MOLECULAR ANTIGEN ARRAYS

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention is in the fields of medicine, immunology, virology and molecular biology.

Related Art

Vaccination has provided one of the most effective ways of fighting infectious diseases and has led to the most significant benefits for public health in the last century. Early vaccination strategies used live, attenuated or inactivated pathogens as the immunogen. Safety concerns within the public and the authorities have fostered a search for more defined and safer vaccines.

This search stimulated a new direction of research, where individual antigens were isolated or recombinantly expressed and injected as immunogens. Examples of these include the development and use of subunit vaccines. Such vaccines, however, often require the addition of an adjuvant to generate a sufficient immune response against the antigen, as an isolated protein is typically not sufficiently immunogenic to generate a protective immune response. Although several strong adjuvants are known, such as complete Freund's adjuvant, they are generally toxic and cannot be used in humans. Great efforts are therefore being made in the search for new adjuvants.

Recently, research on the principles of discrimination by the immune system between self and foreign has revealed that the degree of organization and the repetitiveness of the antigens on the surfaces of viruses are a very strong signal for an antigen to be recognized as foreign (Bachmann & Zinkernagel, *Immunol. Today 17:553-558* (1996)). This property of viral structures was made use of in the design of new vaccines based on virus-like particles (VLPs), which combined the immunogenicity of viral structures and the improved safety profile of non-replicable vaccines. In those vaccines, the antigen is either fused or chemically attached to virus-like particles, the chemical attachment being covalent or non-covalent. Thus, the

immunogenic property of the viral structure is transferred to the antigen by linking the antigen to virus-like particles.

A variety of VLPs have been used for the attachment of antigens. For example, WO 00/32227 describes the use of Hepatitis B core antigen in the production of certain types of vaccines.

A new class of highly expressable and highly immunogenic VLPs has been disclosed in WO 03/056905, which is incorporated herein by reference in its entirety. These VLPs are composed of the coat protein of RNA bacteriophages. The coat proteins are expressed recombinantly in bacteria, and the VLP does not contain the phage RNA genome and therefore cannot replicate.

A new RNA bacteriophage, AP205, has been recently identified (Klovins,J., *et al., J. Gen. Virol. 83*: 1523-33 (2002).) The AP205 RNA phage (Taxonomy ID: 154784) is a single-stranded, positive-strand RNA (no DNA stage) virus, which belongs to the *Leviviridae* family, *Levivirus* genus, *Unclassified Levivirus* subgroup. The other members of this subgroup are RNA phages BO1, fr1, TW19, and PP7. Two described *Levivirus* subgroups include following RNA phages: fr, JP501, f2, M12, MS2, and R17 (subgroup I) and BZ13, JP34, TH1, GA, and KU1 (subgroup II). The AP205 genome is 4267 nucleotides (nt) in length. Full-length genomic sequence: accessions AF334111, NC_002700. The natural host of the AP205 phage is *Acinetobacter spp.* (Klovins,J., *et al., J. Gen. Virol. 83*: 1523-33 (2002)). The genome of the AP205 phage comprises three large open reading frames (ORFs), which code for the maturation, the coat and the replicase proteins. In addition, two additional small ORFs are present at the 5' terminus, preceding the maturation gene. The function of the proteins coded by these ORFs is unknown. It has been postulated that one of these ORFs might code for a lysis protein (Klovins,J., *et al., J. Gen. Virol. 83*: 1523-33 (2002)).

SUMMARY OF THE INVENTION

A first aspect provides a virus-like particle comprising at least one protein selected from the group consisting of:

(a) a protein having an amino acid sequence as set forth in SEQ ID NO:1;

(b) a protein having an amino acid sequence as set forth in SEQ ID NO:3; and

(c) a mutein of said protein of (a) or (b).

A second aspect provides a virus-like particle comprising at least one protein selected from the group consisting of:

(a) a protein comprising amino acids 2-131 of SEQ ID NO:1;

(b) a protein comprising amino acids 2-131 of SEQ ID NO: 3; and

(c) a mutein of SEQ ID NO:1, wherein said mutein consists of an addition, deletion or substitution of one to three amino acids from amino acids 1- 131 of SEQ ID NO: 1.

A third aspect provides the virus-like particle of the second aspect comprising at least one protein comprising amino acids 2-131 of SEQ ID NO:3.

A fourth aspect provides the virus-like particle of the second aspect comprising at least one mutein of SEQ ID NO:1, wherein said mutein consists of an addition, deletion or substitution of one amino acids from amino acids 2-131 of SEQ ID NO: 1.

A fifth aspect provides a vector for producing an AP205 virus-like particle, wherein said vector comprises a nucleotide sequence encoding a protein comprising amino acids 2-131 of SEQ ID NO:3.

A sixth aspect provides a vector for producing an AP205 virus-like particle, wherein said vector comprises a nucleotide sequence encoding a mutein of SEQ ID NO:1, wherein said mutein consists of an addition, deletion or substitution of one to three amino acids from amino acids 2- 131 of SEQ ID NO: 1.

A seventh aspect provides a host cell comprising the vector of the fifth or the sixth aspect.

An eighth aspect provides a method of producing a virus-like particle comprising:

(a) providing a vector according to the fifth or the sixth aspect;

(b) introducing said vector into a host cell; and

(c) expressing the sequence of said vector in said host cell to obtain a protein or a mutein capable of forming a virus-like particle.

A ninth aspect provides a mutein having an amino acid sequence as set forth in SEQ ID NO:3.

A tenth aspect provides a mutein of the recombinant protein of SEQ ID NO: 1.

An eleventh aspect provides a vector for producing a AP205 virus like particle comprising a nucleotide sequence being at least 80% identical to that of SEQ ID NO:2 or SEQ ID NO:4.

A twelfth aspect provides a vector for producing a AP205 virus like particle comprising a nucleotide sequence being at least 90% identical to that of SEQ ID NO:2 or SEQ ID NO: 4.

A thirteenth aspect provides a vector for producing a AP205 virus like particle comprising a nucleotide sequence being at least 95% identical to that of SEQ ID NO:2 or SEQ ID NO: 4.

A fourteenth aspect provides a vector for producing a AP205 virus like particle comprising a nucleotide sequence being at least 99% identical to that of SEQ ID NO:2 or SEQ ID NO: 4.

A fifteenth aspect provides a vector for the production of a recombinant protein comprising a nucleotide sequence encoding a polypeptide fused to a protein, wherein said protein is selected from the group consisting of:

(a) a protein having an amino acid sequence as set forth in SEQ ID NO:1;

(b) a protein having an amino acid sequence as set forth in SEQ ID NO:3; and

(c) a mutein of said polypeptide of (a) or (b).

A sixteenth aspect provides a composition comprising:

(a) a core particle selected from the group consisting of

(i) an AP205 virus particle; and

(ii) AP205 virus-like particles; and

(b) an organic molecule

wherein the organic molecule is bound to the core particle.

A seventeenth aspect provides a composition comprising:

(a) a core particle selected from the group consisting of

(i) an AP205 virus-like particle comprising at least one protein comprising amino acids 2- 131 of SEQ ID NO:1;

(ii) an AP205 virus-like particle comprising at least one protein comprising amino acids 2-131 of SEQ ID NO:3; and

(iii) a mutein of SEQ ID NO:1, wherein said mutein consists of an addition, deletion or substitution of one to three amino acids from amino acids 1-131 of SEQ ID NO:1; and

(b) an organic molecule, wherein said organic molecule is bound to said core particle.

An eighteenth aspect provides the composition as defined above, wherein said composition comprises an amino acid linker.

A nineteenth aspect provides a pharmaceutical composition comprising:

a) the composition as defined above and

b) an acceptable pharmaceutical carrier.

A twentieth aspect provides a vaccine composition comprising an immunologically effective amount of the composition as defined above.

A twenty-first aspect provides the vaccine composition of the twentieth aspect further comprising an adjuvant.

A twenty-second aspect provides a process for producing a non-naturally occurring, ordered and repetitive antigen array comprising:

(a) providing a molecular scaffold comprising a core particle selected from the group of

(i) AP205 virus; and

(ii) AP205 virus-like particles; and

(b) providing an organic molecule suitable for inducing an immune response;

(c) providing a means of associating (a) and (b), said means optionally contained within (a) and/or (b), or as a separate molecule; and

(d) combining the elements of (a) through (c), such that said organic molecule associates with said scaffold to form an ordered and repetitive antigen array.

A twenty-third aspect provides a process for producing a non-naturally occurring, ordered and repetitive array comprising:

(a) providing a molecular scaffold comprising a virus-like particle selected from the group consisting of:

(i) a protein comprising amino acids 2-131 of SEQ ID NO:1;

(ii) a protein comprising amino acids 2-131 of SEQ ID NO:3; and

(iii) a mutein of SEQ ID NO:1, wherein said mutein consists of an addition, deletion or substitution of one to three amino acids from amino acids 1-131 of SEQ ID NO:1;

(b) providing an organic molecule suitable for inducing an immune response;

(c) providing a means of associating (a) and (b), said means optionally contained within (a) and/or (b), or as separate molecule; and

(d) combining the elements of (a) through (c), such that said organic molecule associates with said scaffold to form said ordered and repetitive array.

A twenty-fourth aspect provides a nucleic acid molecule comprising a nucleotide sequence as set forth in SEQ ID NO:125.

A twenty-fifth aspect provides a host cell containing a nucleic acid according to the twenty-fourth aspect or a vector according to any one of the eleventh to fourteenth aspects.

A twenty-sixth aspect provides a method of producing a virus-like particle according to any of the first to fourth aspects comprising the steps of

(a) providing a nucleic acid according to the twenty-fourth aspect or a vector according to any one of the eleventh to fourteenth aspects;

(b) introducing said nucleic acid or said vector into a host cell;

(c) expressing said nucleic acid or the sequence of said vector in said host cell to obtain a protein or a mutein capable of forming a virus-like particle according to the first or second aspect.

A twenty-seventh aspect provides a vaccine composition according to the twentieth aspect for use in a method of immunization.

A twenty-eighth aspect provides the vaccine composition for use in a method according to the twenty-seventh aspect wherein the composition further comprises an adjuvant.

A twenty-ninth aspect provides the use of the vaccine composition according to the twentieth aspect for the preparation of a vaccine, wherein said vaccine is suitable for being administered to a subject.

A thirtieth aspect provides the use of the vaccine composition according to the twenty-ninth aspect, wherein said composition further comprises an adjuvant.

A thirty-first aspect provides the composition as defined above for use in the treatment or prevention of a disease, disorder or physiological condition in an individual, wherein said composition is suitable for being administered to said individual.

A thirty-second aspect provides the use of the composition as defined above for the preparation of a composition for treating or preventing a disease, disorder or physiological condition in an individual, wherein said composition is suitable for being administered to said individual.

A thirty-third aspect provides a vaccine composition according to the twentieth aspect for use the treatment or prevention of a disease, disorder or physiological condition

in an individual, wherein said composition is suitable for being administered to said individual.

A thirty-fourth aspect provides the use of the vaccine composition according to the twentieth aspect for the preparation of a vaccine for treating or preventing a disease, disorder or physiological condition in an individual, wherein said vaccine composition is suitable for being administered to said individual.

We have discovered that AP205 coat protein can be recombinantly expressed in bacteria using the vectors of the invention. We have also developed methods for

purification of AP205 virus-like particles. Moreover, the AP205 coat proteins produced by the present method spontaneously formed capsids, as evidenced by Electron Microscopy (EM) and immunodiffusion, and therefore the coat protein alone together with RNA are sufficient for assembly of the capsid in E. coli. This rules out any role of the proteins coded by the two ORFs of unknown function. A surprising feature of the invention is that no sequence homology exists between the sequence of the AP205 coat protein and other RNA phage coat proteins from which the structure has been elucidated, yet the structural properties of the capsid formed by the AP205 coat protein and those formed by the coat protein of those RNA phages are nearly indistinguishable when seen in EM. We have discovered that AP205 VLPs are highly immunogenic, and can be linked with organic molecules to generate vaccine constructs displaying the organic molecules oriented in a repetitive manner. High titers were elicited against the so displayed organic molecules showing that bound organic molecules are accessible for interacting with antibody molecules and are immunogenic.

Disclosed are recombinantly expressed virus-like particles (VLPs), spontaneously assembled from at least one coat protein of bacteriophage AP205 recombinantly expressed in *E. coli*. In connection with the aforesaid, assembly-competent mutant forms of AP205 VLPs, including AP205 coat protein with the substitution of praline at amino acid 5 to threonine (SEQ ID NO: 3) are disclosed. These VLPs, AP205 VLPs derived from natural sources (SEQ ID NO: 1)., or AP205 viral particles, may be bound to at least one organic molecule to produce ordered repetitive arrays of the organic molecules. Organic molecules disclosed include antigens and antigen determinants, allergens, self antigens, haptens, cancer antigens and infectious disease antigens as well as small organic molecules such as drugs of abuse like nicotine and derivatives thereof. Immunisation of animals using antigen-AP205 VLP conjugates, or compositions comprising such conjugates as disclosed, induce a strong immune response against the displayed antigen. The VLP disclosed is thus useful for the attachment and display of molecules and in particular of antigens. Hence, the conjugates, compositions and methods disclosed are useful for the stimulation of an immune response against a variety of displayed antigens, and thus for the use in animals.

In a first embodiment, the present invention provides for a virus-like particle comprising, alternatively or preferably consisting essentially of, or alternatively of preferably consisting of at least one protein selected from the group consisting of: (a) a protein having an amino acid sequence as set forth in SEQ ID NO:1; (b) a protein having an amino acid sequence as set forth in SEQ ID NO:3; and (c) a mutein of said protein of (a) or (b). Preferably, said protein is recombinant. Thus, disclosed is a capsid formed by at least one protein selected from the group consisting of: (a) a protein having an amino acid sequence as set forth in SEQ ID NO:1; (b) a protein having an amino acid sequence as set forth in SEQ ID NO:3; and (c) a mutein of said protein of (a) or (b). In a preferred embodiment, said mutein has an amino acid sequence as set forth in SEQ ID NO:1 or as set forth in SEQ ID NO:3, wherein at least one amino acid residue, preferably three amino acid residues, more preferably two amino acid residues, and even more preferably one amino acid residue of SEQ ID NO: 1 or SEQ ID NO:3 is added, deleted or substituted, wherein preferably said at least one substitution is a conservative substitution. In a further preferred embodiment, said mutein has an amino acid sequence as set forth in SEQ ID NO:1 or as set forth in SEQ ID NO:3, wherein at least one cysteine residue, preferably two cysteine residues of SEQ ID NO: 1 or SEQ ID NO:3, is deleted or substituted, wherein preferably said at least one, preferably two, substitution is a conservative substitution. In a still further preferred embodiment, said mutein has an amino acid sequence as set forth in SEQ ID NO:1 or as set forth in SEQ ID NO:3, wherein at least one lysine residue, preferably three lysine residues, more preferably two lysine residues, and even more preferably one lysine of SEQ ID NO: 1 or SEQ ID NO:3 is added, deleted or substituted, wherein preferably said at least one substitution is a conservative substitution.

In a second embodiment, the invention provides for a mutein having an amino acid sequence as set forth in SEQ ID NO:3. Alternatively, disclosed is a mutein of the recombinant protein of SEQ ID NO: 1 or SEQ ID NO:3, wherein at least one amino acid residue, preferably three amino acid residues, more preferably two amino acid residues, and even more preferably one amino acid residue of SEQ ID NO: 1 or SEQ ID NO:3 is added, deleted or substituted, wherein preferably said

at least one substitution is a conservative substitution.

In a still further embodiment, the invention provides for a mutein of the recombinant protein of SEQ ID NO: 1 or SEQ ID NO: 3, wherein at least one cysteine residue, preferably two cysteine residues of SEQ ID NO: 1 or SEQ ID NO:3, is deleted or substituted, wherein preferably said at least one, preferably two, substitution is a conservation substitution. Alternatively, disclosed is a mutein of the recombinant protein of SEQ ID NO: 1 or SEQ ID NO:3, wherein at least one lysine residue, preferably three lysine residues, more preferably two lysine residues, and even more preferably one lysine of SEQ ID NO: 1 or SEQ ID NO:3 is added, deleted or substituted, wherein preferably said at least one substitution is a conservative substitution.

In another embodiment, the invention provides for a vector for producing a AP205 virus like particle whose sequence is at least 80%, preferably at least 90%, more preferably at least 95%, and even more preferably 99% identical to that of SEQ ID NO:2 or SEQ ID NO: 4. Alternatively, disclosed is a vector for the production of a recombinant protein comprising a polypeptide fused to a protein, wherein said protein is selected from the group consisting of: (a) a protein having an amino acid sequence as set forth in SEQ ID NO:1; (b) a protein having an amino acid sequence as set forth in SEQ ID NO:3; and (c) a mutein of said polypetide of (a) or (b).

In a further embodiment, the invention provides for a method of producing a AP205 virus-like particle comprising the steps of: (a) providing a nucleic acid comprising a nucleotide sequence being at least 80%, preferably at least 90%, more preferably at least 95%, and even more preferably 100% identical to that of SEQ ID NO:2 or SEQ ID NO: 4, or providing a vector comprising a nucleotide sequence being at least 80%, preferably at least 90%, more preferably at least 95%, and even more preferably 99% identical to that of SEQ ID NO:2 or SEQ ID NO: 4; (b) introducing said nucleic acid or said vector into a host cell; (c) expressing said nucleic acid or the sequence of said vector in said host cell to obtain a protein or a mutein capable of forming a AP205 virus-like particle. Preferably, said host cell is *E.coli*.

In still a further embodiment, the invention provides for a method of producing a AP205 virus-like particle comprising the steps of: (a) providing a nucleic acid or a vector encoding at least one protein selected from the group consisting of: (i) a protein having an amino acid sequence as set forth in SEQ ID NO:1; (ii) a protein having an amino acid sequence as set forth in SEQ ID NO:3; and (iii) a mutein of said protein of (i) or (ii); (b) introducing said nucleic acid or said vector into a host cell; (c) expressing said nucleic acid or the sequence of said vector in said host cell to obtain said protein or said mutein capable of forming a AP205 virus-like particle. Preferably, said host cell is *E.coli*. Preferred embodiments of the proteins and muteins indicated under (i) and (ii) have already been indicated above.

In one embodiment, the invention provides a composition comprising one or more recombinant VLPs of RNA bacteriophage AP205 or mutants thereof. In a further embodiment, the invention provides compositions comprising one or more AP205 VLPs and one or more organic molecules, wherein the molecule is attached, linked, coupled or fused i.e. bound, to the AP205 VLPs. In another embodiment, the organic molecule is an antigen.

In certain other embodiments, the organic molecule is selected from the group consisting of: (a) an organic molecule suited to induce an immune response against cancer cells; (b) an organic molecule suited to induce an immune response against infectious diseases; (c) an organic molecule suited to induce an immune response against allergens; (d) an organic molecule suited to induce an improved response against self-antigens; (e) an organic molecule suited to induce an immune response in farm animals or pets; and (f) an organic molecule suited to induce a response against a drug, a hormone or a toxic compound and (g) fragments (e.g. an epitope or antigenic domain of any of the molecules set out in (a)-(f).

In another embodiment, the organic molecules are one or more antigens. In one such embodiment, the antigens are recombinant polypeptides. In another embodiment, the antigens are extracted from a natural source, such as pollen, bees, pathogens or tumors. In yet another embodiment, the antigen is selected from the group consisting of: (a) a polypeptide suited to induce an immune response against cancer cells; (b) a polypeptide suited to induce an immune response against infectious diseases; (c) a

polypeptide suited to induce an immune response against allergens; (d) a polypeptide suited to induce an immune response against self-antigens; and (e) a polypeptide suited to induce an immune response in farm animals or pets.

In a particular embodiment, the antigen comprises an epitope of cytotoxic T-cells or helper T-cells. In a related embodiment the antigen comprises a B cell epitope.

In a related aspect, the invention provides methods for attaching, i.e. binding, organic molecules to the AP205 VLP. In certain embodiments, the organic molecules are bound in an oriented fashion to the AP205 VLP.

In another embodiment of the invention, the conjugates or compositions are used in methods of immunizating an animal by introducing it into an animal subcutaneously, intramuscularly, intranasally, intradermally, intravenously, transdermally, transmucosally, orally, or directly into a lymph node. In another embodiment, the composition is applied locally, near a tumor or local viral reservoir against which one would like to vaccinate.

Disclosed further is a vaccine comprising an immunologically effective amount of the composition of the present invention together with a pharmaceutically acceptable diluent, carrier or excipient. In a futher embodiment, the vaccine further comprises at least one adjuvant, such as Alum or incomplete Freund's adjuvant. The invention also provides methods of immunizing and/or treating an animal comprising administering to the animal an immunologically effective amount of conjugates, compositions, or vaccines of the invention.

The AP205 VLPs conjuates or compositions can be used to vaccinate against tumors, viral diseases, self-molecules or non-peptidic small molecules, for example. The vaccination can be for prophylactic or therapeutic purposes, or both. AP205 VLPs conjuates or compositions can be used to vaccinate against allergies in order to induce immune-deviation and/or antibody responses against the allergen, suitable for the treatment or prevention of allergies.

Disclosed yet further are methods of treating or preventing diseases, physical disorders or conditions in an individual or a population of individuals, by the administration of compositions comprising or, alternatively, consisting essentially of, an AP205 VLP bound to an organic molecule. In a related aspect immune molecules

and antibodies, respectively, such as antibodies, generated against such compositions may be used for treatment, prophalaxis or diagnosis of a disease, condition or disorder.

In another embodiment of the invention, compositions comprising an AP205 VLP bound to an organic molecule are provided in the form of a kit. In another embodiment of the invention, compositions comprising an immune molecule and antibody, respectively, isolated by the use of an AP205 VLP bound to an organic molecule are also provided in the form of a kit. Such kits are useful for a variety of purposes including but not limited to the detection of immune molecules and antibodies, respectively, reacting to organic molecules presented on the VLP, for detection of organic molecules, for the screening of immune molecules and antibodies, respectively, and/or for the diagnosis of conditions characterized by the presence or absence of the immune molecules and antibodies, respectively. In certain related embodiments, the kits of the invention may comprise one or more additional components such as buffers, carriers, excipients, adjuvants, detection reagents *etc.*

In another embodiment, the invention also provides for vectors and host cells for the expression of the coat protein of RNA bacteriophage AP205 forming the virus-like particles. Host cells include prokaryotes including *E. coli*; and eukaryotes including yeast, animals, cell lines, *etc.*

In another embodiment, the invention provides methods for expressing the coat protein of RNA bacteriophage AP205 and the virus-like particles thereof. In another aspect, the invention provides methods for purifying and isolating virus-like particles of bacteriophage AP205.

Other embodiments of the present invention will be apparent to one of ordinary skill in light of what is known in the art, the following drawings and description of the invention, and the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-B depict electron micrographs comparing AP205 phage particles to AP205 virus like particles spontaneously assembled from recombinant protein

expressed in *E. coli* and purified. FIG. 1 A shows an electron micrograph picture of AP205 phage particles, while an electron micrograph picture of self assembled particles of recombinant AP205 VLP is shown in FIG. 1 B.

Figure 2 shows the SDS-PAGE analysis of the coupling reaction of AP205 VLP and Qβ VLP to Derp1.2 peptide. The samples were run under reducing conditions on a 16% Tris-glycine gel. Lane 1 is the protein marker, with corresponding molecular weights indicated on the left border of the gel; lane 2, derivatized Qβ capsid protein; lane 3, the supernatant of the coupling reaction of Qβ capsid protein to the Derp1.2 peptide; lane 4, the pellet of the coupling reaction of Qβ capsid protein to the Derp1.2 peptide; lane 5, derivatized AP205 VLP; lane 6, the supernatant of the coupling reaction of AP205 VLP to the Derp1.2 peptide; lane 7, the pellet of the coupling reaction of AP205 VLP to the Derp1.2 peptide. Coupling products corresponding to the coupling of 1, 2, 3 ,4 and respectively 5 peptides per monomer are indicated by arrows in the Figure. A higher number of epitopes could be coupled to AP205 VLP than to Qβ capsid protein.

Figure 3 shows an ELISA analysis of the IgG antibodies specific for "Derp 1.2" in sera of mice immunized against the Derp1.2 peptide coupled to AP205 VLP or Qβ capsid protein respectively. Total IgG titers, as well as IgG subtype titers were determined. No antibodies specific for Derp1.2 could be detected in any of the preimmune sera analysed for each of the IgG subtypes. The figure shows that for both AP205 and Qβ, subtypes typical of a Th1 immune response are induced, as the IgG2a titer is much higher than the IgG1 titer. A strong specific anti-peptide immune response was obtained with the peptide coupled to both VLPs. Antibodies specific for the carrier were also measured by ELISA, and these were comparable for both carriers.

Figure 4A-4C shows partial sequences of the different eukaryotic expression vectors used. Only the modified sequences are shown. FIG. 4A: pCep-Xa-Fc*: the sequence is shown from the Bam HI site onwards and different features are shown above the translated sequence (SEQ ID NO: 103 and SEQ ID NO: 104). The arrow indicates the cleavage site of the factor Xa protease. FIG. 4B: pCep-EK-Fc*: the sequence is shown from the Bam HI site onwards and different features are shown above the translated sequence (SEQ ID NO: 105 and SEQ ID NO: 106). The arrow

indicates the cleavage site of the enterokinase. The sequence downstream of the Hind III site is identical to the one shown in FIG. 4A. FIG. 4C: pCep-SP-EK-Fc*: the sequence is shown from the beginning of the signal peptide on and different features are shown above the translated sequence (SEQ ID NO: 107 and SEQ ID NO: 108). The signal peptide sequence which is cleaved of by the signal peptidase is shown in bold The arrow indicates the cleavage site of the enterokinase. The sequence downstream of the Hind III site is identical to the one shown in FIG. 4A.

Figure 5 A-B depicts rMIF constructs, and an SDS-PAGE depicting expression and purification of rMIF constructs, for coupling to AP205 VLP. FIG. 5A shows a schematic description of the MIF constructs, with added amino acid linker containing a cysteine residue. FIG. 5B shows an SDS-PAGE analysis of the purified MIF constructs, run under reducing conditions and stained with Coomassie-brillant blue. Loaded on the gels are the purified rat constructs rMIF-C1 (SEQ ID NO: 114), rMIF-C2 (SEQ ID NO: 115), and rMIF-C3 (SEQ ID NO: 117), described in FIG. 5A.

Figure 6 shows the result of the coupling reaction of rMIF-C1 to AP205 VLP. Lane 1: Molecular Marker. Lane 2: AP205 VLP. Lane 3: derivatized AP205 VLP. Lane 4: dialyzed, derivatized AP205 VLP. Lane 5: dialyzed, derivatized AP205 VLP. Lane 6: Coupling reaction of rMIF-C1 to AP205 VLP. The coupling product is indicated by an arrow in the figure. The molecular weights of the marker proteins are indicated on the left border of the gel.

Figure 7 shows the analysis by ELISA of the IgG response specific for rMIF-C1 in the sera of mice immunized with rMIF-C1 coupled to AP205 VLP.

Figure 8 shows an ELISA analysis of the IgG antibodies specific for Angio I peptide in the sera of the three mice (1-3) immunized on day 0 and 14 against the Angio I peptide coupled to AP205 VLP. Total IgG titers were determined in the day 21 sera.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

The following definitions are summaries of concepts commonly understood by

one of ordinary skill in the relevant art and are provided for the purposes of comprehension of the following disclosure but are not meant to be a limitation of the disclosure.

Amino acid linker: An "amino acid linker", or also just termed "linker" within this specification, as used herein, either associates the antigen or antigenic determinant with the second attachment site, or more preferably, already comprises or contains the second attachment site, typically - but not necessarily - as one amino acid residue, preferably as a cysteine residue. The term "amino acid linker" as used herein, however, does not intend to imply that such an amino acid linker consists exclusively of amino acid residues, even if an amino acid linker consisting of amino acid residues is a preferred embodiment of the present invention. The amino acid residues of the amino acid linker are, preferably, composed of naturally occuring amino acids or unnatural amino acids known in the art, all-L or all-D or mixtures thereof. However, an amino acid linker comprising a molecule with a sulfhydryl group or cysteine residue is also encompassed within the invention. Such a molecule comprise preferably a C1-C6 alkyl-, cycloalkyl (C5,C6), aryl or heteroaryl moiety. However, in addition to an amino acid linker, a linker comprising preferably a C1-C6 alkyl-, cycloalkyl- (C5, C6), aryl- or heteroaryl- moiety and devoid of any amino acid(s) shall also be encompassed within the scope of the invention. Association between the antigen or antigenic determinant or optionally the second attachment site and the amino acid linker is preferably by way of at least one covalent bond, more preferably by way of at least one peptide bond.

Animal: As used herein, the term "animal" is meant to include, for example, humans, sheep, elks, deer, mule deer, minks, mammals, monkeys, horses, cattle, pigs, goats, dogs, cats, rats, mice, birds, chicken, reptiles, fish, insects and arachnids.

Antibody: As used herein, the term "antibody" refers to molecules which are capable of binding an epitope or antigenic determinant. The term is meant to include whole antibodies and antigen-binding fragments thereof, including single-chain antibodies. Such antibodies include human antigen binding antibody fragments and include, but are not limited to, Fab, Fab' and F(ab')2, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a V_L or V_H domain. The antibodies can be from any animal origin including birds and

mammals. Preferably, the antibodies are mammalian *e.g.* human, murine, rabbit, goat, guinea pig, camel, horse and the like, or other suitable animals *e.g.* chicken. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulins and that do not express endogenous immunoglobulins, as described, for example, in U.S. Patent No. 5,939,598, the disclosure of which is incorporated herein by reference in its entirety.

Antigen: As used herein, the term "antigen" refers to a molecule capable of being bound by an antibody or a T cell receptor (TCR) if presented by MHC molecules. The term "antigen", as used herein, also encompasses T-cell epitopes. A T-cell epitope is recognized by a T-cell receptor in the context of a MHC class I, present on all cells of the body except erythrocytes, or class II, present on immune cells and in particular antigen presenting cells. This recognition event leads to activation of T-cells and subsequent effector mechanisms such as proliferation of the T-cells, cytokine secretion, perforin secretion etc. An antigen is additionally capable of being recognized by the immune system and/or being capable of inducing a humoral immune response and/or cellular immune response leading to the activation of B- and/or T-lymphocytes. This may, however, require that, at least in certain cases, the antigen contains or is linked to a T_H cell epitope and is given in adjuvant. An antigen can have one or more epitopes (B- and T- epitopes). The specific reaction referred to above is meant to indicate that the antigen will preferably react, typically in a highly selective manner, with its corresponding antibody or TCR and not with the multitude of other antibodies or TCRs which may be evoked by other antigens. Antigens as used herein may also be mixtures of several individual antigens. Antigens, as used herein, include but are not limited to allergens, self antigens, haptens, cancer antigens and infectious disease antigens as well as small organic molecules such as drugs of abuse (like nicotine) and fragments and derivatives thereof. Furthermore, antigens used for the present invention can be peptides, proteins, domains, carbohydrates, alkaloids, lipids or small molecules such as, for example, steroid hormones and fragments and derivatives thereof

Antigenic determinant: As used herein, the term "antigenic determinant" is meant to refer to that portion of an antigen that is specifically recognized by either B- or T-lymphocytes. B-lymphocytes respond to foreign antigenic determinants via antibody production, whereas T-lymphocytes are the mediator of cellular immunity. Thus, antigenic determinants or epitopes are those parts of an antigen that are recognized by antibodies, or in the context of an MHC, by T-cell receptors. An antigenic determinant contains one or more epitopes. Allergens also serve as antigens in vertebrate animals.

Allergens: As used herein, the term "allergen" refers to antigens associated with allergies. An allergic response is characterized by the release of inflammatory factors, particularly histamine, leading to pathologic inflammation in an individual. Allergies are, typically, also associated with IgE antibodies directed against the allergens. The term "allergen", as used herein, also encompasses "allergen extracts" and "allergenic epitopes." Examples of allergens include, but are not limited to: pollens (*e.g.* grass, ragweed, birch and mountain cedar); house dust and dust mites; mammalian epidermal allergens and animal danders; mold and fungus; insect bodies and insect venom; feathers; food; and drugs (*e.g.*, penicillin).

AP205 virus-like particle or AP205 VLP: As used herein, the terms "AP205 virus-like particle" or "AP205 VLP" refer to compositions and virus-like particles, respectively, comprising, or alternatively consisting essentially of, or alternatively and preferably consisting of at least one protein and coat protein, respectively, of the bacteriophage AP205, or a fragment or a mutein thereof, wherein said at least one coat protein, or fragment or mutein thereof, is typically and preferably able to assemble forming a virus-like particle. In alternative and preferred embodiments the terms "AP205 virus-like particle" or "AP205 VLP", as used herein, refer to compositions and virus-like particles, respectively, comprising, or alternatively consisting essentially of, or alternatively and preferably consisting of at least one protein and coat protein, respectively, of the bacteriophage AP205, or a mutein thereof,,wherein said at least one coat protein of the bacteriophage AP205 or said mutein thereof is able to assemble forming a virus-like particle. In a very preferred embodiment of the present invention, the terms "AP205 virus-like particle" or "AP205 VLP" refer to compositions and virus-like particles, respectively, comprising, or alternatively consisting essentially of,

or alternatively and preferably consisting of at least one coat protein of the bacteriophage AP205 having an amino acid sequence as set forth in SEQ ID NO:1, wherein typically and preferably said at least one coat protein is able to assemble to a virus-like particle and capsid, respectively. In a further alternative very preferred embodiment of the present invention, the terms "AP205 virus-like particle" or "AP205 VLP" refer to compositions comprising, or alternatively consisting essentially of, or alternatively and preferably consisting of at least one mutein of a coat protein of the bacteriophage AP205 having an amino acid sequence as set forth in SEQ ID NO:3, wherein said at least one mutein of said coat protein is able to assemble to a virus-like particle. In a further alternative very preferred embodiment of the present invention, the terms "AP205 virus-like particle" or "AP205 VLP" refer to compositions and virus-like particles, respectively, comprising, or alternatively consisting essentially of, or alternatively and preferably consisting of at least one mutein of a coat protein of the bacteriophage AP205 having an amino acid sequence as set forth in SEQ ID NO:1 or SEQ ID NO:3, wherein said at least one mutein of said protein is able to assemble to a virus-like particle. In a further alternative very preferred embodiment of the present invention, the terms "AP205 virus-like particle" or "AP205 VLP" refer to compositions and virus-like particles, respectively, comprising, or alternatively consisting essentially of, or alternatively and preferably consisting of at least one mutein having an amino acid sequence as set forth in SEQ ID NO:1 or as set forth in SEQ ID NO:3, wherein at least one amino acid residue, preferably three amino acid residues, more preferably two amino acid residues, and even more preferably one amino acid residue is added, deleted or substituted, wherein preferably said at least one substitution is a conservative substitution. In a still further alternative very preferred embodiment of the present invention, the terms "AP205 virus-like particle" or "AP205 VLP" refer to compositions and virus-like particles, respectively, comprising, or alternatively consisting essentially of, or alternatively and preferably consisting of at least one mutein having an amino acid sequence as set forth in SEQ ID NO:1 or as set forth in SEQ ID NO:3, wherein at least one cysteine residue, preferably three cysteine residues, more preferably two cysteine residues, and even more preferably one cysteine is deleted or substituted, wherein preferably said at least one substitution is a conservative substitution. In again another alternative very preferred embodiment of

the present invention, the terms "AP205 virus-like particle" or "AP205 VLP" refer to compositions and virus-like particles, respectively, comprising, or alternatively consisting essentially of, or alternatively and preferably consisting of at least one mutein having an amino acid sequence as set forth in SEQ ID NO:1 or as set forth in SEQ ID NO:3, wherein at least one lysine residue, preferably three lysine residues, more preferably two lysine residues, and even more preferably one lysine is added, deleted or substituted, wherein preferably said at least one substitution is a conservative substitution. Further preferred embodiments of the AP205 VLP become apparent as this specification proceeds. The AP205 subunits composing the AP205 VLP may all be linked to other subunits within the particle by disulfide briges, or alternatively a majority of the AP205 VLP subunits are linked to other AP205 VLP subunits within the particle by disulfide bridges. In some embodiments, a minority or none of the AP205 VLP subunits are linked by disulfide bridges to other AP205 VLP subunits within the particle.

Association: As used herein, the term "association" as it applies to the first and second attachment sites, refers to the binding of the first and second attachment sites that is preferably by way of at least one non-peptide bond. The nature of the association may be covalent, ionic, hydrophobic, polar or any combination thereof, preferably the nature of the association is covalent.

Attachment Site, First: As used herein, the phrase "first attachment site" refers to an element of non-natural or natural origin, to which the second attachment site located on the antigen or antigenic determinant may associate. The first attachment site may be a protein, a polypeptide, an amino acid, a peptide, a sugar, a polynucleotide, a natural or synthetic polymer, a secondary metabolite or compound (biotin, fluorescein, retinol, digoxigenin, metal ions, phenylmethylsulfonylfluoride), or a combination thereof, or a chemically reactive group thereof. The first attachment site is located, typically and preferably on the surface, of the core particle such as, preferably the virus-like particle. Multiple first attachment sites are present on the surface of the core and virus-like particle, respectively, typically in a repetitive configuration.

