypeptide, homolog, ortholog, variant, or modified form thereof, or of an endocytic receptor, for targeting the antigen to an endosomal/lysosomal compartment or lysosome-related organelle and for association with an MHC class II molecule either within the compartment/organelle or within another compartment/organelle to which the antigen is delivered. Such chimeric DNA molecules can encode additional domain sequences as described above (e.g., sequences encoding transmembrane domains, signal sequences, cytoplasmic domains for targeting to an endosomal/lysosomal compartment or lysosome-related organelles, di-leucine domains, Tyr motif domains, proline rich domains, Ser-Val-Val domains, and the like).

Kits

[0249] The invention further comprises kits to facilitate performing the methods described herein. In one aspect, a kit comprises a nucleic acid vector as described above and a cell for receiving the vector. The kit may additionally comprise one or more nucleic acids for engineering the cell into a professional APC. In one aspect, however, the cell is a professional APC. The cell may or may not express co-stimulatory molecules. In a preferred aspect, when the cell does not express co-stimulatory molecules, the antigen encoded by the vector is an autoantigen. In another aspect, a panel of cells is provided expressing different MHC molecules (e.g., known to be expressed in human beings). In a

further aspect, the kit comprises reagents to facilitate entry of the vectors into a cell (e.g., lipid-based formulations, viral packaging materials, cells, and the like). In still a further aspect, one or more T cell lines specific for the antigen encoded by the vector is provided, to verify the ability of the vector to elicit, modulate, or enhance an immune response.

EXAMPLES

[0250] The invention will now be further illustrated with reference to the following examples. It will be appreciated that what follows is by way of example only and that modifications to detail may be made while still falling within the scope of the invention.

Example 1 – Construction of Allergen X Constructs

[0251] A number of different nucleic acid constructs were constructed encoding chimeric Allergen X vaccines as described in Table 1. Plasmids comprising the polynucleotides of SEQ ID NO:Z were designed using techniques well known in the art. Specifically, a plasmids comprising polynucleotides encoding gene fusions comprising a portion of the LAMP-1 luminal domain (SEQ ID NO:2) to Allergen X (SEQ ID NO:Y) and then to a portion of the LAMP-1 Transmembrane/Cytoplasmic Tail (SEQ ID NO:1) were inserted into the NTC 8382 vector. Representative examples of such constructed polynucleotides are provided as SEQ ID NO:W. The resulting constructs were sequence confirmed and protein expression was confirmed.

Example 2 - Evaluation of the Immune Response of Mice to Plasmids Encoding Allergen X

[0252] Allergen X antigen constructs as described in Example 1 were tested for their ability to modulate an immune response using the assay described in Su, Yan, Michael Connolly, Anthony Marketon, and Teri Heiland. "CryJ-LAMP DNA Vaccines for Japanese Red Cedar Allergy Induce Robust Th1-Type Immune Responses in Murine Model." *Journal of Immunology Research* 2016 (2016): 1-15.

[0253] Results for each construct is are reported in Table 3. Unless otherwise indicated, the "control" construct is the vector backbone lacking allergen sequence.

Table 3

Active	Weakly/Non-Active
Cor a 1 (SEQ ID NO:7) IgG1: Vaccinated group: 4×10^5 endpoint titer IgG2a: Vaccinated group: 3.5×10^6 endpoint titer	IgG1: Control group: 2.1×10^4 endpoint titer IgG2a: Control group: Not detected
After removal of amino acids 319-480aa of full length Cor a 9 to generate SEQ ID NO:9: IgG1 Vaccinated group: 5.7×10^5 endpoint titer Control group: Not detected IgG2a Vaccinated group: 6.2×10^5 endpoint titer Control group: Not detected Removal specifically enhanced the IgG2a:IgG1 ratio	Before Removal (full length Cor a 9 sequence that includes amino acids 319-480aa) IgG1 Vaccinated group: 1.2×10^6 endpoint titer Control group: 2.7×10^4 endpoint titer IgG2a Vaccinated group: 7.5×10^5 endpoint titer Control group: Not detected
Cor a 1 – Gly4 – Cor a 9 (SEQ ID NO:11) IgG1: Vaccinated group: 1×10^5 endpoint titer IgG2a: Vaccinated group: 3.3×10^5 endpoint titer	IgG1: Control group: 2.2×10^3 endpoint titer IgG2a: Control group: 2.7×10^3 endpoint titer

Removal of aa 368-551 of Pru du 6 sequence to generate SEQ ID NO:13: After removal: IgG1 Vaccinated group: 1.6x10⁵ endpoint titer Control group: 1.3x10⁴ endpoint titer IgG2a Vaccinated group: 5.5x10⁵ endpoint titer Control group: Not detected Removal of the basic chain (368-551 aa) improved the antibody responses and specifically enhanced the IgG2a:IgG1 ratio	Full length Pru du 6 sequence with aa 368-551. Before removal: IgG1 Vaccinated group: 9.9x10⁵ endpoint titer Control group: 1.3x10⁴ endpoint titer IgG2a Vaccinated group: 2.8x10⁵ endpoint titer Control group: Not detected
Ana o 1 (SEQ ID NO:15) IgG1: Vaccinated group: 1.2x10⁶ endpoint titer IgG2a: Vaccinated group: 6x10⁶ endpoint titer	IgG1: Control group: 4.9x10⁴ endpoint titer IgG2a: Control group: Not detected
Ana o 2 (SEQ ID NO:17) IgG1: Vaccinated group: 8.1x10⁵ endpoint titer IgG2a: Vaccinated group: 4x10⁶ endpoint titer	IgG1: Control group: 1.6x10⁴ endpoint titer IgG2a: Control group: Not detected
Ana o 3 (SEQ ID NO:19) IgG1: Vaccinated group: 2x10⁵ endpoint titer IgG2a: Vaccinated group: 1.4x10⁶ endpoint titer	IgG1: Control group: 5.4x10³ endpoint titer IgG2a: Control group: Not detected
Ana o 2 -Gly4- Ana o 1 - Gly4- Ana o 3 (SEQ ID NO:21) IgG1: Vaccinated group: 5.5x10⁵ endpoint titer IgG2a: Vaccinated group: 3.4x10⁶ endpoint titer	IgG1: Control group: 3.7x10⁴ endpoint titer IgG2a: Control group: Not detected
Jug n 1 (SEQ ID NO:23) IgG1: Vaccinated group: 6x10⁵ endpoint titer IgG2a: Vaccinated group: 1.7x10⁶ endpoint titer	IgG1: Control group: 7x10⁴ endpoint titer IgG2a: Control group: 1x10³ endpoint titer

Jug r 2 (SEQ ID NO:25) IgG1: Vaccinated group: 9.9x10 ⁵ endpoint titer IgG2a: Vaccinated group: 4.2x10 ⁶ endpoint titer	IgG1: Control group: 7x10 ⁴ endpoint titer IgG2a: Control group: 2x10 ³ endpoint titer
Jug n 1 – Gly4 – Jug r 2 (SEQ ID NO:27) IgG1: Vaccinated group: 2x10 ⁵ endpoint titer IgG2a: Vaccinated group: 1.7x10 ⁵ endpoint titer	IgG1: Control group: 1x10 ⁴ endpoint titer IgG2a: Control group: Not detected
Amb a 1 (SEQ ID NO:29) IgG1: Vaccinated group: 4x10 ⁵ endpoint titer IgG2a: Vaccinated group: 3.5x10 ⁶ endpoint titer	IgG1: Control group: 2.1x10 ⁴ endpoint titer IgG2a: Control group: Not detected
Bet v 1-A (SEQ ID NO:33) IgG1: Vaccinated group: 4x10 ⁵ endpoint titer IgG2a: Vaccinated group: 2x10 ⁶ endpoint titer	IgG1: Control group: 5x10 ³ endpoint titer IgG2a: Control group: 5 x 10 ³ endpoint titer
Can f 1 (SEQ ID NO:37) IgG1: Vaccinated group: 4x10 ⁵ endpoint titer IgG2a: Vaccinated group: 2x10 ⁶ endpoint titer	IgG1: Control group: 1.8x10 ⁴ endpoint titer IgG2a: Control group: 5 x 10 ³ endpoint titer
Cyn d 1 (SEQ ID NO:41) IgG1: Vaccinated group: 5x10 ⁵ endpoint titer IgG2a: Vaccinated group: 6x10 ⁶ endpoint titer	IgG1: Control group: 4x10 ⁴ endpoint titer IgG2a: Control group: 5 x 10 ³ endpoint titer
Der F 1 (19-321) (SEQ ID NO:45) IgG1: Vaccinated group: 1.5x10 ⁵ endpoint titer IgG2a: Vaccinated group: 2x10 ⁶ endpoint titer	IgG1: Vaccinated group with Der F 1 (99-321) (SEQ ID NO:49): 5x10 ⁴ endpoint titer Control group: Not detected IgG2a Vaccinated group with Der F 1 (99-321) (SEQ ID NO:49): 1x10 ⁶ endpoint titer Control group: Not detected

Der P2 (SEQ ID NO:53) IgG1: Vaccinated group: 1×10^5 endpoint titer IgG2a: Vaccinated group : 1×10^6 endpoint titer	IgG1: Control group: 8×10^3 endpoint titer IgG2a: Control group: Not detectable
DerF2 (SEQ ID NO:57) IgG1: Vaccinated group: 6×10^5 endpoint titer IgG2a: Vaccinated group : 1.2×10^6 endpoint titer	IgG1: Control group: Not detectable IgG2a: Control group: Not detectable
After deletion of amino acids 27-50 of DerP1 sequence (SEQ ID NO:61): IgG1 Vaccinated group: 3.5×10^3 endpoint titer Control group: Not detectable IgG2a Vaccinated group : 4×10^5 endpoint titer Control group: Not detectable Removal sequence improved the antibody responses and specifically enhanced the IgG2a:IgG1 ratio	Before deletion of amino acids 27-50 of DerP1 sequence: IgG1 Vaccinated group: 2.3×10^5 endpoint titer Control group: 1.1×10^4 IgG2a Vaccinated group : 9.3×10^4 endpoint titer Control group: Not detectable
Fel D 1 (SEQ ID NO:66) IgG1: Vaccinated group: 4×10^5 endpoint titer IgG2a: Vaccinated group : 2×10^6 endpoint titer	IgG1: Control group: 1.4×10^3 endpoint titer IgG2a: Control group: Not detectable
Fel d 4 (SEQ ID NO:74) IgG1: Vaccinated group: 2.5×10^4 endpoint titer IgG2a: Vaccinated group: 7×10^4 endpoint titer	IgG1: Control group: Not detectable IgG2a: Control group: Not detectable
Lit v 1 (SEQ ID NO:78) IgG1: Vaccinated group: 6×10^5 endpoint titer IgG2a: Vaccinated group : 1.1×10^6 endpoint titer	IgG1: Control group: 4.2×10^4 endpoint titer IgG2a: Control group: 1.9×10^4 endpoint titer

Lol p 5a (SEQ ID NO:82) IgG1: Vaccinated group: 1x10 ⁶ endpoint titer IgG2a: Vaccinated group: 1.5x10 ⁶ endpoint titer	IgG1: Control group: 1.8x10 ⁵ endpoint titer IgG2a: Control group: Not detected
Phl p 1 (SEQ ID NO:86) IgG1: Vaccinated group: 5x10 ⁵ endpoint titer IgG2a: Vaccinated group: 1.5x10 ⁶ endpoint titer	IgG1: Control group: No detected IgG2a: Control group: Not detected
Phl p 5 (SEQ ID NO:90) IgG1: Vaccinated group: 1x10 ⁶ endpoint titer IgG2a: Vaccinated group: 3x10 ⁶ endpoint titer	IgG1: Control group: 5x10 ⁴ endpoint titer IgG2a: Control group: Not detected
Der f 15 (SEQ ID NO:93) IgG1: Vaccinated group: 1.2x10 ⁶ endpoint titer IgG2a: Vaccinated group: 4.4x10 ⁶ endpoint titer	IgG1: Control group: 1x10 ⁵ endpoint titer IgG2a: Control group: 2.7x10 ³ endpoint titer
Der f 18 (SEQ ID NO:94) IgG1: Vaccinated group: 1.2x10 ⁶ endpoint titer IgG2a: Vaccinated group: 4.4x10 ⁶ endpoint titer	IgG1: Control group: 1x10 ⁵ endpoint titer IgG2a: Control group: 2.7x10 ³ endpoint titer
Cte f 1 (SEQ ID NO:96) IgG1: Vaccinated group: 1.3x10 ⁶ endpoint titer IgG2a: Vaccinated group: 2.9x10 ⁶ endpoint titer	IgG1: Control group: 1x10 ⁶ endpoint titer IgG2a: Control group: 2.3x10 ⁵ endpoint titer
Der F1-Der F2 (SEQ ID NO:110) F1 IgG1: Vaccinated group: 1.8x10 ⁵ endpoint titer IgG2: Vaccinated group: 1.0x10 ⁶ endpoint titer F2 IgG1: Vaccinated group: 9.4 x10 ³ endpoint titer IgG2: Vaccinated group: 6.0x10 ⁵ endpoint titer	

Der F15 – Der F18 (SEQ ID NO:109) F15 IgG1: Vaccinated group: 3.8×10^4 endpoint titer IgG2: Vaccinated group: 4.0×10^5 endpoint titer F18 IgG1: Vaccinated group: 1.2×10^5 endpoint titer IgG2: Vaccinated group: 1.1×10^6 endpoint titer	
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[0254] Six to eight week old female Balb/c mice were treated with either control vector or vaccines and immunized by Bioject intradermal delivery at days 0, 7, and 14. Mice were boosted with recombinant allergen (5µg/mouse) in the presence of Alum adjuvant on day 42 and bled on day 21, 35, and 56.

[0255] Serum samples were diluted 1:100 (day 21), 1:2000 (day 35) or 1:5000 (day 56) fold in 1% BSA in PBS. Day 56 samples were further diluted by a 7 point 1:3 serial dilution to measure the endpoint antibody titers. To detect IgE, sera were treated with Agarose-Protein G (Thermo Fisher Scientific, Rockford, IL) 50 minutes and then 1:20 diluted samples were loaded to ELISA plates. Samples were detected with goat anti-mouse IgG1-HRP, goat anti-mouse IgG2a-HRP (Southern Biotech, Birmingham, Al), or rat anti-mouse-IgE-biotin (R35-118, BD Pharmingen, San Jose, CA) followed Pierce Streptavidin-HRP (Thermo Fisher Scientific, Rockford, IL). Reaction was developed with SureBlue TMB Substrate and stopped with TMB Stop Solution. Plates were read (OD₄₅₀) by using Epoch ELISA reader (BioTek, Winooski, VT). Endpoint titers were determined by subtracting twice above the of background average (PBS) reading. The means and standard errors of endpoint titers or OD₄₅₀ values per group were analyzed by using Excel statistic function. IgE data were analyzed by using Student T test. Tests were two tailed, and p values ≤ 0.05 were considered significant.

[0256] Variations, modifications, and other implementations of what is described herein will occur to those of ordinary skill in the art without departing from the spirit and scope of the invention and the claims. All of the patents, patent applications, international applications, and references identified are expressly incorporated herein by reference in their entireties.

CLAIMS

What is Claimed:

1. An isolated nucleic acid encoding a polypeptide comprising:
 - a. a lysosomal associated membrane protein (LAMP) luminal domain;
 - b. at least one Allergen X as described in Table 1; and
 - c. a LAMP transmembrane domain/cytoplasmic tail.
2. The nucleic acid of claim 1, wherein LAMP is selected from LAMP polypeptide (LAMP-1), DC-LAMP, LAMP-2, LAMP-3, LIMP II, or ENDOLYN.
3. The nucleic acid of claim 2, wherein the LAMP luminal domain is derived from LAMP-1.
4. The nucleic acid of claim 3, wherein the LAMP luminal domain comprises the amino acid sequence as shown as:
 - (a) SEQ ID NO:2;
 - (b) SEQ ID NO:3;
 - (c) amino acids 29-381 of SEQ ID NO:4 or 25-370 of SEQ ID NO:5; or
 - (d) variants of (a)-(c), wherein the variants are at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, 96%, 97%, 98% or 99% identical to (a)-(c).
5. The nucleic acid of any one of claims 1-4, wherein the LAMP transmembrane domain / cytoplasmic tail comprises:
 - (a) SEQ ID NO:1;
 - (b) amino acids 382-417 of SEQ ID NO:4 or 371-406 of SEQ ID NO:5; or
 - (c) variants of (a)-(b), wherein the variants are at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, 96%, 97%, 98% or 99% identical to (a)-(b).
6. The nucleic acid of any one of claims 1-5, wherein Allergen X comprises:

- (a) amino acid sequence of at least one of SEQ ID NO:Y or SEQ ID NO:V;
or
(b) a variant of (a) wherein said variant is at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, 96%, 97%, 98% or 99% identity to (a);
and wherein said variant retains either (a) the ability to raise an antibody that cross-reacts with the Allergen X from which it was derived and/or (b) Allergen X biological activity.

