

INTERLEUKIN-1 CONJUGATES AND USES THEREOF

FIELD OF THE INVENTION

[0001] The present invention is in the fields of medicine, public health, immunology, molecular biology and virology. The invention provides compositions comprising a virus-like particle (VLP) or a virus particle and at least one antigen, wherein said antigen is an Interleukin-1 (IL-1) protein, an IL-1 fragment or peptide or an IL-1 mutein covalently linked to the VLP or the virus particle. The invention also provides a process for producing the compositions. The compositions of this invention are useful in the production of vaccines for the treatment of various human disorders, including rheumatoid arthritis osteoarthritis and others. The compositions of the invention hereby induce efficient immune responses, in particular antibody responses.

RELATED ART

[0002] IL-1 is a potent proinflammatory cytokine produced by various cell types, including macrophages, dendritic cells, B-cells and T-cells (Dinarello C.A., 1991. Blood 77(8):1627-1652). It consists of two molecular species, IL-1 α and IL-1 β , which share only limited sequence identity but exert similar biological activities through binding to IL-1 receptor type I (IL-1RI) (Dinarello C.A. et al., 1997, Cytokine & Growth Factor Rev. 8:253). Both IL-1 molecules also bind to a second IL-1 receptor (IL-1RII), which lacks the intracellular signalling domain, and is believed to play a regulatory role as a decoy receptor (Dinarello C.A. et al., 1997, Cytokine & Growth Factor Rev. 8:253). In addition, a third member of the IL-1 family, the IL-1 receptor antagonist (IL-1ra), binds to both receptors without exerting any agonistic activity. IL-1ra together with IL-1RII and the shed forms of IL-1RI and IL-1RII counteract the activity of IL-1 α and IL-1 β and ensure a tight regulation of the inflammatory response.

[0003] A dysregulation of the IL-1-mediated inflammatory response is observed in many human disorders, including rheumatoid arthritis, inflammatory bowel disease, kidney diseases, osteoporosis and others. In each of these diseases either overproduction of IL-1 and/or underproduction of IL-1ra predisposes to the development of disease (Arend W.P., 2002, Cytokine & Growth Factor Reviews 13:323-340). A recombinant version of IL-1ra (anakinra, Kineret®) is efficacious in reducing inflammation and preventing tissue damage in several inflammatory disorders, but the need for high systemic concentrations and the short half life of the drug require frequent (daily) administrations of high doses (~100 mg),

resulting in high cost of goods and potential patient compliance problems (Kineret[®] prescribing information, Amgen; Granowitz E.V. et al. 1992, Cytokine 4:353). In addition, a large proportion of patients develop antibodies against Kineret[®], which potentially neutralize the biological activity of the drug (Fleischmann R.M., et al., 2003, Arthritis Rheum 46:2287).

[0004] New therapeutic techniques therefore focus on active immunization strategies, which induce the production of IL-1-neutralizing antibodies by the immune system of the patient. Svenson and co-workers (2000, J. Immunol. Methods 236:1-8) immunized mice with recombinant IL-1 α chemically crosslinked to purified protein derivative of tuberculin (PPD), and observed the induction of antibodies which neutralized the biological activity of IL-1 α . This strategy relies on the delivery of T-cell help to autoreactive B-cells by physical linkage of the self-antigen to a foreign antigen.

[0005] US patent 6,093,405 discloses a method of reducing the level of a circulating cytokine by immunization with an immunogenic composition containing the chemically or physically inactivated cytokine itself. Whereas in this method native cytokines are rendered immunogenic by physical or chemical treatment, the present invention discloses a method for making native cytokines immunogenic by presenting them in a highly repetitive fashion on the surface of VLPs. WO2003/084979 furthermore describes the use of immunogenic compounds containing cytokine-derived peptides of 5-40 amino acids length for the treatment of diseases associated with an overproduction of cytokines.

SUMMARY OF THE INVENTION

[0006] We have, now, surprisingly found that the inventive compositions and vaccines, respectively, comprising at least one IL-1 molecule, are not only capable of inducing immune responses against IL-1, and hereby in particular antibody responses, but are, furthermore, capable of neutralizing the pro-inflammatory activity of IL-1 in vivo. In addition we have surprisingly found that IL-1 molecule, when covalently linked to the VLP in accordance with the invention, can protect from inflammation and from clinical signs of arthritis in a mouse model of rheumatoid arthritis. Moreover, we have found that the inventive compositions protected mice better from the development of arthritis symptoms than the recombinant IL-1 receptor antagonist Kineret[®], which is approved for the treatment of human rheumatoid arthritis (Example 7). Furthermore, we surprisingly found that compositions of the invention were able to inhibit the development of atherosclerotic symptoms, when injected into genetically susceptible mice (Example 4) and therefore are an efficient treatment for

atherosclerosis. Furthermore, we demonstrated that IL-1 α is involved in the pathogenesis of atherosclerosis.

[0007] Thus, in the first aspect, the present invention provides a composition which comprises (a) a virus-like particle (VLP) with at least one first attachment site; and (b) at least one antigen with at least one second attachment site, wherein said at least one antigen an IL-1 molecule, preferably selected from the group consisting of IL-1 protein, IL-1 mature fragment, IL-1 peptide and IL-1 mutein, wherein (a) and (b) are linked through said at least one first and said at least one second attachment site, preferably to form an ordered and repetitive antigen array. In preferred embodiments of the invention, the virus-like particles suitable for use in the present invention comprises recombinant protein, preferably recombinant coat protein, mutants or fragments thereof, of a virus, preferably of an RNA bacteriophage. In one preferred embodiment, the inventive composition comprises at least one IL-1 mature fragment, preferably comprising the biological activity of IL-1. Thus, the present invention uses the presentation of the self-antigen in a highly repetitive fashion on virus-like particles to stimulate autoreactive B-cells.

[0008] In another aspect, the present invention provides a vaccine composition.

[0009] Furthermore, the present invention provides a method to administering the vaccine composition to a human or an animal, preferably a mammal. The inventive vaccine composition is capable of inducing strong immune response, in particular antibody response, typically and preferably without the presence of at least one adjuvant. Thus, in one preferred embodiment, the vaccine is devoid of an adjuvant. The avoidance of using adjuvant may reduce a possible occurrence of unwanted inflammatory T cell responses.

[0010] In one preferred embodiment, the VLP is a VLP of an RNA bacteriophage. In a further preferred embodiment said RNA bacteriophage is an RNA bacteriophage selected from the group consisting of: Q β , fr, GA and AP205. In a further preferred embodiment said VLP of an RNA bacteriophage comprised by the composition and the vaccine composition, respectively, is recombinantly produced in a host and the VLP of an RNA bacteriophage is essentially free of host RNA, preferably host nucleic acid. It is advantageous to reduce, or preferably to eliminate, the amount of host RNA to avoid unwanted T cell responses as well as other unwanted side effects, such as fever.

[0011] In one aspect, the present invention provides a method of treating a disease selected from the group consisting of: (a) vascular diseases; (b) inherited IL-1-dependent inflammatory diseases; (c) chronic autoimmune inflammatory diseases; (d) bone and cartilage degenerative diseases; (e) allergic diseases; and (f) neurological disease; in which diseases IL-1 protein mediates, or contributes to the condition, wherein the method comprises

administering the inventive composition or the invention vaccine composition, respectively, to an animal, preferably human. Diseases, in which IL-1 protein mediates, or contributes to the condition, are, for example, atherosclerosis, familial Mediterranean fever, rheumatoid arthritis, osteoarthritis, and allergy.

[0012] In a further aspect, the present invention provides a pharmaceutical composition comprising the inventive composition and an acceptable pharmaceutical carrier.

[0013] In again a further aspect, the present invention provides for a method of producing the composition of the invention comprising (a) providing a VLP with at least one first attachment site; (b) providing at least one antigen, wherein said antigen is an IL-1 molecule, an IL-1 protein, an IL-1 mature fragment, an IL-1 peptide or an IL-1 mutein, with at least one second attachment site; and (c) combining said VLP and said at least one antigen to produce said composition, wherein said at least one antigen and said VLP are linked through said at least one first and said at least one second attachment sites.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Coupling of mIL-1 β ₁₁₉₋₂₆₉ protein to Q β capsid protein

[0014] Proteins were analyzed on a 12 % SDS-polyacrylamide gel under reducing conditions. The gel was stained with Coomassie Brilliant Blue. Molecular weights of marker proteins are given in kDa on the left margin, identities of protein bands are indicated on the right margin. Lane 1: Pre stained protein marker (New England Biolabs). Lane 2: derivatized Q β capsid protein. Lane 3: free reduced mIL-1 β ₁₁₉₋₂₆₉ protein. Lane 4: Q β -mIL-1 β ₁₁₉₋₂₆₉ coupling reaction.

Figure 2: Coupling of mIL-1 α ₁₁₇₋₂₇₀ protein to Q β capsid protein

[0015] Proteins were analyzed on a 12 % SDS-polyacrylamide gel under reducing conditions. The gel was stained with Coomassie Brilliant Blue. Molecular weights of marker proteins are given in kDa on the left margin, identities of protein bands are indicated on the right margin. Lane 1: Prestained protein marker (New England Biolabs). Lane 2: derivatized Q β capsid protein. Lane 3: free reduced mIL-1 α ₁₁₇₋₂₇₀ protein. Lane 4: Q β -mIL-1 α ₁₁₇₋₂₇₀ coupling reaction.

DETAILED DESCRIPTION OF THE INVENTION

[0016] Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs.

[0017] Adjuvant: The term “adjuvant” as used herein refers to non-specific stimulators of the immune response or substances that allow generation of a depot in the host which when combined with the vaccine and pharmaceutical composition, respectively, of the present invention may provide for an even more enhanced immune response. Preferred adjuvants are complete and incomplete Freund’s adjuvant, aluminum containing adjuvant, preferably aluminium hydroxide, and modified muramyl dipeptide. Further preferred adjuvants are mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and human adjuvants such as BCG (bacille Calmette Guerin) and *Corynebacterium parvum*. Such adjuvants are also well known in the art. Further adjuvants that can be administered with the compositions of the invention include, but are not limited to, Monophosphoryl lipid immunomodulator, AdjuVax 100a, QS-21, QS-18, CRL1005, Aluminum salts (Alum), MF-59, OM-174, OM-197, OM-294, and Viroosomal adjuvant technology. The adjuvants can also comprise a mixture of these substances. VLP have been generally described as an adjuvant. However, the term “adjuvant”, as used within the context of this application, refers to an adjuvant not being the VLP used for the inventive compositions, rather it relates to an additional, distinct component.