Attachment Site, Second: As used herein, the phrase "second attachment site" refers to an element associated with the antigen or antigenic determinant to which the first attachment site located on the surface of the core particle and virus-like particle,

respectively, may associate. The second attachment site of the antigen or antigenic determinant may be a protein, a polypeptide, a peptide, a sugar, a polynucleotide, a natural or synthetic polymer, a secondary metabolite or compound (biotin, fluorescein, retinol, digoxigenin, metal ions, phenylmethylsulfonylfluoride), or a combination thereof, or a chemically reactive group thereof. At least one second attachment site is present on the antigen or antigenic determinant. The term "antigen or antigenic determinant with at least one second attachment site" refers, therefore, to an antigen or antigenic construct comprising at least the antigen or antigenic determinant and the second attachment site. However, in particular for a second attachment site, which is of non-natural origin, i.e. not naturally occurring within the antigen or antigenic determinant, these antigen or antigenic constructs comprise an "amino acid linker".

Bound: As used herein, the term "bound" refers to binding or attachment that may be covalent, *e.g.*, by chemically coupling, or non-covalent, *e.g.*, ionic interactions, hydrophobic interactions, hydrogen bonds, etc. Covalent bonds can be, for example, ester, ether, phosphoester, amide, peptide, imide, carbon-sulfur bonds, carbon-phosphorus bonds, and the like. The term "bound" is broader than and includes terms such as "coupled," "fused" and "attached."

Coat protein(s): As used herein, the term "coat protein(s)" refers to the protein(s) of a bacteriophage or an RNA-phage capable of being incorporated within the capsid assembly of the bacteriophage or the RNA-phage. The coat protein is also referred to as CP. In the current invention the term more usually refers to the coat protein(s) of the RNA-phage AP205.

Core particle: As used herein, the term "core particle" refers to a rigid structure with an inherent repetitive organization. A core particle as used herein may be the product of a synthetic process or the product of a biological process.

Disease, disorder, condition: As used herein, the terms "disease" or "disorder" refer to any adverse condition of an individual including tumors, cancer, allergies, addiction, autoimmunity, poisoning or impairment of optimal mental or bodily function. "Conditions" as used herein includes diseases and disorders but also refers to physiologic states. For example, fertility is a physiologic state but not a disease or disorder. Compositions of the invention suitable for preventing pregnancy by decreasing fertility would therefore be described as a treatment of a condition

(fertility), but not a treatment of a disorder or disease. Other conditions are understood by those of ordinary skill in the art.

Epitope: As used herein, the term "epitope" refers to basic element or smallest unit of recognition by an individual antibody or T-cell receptor, and thus the particular domain, region or molecular structure to which the antibody or T-cell receptor binds. An antigen may consist of numerous epitopes while a hapten, typically, possesses few epitopes.

Immune response: As used herein, the term "immune response" refers to any action by the immune system of an individual that is directed against a molecule or compound, such as an antigen. In mammals, the immune response includes both the activities of cells and the production of soluble molecules such as cytokines and antibodies. The term thus includes a humoral immune response and/or cellular immune response leading to the activation or proliferation of B- and/or T-lymphocytes. In some instances, however, the immune responses may be of low intensity and become detectable only when using at least one substance in accordance with the invention. "Immunogenic" refers to an agent used to stimulate the immune system of a living organism, so that one or more functions of the immune system are increased and directed towards the immunogenic agent. An "immunogenic polypeptide" is a polypeptide that elicits a cellular and/or humoral immune response, whether alone or linked to a carrier in the presence or absence of an adjuvant.

Immune Deviation: As used herein, the term immune deviation refers to the stimulation of an immune response that is of a different nature to a preexisting immune response. For example, an individual possessing a T_H2 immune response against an allergen such that IgE antibodies are produced upon exposure to the allergen may be induced, by embodiments of the present invention, to produce a T_H1 immune response against the allergen. Such T_H1 response will counteract the allergy inducing T_H2 response and so alleviate allergic disease.

Immunotherapeutic: As used herein, the term "immunotherapeutic" refers to a composition for the treatment of diseases, disorders or conditions. More specifically, the term is used to refer to a method of treatment wherein a beneficial immune response is generated by vaccination.

Immunologically effective amount: As used herein, the term "Immunologically effective amount" refers to an amount of a composition sufficient to induce an immune response in an individual when introduced into that individual. The amount of a composition necessary to be immunologically effective varies according many factors including to the composition, the presence of other components in the composition (*e.g.* adjuvants), the antigen, the route of immunization, the individual, the prior immune or physiologic state *etc.*

Individual: As used herein, the term "individual" refers to multicellular organisms and includes both plants and animals. Preferred multicellular organisms are animals, more preferred are vertebrates, even more preferred are mammals, and most preferred are humans.

Low or undetectable: As used herein, the phrase "low or undetectable," when used in reference to gene expression level, refers to a level of expression which is either significantly lower than that seen when the gene is maximally induced (*e.g.*, at least five fold lower) or is not readily detectable by the methods used in examples herein.

Mimotope: As used herein, the term "mimotope" refers to a substance which induces an immune response to an antigen or antigenic determinant. Generally, the term mimotope will be used with reference to a particular antigen. For example, a peptide which elicits the production of antibodies to a phospholipase A_2 (PLA_2) is a mimotope of the antigenic determinant to which the antibodies bind. A mimotope may or may not have substantial structural similarity to or share structural properties with an antigen or antigenic determinant to which it induces an immune response. Methods for generating and identifying mimotopes which induce immune responses to particular antigens or antigenic determinants are known in the art and are described elsewhere herein.

Mutein: As used herein, the term "mutein" refers to a protein or polypeptide differing by one or more amino acids from a given reference (*e.g.* natural, wild type, *etc.*) polypeptide, wherein such difference is caused by addition, substitution or deletion of at least one amino acid or a combination thereof. Preferred embodiments comprise mutations derived from substitution of at least one amino acid, preferably derived from conservative substitution of at least one amino acid. Conservative

substitutions include isosteric substitutions, substitutions where the charged, polar, aromatic, aliphatic or hydrophobic nature of the amino acid is maintained. For example, substitution of a cysteine residue with a serine residue is a conservative substitution. In preferred embodiments of the present invention, the term "mutein" refers to a protein or polypeptide differing by three, preferably two and most preferably one amino acid from a given reference (*e.g.* natural, wild type, *etc.*) polypeptide, wherein such difference is caused by addition, substitution or deletion or a combination thereof. . In further preferred embodiments of the present invention, the term "mutein" refers to a protein or polypeptide differing by three, preferably two and most preferably one amino acid from a given reference (*e.g.* natural, wild type, *etc.*) polypeptide, wherein such difference is derived from substitution of three, preferably two and most preferably one amino acid, preferably derived from conservative substitution of three, preferably two and most preferably one amino acid.

Natural origin: As used herein, the term "natural origin" means that the whole or parts thereof are not synthetic and exist or are produced in nature. Preferably, as used herein, the term "natural origin" means that the whole is not synthetic and exist or is produced in nature.

Non-natural: As used herein, the term generally means not from nature, more specifically, the term means from the hand of man.

Non-natural origin: As used herein, the term "non-natural origin" generally means synthetic or not from nature; more specifically, the term means from the hand of man.

Ordered and repetitive antigen or antigenic determinant array: As used herein, the term "ordered and repetitive antigen or antigenic determinant array" generally refers to a repeating pattern of antigen or antigenic determinant, characterized by a typically and preferably uniform spacial arrangement of the antigens or antigenic determinants with respect to the core particle and virus-like particle, respectively. In one embodiment of the invention, the repeating pattern may be a geometric pattern. Typical and preferred examples of suitable ordered and repetitive antigen or antigenic determinant arrays are those which possess strictly repetitive paracrystalline orders of antigens or antigenic determinants, preferably with spacings of 1 to 30 nanometers, preferably 5 to 15 nanometers.

Organic molecule: As used herein, the term "organic molecule" or „organic molecules" referring to the present invention include preferably antigens and antigen determinants, allergens, self antigens, haptens, cancer antigens and infectious disease antigens as well as small organic molecules such as drugs of abuse (like nicotine) and fragments and derivatives thereof.

Polypeptide: As used herein the term "polypeptide" refers to a polymer composed of amino acid residues, generally natural amino acid residues, linked together through peptide bonds. A polypeptide may not necessarily be limited in size, and include both proteins and peptides. A peptide is a polypeptide of a typical size of about five to about 50 amino acids, or any number amino acids within this general range. A peptide may, however, also be of longer length, for example up to 120-150 amino acids.

Protein: As used herein, the term protein refers to a polypeptide generally of a size of above about 5 or more, 10 or more 20 or more, 25 or more, 50 or more, 75 or more, 100 or more, 200 or more, 500 or more, 1000 or more, 2000 or more amino acids. Proteins generally have a defined three dimensional structure although they do not necessarily need to, and are often referred to as folded, as opposed to peptides and polypeptides which often do not possess a defined three-dimensional structure, but rather can adopt a large number of different conformations, and are referred to as unfolded. Peptides may, however, also have a defined three-dimensional structure.

Purified: As used herein, when the term "purified" is used in reference to a molecule, it means that the concentration of the molecule being purified has been increased relative to molecules associated with it in its natural environment, or environment in which it was produced, found or synthesized. Naturally associated molecules include proteins, nucleic acids, lipids and sugars but generally do not include water, buffers, and reagents added to maintain the integrity or facilitate the purification of the molecule being purified. For example, even if mRNA is diluted with an aqueous solvent during oligo dT column chromatography, mRNA molecules are purified by this chromatography if naturally associated nucleic acids and other biological molecules do not bind to the column and are separated from the subject mRNA molecules. According to this definition, a substance may be 5% or more, 10% or more, 20% or more, 30% or more, 40% or more, 50% or more, 60% or more, 70%

or more, 80% or more, 90% or more, 95% or more, 98% or more, 99% or more, or 100% pure when considered relative to its contaminants.

Receptor: As used herein, the term "receptor" refers to proteins or glycoproteins or fragments thereof capable of interacting with another molecule, called the ligand. The ligand may belong to any class of biochemical or chemical compounds. The receptor need not necessarily be a membrane-bound protein. Soluble protein, like *e.g.*, maltose binding protein or retinol binding protein are receptors as well.

Residue: As used herein, the term "residue" is meant to mean a specific amino acid in a polypeptide backbone or side chain.

Recombinant host cell: As used herein, the term "recombinant host cell" refers to a host cell into which one ore more nucleic acid molecules of the invention have been introduced. Host cells include eukaryotes include *e.g.* mammalian, insect, plant, avian, yeast; and prokaryotic *e.g. E.coli, B.subtilis, etc.*

RNA-phage: As used herein, the term "RNA-phage" refers to RNA viruses infecting bacteria, more specifically to single-stranded positive-sense RNA viruses infecting bacteria.

Self antigen: As used herein, the tem "self antigen" refers to molecules or compounds capable of being encoded by the host`s DNA. These include peptides, proteins, carbohydrates, nucleic acids, lipids and other biological molecules. More typically and preferably, the tem "self antigen" refers to polypeptides or proteins encoded by the host`s DNA. Products generated by proteins or RNA encoded by the host`s DNA are also defined as self. Proteins modified through post translational modifications and proteolytic processing or by alternative splicing of a self-gene product are also defined as self. Products generated by proteins or RNA encoded by the host`s DNA are defined as self. In addition, proteins that result from a combination of two or several self-molecules or that represent a fraction of a self-molecule and proteins that have a high homology to self-molecules as defined above (>95%, preferably >97%, more preferably >99%) may also be considered self.

Vector: As used herein, the term "vector" refers to an agent (*e.g.*, a plasmid or virus) used to transmit genetic material to a host cell. A vector may be composed of either DNA or RNA.

Virus-like particle (VLP): As used herein, the term "virus-like particle" refers to a structure resembling a virus particle. Moreover, a virus-like particle in accordance with the invention is non replicative and noninfectious since it lacks all or part of the viral genome, in particular the replicative and infectious components of the viral genome. A virus-like particle in accordance with the invention may contain nucleic acid distinct from their genome. A typical and preferred embodiment of a virus-like particle in accordance with the present invention is a viral capsid such as the viral capsid of the corresponding virus, bacteriophage, or RNA-phage. The terms "viral capsid" or "capsid", as interchangeably used herein, refer to a macromolecular assembly composed of viral protein subunits. Typically and preferably, the viral protein subunits assemble into a viral capsid and capsid, respectively, having a structure with an inherent repetitive organization, wherein said structure is, typically, spherical or tubular. For example, the capsids of RNA-phages have a spherical form of icosahedral symmetry. The term "capsid-like structure" as used herein, refers to a macromolecular assembly composed of viral protein subunits ressembling the capsid morphology in the above defined sense but deviating from the typical symmetrical assembly while maintaining a sufficient degree of order and repetitiveness.

Virus-like particle of a bacteriophage: As used herein, the term "virus-like particle of a bacteriophage" refers to a virus-like particle resembling the structure of a bacteriophage, being non replicative and noninfectious, and lacking at least the gene or genes encoding for the replication machinery of the bacteriophage, and typically also lacking the gene or genes encoding the protein or proteins responsible for viral attachment to or entry into the host. This definition should, however, also encompass virus-like particles of bacteriophages, in which the aforementioned gene or genes are still present but inactive, and, therefore, also leading to non-replicative and noninfectious virus-like particles of a bacteriophage.

Virus particle: The term "virus particle" as used herein refers to the morphological form of a virus. In some virus types it comprises a genome surrounded by a protein capsid; others have additional structures (*e.g.*, envelopes, tails, etc.).

One, a, or an: When the terms "one," "a," or "an" are used in this disclosure, they mean "at least one" or "one or more," unless otherwise indicated.

As used herein when referring to any numerical value, the term "about" means a

value of ±10% of the stated value (e.g., "about 50°C" encompasses a range of temperatures from 45°C to 55°C, inclusive; similarly, "about 100 mM" encompasses a range of concentrations from 90 mM to 110 mM inclusive).

Overview

We have discovered that recombinant AP205 coat proteins can be expressed in bacteria using the vectors of the invention and obtained in purified form. Recombinant AP205 coat proteins hereby spontaneously self-assemble within the bacteria into AP 205 virus-like particles. The invention provides for host cells and vectors suitable for expression of AP205 VLPs and also assembly competent variant forms of the AP205 coat protein. These expressed VLPs, AP205 VLPs derived from natural sources, or AP205 viral particles, may be bound to organic molecules to produce ordered repetitive arrays of the organic molecules. Organic molecules of the invention include antigens, allergens, self antigens, haptens, cancer antigens and infectious disease antigens. In one embodiment, the organic molecules are polypeptides or proteins.

Formation of conjugates of the invention, *i.e.* binding organic molecules to the VLP, is achieved by attachment, linkage, fusion or other binding, including covalent and non covalent bonds. In one embodiment, the VLP contains a first attachment site, the organic molecule contains a second attachment site. Association between the organic molecule occurs by linking the first and second attachment sites directly, or via a third molecule, typically and preferably via a cross-linker. Attachment sites may occur naturally, or may be introduced. In a preferred embodiment, the binding comprises at least one covalent bond, preferably comprises a peptide bond or alternatively and preferably comprises a non-peptide bond.

Immunization of animals with AP205 VLP conjugates, or with compositions comprising such conjugates as provided by the invention, induce a strong immune response against the displayed organic molecule. Hence, the conjugates and compositions of the invention are useful for the stimulation of an immune response against a variety of displayed antigens, and thus for the use in animals. The present invention also relates to a vaccine comprising an immunologically effective amount of

the composition of one or more conjuagtes of the present invention together with a pharmaceutically acceptable diluent, carrier or excipient. The AP205 VLPs can be used to vaccinate against haptens, allergens, tumors, viral diseases, or self-molecules or non-peptidic small molecules, for example. The vaccination can be for prophylactic or therapeutic purposes, or both. In a related aspect immune molecules and antibodies, respectively, such as antibodies, generated against such compositions may be used for treatment, prophalaxis or diagnosis of a disease, condition or disorder. Such antibodies, and compositions of the invention are also useful as kits.

Cloning of the AP205 Bacteriophage Coat Protein

The AP205 genome consists of a maturation protein, a coat protein, a replicase and two open reading frames not present in related phages; a lysis gene and an open reading frame playing a role in the translation of the maturation gene (Klovins,J., *et al.*, *J. Gen. Virol. 83*: 1523-33 (2002)). In one aspect of the invention the coat protein cDNA was isolated by reverse transcription of AP205 bacteriophage RNA followed by PCR, using known methods in the art. The cDNA of the coat protein including a ribosomal binding site upstream of the coat protein gene was cloned into vector pQb10 (Kozlovska, T. M.. *et al.*, *Gene 137*:133-37 (1993)). In another approach the cDNA of AP205 coat protein may be cloned in vector pQb185, replacing the bacteriophage Qβ coat protein gene, and thus downstream of the ribosomal binding site present in the vector. Both approaches lead to expression of the protein and formation of capsids. Thus, in the present invention, the coat protein may be expressed from vectors containing a ribosomal binding site which is not an AP205 ribosomal binding site, such as in the pQb185 vector.

Vectors pQb10 and pQb185 are vectors derived from pGEM vector, and expression of the cloned genes in these vectors is controlled by the *trp* promoter (Kozlovska, T. M. *et al.*, *Gene 137*:133-37 (1993)). pAP283-58 (SEQ ID No. 2) comprises a putative AP205 ribosomal binding site in the following sequence, which is downstream of the XbaI site, and immediately upstream of the ATG start codon of the AP205 coat protein: *tctaga*ATTTTCTGCGCACCCAT CCCGGGTGGCGCCCAAAGTGAGGAAAATCAC*atg* (SEQ ID NO: 5). The vector pQb185 comprises a Shine Delagarno sequence downstream from the XbaI site and

upstream of the start codon (*tctaga*TTAACCCAACGCGTAGGAG TCAGGCC*atg* (SEQ ID NO: 6), Shine Delagarno sequence underlined), which is also present in vector pAP281-32 (SEQ ID No. 4). Other vectors known to the Art include, *e.g.*, pKK 223.3, pET vector family, pBR322 (Sutcliffe, J.G. *Cold Spring Harb. Symp. Quant. Biol. 43 Pt 1*: 77-90 (1979)), pUC 18, pUC19, which are all modified to comprise a suitable promoter and ribosomal binding sites if not present or not suitable for expression of the coat protein and subsequent formation of virus-like particles as would be recognized by one skilled in the art. Other vectors derived from the aforementioned vectors and other vectors suitable for expression of proteins in *E. coli* or other hosts known to one skilled in the art and in general any vector suitable for expression of proteins in *E.coli* or other hosts are suitable for practicing the invention, provided they allow expression of the coat protein and subsequent formation of virus-like particles. In one aspect of the present invention, vectors for expression of the gene of AP205 coat protein are transfected into *E. coli*. Suitable *E. coli* strains include, but are not limited to, *E. coli* K802 , JM 109, RR1. Other *E. coli* strains are known to one of ordinary skill in the Art, and suitable combinations of vectors and strains can be identified by testing expression of the coat protein by SDS-PAGE and capsid formation and assembly by optionally first purifying the capsids by gel filtration and subsequently testing them in an immunodiffusion assay (Ouchterlony test) or Electron Microscopy (Kozlovska, T. M.. *et al.*, *Gene 137*:133-37 (1993)).

AP205 coat proteins expressed from the vectors pAP283-58 and pAP281-32 may be devoid of the initial Methionine amino-acid, due to processing in the cytoplasm of *E. coli*. The methionine-cleaved polypeptides, and hereby in particular the methionine-cleaved polypeptide of SEQ ID NO:1 and SEQ ID NO:3, as well as the uncleaved forms of the AP205 polypeptides leading to AP205 VLPs in accordance with the present invention, or mixtures thereof also leading to AP205 VLPs in accordance with the present invention are embodiments and within the scope of the invention.

AP205 Virus Like Particles

In one embodiment, the invention provides AP205 coat proteins that form capsids. Such proteins are recombinantly expressed, or prepared from natural sources.

Recombinant AP205 coat protein fragments able to assemble into a VLP are further embodiments of the invention. These fragments may be generated by deletion, either internally or at the termini of the coat protein. Insertions in the coat protein sequence or fusions to the coat protein sequence compatible with assembly into a VLP are further embodiments of the invention. The outcome of insertions, deletions and fusions to the coat protein sequence and whether it is compatible with assembly into a VLP can be determined by electron microscopy.

The present invention provides methods of purification for the recombinant AP205 coat protein. The particles formed by the AP205 coat protein can be isolated in pure form by a combination of fractionation steps by precipitation and of purification steps by gel filtration. Other methods of isolating virus-like particles are known in the art, and may be used to isolate the virus-like particles (VLPs) of bacteriophage AP205. For example, the use of ultracentrifugation to isolate VLPs of the yeast retrotransposon Ty is described in U.S. Patent No. 4,918,166, which is incorporated by reference herein in its entirety. In addition to gel filtration, other chromatographic steps such as ion exchange, hydrophobic interaction or affinity chromatography may also be used.

Expression of the recombinant AP205 coat protein leads to assembly into virus-like particles which, when analyzed by electron microscopy, have identical appearance and size as phage particles. It is a finding of the present invention, that the VLPs can be purified. This invention therefore provides a new VLP, which can be obtained in high amounts in a pure form.

AP205 VLPs self-assembled in E. coli have identical appearance and size as AP205 phage particles. As has been shown for other VLPs (Polyoma VP1 VLPs and Papilloma L1 VLPs, Chackerian B. et al., *PNAS 96*: 2373-2378 (1999)), manipulation of experimental conditions or fusion of epitopes to the VLP may lead to VLPs with a different state of assembly. For example, particles of lower triangulation number than the wt or major particle form may be obtained. Therefore, AP205 VLPs of smaller size than AP205 phage particles, or mixtures of AP205 VLPs of same size and smaller size than AP205 phage particles are also embodiments of the invention.

When analyzed in non-reducing PAGE, the AP205 VLP subunits runs at a higher apparent molecular weight than when analyzed by reducing PAGE, showing that the subunits are associated by disulfide bridges as described in Example 17 of

WO03/024481.

In a further aspect, the present invention provides a vector containing an open reading frame suitable for the production of an AP205 virus like particle, said vector further comprising an additional nucleic acid such that the resulting vector is capable of producing a recombinant AP205 virus-like particle comprising amino acids encoded by said additional nucleic acid.

In another aspect, the present invention provides a vector containing an open reading frame suitable for the production of an AP205 virus like particle, said vector further comprising a restriction enzyme site suitable for the introduction of additional nucleic acid such that the resulting vector is capable of producing a recombinant virus-like particle comprising amino acids encoded by said additional nucleic acid.

Organic Molecules, Haptens and Antigens

Organic molecules used in the methods, conjugates and compositions of the present invention include any antigen, hapten, organic molecule, or fragment thereof. Molecules of the invention include haptens, organic molecules, and antigen fragments that are themselves (*ie* not bound to AP205 virus or virus like paticle) not capable of inducing an immune response in an animal. Organic molecule include for example: (a) organic molecule suited to induce an immune response against cancer cells; (b) organic molecules suited to induce an immune response against infectious diseases; (c) organic molecules suited to induce an immune response against allergens, (d) organic molecules suited to induce an immune response against self-antigens, (e) antigens or haptens suited to induce an immune response against drugs, hormones or toxins, particularly drugs of abuse and (f)fragments (*e.g.*, a domain) of any of the organic molecule, antigens or haptens set out in (a)-(e).

Infectious Diseases

In one specific embodiment of the invention, the organic molecule, antigen or antigenic determinant is one that is useful for the prevention of infectious disease. Such treatment will be useful to treat a wide variety of infectious diseases affecting a wide range of hosts, *e.g.*, human, cow, sheep, pig, dog, cat, other mammalian species and non-mammalian species as well. Such vaccines may be used prophylactically, to

prevent an infection in an individual or population, or therapeutically, to mitigate an ongoing infection. Infectious diseases for which a vaccine is known or desired are well known to those skilled in the art, examples include infections of viral etiology such as HIV, influenza, *Herpes*, viral hepatitis, Epstein Barr, polio, viral encephalitis, measles, chicken pox, *etc.*; infections of bacterial etiology such as pneumonia, tuberculosis, syphilis, lyme disease, cholera, salmonellosis, meningitis, sepsis etc.; or infections of parasitic etiology such as malaria, trypanosomiasis, leishmaniasis, trichomoniasis, amoebiasis, etc. The antigens or antigenic determinants used in the conjugates, compositions and methods of the invention are known to those of ordinary skill in the relevant arts. Examples of antigens or antigenic determinants include the following: the HIV antigens gp140 and gp160; the influenza antigens hemagglutinin, M2 protein and neuraminidase, Hepatitis B surface antigen, and circumsporozoite protein of malaria.

In one such embodiment of the invention, the antigen or antigenic determinant is selected from the group consisting of: (a) a recombinant protein of HIV, (b) a recombinant protein of Influenza virus (*e.g.*, an Influenza virus M2 protein or a fragment thereof), (c) a recombinant protein of Hepatitis C virus, (d) a recombinant protein of *Toxoplasma*, (e) a recombinant protein of *Plasmodium falciparum*, (f) a recombinant protein of *Plasmodium vivax*, (g) a recombinant protein of *Plasmodium ovale*, (h) a recombinant protein of *Plasmodium malariae*, (i) a recombinant protein of *Chlamydia*, and (j) a fragment of any of the proteins set out in (a)-(i).

In another embodiment, the invention is drawn to vaccine compositions comprising at least one antigen or antigenic determinant encoded by an Influenza viral nucleic acid, and the use of such vaccine compositions to elicit immune responses. In specific such embodiment, the Influenza antigen or antigenic determinant is an M2 protein (*e.g.*, an M2 protein having the amino acids shown in GenBank Accession No. P06821, PIR Accession No. MFIV62, or fragment thereof (*e.g.*, amino acids from about 2 to about 24). Portions of an M2 protein, as well as other proteins against which an immunological response is sought, suitable for use with the invention comprise peptides of any number of amino acids in length but will generally be at least 6 amino acids in length (*e.g.*, peptides 6, 7, 8, 9, 10, 12, 15, 18, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 97 amino acids in length).

Hormones, Toxins and Drugs, Especially Drugs of Abuse

In an additional aspect, the invention provides compositions suitable for stimulating immune responses against haptens. These haptens include, but are not limited to, hormones, drugs and toxic compounds. Immune responses against a variety of drugs, hormones and toxic compounds are used to protect an individual at risk of exposure to such compounds, as therapy in an individual exposed to such compounds, or to prevent or treat addictions to such compounds.

Representative toxic compounds include, but are not limited to, the natural products of toxic plants, animals, and microorganisms. Such products include aflatoxin, ciguautera toxin, and tetrodotoxin. Other representative toxic compounds produced artificially, or as a result of metabolism include antibiotics (*e.g.* vancomycin), anticancer compounds (*e.g.* vinblastine) and chemical warfare agents (*eg.* Sarin, mustard gas, VX). One aspect of the invention includes the production of antibodies against toxic metabolites of commonly used pharmaceutical agents, such that an individual may continue to receive the beneficial effects of a pharmaceutical agent without side effects associated with toxic metabolites. Thus, in a preferred embodiment, the toxin is a metabolite generated in the body of an individual, wherein said metabolite is a metabolite of a pharmaceutical agent. In a further preferred embodiment, the toxin is a chemical warfare agent.

Organic molecules, antigens or antigenic determinants suitable for use in conjugates, compositions and methods of treatment of drug addiction, in particular recreational drug addiction, will be known to those of ordinary skill in the relevant arts. Representative examples of organic molecules, antigens or antigenic determinants include, for example, opioids and morphine derivatives such as codeine, fentanyl, heroin, morphine and opium; stimulants such as amphetamine, cocaine, MDMA (methylenedioxymethamphetamine), methamphetamine, methylphenidate, and nicotine; hallucinogens such as LSD, mescaline and psilocybin; cannabinoids such as hashish and marijuana, other addictive drugs or compounds; and derivatives, by-products, variants and complexes of such compounds.

Allergies and Cancer

In related embodiments, the invention provides compositions suitable for use as immunotherapeutics that may be used for the treatment or prevention of allergies or cancer.

Antigens or antigenic determinants suitable for use in conjugates, compositions and methods of treatment or prevention of allergies would be known to those of ordinary skill in the relevant art. Representative examples of such antigens or antigenic determinants include the following: bee venom phospholipase A_2, Bet v I (birch pollen allergen), 5 Dol m V (white-faced hornet venom allergen), Mellitin and Der p I (House dust mite allergen), gluten, gliadin, shellfish allergens, cockroach allergens, peanut and other nut allergens, ragweed and other pollen allergens, grevillea allergen, as well as fragments of each which can be used to elicit immunological responses. In a particular embodiment of the invention, the allergen is selected from the group consisting of (a) a recombinant protein of bee sting allergy, (b) a recombinant proteins of nut allergy, (c) recombinant proteins of food allergies, (d) recombinant proteins of asthma, (e) a recombinant protein of *Chlamydia*, and (f) a fragment of any of the allergens of (a) through (e).

As noted above, a suitable antigen or antigenic determinant for use in the conjugates, compositions or methods of the present invention is Der p I. Der p I is a 25kD protease found in house dust mite faecal particles and represents the major allergen of house dust mite. Accordingly, 80% of mite allergic patients have anti-Der p I IgE antibodies. In particular, the Der pI peptides p52-72 and p117-133 (SEQ ID NO: 64), among others, are known to comprise epitopes which are recognized by antibodies specific for the native Der p I. IgE antibodies raised in a polyclonal response to the whole antigen bind with high affinity to the peptide region 59-94 (L. Pierson-Mullany *et al.* (2000) Molecular Immunology). Other regions also bind IgE with high affinity. The peptide p117-133 contains a cysteine at its N-terminus representing the second attachment site in accordance with the invention. 3D modelling assigns peptides p52-72 and p117-133 to the surface of the whole protein (Jeannin, P. et al., Molecular Immunology *30:1511-1518* (1993)).However, other fragments of the Der p I protein may comprise B cell epitopes suitable for the present invention.

In a preferred embodiment of the inventive composition, the antigen or

antigenic determinant is a Der p I peptide, and wherein said Der p I peptide with said second attachment site has an amino acid sequence selected from the group consisting of: a) CGNQSLDLAEQELVDCASQHGCH (SEQ ID NO: 97); and b) CQIYPPNANKIREALAQTHSA (SEQ ID NO: 64).

In additional embodiments, the invention provides vaccine compositions suitable for use in methods for preventing and/or attenuating allergic reactions, such as allergic reactions which lead to anaphylaxis. As disclosed elsewhere herein, allergic reactions may be characterized by the T_H2 responses against an antigen leading to the presence of IgE antibodies. Stimulation of T_H1 immune responses and the production of IgG antibodies may alleviate allergic disease. Thus, vaccine compositions of the invention include compositions which lead to the production of antibodies that prevent and/or attenuate allergic reactions. Thus, in certain embodiments, vaccine compositions of the invention include compositions which elicit an immunological response against an allergen. Examples of such allergens include phospholipases such as the phospholipase A_2 (PLA_2) proteins of *Apis mellifera* (GenBank Accession No. 443189, GenBank Accession No. 229378), *Apis dorsata* (GenBank Accession No. B59055), *Apis cerana* (GenBank Accession No. A59055), *Bombus pennsylvanicus* (GenBank Accession No. B56338), and *Heloderma suspectum* (GenBank Accession No. P80003; GenBank Accession No. S14764; GenBank Accession No. 226711).

Using the amino acid sequence of a PLA_2 protein of *Apis mellifera* (GenBank Accession No. 443189, GenBank Accession No. 229378) for illustration, peptides of at least about 60 amino acids in length, which represent any portion of the whole PLA_2 sequence, may also be used in compositions for preventing and/or attenuating allergic reactions. Examples of such peptides include peptides which comprise amino acids 1-60, amino acids 1-70, amino acids 10-70, amino acids 20-80, amino acids 30-90, amino acids 40-100, amino acids 47-99, amino acids 50-110, amino acids 60-120, amino acids 70-130, or amino acids 90-134, as well as corresponding portions of other PLA_2 proteins (*e.g.*, PLA_2 proteins described above). Further examples of such peptides include peptides which comprise amino acids 1-10, amino acids 5-15, amino acids 10-20, amino acids 20-30, amino acids 30-40, amino acids 40-50, amino acids 50-60, amino acids 60-70, amino acids 70-80, amino acids 80-90, amino acids 90-100,

amino acids 100-110, amino acids 110-120, or amino acids 120-130, as well corresponding portions of other PLA_2 proteins (*e.g.*, PLA_2 proteins described above).

Portions of PLA_2, as well as portions of other proteins against which an immunological response is sought, suitable for use with the invention may comprise, or alternatively consist of, peptides which are generally at least 6 amino acids in length (*e.g.*, peptides 6, 7, 8, 9, 10, 12, 15, 18, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acids in length).

PLA_2 peptides (*e.g.*, the full length PLA_2 proteins discussed above, as well as subportions of each) may also be coupled to any substance (*e.g.*, an AP205 capsid protein or fragment thereof) which allows for the formation of ordered and repetitive antigen arrays.

The selection of antigens or antigenic determinants for compositions and methods of treatment for cancer would be known to those of ordinary skill in the relevant arts. In a particular embodiment of the invention, the antigen or antigenic determinant is selected from the group consisting of: (a) a recombinant protein of breast cancer cells; (b) a recombinant protein of kidney cancer cells; (c) a recombinant protein of prostate cancer cells; (d) a recombinant protein of skin cancer cells; (e) a recombinant protein of brain cancer cells; (f) a recombinant protein of leukemia cells; (g) a recombinant profiling; and (h) a fragment of any of the proteins set out in (a)-(g).

Representative examples of such types of antigens or antigenic determinants include the following: Her2 (breast cancer), GD2 (neuroblastoma), EGF-R (malignant glioblastoma), CEA (medullary thyroid cancer), and CD52 (leukemia), human melanoma protein gp100, human melanoma protein melan-A/MART-1, tyrosinase, NA17-A nt protein, MAGE-3 protein, p53 protein, and HPV16 E7 protein, as well as fragments of each which can be used to elicit immunological responses. Further antigenic determinants useful for compositions and methods of treatment for cancer are molecules and antigenic determinants involved in angiogenesis. Angiogenesis, the formation of new blood vessels, plays an essential role in physiological and pathophysiological processes such as wound healing and solid tumor growth, respectively (Folkman, J. (1995) *Nat. Medicine* 1, 27-31; Folkman, J., and Klagsburn, M. (1987) *Science* 235, 442-446; Martiny-Baron, G., and Marmé, D. (1995) *Curr. Opin. Biotechnol.* 6, 675-680; Risau, W. (1997) *Nature* 386, 671-674). Rapidly

growing tumors initiate and depend on the formation of blood vessels to provide the required blood supply. Thus, it is believed that antiangiogenic agents are effective as anticancer therapeutics.

Among several putative angiogenic factors that have been identified, vascular endothelial growth factor (VEGF) is a potent endothelial cell specific mitogen and a primary stimulant of the vascularization of many solid tumors. Thus, blockage of VEGF action is a target for intervention in tumor-induced angiogenesis, as a means of blocking the endothelium rather than the tumor as a strategy to fight tumors (Millauer, B. *et al.* (1994) *Nature* 367, 576-579; Kim, J *et al.* (1993) *Nature* 362, 841-844).

An anti-VEGFR-II antibody (IMC-1C11) and an anti-VEGF antibody have been disclosed (Lu, D. et al. (2000) *J. Biol. Chem.* 275, 14321-14330; Presta, L.G, et al (1997) *Cancer Res.* 47, 4593–4599). The former neutralizing monoclonal anti-VEGFR-2 antibody recognizes an epitope that has been identified as putative VEGF/VEGFR-II binding site (Piossek, C. *et al.* (1999) *J Biol Chem. 274,* 5612-5619).

Thus, in one embodiment of the invention, the antigen or antigenic determinant used in conjugates, compositions or methods of the invention is a peptide derived from the VEGFR-II contact site. This provides a composition and a vaccine composition in accordance with the invention, which may have antiangiogenic properties useful for the treatment of cancer. Inhibition of tumor growth in mice using sera specific for VEGFR-2 has been demonstrated (Wei, YQ et al. (2000) Nature Medicine 6, 1160-1165). Therefore, further antigenic determinants suitable for inventive compositions and antiangiogenic vaccine compositions in accordance with the invention comprise either the human VEGFR-II derived peptide with the amino acid sequence CTARTELNVGI DFNWEYPSSKHQHKK (SEQ ID NO: 99), and/or the murine VEGFR-II derived peptide having the amino acid sequence CTARTELNVGLDFTWHSPPSKSHHKK (SEQ ID NO: 113), and/or the relevant extracellular globular domains 1-3 of the VEGFR-II.

Self-Antigens

In specific embodiments, the invention provides vaccine compositions suitable for use in methods for preventing and/or attenuating diseases or conditions which are caused or exacerbated by "self" gene products (*e.g.*, tumor necrosis factors). It is

usually difficult if not impossible to induce antibody responses to self-molecules by conventional vaccination. The present invention provides one way to improve the efficiency of vaccination by increasing the degree of repetitiveness of the antigen to be used for immunization, through binding of the antigen to the AP205 VLP.