7. The nucleic acid of any one of claims 1-6, wherein the nucleic acid comprises:
(a) any one of the polynucleotides of SEQ ID NO:Z or SEQ ID NO:W as shown in Table 1;
(b) a polynucleotide encoding a polypeptide encoded by any one of the polynucleotides of SEQ ID NO:Z or SEQ ID NO:W as shown in Table 1;
(c) a polynucleotide that encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: Y or SEQ ID NO:V as shown in Table 1;
(d) a polynucleotide encoding a luminal domain (such as, for example, SEQ ID NOs:2 or 3 or as shown in SEQ ID Nos: 4 or 5), one or more of SEQ ID NO:Y as shown in Table 1, and a trafficking domain (such as SEQ ID NO:1, or as shown in SEQ ID Nos: 4 or 5);
(e) a polynucleotide encoding a luminal domain (such as, for example, SEQ ID NOs:2 or 3 or as shown in SEQ ID Nos: 4 or 5) and one or more of SEQ ID NO:Y as shown in Table 1;
(f) a polynucleotide encoding one or more of SEQ ID NO:Y as shown in Table 1 and a trafficking domain (such as SEQ ID NO:1, or as shown in SEQ ID Nos: 4 or 5)
or
(g) polynucleotides with at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, 96%, 97%, 98% or 99% identity to any one of the polynucleotides of (a)-(f).

8. A vector comprising the nucleic acid of any one of claims 1-7.

9. A host cell comprising the nucleic acid of any one of claims 1-7 or the vector of claim 8.

10. A polypeptide encoded by the nucleic acid of any one of claims 1-7, the vector of claim 8, or the host cell of claim 9.

11. A vaccine comprising the nucleic acid of any one of claims 1-7, the vector of claim 8, the host cell of claim 9, or the polypeptide of claim 10.

12. A method of treating or preventing an allergic response, wherein the method comprises administering to a subject the vaccine of claim 11 in an effective amount sufficient to reduce, inhibit or prevent an allergic response.

13. The method of claim 12, wherein the vaccine is administered therapeutically.

14. The method of claim 12, wherein the vaccine is administered prophylactically.

15. The method of any one of claims 12-14, wherein the method comprises a priming step and at least one boosting step.

16. The method of claim 15, wherein:

- (a) the LAMP Construct is used to prime the subject; and/or
- (b) the boosting step comprises administration of an allergen, the LAMP Construct, a polypeptide encoded by the LAMP Construct, or a cell comprising the LAMP Construct.

17. The method of either claim 15 or 16, wherein the allergen used to prime is the same that is used to boost.

18. The method of any one of claims 15-17, wherein the allergen used to prime is derived from the same protein as the allergen used to boost.

19. The method of any one of claims 15-18, wherein more than one allergen is used to prime and/or boost.

Figure 1

Portion of Human LAMP-1 Transmembrane/Cytoplasmic Tail Used in Constructs (amino acids 383-417 of Human Full Length Lamp-1 Sequence) (SEQ ID NO:1)

LIPIAVGGALAGLVLLIVLIAYLVGKRKSHAGYQTI

Portion of Human LAMP-1 Luminal Domain Used in Constructs (amino acids 3-381 of Full Length Lamp-1 Sequence) (SEQ ID NO:2)

APRSARRPLLLLLLLLLLLLLGLMHCAAAAMFMVKNNGTACIMANFSAAFVSVNYDTKSGPK
NMTLDLP SDATVVLNRSSCGKENTSDPSLVIAFGRGHTLTNLNFTRNATRYSVQLMSFVY
NLSDTHLFPNASSKEIKTVESITDIRADIDKKYRCVSGTQVHMNNVTVTLHDATI QAYL
SNSFSRGETRCEQDRPSPTTAPPAPPSPSPSPVVKSPSVDKYNVSGTNGTCLLASMGL
QLNLTYERKDNTTVTRLLNINPNKTSASGSCGAHLVTLELHSEGTTVLLFQFGMNASSS
RFFLQGIQLNTILPDARDPAFKAANGSLRALQATVGNSYKCNAAEEHVRVTKAFSVNIFK
VWVQAFKVEGGQFGSVEECLLDENS

5' Sequence Included in Constructs (SEQ ID NO:3)

MAPRSARRPLLLLLLLLLLLLLGLMHCAAAAMFMVKNNGTACIMANFSAAFVSVNYDTKSGP
KNMTLDLP SDATVVLNRSSCGKENTSDPSLVIAFGRGHTLTNLNFTRNATRYSVQLMSFV
YNLSDTHLFPNASSKEIKTVESITDIRADIDKKYRCVSGTQVHMNNVTVTLHDATI QAY
LSNSFSRGETRCEQDRPSPTTAPPAPPSPSPSPVVKSPSVDKYNVSGTNGTCLLASMGL
LQLNLTYERKDNTTVTRLLNINPNKTSASGSCGAHLVTLELHSEGTTVLLFQFGMNASS
SRFFLQGIQLNTILPDARDPAFKAANGSLRALQATVGNSYKCNAAEEHVRVTKAFSVNIF
KVWVQAFKVEGGQFGSVEECLLDENSLE

Human LAMP-1 Protein Sequence (SEQ ID NO:4)

Second Luminal Domain
(228-381 aa)

Hinge Region (195-227 aa)

Transmembrane Domain
(382-405 aa)

Cytoplasmic Tail
(406-417aa)

Figure 3

Mouse LAMP-1 Protein Sequence (SEQ ID NO:5)

1 MAAPGARRPL LLLLLAGLAH GASAlfevkn **N**gtt**C**imasf sasflttyet a**N**gsqiv**N**is
Signal sequence (1-24aa)
61 lpasaevlk**N** gss**C**gke**N**vs dpslttitfgr gylltl**N**ftk **N**tttrysvqhm yfty**N**lsdte
(25-188 aa)
121 hfpnaiskei ytmdsttdik adinkayr**C**v sdirvymk**N**v tvvlrdatiq aylssg**N**fsk
181 eeth**C**tqdgp spttgppsp *pplvptnptv sky**N**vtgn**N**g tcllasmalq lNitylkkd**N***
Hinge Region (189-218 aa)
241 ktvtrafnis p**N**dtssgs**C**g inlvtlkven knralelqfg m**N**assslffl qgvrl**N**mtlp
(219-370 aa)
301 dalvptfsis **N**hslkalqat vgsy**k**Cnte ehifvskmls lnvfsvqvqa fkvd sdrfgs
361 vee**C**vqdgnn MLIPIAVGGA LAGLVLVLI AYLIgrkrsh aGYQTI
Transmembrane Domain (371-394 aa) Cytoplasmic Tail (395-406aa)

For the constructs, the sequences are highlighted as follows: *hLAMP Signal peptide*-*hLAMP Luminal domain*-CTCGAG-*Antigen*-GAATTC-*hLAMP TM and Cyto domain*

Figure 4

SEQ ID NO:7 Cor a 1 amino acids(1-159)

MGVFCYDEATSVIPPARLFKSFVLADNLI PKVAPQHFTSAENLEGNGGPGTIKKITFAEGNEFKYMKH
KVEEIDHANFKYCYSII EGGPLGHTLEKISYEIKMAAAPHGGGSILKITSKYHTKGNASINEEEIKAGKE
KAAGLKFKA VEAYLLAHPDAY

Polynucleotide sequence encoding Cor a 1(flanking XhoI-EcoRI uppercase) (SEQ ID NO:6)

CTCGAGatggggcgtgttctgctacgaggacgaggccacaagcgtgatccctcccgcagactgttcaaga
gcttcgtgctggacgcccgaacaatctgatcccaaaagtggccccccagcacttcaccagcgcgcgagaatct
ggaaggcaatggcggacccggcaccatcaagaagatcacattcgccgagggcaacgagttcaagtacatg
aagcacaagtggaagagatcgaccacgccaacttcaagtactgctacagcatcatcgaaggcggccctc
tgggccacacactggaaaagatcagctacgagatcaagatggccgctgcccctcacggcggaggcagcat
tctgaagatcaccagcaagtaccacaccaagggcaacgccagcatcaacgaggaagaaatcaaggccggc
aaagagaaagccgcgcgactgtttaaggccgtggaagcctatctgctggcccaccccgatgcctacGAAT
TC

SEQ ID NO:9 Cor a 9 amino acids (24-320)

INVGLRRQQRYFGECNLDRLNALEPTNRIEAEACQIESWDHNDQQFQCAGVAVIRRTIEPNGLLLPQYS
NAPELIYIERGRGITGVLFPGCPETFEDPQQQSQQGQRQGQGSQRSEQDRHQKIRHFRREGDIIALPAGV
AHWCYNDGDSPPVTVSLLHTNNYANQLDENPRHFYLAGNPDDHEHQRRQQQFQRRRQQQSHGEQGEQE
QQGEGNNVFSGFDAEFLADAFNVDVDTARRLQSNQDKRRNIVKVEGRLQVVRPERSRQEWERQERQERES
EQERERQRRQGGGRDGN

Polynucleotide encoding Cor a 9 (revised) (flanking XhoI-EcoRI uppercase) (SEQ ID NO:8)

CTCGAGatcaacgtgggactgcgagacagcagcagcggtaacttcggcgagtgcaatctggaccggctga
acgctctggaaccaccaacagaatcgaggccgaggttgccagatcgagagctgggaccacaacgacca
gcagttccagtgtgctggcgtggcgtgatcagacggaccatcgagcccaacggactgctgctgccccag
tacagcaatgcccccgagctgatctacatcgagcggggcagaggaatcaccggcgtgctgtttcccggt
gccccgagacattcgaggaccctcagcagcagagccagcaaggccagagacaaggccaaggccagtcca
gagaagcgagcaagaccggcaccagaagatccggcacttcagagagggcgacatcattgctctgccagcc
ggcgtggccactggtgctacaatgatggcgatagccccgtcgtgaccgtgtctctgctgcacaccaaca
actacgccaaccagctggacgagaaccccagacacttctatctggccggcaacccccgacgacgagacca
gaggcaagggcagcagcagtttcggccagagaagaaggcagcagcagcagccatggcgagcaaggcgag
caagagcagcaaggcgagggcaacaacgtgttcagcggcttcgacgccgagtttctggccgacgccttca
acgtggacgtggacacagccagacggctgcagttccaaccaagacaagcggcggaacatcgtgaaagtgga
aggccggctccaagtcgtgcgcccgagagatctagacaagagtgggagcgggaagagcgggaagaacgc
gagagcgagcaagagagagagcggcagagaaggcaaggcggcagaggcagagatgtgaacGAATTC

SEQ ID NO:11 Cor a 1-Gly 4-Cor a 9 amino acids

MGVFCYDEATSVIPPARLFKSFVLADNLI PKVAPQHFTSAENLEGNGGPGTIKKITFAEGNEFKYMKH
KVEEIDHANFKYCYSII EGGPLGHTLEKISYEIKMAAAPHGGGSILKITSKYHTKGNASINEEEIKAGKE

For the constructs, the sequences are highlighted as follows: **hLAMP Signal peptide**-**hLAMP Luminal domain**-CTCGAG-**Antigen**-GAATTC-**hLAMP TM and Cyto domain**

KAAGLFLKAVEAYLLAHPDAYGGGGINVGLRRQQQRYFGECNLDRLNALEPTNRIEAEACQIESWDHNDQQ
FQCAGVAVIRRTIEPNGLLLPQYSNAPELIYIERGRGITGVLFPGCPETFEDPQQQSQQGQRQGGQSQR
SEQDRHQKIRHFREGDIIALPAGVAHWCYNDGDSFVTVSLLHTNNYANQLDENPRHFYLAGNPDDHQR
QQQQQFGQRRRQQQSHSGEQGEQEQGEGNNVFSGFDAEFLADAFNVDVDTARRLQSNQDKRRNIVKVEG
RLQVVRPERSRQEWERQERQERESEQERERQRRQGGGRDGVN

Polynucleotide encoding Cor a 1-GLY4-Cor a 9 (SEQ ID NO:10) (XhoI-EcoRI and Gly4 linker uppercase)

CATATGCTCGAGatggggcgtgttctgctacgaggacgaggccacaagcgtgatccctcccgccagactgt
tcaagagcttcgtgctggacgcgcgacaatctgatccccaaagtggccccccagcacttcaccagcgccga
gaatctggaaggcaatggcggacccggcaccatcaagaagatcacattcgcccgaggggcaacgagttcaag
tacetgaagcacaaagtggagagatcgaccacgccaacttcaagtactgctacagcatcatcgaaggcg
gccctctggggcacacactggaaaagatcagctacgagatcaagatggcgcgtgccctcacgccggagg
cagcattctgaagatcaccagcaagtaccacaccaaggggcaacgccagcatcaacgaggaagaaatcaag
gccggcacaagagaaaagccgcggactgtttaaggccgtggaagcctatctgctggcccaccccgatgect
acGGCGGAGGGGGCcatcaacgtgggactgaggagacagcagcagcgtacttcggcgagtgaatctgga
ccggctgaacgctctggaaccaccaaagaatcgaggccgaggcttgccagatcgagagctgggaccac
aacgaccagcagttccagtgtgctggcgtggccgtgatcagacggaccatcgagcccaacggactgctgc
tgccccagtagcagaatgcccccgagctgatctacatcgagcggggcagaggaatcacccggcgtgctgtt
tccccggtgccccgagacattcgaggaccctcagcagcagagccagcaaggccagagacaaggccaaggc
cagtcacagagaagcgagcaagaccggcaccagaagatccggcacttcagagagggcgacatcattgctc
tgccagccggcgtggcccactggtgctacaatgatggcgatagcccgtcgtgaccgtgtctctgctgca
caccacaactacgccaaccagctggacgagaaccccagacacttctatctggccggcaaccccgacgac
gagcaccagaggcaaggcgagcagcagcttcggccagagaagaaggcagcagcagcacagccatggcgagc
aaggcgagcaagagcagcaaggcgagggaacaacgtgttcagcggcttcgacgccgagtttctggccga
cgcttcaacgtggacgtggacacagccagacggctgcagtccaaccaagacaagcggcggaacatcgtg
aaagtggaaaggccggctccaagtcgtgcccggcagagatctagacaagagtgaggcggcaagagcggc
aagaacgcgagagcgagcaagagagagagcggcagagaaggcaaggcggcagaggcagagatgtgaacGA
ATTCGTCGAC

SEQ ID NO:13 Pru du 6 amino acids (26-272)

ARQSQSPQNQCQLNQLQAREPDNRIQAEAGQIETWNFNQEDFQCAGVAASRITIQRNGLHLPSYSNAPQ
LIYIVQGRGVLGAVFSGCPETFEESSQSSSQGRRQEQEQEQERQQQQQGEQGRQQGQQEQEQERQGRQQGRQ
QQEEGRQQEQQQGQQGRPQQQQQFRQFDRHQKTRRIREGDVVAIPAGVAYWSYNDGDQELVAVNLFHVSS
DHNQLDQNPFRKFLYLAGNPENEFNQGGQSQRPRQQGEQGRPGHQHQPFGPRPRQQEQQSGNNVFSGFNTQLL
AQALNVNEETARNLQGQNDNRNQIIRVRGNLDFVQPPRGRQEREHEERQQEQQLQERQQQGGQLMAN

Polynucleotide encoding Pru du 6 (SEQ ID NO:12) (flanking XhoI-EcoRI-uppercase)

CTCGAGgccagacagagccagctgagccccagaatcagtgccagctgaaccagctgcaagccagagagc
ccgacaaccggattcaagccgaggccggccagatcgagacatggaacttcaaccaagaggacttcagtg
tgccggcgtggccggcagcagaatccatccagcggaacggactgcatctgccagctacagcaacgcc
ccccagctgatctacatcgtgcaaggcagaggcgtgctggcgccgtgtttagcggatgccccgagacat
tcgaggaaagccagcagagcagccagcaaggccggcagcaagagcaagaacaagagagacaacagcagca
gcaaggggagcaaggcagacagcaaggacagcaagagcagcagcaagagcgccaaggacggcagcaagg
cgccagcagcaagaagaggggcagacagcaagaacagcagcaaggccagcaaggcgccctcagcagcagc