[0018] 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 refers to T-cell epitopes. 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 Th 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.

[0019] epitope: The term epitope refers to continuous or discontinuous portions of an antigen, preferably a polypeptide, wherein said portions can be specifically bound by an

antibody or by a T-cell receptor within the context of an MHC molecule. With respect to antibodies, specific binding excludes non-specific binding but does not necessarily exclude cross-reactivity. An epitope typically comprise 5-10 amino acids in a spatial conformation which is unique to the antigenic site.

[0020] Specific binding (antibody / antigen): Within this application, antibodies are defined to be specifically binding if they bind to the antigen with a binding affinity (K_a) of 10^6 M^{-1} or greater, preferably 10^7 M^{-1} or greater, more preferably 10^8 M^{-1} or greater, and most preferably 10^9 M^{-1} or greater. The affinity of an antibody can be readily determined by one of ordinary skill in the art (for example by Scatchard analysis, by ELISA or by Biacore analysis).

[0021] Specific binding (IL-1 / IL-1 receptor): The interaction between a receptor and a receptor ligand can be characterized by biophysical methods generally known in the art, including, for example, ELISA or Biacore analysis. An IL-1 molecule is regarded as capable of specifically binding an IL-1 receptor, when the binding affinity (K_a) of said IL-1 to said IL-1 receptor is at least 10^5 M^{-1} , preferably at least 10^6 M^{-1} , more preferably at least 10^7 M^{-1} , still more preferably at least 10^8 M^{-1} , and most preferably at least 10^9 M^{-1} ; wherein preferably said IL-1 receptor is an IL-1 receptor from mouse or human, most preferably human. Further preferably, said IL-1 receptor comprises or more preferably consists of any one of the sequences SEQ ID NO:166 to SEQ ID NO:169, most preferably said IL-1 receptor comprises or preferably consists of any one of the sequences SEQ ID NO:166 and SEQ ID NO:167.

[0022] Associated: The terms “associated” or “association” as used herein refer to all possible ways, preferably chemical interactions, by which two molecules are joined together. Chemical interactions include covalent and non-covalent interactions. Typical examples for non-covalent interactions are ionic interactions, hydrophobic interactions or hydrogen bonds, whereas covalent interactions are based, by way of example, on covalent bonds such as ester, ether, phosphoester, amide, peptide, carbon-phosphorus bonds, carbon-sulfur bonds such as thioether, or imide bonds.

[0023] Attachment Site, First: As used herein, the phrase “first attachment site” refers to an element which is naturally occurring with the VLP or which is artificially added to the VLP, and to which the second attachment site may be linked. The first attachment site preferably is 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 chemically reactive group such as an amino group, a carboxyl group, a sulfhydryl group, a hydroxyl group, a guanidinyl group, histidinyl group, or a combination thereof. A preferred embodiment of a chemically reactive group being the first attachment site is the amino group of an amino acid, preferably

of lysine. The first attachment site is located, typically on the surface, and preferably on the outer surface of the VLP. Multiple first attachment sites are present on the surface, preferably on the outer surface of virus-like particle, typically in a repetitive configuration. In a preferred embodiment the first attachment site is associated with the VLP, through at least one covalent bond, preferably through at least one peptide bond. In a further preferred embodiment the first attachment site is naturally occurring with the VLP. Alternatively, in a preferred embodiment the first attachment site is artificially added to the VLP.

[0024] Attachment Site, Second: As used herein, the phrase “second attachment site” refers to an element which is naturally occurring with or which is artificially added to the IL-1 molecule and to which the first attachment site may be linked. The second attachment site of the IL-1 molecule preferably is a protein, a polypeptide, a peptide, an amino acid, a sugar, a polynucleotide, a natural or synthetic polymer, a secondary metabolite or compound (biotin, fluorescein, retinol, digoxigenin, metal ions, phenylmethylsulfonylfluoride), or a chemically reactive group such as an amino group, a carboxyl group, a sulfhydryl group, a hydroxyl group, a guanidinyll group, histidinyll group, or a combination thereof. A preferred embodiment of a chemically reactive group being the second attachment site is the sulfhydryl group, preferably of an amino acid cysteine. The term “IL-1 molecule with at least one second attachment site” refers, therefore, to a construct comprising the IL-1 molecule and at least one second attachment site. However, in particular for a second attachment site, which is not naturally occurring within the IL-1 molecule, such a construct typically and preferably further comprises a “linker”. In another preferred embodiment the second attachment site is associated with the IL-1 molecule through at least one covalent bond, preferably through at least one peptide bond. In a further embodiment, the second attachment site is naturally occurring within the IL-1 molecule. In another further preferred embodiment, the second attachment site is artificially added to the IL-1 molecule through a linker, wherein said linker comprises or alternatively consists of a cysteine. Preferably the linker is fused to the IL-1 molecule by a peptide bond.

[0025] Coat protein: The term “coat protein” and the interchangeably used term “capsid protein” within this application, refers to a viral protein, preferably a subunit of a natural capsid of a virus, preferably of a RNA-phage, which is capable of being incorporated into a virus capsid or a VLP.

[0026] IL-1 molecule: The term “IL-1 molecule” or shortly “IL-1”, as used herein, refers to any polypeptide having an amino acid sequence having at least 80 %, preferably at least 90 %, more preferably at least 95 %, even more preferably at least 99 % and most preferably 100 % sequence identity with any one of the sequences selected from the group consisting of SEQ

ID NO:36 to SEQ ID NO:116, SEQ ID NO:130 to SEQ ID NO:140 and SEQ ID NO:163 to SEQ ID NO:165. The term “IL-1-molecule”, as used herein, preferably refers to any IL-1 protein, IL-1 fragment, IL-1 mature fragment, IL-1 peptide or IL-1 mutein comprising or alternatively consisting of a polypeptide having an amino acid sequence having at least 80 %, preferably at least 90 %, more preferably at least 95 %, even more preferably at least 99 % and most preferably 100 % sequence identity with any one of the sequences selected from the group consisting of SEQ ID NO:36 to SEQ ID NO:116, SEQ ID NO:130 to SEQ ID NO:140 and SEQ ID NO:163 to SEQ ID NO:165. The term IL-1 molecule, as used herein, also typically and preferably refers to orthologs of IL-1 proteins of any animal species. An IL-1 molecule is preferably, but not necessarily, capable of binding to the IL-1 receptor and further preferably comprises biological activity.

[0027] IL-1 alpha molecule: The term “IL-1 alpha molecule” or shortly “IL-1 alpha”, as used herein, refers to an IL-1 alpha protein, IL-1 alpha fragment, IL-1 alpha mature fragment, IL-1 alpha peptide or IL-1 alpha mutein comprising or alternatively consisting of a polypeptide having an amino acid sequence having at least 80 %, preferably at least 90 %, more preferably at least 95 %, even more preferably at least 99 % and most preferably 100 % sequence identity with any one of the sequences selected from the group consisting of SEQ ID NO:36 to 48, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67 to SEQ ID NO:88, and SEQ ID NO:163. A specifically preferred embodiment of IL-1 alpha is human IL-1 alpha 119-271 (SEQ ID NO:63).

[0028] IL-1 beta molecule: The term “IL-1 beta molecule” or shortly “IL-1 beta”, as used herein, refers to an IL-1 beta protein, IL-1 beta fragment, IL-1 beta mature fragment, IL-1 beta peptide or IL-1 beta mutein comprising or alternatively consisting of a polypeptide having an amino acid sequence having at least 80 %, preferably at least 90 %, more preferably at least 95 %, even more preferably at least 99 % and most preferably 100 % sequence identity with any one of the sequences selected from the group consisting of SEQ ID NO:49 to SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:89 to SEQ ID NO:116, SEQ ID NO:130 to SEQ ID NO:140, SEQ ID NO:164, and SEQ ID NO:165. A specifically preferred embodiment of IL-1 beta is human IL-1 beta 117-269 (SEQ ID NO:64).

[0029] IL-1 protein: The term “IL-1 protein”, as used herein, refers to a naturally occurring protein, wherein said naturally occurring protein has an amino acid sequence having at least 80 %, preferably at least 90 %, more preferably at least 95 %, even more preferably at least 99 % and most preferably 100 % sequence identity with any one of SEQ ID NO:36 to SEQ ID NO:62; or wherein said naturally occurring protein is capable of binding the IL-1 receptor and preferably comprises biological activity. The term “IL-1 protein”, as used herein, preferably

refers to a naturally occurring protein, wherein said naturally occurring protein has an amino acid sequence having at least 80 %, preferably at least 90 %, more preferably at least 95 %, even more preferably at least 99 % and most preferably 100 % sequence identity with any one of SEQ ID NO:36 to SEQ ID NO:62; and wherein said naturally occurring protein is capable of binding the IL-1 receptor and preferably comprises biological activity. Typically and preferably, the term “IL-1 protein”, as used herein, refers to at least one naturally occurring protein, wherein said protein is capable of binding the IL-1 receptor and comprises biological activity, and wherein further said protein comprises or alternatively consists of a polypeptide having an amino acid sequence having at least 80 %, preferably at least 90 %, more preferably at least 95 %, even more preferably at least 99 % and most preferably 100 % sequence identity with any one of SEQ ID NO:36 to SEQ ID NO:62. Accordingly, the term “IL-1 alpha protein” relates to an IL-1 protein comprising or alternatively consisting of a polypeptide having an amino acid sequence having at least 80 %, preferably at least 90 %, more preferably at least 95 %, even more preferably at least 99 % and most preferably 100 % sequence identity with any one of SEQ ID NO:36 to SEQ ID NO:48, whereas the term “IL-1 beta protein” relates to an IL-1 protein comprising or alternatively consisting of a polypeptide having an amino acid sequence having at least 80 %, preferably at least 90 %, more preferably at least 95 %, even more preferably at least 99 % and most preferably 100 % sequence identity with any one of SEQ ID NO:49 to SEQ ID NO:62.