Unlike isolated proteins, viruses induce prompt and efficient immune responses in the absence of any adjuvants both with and without T-cell help (Bachmann & Zinkernagel, *Ann. Rev. Immunol*: 15:235-270 (1997)). Although viruses often consist of few proteins, they are able to trigger much stronger immune responses than their isolated components. For B-cell responses, it is known that one crucial factor for the immunogenicity of viruses is the repetitiveness and order of surface epitopes. Many viruses exhibit a quasi- crystalline surface that displays a regular array of epitopes which efficiently crosslinks epitope-specific immunoglobulins on B cells (Bachmann & Zinkernagel, *Immunol. Today* 17:553-558 (1996)). This crosslinking of surface immunoglobulins on B cells is a strong activation signal that directly induces cell-cycle progression and the production of IgM antibodies. Further, such triggered B cells are able to activate T helper cells, which in turn induce a switch from IgM to IgG antibody production in B cells and the generation of long-lived B cell memory - the goal of any vaccination (Bachmann & Zinkernagel, *Ann. Rev. Immunol.* 15:235-270 (1997)). Viral structure is even linked to the generation of anti-antibodies in autoimmune disease and as a part of the natural response to pathogens (see Fehr, T., *et al., J Exp. Med. 185*:1785-1792 (1997)). Thus, antibodies presented by a highly organized viral surface are able to induce strong anti-antibody responses.

The immune system usually fails to produce antibodies against self-derived structures. For soluble antigens present at low concentrations, this is due to tolerance at the Th cell level. Under these conditions, coupling the self-antigen to a carrier that can deliver T help may break tolerance. For soluble proteins present at high concentrations or membrane-associated proteins at low concentration, B and T_H cells may be tolerant. However, B cell tolerance may be reversible (anergy) and can be broken by administration of the antigen in a highly organized fashion coupled to a foreign carrier (Bachmann & Zinkernagel, *Ann. Rev. Immunol.* 15:235-270 (1997).

Thus, vaccine compositions of the invention include conjugates, compositions and methods which lead to the production of antibodies that prevent and/or attenuate

diseases or conditions caused or exacerbated by "self" gene products. Examples of such diseases or conditions include graft versus host disease, IgE-mediated allergic reactions, anaphylaxis, adult respiratory distress syndrome, Crohn's disease, allergic asthma, acute lymphoblastic leukemia (ALL), diabetes, non-Hodgkin's lymphoma (NHL), Graves' disease, systemic lupus erythematosus (SLE), inflammatory autoimmune diseases, myasthenia gravis, immunoproliferative disease lymphadenopathy (IPL), angioimmunoproliferative lymphadenopathy (AIL), immunoblastive lymphadenopathy (IBL), rheumatoid arthritis, diabetes, multiple sclerosis, Alzheimer disease, osteoporosis, and autoimmune conditions associated with certain infections including rheumatic fever, scarlet fever, lyme disease, and infectious polyarthritis.

The selection of antigens or antigenic determinants for conjugates, compositions and methods of treatment for other diseases or conditions associated with self antigens would be also known to those of ordinary skills in the relevant art. Representative examples of such antigens or antigenic determinants are, for example, lymphotoxins (*e.g.* Lymphotoxin α (LT α), Lymphotoxin β (LT β)), and lymphotoxin receptors, Receptor activator of nuclear factor kB ligand (RANKL), vascular endothelial growth factor (VEGF), vascular endothelial growth factor receptor (VEGF-R), Interleukin-5, Interleukin-17, Interleukin-13, CCL21, CXCL12, SDF-1, MCP-1, Endoglin, Resistin, GHRH, LHRH, TRH, MIF, Eotaxin, Bradykinin, BLC, Tumor Necrosis Factor α and amyloid beta peptide ($A\beta_{1-42}$), as well as fragments of each which can be used to elicit immunological responses.

In one embodiment, the antigenic determinant is the amyloid beta peptide ($A\beta_{1-42}$) (DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA (SEQ ID NO: 7), or a fragment thereof. Aβ peptide has a central role in the neuropathology of Alzheimers disease. Region specific, extracellular accumulation of Aβ peptide is accompanied by microgliosis, cytoskeletal changes, dystrophic neuritis and synaptic loss. These pathological alterations are thought to be linked to the cognitive decline that defines the disease.

In a mouse model of Alzheimer disease, transgenic animals engineered to produce $A\beta_{1-42}$ (PDAPP-mice), develop plaques and neuron damage in their brains.

Recent work has shown immunization of young PDAPP-mice, using $A\beta_{1-42}$, resulted in inhibition of plaque formation and associated dystrophic neuritis (Schenk, D. *et al.*, *Nature 400*:173-77 (1999)).

Furthermore immunization of older PDAPP mice that had already developed AD-like neuropathologies, reduced the extent and progression of the neuropathologies. In another study, peripherally administered antibodies raised against $A\beta_{1-42}$, were able to induce clearance of pre-existing amyloid (Bard, F. *et al.*, *Nature Medicine 6*:916-19 (2000)). This study utilized either polyclonal antibodies raised against $A\beta_{1-42}$, or monoclonal antibodies raised against synthetic fragments derived from different regions of Aβ. Thus induction of antibodies against Aβ using the conjugates, compositions and methods of the present invention can be considered as a potential therapeutic treatment for Alzheimer disease.

It is well established that the administration of purified proteins alone is usually not sufficient to elicit a strong immune response; isolated antigen generally must be given together with helper substances called adjuvants. Within these adjuvants, the administered antigen is protected against rapid degradation, and the adjuvant provides an extended release of a low level of antigen. In the present invention, Aβ peptide or fragments thereof are made immunogenic through binding to AP205 VLP and do not necessarily require an adjuvant.

As indicated, one of the key events in Alzheimer's Disease (AD) is the deposition of amyloid as insoluble fibrous masses (amyloidogenesis) resulting in extracellular neuritic plaques and deposits around the walls of cerebral blood vessels (for review see Selkoe, D. J. (1999) Nature. 399, A23-31). The major constituent of the neuritic plaques and congophilic angiopathy is amyloid ß (Aß), although these deposits also contain other proteins such as glycosaminoglycans and apolipoproteins. Aß is proteolytically cleaved from a much larger glycoprotein known as Amyloid Precursor Proteins (APPs), which comprises isoforms of 695-770 amino acids with a single hydrophobic transmembrane region. Aß forms a group of peptides up to 43 amino acids in length showing considerable amino- and carboxy-terminal heterogeneity (truncation) as well as modifications (Roher, A. E. et al (1988) J. Cell Biol. 107, 2703-2716. Roher, A. E. et al (1993) J. Neurochem. 61, 1916-1926). Prominent isoforms are

Aß 1-40 and 1-42. It has a high propensity to form ß-sheets aggregating into fibrils, which ultimately leads to the amyloid. Recent studies have demonstrated that a vaccination-induced reduction in brain amyloid deposits resulted in cognitive improvements (Schenk, D. et al. (1999) Nature. 400, 173-177). Hence, fragments of Aß suitable for generating vaccines of the invention include, but are not limited to: Aβ 1-15 , Aβ 1-27 and Aβ 33-42. An amino acid linker is fused to the aminoacid sequence of Aß or Aß fragments to allow coupling to the AP205 VLP, as described elsewhere herein. Amino acid linkers suitable for fusion to the N-terminus of Aß or Aß fragments include but are not limited to the sequence CGG and CGHGNKS. Linkers suitable for fusion to the C-terminus of Aß or Aß fragments include but are not limited to the sequence GGC. In one embodiment, when a linker is fused to the C-terminus of Aß or Aß fragments, the C-terminal cysteine is amidated. The Aß fragment 1-15 is fused to an amino acid linker and has the sequence: DAEFRHDSGYEVHHQGGC-NH2 (SEQ ID NO: 8), wherein the C.terminal cysteine is amidated, which is indicated by the C-terminal "NH2". The Aß fragment 1-27 is fused to an amino acid linker and has the sequence: DAEFRHDSGYEVHHQKLVFFAEDVGSNGGC-NH2 (SEQ ID NO: 9). The Aß fragment 33-42 is fused to an amino acid linker and has the sequence: CGHGNKSGLMVGGVVIA (SEQ ID NO: 10).

In one embodiment of the invention, the antigen or antigenic determinant comprises, or preferably is, an angiotensin peptide or a fragment thereof. The term "angiotensin peptide" as used herein, encompasses any peptide comprising the sequence, or any fragment thereof, of angiotensinogen, angiotensin I or angiotensin II. Angiotensin is associated with hypertension (Gardiner et al, *Br. J. Pharm. 129*: 1178 (2000)). Therefore, conjugates, compostion and methods of present invention suitable for reducing levels of angiotensin are suitable for the treatment of hypertension. In one embodiment, the conjugate or composition comprises at least one angiotensin peptide. The amino acid sequences sequences of peptides in some embodiments are as follows: Angiotensinogen: DRVYIHPFHLVIHN (SEQ ID NO: 11); Angiotensin I: DRVYIHPFHL (SEQ ID NO: 12); Angiotensin II: DRVYIHPF (SEQ ID NO: 13). Typically, one or more additional amino acids are added at the C- and/or at the N-terminus of the angiotensin peptide sequences. Those additional amino acids are, in particular, valuable for an oriented and ordered association to the AP 205 virus-like

particle. The sequence of the angiotensin peptides corresponds to the human sequence, which is identical to the murine sequence. Therefore, immunization of a human or a mouse with vaccines or compositions, respectively, comprising such angiotensin peptides as an antigen or antigenic determinant in accordance with the invention, is a vaccination against a self-antigen. In some embodiments, the angiotensin peptide with said second attachment site has an amino acid sequence selected from the group consisting of a) the amino acid sequence of CGGDRVYIHPF (SEQ ID NO: 14); b) the amino acid sequence of CGGDRVYIHPFHL (SEQ ID NO: 15); c) the amino acid sequence of DRVYIHPFHLGGC (SEQ ID NO: 16); and d) the amino acid sequence of CDRVYIHPFH (SEQ ID NO: 98).

In another embodiment of the invention, the antigenic determinant is RANKL (Receptor Activator of NFkB Ligand). RANKL is also known as TRANCE (TNF-related Activation Induced Cytokine), ODF (Osteoclast Differentiation Factor) or OPGL (Osteoprotegerin Ligand). The amino acid sequence of the extracellular part of human RANKL is shown in EMBL database deposit RANKL_human: TrEMBL:O14788. Sequences for the extracellular part of murine RANKL and an isoform are shown in EMBL database deposits RANKL_mouse: TrEMBL:O35235, and in RANKL_mouse splice forms: TrEMBL:Q9JJK8 and TrEMBL:Q9JJK9, respectively.

It has been shown that RANKL is an essential factor in osteoclastogenesis. Inhibition of the interaction of RANKL with its receptor RANK can lead to a suppression of osteoclastogenesis and thus provide a means to stop excessive bone resorption as seen in osteoporosis and other conditions. The RANKL/RANK interaction was inhibited either by a RANK-Fc fusion protein or the soluble decoy receptor of RANKL, termed osteoprotegerin (OPG.)

In bone, RANKL is expressed on stromal cells or osteoblasts, while RANK is expressed on the osteoclast precursor. The interaction of RANK and RANKL is crucial for the development of osteoclast precursors to mature osteoclasts. The interaction can be blocked by OPG. OPG-deficient mice develop osteoporosis that can be rescued by injection of recombinant OPG, suggesting that OPG is able to reverse osteoporosis. Thus, inhibition of the RANK-RANKL interaction by providing suitable conjugates,

compositions and methods of the invention (*eg* RANKL-AP205 VLP conjugates) are effective in reversing or preventing osteoporosis.

In addition, arterial calcification was observed in OPG knockout. mice which could be reversed by injection of OPG (Min *et al., J. Exp. Med. 4*: 463 (2000)). In an adjuvant-induced arthritis model OPG injection was able to prevent bone loss and cartilage destruction, but not inflammation (paw swelling). It is assumed that activated T cells lead to a RANKL-mediated increase of osteoclastogenesis and bone loss. OPG inhibits prostate cancer-induced osteoclastogenesis and prevents prostate tumor growth in the bone of mice. OPG diminishes advanced bone cancer pain in mice.

RANKL is a transmembrane protein of 245 aa belonging to the TNF-superfamily. Part of the extracellular region (178 aa) can be cleaved by a TACE-like protease (Lum *et al., J Biol Chem. 274*:13613 (1999)). In addition splice variants lacking the transmembrane domain have been described (Ikeda *et al., Endocrinology142*: 1419 (2001)). The cleaved extracellular portion contains the domain highly homologous to soluble TNF-α and forms homotrimers as seen for TNF-α. The C-terminus seems to be involved in the trimer contact site. One cysteine is present in this region of the sequence.

We have built a model for the 3-dimensional structure of the corresponding region of RANKL and found that the naturally present cysteine may not be accessible in the folded structure for interaction with a first attachment site on the carrier in accordance with the present invention. The N-terminus is one site suitable for attaching a second attachment site comprising an amino acid linker with an additional cysteine residue. A human-RANKL construct with an N terminal amino acid linker containing a cysteine residue fused to the extracellular part of RANKL is one embodiment of the invention. However, an amino-acid linker containing a cysteine residue as second attachment site and being fused at the C-terminus of the RANKL sequence or the extracellular part of RANKL leads to further embodiments of the invention.

Human-RANKL constructs are generated according to the teachings disclosed in herein and one of ordinary skill in the art is able to compare murine and human RANKL sequences in an amino acid sequence alignment to identify the part of the sequence of human-RANKL to be cloned in the vectors. Fragments containing amino acids 138-317 and corresponding to the C-terminal region of the extracellular domain

of human RANKL, comprise one embodiment of the invention, and are modified for coupling to AP205 VLP as required according to the teachings of the present invention. The invention also embodies other suitable vectors used for expression in the suitable host described below. Additional human-RANKL constructs that are intended to be encompassed within the scope of the present invention include those comprising the part of the extracellular region (178 aa) or fragments thereof that can be shed by a TACE-like protease (Lum *et al., J Biol Chem. 274*:13613 (1999)), or that comprise the sequence corresponding to the alternative splice variants lacking the transmembrane domain, as well as conservative fragments thereof. Human C-terminal fragments of RANKL comprising amino acids 165-317 are also embodiments of the invention. Fragments of RANKL which encompass the entire extracellular region (amino acids 71-317) and can be modified for coupling to AP205 VLP and as required according to the teaching of the present invention, are also within the scope of the invention.

RANKL has been expressed in different systems (e.g *E.coli*, insect cells, mammalian cells) and has been shown to be active. Therefore, several expression systems can be used for production of suitable RANKL antigens of the composition. In the case where expression of the protein is directed to the periplasm of *E. coli*, the signal peptide of RANKL, or of RANKL constructs consisting of the extracellular part of the protein, and both possibly modified to comprise a second attachment site in accordance with the invention, is replaced with a bacterial signal peptide. For expression of the protein in the cytoplasm of *E. coli*, RANKL constructs are devoid of signal peptide.

In one embodiment of the invention, the antigenic determinant is MIF or a fragment thereof. MIF is a cytokine that functions as an inhibitor of macrophage migration. It is also known as delayed early response protein 6 (DER6), glycosylation inhibiting factor or phenylpyruvate tautomerase.

MIF has been shown to be implicated in a wide range of conditions. MIF (mRNA and protein) is upregulated in delayed type hypersensitivity (DTH) reaction induced by tuberculin, and anti-MIF antibody inhibits this DTH reaction. MIF is also upregulated in renal allograft rejection. In a model for ocular autoimmune disease, experimental autoimmune uveoretinitis (EAU), anti-MIF treatment caused delay of

EAU development. In patients, there is an increase in serum of MIF, which is also the case in Behcet's disease patients and patients suffering from iridocyclitis. Immunization against MIF may provide a way of treatment against rheumatoid arthritis. Thus conjugates, composites and methods of the invention suitable for immunizing againts MIF, or for reducing serum MIF, are useful in the treatment or prevention of those diseases, disorders and conditons associated with overproduction of MIF.

High serum MIF concentration has been found in atopic dermatitis patients. In skin lesions, MIF is diffusely expressed instead of being found in the basal cell layer in controls. MIF concentration is decreased after steroid treatment, consistent with a role of MIF in inflammation. MIF has also been found to contribute to the establishment of glomerulonephritis. Animals treated with anti-MIF antibody show significantly reduced glomerulonephritis. MIF is pituitary derived, secreted e.g. upon LPS stimulation, and potentiates endotoxemia. Accordingly, anti-MIF mAb inhibits endotoxemia and septic shock, while recombinant MIF markedly increases lethality of peritonitis. MIF is also a glucocorticoid-induced modulator of cytokine production, and promotes inflammation.

MIF is also produced by T-cells (T_H2), supports proliferation of T-cells, and anti-MIF-treatment reduces T-cell proliferation and IgG levels. There is an increased MIF concentration in the cerebrospinal fluid of multiple sclerosis and neuro-Behcet's disease patients. High MIF levels were also found in sera of patients with extended psoriasis. High MIF levels are found in sera of ulcerative colitis patients but not Crohn's disease patients.

High MIF levels have been found in sera of patients with bronchic asthma. MIF is also upregulated in synovial fluid of rheumatoid arthritis patients. Anti-MIF treatment was effectivly decreasing rheumatoid arthritis in mouse and rat models (Mikulowska *et al., J. Immunol. 158*:5514-7(1997); Leech *et al., Arthritis Rheum. 41*:910-7 (1998), Leech *et al. Arthritis Rheum. 43*:827-33 (2000), Santos *et al., Clin. Exp. Immunol. 123*:309-14 (2001)). Thus, treatment directed at inhibiting MIF activity using a composition comprising MIF as an antigenic determinant are beneficial for the conditions mentioned above.

MIF from mouse, rat and human consists of 114 amino acid and contains three conserved cysteines, as shown in MIF_rat: SEQ ID NO: 120, MIF_mouse: SEQ ID NO: 121 and in MIF_human: SEQ ID NO: 119 SwissProt. Three subunits form a homotrimer that is not stabilized by disulfide bonds. The X-ray structure has been solved and shows three free cysteines (Sun *et al., PNAS 93*: 5191-96 (1996)), while some literature data claim the presence of a disulfide bond. Nonetheless, none of the cysteines are exposed enough for optimal interaction with a possible first attachment site present on the carrier. Thus, as the C-terminus of the protein is exposed in the trimer structure, an amino acid linker containing a cysteine residue is, in one aspect, added to the C-terminus of the protein, for generation of the second attachment site in this embodiment of the invention. There is only one amino acid change between mouse- and rat-MIF, and similarly a very high sequence homology (about 90 % sequence identity) between human- and rat-MIF or human- and mouse-MIF. The invention embodies conjugates, compositions and methods comprising human- and mouse-MIF constructs associated to the AP205 VLP.

An amino acid linker containing a cysteine that is added at the N-terminus of the sequence of MIF leads to further embodiments of the present invention. MIF has been expressed in *E.coli*, purified and shown to be fully functional (Bernhagen *et al., Biochemistry 33*: 14144-14155 (1994). Thus, MIF may be expressed in *E. coli* for generating the useful embodiments of the invention.

Tautomerase activity of MIF is inhibited if the start methionine is not cleaved from the construct. MIF constructs expressed in *E.coli* show tautomerase activity. Mutants of MIF where the start methionine is cleaved and where the proline residue right after the start methionine in the sequence is mutated to alanine also do not show tautomerase activity and represent further embodiments of the invention and are intended to be encompassed within the scope of the invention. In one specific embodiment, AP205 is conjugated to MIF mutants devoid of tautomerase activity.

In one embodiment of the invention, the antigen or antigenic determinant is Interleukin-17 (IL-17). Human IL-17 is a 32-kDa, disulfide-linked, homodimeric protein with variable glycosylation (Yao, Z. *et al., J. Immunol. 155*: 5483-5486 (1995); Fossiez, F. *et al., J. Exp. Med. 183*: 2593-2603 (1996)). The protein comprises 155 amino acids and includes an N-terminal secretion signal sequence of 19-23 residues.

The amino acid sequence of IL-17 is similar only to a *Herpesvirus* protein (HSV13) and is not similar to other cytokines or known proteins. The amino acid sequence of human IL-17 is shown in GenBank ACCESSION #: AAC50341. The mouse protein sequence is shown in GenBank ACCESSION #: AAA37490. Of the large number of tissues and cell lines evaluated, the mRNA transcript encoding IL-17 has been detected only in activated T cells and phorbol 12-myristate 13-acetate/ionomycin-stimulated peripheral blood mononuclear cells (Yao, Z. *et al., J. Immunol. 155*: 5483-5486 (1995); Fossiez, F. *et al., J. Exp. Med. 183*: 2593-2603 (1996)). Both human and mouse sequences contain 6 cysteine residues.

The receptor for IL-17 is widely expressed in many tissues and cell types (Yao, Z. *et al., Cytokine 9*: 794-800 (1997)). Although the amino acid sequence of the human IL-17 receptor (866 aa) predicts a protein with a single trans-membrane domain and a long, 525 aa intracellular domain, the receptor sequence is unique and is not similar to that of any of the receptors from the cytokine/growth factor receptor family. This coupled with the lack of similarity of IL-17 itself to other known proteins indicates that IL-17 and its receptor may be part of a novel family of signalling protein and receptors. Clinical studies indicate IL-17 may be involved in many inflammatory diseases. IL-17 is secreted by synovial T cells from rheumatoid arthritis patients and stimulates the production of inflammatory mediators (Chabaud, M. *et al., J. Immunol. 161*: 409-414 (1998); Chabaud, M. *et al., Arthritis Rheum. 42*: 963-970 (1999)). High levels of IL-17 have been reported in patients with rheumatoid arthritis (Ziolkowska M. *et al., J Immunol. 164*:2832-8 (2000)).

Interleukin-17 has been shown to have an effect on proteoglycan degradation in murine knee joints (Dudler J. *et al., Ann Rheum Dis. 59*: 529-32 (2000)) and contribute to destruction of the synovium matrix (Chabaud M. *et al., Cytokine. 12*:1092-9 (2000)). There are relevant arthritis models in animals for testing the effect of immunization against IL-17 (Chabaud M. *et al., Cytokine. 12*:1092-9 (2000)). Elevated levels of IL-17 mRNA have been found in mononuclear cells from patients with multiple sclerosis (Matusevicius, D. *et al., Mult. Scler. 5*: 101-104 (1999)). Elevated serum levels of IL-17 are observed in patients suffering Systemic Lupus Erythematosus (Wong C.K. *et al., Lupus 9*: 589-93 (2000)). In addition, IL-17 mRNA levels are

increased in T cells isolated from lesional psoriatic skin (Teunissen, M. B. *et al., J. Invest. Dermatol. 111*: 645-649 (1998)).

The involvement of IL-17 in rejection of kidney graft has also been demonstrated (Fossiez F. *et al., Int. Rev. Immunol.16*:541-51 (1998)). Evidence for a role of IL-17 in organ allograft rejection has also been presented by Antonysamy *et al.* (*J. Immunol. 162*:577-84 (1999)) who showed IL-17 promotes the functional differentiation of dendritic cell progenitors. Their findings suggest a role for IL-17 in allogeneic T cell proliferation that may be mediated in part via a maturation-inducing effect on DCs. Furthermore the same group reports (Tang J.L. *et al., Transplantation 72*:348-50 (2001)) a role for IL-17 in the immunopathogenesis of acute vascular rejection where Interleukin-17 antagonism inhibits acute but not chronic vascular rejection. IL-17 appears to have potential as a novel target for therapeutic intervention in allograft rejection.

The anti-IL-17 monoclonal antibody mAb5 (Schering-Plough Research Institute) is able to completely inhibit the production of IL-6 from rheumatoid arthritis (RA) synovium supernatants following induction by 50 ng/ml of IL-17. An irrelevant mAb MX1 had no effect in this assay. mAb5 is a mouse IgG1 obtained after immunization with human rIL-17 (r = recombinant). A concentration of 1 μg/ml of mAb5 was able to completely inhibit the IL-6 production in the assay system (Chabaud, M. et al., *J. Immunol. 161*: 409-414 (1998)). Thus, immunization against IL-17 provides a way of treatment for the various conditions described above.

Thus, in one embodiment of the invention the composition comprises a linker containing a second attachment site fused to the C-terminus of recombinant IL-17. In further embodiments an amino acid linker containing a cysteine is fused to the N-terminus of the sequence corresponding to the sequence of the processed protein, or inserted at the N-terminus of the sequence of the mature form of the protein, C-terminally of the signal peptide. For eukaryotic expression systems, the signal peptide of the IL-17 gene, as it is the case for the other self-antigens indicated herein, may be replaced by another signal peptide, for example originating from a specific eukaryotic expression vector. For expression in bacteria, the signal peptide is replaced by a bacterial signal peptide for soluble expression in the periplasm. For expression in the cytoplasm, the construct is devoid of signal peptide. Constructs of human IL-17 devoid

of signal peptide will, in some embodiments, comprise residues 24-155, 22-155, 21-155 or 20-155. Constructs of mouse IL-17 devoid of signal peptide will, in some embodiments, comprise residues 26-158, 25-158, 24-158 or 27-155. Human IL-17 may be expressed in CV1/EBNA cells; recombinant hIL-17 has been shown to be secreted in both glycosylated and nonglycosylated forms (Yao, Z. *et al., J. Immunol. 155*: 5483-5486 (1995)). IL-17 can also be expressed as hIL-17/Fc fusion protein, with subsequent cleavage of the IL-17 protein from the fusion protein. IL-17 may also be expressed in the yeast *Pichia pastoris* (Murphy K.P. *et. al., Protein Expr Purif. 12*: 208-14 (1998)). Human IL-17 may also be expressed in *E. coli.* When expression of IL-17 in *E. coli* is directed to the periplasm, the signal peptide of IL-17 is replaced by a bacterial signal peptide. In one embodiment , IL-17 constructs are devoid of signal peptide for expression of the protein in the cytoplasm of *E. coli*,

In another embodiment of the invention the antigenic determinant is Interleukin-13 (IL-13). IL-13 is a cytokine that is secreted by activated T lymphocytes and primarily impacts monocytes, macrophages, and B cells. The first 20 amino acids of the precursor protein correspond to the signal peptide, and are absent of the processed protein. The mouse sequence has also been described (Brown K.D. *et al., J. Immunol. 142*:679-687 (1989)). Depending on the expression host, the IL-13 construct will comprise the sequence of the precursor protein, e.g. for expression and secretion in eukaryotic hosts, or consist of the mature protein, e.g. for cytoplasmic expression in *E.coli*. For expression in the periplasm of E. coli, the signal peptide of IL-13 is replaced by a bacterial signal peptide.

IL-13 is a T helper 2-derived cytokine (like IL-4, IL-5) that has recently been implicated in allergic airway responses (asthma). Upregulation of IL-13 and IL-13 receptor has been found in many tumour types (e.g. Hodgkin lymphoma). Interleukin 13 is secreted by and stimulates the growth of Hodgkin and Reed-Sternberg cells (Kapp U. *et al., J Exp Med. 189*:1939-46 (1999)). Thus, immunization against IL-13 provides a way of treating among others the conditions described above, such as Asthma or Hodgkins Lymphoma.

In one embodiment, the composition comprises an amino acid linker containing a cysteine residue and being fused to the N or C-terminus of the sequence of mature IL-13 to introduce a second attachment site within the protein. In other embodiments, an

amino acid linker containing a cysteine is added to the N-terminus of the mature form of IL-13, since it is freely accessible according to the NMR structure of IL-13 (Eisenmesser, E. Z. *et al., J.Mol.Biol. 310*: 231 (2001)). In other embodiments, the amino acid linker containing a cysteine is fused to the N-terminus of the sequence corresponding to the sequence of the processed protein, or inserted at the N-terminus of the sequence of the mature form of the protein, C-terminally of the signal peptide. In other embodiments, an amino acid linker containing a cysteine residue is added to the C-terminus of the protein.

IL-13 may be expressed in E.coli (Eisenmesser E.Z. et al., *Protein Expr. Purif. 20*:186-95 (2000)), or in NS-0 cells (eukaryotic cell line) (Cannon-Carlson S. *et al., Protein Expr. Purif.12*:239-48 (1998)).

In one embodiment of the invention, the antigenic determinant is Interleukin-5 (IL-5). IL-5 is a lineage-specific cytokine for eosinophilopoiesis and plays an important part in diseases associated with increased number of eosinophils, such as asthma.

The biological function of IL-5 has been shown in several studies (Coffman R.L. *et al., Science 245*: 308-10 (1989); Kopf *et al., Immunity 4*:15-24 (1996)), which point to a beneficial effect of inhibiting IL-5 function in diseases mediated through eosinophils. Inhibition of the action of IL-5 provides thus a way of treatment against asthma and other diseases associated with eosinophils.

IL-5 forms a dimer, covalently linked by a disulfide bridge. A single chain (sc) construct has been reported wherein two monomers of IL-5 are linked by a peptide linker.

In one embodiment of the invention, a peptide linker containing a cysteine is added at the N-terminus of the sequence of the processed form of IL-5. Addition of a linker containing a cysteine is also envisaged at the N-terminus of the sequence of the processed form of a scIL-5. In other embodiments, the amino acid linker containing a cysteine is fused to the N-terminus of the sequence corresponding to the sequence of the processed protein, or inserted at the N-terminus of the sequence of the mature form of the protein, C-terminally of the signal peptide.

In other embodiments, a linker containing a cysteine is fused to the C- terminus of the sequence of IL-5, or to the C-terminus of a scIL-5 sequence.

A number of expression systems have been described for IL-5 and can be used in preparing the compositions of the invention. A bacterial expression system using *E.coli* has been described by Proudfoot *et al., (Biochem J. 270*:357-61 (1990)). In the case where IL-5 is expressed in the cytoplasm of *E. coli*, the IL-5 construct is devoid of a signal peptide. Insect cells may also be used for producing IL-5 constructs for making the compositions of the invention (Pierrot C. *et al., Biochem. Biophys. Res. Commun. 253*:756-60 (1998)). Likewise, Baculovirus expression systems (sf9 cells; Ingley E. *et al., Eur. J. Biochem. 196*:623-9 (1991) and Brown P.M. *et al., Protein Expr. Purif. 6*: 63-71 (1995)) can also be used. Finally, mammalian expression systems have also been reported (CHO cells) and can be used in preparing these compositions of the invention (Kodama S et al., J. Biochem. (Tokyo) 110:693-701 (1991)).

Baculovirus expression systems (Mitchell *et al., Biochem. Soc. Trans. 21*:332S (1993); Kunimoto DY *et al., Cytokine 3*:224-30 (1991)) and a mammalian cell expression system using CHO cells (Kodama S *et al., Glycobiology 2*:419-27 (1992)) have also been described for mouse IL-5.

The expression of murine IL-5 constructs wherein the IL-5 sequence is fused at its N-terminus to amino acid linkers containing a cysteine residue are suitable for coupling of IL-5 to AP205 VLP. Human constructs can be generated according to the teachings herein yield the proteins human C-IL-5-E, human C-IL-5-F and human C-IL-5-S suitable for coupling to AP205 VLP and leading to other embodiments of the invention.

In one embodiment of the invention, the antigenic determinant is CCL-21. CCL-21 is a chemokine of the CC subfamily that is also known as small inducable cytokine A21, as exodus-2, as SLD (secondary lymphocyte cytokine), as TCA4 (thymus-derived chemotactic agent 4) or 6Ckine.

CCL21 inhibitis hemopoiesis and stimulates chemotaxis for thymocytes, activated T-cells and dendritic cells, but not for B cells, macrophages or neutrophils. It shows preferential activitiy towards naive T cells. It is also a potent mesangial cell chemoattractant. CCL21 binds to chemokine receptors CCR7 and to CXCR3 (depending on the species). It can trigger rapid integrin-dependent arrest of lymphocytes rolling under physiological shear and is highly expressed by high endothelial venules.

Murine CCL21 inhibited tumor growth and angiogenesis in a human lung cancer SCID mouse model (Arenberg *et al., Cancer Immunol. Immunother. 49*: 587-92 (2001)) and a colon carcinoma tumor model in mice (Vicari *et al., J. Immunol. 165*: 1992-2000 (2001)). The angiostatic activity of murine CCL21 was also detected in a rat corneal micropocket assay (Soto *et al., Proc. Natl. Acad. Sci. USA 95*: 8205-10 (1998).

It has been shown that chemokine receptors CCR7 and CXCR4 are upregulated in breast cancer cells and that CCL21 and CXCL12, the respective ligands, are highly expressed in organs representing the first destinations of breast cancer metastasis (Müller et al. (*Nature 410*: 50-6 (2001)). In vitro CCL21-mediated chemotaxis could be blocked by neutralizing anti-CCL21 antibodies as was CXCR4-mediated chemotaxis by the respective antibodies. Thus, immunization against CCL21 provides a way of treatment against metastatis spread in cancers, more specifically in breast cancer.

Secreted CCL21 consist of 110 or 111 aa in mice and humans, respectively. The respective sequences are shown in Swissprot: SY21_human and in Swissprot: SY21_mouse. In contrast to other CC cytokines does CCL21 contain two more cysteines within an extended region at the C-terminus. It is assumed that all cysteines are engaged in disulfide bonds.

In the following, constructs and expression systems are described for making compositions of the invention comprising the CCL21 antigenic determinant. In the NMR structure of the homologous protein eotaxin, both N- and C-terminus are exposed to the solvent. In some embodiments, an amino acid linker containing a cysteine residue as a second attachment site is added at the C-terminus of the protein. A fusion protein with alkaline phosphatase (at the C-terminus of CCL21) has been expressed and was shown to be functional, showing that fusions at the C-terminus of CCL21 are compatible with receptor binding. In specific embodiments, the amino acid linker containing a cysteine is fused to the N-terminus of the sequence corresponding to the sequence of the processed protein, or inserted at the N-terminus of the sequence of the mature form of the protein, C-terminally of the signal peptide.

Several expression systems have been described for production of CCL21 (e.g. Hedrick *et al., J Immunol. 159*: 1589-93 (1997)). For example, it may expressed in a baculovirus system (Nagira et al., *J. Biol. Chem. 272*: 19518-24 (1997)).

In a related embodiment, the antigenic determinant is Stromal derived factor-1 (SDF-1), now termed CXCL12. CXCL12 is a chemokine produced by bone marrow stromal cells and was originally identified as a stimulatory factor for pre-B cells.

As stated above, it has been shown that chemokine receptors CCR7 and CXCR4 are upregulated in breast cancer cells and that CCL21 and SDF-1, the respective ligands, are highly expressed in organs representing the first destinations of breast cancer metastasis (Müller et al. *Nature 410*: 50-6 (2001)). In vitro SDF-1 / CXCR4-mediated chemotaxis could be inhibitied by neutralizing anti-SDF-1 and anti-CXCR4 antibodies.

In a breast cancer metastasis model in SCID mice using the human MDA-MB-231 breast cancer cell line, a significant decrease in lung metastasis was observed when mice were treated with anti-CXCR4 antibodies. In the draining lymph nodes, a reduction of metastasis to the inguinal and axillary lymph nodes (38% instead of 100% metastasis in controls) was observed. Thus, immunization against CXCL12 provides a way of treatment against metastatis of cancers, more specifically of breast cancers.

The SDF-1 / CXCR4 chemokine-receptor pair has been shown to increase the efficacy of homing of more primitive hematopoietic progenitor cells to be bone marrow. In addition, CXCR4 and SDF-1 are supposed to influence the distribution of chronic lymphocytic leukemia cells. These cells invariably infiltrate the bone marrow of patients and it was shown that their migration in the bone marrow was CXCR4 dependent. Chronic lymphocytic leukemia cells undergo apoptosis unless they are cocultured with stromal cells. SDF-1 blocking antibodies could inhibit this protective effect of stromal cells (Burger *et al., Blood 96:* 2655-63 (2000)). Immunizing against CXCL12 thus provides a way of treatment against chronic lymphocytic leukemia.

CXCR4 has been shown to be a coreceptor for entry of HIV into T-cells. SDF-1 inhibits infection of CD4+ cells by X4 (CXCR4-dependent) HIV strains (Oberlin et al., *Nature 382*:833-5 (1996); Bleul *et al., Nature 382*:829-33 (1996), Rusconi *et al., Antivir. Ther. 5*:199-204 (2000)). Synthetic peptide analogs of SDF-1 have been shown to effectively inhibit HIV-1 entry and infection via the CXCR4

receptor(WO059928A1). Thus, immunization against CXCL12 provides a way to block HIV entry in T-cells, and therefore a way of treating AIDS.

SDF-1-CXCR4 interactions were also reported to play a central role in CD4+ T cell accumulation in rheumatoid arthritis synovium (Nanki et al., 2000). Immunization against SDF-1 thus provides a way of treatment against rheumatoid arthritis.

Human and murine SDF-1 are known to arise in two forms, SDF-1α and SDF-1β, by differential splicing from a single gene. They differ in four C-terminal amino acids that are present in SDF-1β (74 aa) and absent in SDF-1α (70 aa). The sequence of human SDF-1 is shown in Swissprot: SDF1_human and the sequence of mouse SDF-1 is shown inSwissprot: SDF1_mouse. SDF-1 contains four conserved cysteines that form two intra-molecular disulfide bonds. The crystal structure of SDF shows a non covalently-linked dimer (Dealwis *et al., PNAS 95*: 6941-46 (1998)). The SDF-1 structure also shows a long N-terminal extension.