For the constructs, the sequences are highlighted as follows: **hLAMP Signal peptide**-**hLAMP Luminal domain**-CTCGAG-**Antigen**-GAATTC-**hLAMP TM and Cyto domain**

agcagttccggcagttcgaccggcaccagaaaaccggcgatcagagaaggcgacgtggtggctattcc
agccgggggtggcctactggtcctacaacgacggcgaccaagaactggtggccgtgaatctgttccacgtg
tccagcgaccacaaccagctggaccagaacccccggaagtctctatctggctggcaaccccgagaacgagt
tcaaccagcaagggcagagccagccagacagcaaggcgaacaaggacggcccgacagcaccagcagcc
tttcggcagaccacggcagcaagagcagcaaggcagcggcaacaacgtgttcagcggcttcaacaccag
ctgctggcccaagctctgaacgtgaacgaggaaacccggccgaatctgcaaggccagaacgacaacagaa
accagatcatcagagtgccggggcaatctggacttcgtgcagccccctagagggcggaagagagagagca
cgaagagaggcagcaagaacagctgcagcaagagcggcagcagcaaggcggacagctgatggccaacGAA
TTC

SEQ ID NO:15 Ana o 1 amino acids (27-538)

KIDPELKQCKHQCKVQRQYDEQQKEQCVKECEKYYKEKKGREREHEEEEEEWGTGGVDEPSTHEPAEKHL
SQCMRQCERQEGGQQKQLCRFRQCERYKKERGQHNYKREDEDEDEDEAEEDENPYVFEDEDFTTKVKT
EQGKVLLPKFTQKSKLLHALEKYRLAVLVANPQAFVVP SHMDADS IFFVSWGRGTITKILENKRESINV
RQGDIVSISSGTPFYIANNDENEKLYLVQFLRPVNLPGHFVVFHGPGENPESFYRAFSWEILEALKTS
KDTLEKLFQKQDQGTIMKASKEQIRAMSRREGGPKIWPFTTEESTGSFKLFKKDPSQSNKYGQLFEAERID
YPPLEKLDMMVSYANITKGGMSVPFYNSRATKIAIVS GEGCVEIACPHLSSSKSSHPSYKKLRARIRKD
TVFIVPAGHPFATVASGNENLEIVCFEVNAEGNIRYTLAGKKNI IKVMEKEAKELAFKMEGEEVDKVF GK
QDEEFFFFQGPWRKEKEGRADE

**Polynucleotide encoding Ana o 1 (SEQ ID NO:14) (flanking XhoI-EcoRI
uppercase)**

CTCGAGaagatcgaccccgagctgaagcagtgcaagcaccagtgcaaagtgcagcggcagtacgacgagc
agcagaaaagaacagtgcgtagaagagtgcgagaagtactacaaagagaagaaggccgcgagcgcgagca
cgaagaggaagaggaagaatggggcaccggcgagtgtagcagagccttctacacacgagcccgccgagaaa
catctgagccagtgcatgagacagtgcgaaacggcaagagggcgccagcagaaaacagctgtgcccgttcc
ggtgccaagagcgggtacaagaaagagcggggccagcacaactacaagagagaggacgacgaggacgaaga
tgaggacgaggctgaggaagaggacgagaacccctacgtgttcgaggatgaggacttcaccaccaaaagtg
aaaaccgagcaaggcgaagtgggtgctgctgcccaagttcaccacagaagtccaagctgctgcacgctctgg
aaaagtaccggctggccgttctgggtggccaaccctcaagccttcgtgggtgccagccacatggacgccga
cagcatcttcttcgtgtcttggggcagaggcaccatcaccaagattctggaaaacaagcgcgagagcatc
aacgtgcccgaaggcgacatcggtgtccatcagcagcggcacccttctacattgccaacaacgacgaga
acgagaagctgtatctggtgcagtttctgcccggccgtgaatctgcccggccactttgaagtgttccacgg
accggcgagagagaaccccgagagcttctacagagccttcagctgggaaattctggaagccgctctgaaa
acatccaaggacacactggaaaagctgttcgagaagcaagaccaaggaccatcatgaaggccagcaaaag
aacagatccgggccatgagcagaagaggcgaggggcccaagatctggcccttcaccgaggaaagcaccgg
cagcttcaagctgtttaagaaggaccccagccagagcaacaaatacgggcagctgtttgaggccgagcgg
atcgactacccccactggaaaagctggacatggtggtgtcctacgccaatatcaccaaggcgccatga
gcgtgcccttttacaacagcagagccaccaagatcgccatcggtggtgtccggcgagggtgcgtggaaat
cgcttgccctcatctgagcagcagcaagtccagccaccccgctacaagaagctgcccggccagaatccgg
aaggacaccgtgttcatcggtgccagccggccacccttttgccacagtgggccagcggcaacgagaatctgg
aaatcggtgtgcttgaagtgaacgccgagggcaacatccggtacacactggccggcaagaagaacatcat
caaagtgatggaaaaggaagccaaagaactggcctttaagatggaaggcgagggaagtggacaaagtgttc
ggcaagcaagatgaagagttcttctttcaaggccccgagtgggcgaaagagaaagagggcgagagccgacg
agGAATTC

SEQ ID NO:17 Ana o 2 amino acids (1-457)

For the constructs, the sequences are highlighted as follows: **hLAMP Signal peptide**-**hLAMP Luminal domain**-CTCGAG-**Antigen**-GAATTC-**hLAMP TM and Cyto domain**

LSVCFLLILFHGCLASRQEWQQQDECQIDRLDALEPDNRVEYEAGTVEAWDPNHEQFRCAGVALVRHTIQP
NGLLLPQYSNAPQLIYVVQGEGMTGISYPGCPETYQAPQQGRQQQSGRFQDRHQKIRRFRRGDIIAIPA
GVAHWCYNEGNPVTLLDVSNQNLDRTPRKFLAGNPKDVFQQQQQHQSRRNLFSGFDELLAE
AFQVDERLIKQLKSEDNRRGIVKVKDDELVRIRPSRSQSERGSESEEESEDEKRRWGQRDNGLIETICTM
RLKENINDPARADIYTPEVGRLTTLNSLNLPIKWLQLSVEKGVLYKNALVLPHWNLNSHSIIYGCKGKG
QVQVVDNFGNRVFDGEVREGQMLVVPQNFVAVVKRAREERFEWISFKTNDRAMTSPLAGRTSVLGGMPEEV
LANAFQISREDARKIKFNNQQTTLTSGESSHMRDDA

Polynucleotide encoding Ana o 2 (SEQ ID NO:16) (flanking XhoI-EcoRI uppercase)

CTCGAGctgagcgtgtgctttctgattctgttccacggctgtctggccagccggcaagaatggcagcagc
aagacgagtgccagatcgaccggctggacgctctggaaccgcacaaccgggtggaatacgaggccggcac
agtggaagcttgggacccaaccacgagcagttcagatgtgccggcgtggcactcgtgcccacaccatc
cagccaaacggactgctgctgccccagtacagcaacgccccccagctgatctatgtggtgcaaggcgagg
gcatgaccggcatcagctatcccggctgccccgagacatatcaagcccctcagcaaggcagacagcaagg
ccagagcggccgggttccaagaccggcaccagaagatccggcgggttcagacggggcgacatcattgccatt
ccagccgggggtggcccactgggtgctacaacgagggcaatagccccgtcgtgaccgtgacactgctggacg
tgtccaacagccagaaccagctggaccggaccccccggaagtttcatctggccggcaacccaaggacgt
gttccagcaacagcagcagcaccagagccggggcagaaatctgttcagcggcttcgacaccgagctgctg
gccgaggttttcaagtggacgagcggctgatcaagcagctgaagtccgaggacaacagaggcggcatcg
tgaaagtgaaggacgacgagctgagagtgtatccggcccagcagaagccagagcgagagaggcagcgagag
cgaggaagagtctgaggacgagaagcggagatggggccagcgggacaacggcatcgaagagacaatctgc
accatgcccgtgaaagagaacatcaacgaccccgccagagccgacatctacacccccgaagtgggcccggc
tgacaactctgaactctctgaatctgccattctgaagtggctgcagctgtccgtggaaaaggggtgct
gtacaagaacgctctggtgctgcctcactggaatctgaacagccacagcatcatctacggctgcaagggc
aagggccaagtccaagtgggtggacaacttcggcaacagagtgttcgacggcggaagtgcgcgagggccaga
tgctcgtggtgccccagaatttcgccgtcgtgaagcgggcccagagaagaaagattcgagtggatcagctt
caagaccaacgaccgggcatgaccagccctctggccggaagaacatctgtgctgggcccagatgcccgag
gaagtgctggctaacgccttcagatcagcagagaggacgcccgaagatcaagttcaacaaccagcaga
ccacactgaccagcggcgagagcagccaccacatgagagatgacgccGAATTC

SEQ ID NO:19 Ana o 3 amino acids (21-138)

SIYRAIVEVEEDSGREQSCQRQFEEQQFRNCQRYVKQEVQRGGRYNQREQSLRECCQELQEVDRRCRCQ
NLEQMVRQLQQEQIKGEEVRELYETASELPRICISISPSQGCQFQSSY

Polynucleotide encoding Ana o 3 (SEQ ID NO:18) (flanking XhoI-EcoRI uppercase)

CTCGAGagcatctaccgggccatcgtggaagtggaagaggacagcggcagagagcagagctgccagcggc
agttcgaggaacagcagcgggttcagaaactgccagagatacgtgaagcaagaagtgcagagaggcggcag
atacaaccagagacaagagtctctgagagagtgtgccaagagctgcaagaagtggaccggcgctgccgg
tgccagaatctggaacagatggtgcgccagctgcagcagcaagagcagatcaagggcgaggaagtgcgcg
agctgtacgagacagccagcagctgcctcggatctgcagcatcagcccaagccaaggctgccagttcca
gacagctacGAATTC

SEQ ID NO:21 Ana o 2-Gly4-Ana o 1-Gly4-Ana o 3 amino acids

LSVCFLLILFHGCLASRQEWQQQDECQIDRLDALEPDNRVEYEAGTVEAWDPNHEQFRCAGVALVRHTIQP
NGLLLPQYSNAPQLIYVVQGEGMTGISYPGCPETYQAPQQGRQQQSGRFQDRHQKIRRFRRGDIIAIPA

For the constructs, the sequences are highlighted as follows: *hLAMP Signal peptide*-*hLAMP Luminal domain*-CTCGAG-Antigen-GAATTC-*hLAMP TM and Cyto domain*

GVAHWCYNEGNSPVVTVTLLDVSNSQNQLDRTPRKFLAGNPKDVFOQQQQHQSRGRNLFSGFDTELLAE
AFQVDERLIKQLKSEDNRGGIVKVKDELRVIRPSRSQSERGSESEEESEDEKRRWGQORDNGIEETICTM
RLKENINDPARADIYTPEVGRLLTTLNSLNLPIKWLQLSVEKGVLYKNALVLPWHNLNSHSIIYGCKGKC
QVQVVDNFGNRFVDGEVREGOMLVVPQNFVVKRAREERFEWISFKTNDRAMTSPLAGRTSVLGGMPEEV
LANAFQISREDARKIKFNNQOTTLTSGESSHHMRDDA **GGGG**KIDPELKQCKHQCKVQRQYDEQQKEQCV
KECEKYKKEKKGREREHEEEEEEWGTGGVDEPSTHEPAEKHLSQCMRQCERQEGGQQQLCRFRQCERYK
KERGQHNYKREDDDEDEDEAEEDENPYVFEDEDEFTTKVKTEQKQVLLPKFTQKSKLLHALEKYRLAV
LVANPQAFVVP SHMDADSIFFVSWGRGTITKILENKRESINVRQGDIVSISGTFYIANNDENEKLYLV
QFLRPVNLPGHFVFGPGGENPESFYRAFSWEILEAALKTSKDTLEKLFQKQDQGTIMKASKEQIRAMS
RRGEGPKIWPFTTEESTGSFKLFKKDPSQSNKYQLFEAERIDYPPLEKLDMVVSYANITKGMSVPFYNS
RATKIAIVS GEGCVEIACPHLSSSKSSHPSYKKLRARIRKDTVFI VPAGHPFATVASGNENLEIVCFEV
NAEGNIRYTLAGKKNIIKVMKEKAKE LAFKMEGEEVDKVFQKQDEEFFQGPWRKEKEGRADE **GGGG**S
IYRAIVEVEEDSGREQSCQRQFEEQQRFNRNCQRYVKQEVQRGGRYNQREQESLRECCQELQEVDRRCRCQN
LEQMVRQLQQQEIQKEEVRELYETASELPRICSI SPSQGCQFQSSY

Polynucleotide encoding Ana 0 2-Gly4-Ana 0 1-Gly4-Ana 0 3 (SEQ ID NO: 20)

CTCGAGctgagcgtgtgctttctgattctgttccacggctgtctggccagccggcaagaatggcagcagc
aagacgagtgccagatcgaccggctggacgctctggaacccgacaaacgggtggaatcagagggccggcac
agtggaagcttgggaccccaaccacgagcagttcagatgtgccggcgtggcactcgtgcccacaccatc
cagccaaacggactgctgctgcccagtcacagcaacgccccccagctgatctatgtggtgcaaggcgagg
gcatgaccggcctcagctatcccggtgcccagagacatatcaagccctcagcaaggcagacagcaagg
ccagagcggcgggttccaagaccggcaccagaagatccggcggttcagacggggcgacatcattgccatt
ccagccgggtgcccactggtgctacaacgagggcaatagcccgctcgtgaccgtgacactgctggacg
tgtccaacagccagaaccagctggaccggaccccccggaagtttcattctggccggcaaccccaaggacgt
gttccagcaacagcagcagcaccagagccggggcagaaatctgttcagcggcttcgacaccgagctgctg
gccgagggctttcaagtggacgagcggctgatcaagcagctgaagtcgaggacaacagaggcggcatcg
tgaaagtgaaggacgacgagctgagagtgtatccggcccagcagaagccagagcgagagagggcagcgagag
cgaggaagagtctgaggacgagaagcggagatggggccagcgggacaacggcatcgaagagacaatctgc
accatgcccgtgaaagagaacatcaacgaccccgccagagccgacatctacacccccgaagtgggcccgc
tgacaactctgaactctctgaatctgcccattctgaagtggctgcagctgtccgtggaaaaggggggtgct
gtacaagaacgctctgggtgctgctcactggaatctgaacagccacagcatcatctacggctgcaagggc
aagggccaagtccaagtgggtggacaacttcggcaacagagtgttcgacggcgaagtgcgcgagggccaga
tgctcgtggtgcccagaatttcgcccgtcgtgaagcgggcccagagaagaaagattcgagtggatcagctt
caagaccaacgacccgggcatgaccagccctctggccgggaagaacatctgtgctgggcccagatgcccgag
gaagtgtggttaacgccttcagatcagcagagaggacgcccgaagatcaagttcaacaaccagcaga
ccacactgaccagcggcgagagcagccaccacatgagagatgacgccc **GGCGGAGGGGGC**aagatcgacc
cgagctgaagcagtgcaagcaccagtgcaaagtgcagcggcagtagcagcagcagcagaaagaacagtg
gtgaaagagtgcgagaagtaactacaaagagaagaagggccgagcgcgagcagcagcaggaagaggaag
aatggggcaccggcggagtggacgagccttctacacacgagcccgagagaacatctgagccagtgc
gagacagtgcaacggcaagagggcgccagcagaaacagctgtgccggttcgggtgccaagagcggta
aagaaagagcggggccagcacaactacaagagagaggacgacgaggacgaagatgaggacgaggctgagg
aagaggacgagaacccctacgtgttcgaggatgaggacttcaccaccaaagtgaacaccgagcaaggcaa
agtgggtgctgctgcccagttcaccacagaagtccaagctgctgcacgctctggaaaagtaccggctggcc
gttctggtggccaaccctcaagccttcgtgggtgccagccacatggacgcccagacagcatcttcttcgtgt
cttggggcagaggcaccatcaccaagattctggaaaacaagcgcgagagcatcaacgtgcggcgaaggcga