[0030] IL-1 fragment: The term “IL-1 fragment”, as used herein, relates to a polypeptide comprising a consecutive stretch of an IL-1 protein, wherein said polypeptide is at least 50, preferably at least 100, most preferably at least 150 amino acids in length. Typically and preferably said IL-1 fragment is at most 300, more preferably at most 250, and most preferably at most 200 amino acids in length. Typically and preferably, IL-1 fragments are capable of binding the IL-1 receptor and further preferably comprises biological activity. Accordingly, the terms “IL-1 alpha fragment” and “IL-1 beta fragment” relate to an IL-1 fragment as defined, wherein said IL-1 protein is an IL-1 alpha protein or an IL-1 beta protein, respectively.

[0031] IL-1 mature fragment: The term “IL-1 mature fragment”, as used herein, relates to a IL-1 fragment, wherein said IL-1 fragment is a naturally occurring maturation product of an IL-1 protein. Accordingly, the terms “IL-1 alpha mature fragment” and “IL-1 beta mature fragment”, as used herein relate to IL-1 mature fragments as defined, wherein said IL-1 protein is an IL-1 alpha protein or an IL-1 beta protein, respectively. Preferred embodiments of IL-1 alpha mature fragments are SEQ ID NO:63, SEQ ID NO:65 and SEQ ID NO:163.

Preferred embodiments of IL-1 beta mature fragments are SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:130, SEQ ID NO:164, and SEQ ID NO:165.

[0032] Preferred IL-1 alpha mature fragments comprise or preferably consist of an amino acid sequence selected from the group consisting of: (a) human IL-1 alpha 119-271 (SEQ ID NO:63); (b) mouse IL-1 alpha 117-270 (SEQ ID NO:65); (c) mouse IL-1 alpha 117-270s (SEQ ID NO:163); and (e) an amino acid sequence which is at least 80 %, or preferably at least 90 %, more preferably at least 95 %, or most preferably at least 99 % identical with any one of SEQ ID NO:63, SEQ ID NO:65 and SEQ ID NO:163.

[0033] Preferred IL-1 beta mature fragments comprise or preferably consist of an amino acid sequence selected from the group consisting of: (a) human IL-1 beta 117-269 (SEQ ID NO:64); (b) human IL-1 beta 116-269 (SEQ ID NO:165); (c) mouse IL-1 beta 119-269 (SEQ ID NO:66); (d) mouse IL-1 beta 119-269s (SEQ ID NO:164); and (e) an amino acid sequence which is at least 80 %, or preferably at least 90 %, more preferably at least 95 %, or most preferably at least 99 % identical with any one of SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:164 and SEQ ID NO:165.

[0034] IL-1 peptide: The term "IL-1 peptide", as used herein, relates to a polypeptide comprising a consecutive stretch of a naturally occurring protein, wherein said protein is capable of binding the IL-1 receptor and preferably comprises biological activity, wherein said polypeptide is 4 to 49, preferably 6 to 35, most preferably 10 to 25 amino acids in length. The IL-1 peptide may be, but typically is not, capable of binding the IL-1 receptor and typically has no biological activity. Accordingly, the terms "IL-1 alpha peptide" and "IL-1 beta peptide", as used herein relate to IL-1 peptides as defined, wherein said naturally occurring protein is an IL-1 alpha protein or an IL-1 beta protein, respectively. Preferred IL-1 peptides are SEQ ID NO:82 to SEQ ID NO:116.

[0035] IL-1 mutein: The term "IL-1 mutein" as used herein comprise or preferably consist of any polypeptide derived from an IL-1 molecule, preferably from an IL-1 alpha or an IL-1 beta protein, an IL-1 alpha or an IL-1 beta fragment, an IL-1 alpha or an IL-1 beta mature fragment or an IL-1 alpha or an IL-1 beta peptide, wherein preferably said polypeptide exhibits reduced biological activity as compared to the IL-1 molecule it is derived from. Accordingly, IL-1 alpha muteins and IL-1 beta muteins are IL-1 muteins as defined, wherein said polypeptide is derived from an IL-1 alpha molecule or an IL-1 beta molecule, respectively.

[0036] In preferred IL-1 muteins, said biological activity is less than 80 %, more preferably less than 60 %, still more preferably less than 40 %, still more preferably less than 20 % of the biological activity of the IL-1 molecule it is derived from. Further preferred IL-1 muteins

are derived from an IL-1 mature fragment, wherein the biological activity of said IL-1 mutein is less than 80 %, more preferably less than 60 %, still more preferably less than 40 %, still more preferably less than 20 % of the biological activity of the IL-1 mature fragment said IL-1 mutein is derived from. Very preferred IL-1 muteins do not exhibit biological activity. Further preferably, but not necessarily, IL-1 muteins are capable of specifically binding an IL-1 receptor. Very preferred are IL-1 muteins derived from (i) an IL-1 protein, preferably from SEQ ID NO:36 to SEQ ID NO:62; or (ii) more preferably of an IL-1 mature fragment, preferably from any one of SEQ ID NO:63 to SEQ ID NO:66, SEQ ID NO:130, and SEQ ID NO:163 to SEQ ID NO:165.

[0037] IL-1 muteins useful in the context have been described in Kamogashira et al. (1988) J. Biochem. 104:837-840; Gehrke et al. (1990) The Journal of Biological Chemistry 265(11):5922-5925; Conca et al. (1991) The Journal of Biological Chemistry 266(25):16265-16268; Ju et al. (1991) PNAS 88:2658-2662; Auron et al. (1992) Biochemistry 31:6632-6638; Guinet et al. (1993) Eur. J. Biochem 211:583-590; Camacho (1993) Biochemistry 32:8749-8757; Baumann (1993) Journal of Receptor Research 13(1-4):245-262; Simon (1993) The Journal of Biological Chemistry 268(13):9771-9779; and Simoncsits (1994) Cytokine 6(2):206-214, the disclosure of which is incorporated herein by reference.

[0038] Preferred IL-1 muteins comprise or preferably consist of a polypeptide having an amino acid sequence which differs from the amino acid sequence of an IL-1 protein, an IL-1 fragment, an IL-1 mature fragment or an IL-1 peptide in 1 to 10, preferably 1 to 6, more preferably 1 to 5, still more preferably 1 to 4, still more preferably 1 to 3, still more preferably 1 to 2, and most preferably in exactly 1 amino acid residue(s), wherein preferably said amino acid residue(s) are (i) deleted from said polypeptide, (ii) inserted into said polypeptide, (iii) exchanged by another amino acid residue, or (iv) any combination of (i) to (iii). In a preferred embodiment, said amino acid residues are in one consecutive stretch. Further preferred IL-1 muteins comprise or preferably consist of a polypeptide having an amino acid sequence which differs from the amino acid sequence of an IL-1 protein, an IL-1 fragment, or an IL-1 mature fragment, preferably of an IL-1 mature fragment, in 1 to 10, preferably 1 to 6, more preferably 1 to 5, still more preferably 1 to 4, still more preferably 1 to 3, still more preferably 1 to 2, and most preferably in exactly 1 amino acid residue(s), wherein preferably said amino acid residue(s) are (i) deleted from said polypeptide, (ii) inserted into said polypeptide, (iii) exchanged by another amino acid residue, or (iv) any combination of (i) to (iii).

[0039] Further preferred IL-1 muteins comprise or more preferably consist of a polypeptide having an amino acid sequence which differs from the amino acid sequence of any one of SEQ ID NO:36 to SEQ ID NO:48 or SEQ ID NO:49 to SEQ ID NO:62 in 1 to 10, preferably

1 to 6, more preferably 1 to 5, still more preferably 1 to 4, still more preferably 1 to 3, still more preferably 1 to 2, and most preferably in exactly 1 amino acid residue(s), wherein preferably said amino acid residue(s) are (i) deleted from said polypeptide, (ii) inserted into said polypeptide, (iii) exchanged by another amino acid residue, or (iv) any combination of (i) to (iii). Further preferred IL-1 muteins comprise or preferably consist of a polypeptide having an amino acid sequence which differs from the amino acid sequence selected from the group consisting of (i) any one of SEQ ID NO:63, SEQ ID NO:65, and SEQ ID NO:163, most preferably SEQ ID NO:63; or (ii) of any one selected from the group consisting of SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:130, SEQ ID NO:164, and SEQ ID NO:165, most preferably SEQ ID NO:64 in 1 to 10, preferably 1 to 6, more preferably 1 to 5, still more preferably 1 to 4, still more preferably 1 to 3, still more preferably 1 to 2, and most preferably in exactly 1 amino acid residue(s), wherein preferably said amino acid residue(s) are (i) deleted from said polypeptide, (ii) inserted into said polypeptide, (iii) exchanged by another amino acid residue, or (iv) any combination of (i) to (iii).

[0040] Further preferred IL-1 muteins are IL-1 alpha muteins, wherein said IL-1 alpha muteins comprise or more preferably consist of a polypeptide having an amino acid sequence which differs from the amino acid sequence of any one of SEQ ID NO:36 to SEQ ID NO:48 in 1 to 6, preferably 1 to 5, more preferably 1 to 4, still more preferably 1 to 3, still more preferably 1 to 2, and most preferably in exactly 1 amino acid residue(s), wherein preferably said amino acid residue(s) are (i) deleted from said polypeptide, (ii) inserted into said polypeptide, (iii) exchanged by another amino acid residue, or (iv) any combination of (i) to (iii). Further preferred IL-1 alpha muteins comprise or preferably consist of a polypeptide having an amino acid sequence which differs from the amino acid sequence selected from the group consisting of (i) any one of SEQ ID NO:63, SEQ ID NO:65, and SEQ ID NO:163, most preferably SEQ ID NO:63, in 1 to 6, preferably 1 to 5, more preferably 1 to 4, still more preferably 1 to 3, still more preferably 1 to 2, and most preferably in exactly 1 amino acid residue(s), wherein preferably said amino acid residue(s) are (i) deleted from said polypeptide, (ii) inserted into said polypeptide, (iii) exchanged by another amino acid residue, or (iv) any combination of (i) to (iii). Very preferred IL-1 alpha muteins comprise or preferably consist of a polypeptide having an amino acid sequence which differs from the amino acid sequence of SEQ ID NO:63 in 1 to 10, preferably 1 to 6, more preferably 1 to 5, still more preferably 1 to 4, still more preferably 1 to 3, still more preferably 1 to 2, and most preferably in exactly 1 amino acid residue(s), wherein preferably said amino acid residue(s) are (i) deleted from said polypeptide, (ii) inserted into said polypeptide, (iii) exchanged by another amino acid residue, or (iv) any combination of (i) to (iii).