Alanine-scanning mutagenesis was used to identify (part of) the receptor-binding site on SDF-1 (Ohnishi *et al., J. Interferon Cytokine Res. 20*: 691-700 (2000)) and Elisseeva *et al.* (*J. Biol. Chem. 275*:26799-805 (2000)) and Heveker et al. (*Curr. Biol. 8*:369-76 (1998)) described SDF-1 derived peptides inhibiting receptor binding (and HIV entry).

In the following, constructs and expression systems suitable for the generation of the compositions of the invention related to SDF-1 are described. The N- and C-terminus of SDF-1 are exposed to the solvent. In specific embodiments, an amino acid linker containing a cysteine as second attachment site is thus fused to the C- terminus of the protein sequence, while in other specific embodiments an amino acid linker containing a cysteine as second attachment site is fused to the N-terminus of the protein sequence. The amino acid linker containing a cysteine is fused to the N-terminus of the sequence corresponding to the sequence of the processed protein, or inserted at the N-terminus of the sequence of the mature form of the protein, C-terminally of the signal peptide. The genes coding for these specific constructs may be cloned in a suitable expression vector.

Expression of SDF-1 in a sendai virus system in chicken embryonic fibroblasts (Moriya *et al., FEBS Lett. 425*:105-11 (1998)) has been described as well as expression

in *E.coli* (Holmes *et al., Prot. Expr. Purif. 21*: 367-77 (2001)) and chemical synthesis of SDF-1 (Dealwis *et al., PNAS 95*: 6941-46 (2001)).

In another embodiment of the invention, the antigenic determinant is B-lymphocyte chemoattractant (BLC, CXCL13). BLC is expressed in the spleen, Peyer's patches and lymph nodes (Gunn et al., 1998). Its expression is strongest in the germinal centres, where B cells undergo somatic mutation and affinity maturation. It belongs to the CXC chemokine family, and its closest homolog is GROα (Gunn *et al., Nature 391*:799-803 (1998)). Human BLC is 64% homologous to murine BLC. Its receptor is CXCR5. BLC also shares homology with IL-8. BLC recruits B-cells to follicles in secondary lymphoid organs such as the spleen and peyer's patches. BLC is also required for recruitment of B-cells to compartment of the lymph nodes rich in follicular Dendritic Cells (FDCs) (Ansel *et al., Nature 406*:309-314 (2000)). BLC also induces increased expression of Lymphotoxinα1β2 (LTα1β2) on the recruited B-cells. This provides a positive feed-back loop, since LTα1β2 promotes BLC expression (Ansel *et al., Nature 406*:309-314 (2000)). BLC has also been shown to be able to induce lymphoid neogenesis (Luther *et al., Immunity 12*:471-481(2000)). It appears that FDCs also express BLC. Thus immunization against BLC may provide a way of treatment against autoimmune diseases where lymphoid neogenesis is involved, such as Rheumatoid synovitis and Rheumatoid arthritis or Type I diabetes. A construct of BLC bearing a C-terminal His-tag has been described, and is functional (Ansel, K.M. *et al., J. Exp. Med. 190*: 1123-1134 (1999)).

In one embodiment of the present invention, the composition comprises a linker containing a cysteine residue as second attachment site and being fused at the C-terminus of the BLC sequence.

In IL-8, which is homologous to BLC, both N- and C-termini are free. Fusion of an amino acid linker containing a cysteine residue as second attachment site to the N-terminus of BLC leads to one embodiment of the invention.

In other embodiments of the present invention, the composition comprises an amino acid linker containing a cysteine and being fused to the N-terminus of the sequence corresponding to the sequence of the processed protein, or inserted at the N-terminus of the sequence of the mature form of the protein, C-terminally of the signal

peptide. The genes coding for these specific constructs may be cloned in a suitable expression vector and expressed accordingly. The sequence of human BLC is shown in Accession: NP_006410. Amino acids 1-22 of the sequence are the signal peptide. The mouse sequence is shown in Accession NP_061354. Amino acids 1-21 are the signal peptide. Compositions of the invention with BLC as the antigenic determinant, in some embodiments, use the mature form of the protein for generating the compositions of the invention.

In another specific embodiment, the antigenic determinant is Eotaxin. Eotaxin is a chemokine specific for Chemokine receptor 3, present on eosinophils, basophils and Th2 cells. Eotaxin seems however to be highly specific for Eosinophils (Zimmerman *et al., J. Immunol. 165*: 5839-46 (2000)). Eosinophil migration is reduced by 70% in the eotaxin-1 knock-out mouse, which however can still develop eosinophilia (Rothenberg *et al., J. Exp. Med. 185*: 785-90 (1997)). IL-5 seems to be responsible for the migration of eosinophils from bone-marrow to blood, and eotaxin for the local migration in the tissue (Humbles *et al., J. Exp. Med. 186*: 601-12 (1997)).

The human genome contains 3 eotaxin genes, eotaxin1-3. They share 30% homology to each other. Two genes are known so far in the mouse: eotaxin 1 and eotaxin 2 (Zimmerman *et al., J. Immunol. 165*: 5839-46 (2000)). They share 38% homology. Murine eotaxin-2 shares 59% homology with human eotaxin-2. In the mouse, eotaxin-1 seems to be ubiquitously expressed in the gastro-intestinal tract, while eotaxin-2 seems to be predominantly expressed in the jejunum (Zimmerman *et al., J. Immunol. 165*: 5839-46 (2000)). Eotaxin-1 is present in broncho-alveolar fluid (Teixeira *et al., J. Clin. Invest. 100*: 1657-66 (1997)). Eotaxin has a MW of 8.3 kDa. It is in equilibrium between monomers and dimers over a wide range of conditions, with an estimated Kd of 1.3 mM at 37°C (Crump *et al., J. Biol. Chem. 273*: 22471-9 (1998)). The monomer form is however predominant. The structure of Eotaxin has been elucidated by NMR spectroscopy. Binding site to its receptor CCR3 is at the N-terminus, and the region preceding the first cysteine is crucial (Crump *et al., J. Biol. Chem. 273*: 22471-9 (1998)). Peptides of chemokine receptors bound to Eotaxin confirmed this finding. Eotaxin has four cysteines forming two disulfide bridges. Therefore, in one embodiment, the inventive composition comprises an amino-acid linker containing a cysteine residue as second attachment site and being in one

embodiment, fused to the C-terminus of the Eotaxin sequence. In further embodiments, an amino acid linker containing a cysteine is fused to the N-terminus of the sequence corresponding to the sequence of the processed protein, or inserted at the N-terminus of the sequence of the mature form of the protein, C-terminally of the signal peptide. The genes coding for these specific constructs are cloned in a suitable expression vector.

Eotaxin can be chemically synthesized (Clark-Lewis *et al., Biochemistry 30*:3128-3135 (1991)). Expression in E. coli has also been described for Eotaxin-1, in the cytoplasm (Crump *et al., J. Biol. Chem. 273*: 22471-9 (1998)). Expression in *E. coli* as inclusion bodies with subsequent refolding (Mayer *et al., Biochemistry 39:* 8382-95 (2000)), and Insect cell expression (Forssmann *et al., J. Exp. Med. 185*: 2171-6 (1997)) have been described for Eotaxin-2, and may, moreover, be used to arrive at the specific embodiments of the invention.

In yet another specific embodiment of the invention, the antigenic determinant is Macrophage colony-stimulating factor (M-CSF or CSF-1). M-CSF or CSF-1 is a regulator of proliferation, differentiation and survival of macrophages and their bone-marrow progenitors. The receptor for M-CSF is a cell surface tyrosine kinase receptor, encoded by the protooncogene cFMS. An elevated expression of M-CSF and its receptor has been associated with poor prognosis in several epithelial cancers such as breast, uterine and ovarian cancer. Tumor progression has been studied in a mouse strain resulting from the crossing of a transgenic mouse susceptible to mammary cancer (PyMT) with a mouse containing a recessive null mutation in the csf-1 gene. These mice show attenuated late stage invasive carcinoma and pulmonary metastasis compared to the PyMT mouse (Lin *et al., J. Exp. Med. 193*:727-739 (2001)). The cause seems to be the absence of macrophage recruitment to neoplastic tissues. Subcutaneous growth of Lewis lung cancer is also impaired in csf.1 null mice. It is postulated that the mechanism of macrophage enhancement of tumor growth would be through angiogenic factors, growth factors and proteases produced by the macrophages.

Structural data on the soluble form of M-CSF are available (crystal structure: Pandit *et al., Science 258*:1358-62 (1992)), and show that both the N- and C-termini of the protein are accessible. However, the N-terminus is close to the site of interaction with the receptor. In addition, M-CSF is present both in a soluble and cell surface form, where the transmembrane region is at its C-terminus. Therefore certain

embodiment of the present invention comprise an amino acid linker containing a cysteine and being, in one embodiment, added at the C-terminus of M-CSF or fragments thereof, at the C-terminus of the soluble form of M-CSF. In other embodiments, the amino acid linker containing a cysteine is fused to the N-terminus of the sequence corresponding to the sequence of the processed protein or of the soluble form of the protein, or inserted at the N-terminus of the sequence of the mature form of the protein or of the soluble form of the protein, C-terminally of the signal peptide. M-CSF is a dimer, where the two monomers are linked via an interchain disulfide bridge.

An expression system in E. coli has been described for an N-terminal 149 amino acid fragment (functional) of M-CSF (Koths *et al., Mol. Reprod. Dev. 46:*31-37 (1997)). This fragment of M-CSF, modified as outlined above, represents a one antigenic determinant in accordance with the embodiments of the invention. The human sequence is shown in Accession: NP_000748. Other antigenic determinants of the present invention comprise the N-terminal fragment consisting of residue 33 -181 or 33 –185 of the above sequence, corresponding to the soluble form of the receptor.

The mouse sequence is shown in Accession. NP_031804. The mature sequence starts at amino acid 33. Thus, one antigenic determinant in accordance with the present invention comprises amino-acid 33 -181 or 33 –185.

In one embodiment, the antigenic determinant is Resistin (Res). Passive immunization studies were performed with a rabbit polyclonal antibodies generated against a fusion protein of mouse Resistin (mRes) fused to GST, expressed in bacteria. This passive immunization lead to improved glucose uptake in an animal obesity/ Type II diabetes model (Steppan *et al., Nature* 409: 307-12 (2001)).

Resistin (Res) is a 114 aa peptide hormone of approximately 12 KD. It contains 11 cysteine of which the most N-terminal one was shown to be responsible for the dimerisation of the protein and the other 10 are believed to be involved in intramolecular disulfide bonds (Banerjee and Lazar, *J. Biol. Chem. 276*: 25970-3 (2001)). Mutation of the first cysteine to alanine abolishes the dimerisation of mRes.

mRes with a FLAG tag at its C-terminus remains active in an animal model (Steppan *et al., Nature* 409: 307-12 (2001)). Similarly a C-terminally HA taged (Haemagglutinin tag) version of resistin was shown to be active in a tissue culture assay (Kim *et al., J. Biol. Chem. 276*: 11252-6 (2001)). Therefore, in one embodiment,

the present compositions comprise an amino-acid linker containing a cysteine residue as second attachment site and being fused at the C-terminus of the resistin sequence. In another embodiment, the amino acid linker containing a cysteine is fused to the N-terminus of the sequence corresponding to the sequence of the processed protein, or inserted at the N-terminus of the sequence of the mature form of the protein, C-terminally of the signal peptide.

In one embodiment of the present invention, MRes or huRes may also be expressed as Fc fusion molecules with a protease cleavage site inserted between Resistin and the Fc part of the construct, such as C-terminally to one or more cysteine residues of the hinge region of the Fc part of the fusion protein in a eukaryotic expression system, or such as according to the descriptions herein. Cleavage of the fusion protein releases Resistin additionally comprising either an aminoacid linker containing a cysteine residue or part or all of the hinge region of the Fc part of the fusion protein which comprises a cysteine residue at its C-terminus, which is suitable for coupling to AP205 VLP. The human Resistin sequence is shown in Accession AF323081. The mouse sequence is shown in Accession AF323080. A favored embodiment of the invention is human resistin protein fused at its C-terminus to an amino acid linker containing a cysteine residue. Human resistin construct can be generated according to the teachings disclosed herein and by comparing murine and human Resistin sequences in a protein sequence alignment to identify the part of the sequence of human Resistin to be cloned into vectors herein or in other suitable expression vectors. Example of human resistin constructs suitable for generating compositions of the inventions are human resistin-C-Xa, human resistin-C-EK and human resistin-C.

Human Resistin constructs so generated are a one embodiment of the invention. Vaccination against Resistin using the aforementioned compositions of the invention may thus provide a way of treating Type II Diabetes and obesity.

In another embodiment the antigenic determinant is Lymphotoxin-β. Immunization against lymphotoxin-β may be useful in treating Prion mediated disease. Prions are cellular proteins existing in most mammalian species. Prion proteins exist in two forms, a normally folded form that is usually present in healthy individuals (PrP^{c}) and a misfolded form that causes disease (Prp^{Sc}). The current prion hypotheses

postulates that the misfolded prion form Prp^{Sc} can catalyse the refolding of healthy prion PrP^{c} into disease causing Prp^{Sc} (A. Aguzzi, *Haematologica 85*, 3-10 (2000)). In some rare instances, this transition may also occur spontaneously, causing classical CJD in humans. Some mutations in PrP^{c} are associated with an increase in this spontaneous transition, causing the various forms of familial CJD. However, Prp^{Sc} may also be infectious and may be transmitted by blood transfusion or via the food chain. The latter form of prion mediated disease is known as Kuru Kuru and used to occur in human cannibals. However, since species that are feeding on their own individuals are not abundant, this form of orally transmitted disease was too rare to be documented for other species.

The massive feeding of cows with beef-products throughout Europe changed the situation and numbers of cows infected with a transmissible form of BSE-causing Prp^{Sc}, dramatically increased in recent years, afflicting hundreds of thousands of cows. This sudden appearance of massive numbers of BSE-diseased cows caused great fear in the human population that a similar disease may be induced in humans. Indeed, in 1996, the first case of a variant form of CJD was reported that could be attributed to the consumption of Prp^{Sc} infected beef. Until now, this fear has further increased, since the number of infected humans has constantly increased during the following years and no cure is in sight. Moreover, since sheep succumb to a prion-mediated disease called scrapie and since other mammalian species can be infected with Prp^{Sc}, it is possible that BSE-like diseases may occur also in other species.

Scrapie (a prion-mediated disease) agent replication is believed to take mainly place in lymphoid tissues and was shown to depend on prion-protein expressing follicular dendritic cells (FDCs) (Brown *et al., Nature Med. 11*: 1308-1312 (1999)). The mechanism of prion transmission has been studied in great detail. It is now clear that prions first replicate in the lymphoid organs of infected mice and are subsequently transported to the central nervous system. It was shown that mice lacking functional follicular dendritc cells show an impaired prion replication in spleens and a (small) retardation of neuroinvasion (Montrasio *et al., Science 288*: 1257-1259 (2000)). This was achieved by injecting the mice with a soluble lymphotoxin-β receptor-Fc-fusion protein (LTβR-Fc). This soluble receptor construct inhibits the development of FDCs by interfering with the crucial interaction of lymphotoxin-β on T, B or NK cells with

the lymphotoxin-β receptor on the FDC precursor cells. FDCs are a poorly studied cell type but it is now clear that they depend upon the production of lymphotoxin and/or TNF by B cells for their development (F. Mackay, J. L. Browning, *Nature* 395, 26-27 (1998)). Indeed, mice deficient for lymphotoxin do not exhibit FDCs (M. S. Matsumoto, et al., *Science* 264, 703-707 (1996)). In addition to FDCs, antibodies may also play a role in disease progression (S. Brandner, M. A. Klein, A. Aguzzi, *Transfus Clin Biol* 6, 17-23 (1999)).

Thus, vaccination against lymphotoxin-β (also called TNFγ), LTα or LTβ receptor, thereby eliminating FDCs from lymphoid organs, may provide a vaccine for treatment or prevention of Creutzfeld-Jakob (variant form) or other prion-mediated diseases such as bovine spongioform encephalopathy (BSE) and thus prevent prion replication and neuroinvasion.

Immunization against Lymphotoxin-β may also provide a way of treating diabetes. Transgene expression of soluble LTβR-Fc fusion protein in nonobese diabetic NOD mice blocked diabetes development but not insulitis (Ettinger *et al., J. Exp. Med. 193*: 1333-40 K (2001)). Wu *et al.* (*J. Exp. Med. 193*: 1327-32 (2001)) also used NOD mice to study the involvement of lymphotoxin-β, but instead of transgenic animals they did inject the LTβR-Fc fusion protein. They saw a strong inhibition of diabetes development and inhibition of insulitis. Most interestingly, they could even reverse preexisting insulitis by the fusion protein treatment. In the pancreas the formation of lymphoid follicular structures could thus be reversed. Vaccination against lymphotoxin-β may thus provide a way of treatment against type-I diabetes.

In one embodiment, the inventive composition comprises an amino acid linker containing a cysteine and being added to the N-terminus of the sequence corresponding to the processed form of lymphotoxin-β, or inserted between the N-terminus of the sequence corresponding to the mature form of the protein, and the signal peptide, C-terminally to the signal peptide. In related embodiments of the invention, the extracellular part of lymphotoxin-β is expressed as a fusion protein either with glutathione-S-transferase, fused N-terminally to lymphotoxin-β, or with a 6 histidine-tag followed by a myc-tag, fused again N-terminally to the extracellular part of lymphotoxin-β. An amino acid spacer containing a protease cleavage site as well as a

linker sequence containing a cysteine as attachment site, C-terminally to the protease cleavage site, are fused to the N-terminus of the sequence of the extracellular part of lymphotoxin-β. In one embodiment the extracellular part of lymphotoxin-β consists of fragments corresponding to amino acids 49-306 or 126-306 of lymphotoxin-β. These specific compositions of the invention may be cloned and expressed in the pCEP-Pu eukaryotic vector. In certain embodiments, the inventive compositions comprise an aminoacid linker containing a cysteine residue suitable as second attachment site, and being fused to the C-terminus of lymphotoxin-β or lymphotoxin-β fragments. In a particularly favored embodiment, the amino acid sequence LACGG, comprising the amino acid linker ACGG which itself contains a cysteine residue for coupling to AP205 VLP is fused to the N-terminus of the extracellular part of lymphotoxin-β or of a fragment of the extracellular part of lymphotoxin-β, yielding the proteins human C-LTβ_{49-306} and human C-LT$\beta_{126-306}$ after cleavage with enterokinase of the corresponding fusion proteins expressed either in vector pCEP-SP-GST-EK or vector pCP-SP-his-myc-EK.

In one embodiment, the antigen or antigenic determinant is the prion protein, fragments thereof and in particular peptides of the prion protein. In an embodiment of the invention, the antigenic determinant is the prion protein or fragments thereof. Immunization against prion protein may provide a way of treatment or prevention of Creutzfeld-Jakob (variant form) or other prion-mediated diseases. Murine peptides corresponding to fragments of the murine prion protein and of sequence CSAMSRPMIHFGNDWEDRYYRENMYR (SEQ ID NO: 17) ("cprplong") and CGNDWEDRYYRENMYR ("cprpshort") (SEQ ID NO: 18) comprise an added N-terminal cysteine residue for for chemical coupling to AP205 VLP and lead to one embodiment of the invention. In one embodiment the prion protein is the human prion protein. Guidance on how to modify human prion protein for association with the AP205 VLP is given throughout the application. Mouse prion protein constructs are disclosed, and human prion protein constructs can also be generated. Further constructs comprise the whole human prion protein sequence, and other fragments of the human prion protein, which are further compositions of the invention. Immunization against prion protein may provide a way of treatment or prevention of Creutzfeld-Jakob

(variant form) or other prion-mediated diseases. Immunization using the compositions of the invention comprising the prion protein may provide a way of treatment against prion mediated diseases in other animals. The peptides of the human prion protein corresponding to the murine peptides described above and of amino acid sequence CSAMSRPIIHFGSDYEDRYYRENMHR ("human cprplong") (SEQ ID NO: 19) and CGSDYEDRYYRENMHR ("human cprpshort") (SEQ ID NO: 20) lead to embodiments of the invention. These peptides comprise an N-terminal cysteine residue added for coupling to AP205 VLP. Corresponding bovine and sheep peptides are CSAMSRPLIHFGNDYEDRYYRENMHR ("bovine cprplong") (SEQ ID NO: 21) and CGNDYEDRYYRENMHR ("bovine cprpshort") (SEQ ID NO: 22) CSAMSRPLIHFGNDYEDRYYRENMYR ("sheep cprplong") (SEQ ID NO: 23) and CGNDYEDRYYRENMYR ("sheep cprpshort") (SEQ ID NO: 24), all leading to embodiments of the invention.

In one embodiment of the invention, the antigenic determinant is tumor necrosis factor α (TNF-α), fragments thereof or peptides of TNF-α. In particular, peptides or fragments of TNF-α can be used to induce a self-specific immune response directed towards the whole protein by immunizing a human or an animal with vaccines and compositions, respectively, comprising such peptides or fragments in accordance with the invention. The following murine peptides are the murine homologs to human peptides that have been shown to be bound by antibodies neutralizing the activity of TNF-α (Yone *et al. J. Biol. Chem.270*: 19509-19515) and were, in another embodiment of the invention, modified with cysteine residues for coupling to AP205 VLP.

MuTNFα peptide; the sequence CGG was added at the N-terminus of the epitope consisting of amino acid residues 22-32 of mature murine TNF-α, giving the sequence: CGGVEEQLEWLSQR (SEQ ID NO: 25).

3'TNF II peptide; the sequence GGC was fused at the C-terminus of the epitope consisting of amino acid residues 4-22 of mature murine TNF-α and glutamine 21 was mutated to glycine. The sequence of the resulting peptide is: SSQNSSDKPVAHVVANHGVGGC (SEQ ID NO: 26).

5'TNF II peptide: a cysteine residue was fused to the N-terminus of the epitope consisting of amino acid residues 4-22 of mature murine TNF-α and glutamine 21 was mutated to glycine. The sequence of the resulting peptide is: CSSQNSSDKPVAHVVANHGV (SEQ ID NO: 27).

The corresponding human sequence of the 4-22 epitope is SSRTPSDKPVAHVVANPQAEGQ (SEQ ID NO: 28). As for the murine sequence a cysteine is, in one embodiment, fused at the N-terminus of the epitope, or the sequence GGC is fused at the C-terminus of the epitope for covalent coupling to AP205 VLP according to the invention. It is, however, within the scope of the present invention that other cysteine containing sequences are fused at the N- or C-termini of the epitopes. In general, one or two glycine residues are, in one embodiment, inserted between the added cysteine residue and the sequence of the epitope. Other amino acids may, however, also be inserted instead of glycine residues, and these amino acid residues include small amino acids such as serine.

In a further preferred embodiment of the inventive composition, the antigen or antigenic determinant is tumor necrosis factor α (TNF-α), fragments or muteins thereof or peptides of TNF -α or fragments or muteins thereof, wherein said antigen or antigenic determinant with said second attachment site has an amino acid sequence selected from the group consisting of: a) the amino acid sequence of CSSRTPSDKPVAHVVANPQAEGQ (SEQ ID NO: 100); b) the amino acid sequence of SSRTPSDKPVAHVVANPQAEGQGGC (SEQ ID NO: 101); and c) the amino acid sequence of CGGQLQWLNRRANA (SEQ ID NO: 102).

The human sequence corresponding to amino acid residues 22-32 is QLQWLNRRANA (SEQ ID NO: 29). In one related embodiment, the sequence CGG is fused at the N-terminus of the epitope for covalent coupling to AP205 VLP according to the invention. Other TNF-α epitopes suitable for using in the present invention have been described and are disclosed for example by Yone *et al.* (*J. Biol. Chem.270*: 19509-19515). The invention further includes compositions which contain mimotopes of the antigens or antigenic determinants described herein.

One specific composition of the invention comprises an antibody or an antibody fragment presented on a virus-like particle for induction of an immune

response against that antibody. In one embodiment, antibodies or antibody fragments which are produced by lymphoma cells, are selected for attachment to the virus-like particle for immunization in order to induce a protective immune response against the lymphoma.

In other further embodiments, an antibody or antibody fragment mimicking an antigen is attached to the AP205 VLP. The mimicking antibody or antibody fragment is generated by immunization and subsequent isolation of the mimicking antibody or antibody fragment , or by any known method known to the art such as e.g. hybridoma technology (Gherardi, E. et al., J. Immunol. Methods 126: 61-68 (1990)), phage display (Harrison *et al., Methods Enzymol. 267*: 83-109 (1996)), ribosome display (Hanes, J. *et al., Nat. Biotechnol. 18*: 1287-1292 (2000), yeast two-hybrid (Visintin, M. et *al., Proc. Natl. Acad. Sci. USA 96*: 11723-11728 (1999)), yeast surface display (Boder, ET. & Wittrup, KD. *Methods. Enzym. 328*: 430-444 (2000)), bacterial surface display (Daugherty, PS. *et al., Protein Eng. 12*: 613-621 (1999)). The mimicking antibody may also be isolated from an antibody library or a naïve antibody library using methods known to the art.

In a further embodiment, an antibody recognizing the combining site of another antibody, i.e. an anti-idiotypic antibody, further called the immunizing antibody, is used. The antibody recognized by the anti-idiotypic antibody will be further referred to as the neutralizing antibody. Thus, by immunizing against the anti-idiotypic antibody, molecules with the specificity of the neutralizing antibody are generated in situ; we will further refer to these generated antibodies as the induced antibodies. In another embodiment, the immunizing antibody is selected to interact with a ligand molecule of the target molecule against which immunization is sought. The ligand molecule may be any molecule interacting with the target molecule but will, in one embodiment, preferentially interact with the site of the target molecule against which antibodies should be generated for inhibition of its function. The ligand molecule may be a natural ligand of the target molecule, or may be any engineered, designed or isolated ligand having suitable binding properties.

The immunizing antibodies may be of human origin, such as isolated from a naïve or immune human antibody library, or may have been isolated from a library generated from another animal source, for example of murine origin.

Coupling of the antibody or antibody fragment to AP205 VLP is achieved either by limited reduction of exposed disulfide bridges (for example of the interchain disulfide bridge between CH1 and Cκ or Cλ in a Fab fragment) or, in another embodiment, by fusion of a linker containing a cysteine residue at the C-terminus of the antibody or antibody fragment. In a further embodiment, a linker containing a cysteine residue is fused to the N-terminus of the antibody or antibody fragment for attachment to a VLP or pilus protein.

A number of vaccine compositions which employ mimotopes are known in the art, as are methods for generating and identifying mimotopes of particular epitopes. For example, Arnon *et al., Immunology 101*:555-562 (2000), the entire disclosure of which is incorporated herein by reference, describe mimotope peptide-based vaccines against *Schistosoma mansoni*. The mimotopes uses in these vaccines were obtained by screening a solid-phase 8-mer random peptide library to identify mimotopes of an epitope recognized by a protective monoclonal antibody against *Schistosoma mansoni*. Similarly, Olszewska *et al., Virology 272*:98-105 (2000), the entire disclosure of which is incorporated herein by reference, describe f mice. In addition, Zuercher *et al., Eur. J. Immunol. 30*:128-135 (2000), the entire disclosure of which is incorporated herein by reference, describe compositions and methods for oral anti-IgE immunization using epitope-displaying phage. In particular, epitope-displaying M13 bacteriophages are employed as carriers for an oral anti-IgE vaccine. The vaccine compositions tested contain mimotopes and epitopes of the monoclonal anti-IgE antibody BSW17.

Embodiments of the invention include vaccine compositions which contain mimotopes that elicit immunological responses against particular antigens, as well as individual mimotope/AP205 VLP conjugates which make up these vaccine compositions, and the use of these vaccine compositions to elicit immunological responses against specific antigens or antigenic determinants. Mimotopes may also be polypeptides, such as anti-idiotypic antibodies. Therefore, in a further embodiment of the invention, the antigen or antigenic determinant is an anti-idiotypic antibody or anti-idiotypic antibody fragment.

The invention further includes compositions which contain mimotopes of the antigens or antigenic determinants described herein.

Mimotopes of particular antigens may be generated and identified by any number of means including the screening of random peptide phage display libraries (*see, e.g.*, WO 97/31948, the entire disclosure of which is incorporated herein by reference). Screening of such libraries will often be performed to identify peptides which bind to one or more antibodies having specificity for a particular antigen.

Mimotopes suitable for use in vaccine compositions of the invention may be linear or circular peptides. Mimotopes which are linear or circular peptides may be linked to non-natural molecular scaffolds or core particles and VLPs, respectively, by a bond which is not a peptide bond.

As suggested above, a number of human IgE mimotopes and epitopes have been identified which elicit immunological responses against human IgE molecules. (*See, e.g.*, WO 97/31948.) Thus, in certain embodiments, vaccine compositions of the invention include compositions which elicit an immunological response against immunoglobin molecules (*e.g.*, IgE molecules).

Peptides which can be used to elicit such immunological responses include proteins, protein subunits, domains of IgE molecules, and mimotopes which are capable of eliciting production of antibodies having specificity for IgE molecules. Generally, portions of IgE molecules used to prepare vaccine compositions will be derived from IgE molecules of the species from which the composition is to be administered. For example, a vaccine composition intended for administration to humans will often contain one or more portions of the human IgE molecule, and/or one or more mimotopes which are capable of eliciting immunological responses against human IgE molecules.

In specific embodiments, vaccine compositions of the invention intended for administration to humans will contain at least one portion of the constant region of the IgE heavy chain set out in Accession No. AAB59424. In more specific embodiments, IgE peptides used to prepare vaccine compositions of the invention comprise, or alternatively consist of, peptides having the following amino acid sequences: CGGVNLTWSRASG (SEQ ID NO: 30).

In additional specific embodiments, vaccine compositions of the invention will contain at least one mimotope which is capable of eliciting an immune response that results in the production of antibodies having specificity for a particular antigen. Examples of

mimotopes of IgE suitable for use in the preparation of vaccine compositions of the invention include peptides having the following amino acid sequences:

Mimotope	SEQ ID NO	Mimotope	SEQ ID NO
INHRGYWV	31	VKLPWRFYQV	39
RNHRGYWV	32	VWTACGYGRM	40
RSRSGGYWLW	33	GTVSTLS	41
VNLTWSRASG	34	LLDSRYW	42
$C_\varepsilon H_3$ epitope		QPAHSLG	43
VNLPWSRASG	35	LWGMQGR	44
VNLTWSFGLE	36	LTLSHPHWVLNHFVS	45
VNLPWSFGLE	37	SMGPDQTLR	46
$C_\varepsilon H_3$ mimotope		VNLTWS	47
VNRPWSFGLE	38	GEFCINHRGYWVCGDPA	48

Preparation of vaccines and immunogens

VLPs of bacteriophage AP205 are useful in generating vaccine constructs, and render antigens attached to it highly immunogenic. The present invention provides methods of attachment of antigens to the AP205 VLP. In one embodiment the antigen is attached to the VLP by way of chemical cross-linking, using a heterobifunctional cross-linker. Several hetero-bifunctional cross-linkers are known to the art. In the some embodiments, the hetero-bifunctional cross-linker contains a functional group which can react with the side-chain amino group of lysine residues of the VLP, and functional group which can react with a cysteine residue naturally present, made available for reaction by reduction, or engineered on the antigen and optionally also made available for reaction by reduction. The first step of the procedure, called the derivatization, is the reaction of the VLP with the cross-linker. The product of this reaction is an activated VLP, also called activated carrier. In the second step, unreacted cross-linker is removed using usual methods such as gel filtration or dialysis. In the third step, the antigen is reacted with the activated VLP, and this step is called the coupling step. Unreacted antigen may be optionally removed in a fourth step. Several hetero-bifunctional cross-linkers are known to the art. These include the cross-linkers SMPH

(Pierce), Sulfo-MBS, Sulfo-EMCS, Sulfo-GMBS, Sulfo-SIAB, Sulfo-SMPB, Sulfo-SMCC, SVSB, SIA and other cross-linkers available, for example from the Pierce Chemical Company (Rockford, IL, USA), and having one functional group reactive towards amino groups and one functional group reactive towards cysteine residues. The above mentioned cross-linkers all lead to formation of a thioether linkage. Another class of cross-linkers suitable in the practice of the invention is characterized by the introduction of a disulfide linkage between the antigen and the VLP upon coupling. Cross-linkers belonging to this class include for example SPDP and Sulfo-LC-SPDP (Pierce). The extent of derivatization of the VLP with cross-linker can be influenced by varying experimental conditions such as the concentration of each of the reaction partners, the excess of one reagent over the other, the pH, the temperature and the ionic strength, as is well known from reaction theory in the field of organic chemistry. The degree of coupling, i.e. the amount of antigens per subunits of AP205 VLP can be adjusted by varying the experimental conditions described above to match the requirements of the vaccine. Solubility of the antigen may impose a limitation on the amount of antigen that can be coupled on each subunit, and in those cases where the obtained vaccine is insoluble, reducing the amount of antigens per subunit is beneficial.

One method of attachment of antigens to AP205 VLP, is the linking of a lysine residue on the surface of the AP205 VLP with a cysteine residue on the antigen. In some embodiments, engineering of an amino acid linker containing a cysteine residue to the antigen for coupling to AP205 VLP is required. Alternatively, a cysteine may be introduced either by insertion or mutation within the antigen.

In a preferred embodiment of the present invention, the composition comprsises an amino acid linker. Preferably, said amino acid linker is bound to the at least one antigen, antigenic determinant and organic molecule, respectively, by way of at least one covalent bond. The selection of the amino acid linker will be dependent on the nature of the antigen, on its biochemical properties, such as pI, charge distribution, or glycosylation. In general, flexible amino acid linkers are favored embodiments. Examples of amino acid linkers are the hinge region of Immunoglobulins, glycine serine linkers $(GGGGS)_n$ (SEQ ID NO: 49), and glycine linkers $(G)_n$ all further containing a cysteine residue as second attachment site and optionally further glycine

residues. (The following are examples of such amino acid linkers:

N-terminal gamma1: CGDKTHTSPP (SEQ ID NO: 50)

C-terminal gamma 1: DKTHTSPPCG (SEQ ID NO: 51)

N-terminal gamma 3: CGGPKPSTPPGSSGGAP (SEQ ID NO: 52)

C-terminal gamma 3: PKPSTPPGSSGGAPGGCG (SEQ ID NO: 53)

N-terminal glycine linker: GCGGGG (SEQ ID NO: 54)

C-terminal glycine linker: GGGGCG (SEQ ID NO: 55)

C-terminal glycine-lysine linker: GGKKGC (SEQ ID NO: 56)

N-terminal glycine-lysine linker: CGKKGG (SEQ ID NO: 57)

For peptides, GGCG (SEQ ID NO: 58), GGC or GGC-NH2 ("NH2" stands for amidation) linkers at the C-terminus of the peptide, or CGG at its N-terminus have shown to be useful. In some embodiments, glycine residues will be inserted between bulky amino acids and the cysteine to be used as second attachment site.

Preferred embodiments of the amino acid linker are selected from the group consisting of: (a) CGG; (b) N-terminal gamma 1-linker; (c) N-terminal gamma 3-linker; (d) Ig hinge regions; (e) N-terminal glycine linkers; (f) $(G)_kC(G)_n$ with n=0-12 and k=0-5 (SEQ ID NO: 93); (g) N-terminal glycine-serine linkers; (h) $(G)_kC(G)_m(S)_l(GGGGS)_n$ with n=0-3, k=0-5, m=0-10, l=0-2 (SEQ ID NO: 94); (i) GGC; (k) GGC-NH2; (l) C-terminal gamma 1-linker; (m) C-terminal gamma 3-linker; (n) C-terminal glycine linkers; (o) $(G)_nC(G)_k$ with n=0-12 and k=0-5 (SEQ ID NO: 95); (p) C-terminal glycine-serine linkers; (q) $(G)_m(S)_l(GGGGS)_n(G)_oC(G)_k$ with n=0-3, k=0-5, m=0-10, l=0-2, and o=0-8 (SEQ ID NO: 96).

In certain embodiments, the antigen or antigen determinant comprises a single second attachment site or a single reactive attachment site capable of association with the first attachment sites on the AP205 VLPs or VLP subunits, respectively. This ensures a defined and uniform binding and association, respectively, of the at least one, but typically more than one, preferably more than 10, 20, 40, 80, 120 antigens to the AP205 VLP. The provision of a single second attachment site or a single reactive attachment site on the antigen, thus, ensures a single and uniform type of binding and association, respectively leading to a very highly ordered and repetitive array. For

example, if the binding and association, respectively, is effected by way of a lysine- (as the first attachment site) and cysteine- (as a second attachment site) interaction, it is ensured, in accordance with this preferred embodiment of the invention, that only one cysteine residue per antigen, independent whether this cysteine residue is naturally or non-naturally present on the antigen, is capable of binding and associating, respectively, with the AP205 VLP and the first attachment site of the AP205 VLP, respectively.

The cysteine residue present on the antigen has to be in its reduced state to react with the hetero-bifunctional cross-linker on the activated VLP, that is a free cysteine or a cysteine residue with a free sulfhydryl group has to be available. In the instance where the cysteine residue to function as second attachment site is in an oxidized form, for example if it is forming a disulfide bridge, reduction of this disulfide bridge with *e.g.* DTT, TCEP or β-mercaptoethanol is required.