For the constructs, the sequences are highlighted as follows: *hLAMP Signal peptide*-*hLAMP Luminal domain*-CTCGAG-Antigen-GAATTC-*hLAMP TM and Cyto domain*

catcggtgtccatcagcagcggcacccttctacattgccaacaacgacgagaacgagaagctgtatctg
gtgcagtttctgcggcccggtgaatctgccggccactttgaagtgtccacggaccggcgagagaacc
ccgagagcttctacagagccttcagctgggaaattctggaagccgctctgaaaacatccaaggacacact
ggaaaagctgttcgagaagcaagaccaagggaaccatcatgaaggccagcaaagaacagatccggggccatg
agcagaagaggcgagggccccaagatctggccttcaccgaggaaagcaccggcagcttcaagctgttta
agaaggaccccagccagagcaacaaatacgggcagctgtttgaggccgagcggatcgactacccccact
ggaaaagctggacatggtggtgtcctacgccaatatcaccaaggcgccatgagcgtgcccttttacaac
agcagagccaccaagatcgccatcggtgttcggcgagggtgctggtggaaatcgcttgccctcatctga
gcagcagcaagtcagccaccccagctacaagaagctgcgggccagaatccggaaggacaccgtgttcac
cgtgccagccggccacccttttgccacagtggccagcggcaacgagaatctggaaatcggtgtgcttcgaa
gtgaacgccgaggggcaacatccggtacacactggccggcaagaagaacatcatcaaagtgatggaaaagg
aagccaaagaactggcctttaagatggaaggcgaggaagtggacaaagtgttcggcgaagcaagatgaaga
gttcttcttcaaggccccgagtgggcgcaaagagaaagaggggcagagccgacgagGGCGGAGGGGGCagc
atctaccggggccatcgtggaagtggaaaggacagcggcagagagcagagctgccagcggcagttcgagg
aacagcagcgggttcagaaactgccagagatacgtgaagcaagaagtgcagagaggcggcagatacaacca
gagacaagagtctctgagagagtgtgccaagagctgcaagaagtggaccggcgctgccggtgccagaat
ctggaacagatggtgcccagctgcagcagcaagagcagatcaaggcgaggaagtgcgcgagctgtacg
agacagccagcagctgcctcgatctgcagcatcagcccaagccaaggctgccagttccagagcagcta
cGAATTC

SEQ ID NO:23 Jug n 1 amino acids (39-161)

RTTITITMEIDEDIDNPRRRGEGCQEIQIRQQNLNHCQYYLRQQSRSGGYDEDNQRQHFRRQCCQQLSQIEE
QCQCEGLRQAVRRQQQQQLRGEEMEEMVQSARDLPKECGISSQRCEIRRSWF

**Polynucleotide encoding Jug n 1 (SEQ ID NO:22) (flanking XhoI-EcoRI
uppercase)**

CTCGAGcggaccaccatcaccaccatggaaatcgacgaggacatcgacaaccccagaagaagaggcgagg
gctgccaagagcagatccagcggcagcagaatctgaaccactgccagtactatctgaggcagcagagcag
aagcggcggtacgacgaggataaaccagagacagcacttcagacagtgtgcccagcagctgagccagatc
gaggaacagtgccagtgcgagggactgagacaagccgtgcggagacaacagcagcagcaaggactgcggg
gcgaagagatggaagaaatggtgcagagcgcagagatctgcccgaagagtgcggcatcagcagccagag
atgcgagatccggcggagttggttcGAATTC

SEQ ID NO:25 Jug r 2 amino acids (1-593)

RGRDDDEENPRDPREYRQCQEYCRQGGQGRQQQQCQIRCEERLEEDQRSQEERERRRGRDVEDDQNP
DPEQRYEQCQQCERQRRGQEQLCRRRCEQRRQQEERERQRGRDRQDPQQYHRCQRRQIQEQSPERQ
RQCQQRCEQYKEQQGRERGPEASPRRESRGREEEQQRHNPYYFHSQSIRSRHESEEGEVKYLERFTERT
ELLRGIENYRVVILDANPNTSMLPHHKDAESVAVVTRGRATLTLVSQETRESFNLECGDVIRVPAGATVY
VINQDSNERLEMVKLLQPVNNPGQFREYYAAGAKSPDQSYLRVFSNDILVAALNTPRDLRERFFDQQEQR
EGVIIRASQEKLRALSQHAMSAGQRPWGRRSSGGPISLKSESPSYSNQFGQFFEACPEEHRQLQEMDVLV
NYAEIKRGAMVPHYNSKATVVVYVVEGTGRYEMACPHVSSQSYEGQGRREQEEEEESTGRFQKVTLARLAR
GDIFVIPAGHPAIATASQENLRLLGFDINGENNQRDFLAGQNNIINQLEREAKELSFNMPREEIEEIFE
SQMESYFVPTERQSRRGQGRDHPLASILDFAFF

**Polynucleotide encoding Jug r 2 (SEQ ID NO:24) (flanking XhoI-EcoRI
uppercase)**

For the constructs, the sequences are highlighted as follows: **hLAMP Signal peptide**-**hLAMP Luminal domain**-CTCGAG-Antigen-GAATTC-**hLAMP TM and Cyto domain**

CTCGAGagaggccgggacgacgacgatgaggaaaaccccagagatccccgcgagcagtaccggcagtgcc
aagagtactgcagaaggcaaggccaaggccagagacagcagcagcagtgccagatcagatgcgaggaacg
gctggaagaggaccagcggagccaagaggaacgcgagcggagaagaggcagagatgtggacgaccagaac
ccccgggaccccgcagcagagatacgcgagcagtgtcagcagcagcagtgtgaacggcagcggagaggccaagagc
agacactgtgtcggcggagatgcgagcagcggcggcagcaagaggaaagagaacgccagcggggcagaga
cagacaagacccccagcagcagtaccaccggtgccagagaagatgccagatccaagaacagagccccgag
cggcagcggcagtgccagcagagatgcgaagacagtaaaagagcagcaaggcagagagagggggcccag
aggccagccctagaagagagtccagaggacgggaagaagaacagcagcggcacaacccctactacttcca
cagccagagcatcagaagccggcacgagagcgaagaggggcgaagtgaagtatctggaacggttcaccgag
cggaccgagctgctgagaggcatcgagaactaccgggtcgtgattctggacgccaacccaacacatcca
tgctgccccaccacaaggacgcgcgagtctgtggccgctcgtgacaaggggcagagccacactgacactggt
gtcccaagagactcgcgagagcttcaatctggaatgcggcgacgtgatccgggtgccagctggggctaca
gtgtacgtgatcaaccaagacagcaacgagcggctggaaatggtcaagctgctgcagcccgatgaacaacc
ccggccagttcagagagtactacgcgcgctggcgccaagtcccccgaccagagctatctgcgggtgttcag
caacgacattctggtggcgcgtctgaataccctcgggacagactggaagattcttcgatcagcaagag
cagcgcgagggcgtgatcatcagagccagccaagagaagctgcgggctctgagccagcagccatgtctg
ctggacagagggccttggggcagaagaagctctggcgccctatctctctgaagtccgagagccctccta
cagcaaccagtttggccagttcttcgaggcttgccccgaggaacaccggcagctgcaagaaatggacgtg
ctcgtgaactacgcgcgagatcaagagggggcgccatgatggtgccccactacaacagcaaggccaccgtgg
tggtgtacgtggtggaaggcacccggcagatacgcgagatggcatgccccacgtgtccagccagctcttaca
gggccaaggacgcagagagcaagaagagggaagagtcaccggacggttccagaaagtgaccgccagactg
gccagaggcgacatcttcgtgatcccgccggacaccctatcgccatcaccgccagccagaacgagaatc
tgcggtgctgggcttcgacatcaacggcgagaacaaccagcgggactttctggccggacagaacaacat
catcaaccagctggaacgggaagccaaagaactgagcttcaacatgccccgcgaggaaatcgaagagatt
ttcgagagccagatggaagctacttcgtgcccaccgagcgcgcagagcagaagggggccaagggcgggac
accactggcctctattctggatttcgccttcttcGAATTC

SEQ ID NO:27 Jug n 1-Gly4-Jug r 2 amino acids

RTTITIMEIDEDIDNPRRRGEGCQEQIQRQQNLNHCQYYLRQQSRSGGYDEDNQHQHFRQCCQQLSQIEE
QCQCEGLRQAVRRQQQQQLRGEEMEMVQSARDLPKECGISSQRCEIRRSWFGGGGRGRDDDEENPRD
PREQYRQCQEYCRRGQGQQRQQQCQIRCEERLEEDQRSQEERERRRGRDQDDQNPRDPEQRYEQCQQQC
ERQRRGQEQTLRRRRCEQRRQQEERERQRGRDRQDPQQQYHRCQRRQCIQEQSPERQRQCQQRRCERQYKE
QQGRERGPEASPRRESRGREEEQQRHNPYYFHSQSIRSRHESEEGEVKYLERFTELLRGIENYRVVI
LDANPNTSMLPHHKDAESVAVVTRGRATLTLSVQETRESFNLECGDVIRVPAGATVYVINQDSNERLEMV
KLLQPVNNPGQFREYYAAGAKSPDQSYLRVFSNDILVAALNTPRDRLERFFDQQEQREGVIRASQEKLR
ALSQHAMSAGQRPWGRSSGGPISLKSESPSYSNQFGQFFEACPEEHRQLQEMDVLVNYAEIKRGAMMVP
HYNKATVVVYVVEGTGRYEMACPHVSSQSYEQGRREQEEEEESTGRFQKV TARLARGDIFVIPAGHP IA
ITASQNNENLRLGLFDINGENNQRDFLAGQNNIINQLEREAKELSFNMPREEIEEIFESQMESYFVPTERQ
SRRGQGRDHPLASILDFAFF

**Polynucleotide encoding Jug n 1-Gly4-Jug r 2 (flanking XhoI-EcoRI
uppercase) (SEQ ID NO:26)**

CTCGAGcgggaccaccatcaccaccatggaaatcgacgaggacatcgacaaccccagaagaagaggcgagg
gctgccaaagagcagatccagcggcagcagaatctgaaccactgccagtactatctgaggcagcagagcag
aagcggcggctacgacgaggataaaccagagacagcaacttcagacagtgctgccagcagctgagccagatc
gaggaacagtgccagtgccagggactgagacaagccgtgcggagacaacagcagcagcaaggactgcggg
gcgaagagatggaagaaatggtgcagagcgcgcagagatctgccccaaagagtgccgcatcagcagccagag

For the constructs, the sequences are highlighted as follows: **hLAMP Signal peptide**-**hLAMP Luminal domain**-CTCGAG-Antigen-GAATTC-**hLAMP TM and Cyto domain**

atgctgagatccggcggagttggttc**GGCGGAGGGGGC**agaggccgggacgacgacgatgaggaaaacccc
agagatccccgcgagcagtagaccggcagtgccaagagtagtcagagaaggcaaggccaaggccagagacagc
agcagcagtgccagatcagatgcgaggaacggctggaagaggaccagcggagccaagaggaaacgcgagcgc
gagaagaggcagagatgtggacgaccagaacccccgggacccccgagcagagatacagagcagtgtcagcag
cagtggtgaacggcagcggagaggccaagagcagacactgtgtcggcggagatgcgagcagcggcggcagc
aagaggaaagagaacgccagcggggcagagacagacaagacccccagcagcagtagaccaccgggtgccagag
aagatgccagatccaagaacagagccccgagcggcagcggcagtgccagcagagatgcgaaagacagtagc
aaagagcagcaaggcagagagagggggccagaggccagccctagaagagagtagcagaggacgggaagaag
aacagcagcggcacaaccccctactacttccacagccagagcatcagaagccggcagcagagcgaagaggg
cgaagtgaagtatctggaacgggttcaccgagcggaccgagctgctgagaggcatcgagaactaccgggtc
gtgattctggacgccaacccccacacatccatgctgccccaccacaaggacgccagtagctgtggcgtcg
tgacaaggggcagagccacactgacactggtgtcccaagagactcgcgagagcttcaatctggaatggcg
cgacgtgatccgggtgccagctggggctacagtgtagctgatcaaccaagacagcaacgagcggctggaa
atgggtcaagctgctgcagcccgtagaacaaccccgccagttcagagagtagtagccgctggcgccaagt
ccccgcaccagagctatctgcggtgttcagcaacgacattctgggtggcgtctgaatacccctcgggga
cagactggaaagattcttcgatcagcaagagcagcgcgagggcgtagcatcagagccagccaagagaag
ctgcgggctctgagccagcagccatgtctgctggacagaggcccttggggcagaagaagctctggcgcc
ctatctctctgaagtcgagagccccctctacagcaaccagtttgccagttcttcgaggcttgccccga
ggaacaccggcagctgcaagaaatggacgtgctcgtgaactacgccgagatcaagagggggcgccatgatg
gtgccccactacaacagcaaggccaccgtggtggtgtacgtggtggaaggcaccggcagatacagagatgg
catgccccacgtgtccagccagctttacgaggggccaaggacgcagagagcaagaagagggaagagtagccac
cggacgggtccagaaagtgaccgccagactggccagaggcgacatcttcgtgatcccagccggacacct
atcgccatcaccgccagccagaaacgagaatctgcggtgctggtggttcgacatcaacggcgagaacaacc
agcgggactttctggccggacagaacaacatcatcaaccagctggaacgggaagccaaagaactgagctt
caacatgccccgcgaggaaatcgaagagattttcgagagccagatggaaagctaacttcgtgcccaccgag
cgccagagcagaagggggccaagggcgggatcaccactggcctctattctggatttcgccttcttcGAAT
TC

SEQ ID NO:29 Amb a 1 amino acids (26-296)

AEDLQEILPVNETRRLTTSAYNIIDGCWRGKADWAENRKALADCAQGFQKGTGKGDKGDIYTVTSELDD
DVANPKEGTLRFGAQNRPLWIIFERDMVIRLDKEMVNSDKTIDGRGAKVEIINAGFTLNGVKNV I IHN
INMHDVKVNPGLIKSNDGPAAPRAGSDGDAISISGSSQIWIHDHCSLSKSVLDGLVDAKLGTTRLTVSNL
FTQHQFVLLFGAGDENIEDRGM LATVAFNTFTDNVDQRMPCRHRGFFQVNNNYDKWGSYAIGGSASPTI
LSQGNRF CAPDERSKKNVLGRHGEAAAESMKWNWRTNKDVLENGAI FVASGVDPVLTPEQSAGMIPAEPG
ESALS LTSSAGVLS CQPGAPC

SEQ ID NO:33 Bet v 1-A amino acids (2-160)

GVFNYETETTSVIPAAARLFKAFILDGDNLFPKVAPQAISSVENIEGNGGPGTIKKISFPEGFPFKYVKDR
VDEV DHTNFKYNYSVIEGGPIGDTLEKISNEIKIVATPDGGSILKISNKYHTKGDHEVKAEQVKASKEMG
ETLLRAVESYLLAHSDAYN

SEQ ID NO:37 Can f 1 amino acids (19-174)

QDTPALGKDTVAVSGKWLKAMTADQEVPEKPDSVTPMILKAQKGGNLEAKITMLTNGQCQNITVVLHKT
SEPGKYTAYEGQRRVFIQPSVRDHYILYCEGELHGRQIRMAKLLGRDPEQSQEALDFREFSRAKGLNQ
EILELAQSETCSPGGQ

SEQ ID NO:41 Cyn d 1 amino acids (1-246)

For the constructs, the sequences are highlighted as follows: **hLAMP Signal peptide**-**hLAMP Luminal domain**-CTCGAG-**Antigen**-GAATTC-**hLAMP TM and Cyto domain**

AIGDKPGPNITATYGSKWLEARATFYGSNPRGAAPDDHGGACGYKDVDKPPFDGMTACGNEPIFKDGLGC
RACYEIKCKEPVECSGEPVLVKITDKNYEHIAAYHFDLSGKAFGAMAKKGQEDKLRKAGELTLQFRRVKC
KYPSTGKITFHIKGSNDHYLALLVKYAAGDGNIVAVDIKPRDSDEFIPMKSSWGAIWRIDPKPLKGP
SIRLTSEGGAHLVQDDVIPANWKPDVTYTSKLQFGA

SEQ ID NO:45 Der f 1 amino acids (19-321)

RPASIKTFEEFKKAFNKNYATVEEEEVARKNFLES�KYVEANKGAINHLSDSLDEFKNRYLMSAEAFEQ
LKTQFDLNAETSACRINSVNVPSELDRSLRTVTPIRMQGGCGSCWAFSGVAATESAYLAYRNTSLDLSE
QELVDCASQHGCHGDTIPRGIEYIQQNGVVEERSYPYVAREQRCRRPNSQHYGISNYCQIYPPDVKQIRE
ALTQHTAIAVIGIKDLRAFQHYDGRITIIQHDNGYQPNYHAVNIVGYGSTQGDDYWIVRNSWDTTWGDS
GYGYFQAGNNLMMIEQYPYVIM