[0041] Further preferred IL-1 muteins are IL-1 beta muteins, wherein said IL-1 beta muteins comprise or more preferably consist of a polypeptide having an amino acid sequence which differs from the amino acid sequence of any one of SEQ ID NO:49 to SEQ ID NO:62 in 1 to 6, preferably 1 to 5, more preferably 1 to 4, still more preferably 1 to 3, still more preferably 1 to 2, and most preferably in exactly 1 amino acid residue(s), wherein preferably said amino acid residue(s) are (i) deleted from said polypeptide, (ii) inserted into said polypeptide, (iii) exchanged by another amino acid residue, or (iv) any combination of (i) to (iii). Further preferred IL-1 beta muteins comprise or preferably consist of a polypeptide having an amino acid sequence which differs from the amino acid sequence selected from the group consisting of SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:130, SEQ ID NO:164, and SEQ ID NO:165, most preferably SEQ ID NO:64, in 1 to 6, preferably 1 to 5, more preferably 1 to 4, still more preferably 1 to 3, still more preferably 1 to 2, and most preferably in exactly 1 amino acid residue(s), wherein preferably said amino acid residue(s) are (i) deleted from said polypeptide, (ii) inserted into said polypeptide, (iii) exchanged by another amino acid residue, or (iv) any combination of (i) to (iii). Very preferred IL-1 beta muteins comprise or preferably consist of a polypeptide having an amino acid sequence which differs from the amino acid sequence of SEQ ID NO:64 in 1 to 10, preferably 1 to 6, more preferably 1 to 5, still more preferably 1 to 4, still more preferably 1 to 3, still more preferably 1 to 2, and most preferably in exactly 1 amino acid residue(s), wherein preferably said amino acid residue(s) are (i) deleted from said polypeptide, (ii) inserted into said polypeptide, (iii) exchanged by another amino acid residue, or (iv) any combination of (i) to (iii). Still more preferred IL-1 beta muteins comprise or preferably consist of a polypeptide having an amino acid sequence selected from any one of the group consisting of SEQ ID NO:131 to SEQ ID NO:140.

[0042] Agonistic effect/biological activity of the IL-1: The terms “biological activity” or “biologically active” as used herein with respect to IL-1 refer to the ability of the IL-1 molecule to induce the production of IL-6 after systemical administration into animals, preferably as outlined in Example 2 E. and in Example 3 E. By biological activity of the IL-1 molecule is also meant the ability to induce the proliferation of thymocytes (Epps et al., Cytokine 9(3):149-156 (1997), D10.G4.1 T helper cells (Orencole and Dinarello, Cytokine 1(1):14-22 (1989), or the ability to induce the production of IL-6 from MG64 or HaCaT cells (Boraschi et al., J. Immunol. 155:4719-4725 (1995) or fibroblasts (Dinarello et al., Current Protocols in Immunology 6.2.1-6-2-7 (2000)), or the production of IL-2 from EL-4 thymoma cells (Simon et al., J. Immunol. Methods 84(1-2):85-94 (1985)), or the ability to inhibit the growth of the human melanoma cell line A375 (Nakai et al., Biochem. Biophys. Res. Commun. 154:1189-1196 (1988)).

[0043] Linked: The terms “linked” or “linkage” as used herein, refer to all possible ways, preferably chemical interactions, by which the at least one first attachment site and the at least one second attachment site are joined together. Chemical interactions include covalent and non-covalent interactions. Typical examples for non-covalent interactions are ionic interactions, hydrophobic interactions or hydrogen bonds, whereas covalent interactions are based, by way of example, on covalent bonds such as ester, ether, phosphoester, amide, peptide, carbon-phosphorus bonds, carbon-sulfur bonds such as thioether, or imide bonds. In certain preferred embodiments the first attachment site and the second attachment site are linked through at least one covalent bond, preferably through at least one non-peptide bond, and even more preferably through exclusively non-peptide bond(s). The term “linked” as used herein, however, shall not only refer to a direct linkage of the at least one first attachment site and the at least one second attachment site but also, alternatively and preferably, an indirect linkage of the at least one first attachment site and the at least one second attachment site through intermediate molecule(s), and hereby typically and preferably by using at least one, preferably one, heterobifunctional cross-linker. In other preferred embodiments the first attachment site and the second attachment site are linked through at least one covalent bond, preferably through at least one peptide bond, and even more preferably through exclusively peptide bond(s). In a very preferred embodiment the first attachment site and the second attachment site are linked exclusively by peptide bonds, preferably by genetic fusion, either directly, or, preferably, via an amino acid linker. In a further preferred embodiment the second attachment site is linked to the C-terminus of said first attachment site exclusively by peptide bonds, preferably by genetic fusion.

[0044] Linker: A “linker”, as used herein, either associates the second attachment site with the IL-1 molecule or already comprises, essentially consists of, or consists of the second attachment site. Preferably, a “linker”, as used herein, already comprises the second attachment site, typically and preferably - but not necessarily - as one amino acid residue, preferably as a cysteine residue. A “linker” as used herein is also termed “amino acid linker”, in particular when a linker according to the invention contains at least one amino acid residue. Thus, the terms “linker” and “amino acid linker” are interchangeably used herein. However, this does not imply that such a linker consists exclusively of amino acid residues, even if a linker consisting of amino acid residues is a preferred embodiment of the present invention. The amino acid residues of the linker are, preferably, composed of naturally occurring amino acids or unnatural amino acids known in the art, all-L or all-D or mixtures thereof. Further preferred embodiments of a linker in accordance with this invention are molecules comprising a sulfhydryl group or a cysteine residue and such molecules are, therefore, also encompassed

within this invention. Further linkers useful for the present invention are molecules comprising a C1-C6 alkyl-, a cycloalkyl such as a cyclopentyl or cyclohexyl, a cycloalkenyl, aryl or heteroaryl moiety. Moreover, linkers comprising preferably a C1-C6 alkyl-, cycloalkyl- (C5, C6), aryl- or heteroaryl- moiety and additional amino acid(s) can also be used as linkers for the present invention and shall be encompassed within the scope of the invention. Association of the linker with the IL-1 molecule is preferably by way of at least one covalent bond, more preferably by way of at least one peptide bond. In the context of linkage by genetic fusion, a linker may be absent or preferably is an amino acid linker, more preferably an amino acid linker consisting exclusively of amino acid residues. Very preferred linkers for genetic fusion are flexible amino acid linkers. In the context of linkage by genetic fusion linkers preferred consist of 1 to 20, more preferably of 2 to 15, still more preferably of 2 to 10, still more preferably of 2 to 5, and most preferably of 3 amino acids. Very preferred linkers for genetic fusion comprise or preferably consist of GSG (SEQ ID NO:189).

[0045] Ordered and repetitive antigen array: As used herein, the term “ordered and repetitive antigen array” generally refers to a repeating pattern of antigen or, characterized by a typically and preferably high order of uniformity in spacial arrangement of the antigens with respect to virus-like particle, respectively. In one embodiment of the invention, the repeating pattern may be a geometric pattern. Certain embodiments of the invention, such as antigens coupled to the VLP of RNA bacteriophages, are typical and preferred examples of suitable ordered and repetitive antigen arrays which, moreover, possess strictly repetitive paracrystalline orders of antigens, preferably with spacing of 1 to 30 nanometers, preferably 2 to 15 nanometers, even more preferably 2 to 10 nanometers, even again more preferably 2 to 8 nanometers, and further more preferably 1.6 to 7 nanometers.

[0046] Packaged: The term “packaged” as used herein refers to the state of a polyanionic macromolecule or immunostimulatory substances in relation to the VLP. The term “packaged” as used herein includes binding that may be covalent, e.g., by chemically coupling, or non-covalent, e.g., ionic interactions, hydrophobic interactions, hydrogen bonds, etc. The term also includes the enclosement, or partial enclosement, of a polyanionic macromolecule. Thus, the polyanionic macromolecule or immunostimulatory substances can be enclosed by the VLP without the existence of an actual binding, in particular of a covalent binding. In preferred embodiments, the at least one polyanionic macromolecule or immunostimulatory substances is packaged inside the VLP, most preferably in a non-covalent manner. In case said immunostimulatory substances is nucleic acid, preferably a DNA, the term packaged implies that said nucleic acid is not accessible to nucleases hydrolysis, preferably not accessible to DNase hydrolysis (e.g. DNaseI or Benzonase), wherein

preferably said accessibility is assayed as described in Examples 11-17 of WO2003/024481A2.

[0047] Polypeptide: The term “polypeptide” as used herein refers to a molecule composed of monomers (amino acids) linearly linked by amide bonds (also known as peptide bonds). It indicates a molecular chain of amino acids and does not refer to a specific length of the product. Thus, peptides, dipeptides, tripeptides, oligopeptides and proteins are included within the definition of polypeptide. Post-translational modifications of the polypeptide, for example, glycosylations, acetylations, phosphorylations, and the like are also encompassed.

[0048] Recombinant VLP: The term “recombinant VLP”, as used herein, refers to a VLP that is obtained by a process which comprises at least one step of recombinant DNA technology. The term “VLP recombinantly produced”, as used herein, refers to a VLP that is obtained by a process which comprises at least one step of recombinant DNA technology. Thus, the terms “recombinant VLP” and “VLP recombinantly produced” are interchangeably used herein and should have the identical meaning.

[0049] 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.).

[0050] Virus-like particle (VLP), as used herein, refers to a non-replicative or non-infectious, preferably a non-replicative and non-infectious virus particle, or refers to a non-replicative or non-infectious, preferably a non-replicative and non-infectious structure resembling a virus particle, preferably a capsid of a virus. The term “non-replicative”, as used herein, refers to being incapable of replicating the genome comprised by the VLP. The term “non-infectious”, as used herein, refers to being incapable of entering the host cell. Preferably a virus-like particle in accordance with the invention is non-replicative and/or non-infectious since it lacks all or part of the viral genome or genome function. In one embodiment, a virus-like particle is a virus particle, in which the viral genome has been physically or chemically inactivated. Typically and more preferably a virus-like particle lacks all or part of 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, preferably RNA bacteriophage. The terms “viral capsid” or “capsid”, refer to a macromolecular assembly composed of viral protein subunits. Typically, there are 60, 120, 180, 240, 300, 360 and more than 360 viral protein subunits. Typically and preferably, the interactions of these subunits lead to the formation of viral capsid or viral-capsid like structure with an inherent

repetitive organization, wherein said structure is, typically, spherical or tubular. For example, the capsids of RNA bacteriophages or HBcAgs 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 resembling the capsid morphology in the above defined sense but deviating from the typical symmetrical assembly while maintaining a sufficient degree of order and repetitiveness. One common feature of virus particle and virus-like particle is its highly ordered and repetitive arrangement of its subunits.