Attachment of the antigen to the AP205 VLP by using a hetero-bifunctional cross-linker according to the method described above, allows coupling of the antigen to the AP205 VLP in an oriented fashion. Other methods of binding the antigen to the AP205 VLP include methods wherein the antigen is cross-linked to the AP205 VLP using the carbodiimide EDC, and NHS. The antigen or antigen determinant may also be first thiolated through reaction, for example with SATA, SATP or iminothiolane. The antigen or antigen determinant, after deprotection if required, may then be coupled to the AP205 VLP as follows. After separation of the excess thiolation reagent, the antigen or antigen determinant is reacted with the AP205 VLP previously activated with a hetero-bifunctional cross-linker comprising a cysteine reactive moiety, and therefore displaying at least one or several functional groups reactive towards cysteine residues, to which the thiolated antigen or antigen determinant can react, such as described above. Optionally, low amounts of a reducing agent are included in the reaction mixture. In other methods, the antigen is attached to the AP205 VLP using a homo-bifunctional cross-linker such as glutaraldehyde, DSG, $BM[PEO]_4$, BS^3, (Pierce Chemical Company, Rockford, IL, USA) or other known homo-bifunctional cross-linkers whith functional groups reactive towards amine groups or carboxyl groups of the AP205 VLP.

In a further embodiment, the antigen or antigen determinant is bound to the AP205 VLP through modification of the carbohydrate moieties present on glycosylated antigen or antigen determinant and subsequent reaction with the AP205 VLP. In one embodiment, the glycosylated antigen or antigen determinant is reacted with sodium periodate in a mild oxidation reaction of the carbohydrate moiety, to yield an activated antigen or antigen determinant with one or more aldehyde functional groups. The so activated antigen or antigen determinant is separated from excess sodium periodate, and further reacted with the AP205 VLP, wherein lysine residues of the AP205 VLP or of at least one AP205 VLP subunit are reacting with the previously formed aldehyde functional group on the antigen or antigen determinant, for example as described by Hermanson, G.T. in *Bioconjugate Techniques*, Academic Press Inc., San Diego, CA, USA. Self polymerization of the activated antigen or antigen determinant may be controlled by adjusting the pH as described in the aforementioned publication. The formed Schiff base is preferably further reduced with sodium cyanoborohydride, which is subsequently removed by gel filtration or dialysis. Alternatively, carboxyl groups of the AP205 VLP or of at least one of the AP205 VLP subunit may be reacted with EDC and a dihydrazyde, such as adipic acid dihydrazyde, to yield a hydrazide moiety available for reaction with the one or more aldehyde functional groups present on the activated antigen or antigen determinant. The so formed hydrazone may be further reduced with sodium cyanoborohydride. Alternatively, the activated antigen or antigen determinant with one or more aldehyde functional groups is reacted with cysteamine, resulting in the introduction of a cysteine group in the antigen or antigen determinant. Additional cross-linking methods and cross-linkers, suitable for binding an antigen or antigen determinant to a AP205 VLP, as well as guidance on performing the coupling reactions and on the use of chemical cross-linkers and chemical cross-linking procedures can be found in Hermanson, G.T. in *Bioconjugate Techniques*, Academic Press Inc., San Diego, CA, USA.

Other methods of binding the VLP to an antigen include methods where the VLP is biotinylated, and the antigen expressed as a streptavidin-fusion protein, or methods wherein both the antigens and the VLP are biotinylated. In this case, the antigen may be first bound to streptavidin or avidin by adjusting the ratio of antigen to streptavidin such that free binding sites are still available for binding of the VLP,

which is added in the next step. Alternatively, all components may be mixed in a "one pot" reaction. Other ligand-receptor pairs, where a soluble form of the receptor and of the ligand is available, and are capable of being cross-linked to the VLP or the antigen, may be used as binding agents for binding the antigen to the VLP. In a preferred embodiment of the present invention, said first and/or said second attachment sites are selected from the group consisting of: (a) an antigen and an antibody or antibody fragment thereto; (b) biotin and avidin; strepavidin and biotin; (c) a receptor and its ligand; (d) a ligand-binding protein and its ligand; (e) interacting leucine zipper polypeptides; (f) an amino group and a chemical group reactive thereto; (g) a carboxyl group and a chemical group reactive thereto; (h) a sulfhydryl group and a chemical group reactive thereto; and (i) a combination thereof.

Immune Responses

The nature or type of immune response is not a limiting factor of this disclosure. The desired outcome of a therapeutic or prophylactic immune response may vary according to the disease, according to principles well known in the art. For example, an immune response against an infectious agent may completely prevent colonization and replication of an infectious agent, affecting "sterile immunity" and the absence of any disease symptoms. However, a vaccine against infectious agents may be considered effective if it reduces the number, severity or duration of symptoms; if it reduces the number of individuals in a population with symptoms; or reduces the transmission of an infectious agent. Similarly, immune responses against cancer, allergens or self antigens may completely treat a disease, may alleviate symptoms, or may be one facet in an overall therapeutic intervention against a disease. For example, the stimulation of an immune response against a cancer may be coupled with surgical, chemotherapeutic, radiologic, hormonal and other immunologic approaches in order to affect treatment.

Furthermore, it may be desired to stimulate different types of immune response depending on the disease, and according to principles known in the art. It is well known, for example, that some immune responses are more appropriate for a particular antigen than other immune responses. Some immune responses are, indeed, inappropriate and can cause pathology, such as pathologic inflammation in response to

infection, allergies and autoimmune disease, further described herein. While not desiring certain specific immune responses, the present invention stimulates immune responses towards therapeutic or prophylactic goals. A further particular embodiment of the invention includes the generation of an immune response able to counter the effects of a pathologic immune response.

The nature of the immune response can be affected by the nature of the antigen, route of introduction into the body, dose, dosage regimen, reptitive nature of the antigen, host background, and signalling factors of the immune system. Such knowledge is well known in the art. As such, an immune response may be tailored by the application of both art known theory and routine experimentation.

While not wishing to be bound by theory, the current invention presents particular novel and surprising advantages as a component of a pharmaceutical composition to generate an immune response, and particularly as a vaccine.

Firstly, other carriers known in the art including BSA, keyhole limpet hemocyanin, tetanus toxoid, bacterial outermembrane proteins, cholera toxin, *Pseudomonas aeruginosa* Exotoxin A and bacterial pili may be inappropriate for use in an individual, and in particular a human. The aforementioned carriers may induce allergic reactions, or stimulate pathologic immune responses (for example, cholera toxin, KLH, BSA). The aforementioned carriers may require the presence of adjuvants such as complete Freunds adjuvant, now considered innappropriate for use in humans. A number of the carriers may be components of current vaccines (for example, tetanus toxoid, cholera toxin, Exotoxin A), or represent antigens that are commonly encountered (for example, bacterial pili, exotoxin A, outermembrane proteins). As such an individual may possess a high level of pre-existing immunity to these carriers, such that immunization with an antigen-carrier conjugate will induce a relatively greater immune response to the carrier than to the novel antigen. For these reasons, individually or as a whole, the use of AP205 as a carrier protein may represent a useful improvement over current carrier proteins. An AP205-DerP1 conjugate composition is able to stimulate an immune response against DerP1 without the use of complete Freund's adjuvant and without evidence of pathologic immune responses.

In the use of AP205 as a carrier protein, it is possible that AP205-antigen antigens conjugated to AP205 can be taken up by antigen presenting cells and thereby

stimulate T-cell help to induce immune response. Further, haptens, which are normally non immunogenic, may be coupled to AP205 thereby generating an immune response against such haptens.

A further advantageous feature of the invention is that antigens may be presented on the surface of a VLP in regular, repetitive arrays that are able to induce efficient immune responses both with and without T-cell help. This feature of the invention is particularly advantageous.

The present invention thus provides methods for improving the efficiency of vaccination, particularly against self-antigens, by increasing the degree of repetitiveness of the antigen to be used for immunization, through binding of the antigen to the AP205 VLP.

Compositions, Vaccines, and the Administration Thereof, and Methods of Treatment

The invention provides vaccine compositions which may be used for preventing and/or attenuating diseases or conditions. The invention further provides vaccination methods for preventing and/or attenuating diseases or conditions in individuals.

In one embodiment, the invention provides vaccines for the prevention of infectious diseases in a wide range of species, particularly mammalian species such as human, monkey, cow, dog, cat, horse, pig, etc. Vaccines may be designed to treat infections of viral etiology such as HIV, influenza, *Herpes*, viral hepatitis, Epstein Barr, polio, viral encephalitis, measles, chicken pox, etc.; or infections of bacterial etiology such as pneumonia, tuberculosis, syphilis, etc.; or infections of parasitic etiology such as malaria, trypanosomiasis, leishmaniasis, trichomoniasis, amoebiasis, etc.

In another embodiment, the invention provides vaccines for the prevention and treatment of cancer in a wide range of species, particularly mammalian species such as human, monkey, cow, dog, cat, horse, pig, etc. Vaccines may be designed to treat all types of cancer: lymphomas, carcinomas, sarcomas, melanomas, etc.

In another embodiment of the invention, conjugates, compositions and methods of the invention may be used in the design of vaccines for the treatment of allergies. Antibodies of the IgE isotype are important components in allergic reactions. Mast

cells bind IgE antibodies on their surface and release histamines and other mediators of allergic response upon binding of specific antigen to the IgE molecules bound on the mast cell surface. Inhibiting production of IgE antibodies, therefore, is a promising target to protect against allergies. This should be possible by attaining a desired T helper cell response. T helper cell responses can be divided into type 1 (T_H1) and type 2 (T_H2) T helper cell responses (Romagnani, *Immunol. Today 18*:263-266 (1997)). T_H1 cells secrete interferon-gamma and other cytokines. In contrast, a critical cytokine produced by T_H2 cells is IL-4, which drived B cells to produce IgE. In many experimental systems, the development of T_H1 and T_H2 responses is mutually exclusive sinceT_H1 cells suppress the induction of T_H2 cells and *vice versa.* Thus, antigens that trigger a strong T_H1 response simultaneously suppress the development of T_H2 responses and hence the production of IgE antibodies. It is finding of the present invention that AP205 VLP induce a T_H1-type immune response. Thus, by using the processes of the invention, AP205 VLPs can be decorated with various allergens and used for immunization. Due to the coupling of the allergen to AP205 VLP, a T_H1 response will be elicited, "protective" IgG antibodies will be produced, and the production of IgE antibodies which cause allergic reactions will be prevented. Since the allergen is presented by VLPs which are recognized by a different set of helper T cells than the allergen itself, it is likely that the allergen-specific IgG antibodies will be induced even in allergic individuals harboring pre-existing T_H2 cells specific for the allergen. The presence of high concentrations of IgG antibodies may prevent binding of allergens to mast cell bound IgE, thereby inhibiting the release of histamine. Thus, presence of IgG antibodies may protect from IgE mediated allergic reactions. Typical substances causing allergies include but are not limited to: pollens (e.g. grass, ragweed, birch or mountain cedar pollens), house dust, mites, mammalian epidermal allergens and animal danders, mold and fungus, insect bodies and insect venom, hair, saliva, serum, feathers, food or drugs (*e.g.*, penicillin) *See* Shough, H. *et al.,* REMINGTON'S PHARMACEUTICAL SCIENCES, 19th edition, (Chap. 82), Mack Publishing Company, Mack Publishing Group, Easton, Pennsylvania (1995), the entire contents of which is hereby incorporated by reference. .

In specific embodiments, the invention provides methods for preventing and/or attenuating diseases or conditions which are caused or exacerbated by "self" gene

products (*e.g.*, tumor necrosis factors), i.e. "self antigens" as used herein. In related embodiments, the invention provides methods for inducing immunological responses in individuals which lead to the production of antibodies that prevent and/or attenuate diseases or conditions are caused or exacerbated by "self" gene products. Examples of such diseases or conditions include graft versus host disease, IgE-mediated allergic reactions, anaphylaxis, adult respiratory distress syndrome, Crohn's disease, allergic asthma, acute lymphoblastic leukemia (ALL), non-Hodgkin's lymphoma (NHL), Graves' disease, inflammatory autoimmune diseases, myasthenia gravis, systemic lupus erythematosus (SLE), immunoproliferative disease lymphadenopathy (IPL), angioimmunoproliferative lymphadenopathy (AIL), immunoblastive lymphadenopathy (IBL), rheumatoid arthritis, diabetes, multiple sclerosis, osteoporosis and Alzheimer's disease.

As would be understood by one of ordinary skill in the art, when compositions of the invention are administered to an individual, they may be in a composition which contains salts, buffers, adjuvants, or other substances which are desirable for improving the efficacy of the composition. Examples of materials suitable for use in preparing pharmaceutical compositions are provided in numerous sources including REMINGTON'S PHARMACEUTICAL SCIENCES (Osol, A, ed., Mack Publishing Co., (1990)).

Compositions of the invention are said to be "pharmacologically acceptable" if their administration can be tolerated by a recipient individual. Further, the compositions of the invention will be administered in a "therapeutically effective amount" (*i.e.*, an amount that produces a desired physiological effect).

The compositions of the present invention may be administered by various methods known in the art, but will normally be administered by injection, infusion, inhalation, oral administration, or other suitable physical methods. The compositions are, alternatively, administered intramuscularly, intravenously, transmucosally, transdermally or subcutaneously. Components of compositions for administration include sterile aqueous (*e.g.*, physiological saline) or non-aqueous solutions and suspensions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl

oleate. Carriers or occlusive dressings can be used to increase skin permeability and enhance antigen absorption.

Other embodiments of the invention include processes for the production of the compositions of the invention and methods of medical treatment using these compositions. It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are intended to provide further explanation of the invention as claimed.

In addition to vaccine technologies, other embodiments of the invention are drawn to methods of medical treatment for cancer and allergies, and for methods of treatment of diseases or conditions which are caused or exacerbated by "self" gene products .

All patents and publications referred to herein are expressly incorporated by reference in their entirety.

Kits

In other embodiments, the compositions of the present invention may be assembled into kits for use in detection in assays or industrial settings, in diagnosis or detection of diseases, conditions or disorders. Such kits according to the present invention may comprise at least one container containing one or more of the above-described conjugates or compositions, including AP205 conjugates and immune molecules and antibodies, respectively, directed against such conjugates. The kits of the invention may optionally further comprise at least one additional container which may contain, for example, one or more antigens, one or more haptens, one or more core particles, one or more conjugates/compositions of the invention, one or more pharmaceutically acceptable carriers or excipients, one or more buffers, one or more proteins, one or more nucleic acid molecules, and the like.

It will be understood by one of ordinary skill in the relevant arts that other suitable modifications and adaptations to the methods and applications described herein are readily apparent and may be made without departing from the scope of the invention or any embodiment thereof. Having now described the present invention in detail, the same will be more clearly understood by reference to the following

examples, which are included herewith for purposes of illustration only and are not intended to be limiting of the invention.

EXAMPLES

EXAMPLE 1:

Cloning of the AP205 Coat Protein gene

The cDNA of AP205 coat protein (CP) was assembled from two cDNA fragments generated from phage AP205 RNA by using a reverse transcription-PCR technique and cloning in the commercial plasmid pCR 4-TOPO for sequencing. Reverse transcription techniques are well known to those of ordinary skill in the relevant art. The first fragment, contained in plasmid p205-246, contained 269 nucleotides upstream of the CP sequence and 74 nucleotides coding for the first 24 N-terminal amino acids of the CP. The second fragment, contained in plasmid p205-262, contained 364 nucleotides coding for amino acids12-131of CP and an additional 162 nucleotides downstream of the CP sequence

The plasmid 283.-58 was designed by two-step PCR, in order to fuse both CP fragments from plasmids p205-246 and p205-262 in one full-length CP sequence. An upstream primer p1.44 containing the *NcoI* site for cloning into plasmid pQb185, or p1.45 containing the *XbaI* site for cloning into plasmid pQb10, and a downstream primer p1.46 containing the *HindIII* restriction site were used (recognition sequence of the restriction enzyme underlined):

p1.44 5'-AACC ATG GCA AAT AAG CCA ATG CAA CCG-3' (SEQ ID NO: 59)

p1.45 5'-AATCTAGAATTTTCTGCGCACCCATCCCGG-3' (SEQ ID NO: 60)

p1.46 5'-AAAAGC TTA AGC AGT AGT ATC AGA CGA TAC G-3' (SEQ ID NO: 61)

Two additional primers, p1.47, annealing at the 5' end of the fragment contained in p205-262, and p1.48, annealing at the 3' end of the fragment contained in plasmid p205-246 were used to amplify the fragments in the first PCR. Primers p1.47

and p1.48 are complementary to each other.

p1.47: 5'-GAGTGATCCAACTCGTTTATCAACTACATTT-TCAGCAAGTCTG-3' (SEQ ID NO: 62)

p1.48: 5'-CAGACTTGCTGAAAATGTAGTTGATAAACGA-GTTGGATCACTC-3' (SEQ ID NO: 63)

In the first two PCR reactions, two fragments were generated. The first fragment was generated with primers p1.45 and p1.48 and template p205-246. The second fragment was generated with primers p1.47 and p1.46, and template p205-262. Both fragments were used as templates for the second PCR reaction, a splice-overlap extension, with the primer combination p1.45 and p1.46 or p1.44 and p1.46 . The product of the two second-step PCR reactions were digested with *XbaI* or *NcoI* respectively, and *HindIII,* and cloned with the same restriction sites into pQb10 or pQb185 respectively, two pGEM-derived expression vectors under the control of *E.coli* tryptophan operon promoter.

Two plasmids were obtained, pAP283-58 (SEQ ID NO.2) containing the gene coding for wt AP205 CP (SEQ ID NO: 1) in pQb10, and pAP281-32 (SEQ ID NO: 4) containing the nucleotide sequence of SEQ ID NO: 125 encoding for the mutein with mutation Pro5→Thr having the amino acid sequence of SEQ ID NO: 3 in pQb185 . The coat protein sequences were verified by DNA sequencing. PAP283-58 contains 49 nucleotides upstream of the ATG codon of the CP, downstream of the XbaI site, and contains the putative original ribosomal binding site of the coat protein mRNA.

EXAMPLE 2:

Expression and Purification of Recombinant AP205 VLP

A. Expression of recombinant AP205 VLP

E.coli JM109 was transformed with plasmid pAP283-58. 5 ml of LB liquid medium with 20 µg/ml ampicillin were inoculated with a single colony, and incubated at 37 °C for 16-24 h without shaking.

The prepared inoculum was diluted 1:100 in 100-300 ml of LB medium, containing 20 µg/ml ampicillin and incubated at 37 °C overnight without shaking. The

resulting second inoculum was diluted 1:50 in 2TY medium, containing 0.2 % glucose and phosphate for buffering, and incubated at 37 °C overnight on a shaker. Cells were harvested by centrifugation and frozen at -80°C.

B. Purification of recombinant AP205 VLP

Solutions and buffers:

1. Lysis buffer
   50mM Tris-HCl pH 8.0 with 5mM EDTA , 0.1% tritonX100 and PMSF at 5 micrograms per ml.
2. SAS
   Saturated ammonium sulphate in water
3. Buffer NET.
   20 mM Tris-HCl, pH 7.8 with 5mM EDTA and 150 mM NaCl.
4. PEG
   40% (w/v) polyethylenglycol 6000 in NET

Lysis:

Frozen cells were resuspended in lysis buffer at 2 ml/g cells. The mixture was sonicated with 22 kH five times for15 seconds, with intervals of 1min to cool the solution on ice. The lysate was then centrifuged for 20 minutes at 12 000 rpm, using a F34-6-38 rotor (Ependorf). The centrifugation steps described below were all performed using the same rotor, except otherwise stated. The supernatant was stored at 4° C, while cell debris were washed twice with lysis buffer. After centrifugation, the supernatants of the lysate and wash fractions were pooled.

Ammonium-sulphate precipitation can be further used to purify AP205 VLP. In a first step, a concentration of ammonium-sulphate at which AP205 VLP does not precipitate is chosen. The resulting pellet is discarded. In the next step, an ammonium sulphate concentration at which AP205 VLP quantitatively precipitates is selected, and AP205 VLP is isolated from the pellet of this precipitation step by centrifugation (14 000 rpm, for 20 min). The obtained pellet is solubilised in NET buffer.

Chromatography:

The capsid protein from the pooled supernatants was loaded on a Sepharose 4B column (2.8 X 70 cm), and eluted with NET buffer, at 4 ml/hour/fraction. Fractions 28-40 were collected, and precipitated with ammonium sulphate at 60% saturation. The fractions were analyzed by SDS-PAGE and Western Blot with an antiserum specific for AP205 VLP coat protein prior to precipitation. The pellet isolated by centrifugation was resolubilized in NET buffer, and loaded on a Sepharose 2B column (2.3 X 65 cm), eluted at 3 ml/h/fraction. Fractions were analysed by SDS-PAGE, and fractions 44-50 were collected, pooled and precipitated with ammonium sulphate at 60% saturation. The pellet isolated by centrifugation was resolubilized in NET buffer, and purified on a Sepharose 6B column (2.5 X 47 cm), eluted at 3 ml/hour/fraction. The fractions were analysed by SDS-PAGE. Fractions 23-27 were collected, the salt concentration adjusted to 0.5 M, and precipitated with PEG 6000, added from a 40% stock in water and to a final concentration of 13.3%. The pellet isolated by centrifugation was resolubilized in NET buffer, and loaded on the same Sepharose 2B column as above, eluted in the same manner. Fractions were analyzed by SDS-PAGE. Fractions 43-53 were collected, and precipitated with ammonium sulphate at a saturation of 60%. The pellet isolated by centrifugation was resolubilized in water, and the obtained protein solution was extensively dialyzed against water. About 10 mg of purified protein per gram of cells could be isolated. Purification of AP 205 VLPs could be shown, which was analysed by SDS PAGE and Western-blotting. Fractions of recombinant AP205 VLP of the first Sepharose 4B chromatography step as well as of the last Sepharose 2B chromatography step could be shown on the silver-stained SDS-PAGE run under reducing conditions as well as by Western blotting with an anti-serum specific for AP205 VLP coat protein.

Examination of the virus-like particles in Electron microscopy showed that they were identical to the phage particles (FIG. 1A and 1 B).

FIG. 1A shows an EM picture of AP205 phage particles, while an EM picture of self assembled particles of recombinant AP205 VLP is shown in FIG. 1 B.

For the sake of simplicity, the term „AP205 virus-like particle" and the term

„AP205 VLP", as used within the Example Section, refers to a virus-like particle expressed and purified as described in Example 2 and composed of coat proteins having an amino acid sequence as set forth in SEQ ID NO:1.

EXAMPLE 3:

Coupling of Derp1.2 and Flag Peptide Antigen to AP205 VLP, and Immunization of Mice with Derp1.2 Peptide Coupled to AP205 VLP

A. Coupling of Derp1.2 peptide and Flag peptide to recombinant AP205 VLP

The peptide Derp1.2 (sequence: CQIYPPNANKIREALAQTHSA "Der p 1 p117"; aa 117-137 (SEQ ID NO: 64)) and Flag (sequence: CGGDYKDDDDK (SEQ ID NO: 65)) were chemically synthesized according to art-known methods. AP205 VLP, expressed and purified as described in example 2, was resolubilized in 20 mM Hepes, 150 mM NaCl, pH 7.4 buffer (HBS buffer). Resolubilized AP205 VLP was then reacted at a concentration of 2 mg/ml (determined in a Bradford assay), with 2.85 mM SMPH (Pierce) for 30 minutes at room temperature (RT). The reaction mixture was then dialyzed against HBS buffer, and reacted with 0.714 mM Derp1.2 or FLAG, diluted in the reaction mixture from a 50 mM stock in DMSO. The coupling reaction was left to proceed for 2 hours at 15°C, and the reaction mixture dialyzed 2 X 2 hours against a 1000-fold volume HBS, and flash frozen in liquid nitrogen in aliquots for storage at -80°C until further use.

An aliquot was thawed, and coupling of the antigen to an AP205 subunit assessed by SDS-PAGE and the protein concentration measured in a Bradford assay. The result of the coupling reaction of Derp 1.2 to AP205 VLP is shown in FIG. 2. The monomer subunit of AP205 VLP has a molecular weight of 14 kDa. Upon derivatization of AP205 VLP with the cross-linker, dimers, trimers, tetramers, pentamers and hexamers produced by cross-linking, are detected in SDS-PAGE in addition to the monomer form of the subunit.

FIG. 2 shows the SDS-PAGE analysis of the coupling reaction of AP205 VLP and Qβ VLP to Derp1.2 peptide. The samples were run under reducing conditions on a 16% Tris-glycine gel. Lane 1 is the protein marker, with corresponding molecular

weights indicated on the left border of the gel; lane 2, derivatized Qβ capsid protein; lane 3, the supernatant of the coupling reaction of Qβ capsid protein to the Derp1.2 peptide; lane 4, the pellet of the coupling reaction of Qβ capsid protein to the Derp1.2 peptide; lane 5, derivatized AP205 VLP; lane 6, the supernatant of the coupling reaction of AP205 VLP to the Derp1.2 peptide; lane 7, the pellet of the coupling reaction of AP205 VLP to the Derp1.2 peptide. Coupling products corresponding to the coupling of 1, 2, 3 ,4 and respectively 5 peptides per monomer are indicated by arrows in the Figure. A higher number of epitopes could be coupled to AP205 VLP than to Qβ capsid protein.

B. Immunization of mice with Derp1.2 peptide coupled to recombinant AP205 VLP analysis of immune response and IgG subtype determination

AP205 VLP coupled to Derp1.2 peptide or Qβ VLP coupled to Derp1.2 peptide were injected s.c. in mice (3 mice each) at day 0 and 14. Derp1.2 peptide was coupled to Qβ capsid protein using the same conditions as described under A for the coupling to AP205 VLP. Each mice was immunized with 10 μg of vaccine diluted in PBS to 200 μl. Mice were retroorbitally bled on day 20, and the titer of the antibodies specific for the Derp1.2 peptide were measured in an ELISA against Derp1.2 peptide. The Der p I peptide "Der p I p52" was coupled to bovine RNAse A using the chemical cross-linker sulfo-SPDP. ELISA plates were coated with coupled RNAse preparations at a concentration of 10 μg/ml. The plates were blocked and then incubated with serially diluted mouse sera. Bound antibodies were detected with enzymatically labeled anti-mouse IgG antibodies specific for the respective subtypes. As a control, preimmune sera of the same mice were also tested (data not shown). The results are shown in FIG. 3.

FIG. 3 shows an ELISA analysis of the IgG antibodies specific for "Derp 1.2" in sera of mice immunized against the Derp1.2 peptide coupled to AP205 VLP or Qβ capsid protein respectively. Total IgG titers, as well as IgG subtype titers were determined. No antibodies specific for Derp1.2 could be detected in any of the preimmune sera analysed for each of the IgG subtypes. The figure shows that for both AP205 and Qβ, subtypes typical of a Th1 immune response are induced, as the IgG2a

titer is much higher than the IgG1 titer. A strong specific anti-peptide immune response was obtained with the peptide coupled to both VLPs. Antibodies specific for the carrier were also measured by ELISA, and these were comparable for both carriers.

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EXAMPLE 4:

Modular Eukaryotic Expression System for Coupling of Antigens to VLPs

10 This system was generated in order to add various amino acid linker sequences containing a cysteine residue to antigens for chemical coupling to VLPs. A. Construction of an EBNA-derived expression system encoding a cysteine-containing amino acid linker and cleavable Fc-Tag:

pCep-Pu (Wuttke *et al. J. Biol. Chem. 276*: 36839-48 (2001)) was digested with 15 Kpn I and Bam HI and a new multiple cloning site was introduced with the annealed oligonucleotides PH37 and PH38leading to pCep-MCS.

A modular system containing a free cysteine flanked by several glycines, a protease cleavage site and the constant region of the human IgG1 was generated as follows. pSec2/Hygro B (Invitrogen Cat. No. V910-20) was digested with Bsp120I and 20 Hind III and ligated with the annealed oligonucleotides SU7 and SU8 leading to construct pSec-B-MCS. pSec-B-MCS was then digested with Nhe I and Hind III and ligated with the annealed oligonucleotides PH29 and PH30 leading to construct pSec 29/30. The construct pSec-FL-EK-Fc* was generated by a three fragment ligation of the following fragments; first pSec 29/30 digested with Eco RI and Hind III, the 25 annealed oligonucleotides PH31 and PH32and the Bgl I/EcoRI fragment of a plasmid (pSP-Fc*-C1) containing a modified version of the human IgG1 constant region (for details of the hu IgG1 sequence see the sequence of the final construct pCep-Xa-Fc* (FIG. 4A-4C). The resulting construct was named pSec-FL-EK-Fc*. From this plasmid the linker region and the human IgG1 Fc part was excised by Nhe I, Pme I digestion 30 and cloned into pCep-MCS digested with Nhe I and Pme I leading to construct pCep-FL-EK-Fc*. Thus a modular vector, was created where the linker sequence and the protease cleavage site, which are located between the Nhe I and Hind III sites, can

easily be exchanged with annealed oligonucleotides. For the generation of cleavable fusion protein vectors pCep-FL-EK-Fc* was digested with Nhe I and Hind III and the Factor Xa cleavage site N-terminally flanked with amino acids GGGGCG (SEQ ID NO: 55) was introduced with the annealed oligonuclotides PH35 and PH36and the enterokinase site flanked n-terminally with GGGGCG (SEQ ID NO: 55) was introduced with the annealed oligonucleotides PH39 and PH40 leading to the constructs pCep-Xa-Fc* (FIG. 4A, nucleotide sequence as set forth in SEQ ID NO: 103, amino acid sequence as set forth in SEQ ID NO: 104) and pCep-EK-Fc* (FIG. 4B, nucleotide sequence as set forth in SEQ ID NO: 105, amino acid sequence as set forth in SEQ ID NO: 106) respectively. The construct pCep-SP-EK-Fc* (FIG. 4C, , nucleotide sequence as set forth in SEQ ID NO: 107, amino acid sequence as set forth in SEQ ID NO: 108) which in addition contains a eukaryotic signal peptide was generated by a three fragment ligation of pCep-EK-Fc* digested Kpn I/ Bam HI, the annealed oligos PH41 and PH42 and the annealed oligos PH43 and PH44.

B. Large Scale production of fusion proteins:

For the large scale production of the different fusion proteins 293-EBNA cells (Invitrogen) were transfected with the different pCep expression plasmids with Lipofectamine 2000 reagent (Invitrogen Corporation; Carlsabad, CA) according to the manufacturer's recommendation. 24-36 h post transfection the cells were split at a 1 to 3 ratio under puromycin selection (1μg/ml) in DMEM supplemented with 10 % FCS. The resistant cells were then expanded in selective medium. For the harvesting of the fusion proteins the resistant cell population were passed onto poly-L-lysine coated dishes. Once the cells had reached confluence, they were washed 2 times with PBS and serum free medium (DMEM) was added to the plates. The tissue culture supernatant were harvested every 2 to 4 days and replaced with fresh DMEM medium during a period of up to one month. The harvested supernatants were kept at 4 °C.

C. Purification of the fusion proteins:

The recombinant Fc-fusion proteins were purified by affinity chromatography using protein A sepharose CL-4B (Amersham Pharmacia Biotech AG). Briefly chromatography columns were packed with 1-3 ml protein A resin and the tissue

culture supernatants containing the recombinant proteins were applied to the column with a peristaltic pump at a flow rate of 0.5 – 1.5 ml/min. The column was then washed with 20-50 ml PBS. Depending on the fusion protein the protease cleavage was performed on the column or the protein was eluted as described below. Recombinant fusion proteins were eluted with a citrate/ phosphate buffer (pH 3.8) supplemented with 150 mM NaCl and the fractions containing the protein were pooled and concentrated with ultrafree centrifugal filters (Millipore Corporation; Bedford, MA).

D. Protease cleavage of recombinant fusion proteins (Factor Xa, enterokinase):

Eluted recombinant fusion proteins containing the enterokinase (EK) cleavage site were cleaved using the EKmax system (Invitrogen) according to the manufacturer's recommendation. The cleaved Fc part of the fusion protein was removed by incubation with protein A. The enterokinase was then removed with the EK-Away system (Invitrogen Corporation; Carlsbad, CA) according to the manufacturers recommendation. Similarly fusion proteins containing the factor Xa (Xa) cleavage site were cleaved using the restriction protease factor Xa cleavage and removal kit (Roche) according to the manufacturer's recommendation. The cleaved Fc part was removed by incubation with protein A and the protease was removed with the streptavidin resin provided with the kit.

The different fusion proteins were concentrated with ultrafree centrifugal filters (Millipore Corporation; Bedford, MA), quantitated by UV spectrophotometrie and used for subsequent coupling reactions.

FIG. 4A-4C shows partial sequences of the different eukaryotic expression vectors used. Only the modified sequences are shown.

FIG. 4A: pCep-Xa-Fc*: the sequence is shown from the Bam HI site onwards and different features are shown above the translated sequence (SEQ ID NO: 103 and SEQ ID NO: 104). The arrow indicates the cleavage site of the factor Xa protease.

FIG. 4B: pCep-EK-Fc*: the sequence is shown from the Bam HI site onwards and different features are shown above the translated sequence (SEQ ID NO: 105 and SEQ ID NO: 106). The arrow indicates the cleavage site of the enterokinase. The sequence downstream of the Hind III site is identical to the one shown in FIG. 4A.

FIG. 4C: pCep-SP-EK-Fc*: the sequence is shown from the beginning of the signal peptide on and different features are shown above the translated sequence (SEQ ID NO: 107 and SEQ ID NO: 108). The signal peptide sequence which is cleaved of by the signal peptidase is shown in bold The arrow indicates the cleavage site of the enterokinase. The sequence downstream of the Hind III site is identical to the one shown in FIG. 4A.

EXAMPLE 5:

Eukaryotic Expression and Coupling of Mouse Resistin to AP205 VLP

A. Cloning of mouse Resistin:

Total RNA was isolated from 60 mg mouse adipose tissue using a Qiagen RNeasy kit according to the manufacturer's recommendation. The RNA was eluted in 40 µl H_2O. This total RNA was than used for the reverse transcription with an oligo dT primer using the ThermoScript™ RT-PCR System (Invitrogen Corporation; Carlsbad, CA) according to the manufacturer's recommendation. The sample was incubated at 50 °C for 1h, heated to 85 °C for 5 minutes and treated for 20 minutes at 37 °C with RNAseH.

2 µl of the RT reaction were used for the PCR amplification of mouse resistin. The PCR was performed using Platinium TAQ (Invitrogen Corporation; Carlsbad, CA) according to the manufacturer's recommendation using primers PH19 and PH20. Primer PH19 corresponds to positions 58-77 and primer PH20 to positions 454-435 of the mouse Resistin sequence. The PCR mix was first denatured at 94 °C for 2 minutes and than 35 cycles were performed as follows: 30 seconds 94 °C, 30 seconds 56 °C and 1 minute 72 °C, at the end the samples were left for 10 minutes at 72 °C. The PCR fragment was purified and subcloned by TA cloning into the pGEMTeasy vector (Invitrogen Corporation; Carlsbad, CA) leading to pGEMT-mRes. In order to add appropriate restriction sites a second PCR was performed on pGEMT-mRes with the primers PH21 and PH22 primers using the same cycling program as described above. The forward primer PH21 contains a Bam HI site and nucleotides 81-102 of the mouse Resistin sequence. The reverse primer PH22 contains an Xba I site and nucleotides

426-406 of the mouse Resistin sequence. The indicated positions refer to the mouse resistin sequence Gene Accession No. AF323080. The PCR product was purified and digested with Bam HI and Xba I and subcloned into pcmv-Fc*-C1 digested with Bam HI and Xba I leading to the construct pcmv-mRes-Fc*.

The Resistin open reading frame was excised from pcmv-Res-Fc* by Bam HI/ Xba I digestion and cloned into pCep-Xa-Fc* and pCep-EK-Fc* (see EXAMPLE 4, section B) digested with Bam HI and Nhe I leading to the constructs pCep-mRes-Xa-Fc* and pCep-mRes-EK-Fc* respectively.

B. Production, purification and cleavage of Resistin

pCep-mRes-Xa-Fc* and pCep-mRes-EK-Fc* constructs were then used to transfect 293-EBNA cells for the production of recombinant proteins as described in EXAMPLE 4, section B. The tissue culture supernatants were purified as described in EXAMPLE 4, section C. The purified proteins were then cleaved as described in EXAMPLE 4, section D. The resulting recombinant proteins were termed "resistin-C-Xa" or "Res-C-Xa" and "resistin-C-EK" or "Res-C-EK" according to the vector used. The purified proteins were analyzed by SDS PAGE. Bands corresponding to purified resistin-C-EK and purified resistin-C-Xa were clearly visible on the gel.
SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, and SEQ ID NO: 112 show sequences of precursor recombinant mouse Resistin proteins used for expression. Processed recombinant mouse resistin used for coupling, i.e. Res-C-Xa and Res-C-EK, are shown in Figure 2A and 2B of WO 02/056905. The resistin signal sequence which is cleaved upon protein secretion by the signal peptidase is shown in italic. The amino acid sequences which result form signal peptidase and specific protease (factor Xa or enterokinase) cleavage are shown bold.