SEQ ID NO:49 Der f 1 amino acids (99-321)

TSACRINSVNVPSELDRSLRTVTPIRMQGGCGSCWAFSGVAATESAYLAYRNTSLDLSEQELVDCASQ
GCHGDTIPRGIEYIQQNGVVEERSYPYVAREQRCRRPNSQHYGISNYCQIYPPDVKQIREALTQHTAIA
VIGIKDLRAFQHYDGRITIIQHDNGYQPNYHAVNIVGYGSTQGDDYWIVRNSWDTTWGDSGYGYFQAGNN
LMMIEQYPYVIM

SEQ ID NO:53 Der p 2 amino acids (18-146)

DQVDVKDCANHEIKKVLVPGCHGSEPCIHRGKPFQLEAVFEANQNTKTAKIEIKASIDGLEVDVPGIDP
NACHYMKCPLVKGGQYDIKYTWNVPKIAPKSENVVTVKVMGDDGVLACAIATHAKIRD

SEQ ID NO:57 Der f 2 amino acids (18-146)

DQVDVKDCANNEIKKVMVDGCHGSDPCIHRGKPFTEALFDANQNTKTAKIEVKASLDGLEIDVPGIDT
NACHFVKCPLVKGGQYDIKYTWNVPKIAPKSENVVTVKVLIGDNGVLACAIATHGKIRD

SEQ ID NO:61 Der p 1 (DEL) amino acids (1-26:38-223)

TNACSINGNAPAEIDLQMRVTPIRSGVAATESAYLAYRNQSLDLAEQELVDCASQHNGCHGDTIPRGI
EYIQHNGVVQESYYRYVAREQSCRRPNAQRFGISNYCQIYPQNVNKIREALAQTHSAIAVIGIKDLDAF
RHYDGRITIIQRDNGYQPNYHAVNIVGYSNAQGVQYDWIVRNSWDTNWGDNGYGYFAANIDLMMIEEYPYV
IL

SEQ ID NO:66 Fel d 1 chain 1-chain 2 amino acids (23-92/18-109)

EICPAVKRDVDLFLTGTTPDEYVEQVAQYKALPVVLENARILKNCVDAKMTEEDKENALSLLDKIYTSPLC
VKMAETCPIFYDVFFAVANGNELLLDLSTKVNATEPERTAMKKIQDCYVENGLISRVLDGLVMTTISS
KDCMGEAVQNTVEDLKLNTLGR

SEQ ID NO:65 Fel d 1 chain 1-chain 2 amino acids (23-92/18-109)

EICPAVKRDVDLFLTGTTPDEYVEQVAQYKALPVVLENARILKNCVDAKMTEEDKENALSLLDKIYTSPLC
GGGGSGGGSGGGGSVKMAETCPIFYDVFFAVANGNELLLDLSTKVNATEPERTAMKKIQDCYVENGLI
SRVLDGLVMTTISSSKDCMGEAVQNTVEDLKLNTLGR

SEQ ID NO:70 Fel d 2 amino acids (25-608)

EAHQSEIAHRFNDLGEHFRGLVLVAFSQYLQQCPFEDHVKLNVNEVTEFAKGCVADQSAANCEKSLHELL
GDKLCTVASLRDKYGEMADCCEKKEPERNECFHQHDDNPGFGQLVTPEADAMCTAFHENEQRFLGKYLY
EIARRHPYFYAPELLYYAEEYKGVFTECCEAADKAACTPKVDALREKVLASSAKERLKCASLQKFGERA

For the constructs, the sequences are highlighted as follows: **hLAMP Signal peptide**-hLAMP Luminal domain-CTCGAG-Antigen-GAATTC-hLAMP TM and Cyto domain

FKAWSVARLSQKFPKAEFAEISKLVTDLAKIHKECCHGDLLECADDRADLAKYICENQDSISTKLKECCG
KPVLEKSHCISEVERDELPADLPPLAVDFVEDKEVCCKNYQEAKDVFLGTFLYEYSRRHPEYSVSLLLRLA
KEYEATLEKCCATDDPPACYAHVFDEFKPLVEEPHNLVKTNCELFEKLGEYGFQNALLVRYTKKVPQVST
PTLVEVSRSLGKVGSKCCTHPEAERLSAEDYLSVVLNRLCVLHEKTPVSESVTKCCTESLVNRRPCFSA
LQVDETYVPKEFSAETFTFHADLCTLPEAEKQIKKQSALVELLKHKPKATEEQLKTVMGDFGSFVDKCCA
AEDKEACFAEEGPKLVAAAQAALA

SEQ ID NO:74 Fel d 4 amino acids (16-186)

HEENVVRSNIDISKISGEWYSILLASDVKEKIEENGSMRVFVEHIKALDNSSLFVFHTKENGKCTEIF
LVADKTKDGVYTVVYDGYNVFSIVETVYDEYILLHLLNFDKTRPQLVEFYAREPDVSQKLKEKFVKYCQ
EHGIVNILDLTEVDRCLQARGSEVAQDSSVE

SEQ ID NO:78 Lit v 1 amino acids (1-284)

MDAIIKKKMQAMKLEKDNAMDRADTLEQQNKEANNRAEKSEEEVHNLQKRMQQLENDLDQVQESLLKANIQ
LVEKDKALSNAEGEVAALNRRIQLLEEDLERSEERLNTATTKLAEASQAADESERMKVLENRSLSDDEER
MDALENQLKEARFLAEADRKYDEVARKLAMVEADLERAEERAETGESKIVELEELRVVGNLNLSLEVS
EELKANQREEAYKEQIKTLTNKLKAAEARAFAERSVQKLQKEVDRLEDELVNEKEKYKSI TDELDQTFSE
LSGY

SEQ ID NO:82 Lol p 5a amino acids (26-272)

ADAGYTPAAAATPATPAATPAAAGGKATTDEQKILLEDVNA GFKA AVAAAAANAPPADKFKIFEAAFSSESK
 GLLATSAAKAPGLIPKLDTA YDVAYKAAEATPEAKYDAFVTALTEALRVIAGALEVHAVKPAATEEVLAAK
 IPTGELQIVDKIDA AFKIAATAANAAPTNDKFTVFESAFN KALNECTGGAYETYKF IPSLEAAVKQAYAA
 TVAAAPEVKYAVFEAALTKA ITAMTQAOKAGKPAAAA

SEQ ID NO:86 Ph1 p 1 amino acids (24-263)

IPKVPPGPNI TATYGDKWLD AKSTWYGKPTGAGPKDNGGACGYKD VDKPPFSGMTGCGNTP IFKSGRGCG
SCFEIKCTKPEACSGEPVVVHI TDDNEEPI APYHFDLSGHAFGAMAKKGDE QKLRSAGELELQFRRVCKC
YPEGTKVTFHVEKGSNP NYLALLVKYVNGDGDVVAVDI KEKGKDKWIELKESWGAIWRIDTPDKLTGPFT
VRYTTEGGTKTEAEDVIPEGWKADTSYESK

SEQ ID NO:90 Ph1 p 5 amino acids (25-288)

AADLGYPATPAAPAAGYTPATPAAPAEAAPAGKATTEEQKLIKINAGFKAALAAAAGVQPADKYRTFV
ATFGAASNKAFAEGLSGEPKGAAESSKAALTSKLDAAKLYKTAEGATPEAKYDAYVATLSEALRIIA
GTLEVHAVKPAEEVKVIPAGELQVIEKVDAAFKVAATAANAAPANDKFTVFEEAFNDAIKASTGGAYES
YKFIPALEAAVKOAYAATVATAPEVKYTVFETALKKAITAMSEAOKAAKPAAAA

SEQ ID NO:93 Der f 15 amino acids (20-555)

SIKRDHNDYSKNPMRIVCYVGTWSVYHKVDPYTIEDIDPFKCTHLMYGFAKIDEYKYTIQVFDPYQDDNH
NSWEKRGYERFNNLRLKNPELTTMISLGGWYEGSEKYSDMAANPTYRQQFIQSVLDLFLQEYKFDGLDLDW
EYPGSRGLGNPKIDKQNYLALVRELKDAFEPHGYLLTAAVSPGKDKIDRAYDIKELNKLFDWMNVMTYDYH
GGWENFYGHNAPLYKRPDETDELHTYFNVNYTMHYLLNNGATRDKLVMGVFPFYGRAWSIEDRSKCLKLGP
AKGMSPPGFISGEEGVLSYIELCQLFQKEEWHIQYDEYYNAPYGYNDKIWVGYYDDLASISCKLAFLKELG
VSGVMVWSLENDDFKGHCGRPKNPLLNKVNHNMGDEKNSFECILGPSTTTPTTTPTTTPTTTPTTPTTPT
TPTTTPTTPTTTPTTPTTPTTPTTPTTPTTPTTPTTPTTPTTPTTPTTPTTPTTPTTPTTPTTPTTPTTPT
EGDIPHPTNIHKYLVCFEVNGGWVHIMPCPPGTIWCOEKLTCIGE

For the constructs, the sequences are highlighted as follows: **hLAMP Signal peptide**-hLAMP Luminal domain –CTCGAG-Antigen-GAATTC-hLAMP TM and Cyto domain

SEQ ID NO:94 Der f 18 amino acids (18-462)

SNIRPNVATLEPKTVCCYYESVWHWRQGEKGMDPEDIDTSLCTHIVYSYFGIDAATHEIKLLDEYLMKDLH
DMEHFTQHKGNAMIAVGGSTMSDQFSKTAAVEHYRETFVSTVDLMTRYGFDGVMIDWSGMQAKDSND
FIKLLDKFDENYNIPAI SNYVDFMNVLSLDYTGSWAHTVGHASPFPEQLKLTLEAYHKRGAPRHKMVMVAVP
FYARTWILEKMNKQDIGDKASGPGPRGQFTQTDGFLSYNELCVQIQAEFTNAFTITRDHDNTAIYAVYVHS
NHAEWISFEDRHTLGEKAKNITQQGYAGMSVYTL SNEDVHGVCGDKNPL LHAIQSNYYHGVVTEPTVVTL
PPVTHTEHVTDIPGVFHCHEEGFFRDKTYCATYYECKKGDFGLEKTVHHCANHLQAFDEVSRCTCIDHTK
IPGC

SEQ ID NO:95 Zen-1 amino acids (23-500)

NPRFKRDNRDDVLKQTEELIKSAQDVLEKLPDSDLKDEIAEKLATMKHYKHELENAKNPiKIAHFELELL
 TMFKKFQSLLEANEIiKSLTTTTTEPTTPTPEPTTTTTPEPTTKTPEPTTKTPEPTTPTPEPTTKTPEPT
 TKTPEPTTPTPEPTTKTPEPTTPTPEPTTKTPEPTTKTPEPSTPTPEPTTKTPEPTTKTPEPSTPTPEPT
 TKTPEPSTPTPEPTTKTPEPTTKTPEPSTPTPEPTTKTPEPSTPTPEPTTKTPEPSTTKKPNRDDVLKQA
 EELIKRAEDVFEKLPDSDLKNEIAEKLATMKNYKHELENAKNPiKIAHLESELLTMFKMFQSLLEADEI
 IRSLLLLTEPTTLNSTTPEPTTLNSTTPEPTTLNSTTPEPTTLNSTTPEPTTLNSTTPEPTTLNSTTPEP
 TTLNSTTPEPTTLNSTTPEPTTNSNTTSEPTNSINRKTSEISFLSDWFHKIRTRFNIF

SEQ ID NO:96 Cte f 1 amino acids (19-176)

EDIWKVNKKCTSGGKNQDRKLDQIIQKGQQVKIQNICKLIRDKPHTNQEKEKCMKFCKKVCKGYRGACDG
NICYCSRPSNLGPDWKSKECKDPNNKDSRPTEIVPYRQQLAIPNICKLKNSETNEDSKCKKHCKEKCGRG
GNDAGCDGNFCYCRPKNK

SEQ ID NO:109: Der F15 **GPGPG** Der F18 (GPGPG is one example of a linker that can be used)

[illegible]

Der F1 **GP**GP Der F2 (SEQ ID NO:110) (with exemplified GP linker)

SIKRDHNDYSKNPMRIVCYVGTWSVYHKVDPYTIEDIDPFKCTHLMYGFAKID EYKTIQVDFDPYQDDNHNSWEKRGY
ERFNNLRLKNPELTTMISLGGWYEGSEKYSDMAANPTYRQQFIQSVLDFLQEYKFDGLDLDW EYPGSRLGNPKIDKQN
YLALVRELKDAFEPHGYLLTAAVSPGKDKIDRAYDIKELNKLFDWMNVMTYDYHGGWENFYGH NAPLYKRPEDETDEL
HTYFENVNYTMHYLLNNGATRDKLVMGVPFYGRAWSIEDRSKLLGDPAKGMSPPGFISGEEGVLSYIELCOLFQKEFW

For the constructs, the sequences are highlighted as follows: *hLAMP Signal peptide*-hLAMP Luminal domain-CTCGAG-Antigen-GAATTC-hLAMP TM and Cyto domain

HIQYDEYYNAPYGYNDKIWVGYYDDLASISCKLAFLKELGVSGVMVWSLENDDFKGHCGRPKNPLLNKVHNMINGDEKN
SFECILGPSTTTPTPTTTPTPTTTPTTSPPTTTPTTSPPTTTPTTSPPTTTPTTPTPAPTSTPSPTTTEHTSET
PKYTTYVDGHLIKCYKEGDIPHPTNIHKYLVCEVNGGWVWHIMPCPPGTIWCQEKLTICIGEGPGPGSNIRPNVATLEP
KTVCIYESWVHWRQGEKMDPEDIDTSLCTHIVSYFGIDAATHEIKLLDEYLMKDLHDMEHFTQHKGNAMIAVG
GSTMSDQFSKTAAVEHYRETFVSTVDLMTRYGFDGVMIDWSGMQAKSDNFIKLLDKFDENYNIPAINYVDFMNV
LSLDYTGSWAHTVGHASPFPEQLKLEAYHKRGAPRHKMVMVAVPFYARTWILEKMNKQDIGDKASGPGPRGQFTQT
DGFLSYNELCVQIQAEETNAFTITRDHDNTAIYAVVHSNHAEWISFEDRHTLGEKAKNITQQGYAGMSVYTLNEDVH
GVC GDKNPLLHAIQSNNYHGVVTEPTVVTLPVTHTEHVTIDIPGVFHCHEEGFFRDKTYCATYYECKGDFGLEKTVH
HCANHLQAFDEVSRCTCIDHTKIPGC

Allergen X is Amb a 1

atggcgccccgcagcgccggcgacccctgctgctgctactgctgttgctgctgctcggcctcatgcattgtgctcagcagcaatgtttatggtg
aaaaatggcaacgggacgcgtgcataatggccaacttctctgctgccttctcagtgaactacgacaccaagagtggccctaagaacatgaccttga
cctgccatcagatgccacagtgggtgctcaaccgcagctcctgtggaaaagagaacacttctgacccagctcctgtgattgcttttgaagaggacatac
actcactctcaatttcagagaaatgcaacacgttacagcgtccagctcatgagttttgtttataactgtcagacacacaccttttcccaatgcgagct
ccaaagaaatcaagactgtggaatctataactgacatcagggcagatatagataaaaaatacagatgtgttagtggcaccaggtccacatgaaca
acgtgaccgtaacgctccatgatgccaccatccaggcgtacctttccaacagcagcttcagccgggagagacacgctgtgaacaagacaggccttc
cccaaccacagcggccctgcgccaccagccctcgcctcaccgctgcccagagccctctgtggacaagtacaacgtgagcggcaccaacggg
acctgcctgctggcagcatggggctgcagctgaacctcacctatgagagggaaggacaacacgacggtgacaaggcttccaacatcaacccaaca
agacctggccagcgggagctgcggcgccacctgggtgactctggagctgcacagcagggcaccacgctcctgctcttcagttcgggatgaatga
agttctagccggttttctacaaggaaatccagttgaatacaattcttctgacgccagagacctgcctttaagctgccaacggctccctgcgagcgc
tgcaggccacagtcggcaattcctacaagtgaacgcggaggagcacgtccgtgtcacgaaggcgttttcagtcataatattcaagtggtggtccag
gctttcaaggtggaaggtggccagtttgctctgtggaggagtgtctgctggacgagaacagcCTCGAGgcccaggatctgcaagagattctcccc
gtgaacgagacacggcggctgacaacaagcggcgctacaacatcatcgacggctgctggcggggcaaggccgattgggcccagagaacagaaaaggc
tctggccgattgcgccaaggtctcggaagggaacagtggcggaaggacggcgacatctacaccgtgaccagcgagctggacgacgacgtggc
caatcccaaagagggcacactgagattcgagccgccagaaccggcctctgtgagatcatcttcagcgggacatggtcatccggctggacaaagaa
atggtcgtgaacagcgacaagaccatcgacggaagaggcgccaaagtggaaatcatcaacgccggcttcacactgaacggcgtgaagaacgtgatc
atccacaacatcaacatgcacgacgtgaaagtgaatccggcgactgatcaagagcaacgatggcccagccgcccctagagccggatctgatggc
gagccatttccatcagcggcagctctcagatctggatcgaccactgctctctgagcaagagcgtggacggactcgtggacgccaagctgggcaccac
aagactgacctgtccaactctctgttcacccagcaccagttcgtgctgctgttcggcgctggcgacgagaacatcgaggataggggcatgctggcca
ccgtggccttcaacacattcaccgacaacgtggaccagcggatgccagatgccggcacggcttctccaagtcgtgaacaacaactacgataagt
gggcagctacgcatcgcggtctgcccagccctaccattctgagccaaggcaaccgggtctgcgcccagacgagcggagcaagaagaatgtgctg
ggacggcacggcggaagccgctgaatccatgaagtggaaactggcggaaccaaggacgtgctggaaaacggcgccatcttctggcctctggc
gtggaccagtgctgacaccagaacagagcggcgcatgattccagccgagccggcggaatctgctctgtctgacaagctctcgccggcgtgctgag