[0051] Virus-like particle of an RNA bacteriophage: As used herein, the term “virus-like particle of an RNA bacteriophage” refers to a virus-like particle comprising, or preferably consisting essentially of or consisting of coat proteins, mutants or fragments thereof, of an RNA bacteriophage. In addition, virus-like particle of an RNA bacteriophage resembling the structure of an RNA bacteriophage, being non replicative and/or non-infectious, and lacking at least the gene or genes encoding for the replication machinery of the RNA 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 RNA bacteriophages, in which the aforementioned gene or genes are still present but inactive, and, therefore, also leading to non-replicative and/or non-infectious virus-like particles of an RNA bacteriophage. Preferred VLPs derived from RNA bacteriophages exhibit icosahedral symmetry and consist of 180 subunits (monomers). Preferred methods to render a virus-like particle of an RNA bacteriophage non replicative and/or non-infectious is by physical, chemical inactivation, such as UV irradiation, formaldehyde treatment, typically and preferably by genetic manipulation.

[0052] 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.

[0053] The amino acid sequence identity of polypeptides can be determined conventionally using known computer programs such as the Bestfit program. When using Bestfit or any other sequence alignment program, preferably using Bestfit, to determine whether a particular sequence is, for instance, 95 % identical to a reference amino acid sequence, the parameters are set such that the percentage of identity is calculated over the full length of the reference amino acid sequence and that gaps in homology of up to 5% of the total number of amino acid residues in the reference sequence are allowed. This aforementioned method in determining the percentage of identity between polypeptides is applicable to all proteins, polypeptides or a fragment thereof disclosed in this invention.

[0054] This invention provides compositions and methods for enhancing immune responses against IL-1 in an animal or in human. Compositions of the invention comprise: (a) a core

particle with at least one first attachment site, wherein said core particle is a virus-like particle (VLP) or a virus particle; and (b) at least one antigen with at least one second attachment site, wherein the at least one antigen is an IL-1 molecule, preferably selected from the group consisting of IL-1 protein, IL-1 mature fragment, IL-1 peptide and IL-1 mutein, wherein (a) and (b) are covalently linked through the at least one first and the at least one second attachment site. Preferably, said IL-1 molecule is linked to the core particle, so as to form an ordered and repetitive antigen-VLP array. In preferred embodiments of the invention, at least 20, preferably at least 30, more preferably at least 60, again more preferably at least 120 and further more preferably at least 180 IL-1 molecules are linked to the core particle.

[0055] Any virus known in the art having an ordered and repetitive structure may be selected as a VLP or a virus particle of the invention. Illustrative DNA or RNA viruses, the coat or capsid protein of which can be used for the preparation of VLPs have been disclosed in WO 2004/009124 on page 25, line 10-21, on page 26, line 11-28, and on page 28, line 4 to page 31, line 4. These disclosures are incorporated herein by way of reference.

[0056] Virus or virus-like particle can be produced and purified from virus-infected cell cultures. The resulting virus or virus-like particle for vaccine purpose should be preferably non-replicative or non-infectious, more preferably non-replicative and non-infectious. UV irradiation, chemical treatment, such as with formaldehyde or chloroform, are the general methods known to skilled person in the art to inactivate virus.

[0057] In one preferred embodiment, the core particle is a virus particle, and wherein preferably said virus particle is a bacteriophage, and wherein further preferably said bacteriophage is an RNA bacteriophage, and wherein even further preferably said RNA bacteriophage is an RNA bacteriophage selected from Q β , fr, GA or AP205.

[0058] In one preferred embodiment, the core particle is a VLP. In a further preferred embodiment, the VLP is a recombinant VLP. Almost all commonly known viruses have been sequenced and are readily available to the public. The gene encoding the coat protein can be easily identified by a skilled artisan. The preparation of VLPs by recombinantly expressing the coat protein in a host is within the common knowledge of a skilled artisan.

[0059] In one preferred embodiment, the virus-like particle comprises, or alternatively consists of, recombinant proteins, mutants or fragments thereof, of a virus selected from the group consisting of: a) RNA bacteriophages; b) bacteriophages; c) Hepatitis B virus, preferably its capsid protein (Ulrich, et al., Virus Res. 50:141-182 (1998)) or its surface protein (WO 92/11291); d) measles virus (Warnes, et al., Gene 160:173-178 (1995)); e) Sindbis virus; f) rotavirus (US 5,071,651 and US 5,374,426); g) foot-and-mouth-disease virus (Twomey, et al., Vaccine 13:1603-1610, (1995)); h) Norwalk virus (Jiang, X., et al., Science

250:1580 1583 (1990); Matsui, S.M., et al., J. Clin. Invest. 87:1456 1461 (1991)); i) Alphavirus; j) retrovirus, preferably its GAG protein (WO 96/30523); k) retrotransposon Ty, preferably the protein p1; l) human Papilloma virus (WO 98/15631); m) Polyoma virus; n) Tobacco mosaic virus; and o) Flock House Virus.

[0060] VLP comprising more than one different recombinant proteins is generally referred, in this application, as mosaic VLP. In one embodiment, the VLP is a mosaic VLP, wherein said mosaic VLP comprises, or consists of, more than one recombinant protein, preferably of two recombinant proteins, most preferably of two recombinant capsid proteins, mutants or fragments thereof.

[0061] The term “fragment of a recombinant protein” or the term “fragment of a coat protein”, as used herein, is defined as a polypeptide, which is of at least 70%, preferably at least 80%, more preferably at least 90%, even more preferably at least 95% the length of the wild-type recombinant protein, or coat protein, respectively and which preferably retains the capability of forming VLP. Preferably, the fragment is obtained by at least one internal deletion, at least one truncation or at least one combination thereof. Further preferably, the fragment is obtained by at most 5, 4, 3 or 2 internal deletions, by at most 2 truncations or by exactly one combination thereof.

[0062] The term “fragment of a recombinant protein” or “fragment of a coat protein” shall further refer to a polypeptide, which has at least 80%, preferably 90%, even more preferably 95% amino acid sequence identity with the “fragment of a recombinant protein” or “fragment of a coat protein”, respectively, as defined above and which is preferably capable of assembling into a virus-like particle.

[0063] The term “mutant coat protein” refers to a polypeptide having an amino acid sequence derived from the wild type recombinant protein, or coat protein, respectively, wherein the amino acid sequence is at least 80 %, preferably at least 85 %, 90 %, 95 %, 97 %, or 99 % identical to the wild type sequence and preferably retains the ability to assemble into a VLP.

[0064] In one preferred embodiment, the virus-like particle of the invention is of Hepatitis B virus. The preparation of Hepatitis B virus-like particles has been disclosed, inter alia, in WO 00/32227, WO 01/85208 and in WO 01/056905. All three documents are explicitly incorporated herein by way of reference. Other variants of HBcAg suitable for use in the practice of the present invention have been disclosed in page 34-39 of WO 01/056905.

[0065] In one further preferred embodiment of the invention, a lysine residue is introduced into the HBcAg polypeptide, to mediate the linking of IL-1 molecule to the VLP of HBcAg. In preferred embodiments, VLPs and compositions of the invention are prepared using a HBcAg comprising, or alternatively consisting of, amino acids 1-144, or 1-149, 1-185 of SEQ

ID NO:1, which is modified so that the amino acids at positions 79 and 80 are replaced with a peptide having the amino acid sequence of Gly-Gly-Lys-Gly-Gly (SEQ ID NO:170). This modification changes the SEQ ID NO:1 to SEQ ID NO:2. In further preferred embodiments, the cysteine residues at positions 48 and 110 of SEQ ID NO:2, or its corresponding fragments, preferably 1-144 or 1-149, are mutated to serine. The invention further includes compositions comprising Hepatitis B core protein mutants having above noted corresponding amino acid alterations. The invention further includes compositions and vaccines, respectively, comprising HBcAg polypeptides which comprise, or alternatively consist of, amino acid sequences which are at least 80%, 85%, 90%, 95%, 97% or 99% identical to SEQ ID NO:2.

[0066] In one preferred embodiment of the invention, the virus-like particle of the invention comprises, consists essentially of, or alternatively consists of, recombinant coat proteins, mutants or fragments thereof, of an RNA bacteriophage. Preferably, the RNA bacteriophage is selected from the group consisting of a) bacteriophage Q β ; b) bacteriophage R17; c) bacteriophage fr; d) bacteriophage GA; e) bacteriophage SP; f) bacteriophage MS2; g) bacteriophage M11; h) bacteriophage MX1; i) bacteriophage NL95; k) bacteriophage f2; l) bacteriophage PP7 and m) bacteriophage AP205.

[0067] In one preferred embodiment of the invention, the composition comprises coat protein, mutants or fragments thereof, of RNA bacteriophages, wherein the coat protein has amino acid sequence selected from the group consisting of: (a) SEQ ID NO:3 referring to Q β CP; (b) a mixture of SEQ ID NO:3 and SEQ ID NO:4 (Q β A1 protein); (c) SEQ ID NO:5 (R17 capsid protein); (d) SEQ ID NO:6 (fr capsid protein); (e) SEQ ID NO:7 (GA capsid protein); (f) SEQ ID NO:8 (SP capsid protein); (g) a mixture of SEQ ID NO:8 and SEQ ID NO:9; (h) SEQ ID NO:10 (MS2 capsid protein); (i) SEQ ID NO:11 (M11 capsid protein); (j) SEQ ID NO:12 (MX1 capsid protein); (k) SEQ ID NO:13 (NL95 capsid protein); (l) SEQ ID NO:14 (f2 capsid protein); (m) SEQ ID NO:15 (PP7 capsid protein); and (n) SEQ ID NO:21 (AP205 capsid protein).

[0068] In one preferred embodiment of the invention, the VLP is a mosaic VLP comprising or alternatively consisting of more than one amino acid sequence, preferably two amino acid sequences, of coat proteins, mutants or fragments thereof, of an RNA bacteriophage.

[0069] In one very preferred embodiment, the VLP comprises or alternatively consists of two different coat proteins of an RNA bacteriophage, said two coat proteins have an amino acid sequence of CP Q β (SEQ ID NO: 3) and CP Q β A1 (SEQ ID NO:4), or of CP SP (SEQ ID NO:8) and CP SP A1 (SEQ ID NO:9).