C. Coupling of resistin-C-Xa and resistin-C-EK to AP205 VLP

A solution of 0.2 ml of 2 mg/ml AP205 VLP in 20 mM Hepes, 150 mM NaCl pH 7.4 is reacted for 30 minutes with 5.6 µl of a solution of 100mM SMPH (Pierce) in DMSO at 25 °C on a rocking shaker. The reaction solution is subsequently dialyzed twice for 2 hours against 1 L of 20 mM Hepes, 150 mM NaCl, pH 7.4 at 4 °C. 8 µl of the dialyzed AP205 capsid protein reaction mixture is

then reacted with 32 µl of resistin-C-Xa solution (resulting in a final concentration of resistin of 0.39 mg/ml) and 13 µl of the AP205 capsid protein reaction mixture is reacted with 27 µl resistin-C-EK solution (resulting in a final concentration of resistin of 0.67 mg/ml) for four hours at 25 °C on a rocking shaker. Coupling products are analysed by SDS-PAGE and Western blot under reducing conditions.

EXAMPLE 6:

Expression, Purification and Coupling of Murine Lymphotoxin-β Constructs to AP205 VLP

A. Introduction of cys-containing linkers, expression and purification of mouse lymphotoxin-β

The extracellular part of mouse lymphotoxin-β (LT-β) was recombinantly expressed with a CGG amino acid linker at its N-terminus. The linker contained one cysteine for coupling to VLP. A long (aa 49-306) and a short version (aa 126-306) of the protein were fused at their N-terminus to either glutathione S-transferase (GST) or a histidin-myc tag for purification. An enterokinase (EK) cleavage-site was inserted for cleavage of the tag.

Construction of C- LT-β49-306 and C- LT-β126-306.

Mouse LT-β49-306 was amplified by PCR with oligos 5' LT-β and 3' LT-β from a mouse spleen cDNA library inserted into pFB-LIB. For the PCR reaction, 0.5 µg of each primer and 200 ng of the template DNA was used in the 50 µl reaction mixture (1 unit of PFX Platinum polymerase, 0.3 mM dNTPs and 2 mM $MgSO_4$). The temperature cycles were as follows: 94°C for 2 minutes, followed by 25 cycles of 94°C (15 seconds), 68°C (30 seconds), 68°C (1 minute) and followed by 68°C for 10 minutes. The PCR product was phosphorylated with T4 Kinase and ligated into pEntry1A (Life technologies) which has been cut with *EcoR*V and has been dephosphorylated. The resulting plasmid was named pEntry1A- LT-β49-306.

A second PCR reaction was performed with oligos 5' LT-βlong-*Nhe*I and 3' LT-βstop-*Not*I resp. 5' LT-βshort-*Nhe*I and 3' LT-βstop-*Not*I using pEntry1A- LT-

β49-306 as a template. Oligos 5' LT-βlong-*Nhe*I and 5' LT-βshort-*Nhe*I had an internal *Nhe*I site and contained codons for a Cys-Gly-Gly linker and 3' LT-βstop-*Not*I had an internal *Not*I site and contained a stop codon. For the second PCR reaction, 0.5 µg of each primer and 150 ng of the template DNA was used in the 50 µl reaction mixture (1 unit of PFX Platinum polymerase, 0.3 mM dNTPs and 2 mM $MgSO_4$). The temperature cycles were as follows: 94°C for 2 minutes, followed by 5 cycles of 94°C (15 seconds), 50°C (30 seconds), 68°C (1 minute), followed by 20 cylces of 94°C (15 seconds), 64°C (30 seconds), 68°C (1 minute) and followed by 68°C for 10 minutes.

The PCR products were digested with *Nhe*I and *Not*I and inserted into either pCEP-SP-GST-EK or pCEP-SP-his-myc-EK (Wuttke *et al. J. Biol. Chem. 276*: 36839-48 (2001)). Resulting plasmids were named pCEP-SP-GST-EK-C- LT-β49-306, pCEP-SP-GST-EK-C- LT-β126-306, pCEP-SP-his-myc-EK-C- LT-β49-306, pCEP-SP-his-myc-EK-C- LT-β126-306, respectively. GST stands for glutathione-S-transferase, EK for enterokinase, his for a hexahistidine tag and myc for anti c-myc epitope. The C indicates the CGG linker containing the additional cysteine.

All other steps were performed by standard molecular biology protocols.

Sequence of the oligonucleotides:

5' LT-β:

5'-CTT GGT GCC GCA GGA TCA G-3' (SEQ ID NO: 66)

3' LT-β:

5'-CAG ATG GCT GTC ACC CCA C-3' (SEQ ID NO: 67)

5' LT-βlong-*Nhe*I:

5'-GCC CGC TAG CCT GCG GTG GTC AGG ATC AGG GAC GTC G-3' (SEQ ID NO: 68)

5' LT-βshort-*Nhe*I:

5'-GCC CGC TAG CCT GCG GTG GTT CTC CAG CTG CGG ATT C -3' (SEQ ID NO: 69)

3' LT-βstop-*Not*I

5'-CAA TGA CTG CGG CCG CTT ACC CCA CCA TCA CCG -3' (SEQ ID NO: 70)

B. Expression and production of GST-EK-C- LT-β_{49-306}, GST-EK-C- LT-$\beta_{126-306}$, his-myc-EK-C- LT-β_{49-306} and his-myc-EK-C- LT-$\beta_{126-306}$

The plasmids pCEP-SP-GST-EK-C- LT-β49-306, pCEP-SP-GST-EK-C- LT-β126-306, pCEP-SP-his-myc-EK-C- LT-β49-306 and pCEP-SP-his-myc-EK-C- LT-β126-306 were transfected into 293-EBNA cells (Invitrogen) for protein production as described in EXAMPLE 4. The resulting proteins were named GST-EK-C- LT-β_{49-306}, GST-EK-C- LT-$\beta_{126-306}$, his-myc-EK-C- LT-β_{49-306} and his-myc-EK-C- LT-$\beta_{126-306}$. The protein sequences of the LT-β fusion proteins were translated from the cDNA sequences:

GST-EK-C- LT-β_{49-306}GST-EK-C- LT-$\beta_{126-306}$

his-myc-EK-C- LT-β_{49-306}

his-myc-EK-C- LT-$\beta_{126-306}$

The fusion proteins were analysed on 12% SDS-PAGE gels under reducing conditions. Gels were blotted onto nitrocellulose membranes. Membranes were blocked, incubated with a monoclonal mouse anti-myc antibody or with an anti-GST antibody. Blots were subsequently incubated with horse radish peroxidase-conjugated goat anti-mouse IgG or horse radish peroxidase-conjugated rabbit anti-goat IgG. The expression of LT-β fusion proteins could be shown. LT-β fusion proteins were analysed on 12% SDS-PAGE gels under reducing conditions. Gels were blotted onto nitrocellulose membranes. Membranes were blocked, incubated either with a monoclonal mouse anti-myc antibody (dilution 1:2000) or with an anti-GST antibody (dilution 1:2000). Blots were subsequently incubated with horse radish peroxidase-conjugated goat anti-mouse IgG (dilutions 1:4000) or horse radish peroxidase-conjugated rabbit anti-goat IgG (dilutions 1:4000).GST-EK-C- LT-β_{49-306} and GST-EK-C- LT-$\beta_{126-306}$ could be detected with the anti-GST antibody at a molecular weight of 62 kDa and 48 kDa, respectively. his-myc-EK-C- LT-β_{49-306} and his-myc-EK-C- LT-$\beta_{126-306}$ could be detected with the anti-myc antibody at 40-56 kDa and 33-39 kDa, respectively.

C. Purification of GST-EK-C- LT-β_{49-306}, GST-EK-C- LT-$\beta_{126-306}$, his-myc-EK-C- LT-β_{49-306} and his-myc-EK-C- LT-$\beta_{126-306}$

GST-EK-C- LT-β_{49-306} and GST-EK-C- LT-$\beta_{126-306}$ are purified on glutathione-sepharose column and his-myc-EK-C- LT-β_{49-306} and his-myc-EK-C- LT-$\beta_{126-306}$ are purified on Ni-NTA sepharose column using standard purification protocols. The purified proteins are cleaved with enterokinase and analysed on a 16% SDS-PAGE gel under reducing conditions

D. Coupling of C- LT-β_{49-306} and C- LT-$\beta_{126-306}$ to AP205 VLP

A solution of 120 μM AP205 VLP in 20 mM Hepes, 150 mM NaCl pH 7.2 is reacted for 30 minutes with a 25 fold molar excess of SMPH (Pierce), diluted from a stock solution in DMSO, at 25 °C on a rocking shaker. The reaction solution is subsequently dialyzed twice for 2 hours against 1 L of 20 mM Hepes, 150 mM NaCl, pH 7.2 at 4 °C. The dialyzed AP205 VLP reaction mixture is then reacted with the C-LT-β_{49-306} and C- LT-$\beta_{126-306}$ solution (end concentrations: 60 μM AP205 VLP, 60 μM C- LT-β_{49-306} and C- LT-$\beta_{126-306}$) for four hours at 25 °C on a rocking shaker. Coupling products are analysed by SDS-PAGE and Western blot under reducing conditions.

EXAMPLE 7:

Cloning, Expression, Purification and Coupling of AP205 VLP to MIF

A. Introduction of cys-containing linkers, expression, purification of rat macrophage migration inhibitory factor MIF

Rat macrophage migration inhibitory factor (rMIF) was recombinantly expressed with three different amino acid linkers C1, C2 and C3 fused at its C-terminus. Each of the linker contained one cysteine for coupling to VLP.

Construction of rMIF-C1, rMIF-C2, and rMIF-C3.

The MCS of pET22b(+) (Novagen, Inc.) was changed to GTTTAACTTT AAGAAGGAGATATACATATGGATCCGGCTAGCGCTCGAGGGTTTAAACGG CGGCCGCATGCACC (SEQ ID NO: 71) by replacing the original sequence from the

NdeI site to XhoI site with annealed oligos primerMCS-1F and primerMCS-1R (annealing in 15 mM TrisHCl pH 8 buffer). The resulting plasmid was termed pMod00, which had NdeI, BamHI, NheI, XhoI, PmeI and NotI restriction sites in its MCS. The annealed pair of oligos Bamhis6-EK-Nhe-F and Bamhis6-EKNhe-R and the annealed pair of oligo1F-C-glycine-linker and oligo1R-C-glycine-linker were together ligated into BamHI-NotI digested pMod00 plasmid to get pModEC1, which had an N terminal hexahistidine tag, an enterokinase cleavage site and a C-terminal amino acid glycine linker containing one cysteine residue. The annealed pair of oligos Bamhis6-EK-Nhe-F and Bamhi6-EKNhe R together with the annealed pair of oligo1F-C-gamma1-linker and oligo1R-C-gamma1-linker were ligated into BamHI-NotI digested pMod00 plasmid to get pModEC2, which had an N terminal hexahistidine tag, an enterokinase cleavage site and a C-terminal γ1 linker, derived from the hinge region of human immunoglobulin γ1, containing one cysteine residue. The annealed pair of oligos Bamhis6-EK-Nhe-F and Bamhis6-EK-Nhe-R, the annealed pair of oligo1FA-C-gamma3-linker and oligo1RA-C-gamma3-linker, and the annealed pair of oligo1FB-C-gamma3-linker and oligo1RB-C-gamma3-linker were together ligated into BamHI-NotI digested pMod00 to get pModEC3, which had an N terminal hexahistidine tag, an enterokinase cleavage site and a C terminal γ3 linker, containing one cysteine residue, derived from the hinge region of mouse immunoglobulin γ3.

pBS-rMIF, which contains the rat MIF cDNA, was amplified by PCR with oligos rMIF-F and rMIF-Xho-R. rMIF-F had an internal NdeI site and rMIF-Xho-R had an internal XhoI site. The PCR product was digested with NdeI and XhoI and ligated into pModEC1, pModEC2 and pModEC3 digested with the same enzymes. Resulting plasmids were named pMod-rMIF-C1, pMod-rMIF-C2 and pMod-rMIF-C3, respectively.

For the PCR reaction, 15 pmol of each oligo and 1 ng of the template DNA was used in the 50 µl reaction mixture (2 units of PFX polymerase, 0.3 mM dNTPs and 2 mM $MgSO_4$). The temperature cycles were as follows: 94°C for 2 minutes, followed by 30 cycles of 94°C (30 seconds), 60°C (30 seconds), 68°C (30 seconds) and followed by 68°C for 2 minutes.

All other steps were performed by standard molecular biology protocols.

Sequence of the oligonucleotides:

primerMCS-1F:

5'-TAT GGA TCC GGC TAG CGC TCG AGG GTT TAA ACG GCG GCC GCA T-3' (SEQ ID NO: 72)

primerMCS-1R:

5'-TCG AAT GCG GCC GCC GTT TAA ACC CTC GAG CGC TAG CCG GAT CCA-3' (SEQ ID NO: 73)

Bamhis6-EK-Nhe-F:

5'-GAT CCA CAC CAC CAC CAC CAC CAC GGT TCT GGT GAC GAC GAT GAC AAA GCG CTA GCC C-3' (SEQ ID NO: 74)

Bamhis6-EK-Nhe-R:

5'-TCG AGG GCT AGC GCT TTG TCA TCG TCG TCA CCA GAA CCG TGG TGG TGG TGG TGG TGT G-3'(SEQ ID NO: 75)

oligo1F-C-glycine-linker:

5'-TCG AGG GTG GTG GTG GTG GTT GCG GTT AAT AAG TTT AAA CGC-3'(SEQ ID NO: 76)

oligo1R-C-glycine-linker:

5'-GGC CGC GTT TAA ACT TAT TAA CCG CAA CCA CCA CCA CCA CCC-3' (SEQ ID NO: 77)

oligo1F-C-gamma1-linker:

5'-TCG AGG ATA AAA CCC ACA CCT CTC CGC CGT GTG GTT AAT AAG TTT AAA CGC-3' (SEQ ID NO: 78)

oligo1R-C-gamma1-linker:

5'-GGC CGC GTT TAA ACT TAT TAA CCA CAC GGC GGA GAG GTG TGG GTT TTA TCC-3' (SEQ ID NO: 79)

oligo1FA-C-gamma3-linker:

5'-TCG AGC CGA AAC CGT CTA CCC CGC CGG GTT CTT CTG-3' (SEQ ID NO: 80)

oligo1RA-C-gamma3-linker:

5'-CAC CAC CAG AAG AAC CCG GCG GGG TAG ACG GTT TCG GC-3' (SEQ ID NO: 81)

oligo2FB-C-gamma3-linker:

5'-GTG GTG CTC CGG GTG GTT GCG GTT AAT AAG TTT AAA CGC-3' (SEQ ID NO: 82)

oligo2RB-C-gamma3-linker:

5'-GGC CGC GTT TAA ACT TAT TAA CCG CAA CCA CCC GGA G-3' (SEQ ID NO: 83)

rMIF-F:

5'-GGA ATT CCA TAT GCC TAT GTT CAT CGT GAA CAC-3' (SEQ ID NO: 84)

rMIF-Xho-R:

5'-CCC GCT CGA GAG CGA AGG TGG AAC CGT TC-3' (SEQ ID NO: 85)

Expression and Purification of rMIF-Cs

Competent E. coli BL21 (DE3) cells were transformed with plasmids pMod-rMIF-C1, pMod-rMIF-C2 and pMod-rMIF-C3. Single colonies from ampicillin (Amp)-containing agar plates were expanded in liquid culture (SB with 150mM MOPS, pH 7.0, 200ug/ml Amp, 0.5% glucose) and incubated at 30°C with 220 rpm shaking overnight. 1 l of SB (150 mM MOPS, pH 7.0, 200ug/ml Amp) was then inoculated 1:50 v/v with the overnight culture and grown to OD600=2.5 at 30°C. Expression was induced with 2 mM IPTG. Cells were harvested after overnight culture and centrifuged at 6000 rpm. Cell pellet was suspended in lysis buffer (10mM Na_2HPO_4, 30mM NaCl, 10mM EDTA and 0.25% Tween-20) with 0.8 mg/ml lysozyme, sonicated and treated with benzonase. 2ml of the lysate was then run through a 20 ml Q XL- and a 20 ml SP XL-column. The proteins rMIF-C1, rMIF-C2 and rMIF-C3 were in the flow through.

The protein sequences of the rMIF-Cs were translated from the cDNA sequences.

rMIF-C1(SEQ ID NO: 114; C1 is GGGGCG (SEQ ID NO: 55))

rMIF-C2 (SEQ ID NO: 115; C2 is PKPSTPPGSSGGAPGGCG (SEQ ID NO: 116))

rMIF-C3 (SEQ ID NO: 117; C3 is DKTHTSPPCG (SEQ ID NO: 118))

FIG. 5A shows a schematic description of the MIF constructs, with added amino acid linker containing a cysteine residue. MIF can be a protein from any mammal, including without limitation human MIF (SEQ ID NO: 119), rat MIF (SEQ

ID NO: 120) or mouse MIF (SEQ ID NO: 121). The sequences of human MIF containing the C-terminal amino acid linker C1, C2 or C3 are shown in SEQ ID NOs: 122-124). FIG. 5B shows an SDS-PAGE analysis of the purified MIF constructs, run under reducing conditions and stained with Coomassie-brillant blue. Loaded on the gels are the purified rat constructs rMIF-C1 (SEQ ID NO: 114);, rMIF-C2 (SEQ ID NO: 115);, and rMIF-C3 (SEQ ID NO: 117);, described in FIG. 5A.

EXAMPLE 8:

Cloning, Expression, Purification and Coupling of RANKL

A. Introduction of amino acid linkers containing a cysteine residue, expression and purification of mouse RANKL

A fragment of the receptor activator of nuclear factor kappa b ligand (RANKL), which has also been termed osteoclast differentiation factor, osteoprotegerin ligand and tumor necrosis factor-related activation-induced cytokine was recombinantly expressed with an N-terminal linker containing one cysteine for coupling to VLP.

Construction of expression plasmids

The C-terminal coding region of the RANKL gene was amplified by PCR with oligos RANKL-UP and RANKL-DOWN. RANKL-UP had an internal ApaI site and RANKL-DOWN had an internal XhoI site. The PCR product was digested with ApaI and XhoI and ligated into pGEX-6p1 (Amersham Pharmacia). The resulting plasmid was named pGEX-RANKL. All steps were performed by standard molecular biology protocols and the sequence was verified. The plasmid pGEX-RANKL codes for a fusion protein of a glutathione S-transferase-Prescission cleavage site-cysteine-containing amino acid linker-RANKL (GST-PS-C-RANKL). The cysteine-containing amino acid linker had the sequence GCGGG. The construct also contains a hexa-histidine tag between the cysteine containing amino acid linker and the RANKL sequence.

Oligos:

RANKL-UP:

5'CTGCCAGGGGCCCGGGTGCGGCGGTGGCCATCATCACCACCATCACCAG CGCTTCTCAGGAG-3' (SEQ ID NO: 86)

RANKL-DOWN :

5'-CCGCTCGAGTTAGTCTATGTCCTGAACTTTGAAAG-3' (SEQ ID NO: 87)

Protein sequence of GST-PS-C-RANKL and cDNA sequence of GST-PS-C-RANKL

Expression and Purification of C-RANKL

Competent E. coli BL21 (DE3) cells were transformed with the plasmid pGEX-RANKL. Single colonies from kanamycin and chloramphenicol-containing agar plates were expanded in liquid culture (LB medium, 30μg/ml kanamycin, 50μg/ml chloramphenicol) and incubated at 30°C with 220 rpm shaking overnight. 1 l of LB (with 30ug/ml kanamycin) was then inoculated 1:100 v/v with the overnight culture and grown to OD600=1 at 24°C. Expression was induced with 0.4 mM IPTG. Cells were harvested after 16 h and centrifuged at 5000 rpm. Cell pellet was suspended in lysis buffer (50 mM Tris-HCl, pH=8; 25 % sucrose; 1 mM EDTA, 1% NaN_3; 10 mM DTT; 5 mM $MgCl_2$; 1 mg/ml Lysozyme; 0.4u/ml DNAse) for 30 min. Then 2.5 volumes of buffer A (50 mM Tris-HCl, pH=8.0; 1% Triton X100; 100 mM NaCl; 0,1% NaN_3; 10 mM DTT;1 mM PMSF) were added and incubated at 37°C for 15 min. The cells were sonicated and pelleted at 9000 rpm for 15 min. The supernatant was immediately used for GST-affinity chromatography.

A column GST-Trap FF of 5 ml (Amersham Pharmacia) was equilibrated in PBS, pH 7.3 (140 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4). The supernatant was loaded on the 5 ml GST-Trap FF column and subsequently the column was rinsed with 5 column volumes of PBS. The protein GST-PS-C-RANKL was eluted with 50 mM Tris-HCl, pH=8.0 containing GSH 10 mM.

The purified GST-PS-C-RANKL protein was digested using the protease PreScission (Amersham Pharmacia). The digestion was performed at 37°C for 1 hour using a molar ratio of 500/1 of GST-PS-C-RANKL to PreScission.

Furthermore, the reaction of protease digestion was buffer exchanged using a HiPrep 26/10 desalting column (Amersham Pharmacia), the fractions containing the proteins were pooled and immediately used for another step of GST affinity chromatography using the same conditions reported before. Purification of C-RANKL was analysed on a SDS-PAGE gel. The gel was stained with Coomassie Brilliant Blue. The cleaved C-RANKL is present in the flow-through (unbound fraction) while the uncleaved GST-PS-C-RANKL, the cleaved GST-PS and the PreScission remain bound to the column. C-RANKL protein of the expected size of 22 kDa was obtained in high purity.

The samples loaded on a gel were the following:

Lane 1: Low molecular weight marker. Lanes 2 and 3: the supernatant of the cell lysates of the BL21/DE3 cells transformed with the empty vector pGEX6p1 and pGEX-RANKL respectively, after sixteen hours of induction with IPTG 0.4 mM. Lane 4: the purified GST-PS-C-RANKL protein after GST-Trap FF column. Lane 5: the GST-Trap FF column unbound fraction. Lane 6: the purified GST-PS-C-RANKL protein after the cleavage with the PreScission protease. Lane 7: the unbound fraction of the GST-Trap FF column loaded with the GST-RANKL digestion, which contains the purified C-RANKL. Lane 8: the bound fraction of the GST-Trap FF column loaded with the GST-PS-C-RANKL digestion and eluted with GSH.

B. Coupling of C-RANKL to AP205 VLP

A solution of 120 μM AP205 VLP in 20 mM Hepes, 150 mM NaCl pH 7.2 is reacted for 30 minutes with a 25 fold molar excess of SMPH (Pierce), diluted from a stock solution in DMSO, at 25 °C on a rocking shaker. The reaction solution is subsequently dialyzed twice for 2 hours against 1 L of 20 mM Hepes, 150 mM NaCl, pH 7.2 at 4 °C. The dialyzed AP205 VLP reaction mixture is then reacted with the C-RANKL solution (end concentrations: 60 μM AP205 VLP, 60 μM C-RANKL) for four hours at 25 °C on a rocking shaker. Coupling products are analysed by SDS-PAGE and Western blot under reducing conditions.

EXAMPLE 9

Cloning, expression and purification of IL-5 with an N-terminal amino acid linker containing a cysteine residue. Coupling to VLP and elicitation of an immune response in mice.

A. Cloning of mouse His-C-IL-5 and expression as Inclusion bodies in *E. coli*

IL-5 was amplified from an ATCC clone (pmIL5-4G; ATCC number: 37562) by PCR using the following two primers: Spelinker3-F1 (SEQ ID NO: 90) and Il5StopXho-R (SEQ ID NO: 91). The product of this PCR was used as template for a second PCR with the primers SpeNlinker3-F2 (SEQ ID NO: 92) and Il5StopXho-R. The insert was digested with SpeI and NotI. This insert was ligated into a pET vector derivative (pMODEC3-8 vector), previously digested with *Nhe* I and *Not* I, and transformed into *E.coli* TG1 cells. The construct generated by cloning IL5 into pMODEC3-8 comprises, from its N-terminus, a hexa-histidine tag (to facilitate purification), an Enterokinase cleavage site, a gamma 3 derived amino acid linker (flanked N-terminally by the amino acids ALV and C-terminally by AS) containing a cysteine residue and the DNA encoding the mature form of IL-5 protein. Fidelity of the cloning procedure was confirmed by DNA sequencing. The protein released by cleavage with enterokinase is called "mouse C-IL-5-E".

The construct containing IL-5 described above was termed pMODC6-*IL5.2* (also referred to as pMODC6-*IL5*) and transformed into *E.coli* strain BL21-DE3. The recombinant protein expressed in *E. coli* is termed His-C-IL5.

Clonal BL21-DE3 cells harboring pMODC6-IL5 were grown over night in 5 ml of LB containing 1 mg/L Ampicillin. A 2.0 ml aliquot of this culture was diluted into 100 ml terrific broth (TB) containing 1mg/L Ampicillin. The culture was grown to an optical density, OD_{600nm}, of 0.7-1.0 and expression induced for 4 hours by adding 0.1 ml of a 1.0 M stock of Ispropyl β-D-Thiogalactopyranoside (IPTG) Samples were taken every 2 hours. Recombinant His-C-IL5 was expressed in an insoluble form and located in the inclusion body fraction of induced cells. Expression of His-C-IL5 was confirmed in the following manner. A 10 ml sample of culture was taken 4 hours after induction and

centrifuged for 10 min at 4000 x g. The pellet was suspended in 0.5 ml lysis buffer consisting of 50 mM Tris-HCl, 2 mM EDTA, 0.1 % triton X-100 (pH 8.0). To the suspension was added 20 µl of Lysozyme (40 mg/ml) and after 30 min at 4°C sonicated for 2 min. A 1.0 ml aliquot of benzonase and 100 µl aliquot of 50 mM $MgCl_2$ were added and incubated for 30 min at room temperature. After centrifugation for 15 min at 13000 x g the supernatant was discarded and the pellet heated for 5 min at 98°C in 100 µl of SDS loading buffer. Aliquots of 10 µl were then analyzed by SDS-PAGE under reducing conditions. SDS-PAGE analysis demonstrated a protein band of 17 kDa corresponding to the mass of IL-5. As control, BL21-DE2 cells containing pMODC6-IL5 were grown in the absence of IPTG and extracts prepared from the insoluble cell fraction as described above.

B. Purification and refolding of mouse-His-C-IL5.

A larger scale expression of IL-5 from clone pMODC6-IL5 in BL21-DE3 cells was performed in order to obtain sufficient quantities of pure IL-5 for vaccine production. Overnight cultures were grown and diluted into either 100 ml or 1L volumes of TB medium containing 1.0 mg/L Ampicillin. A total of 3 liters of culture was thus prepared and grown at 37°C until OD_{600nm} reached 0.7 at which time IPTG was added to give a final concentration of 1.0 mM. After 4 h incubation cells were harvested by centrifugation for 30 min at 10 000 x g. After harvesting the pellet was resuspended in PBS (5.0 ml/g wet weight) and centrifuged for 15 minutes at 10 000 x g. The washed pellet was stored at –20°C until further use.

The bacterial pellet was suspended in PBS (2.0 ml/ g cell wet weight) using a Dounce homogenizer. Lysozyme (0.8mg/ml) was added to the suspension and incubated for 30 minutes at room temperature. The suspension was sonicated for 1 minute, 3 times on ice then benzonase and $MgCl_2$ (10 mM final concentration) were added and incubated for 30 minutes at room temperature. Triton X-100 was added to a final concentration of 1% (w/v) the mixture gently stirred at room temperature for 30 minutes. The solution was centrifuged for 20 minutes at 20 000 x g (SS34 tubes) and the supernatant discarded. The pellet harbouring the inclusion bodies was suspended (5.0 ml/ g wet weight) in washing buffer (PBS containing 2M Urea and 1% (w/v)

Triton X-100) using a Dounce homogenizer and agitated for 5 minutes. The solution was centrifuged for 20 minutes at 20 000 x g and the supernatant discarded. The pellet was washed and centrifuged as above 2 more times. A final wash of the inclusion bodies was performed with washing buffer in the absence of Triton X-100.

The His-C-IL-5 present in inclusion bodies of the pellet was solubilized in (5.0ml/g cell wet weight) denaturing buffer (100 mM NaH_2PO_4, 10 mM Tris-HCl, 6.0 M Guanidine-hydrochloride, pH 8.0) and gently stirred for 1h at 25°C. The suspension was centrifuged for 20 min. at 20 000 x g and the supernatant mixed with Ni-NTA resin (QIAgen, equilibrated with solubilization buffer). After 3 hours of gentle agitation at 4°C the slurry was poured into a glass column (C10/10) and the resin washed with 100 ml of 100 mM NaH_2PO_4, 10 mM Tris, 6.0 M Guanidine-hydrochloride (pH 6.3). An additional washing step was performed with 15 ml of 100 mM NaH_2PO_4, 10 mM Tris, 6.0 M Guanidine-hydrochloride (pH 5.9). Mouse His-C-IL5 was eluted from the resin by applying 20 ml of 100 mM NaH_2PO_4, 10 mM Tris, 6.0 M Guanidine-hydrochloride (pH 4.5). Purification was anylysed by SDS-PAGE.

Fractions from the elution step containing His-C-IL-5 were pooled and dialysed against buffer comprising 8.0 M Urea 100mM NaH_2PO_4, 10mM Tris-HCl (pH 8.0) at 4°C using a 10 kDa cut-off membrane. Following dialysis, the protein concentration was determined spectrophotometrically using the following formula; Protein (mg/ml) = (1.55 x A_{280nm}) – (0.76 x A_{260nm}). The concentration of the protein was diluted with dialysis buffer to 0.2 mg/ml. The solution was then dialysed with a 3.5kDa membrane for 24 hours at 4°C against refolding buffer 1 comprising 2.0 M urea, 50mM NaH_2PO_4, 5 mM reduced Glutathione, 0.5 mM oxidized Glutathione, 0.5 M Arginine, 10% (v/v) glycerol (pH 8.5) and for a further 24h against another refolding buffer 2 comprising 50mM NaH_2PO_4, 5 mM reduced Glutathione, 0.5 mM oxidized Glutathione, 0.5 M Arginine, 10% (v/v) glycerol, (pH 8.5). At the end the protein was dialysed for 24h at 4°C against PBS pH 8.0 then centrifuged at 10 000 x g for 30 min. The protein content of the supernatant was estimated by Bradford assay.

In order to further purifiy His-C-IL5, anion exchange with Hitrap Q resin (Amersham Pharmacia, Uppsala Sweeden) was performed. His-C-IL5 was concentrated to 1mg/ml using Centrifugal Filters (Ultrafree-15 Millipore, 10 kDa cut-off) and dialyzed for 14h against 50 mM Phosphate buffer pH 8.4. The solution was

loaded onto a Hitrap Q column and washed with 50mM Phosphate pH 8.4 buffer. His-C-IL-5 was eluted from the column by applying a NaCl gradient from 0 – 1 M. His-C-IL5 eluted from the column at 100 mM NaCl. Analysis of the purification was performed by SDS-PAGE and concentration measured by Bradford assay. Quartenary structure of the protein was assessed by SDS-PAGE performed under non-reducing conditions, which revealed that the His-C-IL5 is present as a dimer in the preparation.

C. Vaccine production: Coupling His-C-IL5 to AP205 VLP

A variety of conditions may be tested to optimize the efficiency of the coupling reaction. These include the addition of reducing agent, (TCEP) to His-C-IL5 and varying the molar ratios of AP205 VLP subunit monomer and His-C-IL5 in the coupling reaction. AP205-His-C-IL-5 vaccine is produced as follows. Purified His-C-IL-5 (40μM) is reduced for 1h with an equimolar amount of TCEP in PBS pH 8.0. Reduced IL-5 (20 μM) is incubated for 4 hours at 22°C with 10 μM Qβ derivatized with SMPH in a total volume of 700 μl. The reaction is dialysed 12 hours against PBS pH 8.0 using a 300 kDa cutt-off dialysis membrane. The coupling reaction is analysed by SDS-PAGE and Western-Blot with anti-His and anti-AP205 antibodies (polyclonal rabbit antiserum). Protein concentration is measured by Bradford. The coupling efficiency [i.e. mol Qβ-IL5/ mol Qβ monomer (total)] is measured by densitometric analysis of the corresponding bands on the Coomassie blue stained SDS-PAGE .

D. Assay of IL-5 activity

The ability of the B cell lymphoma line BCL1 to proliferate in response to murine IL-5 was used to check the bioactivity of the re-folded recombinant His-C-IL-5 (Harriman G.R. (1991) Current Protocols in Immunology 6.5.1-6.5.5 John Wiley and Sons Inc). The proliferative activity of His-C-IL5 covalently coupled to AP205 VLP may also be assessed. Recombinant murine IL-5 (R&D systems, Minneapolis USA) was used as a control. The various forms of recombinat IL-5 were incubated in flat bottom 96 well plates with 2 x 10^4 BCL1 cells per well and incubated for 24h at 37°C, 5% CO_2.1 μCi of ^{3}H-Thymidine (Hartmann Analytic, Switzerland) was added to each well and the plates incubated for another 6h at 37°C 5% CO_2. The cells were harvested,

washed and the incorporation of Thymidine determined by counting the β–emission with a liquid scintillation counter. The assay demonstrated that His-C-IL5 is active.

E. Immunization protocol

In order to generate self reactive antibodies to mouse IL-5, four BalbC mice are injected subcutaneously a day 0 and day 14 with 25 µg of AP205-His-C-IL5 vaccine in 200 µl of PBS. To serve as a negative control, five mice are immunized at day 0 and 14 with a simple mixture of 6.4 µg AP205 VLP and 16 µg IL5 i.e. not covalently coupled (AP205 + His-C-IL-5) in PBS. Mice are bled prior to imunisation and at day 21 of the immunisation protocol. Sera are analysed by ELISA.

F. Sera analysis

ELISA. Maxisorp ELISA plates (Nunc) are coated with 50µl of purified His-C- IL-5 (3 µg/ml) for 14h at 4°C. The plates are washed 3 times with PBS and blocked with 2% BSA in PBS for 2h at 37°C then washed twice with PBS. Five-fold dilutions of sera are added in 2% BSA, 0.1% FCS in PBS and incubated at room temperature for 1 hour. The plates are subsequently washed 3 times with PBS and incubated with anti-mouse IgG conjugated with HRP (dilution 1:1000) at room temperature for 1h. The plates are again washed 3 times with PBS and 100 µl/well developing solution (0.066 M Na2HPO4, 0.035 M citric acid, 0.032% H_2O_2, 0.4% 1,2-Phenylenediamine dihydrochloride) is added. After 5 minutes of reaction at room temperature the ELISA is stopped with 50 µl per well 5% H_2SO_4. Absorbance is measured at 450 nm on a Spectramax spectrophotometer (Molecular Devices).

EXAMPLE 10:

Cloning, Expression and Coupling of Mouse Prion Protein

A. Introduction of amino acid linker containing a cysteine residue, expression and purification of a truncated form of the mouse prion protein

A truncated form (aa 121-230) of the mouse prion protein (termed $mPrP_t$) was recombinantly expressed with a GGGGCG amino acid linker (SEQ ID NO: 55) fused at its C-terminus for coupling to AP205 VLP. The protein was fused to the N-terminus

of a human Fc-fragment for purification. An enterokinase (EK) cleavage-site was introduced behind the EK cleavage site to cleave the Fc- part of the fusion protein after purification.

Construction of $mPrP_t$-EK-Fc*.

Mouse PrP_t was amplified by PCR with the primer 5'PrP-*BamHI* and 3'PrP-*NheI* using the plasmid pBP^{CMV}PrP-Fc as a template. pBP^{CMV}PrP-Fc contained the wild-type sequence of the mouse prion protein. 5'PrP-*BamHI* had an internal *BamHI* site and contained an ATG and 3'PrP-*NheI* had an internal *NheI* site.

For the PCR reaction, 0.5 µg of each primer and 200 ng of the template DNA was used in the 50 µl reaction mixture (1 unit of PFX Platinum polymerase, 0.3 mM dNTPs and 2 mM $MgSO_4$). The temperature cycles were as follows: 94°C for 2 minutes, followed by 5 cycles of 94°C (15 seconds), 50°C (30 seconds), 68°C (45 seconds), followed by 20 cycles of 94°C (15 seconds), 64°C (30 seconds), 68°C (45 seconds) and followed by 68°C for 10 minutes.

The PCR product was digested with *BamHI* and *NheI* and inserted into pCEP-SP-EK-Fc* containing the GGGGCG linker sequence (SEQ ID NO: 55) at the 5'end of the EK cleavage sequence. The resulting plasmid was named pCEP-SP-$mPrP_t$-EK-Fc*.

All other steps were performed by standard molecular biology protocols.

Oligos:

Primer 5'PrP-*BamHI*

5'-CGG GAT CCC ACC ATG GTG GGG GGC CTT GG -3' (SEQ ID NO: 88)

Primer 3'PrP-*NheI*

5'-CTA GCT AGC CTG GAT CTT CTC CCG -3' (SEQ ID NO: 89)

Expression and Purification of $mPrP_t$-EK-Fc*

Plasmid pCEP-SP-$mPrP_t$-EK-Fc* was transfected into 293-EBNA cells (Invitrogen) and purified on a Protein A-sepharose column as described in EXAMPLE 4. $mPrP_t$ after cleavage has the sequence as identified in SEQ ID NO:324 with the GGGGCG linker at its C-terminus. The purified fusion protein $mPrP_t$-EK-Fc* was cleaved with

enterokinase and analysed on a 16% SDS-PAGE gel under reducing conditions before and after enterokinase cleavage. The gel was stained with Coomassie Brilliant Blue. The $mPrP_t$-EK-Fc* fusion protein could be detected as a 50 kDa band. The cleaved $mPrP_t$ protein containing the GGGGCG amino acid linker (SEQ ID NO: 55) fused to its C-terminus could be detected as a broad band between 18 and 25 kDa. The identity of $mPrP_t$ was confirmed by western blotting (data not shown). Thus, mPrPt with a C-terminal amino acid linker containing a cysteine residue, could be expressed and purified to be used for coupling to AP205 VLP.