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ctgtcagcccgagcaccatgtGAATTCacgtgatcccatcgctgtgggtggcgccctggcgggctggctctcatgctcctcatgcctacctgt
cggcaggaagaggagtcacgcaggctaccagactatctagtaa (SEQ ID NO:30)

Allergen X is Bet v 1-A

atggcgccccgcagcgcccgcgacccctgctgctgctactgctgttgctgctgctcggcctcatgcattgtgctcagcagcaatgtttatggg
aaaaatggcaacgggacgcgtgcataatggccaacttctctgctgccttctcagtgaactacgacaccaagagtggcctaagaacatgacccctga
cctgccatcagatgccacagtgggtgctcaaccgcagctcctgtggaaaagagaacacttctgacccagctcctgtgattgctttggaagaggacatac
actactctcaatttcacgagaaatgcaacacgttacagcgtccagctcatgagtttgtttataactgtcagacacacacctttcccaatgcgagct
ccaaagaaatcaagactgtggaatctataactgacatcagggcagatatagataaaaaatacagatgtgttagtggcaccagggtccacatgaaca
acgtgaccgtaacgctccatgatgccaccatccaggcgtacctttccaacagcagcttcagccggggagagacacgctgtgaacaagacaggccttc
cccaaccacagcgccccctgcgccaccagccctcgcctcaccctgtgccaaagagccctctgtggacaagtacaacgtgagcggcaccaacggg
acctgcctgtggccagcatggggctgcagctgaacctcacctatgagagggaaggacaacacgacgggtgacaaggcttctcaacatcaacccaaca
agacctggccagcgggagctgcggcgccacctgggtactctggagctgcacagcgagggcaccaccgtcctgctcttcagttcgggatgaatgca
agttctagccggttttctacaaggaaatccagttgaatacaattcttctgacgccagagacctgcctttaagctgccaacgggtccctgcgagcgc
tgcaggccacagtcggcaattcctacaagtgaacgcggaggagcacgtccgtgtcacgaaggcgttttcagtcaatatattcaagtggtgggtccag
gctttcaagtggaaggtggccagtttgctctgtggaggagtgctgctggacgagaacagcCTCGAGggcgtgttcaactacgagacagagaca
accagcgtgatcccagcggccagactgttcaaggccttattctggacggcgacaatctgttcccaaagtggcccccaagccatcagcagcgtgga
aaacatcgagggcaatggcggaccggcaccatcaagaagatcagcttccccgagggttccattcaatacgtgaaggaccgggtggacgaagt
ggaccacaccaacttcaagtacaactactcctgatcgaggcgccgacccatcgcgacacactggaaaagatcagcaacgagatcaagatcgtggc
caccgccagcggcgagcattctgaagatctccaacaagtaccacacaaaggcgaccacgaagtgaaggccgaacaagtgaagccagcaaa
agatggcgagacactgctgcggcgctgaaaagctatctgctgcccacagcgacgcctacaacGAATTCacgtgatcccatcgctgtgggtg
gtgcctggcgggctggctcctcatgctcctcatgcctacctcgtcggcaggaagaggagtcacgcaggctaccagactatctagtaa (SEQ ID
NO:34)

atggcgccccgcagcgcccgcgacccctgctgctgctactgctgttgctgctgctcggcctcatgcattgtgctcagcagcaatgtttatggg
aaaaatggcaacgggacgcgtgcataatggccaacttctctgctgccttctcagtgaactacgacaccaagagtggcctaagaacatgacccctga
cctgccatcagatgccacagtgggtgctcaaccgcagctcctgtggaaaagagaacacttctgacccagctcctgtgattgctttggaagaggacatac
actactctcaatttcacgagaaatgcaacacgttacagcgtccagctcatgagtttgtttataactgtcagacacacacctttcccaatgcgagct
ccaaagaaatcaagactgtggaatctataactgacatcagggcagatatagataaaaaatacagatgtgttagtggcaccagggtccacatgaaca
acgtgaccgtaacgctccatgatgccaccatccaggcgtacctttccaacagcagcttcagccggggagagacacgctgtgaacaagacaggccttc

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cccaaccacagcgcctcctgcgccaccagccctcgcctcaccctgcccagagccctctgtggacaagtacaacgtgagcggcaccaacggg
 acctgcctgctggccagcatggggctgcagctgaacctcacctatgagaggaaggacaacacgacgggtgacaaggcttctcaacatcaacccaaca
 agacctcggccagcgggagctgcggcgccacctggtagctctggagctgcacagcaggggcaccaccgtcctgctctccagttcgggatgaatgca
 agttctagccgggttttctacaaggaatccagttgaatacaattcttctgacgccagagaccctgcctttaaagctgccaacggctcctgcgagcgc
 tgcaggccacagtcggcaattcctacaagtgaacgcggaggagcacgtccgtgtcacgaaggcgttttcagtcaatatattcaagtggtgggtccag
 gctttcaaggtggaaggtggcagtttggtctgtggaggagtgtctgctggacgagaacagcCTCGAGcaagacacccccgctctgggaaggat
 accgtggcctgtccggcaagtggatctgaaggccatgaccgccgaccaagaagtgccgagaagcccgatagcgtgacccccatgattctgaaag
 ccagaaggcgccgaatctggaagccaagatcacctatgctgaccaacggccagtgccagaacatcacctggtgtctgcacaagaccagcgagcccg
 gcaagtacacagcctacgagggccagcgggtggtgttcatccagccttctccagtgccggatcactacattctgtactgcgagggcgagctgcacggc
 cggcagatcagaatggccaagctgctgggcagagatcccgagcagagccaagaggctctggaagatttcagagagttcagccgggccaaggagctg
 aaccaagagattctggaactggctcagagcgagacatgctctcccgcgacaaGAATTCacgctgatccccatcgctgtgggtgggtgcctggcg
 gggctggtcctcatcgtcctcatcgctacctcgtcggcaggaagaggagtcacgcaggctaccagactatctagtaa (SEQ ID NO:38)

Allergen X is Cyn d 1

atggcgccccgagcgcggcgacccctgctgctgctactgctgttggctgctgctggcctcatgcattgtgctcagcagcaatgttatgggt
 aaaaatggcaacgggaccgctgcataatggccaacttctctgctgccttctcagtgaaactacgacaccaagagtggcctaagaacatgaccctga
 cctgccatcagatgccacagtggtgctcaaccgcagctcctgtggaaaagagaacacttctgacccagctcctgtgattgcttttgaagaggacatac
 actcactctcaatttcacgagaaatgaacacgttacagcgtccagctcatgagttttgtttataacttgcagacacacaccttttcccaatgcgagct
 ccaaagaaatcaagactgtggaatctataactgacatcagggcagatatagataaaaaatacagatgtgttagtggcaccaggtccacatgaaca
 acgtgaccgtaacgctccatgatgccaccatccaggcgtacctttcaacagcagcttcagccggggagagacacgctgtgaacaagacaggccttc
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 acctgcctgctggccagcatggggctgcagctgaacctcacctatgagaggaaggacaacacgacgggtgacaaggcttctcaacatcaacccaaca
 agacctcggccagcgggagctgcggcgccacctggtagctctggagctgcacagcaggggcaccaccgtcctgctctccagttcgggatgaatgca
 agttctagccgggttttctacaaggaatccagttgaatacaattcttctgacgccagagaccctgcctttaaagctgccaacggctcctgcgagcgc
 tgcaggccacagtcggcaattcctacaagtgaacgcggaggagcacgtccgtgtcacgaaggcgttttcagtcaatatattcaagtggtgggtccag
 gctttcaaggtggaaggtggcagtttggtctgtggaggagtgtctgctggacgagaacagcCTCGAGgccatcggcgataagcccgacccaa
 catcaccgccacatacggcagcaagtggctggaagccagagccacattctacggctccaacccagaggcggcgtcccgatgatcatggcgagct
 tggcgctacaaggacgtggacaagcccccttcgacggcatgaccgcttgcggcaacgagcccatcttaaggacggactgggctgccgggcttgc
 acgagatcaagtgcaaagaacccgtggaatgcagcggcgagcccgctgctgtgaagatcacgcagaagaactacgagcacattgccgcctaccct
 tcgatctgagcggcaaggccttttggcgccatggccaagaaggccaagaggacaagctgcggaaggccggcgaactgacactgcagtttcggagag
 tgaagtgaagtacccagcggcaccaagatcacattccacatcgagaagggcagcaacgatcactatctggctctgctcgtgaaatacgcgcgtgg

For the constructs, the sequences are highlighted as follows: *hLAMP Signal peptide*-*hLAMP Luminal domain* -CTCGAG-*Antigen*-GAATTC-*hLAMP TM and Cyto domain*

cgacggcaacatcgtggccgtggacatcaagcccagagacagcgacgagttcatccccatgaagtccagctggggcgccatctggcggatcgaccc
aaagaagcctctgaagggcccttctccatccggctgacatctgaggcgaggacacatctggtgcaagacgacgtgatccccccaactggaagccc
gacaccgtgtacaccagcaagctgcagtttggcgccGAATTCcagctgatccccatcgctgtgggtggcgccctggcgggctggcctcatcgctct
catcgctacctcgctggcaggaagaggagtcacgcaggctaccagactatctagtaa (SEQ ID NO:42)

Allergen X is Der F 1 (19-321)

atggcgccccgcagcgccggcgacccctgctgctgctactgctgttctgctgctcgccctcatgcattgtgctcagcagcaatgtttatggtg
aaaaatggcaacgggacgcgtgcataatggcaacttctctgctgccttctcagtgaactacgacaccaagagtggccctaagaacatgacccctga
cctgccatcagatgccacagtggtgctcaaccgcagctcctgtggaaaagagaacacttctgacccagctcctgtgattgcttttgaagaggacatac
actactctcaatttcacgagaaatgcaacacgttacagcgtccagctcatgagttttgttataacttgcagacacacacctttcccaatgcgagct
ccaaagaaatcaagactgtggaatctataactgacatcagggcagatatagataaaaaatacagatgtgttagtggcaccaggtccacatgaaca
acgtgaccgtaacgctccatgatgccaccatccaggcgtacctttcaacagcagcttcagccgggagagacacgctgtgaacaagacaggccttc
cccaaccacagcgccccctgcgccaccagccccctgcctcaccctgcctcagcccaagagccccctctgtggacaagtacaacgtgagcggcaccaacggg
acctgcctgctggccagcatggggctgcagctgaacctcacctatgagaggaaggacaacacgacgggtgacaaggcttctcaacatcaacccaaca
agacctcggccagcgggagctgcggcgccacctggtagctctggagctgcacagcgaggccaccacgtcctgtcttccagttcgggatgaatgca
agttctagccggttttctacaaggaatccagttgaatacaattcttctgacgccagagaccctgcctttaaagctgccaacggctccctgcgagcgc
tgcaggccacagtcggcaattcctacaagtgaacgcggaggagcacgtccgtgtcacgaaggcgttttcagtcaatatattcaagtggtgggtccag
gctttcaaggtggaaggtggcagtttggctctgtggaggagtgctgctggacgagaacagcCTCGAGaggcccgccagcatcaagacattcgaa
gagttcaagaaggcctttaacaagaactacgccaccgtggaagaggaagaagtggcccggaagaactttctggaatctctgaaatcgtggaagcc
aacaaggcgccatcaaccatctgagcgatctgtctctggacgagtttaagaaccggtatctgatgagcgccgaggccttcgagcagctgaaaacc
agttcgatctgaacgccgaaaccagcgttgccgatcaacagcgtgaacgtgccagcgagctggatctgagatctctgagaaccgtgacccccatc
agaatgcaaggcggctgcggcagctgctgggccttttagcggagtgccgcccacagagctgcctatctggcctaccggaacacatctctggatctgtc
cgagcaagaactcgtggactgcgccagccagcacggctgtcacggcgatacaatccccagaggcatcgagtacatccagcagaacggcgtcgtgga
agaacggtcctacccttacgtggcccgagcagagatgcagaaggcccaactctcagcactacggcatcagcaactactgccagatctaccccc
gacgtgaagcagatcagagaggctctgacccagaccacacccgacattgccgtgatcatcggaatcaaggatctcgggccttcagcactatgacg
gccggaccatcatccagcacgacaacggctaccagcccaactaccacgcctgaacatcgtgggtacggcagcacacaaggcgacgactactgga
tcgtcggaacagctgggacaccacatggggcgatagcggtacggctacttccaagccggcaacaatctgatgatgatcgagcagctaccctacgt
cgtgatcatgGAATTCcagctgatccccatcgctgtgggtggcgccctggcgggctggcctcatcgctcctcatcgctacctcgctcgccaggaaga
ggagtcacgcaggctaccagactatctagtaa (SEQ ID NO:46)

For the constructs, the sequences are highlighted as follows: *hLAMP Signal peptide*-*hLAMP Luminal domain*-CTCGAG-*Antigen*-GAATTC-*hLAMP TM and Cyto domain*

Allergen X is Der F 1 (99-321)

atggcgccccgcagcgccggcgaccctgtgctgtactgtgttgctgctgctcggcctcatgcattgtgctcagcagcaatgtttatgggtg
 aaaaatggcaacgggaccgctgcataatggccaacttctctgctgccttctcagtgaactacgacaccaagagtggccctaagaacatgacccttga
 cctgccatcagatgccacagtgggtgctcaaccgcagctcctgtggaaaagagaacacttctgacccagctcctgtgattgcttttgaagaggacatac
 actcactctcaatttcacgagaaatgcaacacgttacagcgtccagctcatgagttttgtttataactgtcagacacacaccttttcccaatgcgagct
 ccaaagaaatcaagactgtggaatctataactgacatcagggcagatatagataaaaaatacagatgtgttagtggcaccaggtccacatgaaca
 acgtgaccgtaacgctccatgatgccaccatccaggcgtacctttccaacagcagcttcagccggggagagacacgctgtgaacaagacaggccttc
 cccaaccacagcgccccctgcgccaccagccctcgccctcaccgtgcccaagagccctctgtggacaagtacaacgtgagcggcaccaacggg
 acctgcctgtggtccagcatggggctgcagctgaacctcacctatgagagggaaggacaacacgacgggtgacaaggcttctcaacatcaacccaaca
 agacctcgccagcgggagctgcggcgccaccctgggtgactctggagctgcacagcgagggcaccaccgtcctgctcttccagttcgggatgaatgca
 agttctagccggttttctacaaggaaatccagttgaatacaattcttctgacgccagagaccctgcctttaaagctgccaacgggtccctgcgagcgc
 tgcagggcacagtcggcaattcctacaagtgaacgcggaggagcacgtccgtgtcacgaaggcgttttcagtcataatattcaaaagtgtgggtccag
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 aacgtgcccagcgagctggatctgagatctctgagaaccgtgacccccatcagaatgcaaggcggctgcggcagctgctgggccttttagcggagtgg
 ccgccacagagtctgcctatctggcctaccggaacacatctctggatctgtccgagcaagaactcgtggactgcgccagccagcacggctgtcacggc
 gatacaatccccagaggcatcgagtacatccagcagaacggcgtcgtggaagaacggctctacccttacgtggcccgagcagagatgcagaagg
 cccaactctcagcactacggcatcagcaactactgccagatctaccccccgacgtgaagcagatcagagaggctctgacccagacccacaccgcca
 ttgccgtgatcatcggaatcaaggatctgcgggccttcagcactatgacggccggaccatcatccagcacgacaacggctaccagcccaactaccac
 gccgtgaacatcgtgggtacggcagcacacaaggcgacgactactggatcgtgcggaacagctgggacaccacatggggcgatagcggctacggc
 tacttccaagccggcaacaatctgatgatgatcagcagctacccctacgtcgtgatcatgGAATTCacgctgatccccatcgctgtgggtgggtgcctt
 ggccggggctggctcctcatcgtcctcatcgctacctcgtcggcagggaagaggagtcacgcaggctaccagactatctagtaa (SEQ ID NO:50)