[0070] In preferred embodiments of the present invention, the virus-like particle of the invention comprises, or alternatively consists essentially of, or alternatively consists of recombinant coat proteins, mutants or fragments thereof, of the RNA-bacteriophage Q β , fr, AP205 or GA.

[0071] In one preferred embodiment, the VLP of the invention is a VLP of RNA bacteriophage Q β . The capsid or virus-like particle of Q β showed an icosahedral phage-like capsid structure with a diameter of 25 nm and T=3 quasi symmetry. The capsid contains 180 copies of the coat protein, which are linked in covalent pentamers and hexamers by disulfide bridges (Golmohammadi, R. et al., Structure 4:543-5554 (1996)), leading to a remarkable stability of the Q β capsid. Capsids or VLPs made from recombinant Q β coat protein may contain, however, subunits not linked via disulfide bonds to other subunits within the capsid, or incompletely linked. The capsid or VLP of Q β shows unusual resistance to organic solvents and denaturing agents. Surprisingly, we have observed that DMSO and acetonitrile concentrations as high as 30 %, and guanidinium concentrations as high as 1 M do not affect the stability of the capsid. The high stability of the capsid or VLP of Q β is an advantageous feature, in particular, for its use in immunization and vaccination of mammals and humans in accordance of the present invention.

[0072] Further preferred virus-like particles of RNA bacteriophages, in particular of Q β and fr in accordance of this invention are disclosed in WO 02/056905, the disclosure of which is herewith incorporated by reference in its entirety. Particular example 18 of WO 02/056905 gave detailed description of preparation of VLP particles from Q β .

[0073] In another preferred embodiment, the VLP of the invention is a VLP of RNA bacteriophage AP205. Assembly-competent mutant forms of AP205 VLPs, including AP205 coat protein with the substitution of proline at amino acid 5 to threonine, may also be used in the practice of the invention and leads to other preferred embodiments of the invention. WO 2004/007538 describes, in particular in Example 1 and Example 2, how to obtain VLP comprising AP205 coat proteins, and hereby in particular the expression and the purification thereto. WO 2004/007538 is incorporated herein by way of reference. AP205 VLPs are highly immunogenic, and can be linked with IL-1 molecule to typically and preferably generate vaccine constructs displaying the IL-1 molecule oriented in a repetitive manner.

[0074] In one preferred embodiment, the VLP of the invention comprises or consists of a mutant coat protein of a virus, preferably an RNA bacteriophage, wherein the mutant coat protein has been modified by removal of at least one lysine residue by way of substitution and/or by way of deletion. In another preferred embodiment, the VLP of the invention comprises or consists of a mutant coat protein of a virus, preferably an RNA bacteriophage,

wherein the mutant coat protein has been modified by addition of at least one lysine residue by way of substitution and/or by way of insertion. The deletion, substitution or addition of at least one lysine residue allows varying the degree of coupling, i.e. the amount of IL-1 molecule per subunits of the VLP of a virus, preferably of an RNA bacteriophages, in particular, to match and tailor the requirements of the vaccine.

[0075] In one preferred embodiment, the compositions and vaccines of the invention have an antigen density being from 0.5 to 4.0. The term “antigen density”, as used herein, refers to the average number of IL-1 molecules which is linked per subunit, preferably per coat protein, of the VLP, and hereby preferably of the VLP of an RNA bacteriophage. Thus, this value is calculated as an average over all the subunits of the VLP, preferably of the VLP of the RNA bacteriophage, in the composition or vaccines of the invention.

[0076] VLPs or capsids of Q β coat protein display a defined number of lysine residues on their surface, with a defined topology with three lysine residues pointing towards the interior of the capsid and interacting with the RNA, and four other lysine residues exposed to the exterior of the capsid. Preferably, the at least one first attachment site is a lysine residue, pointing to or being on the exterior of the VLP.

[0077] Q β mutants, of which exposed lysine residues are replaced by arginines can be used for the present invention. Thus, in another preferred embodiment of the present invention, the virus-like particle comprises, consists essentially of or alternatively consists of mutant Q β coat proteins. Preferably these mutant coat proteins comprise or alternatively consist of an amino acid sequence selected from the group of a) Q β -240 (SEQ ID NO:16, Lys13-Arg of SEQ ID NO: 3) b) Q β -243 (SEQ ID NO:17, Asn10-Lys of SEQ ID NO:3); c) Q β -250 (SEQ ID NO:18, Lys2-Arg of SEQ ID NO:3) d) Q β -251 (SEQ ID NO:19, Lys16-Arg of SEQ ID NO:3); and e) Q β -259 (SEQ ID NO:20, Lys2-Arg, Lys16-Arg of SEQ ID NO:3). The construction, expression and purification of the above indicated Q β mutant coat proteins, mutant Q β coat protein VLPs and capsids, respectively, are described in WO 02/056905. In particular is hereby referred to Example 18 of above mentioned application.

[0078] In another preferred embodiment of the present invention, the virus-like particle comprises, or alternatively consists essentially of, or alternatively consists of mutant coat protein of Q β , or mutants or fragments thereof, and the corresponding A1 protein. In a further preferred embodiment, the virus-like particle comprises, or alternatively consists essentially of, or alternatively consists of mutant coat protein with amino acid sequence SEQ ID NO:16, 17, 18, 19, or 20 and the corresponding A1 protein.

[0079] Further RNA bacteriophage coat proteins have also been shown to self-assemble upon expression in a bacterial host (Kastelein, RA. et al., Gene 23:245-254 (1983),

Kozlovskaya, TM. et al., Dokl. Akad. Nauk SSSR 287:452-455 (1986), Adhin, MR. et al., Virology 170:238-242 (1989), Priano, C. et al., J. Mol. Biol. 249:283-297 (1995)). In particular the biological and biochemical properties of GA (Ni, CZ., et al., Protein Sci. 5:2485-2493 (1996), Tars, K et al., J. Mol.Biol. 271:759-773(1997)) and of fr (Pushko P. et al., Prot. Eng. 6:883-891 (1993), Liljas, L et al. J Mol. Biol. 244:279-290, (1994)) have been disclosed. The crystal structure of several RNA bacteriophages has been determined (Golmohammadi, R. et al., Structure 4:543-554 (1996)). Using such information, surface exposed residues can be identified and, thus, RNA bacteriophage coat proteins can be modified such that one or more reactive amino acid residues can be inserted by way of insertion or substitution. Another advantage of the VLPs derived from RNA bacteriophages is their high expression yield in bacteria that allows production of large quantities of material at affordable cost.

[0080] In one preferred embodiment, the composition of the invention comprises at least one antigen, preferably one to four, more preferably one to three, still more preferably one to two and most preferably exactly one antigen, wherein said antigen is an IL-1 molecule, preferably an IL-1 protein, an IL-1 fragment, an IL-1 mature fragment, an IL-1 peptide or an IL-1 mutein, wherein said IL-1 molecule preferably comprises or even more preferably consists of a polypeptide having an amino acid sequence having at least 80 %, preferably at least 90 %, more preferably at least 95 %, even more preferably at least 99 % and most preferably 100 % sequence identity with any one of SEQ ID NO:36 to SEQ ID NO:116, SEQ ID NO:130 to SEQ ID NO:140 and SEQ ID NO:163 to SEQ ID NO:165.

[0081] In a further preferred embodiment said antigen is an IL-1 molecule derived from an organism selected from the group consisting of: (a) humans; (b) primates; (c) rodents; (d) horses; (e) sheep; (f) cat; (g) cattle; (h) pig; (i) rabbit; (j) dog; (k) mouse; and (l) rat. Most preferably said IL-1 molecule is derived from humans, preferably comprising or even more preferably consisting of a polypeptide having at least 80 %, preferably at least 90 %, more preferably at least 95 %, even more preferably at least 99 % and most preferably 100 % sequence identity with any one of the sequences selected from the group consisting of SEQ ID NO:36, SEQ ID NO:49, SEQ ID NO:63, SEQ ID NO:64, any one of SEQ ID NO:67 to 110, and one of SEQ ID NO:130-140, and SEQ ID NO:165.

[0082] In a further preferred embodiment said IL-1 molecule derived from rat or mouse, preferably mouse, wherein said IL-1 molecule preferably comprises or even more preferably consists of a polypeptide having an amino acid sequence having at least 80 %, preferably at least 90 %, more preferably at least 95 %, even more preferably at least 99 % and most preferably 100 % sequence identity with any one of SEQ ID NO:45, SEQ ID NO:46, SEQ ID

NO:53, SEQ ID NO:54, SEQ ID NO:65, SEQ ID NO:66, any one of SEQ ID NO:111 to SEQ ID NO:116, SEQ ID NO:163, and SEQ ID NO:164.

[0083] In a further preferred embodiment IL-1 molecule is an IL-1 alpha molecule, preferably an IL-1 alpha protein, an IL-1 alpha fragment, an IL-1 alpha mature fragment, an IL-1 alpha peptide or an IL-1 alpha mutein, wherein said IL-1 alpha molecule preferably comprises or even more preferably consists of a polypeptide having an amino acid sequence having at least 80 %, preferably at least 90 %, more preferably at least 95 %, even more preferably at least 99 % and most preferably 100 % sequence identity with any one of the sequences selected from the group consisting of SEQ ID NO:36 to 48, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67 to 88, and SEQ ID NO:165. Specifically preferred embodiments of IL-1 alpha molecules are human IL-1 alpha molecules, preferably human IL-1 alpha proteins, human IL-1 alpha fragments or human IL-1 alpha mature fragments, wherein said IL-1 alpha molecules preferably comprise or even more preferably consist of a polypeptide having an amino acid sequence having at least 80 %, preferably at least 90 %, more preferably at least 95 %, even more preferably at least 99 % and most preferably 100 % sequence identity with any one of SEQ ID NO:36, SEQ ID NO:63, and SEQ ID NO:163, most preferably SEQ ID NO:63.