The samples loaded on the gel were the following.

Lane 1: Molecular weight marker. Lane 2: $mPrP_t$-EK-Fc* before cleavage. Lane 3: $mPrP_t$ after cleavage.

B. Coupling of $mPrP_t$ to AP205 VLP

A solution of 120 µM AP205 VLP in 20 mM Hepes, 150 mM NaCl pH 7.2 is reacted for 30 minutes with a 25 fold molar excess of SMPH (Pierce), diluted from a stock solution in DMSO, at 25 °C on a rocking shaker. The reaction solution is subsequently dialyzed twice for 2 hours against 1 L of 20 mM Hepes, 150 mM NaCl, pH 7.2 at 4 °C. The dialyzed AP205 VLP reaction mixture is then reacted with the $mPrP_t$ solution (end concentrations: 60 µM AP205 VLP, 60 µM $mPrP_t$) for four hours at 25 °C on a rocking shaker. Coupling products are analysed by SDS-PAGE and Western blot under reducing conditions.

EXAMPLE 11:

Coupling of rMIF to AP205 VLP

RMIF-C1 (SEQ ID NO: 114), expressed and purified as described in Example 7, in 20 mM Hepes, 150 mM NaCl pH 7.2, 0.18 mM was incubated with one molar equivalent of TCEP for 1 hour at R.T. before use in the coupling reaction. One ml of a AP205 VLP solution, 2.5 mg/ml, was reacted with a 2.3-fold molar excess of SMPH for 1 hour at R.T. The derivatized AP205 VLP was dialyzed two times 2 hours against 2 l of 20 mM Hepes, 150 mM NaCl, pH 7.2. 820 µl of the dialyzed derivatized AP205 VLP (0.18 mM) were subsequently reacted with 820 µl of a 0.18 mM rMIF-C1

solution previously treated with TCEP as described above, for 3 hours at R.T. No precipitate was observable at the end of the coupling reaction, and the samples were analyzed by SDS-PAGE under reducing conditions, and the gel stained with Coomassie Brillant Blue. The result of the coupling reaction is shown in FIG. 6. MIF has a molecular weight of , while the AP205 VLP subunit has a molecular weight of 14 kDa while rMIF-C1 has a molecular weight of 13. The coupling product is migrating as expected with an apparent molecular weight of 27, as described in Fig. 6.

Shown in FIG. 6 is the result of the coupling reaction of rMIF-C1 to AP205 VLP. Lane 1: Molecular Marker. Lane 2: AP205 VLP. Lane 3: derivatized AP205 VLP. Lane 4: dialyzed, derivatized AP205 VLP. Lane 5: dialyzed, derivatized AP205 VLP. Lane 6: Coupling reaction of rMIF-C1 to AP205 VLP. The coupling product is indicated by an arrow in the figure. The molecular weights of the marker proteins are indicated on the left border of the gel.

EXAMPLE 12:

Immunization of mice and rats with rMIF coupled to AP205 VLP

A. Immunization of mice with AP205 VLP coupled to rMIF-C1

AP205 VLP coupled to rMIF-C1 (from Example 11) was injected subcutaneously in female Balb/c mice (3 mice) at day 0 and 14. Each mouse was immunized with 10 µg of vaccine diluted in PBS to 200 µl. Mice were retroorbitally bled on day 21, and the titer of the antibodies specific for rMIF-C1 were measured in an ELISA specific for rMIF-C1, as follows.

ELISA plates were coated with rMIF-C1 at a concentration of 5 µg/ml. The plates were blocked and then incubated with serially diluted mouse sera. Bound antibodies were detected with enzymatically labeled anti-mouse IgG antibody. As a control, a preimmune serum of the same mice was also tested.

Immunization with rMIF-C1 coupled to AP205 VLP led to a strong specific immune response against rMIF-C1 (Fig. 7), whereby the average titer of the three mice against rMIF-C1, defined as the dilution of the serum giving half-maximal OD was of 1:31 000. rMIF-C1 was therefore properly displayed on the AP205 VLP and accessible to the immune system for generation of a strong immune response.

Shown on Fig. 7 is the analysis by ELISA of the IgG response specific for rMIF-C1 in the sera of mice immunized with rMIF-C1 coupled to AP205 VLP. Analysis of the day 21-sera of the three mice (M1, M2 and M3) was done in duplicate. Three-fold dilutions of the sera were applied to the well. "pre imm" stands for the pre immune serum of one mouse subsequently immunized with rMIF-C1 coupled to AP205 VLP.

B. Immunization of rats with AP205 VLP coupled to rMIF-C1

AP205 VLP coupled to rMIF-C1 (from Example 12) is injected s.c. in rats (3 rats each) at day 0 and 28 in the absence of adjuvants. Each rat is immunized with 50 µg of vaccine diluted in PBS to 200 µl. Rats are bled on day 42, and the titer of the antibodies specific for rMIF-C1 are measured in an ELISA specific for rMIF-C1, as described above, using however an enzymatically labeled anti-Rat IgG secondary antibody.

EXAMPLE 13:

Coupling of the Angio I peptide to AP205 VLP and Immunization of mice

A. Coupling of Angio I peptide to AP205 VLP

The Angio I peptide, having the sequence of Angiotensin II (DRVYIHPF) (SEQ ID NO: 13) fused at its N-terminus to the linker sequence CGG containing a cysteine residue for coupling to the activated VLP, was chemically synthesized. AP205 VLP, expressed and purified as described in example 2, was resolubilized in 20 mM Hepes, 150 mM NaCl, pH 7.4 buffer (HBS buffer). Resolubilized AP205 VLP was then reacted at a concentration of 2 mg/ml (determined in a Bradford assay), with 1.43 mM SMPH (Pierce) for 30 minutes at room temperature (RT). The reaction mixture was then dialyzed twice against HBS buffer for 2 hours at 4°C, and reacted with 1.144 mM Angio I peptide (sequence: CGGDRVYIHPF (SEQ ID NO: 12), free amine and free acid), diluted in the reaction mixture from a 50 mM stock in DMSO. The coupling reaction was left to proceed for 2 hours at 15°C, and the reaction mixture dialyzed 2 X 2 hours against a 1000-fold volume HBS, and flash frozen in liquid nitrogen in aliquots for storage at -80°C until further use.

An aliquot was thawed, and coupling of the antigen to an AP205 subunit assessed by SDS-PAGE and the protein concentration measured in a Bradford assay. Coupling efficiency of the peptide to AP205 VLP, as defined by the sum of the intensities of the bands corresponding to 1, 2 or 3 peptides coupled per monomer subunit, divided by the sum of the intensities of coupled and uncoupled AP205 monomer subunits, was of 88%. Epitope density was measured similarly as coupling efficiency, with the modification that the intensity of the coupling bands are multiplied by the number of coupled peptide per subunit in the respective band, in the numerator. Epitope density was of 1.6 Angio I peptides per AP205 VLP subunit.

B. Immunization of mice with AP205 VLP coupled to Angio I peptide

AP205 VLP coupled to Angio I peptide produced as described in part A was injected s.c. in three mice at day 0 and 14 in the absence of adjuvants. Each mouse was immunized with 25 μg of vaccine diluted in PBS to 200 μl. Mice were retro-orbbitally bled on day 21, and the titer of the antibodies specific for Angio I peptide were measured by ELISA. The Angio I peptide was coupled to bovine RNAse A using the chemical cross-linker sulfo-SPDP. ELISA plates were coated with Angio I-coupled RNAse at a concentration of 10 μg/ml. The plates were blocked and then incubated with serially diluted mouse sera. Bound antibodies were detected with enzymatically labeled anti-mouse IgG antibodies. As a control preimmune sera of the same mice were also tested. The results are shown in FIG. 8.

FIG. 8 shows an ELISA analysis of the IgG antibodies specific for Angio I peptide in the sera of the three mice (1-3) immunized on day 0 and 14 against the Angio I peptide coupled to AP205 VLP. Total IgG titers were determined in the day 21 sera. No antibodies specific for Angio I could be detected in the preimmune serum analysed. A very high specific titer against Angio I of 1:69 000 in average (given as the dilution giving half maximal OD at 450 nm) was obtained, demonstrating that self-tolerance against Angio I (a self peptide in the mouse) had been broken. The data demonstrate that vaccines with very high display of peptides are obtained upon coupling antigens to AP205 VLP. The data also demonstrate that very high titers against self-antigens are obtained upon coupling of these self-antigens to AP205 VLP and subsequent immunization with the resulting vaccine in the absence of adjuvant.

Having now fully described the present invention in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious to one of ordinary skill in the art that the same can be performed by modifying or changing the invention within a wide and equivalent range of conditions, formulations and other parameters without affecting the scope of the invention or any specific embodiment thereof, and that such modifications or changes are intended to be encompassed within the scope of the appended claims.

All publications, patents and patent applications mentioned in this specification are indicative of the level of skill of those skilled in the art to which this invention pertains, and are herein incorporated by reference to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference.

CLAIMS:

1. A virus-like particle comprising at least one protein selected from the group consisting of:

   (a) a protein having an amino acid sequence as set forth in SEQ ID NO:1;

   (b) a protein having an amino acid sequence as set forth in SEQ ID NO:3; and

   (c) a mutein of said protein of (a) or (b).

2. The virus-like particle of claim 1, wherein said protein is recombinant.

3. The virus-like particle of claim 1 or 2, wherein said mutein has an amino acid sequence as set forth in SEQ ID NO:1 or as set forth in SEQ ID NO:3, wherein at least one amino acid residue is added, deleted or substituted.

4. The virus-like particle of claim 1 or 2, wherein said mutein has an amino acid sequence as set forth in SEQ ID NO:1 or as set forth in SEQ ID NO:3, wherein three amino acid residues are added, deleted or substituted.

5. The virus-like particle of claim 1 or 2, wherein said mutein has an amino acid sequence as set forth in SEQ ID NO:1 or as set forth in SEQ ID NO:3, wherein two amino acid residues are added, deleted or substituted.

6. The virus-like particle of claim 1 or 2, wherein said mutein has an amino acid sequence as set forth in SEQ ID NO:1 or as set forth in SEQ ID NO:3, wherein one amino acid residue is added, deleted or substituted.

7. The virus-like particle of any one of the claims 3-6, wherein said at least one substitution is a conservative substitution.

8. The virus-like particle of any of claims 1-7, wherein said mutein has an amino acid sequence as set forth in SEQ ID NO:1 or as set forth in SEQ ID NO:3, wherein at least one cysteine residue is deleted or substituted.

9. The virus-like particle of any of claims 1-7, wherein said mutein has an amino acid sequence as set forth in SEQ ID NO:1 or as set forth in SEQ ID NO:3, wherein two cysteine residues are deleted or substituted.

10. The virus-like particle of any one of the claims 8-9, wherein said at least one substitution is a conservative substitution.

11. The virus-like particle of any of claims 1-10, wherein said mutein has an amino acid sequence as set forth in SEQ ID NO:1 or as set forth in SEQ ID NO:3, wherein at least one lysine residue is added, deleted or substituted.

12. The virus-like particle of any of claims 1-10, wherein said mutein has an amino acid sequence as set forth in SEQ ID NO:1 or as set forth in SEQ ID NO:3, wherein three lysine residues are added, deleted or substituted.

13. The virus-like particle of any of claims 1-10, wherein said mutein has an amino acid sequence as set forth in SEQ ID NO:1 or as set forth in SEQ ID NO:3, wherein two lysine residues are added, deleted or substituted.

14. The virus-like particle of any of claims 1-10, wherein said mutein has an amino acid sequence as set forth in SEQ ID NO:1 or as set forth in SEQ ID NO:3, wherein one lysine is added, deleted or substituted.

15. The virus-like particle of any one of the claims 11-14, wherein said at least one substitution is a conservative substitution.

16. A virus-like particle comprising at least one protein selected from the group consisting of:

(a) a protein comprising amino acids 2-131 of SEQ ID NO:1;

(b) a protein comprising amino acids 2-131 of SEQ ID NO:3; and

(c) a mutein of SEQ ID NO:1, wherein said mutein consists of an addition, deletion or substitution of one to three amino acids from amino acids 1- 131 of SEQ ID NO: 1.

17. The virus-like particle of claim 16, wherein said protein is recombinant.

18. The virus-like particle of claim 16 comprising at least one protein omprising amino acids 2-131 of SEQ ID NO:3.

19. The virus-like particle of claim 16 comprising at least one mutein of SEQ ID NO:1, wherein said mutein consists of an addition, deletion or substitution of one amino acid from amino acids 2-131 of SEQ ID NO: 1.

20. A vector for producing an AP205 virus-like particle, wherein said vector comprises a nucleotide sequence encoding a protein comprising amino acids 2-131 of SEQ ID NO:3.

21. A vector for producing an AP205 virus-like particle, wherein said vector comprises a nucleotide sequence encoding a mutein of SEQ ID NO:1, wherein said mutein consists of an addition, deletion or substitution of one to three amino acids from amino acids 2- 131 of SEQ ID NO: 1.

22. The vector of claim 21, wherein said mutein consists of an addition, deletion or substitution of one amino acid from amino acids 2-131 of SEQ ID NO:1.

23. A host cell comprising the vector of claim 20 or 21.

24. The host cell of claim 23, wherein said host cell is E.coli.

25. A method of producing a virus-like particle comprising:

(a) providing a vector according to claim 20 or claim 21;

(b) introducing said vector into a host cell; and

(c) expressing the sequence of said vector in said host cell to obtain a protein or a mutein capable of forming a virus-like particle.

26. The method of claim 25, wherein said host cell is E.coli.

27. A mutein having an amino acid sequence as set forth in SEQ ID NO:3

28. A mutein of the recombinant protein of SEQ ID NO: 1.

29. The mutein of claim 28, wherein at least one amino acid residue is added, deleted or substituted.

30. The mutein of claim 28, wherein three amino acid residues are added, deleted or substituted.

31. The mutein of claim 28, wherein two amino acid residues are added, deleted or substituted.

32. The mutein of claim 28, wherein one amino acid residue is added, deleted or substituted.

33. The mutein of any one of the claims 29-32, wherein said at least one substitution is a conservative substitution.

34. The mutein of any one of the claims 28-33, wherein at least one cysteine residue is deleted or substituted.

35. The mutein of any one of the claims 28-33, wherein two cysteine residues are deleted or substituted.

36. The mutein of claim 34 or 35, wherein said at least one substitution is a conservative substitution.

37. The mutein of any of claims 28-36, wherein at least one lysine residue is added, deleted or substituted.

38. The mutein of any of claims 28-36, wherein three lysine residues are added, deleted or substituted.

39. The mutein of any of claims 28-36, wherein two lysine residues are added, deleted or substituted.

40. The mutein of any of claims 28-36, wherein one lysine is added, deleted or substituted.

41. The mutein of any of claims 37-40, wherein said at least one substitution is a conservative substitution.

42. A vector for producing a AP205 virus like particle comprising a nucleotide sequence being at least 80% identical to that of SEQ ID NO:2 or SEQ ID NO: 4.

43. A vector for producing a AP205 virus like particle comprising a nucleotide sequence being at least at least 90% identical to that of SEQ ID NO:2 or SEQ ID NO: 4.

44. A vector for producing a AP205 virus like particle comprising a nucleotide

sequence being at least 95% identical to that of SEQ ID NO:2 or SEQ ID NO: 4.

45. A vector for producing a AP205 virus like particle comprising a nucleotide sequence being at least 99% identical to that of SEQ ID NO:2 or SEQ ID NO: 4.

46. A vector for the production of a recombinant protein comprising a nucleotide sequence encoding a polypeptide fused to a protein, wherein said protein is selected from the group consisting of:

(a) a protein having an amino acid sequence as set forth in SEQ ID NO:1;

(b) a protein having an amino acid sequence as set forth in SEQ ID NO:3; and

(c) a mutein of said polypetide of (a) or (b).

47. A composition comprising:

(a) a core particle selected from the group consisting of

(i) an AP205 virus particle; and

(ii) AP205 virus-like particles; and

(b) an organic molecule

wherein the organic molecule is bound to the core particle.

48. A composition comprising:

(a) a core particle selected from the group consisting of

(i) an AP205 virus-like particle comprising at least one protein comprising amino acids 2-131 of SEQ ID NO:1;

(ii) an AP205 virus-like particle comprising at least one protein comprising amino acids 2-131 of SEQ ID NO:3; and

(iii) a mutein of SEQ ID NO:1, wherein said mutein consists of an

addition, deletion or substitution of one to three amino acids from amino acids 1-131 of SEQ ID NO:1; and

(b) an organic molecule, wherein said organic molecule is bound to said core particle.

49. The composition of claim 47 or 48, wherein said organic molecule and core particle forms an ordered and repetitive array of the organic molecule on the surface of the core particle.

50. The composition of any one of the claims 47-49, wherein the organic molecule is bound to the core particle via a third molecule, said third molecule linking the core particle to the organic molecule.

51. The composition of any one of the claims 47-50, wherein said organic molecule is bound to the core particle by at least one covalent bond.

52. The composition of claim 51, wherein said covalent bond comprises a peptide bond.

53. The composition of claim 51, wherein said covalent bond comprises a non-peptide bond.

54. The composition of any one of claims 47-53, wherein the organic molecule comprises a hapten, an antigen or an antigenic determinant.

55. The composition of any of claims 47-53, wherein the organic molecule is a hapten, an antigen or an antigenic determinant.

56. The composition of any of claims 47-53, wherein said organic molecule is an antigen or an antigenic determinant.

57. The composition of any of claims 47-56, wherein the virus like particle contains at least a first attachment site, and the organic molecule contains at least a second attachment site, such that said second attachment site is capable of association with said first attachment site to form an ordered and repetitive antigen array.

58. The composition of claim 57, wherein said second attachment site is capable of association with said first attachment site to form an ordered and repetitive antigen array via at least one non-peptide bond.

59. The composition of any of claims 47-58, wherein said first attachment site comprises an amino group, and wherein said second attachment site comprises, a sulfhydryl group.

60. The composition of any of claims 47-58, wherein said first attachment site is an amino group.

61. The composition of claim 59 or 60, wherein said first attachment site comprises a lysine residue.

62. The composition of claim 59 or 60, wherein said first attachment site is a lysine residue.

63. The composition of any of claims 47-58, wherein said second attachment site is a sulfhydryl group.

64. The composition of any of claim 59 or 63, wherein said second attachment site comprises a cysteine residue.

65. The composition of any of claim 59 or 63, wherein said second attachment site is a cysteine residue.

66. The composition of any of claims 47-65, wherein said second attachment site does not naturally occur within said organic molecule.

67. The composition of any of claims 47-66, wherein said composition comprises an amino acid linker.

68. The composition of claim 67, wherein said amino acid linker is bound to said antigen or said antigenic determinant by way of at least one covalent bond.

69. The composition of claim 67, wherein said amino acid linker is bound to said antigen or said antigenic determinant by way of at least one peptide bond.

70. The composition of any of claims 47-69, wherein said amino acid linker comprises said second attachment site.

71. The composition of any of claims 47-70, wherein said amino acid linker is selected from the group consisting of:

(a) CGG;

(b) N-terminal gamma 1-linker;

(c) N-terminal gamma 3-linker;

(d) Ig hinge regions;

(e) N-terminal glycine linkers;

(f) $(G)_kC(G)_n$ with n=0-12 and k=0-5 (SEQ ID NO: 93);

(g) N-terminal glycine-serine linkers;

(h) $(G)_kC(G)_m(S)_l(GGGGS)_n$ with n=0-3, k=0-5, m=0-10, l=0-2 (SEQ ID NO: 94);

(i) GGC;

(k) GGC-NH2;

(l) C-terminal gamma 1-linker;

(m) C-terminal gamma 3-linker;

(n) C-terminal glycine linkers;

(o) $(G)_nC(G)_k$ with n=0-12 and k=0-5 (SEQ ID NO: 95);

(p) C-terminal glycine-serine linkers; and

(q) $(G)_m(S)_l(GGGGS)_n(G)_oC(G)_k$ with n=0-3, k=0-5, m=0-10, l=0-2, and o=0-8 (SEQ ID NO: 96).

72. The composition of any of claims 47-71, wherein said organic molecule is selected from the group consisting of:

(a) an organic molecule suited to induce an immune response against cancer cells;

(b) an organic molecule suited to induce an immune response against infectious diseases;

(c) an organic molecule suited to induce an immune response against allergens;

(d) an organic molecule suited to induce an improved response against self-antigens;

(e) an organic molecule suited to induce an immune response in farm animals or pets;

(f) an organic molecule suited to induce a response against a drug, a hormone or a toxic compound; and

(g) fragments, muteins or domains of the molecules set out in (a)-(f).

73. The composition of any of claims 47-72, wherein the organic molecule is an antigen or an antigenic determinant, or a fragment or mutein thereof, being selected from the group consisting of

(a) an antigen or an antigenic determinant suited to induce an immune response against cancer cells;

(b) an antigen or an antigenic determinant suited to induce an immune response against infectious diseases;

(c) an antigen or an antigenic determinant suited to induce an immune response against allergens;

(d) an antigen or an antigenic determinant suited to induce an improved response against self-antigens;

(e) an antigen or an antigenic determinant suited to induce an immune response in farm animals or pets;

(f) an antigen or an antigenic determinant suited to induce a response against a drug, a hormone or a toxic compound; and

(h) fragments or domains of the molecules set out in (a)-(f).

74. The composition of any of claims 47-73, wherein said organic molecule is an antigen selected from the group of :

(a) a polypeptide of HIV,

(b) a polypeptide of Influenza virus,

(c) a polypeptide of Hepatitis C virus,

(d) a polypeptide of *Toxoplasma*,

(e) a polypeptide of *Plasmodium falciparum,*

(f) a polypeptide of *Plasmodium vivax*,

(g) a polypeptide of *Plasmodium ovale*,

(h) a polypeptide of *Plasmodium malariae*,

(i) a polypeptide of breast cancer cells,

(j) a polypeptide of kidney cancer cells,

(k) a polypeptide of prostate cancer cells,

(l) a polypeptide of skin cancer cells,

(m) a polypeptide of brain cancer cells,

(n) a polypeptide of leukemia cells,

(o) a recombinant profiling,

(p) a polypeptide of bee sting allergy,

(q) a polypeptide of nut allergy,

(r) a polypeptide of food allergies,

(s) a polypeptide of asthma, or

(t) a polypeptide of *Chlamydia*

(u) Her2,

(v) GD2,

(w) EGF-R,

(x) CEA,

(y) CD52,

(z) Human melanoma gp100,

(aa) Human melanoma melanA/MART-1,

(bb) Tyrosinase,

(cc) NA17-A nt,

(dd) MAGE3,

(ee) P53, and

(ff) HPV16E7; and

(gg) any fragment or mutein of said antigen of (a) to (z) and of (aa) to (ff).

75. The composition of any of claims 47-74, wherein said antigen or antigenic determinant is a peptide, a protein, or a fragment or mutein of a protein or peptide, selected from the group consisting of:

a) a phospholipase A_2 protein;

b) a human IgE;

c) a lymphotoxin;

d) an Influenza M2 protein; and

e) a Der p I peptide.

76. The composition of claim 75, wherein said antigen or said antigenic determinant is an influenza M2 protein, or a fragment thereof.

77. The composition of claim 76, wherein said antigen or antigenic determinant is the M2 protein or a fragment thereof being at least 6 amino acids in length.

78. The composition of claim 77, wherein said antigen or antigenic determinant is a peptide comprising the amino acids 2 to 24 of M2 protein or a fragment thereof being at

least 6 amino acids in length.

79. The composition of claim 77, wherein said antigen or antigenic determinant is a peptide consisting of the amino acids 2 to 24 of M2 protein or a fragment thereof being at least 6 amino acids in length.

80. The composition of any of claims 47-79, wherein said organic molecule is an antigen or antigenic determinant, further that said antigen or said antigenic determinant is a self antigen or an anti-idiotypic antibody, or fragments of either thereof.

81. The composition of any of claims 47-80, wherein said self antigen is a protein, a peptide or any fragments or muteins thereof, selected from the group consisting of:

a) a lymphotoxin;
b) a lymphotoxin receptor;
c) RANKL;
d) VEGF;
e) VEGFR;
f) Interleukin-5;
g) Interleukin-8
h) Interleukin-17;
h) Interleukin-13;
i) Angiotensin;
k) CCL21;
l) CXCL12;
m) SDF-1;
n) MCP-1;
o) Endoglin;
p) Resistin;
q) GHRH;

r) LHRH;

s) TRH;

t) MIF;

u) Eotaxin;

v) Bradykinin;

w) BLC;

x) M-CSF;

x) Tumor Necrosis Factor α (TNFα);

y) amyloid beta peptide ($Aß_{1-42}$); and

z) a human IgE.

82. The composition of any of claims 47-81, wherein said self antigen is a lymphotoxin or a fragment thereof selected from the group consisiting of:

a) lymphotoxin α (LTα);

b) lymphotoxin β (LTβ); and

c) a mixture or combination of LTα and LTβ.

83. The composition of any of claims 47-82, wherein said organic molecule is an organic molecule suited to induce an immune response against a drug, hormone or toxin.

84. The composition of any of claims 47-83, wherein said organic molecule is an organic molecule suited to induce an immune response against a drug.

85. The composition of any of claims 47-84, wherein said drug is selected from the group consisting of:

(a) codeine;

(b) fentanyl;

(c) heroin;

(d) morphine;

(e) amphetamine;

(f) cocaine;
(g) methylenedioxymethamphetamine;
(h) methamphetamine;
(i) methylphenidate;
(j) nicotine;
(k) LSD;
(l) mescaline;
(m) psilocybin; and
(n) tetrahydrocannabinol.

86. The composition of any of claims 47-85, wherein said drug is nicotine.

87. The composition of any of claims 47-86, wherein the organic molecule is suited to induce an immune response against a hormone.

88. The composition of any of claims 47-87, wherein said organic molecule is a hormone being selected from the group consisting of:
(a) Progesterone;
(b) Estrogen;
(c) Testosterone;
(d) follicle stimulating hormone;
(e) melanin stimulating hormone;
(f) adrenalin; and
(g) noradrenalin.

89. The composition of any of claims 47-88, wherein the organic molecule is suited to induce an immune response against a toxin.

90. The composition of any of claims 47-89, wherein said organic molecule is a toxin being selected from the group consisting of:
(a) Aflatoxin;

(b) ciguetera toxin;

(c) tetrodotoxin;

(d) antibiotics; and

(e) anticancer agents.

91. A pharmaceutical composition comprising:

a) the composition of any of claims 47-90 and

b) an acceptable pharmaceutical carrier.

92. A vaccine composition comprising an immunologically effective amount of the composition of any of claims 47-90.

93. The vaccine composition of claim 92 further comprising an adjuvant.

94. A process for producing a non-naturally occurring, ordered and repetitive antigen array comprising:

(a) providing a molecular scaffold comprising a core particle selected from the group of

(i) AP205 virus; and

(ii) AP205 virus-like particles; and

(b) providing an organic molecule suitable for inducing an immune response;

(c) providing a means of associating (a) and (b), said means optionally contained within (a) and/or (b), or as a separate molecule; amd

(d) combining the elements of (a) through (c), such that said organic molecule associates with said scaffold to form an ordered and repetitive antigen array.

95. A process for producing a non-naturally occurring, ordered and repetitive array comprising:

(a) providing a molecular scaffold comprising a virus-like particle selected from the group consisting of:

(i) a protein comprising amino acids 2-131 of SEQ ID NO:1;

(ii) a protein comprising amino acids 2-131 of SEQ ID NO:3; and

(iii) a mutein of SEQ ID NO:1, wherein said mutein consists of an addition, deletion or substitution of one to three amino acids from amino acids 1-131 of SEQ ID NO:1;

(b) providing an organic molecule suitable for inducing an immune response;

(c) providing a means of associating (a) and (b), said means optionally contained within (a) and/or (b), or as a separate molecule; and

(d) combining the elements of (a) through (c), such that said organic molecule associates with said scaffold to form said ordered and repetitive array.

96. A nucleic acid molecule comprising a nucleotide sequence as set forth in SEQ ID NO:125.

97. A host cell containing a nucleic acid according to claim 96 or a vector according to any one of the claims 42-45.

98. The host cell of claim 97, wherein said host cell is *E.coli*.

99. A method of producing a virus-like particle according to any of claims 1-19 comprising the steps of

(a) providing a nucleic acid according to claim 96 or a vector according to any one of the claims 42-45;

(b) introducing said nucleic acid or said vector into a host cell;

(c) expressing said nucleic acid or the sequence of said vector in said host cell to obtain a protein or a mutein capable of forming a virus-like particle according to claim 1 or claim 16.

100. The method of claim 99, wherein said host cell is *E.coli*.

101. A vaccine composition according to claim 92 for use in a method of immunization.

102. The vaccine composition for use in a method according to claim 101, wherein the composition further comprises an adjuvant

103. Use of the vaccine composition according to claim 92 for the preparation of a vaccine, wherein said vaccine is suitable for being administered to a subject.

104. The use of the vaccine composition according to claim 103, wherein said composition further comprises an adjuvant.

105. The composition according to any of claims 47-90 for use in the treatment or prevention of a disease, disorder or physiological condition in an individual, wherein said composition is suitable for being administered to said individual.

106. Use of the composition according to any of claims 47-90 for the preparation of a composition for treating or preventing a disease, disorder or physiological condition in an individual, wherein said composition is suitable for being administered to said individual.

107. A vaccine composition according to claim 92 for use in the treatment or prevention of a disease, disorder or physiological condition in an individual, wherein said composition is suitable for being administered to said individual.

108. Use of the vaccine composition according to claim 92 for the preparation of a vaccine for treating or preventing a disease, disorder or physiological condition in an individual, wherein said vaccine composition is suitable for being administered to said individual.

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Bachmann, Martin F
Tissot, Alain
Pumpens, Paul
Cielens, Indulis
Renhofa, Regina

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ggatcaagag ctaccaactc tttttccgaa ggtaactggc ttcagcagag cgcagatacc 2640
aaatactgtc cttctagtgt agccgtagtt aggccaccac ttcaagaact ctgtagcacc 2700

gcctacatac ctcgctctgc taatcctgtt accagtggct gctgccagtg gcgataagtc 2760

gtgtcttacc gggttggact caagacgata gttaccggat aaggcgcagc ggtcgggctg 2820

aacgggggt tcgtgcacac agcccagctt ggagcgaacg acctacaccg aactgagata 2880

cctacagcgc gagcattgag aaagcgccac gcttcccgaa gggagaaagg cggacaggta 2940

tccggtaagc ggcagggtcg gaacaggaga gcgcacgagg gagcttccag ggggaaacgc 3000

ctggtatctt tatagtcctg tcgggtttcg ccacctctga cttgagcgtc gatttttgtg 3060

atgctcgtca ggggggcgga gcctatggaa aaacgccagc aacgcggcct ttttacggtt 3120

cctggccttt tgctggcctt ttgctcacat gttctttcct gcgttatccc ctgattctgt 3180

ggataaccgt attaccgcct ttgagtgagc tgataccgct cgccgcagcc gaacgaccga 3240

gcgcagcgag tcagtgagcg aggaagcgga agagcgccca atacgcaaac cgcctctccc 3300

cgcgcgttgg ccgattcatt aatgcagctg tggtgtcatg gtcggtgatc gccagggtgc 3360

cgacgcgcat ctcgactgca tggtgcacca atgcttctgg cgtcaggcag ccatcggaag 3420

ctgtggtatg gccgtgcagg tcgtaaatca ctgcataatt cgtgtcgctc aaggcgcact 3480

cccgttctgg ataatgtttt ttgcgccgac atcataacgg ttctggcaaa tattctgaaa 3540

tgagctgttg acaattaatc atcgaactag ttaactagta cgcaagttca cgtaaaaagg 3600

gtatcgcgga att 3613

<210> 5
<211> 57
<212> DNA
<213> Artificial Sequence

<220>
<223> sequence containing putative AP205 ribosomal binding site

<400> 5
tctagaattt tctgcgcacc catcccgggt ggcgcccaaa gtgaggaaaa tcacatg 57

<210> 6
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Shine-Dalgarno sequence of vector pQb185

<400> 6
tctagattaa cccaacgcgt aggagtcagg ccatg 35

<210> 7
<211> 42
<212> PRT
<213> Homo sapiens

<400> 7

Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys
1 5 10 15

Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys Gly Ala Ile Ile
20 25 30

Gly Leu Met Val Gly Gly Val Val Ile Ala
35 40

<210> 8
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
<223> Abeta 1-15 GGC

<400> 8

Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln Gly
1 5 10 15

Gly Cys

<210> 9
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> Abeta 1-27 GGC

<400> 9

Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys
1 5 10 15

Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Gly Gly Cys
20 25 30

<210> 10
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Abeta 33-42 mutant

<400> 10

Cys Gly His Gly Asn Lys Ser Gly Leu Met Val Gly Gly Val Val Ile
1 5 10 15

Ala

<210> 11
<211> 14
<212> PRT
<213> Homo sapiens

<400> 11

Asp Arg Val Tyr Ile His Pro Phe His Leu Val Ile His Asn
1 5 10

<210> 12
<211> 10
<212> PRT
<213> Homo sapiens

<400> 12

Asp Arg Val Tyr Ile His Pro Phe His Leu
1 5 10

<210> 13
<211> 8
<212> PRT
<213> Homo sapiens

<400> 13

Asp Arg Val Tyr Ile His Pro Phe
1 5

<210> 14
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> CGG-angiotensin I

<400> 14

Cys Gly Gly Asp Arg Val Tyr Ile His Pro Phe
1 5 10

<210> 15
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> CGG-Angiotensin I peptide

<400> 15

Cys Gly Gly Asp Arg Val Tyr Ile His Pro Phe His Leu
1 5 10

<210> 16
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Angiotensin-I GGC peptide

<400> 16

Asp Arg Val Tyr Ile His Pro Phe His Leu Gly Gly Cys
1 5 10

<210> 17
<211> 26
<212> PRT
<213> Artificial Sequence

<220>
<223> prion peptide "cprplong"

<400> 17

Cys Ser Ala Met Ser Arg Pro Met Ile His Phe Gly Asn Asp Trp Glu
1 5 10 15

Asp Arg Tyr Tyr Arg Glu Asn Met Tyr Arg
20 25

<210> 18
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> prion peptide "cprpshort"

<400> 18

Cys Gly Asn Asp Trp Glu Asp Arg Tyr Tyr Arg Glu Asn Met Tyr Arg
1 5 10 15

<210> 19
<211> 26
<212> PRT
<213> Artificial Sequence

<220>
<223> prion protein "human cprplong"

<400> 19

Cys Ser Ala Met Ser Arg Pro Ile Ile His Phe Gly Ser Asp Tyr Glu
1 5 10 15

Asp Arg Tyr Tyr Arg Glu Asn Met His Arg
20 25

<210> 20
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> prion peptide "human cprpshort"

<400> 20

Cys Gly Ser Asp Tyr Glu Asp Arg Tyr Tyr Arg Glu Asn Met His Arg
1 5 10 15

<210> 21
<211> 26
<212> PRT

<213> Artificial Sequence

<220>
<223> prion peptide "bovine cprplong"

<400> 21

Cys Ser Ala Met Ser Arg Pro Leu Ile His Phe Gly Asn Asp Tyr Glu
1 5 10 15

Asp Arg Tyr Tyr Arg Glu Asn Met His Arg
20 25

<210> 22
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> prion peptide "bovine cprpshort"

<400> 22

Cys Gly Asn Asp Tyr Glu Asp Arg Tyr Tyr Arg Glu Asn Met His Arg
1 5 10 15

<210> 23
<211> 26
<212> PRT
<213> Artificial Sequence

<220>
<223> prion peptide "sheep cprplong"

<400> 23

Cys Ser Ala Met Ser Arg Pro Leu Ile His Phe Gly Asn Asp Tyr Glu
1 5 10 15

Asp Arg Tyr Tyr Arg Glu Asn Met Tyr Arg
20 25

<210> 24
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> prion peptide "sheep cprpshort"

<400> 24

Cys Gly Asn Asp Tyr Glu Asp Arg Tyr Tyr Arg Glu Asn Met Tyr Arg
1 5 10 15

<210> 25
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> murine TNF- a mutant

<400> 25

Cys Gly Gly Val Glu Glu Gln Leu Glu Trp Leu Ser Gln Arg
1 5 10

<210> 26
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> murine TNF- a mutant

<400> 26

Ser Ser Gln Asn Ser Ser Asp Lys Pro Val Ala His Val Val Ala Asn
1 5 10 15

His Gly Val Gly Gly Cys
20

<210> 27
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> murine TNF-a mutant peptide

<400> 27

Cys Ser Ser Gln Asn Ser Ser Asp Lys Pro Val Ala His Val Val Ala
1 5 10 15

Asn His Gly Val
20

<210> 28
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> human TNF-a peptide mutant

<400> 28

Ser Ser Arg Thr Pro Ser Asp Lys Pro Val Ala His Val Val Ala Asn
1 5 10 15

Pro Gln Ala Glu Gly Gln
20

<210> 29
<211> 11
<212> PRT
<213> Homo sapiens

<400> 29

Gln Leu Gln Trp Leu Asn Arg Arg Ala Asn Ala
1 5 10

<210> 30
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> CGG-IgE peptide mutant

<400> 30

Cys Gly Gly Val Asn Leu Thr Trp Ser Arg Ala Ser Gly
1 5 10

<210> 31
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> IgE mimotope

<400> 31

Ile Asn His Arg Gly Tyr Trp Val
1 5

<210> 32
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> IgE mimotope

<400> 32

Arg Asn His Arg Gly Tyr Trp Val
1 5

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

<220>
<223> IgE mimotope

<400> 33

Arg Ser Arg Ser Gly Gly Tyr Trp Leu Trp
1 5 10

<210> 34
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> IgE mimotope

<400> 34

Val Asn Leu Thr Trp Ser Arg Ala Ser Gly
1 5 10

<210> 35
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> CeH3 epitope

<400> 35

Val Asn Leu Pro Trp Ser Arg Ala Ser Gly
1 5 10

<210> 36
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> CeH3 epitope

<400> 36

Val Asn Leu Thr Trp Ser Phe Gly Leu Glu
1 5 10

<210> 37
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> CeH3 epitope

<400> 37

Val Asn Leu Pro Trp Ser Phe Gly Leu Glu
1 5 10

<210> 38
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> CeH3 mimotope

<400> 38

Val Asn Arg Pro Trp Ser Phe Gly Leu Glu
1 5 10

<210> 39
<211> 10
<212> PRT
<213> Artificial Sequence

<220>

<223> CeH3 mimotope

<400> 39

Val Lys Leu Pro Trp Arg Phe Tyr Gln Val
1 5 10

<210> 40
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> CeH3 mimotope

<400> 40

Val Trp Thr Ala Cys Gly Tyr Gly Arg Met
1 5 10

<210> 41
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> CeH3 mimotope

<400> 41

Gly Thr Val Ser Thr Leu Ser
1 5

<210> 42
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> CeH3 mimotope

<400> 42

Leu Leu Asp Ser Arg Tyr Trp
1 5

<210> 43
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> CeH3 mimotope

<400> 43

Gln Pro Ala His Ser Leu Gly
1 5

<210> 44
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> CeH3 mimotope

<400> 44

Leu Trp Gly Met Gln Gly Arg
1 5

<210> 45
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> CeH3 mimotope.