Allergen X is Der P2

atggcgccccgcagcgccggcgaccctgtgctgtactgtgttgctgctgctcggcctcatgcattgtgctcagcagcaatgtttatgggtg
 aaaaatggcaacgggaccgctgcataatggccaacttctctgctgccttctcagtgaactacgacaccaagagtggccctaagaacatgacccttga
 cctgccatcagatgccacagtgggtgctcaaccgcagctcctgtggaaaagagaacacttctgacccagctcctgtgattgcttttgaagaggacatac
 actcactctcaatttcacgagaaatgcaacacgttacagcgtccagctcatgagttttgtttataactgtcagacacacaccttttcccaatgcgagct
 ccaaagaaatcaagactgtggaatctataactgacatcagggcagatatagataaaaaatacagatgtgttagtggcaccaggtccacatgaaca
 acgtgaccgtaacgctccatgatgccaccatccaggcgtacctttccaacagcagcttcagccggggagagacacgctgtgaacaagacaggccttc
 cccaaccacagcgccccctgcgccaccagccctcgccctcaccgtgcccaagagccctctgtggacaagtacaacgtgagcggcaccaacggg
 acctgcctgtggtccagcatggggctgcagctgaacctcacctatgagagggaaggacaacacgacgggtgacaaggcttctcaacatcaacccaaca

For the constructs, the sequences are highlighted as follows: *hLAMP Signal peptide*-*hLAMP Luminal domain* -CTCGAG-*Antigen*-GAATTC-*hLAMP TM and Cyto domain*

agacctcggccagcgggagctcggcgcccacctggtgactctggagctgcacagcgagggcaccaccgtcctgctcttcagttcgggatgaatgca
agttctagccggtttttcctacaaggaatccagttgaatacaattcttctgacgccagagaccctgcctttaaagctgccaacggctccctgcgagcgc
tgcaggccacagtcggcaattcctacaagtgaacgcggaggagcacgtccgtgtcacgaaggcgttttcagtcaatatattcaagtgtgggtccag
gctttcaagggtggaagggtggcagtttgctctgtggaggagtgctgctggacgagaacagcCTCGAGgaccaagtggacgtgaaggactgcgc
caaccacgagatcaagaaagtgtggtgcccggctgccacggcagcgagccttgatcatccaccggggcaagcccttcagctggaagccgtgttcg
aggccaaccagaacaccaagaccgccaagattgagatcaaggccagcatcgacggactggaagtggatgtgcccgcatcgacccaacgcttgct
actacatgaagtgcctctcgtgaaggccagcagtagacatcaagtacacatggaacgtgcccaagatcgccccaagagcgcgagaacgtcgtcgt
gaccgtgaaagtgtatggcgacgacggcgtgctggtgctgcccattgccacacagccaagatccgggacGAATTCacgctgatcccatcgctgtg
gggtggtgccctggcggggctggtcctcatcgtcctcatcgctacctcgtcggcaggaagaggagtcacgcaggctaccagactatctagtaa
(SEQ ID NO:54)

Allergen X is DerF2

atggcggcccgagcgccggcgacccctgctgctgactgctgttctgctgctcggcctcatgcattgtgctcagcagcaatgtttatggtg
aaaaatggcaacgggaccgctgcataatggccaacttctctgctgccttctcagtgaactacgacaccaagagtggccctaagaacatgacccctga
cctgccatcagatgccacagtggtgctcaaccgcagctcctgtggaaaagagaacacttctgacccagctcctgtgattgcttttgaagaggacatac
actactctcaatttcacgagaaatgaacacgttacagcgtccagctcatgagttttgttataacttgcagacacacaccttttcccaatgcgagct
ccaaagaaatcaagactgtggaatctataactgacatcagggcagatatagataaaaaatacagatgtgttagtggcaccaggtccacatgaaca
acgtgaccgtaacgctccatgatgccaccatccaggcgtacctttccaacagcagcttcagccggggagagacacgctgtgaacaagacaggccttc
cccaaccacagcggccctgcgccaccagccctcgccctaccctgtgcccaagagccctctgtggacaagtacaacgtgagcggcaccaacggg
acctgcctgctggccagcatggggctgcagctgaacctcacctatgagaggaaggacaacacgacggtgacaaggcttctcaacatcaacccaaca
agacctcggccagcgggagctcggcgcccacctggtgactctggagctgcacagcgagggcaccaccgtcctgctcttcagttcgggatgaatgca
agttctagccggtttttcctacaaggaatccagttgaatacaattcttctgacgccagagaccctgcctttaaagctgccaacggctccctgcgagcgc
tgcaggccacagtcggcaattcctacaagtgaacgcggaggagcacgtccgtgtcacgaaggcgttttcagtcaatatattcaagtgtgggtccag
gctttcaagggtggaagggtggcagtttgctctgtggaggagtgctgctggacgagaacagcCTCGAGgatcaggtggatgtcaaggactgtgct
aacaacgaaatcaagaaagtcatggtggacggatgtcacgggagcgacccctgtattatccaccggggaaagcccttcacactggaggccctgtttg
atgctaaccagaataccaagacagcaaaaatcgagggtcaaagccagcctggacggcctggaaatcgatgtgccagggttgacaccaacgcttgcc
attcgtcaagtgtcccctggtgaaaggccagcagtagacatcaagtatacttgaacgtccccaagattgccctaaatccgaaaatgtggtcgtga
ccgtgaaactgattggagacaacggcgtcctggcctgtgctatcgcaactcacgggaagattagagacGAATTCacgctgatcccatcgctgtgg
gtggtgccctggcggggctggtcctcatcgtcctcatcgctacctcgtcggcaggaagaggagtcacgcaggctaccagactatctagtaa (SEQ
ID NO:58)

For the constructs, the sequences are highlighted as follows: *hLAMP Signal peptide*-*hLAMP Luminal domain*-CTCGAG-*Antigen*-GAATTC-*hLAMP TM and Cyto domain*

Allergen X is DerP1 (del)

atggcgccccgcagcgccggcgacccctgtgctgtactgtgttgctgctgctcggcctcatgcattgtgctcagcagcaatgtttatggtg
aaaaatggcaacgggaccgctgcataatggccaacttctctgctgccttctcagtgaactacgacaccaagagtggcctaagaacatgacccttga
cctgccatcagatgccacagtgggtgctcaaccgcagctcctgtggaaaagagaacacttctgacccagctcctgtgattgcttttgaagaggacatac
actcacttcaatttcacgagaaatgcaacacgttacagcgtccagctcatgagttttgtttataacttgcagacacacaccttttcccaatgcgagct
ccaaagaaatcaagactgtggaatctataactgacatcagggcagatatagataaaaaatcacagatgtgttagtggcaccaggtccacatgaaca
acgtgaccgtaacgctccatgatgccaccatccaggcgtacctttccaacagcagcttcagccggggagagacacgctgtgaacaagacaggccttc
cccaaccacagcggccctgcgccaccagccctcgccctcacccgtgcccaagagccctctgtggacaagtacaacgtgagcggcaccaacggg
acctgcctgtggtccagcatggggctgcagctgaacctcacctatgagaggaaggacaacacgacgggtgacaaggcttctcaacatcaacccaaca
agacctggccagcgggagctcgccgcccacctgggtgactctggagctgcacagcagggcaccaccgtcctgctcttcagttcgggatgaatgca
agttctagccggttttctacaaggaaatccagttgaatacaattcttctgacgccagagaccctgcctttaagctgccaacgggtccctgcgagcgc
tgcaggccacagtcggcaattcctacaagtgaacgcggaggagcacgtccgtgtcacgaaggcgttttcagtcaatatattcaagtggtgggtccag
gctttcaagtggaagggtggccagtttgctctgtggaggagtgtctgctggacgagaacagcCTCGAGaccaacgcttgctctatcaacggaac
gccccgccgaaatcgacctgaggcagatgaggactgtcacaccattaggtccggagtggccgctactgagctgcctacctggcttatcgaaatca
gagtctggacctggcagagcaggaactgggtgattgcgccagccagcacaatgggtgtcatggagaccatccaaggggaatcgaatacttca
gcacaacggcgtggtccaggagtcatactatagatatgtggcccgcaacagagctgccgaagaccaaagtctcagaggttcggcatcagtaactac
tgtcagattatcctcagaacgtgaataagatccgagaggcactggcacagaccactccgctatcgagtcattcattgggattaaagacctggatgc
ctttcgacattacgacggcggaacatcattcagagagataacggataaccagccaattatcatgctgtgaacatcgctggctactcattacacagg
gggtggattattggattgtccggaactcttgggacacaaactggggcgataatggatatggctatttcgccccaacattgacctgatgatgattgaag
agtatccttacgtggtgatcctGAATTCacgctgatccccatcgctgtgggtgggtgccctggcggggctggtcctcatgctcctcatgcctacctcg
tcggcaggaagaggagtacgcaggctaccagactatctagtaa (SEQ ID NO:62)

atggcgccccgcagcgccggcgacccctgtgctgtactgtgttgctgctgctcggcctcatgcattgtgctcagcagcaatgtttatggtg
aaaaatggcaacgggaccgctgcataatggccaacttctctgctgccttctcagtgaactacgacaccaagagtggcctaagaacatgacccttga
cctgccatcagatgccacagtgggtgctcaaccgcagctcctgtggaaaagagaacacttctgacccagctcctgtgattgcttttgaagaggacatac
actcacttcaatttcacgagaaatgcaacacgttacagcgtccagctcatgagttttgtttataacttgcagacacacaccttttcccaatgcgagct
ccaaagaaatcaagactgtggaatctataactgacatcagggcagatatagataaaaaatcacagatgtgttagtggcaccaggtccacatgaaca
acgtgaccgtaacgctccatgatgccaccatccaggcgtacctttccaacagcagcttcagccggggagagacacgctgtgaacaagacaggccttc
cccaaccacagcggccctgcgccaccagccctcgccctcacccgtgcccaagagccctctgtggacaagtacaacgtgagcggcaccaacggg
acctgcctgtggtccagcatggggctgcagctgaacctcacctatgagaggaaggacaacacgacgggtgacaaggcttctcaacatcaacccaaca

For the constructs, the sequences are highlighted as follows: *hLAMP Signal peptide*-hLAMP Luminal domain-CTCGAG-Antigen-GAATTC-hLAMP TM and Cyto domain

agacctcgccagcgaggagctgcggcgccacctggtgactctggagctgcacagcgagggcaccaccgtcctgctctccagttcgggatgaatgca
agttctagccggttttctacaaggaatccagttgaatacaattcttctgacgccagagaccctgcctttaaagctgccaacggctcctcgagcg
tgcaggccacagtcggcaattcctacaagtgaacgcggaggagcacgtccgtgtcacgaaggcgttttcagtcaatatattcaagtgtgggtccag
gctttcaagggtgaagggtggcagtttgctctgtggaggagtgtctgctggacgagaacagcCTCGAGgaaatctgccagccgtgaagcgggat
gtggatctgtttctgaccggcaccggcagcagtagctggaacaagtggccagtagacaaggctctgccgtggtgctggaacaccccgattctgaa
gaactgcgtggacgccaagatgaccgaagaggacaaagagaacgctctgtctctgtggacaagatctacaccagccctctgtgtggcgccgagg
atctggcgaggcggaagtggcgagggggctctgtgaagatggccgagacatgccccatcttctacgacgtgttcttccgtggccaacggcaac
gagctgctgctgagctgagctgaccaaagtgaacgccaccgagcccgagcgagccgacccatgaagaagatccaagactgctacgtggaacacgga
ctgatcagccgggtgctggacggactcgtgatgaccaccatcagcagctccaaggactgcatggcgaggccgtgcagaacaccgtggaagatctga
agctgaacacactggcgccggGAATTCacgctgatccccatcgctgtgggtggtgccctggcggggctggtcctcatcgtcctcatgcctacctcgt
ggcaggaagaggagtcacgcaggctaccagactatctagtaa (SEQ ID NO:67)

atggcgccccgagcgccggcgacccctgctgctgactgctgttctgctgctcgccctcatgcattgtgcgtcagcagcaatgtttatggtg
aaaaatggaacgggaccgctgcataatggccaattctctgctgccttctcagtgaaatcagacaccaagagtggcctaagaacatgacccctga
cctgccatcagatgccacagtggtgctcaaccgcagctcctgtggaaaagagaacacttctgacccagctcctgtgattgcttttgaagaggacatac
actcactctcaatttcacgagaaatgaacacgttacagcgtccagctcatgagttttgtttataacttgcagacacacacctttcccaatgagagct
ccaaagaaatcaagactgtggaatctataactgacatcagggcagatatagataaaaaatacagatgtgttagtggcaccaggtccacatgaaca
acgtgaccgtaacgctcatgatgccaccatccaggcgtacctttccaacagcagcttcagccggggagagacacgctgtgaacaagacaggccttc
cccaaccacagcgccccctgcgccaccagccctcgcctcaccgctgcccagagccctctgtggacaagtacaacgtgagcgccaccaacggg
acctgcctgctggcagcatggggctgcagctgaacctcacctatgagagggaaggacaacacgacgggtgacaaggcttctcaacatcaacccaaca
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agttctagccggttttctacaaggaatccagttgaatacaattcttctgacgccagagaccctgcctttaaagctgccaacggctcctcgagcg
tgcaggccacagtcggcaattcctacaagtgaacgcggaggagcacgtccgtgtcacgaaggcgttttcagtcaatatattcaagtgtgggtccag
gctttcaagggtgaagggtggcagtttgctctgtggaggagtgtctgctggacgagaacagcCTCGAGgaaagcccatcagagcgagatcgccca
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cgtgaacgaagtaccgagttcgccaagggtcgtggccgatcagctgcgccaactgcgagaagtctctgcagagctgctggcgacaagctg
tgtaccgtggcctctctcgggataagtacggcgagatggccgactgttcgagaagaaagagcccgagcggaacgagtgcttctgcagcacaagg
acgacaaccccggttcggccagctcgtgacaccagaggccgatgcatgtgcaccgcttccacgagaatgagcagcggtttctgggaagatctg
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cgataaggccgcttctgctgacccccaaagtggacgcactgcgcgagaaagtgtggcctccagcgccaagaacggctgaagtgcgcttctctgcag
aagttcgcgagcgggccttcaaggcttgagcgtggcaagactgagccagaagttcccaaggccgagtttgcgagatcagcaagctcgtgaccg
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cagcatcagcacaagctgaaagagtgttgccgcaagccgctgctggaaaagagccactgcatcagcgaagtgaacgggacgagctgcccgtga
tctgcctcctctggcgtgacttctgtggaggacaaagaagtgtgcaagaactaccaagaggccaagatgtgtttctggggacatttctgtatgagt
ctctcgggcgaccccgagtactccgtgtctgtgctgctgcggctggccaaagagtacgaggccacactggaaaagtgtcgcgccaccgacgatcccc
ccgctgttatgccacgtgttcgacaggtcaagccactcgtggaagaaccccaacaatctcgtgaaaacaaactgcgagctgttcgagaagctgggc
gagtacggcttcagaacgcactgctcgtgcggtacaccaagaagtgtcccaagtgctccacccccacactcgtggaagtgtccagatctctgggcaa