[0084] In a further preferred embodiment said IL-1 molecule is an IL-1 beta molecule, preferably an IL-1 beta protein, an IL-1 beta fragment, an IL-1 beta mature fragment, an IL-1 beta peptide or an IL-1 beta mutein, wherein said IL-1 beta molecule preferably comprises or even more preferably consists of a polypeptide having an amino acid sequence having at least 80 %, preferably at least 90 %, more preferably at least 95 %, even more preferably at least 99 % and most preferably 100 % sequence identity with any one of the sequences selected from the group consisting of SEQ ID NO:49 to 62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:89 to 116, SEQ ID NO:130 to SEQ ID NO:140, SEQ ID NO:164, and SEQ ID NO:165. Specifically preferred embodiments of IL-1 beta molecules are human IL-1 beta molecules, preferably human IL-1 beta proteins, human IL-1 beta fragments or human IL-1 beta mature fragments, wherein said IL-1 beta molecules preferably comprises or even more preferably consists of a polypeptide having an amino acid sequence having at least 80 %, preferably at least 90 %, more preferably at least 95 %, even more preferably at least 99 % and most preferably 100 % sequence identity with any one of SEQ ID NO:49, SEQ ID NO:64, SEQ ID NO:130 to SEQ ID NO:140 and SEQ ID NO:165, most preferably SEQ ID NO:64.

[0085] In a further preferred embodiment said IL-1 molecule is an IL-1 protein, an IL-1 fragment or, preferably, an IL-1 mature fragment, wherein said IL-1 protein, IL-1 fragment or

IL-1 mature fragment preferably are capable of binding to the IL-1 receptor and, still more preferably, additionally also comprise biological activity.

[0086] In a further preferred embodiment said IL-1 molecule is an IL-1 protein, wherein said IL-1 protein preferably comprises or even more preferably consists of a polypeptide having an amino acid sequence having at least 80 %, preferably at least 90 %, more preferably at least 95 %, even more preferably at least 99 % and most preferably 100 % sequence identity with any one of SEQ ID NO:36 to SEQ ID NO:62.

[0087] In a further preferred embodiment said IL-1 protein is an IL-1 alpha protein, wherein said IL-1 alpha protein preferably comprises or even more preferably consists of a polypeptide having an amino acid sequence having at least 80 %, preferably at least 90 %, more preferably at least 95 %, even more preferably at least 99 % and most preferably 100 % sequence identity with any one of the sequences selected from the group consisting of SEQ ID NO:36 to SEQ ID NO:48. Most preferably said IL-1 alpha protein is a human IL-1 alpha protein, wherein said human IL-1 alpha protein preferably comprises or even more preferably consists of a polypeptide having least 80 %, preferably at least 90 %, more preferably at least 95 %, even more preferably at least 99 % and most preferably 100 % sequence identity with SEQ ID NO:36.

[0088] In a further preferred embodiment said IL-1 protein is an IL-1 beta protein, wherein said IL-1 beta protein preferably comprises or even more preferably consists of a polypeptide having an amino acid sequence having at least 80 %, preferably at least 90 %, more preferably at least 95 %, even more preferably at least 99 % and most preferably 100 % sequence identity with any one of the sequences selected from the group consisting of SEQ ID NO:49 to SEQ ID NO:62. Most preferably said IL-1 beta protein is a human IL-1 beta protein, wherein said human IL-1 beta protein preferably comprises or even more preferably consists of a polypeptide having least 80 %, preferably at least 90 %, more preferably at least 95 %, even more preferably at least 99 % and most preferably 100 % sequence identity with SEQ ID NO:49.

[0089] In a further preferred embodiment said IL-1 molecule is an IL-1 fragment, preferably an IL-1 mature fragment, and wherein said IL-1 fragment or said IL-1 mature fragment preferably is derived from mouse or human, most preferably human. Preferably said IL-1 fragment or said IL-1 mature fragment comprises or even more preferably consists of a polypeptide having an amino acid sequence having at least 80 %, preferably at least 90 %, more preferably at least 95 %, even more preferably at least 99 % and most preferably 100 % sequence identity with any one of SEQ ID NO:63 to SEQ ID NO:66, SEQ ID NO:130, and SEQ ID NO:163 to SEQ ID NO:165.

[0090] In a further preferred embodiment said IL-1 mature fragment is an IL-1 alpha mature fragment, wherein said IL-1 alpha mature fragment preferably comprises biological activity and wherein further said IL-1 alpha mature fragment preferably comprises or even more preferably consists of a polypeptide having an amino acid sequence having at least 80 %, preferably at least 90 %, more preferably at least 95 %, even more preferably at least 99 % and most preferably 100 % sequence identity with any one of SEQ ID NO:63 or SEQ ID NO:65, most preferably SEQ ID NO:63.

[0091] In a further preferred embodiment said IL-1 mature fragment is an IL-1 beta mature fragment, wherein said IL-1 beta mature fragment preferably comprises biological activity and wherein further said IL-1 beta mature fragment preferably comprises or even more preferably consists of a polypeptide having an amino acid sequence having at least 80 %, preferably at least 90 %, more preferably at least 95 %, even more preferably at least 99 % and most preferably 100 % sequence identity with any one of SEQ ID NO:64, SEQ ID NO:66, and SEQ ID NO:130, most preferably SEQ ID NO:64.

[0092] In a further preferred embodiment said IL-1 molecule is an IL-1 peptide, wherein said IL-1 peptide is derived from mouse, rat or human, most preferably human. Preferably said IL-1 peptide comprises or even more preferably consists of a polypeptide having an amino acid sequence having at least 80 %, preferably at least 90 %, more preferably at least 95 %, even more preferably at least 99 % and most preferably 100 % sequence identity with any one of SEQ ID NO:67 to SEQ ID NO:116.

[0093] In a further preferred embodiment said IL-1 molecule is an IL-1 mutein, wherein preferably said IL-1 mutein comprises reduced or more preferably no biological activity, and wherein further said IL-1 mutein is capable of binding the IL-1 receptor. In a further preferred embodiment said IL-1 mutein comprises or preferably consists of a polypeptide having an amino acid sequence which differs from the amino acid sequence of an IL-1 mature fragment in 1 to 3, more preferably in 1 to 2, and most preferably in exactly 1 amino acid residue. In a further preferred embodiment said IL-1 mutein is an IL-1 beta mutein, preferably a human IL-1 beta mutein, most preferably a human IL-1 beta mutein selected from SEQ ID NO:131 to SEQ ID NO:140.

[0094] The present invention provides for a method of producing the composition of the invention comprising (a) providing a VLP with at least one first attachment site; (b) providing at least one antigen, wherein said antigen is an IL-1 molecule, an IL-1 protein, an IL-1 fragment, preferably an IL-1 mature fragment, an IL-1 peptide or an IL-1 mutein, with at least one second attachment site; and (c) combining said VLP and said at least one antigen to produce said composition, wherein said at least one antigen and said VLP are linked through

the first and the second attachment sites. In a preferred embodiment, the provision of the at least one antigen, i.e. IL-1 molecule, an IL-1 protein, an IL-1 fragment, preferably an IL-1 mature fragment, an IL-1 peptide or an IL-1 mutein, with the at least one second attachment site is by way of expression, preferably by way of expression in a bacterial system, preferably in *E. coli*. Usually a purification tag, such as His tag, Myc tag, Fc tag or HA tag is added to facilitate the purification process. In another approach particularly the IL-1 peptides or IL-1 muteins with no longer than 50 amino acids are chemically synthesized.

[0095] In one preferred embodiment of the invention, the VLP with at least one first attachment site is linked to the IL-1 molecule with at least one second attachment site via at least one peptide bond. A gene encoding an IL-1 molecule, preferably an IL-1 mature fragment, is in-frame ligated, either internally or preferably to the N- or the C-terminus to the gene encoding the coat protein of the VLP. Fusion may also be effected by inserting sequences of the IL-1 into a mutant coat protein where part of the coat protein sequence has been deleted, that are further referred to as truncation mutants. Truncation mutants may have N- or C-terminal, or internal deletions of part of the sequence of the coat protein. For example for the specific VLP HBcAg, amino acids 79-80 are replaced with a foreign epitope. The fusion protein shall preferably retain the ability of assembly into a VLP upon expression which can be examined by electromicroscopy.

[0096] Flanking amino acid residues may be added to increase the distance between the coat protein and foreign epitope. Glycine and serine residues are particularly favored amino acids to be used in the flanking sequences. Such a flanking sequence confers additional flexibility, which may diminish the potential destabilizing effect of fusing a foreign sequence into the sequence of a VLP subunit and diminish the interference with the assembly by the presence of the foreign epitope.

[0097] In other embodiments, the at least one IL-1 molecule, preferably the IL-1 mature fragment can be fused to a number of other viral coat protein, as way of examples, to the C-terminus of a truncated form of the A1 protein of Q β (Kozlovskaya, T. M., et al., Intervirology 39:9-15 (1996)), or being inserted between position 72 and 73 of the CP extension. As another example, the IL-1 can be inserted between amino acid 2 and 3 of the fr CP, leading to a IL-1-fr CP fusion protein (Pushko P. et al., Prot. Eng. 6:883-891 (1993)). Furthermore, IL-1 can be fused to the N-terminal protuberant β -hairpin of the coat protein of RNA bacteriophage MS-2 (WO 92/13081). Alternatively, the IL-1 can be fused to a capsid protein of papillomavirus, preferably to the major capsid protein L1 of bovine papillomavirus type 1 (BPV-1) (Chackerian, B. et al., Proc. Natl. Acad. Sci. USA 96:2373-2378 (1999), WO 00/23955). Substitution of amino acids 130-136 of BPV-1 L1 with an IL-1 is also an

embodiment of the invention. Further embodiments of fusing an IL-1 molecule to coat protein, mutants or fragments thereof, to a coat protein of a virus have been disclosed in WO 2004/009124 page 62 line 20 to page 68 line 17 and herein are incorporated by way of reference.

[0098] US 5,698,424 describes a modified coat protein of bacteriophage MS-2 capable of forming a capsid, wherein the coat protein is modified by an insertion of a cysteine residue into the N-terminal hairpin region, and by replacement of each of the cysteine residues located external to the N-terminal hairpin region by a non-cysteine amino acid residue. The inserted cysteine may then be linked directly to a desired molecular species to be presented such as an epitope or an antigenic protein.

[0099] We note, however, that the presence of an exposed free cysteine residue in the capsid may lead to oligomerization of capsids by way of disulfide bridge formation. Moreover, attachment between capsids and antigenic proteins by way of disulfide bonds are labile, in particular, to sulfhydryl-moiety containing molecules, and are, furthermore, less stable in serum than, for example, thioether attachments (Martin FJ. and Papahadjopoulos D. (1982) Irreversible Coupling of Immunoglobulin Fragments to Preformed Vesicles. J. Biol. Chem. 257: 286-288).