<400> 45

Leu Thr Leu Ser His Pro His Trp Val Leu Asn His Phe Val Ser
1 5 10 15

<210> 46
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> CeH3 mimotope

<400> 46

Ser Met Gly Pro Asp Gln Thr Leu Arg
1 5

<210> 47
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> CeH3 mimotope

<400> 47

Val Asn Leu Thr Trp Ser
1 5

<210> 48
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> CeH3 mimotope

<400> 48

Gly Glu Phe Cys Ile Asn His Arg Gly Tyr Trp Val Cys Gly Asp Pro
1 5 10 15

Ala

<210> 49
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> glycine serine linker

<220>
<221> REPEAT
<222> (1)..(5)
<223> These residues can be repeated from zero to any times as a group

<400> 49

Gly Gly Gly Gly Ser
1 5

<210> 50
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> N-terminal gamma1 linker

<400> 50

Cys Gly Asp Lys Thr His Thr Ser Pro Pro
1 5 10

<210> 51
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> C-terminal gamma 1 linker

<400> 51

Asp Lys Thr His Thr Ser Pro Pro Cys
1 5

<210> 52
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> N-terminal gamma 3 linker

<400> 52

Cys Gly Gly Pro Lys Pro Ser Thr Pro Pro Gly Ser Ser Gly Gly Ala
1 5 10 15

Pro

<210> 53
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
<223> C-terminal gamma 3 linker

<400> 53

Pro Lys Pro Ser Thr Pro Pro Gly Ser Ser Gly Gly Ala Pro Gly Gly
1 5 10 15

Cys Gly

<210> 54
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> N-terminal glycine linker

<400> 54

Gly Cys Gly Gly Gly Gly
1 5

<210> 55
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> C-terminal glycine linker

<400> 55

Gly Gly Gly Gly Cys Gly
1 5

<210> 56
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> C-terminal glycine-lysine linker

<400> 56

Gly Gly Lys Lys Gly Cys
1 5

<210> 57
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> N-terminal glycine-lysine linker

<400> 57

Cys Gly Lys Lys Gly Gly
1 5

<210> 58
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> C-terminal linker

<400> 58

Gly Gly Cys Gly
1

<210> 59
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> p1.44 primer

<400> 59
aaccatggca ataagccaa tgcaa 25

<210> 60
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> p1.45 primer

<400> 60
aatctagaat tttctgcgca cccatcccgg 30

<210> 61
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> p1.46 primer

<400> 61
aaaagcttaa gcagtagtat cagacgatac g 31

<210> 62
<211> 43
<212> DNA
<213> Artificial Sequence

<220>
<223> p1.47 primer

<400> 62
gagtgatcca actcgtttat caactacatt ttcagcaagt ctg 43

<210> 63
<211> 43
<212> DNA
<213> Artificial Sequence

<220>
<223> p1.48 primer

<400> 63
cagacttgct gaaaatgtag ttgataaacg agttggatca ctc 43

<210> 64
<211> 21
<212> PRT
<213> Artificial Sequence

<220>
<223> Derp1 117-137 peptide mutant

<400> 64

Cys Gln Ile Tyr Pro Pro Asn Ala Asn Lys Ile Arg Glu Ala Leu Ala
1 5 10 15

Gln Thr His Ser Ala
20

<210> 65
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Flag

<400> 65

Cys Gly Gly Asp Tyr Lys Asp Asp Asp Asp Lys
1 5 10

<210> 66
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> 5' LT- b primer

<400> 66
cttggtgccg caggatcag 19

<210> 67
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> 3' LT-b primer

<400> 67
cagatggctg tcaccccac 19

<210> 68
<211> 37
<212> DNA
<213> Artificial Sequence

<220>
<223> 5' LT- blong-NheI primer

<400> 68
gcccgctagc ctgcggtggt caggatcagg gacgtcg 37

<210> 69
<211> 37
<212> DNA
<213> Artificial Sequence

<220>
<223> 5' LT- sbhort-NheI primer

<400> 69
gcccgctagc ctgcggtggt tctccagctg cggattc 37

<210> 70
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> 3' LT- bstop-NotI primer

<400> 70
caatgactgc ggccgcttac cccaccatca ccg 33

<210> 71
<211> 74
<212> DNA
<213> Artificial Sequence

<220>
<223> MCS of vector pET22b(+)

<400> 71
gtttaacttt aagaaggaga tatacatatg gatccggcta gcgctcgagg gtttaaacgg 60

cggccgcatg cacc 74

<210> 72
<211> 43
<212> DNA
<213> Artificial Sequence

<220>
<223> primerMCS-1F

<400> 72
tatggatccg gctagcgctc gagggtttaa acggcggccg cat 43

<210> 73
<211> 45
<212> DNA
<213> Artificial Sequence

<220>

<223> primerMCS-1R

<400> 73
tcgaatgcgg ccgccgttta aaccctcgag cgctagccgg atcca 45

<210> 74
<211> 58
<212> DNA
<213> Artificial Sequence

<220>
<223> Bamhis6-EK-Nhe-F primer

<400> 74
gatccacacc accaccacca ccacggttct ggtgacgacg atgacaaagc gctagccc 58

<210> 75
<211> 58
<212> DNA
<213> Artificial Sequence

<220>
<223> Bamhis6-EK-Nhe-R

<400> 75
tcgagggcta gcgctttgtc atcgtcgtca ccagaaccgt ggtggtggtg gtggtgtg 58

<210> 76
<211> 42
<212> DNA
<213> Artificial Sequence

<220>
<223> oligo1F-C-glycine-linker

<400> 76
tcgagggtgg tggtggtggt tgcggttaat aagtttaaac gc 42

<210> 77
<211> 42
<212> DNA
<213> Artificial Sequence

<220>
<223> oligo1R-C-glycine-linker

<400> 77
ggccgcgttt aaacttatta accgcaacca ccaccaccac cc 42

<210> 78
<211> 51
<212> DNA
<213> Artificial Sequence

<220>
<223> oligo1F-C-gamma1-linker

<400> 78
tcgaggataa aacccacacc tctccgccgt gtggttaata agtttaaacg c 51

<210> 79
<211> 51

<212> DNA
<213> Artificial Sequence

<220>
<223> oligo1R-C-gamma1-linker

<400> 79
ggccgcgttt aaacttatta accacacggc ggagaggtgt gggttttatc c 51

<210> 80
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> oligo1FA-C-gamma3-linker

<400> 80
tcgagccgaa accgtctacc ccgccgggtt cttctg 36

<210> 81
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> oligo1RA-C-gamma3-linker

<400> 81
caccaccaga agaacccggc ggggtagacg gtttcggc 38

<210> 82
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> oligo2FB-C-gamma3-linker

<400> 82
gtggtgctcc gggtggttgc ggttaataag tttaaacgc 39

<210> 83
<211> 37
<212> DNA
<213> Artificial Sequence

<220>
<223> oligo2RB-C-gamma3-linker

<400> 83
ggccgcgttt aaacttatta accgcaacca cccggag 37

<210> 84
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> rMIF-F

<400> 84
ggaattccat atgcctatgt tcatcgtgaa cac 33

<210> 85
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> rMIF-Xho-R

<400> 85
cccgctcgag agcgaaggtg gaaccgttc 29

<210> 86
<211> 62
<212> DNA
<213> Artificial Sequence

<220>
<223> RANKL-UP oligonucleotide

<400> 86
ctgccagggg cccgggtgcg gcggtggcca tcatcaccac catcaccagc gcttctcagg 60

ag 62

<210> 87
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> RANKL-DOWN oligonucleotide

<400> 87
ccgctcgagt tagtctatgt cctgaacttt gaaag 35

<210> 88
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer 5'PrP-BamHI

<400> 88
cgggatccca ccatggtggg gggccttgg 29

<210> 89
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer 3'PrP-NheI

<400> 89
ctagctagcc tggatcttct cccg 24

<210> 90
<211> 55
<212> DNA
<213> Artificial Sequence

<220>
<223> primer Spelinker3-F1

<400> 90
ccccgccggg ttcttctggc ggtgctccgg ctagcatgga gattcccatg agcac 55

<210> 91
<211> 49
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer IL5StopXho-R

<400> 91
ttttgcggcc gcgtttaaac tcgagttatt agccttccat tgcccactc 49

<210> 92
<211> 52
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer SpeNlinker3-F2

<400> 92
ttttactagt tggttgcggc ggcccgaaac cgagcacccc gccgggttct tc 52

<210> 93
<211> 3
<212> PRT
<213> Artificial Sequence

<220>
<223> N-terminal glycine linker

<220>
<221> REPEAT
<222> (1)..(1)
<223> Glycine can be repeated from zero to five times

<220>
<221> REPEAT
<222> (3)..(3)
<223> Glycine can be repeated from zero to twelve times

<400> 93

Gly Cys Gly
1

<210> 94
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> N-terminal glycine-serine linker

<220>
<221> REPEAT

<222> (1)..(1)
<223> Glycine can be repeated from zero to five times

<220>
<221> REPEAT
<222> (3)..(3)
<223> Glycine can be repeated from zero to ten times

<220>
<221> REPEAT
<222> (4)..(4)
<223> Serine can be repeated from zero to two times

<220>
<221> REPEAT
<222> (5)..(9)
<223> These residues can be repeated from zero to three times as a group

<400> 94

Gly Cys Gly Ser Gly Gly Gly Gly Ser
1 5

<210> 95
<211> 3
<212> PRT
<213> Artificial Sequence

<220>
<223> C-terminal glycine linker

<220>
<221> REPEAT
<222> (1)..(1)
<223> Glycine can be repeated from zero to twelve times

<220>
<221> REPEAT
<222> (3)..(3)
<223> Glycine can be repeated from zero to five times

<400> 95

Gly Cys Gly
1

<210> 96
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> C-terminal glycine-serine linker

<220>
<221> REPEAT
<222> (1)..(1)
<223> Glycine can be repeated from zero to ten times

<220>
<221> REPEAT
<222> (2)..(2)
<223> Serine can be repeated from zero to two times

<220>
<221> REPEAT
<222> (3)..(7)
<223> These residues can be repeated from zero to three times as a group

<220>
<221> REPEAT
<222> (8)..(8)
<223> Glycine can be repeated from zero to eight times

<220>
<221> REPEAT
<222> (10)..(10)
<223> Glycine can be repeated from zero to five times

<400> 96

Gly Ser Gly Gly Gly Gly Ser Gly Cys Gly
1 5 10

<210> 97
<211> 23
<212> PRT
<213> Artificial Sequence

<220>
<223> C-Der p1 peptide mutant

<400> 97

Cys Gly Asn Gln Ser Leu Asp Leu Ala Glu Gln Glu Leu Val Asp Cys
1 5 10 15

Ala Ser Gln His Gly Cys His
20

<210> 98
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> C-Angiotensin I peptide

<400> 98

Cys Asp Arg Val Tyr Ile His Pro Phe His
1 5 10

<210> 99
<211> 26
<212> PRT
<213> Homo sapiens

<400> 99

Cys Thr Ala Arg Thr Glu Leu Asn Val Gly Ile Asp Phe Asn Trp Glu
1 5 10 15

Tyr Pro Ser Ser Lys His Gln His Lys Lys
20 25

<210> 100
<211> 23
<212> PRT
<213> Artificial Sequence

<220>
<223> C TNF-a peptide mutant

<400> 100

Cys Ser Ser Arg Thr Pro Ser Asp Lys Pro Val Ala His Val Val Ala
1 5 10 15

Asn Pro Gln Ala Glu Gly Gln
20

<210> 101
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> TNF-a-C mutant

<400> 101

Ser Ser Arg Thr Pro Ser Asp Lys Pro Val Ala His Val Val Ala Asn
1 5 10 15

Pro Gln Ala Glu Gly Gln Gly Gly Cys
20 25

<210> 102
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> C-TNF-a mutant

<400> 102

Cys Gly Gly Gln Leu Gln Trp Leu Asn Arg Arg Ala Asn Ala
1 5 10

<210> 103
<211> 745
<212> DNA
<213> Artificial Sequence

<220>
<223> pCep-Xa-Fc*

<220>
<221> CDS
<222> (1)..(741)

<400> 103
gat cca gca gct ggg ctc gag gtg cta gcg gga ggg ggt gga tgt ggg 48
Asp Pro Ala Ala Gly Leu Glu Val Leu Ala Gly Gly Gly Gly Cys Gly

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1               5                  10                  15

atc gaa ggt cgc aag ctt act cac aca tgc cca ccg tgc cca gca cct      96
Ile Glu Gly Arg Lys Leu Thr His Thr Cys Pro Pro Cys Pro Ala Pro
            20                  25                  30

gaa gcc gag ggg gca ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag     144
Glu Ala Glu Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
        35                  40                  45

gac acc ctc atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg     192
Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
    50                  55                  60

gac gtg agc cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac     240
Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
65                  70                  75                  80

ggc gtg gag gtg cat aat gcc aag aca aag ccg cgg gag gag cag tac     288
Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
                85                  90                  95

aac agc acg tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac     336
Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
            100                 105                 110

tgg ctg aat ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc     384
Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
        115                 120                 125

cca gcc tcc atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga     432
Pro Ala Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
    130                 135                 140

gaa cca cag gtg tac acc ctg ccc cca tcc cgg gat gag ctg acc aag     480
Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
145                 150                 155                 160

aac cag gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac     528
Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
                165                 170                 175

atc gcc gtg gag tgg gag agc aat ggg cag ccg gag aac aac tac aag     576
Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
            180                 185                 190

acc acg cct ccc gtg ttg gac tcc gac ggc tcc ttc ttc ctc tac agc     624
Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
        195                 200                 205

aag ctc acc gtg gac aag agc agg tgg cag cag ggg aac gtc ttc tca     672
Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
    210                 215                 220

tgc tcc gtg atg cat gag gct ctg cac aac cac tac acg cag aag agc     720
Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
225                 230                 235                 240

ctc tcc ctg tct ccg ggt aaa tgac                                   745
Leu Ser Leu Ser Pro Gly Lys
                245
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<210> 104
<211> 247
<212> PRT
<213> Artificial Sequence

<220>
<223> pCep-Xa-Fc*

<400> 104

Asp Pro Ala Ala Gly Leu Glu Val Leu Ala Gly Gly Gly Gly Cys Gly
1 5 10 15

Ile Glu Gly Arg Lys Leu Thr His Thr Cys Pro Pro Cys Pro Ala Pro
20 25 30

Glu Ala Glu Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
35 40 45

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
50 55 60

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
65 70 75 80

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
85 90 95

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
100 105 110

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
115 120 125

Pro Ala Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
130 135 140

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
145 150 155 160

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
165 170 175

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
180 185 190

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
195 200 205

Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
210 215 220

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
225 230 235 240

Leu Ser Leu Ser Pro Gly Lys
245

<210> 105
<211> 96
<212> DNA
<213> Artificial Sequence

<220>
<223> pCep-EK-Fc*

<220>
<221> CDS
<222> (1)..(96)

<400> 105
gat cca gca gct ggg ctc gag gtg cta gcg gga ggg ggt gga tgt ggg 48
Asp Pro Ala Ala Gly Leu Glu Val Leu Ala Gly Gly Gly Gly Cys Gly
1 5 10 15

gac gat gac gac aag ctt act cac aca tgc cca ccg tgc cca gca cct 96
Asp Asp Asp Asp Lys Leu Thr His Thr Cys Pro Pro Cys Pro Ala Pro
20 25 30

<210> 106
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> pCep-EK-Fc*

<400> 106

Asp Pro Ala Ala Gly Leu Glu Val Leu Ala Gly Gly Gly Gly Cys Gly
1 5 10 15

Asp Asp Asp Asp Lys Leu Thr His Thr Cys Pro Pro Cys Pro Ala Pro
20 25 30

<210> 107
<211> 144
<212> DNA
<213> Artificial Sequence

<220>
<223> pCep-SP-EK-Fc*

<220>
<221> CDS
<222> (1)..(141)

<400> 107
atg gag aca gac aca ctc ctg cta tgg gta ctg ctg ctc tgg gtt cca 48
Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1 5 10 15

ggt tcc act ggt gac gcg gat cca gca gct ggg ctc gag gtg cta gcg 96
Gly Ser Thr Gly Asp Ala Asp Pro Ala Ala Gly Leu Glu Val Leu Ala
20 25 30

gga ggg ggt gga tgt ggg gac gat gac gac aag ctt act cac aca tgc 144
Gly Gly Gly Gly Cys Gly Asp Asp Asp Lys Leu Thr His Thr
35 40 45

<210> 108
<211> 47
<212> PRT
<213> Artificial Sequence

<220>
<223> pCep-SP-EK-Fc*

<400> 108

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1 5 10 15

Gly Ser Thr Gly Asp Ala Asp Pro Ala Ala Gly Leu Glu Val Leu Ala
20 25 30

Gly Gly Gly Gly Cys Gly Asp Asp Asp Asp Lys Leu Thr His Thr
35 40 45

<210> 109
<211> 399
<212> DNA
<213> Artificial Sequence

<220>
<223> Res-C-Xa

<220>
<221> CDS
<222> (10)..(399)

<400> 109

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ggatccggg atg aag aac ctt tca ttt ccc ctc ctt ttc ctt ttc ttc ctt     51
          Met Lys Asn Leu Ser Phe Pro Leu Leu Phe Leu Phe Phe Leu
          1               5                  10

gtc cct gaa ctg ctg ggc tcc agc atg cca ctg tgt ccc atc gat gaa       99
Val Pro Glu Leu Leu Gly Ser Ser Met Pro Leu Cys Pro Ile Asp Glu
15                  20                  25                  30

gcc atc gac aag aag atc aaa caa gac ttc aac tcc ctg ttt cca aat      147
Ala Ile Asp Lys Lys Ile Lys Gln Asp Phe Asn Ser Leu Phe Pro Asn
                35                  40                  45

gca ata aag aac att ggc tta aat tgc tgg aca gtc tcc tcc aga ggg      195
Ala Ile Lys Asn Ile Gly Leu Asn Cys Trp Thr Val Ser Ser Arg Gly
            50                  55                  60

aag ttg gcc tcc tgc cca gaa ggc aca gca gtc ttg agc tgc tcc tgt      243
Lys Leu Ala Ser Cys Pro Glu Gly Thr Ala Val Leu Ser Cys Ser Cys
        65                  70                  75

ggc tct gcc tgt ggc tcg tgg gac att cgt gaa gaa aaa gtg tgt cac      291
Gly Ser Ala Cys Gly Ser Trp Asp Ile Arg Glu Glu Lys Val Cys His
    80                  85                  90

tgc cag tgt gca agg ata gac tgg aca gca gcc cgc tgc tgt aag ctg      339
Cys Gln Cys Ala Arg Ile Asp Trp Thr Ala Ala Arg Cys Cys Lys Leu
95                  100                 105                 110

cag gtc gct tcc tct cta gcg gga ggg ggt gga tgt ggg atc gaa ggt      387
Gln Val Ala Ser Ser Leu Ala Gly Gly Gly Gly Cys Gly Ile Glu Gly
                115                 120                 125
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cgc aag ctt act 399
Arg Lys Leu Thr
130

<210> 110
<211> 130
<212> PRT
<213> Artificial Sequence

<220>
<223> Res-C-Xa

<400> 110

Met Lys Asn Leu Ser Phe Pro Leu Leu Phe Leu Phe Phe Leu Val Pro
1 5 10 15

Glu Leu Leu Gly Ser Ser Met Pro Leu Cys Pro Ile Asp Glu Ala Ile
20 25 30

Asp Lys Lys Ile Lys Gln Asp Phe Asn Ser Leu Phe Pro Asn Ala Ile
35 40 45

Lys Asn Ile Gly Leu Asn Cys Trp Thr Val Ser Ser Arg Gly Lys Leu
50 55 60

Ala Ser Cys Pro Glu Gly Thr Ala Val Leu Ser Cys Ser Cys Gly Ser
65 70 75 80

Ala Cys Gly Ser Trp Asp Ile Arg Glu Glu Lys Val Cys His Cys Gln
85 90 95

Cys Ala Arg Ile Asp Trp Thr Ala Ala Arg Cys Cys Lys Leu Gln Val
100 105 110

Ala Ser Ser Leu Ala Gly Gly Gly Gly Cys Gly Ile Glu Gly Arg Lys
115 120 125

Leu Thr
130

<210> 111
<211> 399
<212> DNA
<213> Artificial Sequence

<220>
<223> Res-C-EK

<220>
<221> CDS
<222> (10)..(399)

<400> 111
ggatccggg atg aag aac ctt tca ttt ccc ctc ctt ttc ctt ttc ttc ctt 51
Met Lys Asn Leu Ser Phe Pro Leu Leu Phe Leu Phe Phe Leu

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          1               5                  10

gtc cct gaa ctg ctg ggc tcc agc atg cca ctg tgt ccc atc gat gaa      99
Val Pro Glu Leu Leu Gly Ser Ser Met Pro Leu Cys Pro Ile Asp Glu
15                  20                  25                  30

gcc atc gac aag aag atc aaa caa gac ttc aac tcc ctg ttt cca aat     147
Ala Ile Asp Lys Lys Ile Lys Gln Asp Phe Asn Ser Leu Phe Pro Asn
                35                  40                  45

gca ata aag aac att ggc tta aat tgc tgg aca gtc tcc tcc aga ggg     195
Ala Ile Lys Asn Ile Gly Leu Asn Cys Trp Thr Val Ser Ser Arg Gly
            50                  55                  60

aag ttg gcc tcc tgc cca gaa ggc aca gca gtc ttg agc tgc tcc tgt     243
Lys Leu Ala Ser Cys Pro Glu Gly Thr Ala Val Leu Ser Cys Ser Cys
        65                  70                  75

ggc tct gcc tgt ggc tcg tgg gac att cgt gaa gaa aaa gtg tgt cac     291
Gly Ser Ala Cys Gly Ser Trp Asp Ile Arg Glu Glu Lys Val Cys His
    80                  85                  90

tgc cag tgt gca agg ata gac tgg aca gca gcc cgc tgc tgt aag ctg     339
Cys Gln Cys Ala Arg Ile Asp Trp Thr Ala Ala Arg Cys Cys Lys Leu
95                  100                 105                 110

cag gtc gct tcc tct cta gcg gga ggg ggt gga tgt ggg gac gat gac     387
Gln Val Ala Ser Ser Leu Ala Gly Gly Gly Gly Cys Gly Asp Asp Asp
                115                 120                 125

gac aag ctt act                                                    399
Asp Lys Leu Thr
            130
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<210> 112
<211> 130
<212> PRT
<213> Artificial Sequence

<220>
<223> Res-C-EK

<400> 112

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Met Lys Asn Leu Ser Phe Pro Leu Leu Phe Leu Phe Phe Leu Val Pro
1               5                  10                  15

Glu Leu Leu Gly Ser Ser Met Pro Leu Cys Pro Ile Asp Glu Ala Ile
            20                  25                  30

Asp Lys Lys Ile Lys Gln Asp Phe Asn Ser Leu Phe Pro Asn Ala Ile
        35                  40                  45

Lys Asn Ile Gly Leu Asn Cys Trp Thr Val Ser Ser Arg Gly Lys Leu
    50                  55                  60

Ala Ser Cys Pro Glu Gly Thr Ala Val Leu Ser Cys Ser Cys Gly Ser
65                  70                  75                  80

Ala Cys Gly Ser Trp Asp Ile Arg Glu Glu Lys Val Cys His Cys Gln
                85                  90                  95
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Cys Ala Arg Ile Asp Trp Thr Ala Ala Arg Cys Cys Lys Leu Gln Val
100 105 110

Ala Ser Ser Leu Ala Gly Gly Gly Gly Cys Gly Asp Asp Asp Asp Lys
115 120 125

Leu Thr
130

<210> 113
<211> 26
<212> PRT
<213> Mus musculus

<400> 113

Cys Thr Ala Arg Thr Glu Leu Asn Val Gly Leu Asp Phe Thr Trp His
1 5 10 15

Ser Pro Pro Ser Lys Ser His His Lys Lys
20 25

<210> 114
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> rat MIF-C1

<400> 114

Pro Met Phe Ile Val Asn Thr Asn Val Pro Arg Ala Ser Val Pro Glu
1 5 10 15

Gly Phe Leu Ser Glu Leu Thr Gln Gln Leu Ala Gln Ala Thr Gly Lys
20 25 30

Pro Ala Gln Tyr Ile Ala Val His Val Val Pro Asp Gln Leu Met Thr
35 40 45

Phe Ser Gly Thr Ser Asp Pro Cys Ala Leu Cys Ser Leu His Ser Ile
50 55 60

Gly Lys Ile Gly Gly Ala Gln Asn Arg Asn Tyr Ser Lys Leu Leu Cys
65 70 75 80

Gly Leu Leu Ser Asp Arg Leu His Ile Ser Pro Asp Arg Val Tyr Ile
85 90 95

Asn Tyr Tyr Asp Met Asn Ala Ala Asn Val Gly Trp Asn Gly Ser Thr
100 105 110

Phe Ala Gly Gly Gly Gly Cys Gly
115 120

<210> 115
<211> 132
<212> PRT
<213> Artificial Sequence

<220>
<223> rat MIF-C2

<400> 115

Pro Met Phe Ile Val Asn Thr Asn Val Pro Arg Ala Ser Val Pro Glu
1 5 10 15

Gly Phe Leu Ser Glu Leu Thr Gln Gln Leu Ala Gln Ala Thr Gly Lys
20 25 30

Pro Ala Gln Tyr Ile Ala Val His Val Val Pro Asp Gln Leu Met Thr
35 40 45

Phe Ser Gly Thr Ser Asp Pro Cys Ala Leu Cys Ser Leu His Ser Ile
50 55 60

Gly Lys Ile Gly Gly Ala Gln Asn Arg Asn Tyr Ser Lys Leu Leu Cys
65 70 75 80

Gly Leu Leu Ser Asp Arg Leu His Ile Ser Pro Asp Arg Val Tyr Ile
85 90 95

Asn Tyr Tyr Asp Met Asn Ala Ala Asn Val Gly Trp Asn Gly Ser Thr
100 105 110

Phe Ala Pro Lys Pro Ser Thr Pro Pro Gly Ser Ser Gly Gly Ala Pro
115 120 125

Gly Gly Cys Gly
130

<210> 116
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
<223> amino acid linker C2

<400> 116

Pro Lys Pro Ser Thr Pro Pro Gly Ser Ser Gly Gly Ala Pro Gly Gly
1 5 10 15

Cys Gly

<210> 117
<211> 124

<212> PRT
<213> Artificial Sequence

<220>
<223> rat MIF-C3

<400> 117

Pro Met Phe Ile Val Asn Thr Asn Val Pro Arg Ala Ser Val Pro Glu
1 5 10 15

Gly Phe Leu Ser Glu Leu Thr Gln Gln Leu Ala Gln Ala Thr Gly Lys
20 25 30

Pro Ala Gln Tyr Ile Ala Val His Val Val Pro Asp Gln Leu Met Thr
35 40 45

Phe Ser Gly Thr Ser Asp Pro Cys Ala Leu Cys Ser Leu His Ser Ile
50 55 60

Gly Lys Ile Gly Gly Ala Gln Asn Arg Asn Tyr Ser Lys Leu Leu Cys
65 70 75 80

Gly Leu Leu Ser Asp Arg Leu His Ile Ser Pro Asp Arg Val Tyr Ile
85 90 95

Asn Tyr Tyr Asp Met Asn Ala Ala Asn Val Gly Trp Asn Gly Ser Thr
100 105 110

Phe Ala Asp Lys Thr His Thr Ser Pro Pro Cys Gly
115 120

<210> 118
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> amino acid linker C3

<400> 118

Asp Lys Thr His Thr Ser Pro Pro Cys Gly
1 5 10

<210> 119
<211> 114
<212> PRT
<213> Homo sapiens

<400> 119

Pro Met Phe Ile Val Asn Thr Asn Val Pro Arg Ala Ser Val Pro Asp
1 5 10 15

Gly Phe Leu Ser Glu Leu Thr Gln Gln Leu Ala Gln Ala Thr Gly Lys
20 25 30

Pro Pro Gln Tyr Ile Ala Val His Val Val Pro Asp Gln Leu Met Ala
35 40 45

Phe Gly Gly Ser Ser Glu Pro Cys Ala Leu Cys Ser Leu His Ser Ile
50 55 60

Gly Lys Ile Gly Gly Ala Gln Asn Arg Ser Tyr Ser Lys Leu Leu Cys
65 70 75 80

Gly Leu Leu Ala Glu Arg Leu Arg Ile Ser Pro Asp Arg Val Tyr Ile
85 90 95

Asn Tyr Tyr Asp Met Asn Ala Ala Asn Val Gly Trp Asn Asn Ser Thr
100 105 110

Phe Ala

<210> 120
<211> 114
<212> PRT
<213> Rattus norvegicus

<400> 120

Pro Met Phe Ile Val Asn Thr Asn Val Pro Arg Ala Ser Val Pro Glu
1 5 10 15

Gly Phe Leu Ser Glu Leu Thr Gln Gln Leu Ala Gln Ala Thr Gly Lys
20 25 30

Pro Ala Gln Tyr Ile Ala Val His Val Val Pro Asp Gln Leu Met Thr
35 40 45

Phe Ser Gly Thr Ser Asp Pro Cys Ala Leu Cys Ser Leu His Ser Ile
50 55 60

Gly Lys Ile Gly Gly Ala Gln Asn Arg Asn Tyr Ser Lys Leu Leu Cys
65 70 75 80

Gly Leu Leu Ser Asp Arg Leu His Ile Ser Pro Asp Arg Val Tyr Ile
85 90 95

Asn Tyr Tyr Asp Met Asn Ala Ala Asn Val Gly Trp Asn Gly Ser Thr
100 105 110

Phe Ala

<210> 121
<211> 114
<212> PRT
<213> Mus musculus

<400> 121

Pro Met Phe Ile Val Asn Thr Asn Val Pro Arg Ala Ser Val Pro Glu
1 5 10 15

Gly Phe Leu Ser Glu Leu Thr Gln Gln Leu Ala Gln Ala Thr Gly Lys
20 25 30

Pro Ala Gln Tyr Ile Ala Val His Val Val Pro Asp Gln Leu Met Thr
35 40 45

Phe Ser Gly Thr Asn Asp Pro Cys Ala Leu Cys Ser Leu His Ser Ile
50 55 60

Gly Lys Ile Gly Gly Ala Gln Asn Arg Asn Tyr Ser Lys Leu Leu Cys
65 70 75 80

Gly Leu Leu Ser Asp Arg Leu His Ile Ser Pro Asp Arg Val Tyr Ile
85 90 95

Asn Tyr Tyr Asp Met Asn Ala Ala Asn Val Gly Trp Asn Gly Ser Thr
100 105 110

Phe Ala

<210> 122
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> human MIF-C1

<400> 122

Pro Met Phe Ile Val Asn Thr Asn Val Pro Arg Ala Ser Val Pro Asp
1 5 10 15

Gly Phe Leu Ser Glu Leu Thr Gln Gln Leu Ala Gln Ala Thr Gly Lys
20 25 30

Pro Pro Gln Tyr Ile Ala Val His Val Val Pro Asp Gln Leu Met Ala
35 40 45

Phe Gly Gly Ser Ser Glu Pro Cys Ala Leu Cys Ser Leu His Ser Ile
50 55 60

Gly Lys Ile Gly Gly Ala Gln Asn Arg Ser Tyr Ser Lys Leu Leu Cys
65 70 75 80

Gly Leu Leu Ala Glu Arg Leu Arg Ile Ser Pro Asp Arg Val Tyr Ile
85 90 95

Asn Tyr Tyr Asp Met Asn Ala Ala Asn Val Gly Trp Asn Asn Ser Thr
100 105 110

Phe Ala Gly Gly Gly Gly Cys Gly
115 120

<210> 123
<211> 132
<212> PRT
<213> Artificial Sequence

<220>
<223> human MIF-C2

<400> 123

Pro Met Phe Ile Val Asn Thr Asn Val Pro Arg Ala Ser Val Pro Asp
1 5 10 15

Gly Phe Leu Ser Glu Leu Thr Gln Gln Leu Ala Gln Ala Thr Gly Lys
20 25 30

Pro Pro Gln Tyr Ile Ala Val His Val Val Pro Asp Gln Leu Met Ala
35 40 45

Phe Gly Gly Ser Ser Glu Pro Cys Ala Leu Cys Ser Leu His Ser Ile
50 55 60

Gly Lys Ile Gly Gly Ala Gln Asn Arg Ser Tyr Ser Lys Leu Leu Cys
65 70 75 80

Gly Leu Leu Ala Glu Arg Leu Arg Ile Ser Pro Asp Arg Val Tyr Ile
85 90 95

Asn Tyr Tyr Asp Met Asn Ala Ala Asn Val Gly Trp Asn Asn Ser Thr
100 105 110

Phe Ala Pro Lys Pro Ser Thr Pro Pro Gly Ser Ser Gly Gly Ala Pro
115 120 125

Gly Gly Cys Gly
130

<210> 124
<211> 124
<212> PRT
<213> Artificial Sequence

<220>
<223> human MIF-C3

<400> 124

Pro Met Phe Ile Val Asn Thr Asn Val Pro Arg Ala Ser Val Pro Asp
1 5 10 15

Gly Phe Leu Ser Glu Leu Thr Gln Gln Leu Ala Gln Ala Thr Gly Lys
20 25 30

Pro Pro Gln Tyr Ile Ala Val His Val Val Pro Asp Gln Leu Met Ala
35 40 45

Phe Gly Gly Ser Ser Glu Pro Cys Ala Leu Cys Ser Leu His Ser Ile
50 55 60

Gly Lys Ile Gly Gly Ala Gln Asn Arg Ser Tyr Ser Lys Leu Leu Cys
65 70 75 80

Gly Leu Leu Ala Glu Arg Leu Arg Ile Ser Pro Asp Arg Val Tyr Ile
85 90 95

Asn Tyr Tyr Asp Met Asn Ala Ala Asn Val Gly Trp Asn Asn Ser Thr
100 105 110

Phe Ala Asp Lys Thr His Thr Ser Pro Pro Cys Gly
115 120

<210> 125
<211> 396
<212> DNA
<213> Artificial Sequence

<220>
<223> AP205 P5T mutant

<400> 125
atggcaaata agacaatgca accgatcaca tctacagcaa ataaaattgt gtggtcggat 60

ccaactcgtt tatcaactac attttcagca agtctgttac gccaacgtgt taaagttggt 120

atagccgaac tgaataatgt ttcaggtcaa tatgtatctg tttataagcg tcctgcacct 180

aaaccggaag gttgtgcaga tgcctgtgtc attatgccga atgaaaacca atccattcgc 240

acagtgattt cagggtcagc cgaaaacttg gctaccttaa aagcagaatg ggaaactcac 300

aaacgtaacg ttgacacact cttcgcgagc ggcaacgccg gtttgggttt ccttgaccct 360

actgcggcta tcgtatcgtc tgatactact gcttaa 396