For the constructs, the sequences are highlighted as follows: *hLAMP Signal peptide*-*hLAMP Luminal domain* -CTCGAG-*Antigen*-GAATTC-*hLAMP TM and Cyto domain*

agtgggcagcaagtgtgtcacccaccccgaggccgagagactgtcttgcgcgaggactatctgagcgtggtgctgaaccggctgtgctgtgtcac
gagaaaaaccccggtgtccgagcgcgtgaccaagtgtgtaccgagctctctgtgaacagacggccttgcttcagcgcgtctgcaagtggacgagacata
cgtgcccaaagagttcagcgcgcgagacattcacattccacgccgatctgtcacactgccgaagccgagaaagcagatcaagaaacagtccgctctc
gtggaaactgtgaagcacaagcccaaggccaccgaggaacagctgaaaaccgtgatggcgacttcggcagcttcgtggataagtgtgtgccgctg
aggacaaaagaggcttgcttcgcgaagaggcccaaacctgtggctgtgtctcaagctgtctgtgccGAATTCacgtgatccccatcgctgtgg
gtggtgccctggcggggtgtgtctcatgtctctatcgctctacctcgtcggcaggaagaggagtcacgcaggctaccagactatctagtaa (SEQ
ID NO:71)

atggcgccccgagcgcggcgacccctgtgtgtactgtgttgtgtgtgtcggcctcatgcattgtgcgtcagcagcaatgtttatgggtg
aaaaatggcaacgggaccgctgcataatggccaacttctctgtgtccttctcagtgaactacgacaccaagagtggccctaagaacatgaccttga
cctgccatcagatgccacagtgggtgtcaaccgcagctctgtggaaaagagaacacttctgacccagctctcgtgattgcttttgaagaggacatac
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ccaaagaaatcaagactgtggaatctataactgacatcagggcagatatagataaaaaatcacagatgtgttagtggcaccagggtccacatgaaca
acgtgaccgtaacgctccatgatgccaccatccaggcgtacctttccaacagcagcttcagccggggagagacacgctgtgaacaagacaggccttc
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acctgcctgtgtggcagcatggggctgcagctgaacctcacctatgagaggaaggacacacgacgggtgacaaggcttctcaacatcaacccaaca
agacctcggccagcgggagctgtggcgccacctgggtactctggagctgcacagcagggcaccaccgtcctgtctctccagttcgggatgaatgca
agttctagccggttttctcacaaggaatccagttgaatacaattcttctgacgccagagacctgacctttaaagctgccaacggctcctcgcgagcgc
tgcaggccacagtcggcaattcctacaagtgaacgcggaggagcacgtccgtgtcacgaaggcgttttcagtcaatatattcaaaagtgtgggtccag
gctttcaagggtggaagggtggcagtttggctctgtggaggagtgctgtgtgacgagaaacagcCTCGAGcacgaggaagagaacgtcgtgcggag
caacatcgacatcagcaagatcagcggcgagtggtacagcattctgtgtgcctccgacgtgaaagagaagatcgaagaaaacggcagcatcggggt
gttctgtggaacacatcaaggctctggacaacagctctctgagcttctgttccacaccaagaaaatggcaagtgcaccgagatctttctgtggccga
caagaccaaggacggcgtgtacaccgtggtgtacgacggctacaacgtgttcagcatcgtggaaccgtgtacgatgagtacattctgtcatctgc
tgaacttcgacaagacacggccttccaactcgtggagttctacgccagagaaccggacgtgtccagaagctgaaagaaaagtctgtgaagtactg
ccaagagcacggcatcgtgaacattctggatctgaccgaagtggaccggtgtctgcaagccagaggcagcgaagtggcccaagacagcagcgtgga
aGAATTCacgtgatccccatcgctgtgggtgtgtgccctggcggggtgtgtctcatgtctctacctcgtcggcaggaagaggagtcac
gcaggctaccagactatctagtaa (SEQ ID NO:75)

atggcgccccgagcgcggcgacccctgtgtgtactgtgttgtgtgtgtcggcctcatgcattgtgcgtcagcagcaatgtttatgggtg
aaaaatggcaacgggaccgctgcataatggccaacttctctgtgtccttctcagtgaactacgacaccaagagtggccctaagaacatgaccttga
cctgccatcagatgccacagtgggtgtcaaccgcagctctgtggaaaagagaacacttctgacccagctctcgtgattgcttttgaagaggacatac
actactctcaatttcacgagaaatgaacacgttacagcgtccagctcatgagttttgtttataactgtcagacacacacctttcccaatgcgagct
ccaaagaaatcaagactgtggaatctataactgacatcagggcagatatagataaaaaatcacagatgtgttagtggcaccagggtccacatgaaca
acgtgaccgtaacgctccatgatgccaccatccaggcgtacctttccaacagcagcttcagccggggagagacacgctgtgaacaagacaggccttc

For the constructs, the sequences are highlighted as follows: *hLAMP Signal peptide*-*hLAMP Luminal domain*-CTCGAG-*Antigen*-GAATTC-*hLAMP TM and Cyto domain*

cccaaccacagcgcctcctgcccaccagccctcgcctcaccctgcccagagccctctgtggacaagtacaacgtgagcggcaccaacggg
 acctgcctgctggccagcatggggctgcagctgaacctcacctatgagagggaaggacaacacgacggtgacaaggcttctcaacatcaacccaaca
 agacctcggccagcgggagctgcccgcacactgggtgactctggagctgcacagcagggcaccaccgtcctgctctccagttcgggatgaatgca
 agttctagccggttttctacaaggaatccagttgaatacaattcttctgacgccagagaccctgcctttaaagctgccaacggctcctgagcgc
 tgcaggccacagtcggcaattcctacaagtgaacgcggaggagcacgtccgtgtcacgaaggcgttttcagtcaatatattcaagtggtgggtccag
 gctttcaaggtggaaggtggcagtttggtctgtggaggagtgctgctggacgagaacagcCTCGAGatggacgcatcaagaagaaaatgca
 agccatgaagctggaaggaacacccatggaccggcgacacactggaacagcagaacaagaggccaacaatcgggcccagagaagtccgag
 gaagaagtgcacaaatctgcagaaacggatgcagcagctggaacacgatctggaccaagtgcagagtgctgtgaaggccaacatccaactcgtg
 gaaaaggataaggctctgagcaacccgagggcgaagtggccgctctgaacagacggattcagctgctggaagaggatctggaaggaagcgagga
 acggctgaacaccgccacaacaagctggccgaagccagccaagccgacgagagcgagcggtgctggaagtgctggaacacgctctctga
 cgacagaggaaagaatggacgctctggaacatcagctgaaagaggcccggtttctggccgaggaagccgaccggaagtacgatgaagtggccgg
 aagctggccatggtcagggctgatctggaacggcggaagagagagccgaaacccgagagcaagatcgtggaactggaagaggaaactcgggt
 cgtgggcaacaatctgaagtctctggaagtgtccgaagagaaggccaatcagagagagggaagcctacaagagcagatcaagacactgaccaaca
 agctgaaggctccgagggcagagccgagttcggcagagaagcgtgcagaaactgcagaaagaagtggaccggctggaagtgcagctcgtgaac
 gagaagagaagtacaagagcatcacgcagcagctggaccagacattcagcagctgagcggctacGAATTCacgctgatccccatcgctgtg
 gtgtgcccctggcgggctggtcctcatcgtcctcatcgctacctcgtcggcaggaagaggagtcacgcaggctaccagactatctagtaa (SEQ
 ID NO:79)

Allergen X is Lol p 5a

atggcgccccgagcgcggcgacccctgctgctgctactgctgttgctgctgctcgccctcatgcattgtgcgtcagcagcaatgtttatgggtg
 aaaaatggcaacgggacggcgtgcataatggccaacttctctgctgctctcagtgaaactacgacaccaagagtgccctaagaacatgaccttga
 cctgccatcagatgccacagtggtgctcaaccgcagctcctgtggaagagaacacttctgacccagctcctgattgcttttgaagaggacatac
 actcactctcaatttcacgagaaatgaacacgttacagcgtccagctcatgagttttgtttataactgtcagacacacacctttcccaatgcgagct
 ccaaagaaatcaagactgtggaatctataactgacatcagggcagatatagataaaaaatacagatgtgttagtgccaccaggtccacatgaaca
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 cccaaccacagcgcctcctgcccaccagccctcgcctcaccctgcccagagccctctgtggacaagtacaacgtgagcggcaccaacggg
 acctgcctgctggccagcatggggctgcagctgaacctcacctatgagagggaaggacaacacgacggtgacaaggcttctcaacatcaacccaaca
 agacctcggccagcgggagctgcccgcacactgggtgactctggagctgcacagcagggcaccaccgtcctgctctccagttcgggatgaatgca
 agttctagccggttttctacaaggaatccagttgaatacaattcttctgacgccagagaccctgcctttaaagctgccaacggctcctgagcgc
 tgcaggccacagtcggcaattcctacaagtgaacgcggaggagcacgtccgtgtcacgaaggcgttttcagtcaatatattcaagtggtgggtccag
 gctttcaaggtggaaggtggcagtttggtctgtggaggagtgctgctggacgagaacagcCTCGAGgcccgatgccggctacacaccagccgt
 gctgctacaccagccactccagcagcaacaccagcagccgtggcggcaaggccacaaccgatgagcagaaactgctggaagatgtgaacccgg
 cttcaaggcccgctggtgctgctgcaaatgcccctcccgccgacaagttaagatcttcgaggccgcttcagcagagcagcaaggagctgctgg
 ccacatctgcgccaagccccggactgatccccagctggacaccgcctacgacgtggcctacaaagccgcccaggccacccagaggccaat
 acgatgcctcgtgaccgctctgaccgaggtctgagagtgattgcccgcctctggaagtgcacgccgtgaagccagccaccgaagaagtgtgtggc
 gccaaagattcctaccggcgagctgcagatctggacaagatcgacgccctttaaagatcgccgccaccgcccgaatgcccccctaccaacgata

For the constructs, the sequences are highlighted as follows: *hLAMP Signal peptide*-*hLAMP Luminal domain* -CTCGAG-Antigen-GAATTC-*hLAMP TM and Cyto domain*

agttcaccgtgttcgagagcgccttcaacaaggctctgaacgagtgaccggcggagcctacgagacatacaagttcatcccatctctggaagccgct
gtgaagcaagcctacgcccacagtgccgctgccccgaagtgaagtacgccgtgtttgaggccgactgaccaaggccatcaccgccatgacac
aagcccagaaggccggcaaccagctgctgcagctGAATTCacgtgatcccatcgctgtgggtggcgccctggcggggctggctcctcatcgctct
catcgctacctcgctggcaggaagaggagtcacgcaggctaccagactatctagtaa (SEQ ID NO:83)

Allergen X is Phl p 1

atggcggccgcagcggcggcagccctgctgctgctactgctgttggctgctgctggcctcatgcattgtgctcagcagcaatgtttatgggtg
aaaaatggcaacgggacgcgtgcataatggcaacttctctgctgcttctcagtgaactacgacaccaagagtggccctaagaacatgaccttga
cctgccatcagatgccacagtggtgctcaaccgcagctcctgtggaaaagagaacacttctgacccagctcctgtgattgcttttgaagaggacatac
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ccaaagaaatcaagactgtggaatctataactgacatcagggcagatatagataaaaaatacagatgtgttagtgccaccaggtccacatgaaca
acgtgaccgtaacgctccatgatgccaccatccaggcgtacctttcaacagcagcttcagccgggagagacacgctgtgaacaagacaggccttc
cccaaccacagcggccctgcccaccagccctcgccctcaccgctgcccagagccctctgtggacaagtacaacgtgagcggcaccaacggg
acctgctgctggccagcatgggctgcagctgaacctcacctatgagaggaaggacaacacgacgggtgacaaggcttctcaacatcaacccaaca
agacctggccagcgggagctgcccgcacacctggtagctgtagctgcacagcgggacaccgctcctgctcttccagttcgggatgaatgca
agttctagccggttttctacaaggaatccagttgaatacaattcttctgacgccagagacctgctttaaagctgccaacggctccctgcgagcgc
tgaggccacagtcggcaattcctacaagtgaacgcggaggagcacgtccgtgtcacgaaggcgttttcagtcaatatattcaagtggtgggtccag
gctttcaaggtggaagggtggcagtttgctctgtggaggagtgctgctggacgagaacagcCTCGAGatccccaaagtgcctccggcccaaac
atcaccgccacatacggcgacaagtggctggacgccaagagcacttggtacggcaagcctactggcggcgacccaaggacaatggcggcgcttgt
ggctacaaggacgtggacaagcccccttctctggcatgaccggctgcccgaacacccccatcttcaagagcggcagaggctgtggcagctgcttcca
gatcaagtgcaccaagcccaggcttgacggcggaaccagtcgtggtgcacatcaccgacgacaacgaggaacccattgccccctaccacttcgat
ctgagcggccacgcctttggcgccatggccaagaaaggcgacgagcagaagctgagatctgccggcgagctggaactgcagtttcggagagtgaag
tgcaagtaccccgagggcaccaaagtgcatttcacgtggaaaagggcagcaacccaactatctggctctgctcgtgaaatacgtgaacggcgacg
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gataagctgaccggccctttcaccgtgcggtacacaacagaggcgccaccaagacagaggccgaggatgtgatccagagggtggaaggccga
caccagctacgagagcaaaGAATTCacgtgatcccatcgctgtgggtggcgccctggcggggctggctcctcatgctcctcatgcctacctcgct
ggcaggaagaggagtcacgcaggctaccagactatctagtaa (SEQ ID No:87)

For the constructs, the sequences are highlighted as follows: *hLAMP Signal peptide*-*hLAMP Luminal domain*-CTCGAG-*Antigen*-GAATTC-*hLAMP TM and Cyto domain*

Allergen X is Phl p 5

atggcgccccgcagcgccggcgacccctgctgctgctactgctgttggctgctgctcggcctcatgcattgtgctcagcagcaatgtttatggg
aaaaatggcaacgggacgcgtgcataatggccaacttctgctgccttctcagtgaactacgacaccaagagtggccctaagaacatgacccctga
cctgccatcagatgccacagtgggtgctcaaccgcagctcctgtggaaaagagaacacttctgacccagctcctgattgctttggaagaggacatac
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ccaaagaaatcaagactgtggaatctataactgacatcagggcagatatagataaaaaatacagatgtgttagtggcaccaggtccacatgaaca
acgtgaccgtaacgctccatgatgccaccatccaggcgtacctttccaacagcagcttcagccggggagagacacgctgtgaacaagacaggccttc
cccaaccacagcgccccctgcgccaccagccccctgcctcaccgctgcccaagagccccctgtgtgacaagtacaacgtgagcggcaccacggg
acctgcctgtggccagcatggggctgcagctgaacctcacctatgagaggaaggacaacacgaggtgacaaggcttctcaacatcaacccaaca
agacctcggccagcgggagctcggcgccacctgggtgactctggagctgcacagcgagggcaccaccgtcctgtcttccagttcgggatgaatgca
agttctagccggttttctacaaggaaatccagttgaatacaattcttctgacgccagagacctgcctttaaagctgccaacggctccctgcgagcgc
tgcaggccacagtcggcaattcctacaagtgaacgcggaggagcacgtccgtgtcacgaaggcgttttcagtcaatatattcaagtggtgggtccag
gctttcaagggtggaagggtggccagttggctctgtggaggagtgtctgctggacgagaacagcCTCGAGgccgccgatctgggctatggccagct
acaccagctgccccagccgcccagatacacaccagcaactccagccgctccagctgaagcagccccagctggaaaggccacaaccgaggaacagaa
gctgatcgagaagatcaacgccggttcaaggccgctctggctgcagctgctgggtgcagccagccgacaagtacagaacattcgtggccacattc
ggagccgccagcaacaaggcctttgccgagggactgagcggcgagcctaaaggccgctgagtctagcagcaaggccgactgaccagcaagct
ggagccgcctacaagctggcctacaagacagccgaaggcgcacccccgaggccaaatacgtacgtggcactctgagcgaggctctgag
aatcattgccggcacactggaagtgcacgccgtgaagccagccgctgaggaagtgaagtgatcccagccggcgagctccaagtattgagaaagt
ggatgccgccttcaagtgccgcccaccgctgccaatgccgccccagccaatgacaagttcacctgtttgaggccgcctttaacgacgccatcaagg
cctctaccggcggagcctacgagagctacaagttcatccccgctctggaagccgctgaaacaagcctatgccgccacagtgccacagccccga
agtgaagtacacagtgttcgagacagctctgaagaaagccatcaccccatgtccgaggccagaaggccgccaaccagctgctgctgctGAAT
TCacgctgatccccatcgctgtgggtggcctggcgggctggtcctcatcgtcctcatgcctacctcgtcggcaggaagaggagtacgcaggct
accagactatctagtaa (SEQ ID NO:91)