[00100] Therefore, in a further very preferred embodiment of the present invention, the association or linkage of the VLP and the at least one antigen, i.e. IL-1 molecule, does not comprise a disulfide bond. Further preferred hereby, the at least one second attachment comprise, or preferably is, a sulfhydryl group. Moreover, in again a very preferred embodiment of the present invention, the association or linkage of the VLP and the at least one IL-1 molecule does not comprise a sulphur-sulphur bond. Further preferred hereby, the at least one second attachment comprise, or preferably is, a sulfhydryl group. In a further very preferred embodiment, said at least one first attachment site is not or does not comprise a sulfhydryl group. In again a further very preferred embodiment, said at least one first attachment site is not or does not comprise a sulfhydryl group of a cysteine.

[00101] In a further preferred embodiment said at least one first attachment comprises an amino group and said second attachment comprises a sulfhydryl group.

[00102] In a further preferred embodiment only one of said second attachment sites associates with said first attachment site through at least one non-peptide covalent bond leading to a single and uniform type of binding of said IL-1 molecule to said core particle, wherein said only one second attachment site that associates with said first attachment site is a sulfhydryl group, and wherein said IL-1 molecule and said core particle interact through said association to form an ordered and repetitive antigen array.

[00103] In another preferred embodiment, an IL-1 molecule, preferably an IL-1 protein, more preferably an IL-1 mature fragment, still more preferably an IL-1 mature fragment comprising or consisting of amino acid sequenced SEQ ID NO:63 to SEQ ID NO:66, most preferably SEQ ID NO:63 or SEQ ID NO:64, is fused to either the N- or the C-terminus, preferably the C-terminus, of a coat protein, mutants or fragments thereof, of RNA bacteriophage AP205. VLPs comprising fusion proteins of coat protein of bacteriophage AP205 with an antigen are generally disclosed in WO2006/032674A1 which is incorporated herein by reference. In one further preferred embodiment, the fusion protein further comprises a linker, wherein said linker is fused to the coat protein, fragments or mutants thereof, of AP205 and the IL-1 molecule. In a further preferred embodiment said IL-1 molecule is fused to the C-terminus of said coat protein, fragments or mutants thereof, of AP205 via said linker.

[00104] It has been found that IL-1 molecules, in particular IL-1 proteins and IL-1 fragments comprising at least 100 and up to 300 amino acids, typically and preferably about 140 to 160 amino acids, and most preferably about 155 amino acids, can be fused to coat protein of bacteriophages, preferably to coat protein of AP205, while maintaining the ability of the coat protein to self assemble into a VLP.

[00105] Given the large size of IL-1 proteins, IL-1 fragments and IL-1 mature fragments and also for steric reasons, an expression system producing mosaic VLPs comprising AP205 coat proteins fused to an IL-1 molecule as well as wt coat protein subunits was constructed. In this system, suppression of the stop codon yields the AP205-IL-1 coat protein fusion, while proper termination yields the wt AP205 coat protein. Both proteins are produced simultaneously in the cell and assemble into a mosaic VLP. The advantage of such a system is that large proteins can be displayed without interfering with the assembly of the VLP. As the level of incorporation of AP205-IL-1 fusion protein into the mosaic VLP is depending on the level of suppression, AP205-IL-1 is expressed in *E.coli* cells already containing a plasmid overexpressing a suppressor t-RNA. For opal suppression, plasmid pISM3001 (Smiley, B.K., Minion, F.C. (1993) Enhanced readthrough of opal (UGA) stop codons and production of *Mycoplasma pneumoniae* P1 epitopes in *Escherichia coli*. *Gene* **134**, 33-40), which encodes a suppressor t-RNA recognizing the opal stop codon and introducing Trp is used. Suppression of amber termination can be increased by use of plasmid pISM579, which overexpresses a suppressor t-RNA recognizing the amber stop codon and introducing Trp as well. Plasmid pISM579 was generated by excising the *trpT176* gene from pISM3001 with restriction endonuclease *EcoRI* and replacing it by an *EcoRI* fragment from plasmid pMY579 (gift of Michael Yarus) containing an amber t-RNA suppressor gene. This t-RNA suppressor gene is

a mutant of *trpT*175 (Raftery LA. Et al. (1984) J. Bacteriol. 158:849-859), and differs from *trpT* at three positions: G33, A24 and T35. Expression of the AP205-interleukin-1 α fusion protein in an *E.coli* strain with amber suppression (*supE* or *glnV*) such as *E. coli* JM109 may generate a proportion of AP205-IL-1 fusion proteins with a Gln instead of Trp introduced at the amber stop codon, in addition to AP205-IL-1 fusion proteins with a Trp introduced at the amber stop codon. The identity of the amino acid translated at the stop codon may therefore depend on the combination of suppressor t-RNA overexpressed, and strain phenotype. As described by Miller JH et al. ((1983) J. Mol. Biol. 164: 59-71) and as is well known in the art, the efficiency of suppression is context dependent. In particular, the codon 3' of the stop codon and the first base 3' from the stop codon are particularly important. For example, stop codons followed by a purine base are in general well suppressed.

[00106] Thus, in a preferred embodiment said VLP is a mosaic VLP, wherein said mosaic VLP comprises or preferably consists of at least one, preferably one, first polypeptide and of at least one, preferably one, second polypeptide, wherein said first polypeptide is a recombinant capsid protein, mutant or fragments thereof; and wherein said second polypeptide is a genetic fusion product of a recombinant capsid protein, mutant or fragments thereof, preferably of said first polypeptide, with an IL-1 molecule. In a further preferred embodiment said first polypeptide is a recombinant capsid protein of bacteriophage AP205 or a mutant or fragment thereof. In a further preferred embodiment said first polypeptide is selected from SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23. In a very preferred embodiment said first polypeptide is SEQ ID NO:21. Mosaic VLPs of bacteriophage AP205 comprising an antigen are generally disclosed in WO2006/032674A1, in particular in paragraph 107 of said publication. In a further preferred embodiment said second polypeptide is a genetic fusion product of a recombinant capsid protein, mutant or fragments thereof, preferably of said first polypeptide, with an IL-1 molecule, wherein said IL-1 molecule is fused to the C-terminus of said recombinant capsid protein, mutant or fragments thereof, preferably via an amino acid linker. In a further preferred embodiment said IL-1 molecule comprises or preferably consists of 100 to 300 amino acids, typically and preferably about 140 to 160 amino acids, and most preferably about 155 amino acids. In a very preferred embodiment, the molar ratio of said first polypeptide and said second polypeptide in said mosaic VLP is 10:1 to 5:1, preferably 8:1 to 6:1, most preferably about 7:1.

[00107] In one preferred embodiment of the present invention, the composition comprises or alternatively consists essentially of a virus-like particle with at least one first attachment site linked to at least one antigen, i.e. an IL-1 molecule, with at least one second attachment site via at least one covalent bond, wherein preferably the covalent bond is a non-peptide bond. In

a preferred embodiment of the present invention, the first attachment site comprises, or preferably is, an amino group, preferably the amino group of a lysine residue. In another preferred embodiment of the present invention, the second attachment site comprises, or preferably is, a sulfhydryl group, preferably a sulfhydryl group of a cysteine.

[00108] In a very preferred embodiment of the invention, the at least one first attachment site is an amino group, preferably an amino group of a lysine residue and the at least one second attachment site is a sulfhydryl group, preferably a sulfhydryl group of a cysteine.

[00109] In one preferred embodiment of the invention, the IL-1 molecule is linked to the VLP by way of chemical cross-linking, typically and preferably by using a heterobifunctional cross-linker. In preferred embodiments, the hetero-bifunctional cross-linker contains a functional group which can react with the preferred first attachment sites, preferably with the amino group, more preferably with the amino groups of lysine residue(s) of the VLP, and a further functional group which can react with the preferred second attachment site, i.e. a sulfhydryl group, preferably of cysteine(s) residue inherent of, or artificially added to the IL-1 molecule, and optionally also made available for reaction by reduction. Several hetero-bifunctional cross-linkers are known to the art. These include the preferred 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, and having one functional group reactive towards amino groups and one functional group reactive towards sulfhydryl groups. The above mentioned cross-linkers all lead to formation of an amide bond after reaction with the amino group and a thioether linkage with the sulfhydryl groups. Another class of cross-linkers suitable in the practice of the invention is characterized by the introduction of a disulfide linkage between the IL-1 molecule and the VLP upon coupling. Preferred cross-linkers belonging to this class include, for example, SPDP and Sulfo-LC-SPDP (Pierce).

[00110] In a preferred embodiment, the composition of the invention further comprises a linker. Engineering of a second attachment site onto the IL-1 molecule is achieved by the association of a linker, preferably containing at least one amino acid suitable as second attachment site according to the disclosures of this invention. Therefore, in a preferred embodiment of the present invention, a linker is associated to the IL-1 molecule by way of at least one covalent bond, preferably, by at least one, preferably one peptide bond. Preferably, the linker comprises, or alternatively consists of, the second attachment site. In a further preferred embodiment, the linker comprises a sulfhydryl group, preferably of a cysteine residue. In another preferred embodiment, the amino acid linker is a cysteine residue.

[00111] The selection of a linker will be dependent on the nature of the IL-1 molecule, on its biochemical properties, such as pI, charge distribution and glycosylation. In general, flexible amino acid linkers are favored. In a further preferred embodiment of the present invention, the linker consists of amino acids, wherein further preferably the linker consists of at least one and at most 25, preferably at most 20, more preferably at most 15 amino acids. In an again preferred embodiment of the invention, the amino acid linker contains 1 to 10 amino acids. Preferred embodiments of the linker are selected from the group consisting of: (a) CGG (SEQ ID NO:171); (b) N-terminal gamma 1-linker, preferably CGDKTHTSPP (SEQ ID NO:172); (c) N-terminal gamma 3-linker, preferably CGGPKPSTPPGSSGGAP (SEQ ID NO:173); (d) Ig hinge regions; (e) N-terminal glycine linkers, preferably GCGGGG (SEQ ID NO:174); (f) (G)_kC(G)_n with n=0-12 and k=0-5 (SEQ ID NO:175); (g) N-terminal glycine-serine linkers, preferably (GGGGS)_n, n=1-3 (SEQ ID NO:176) with one further cysteine; (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:177); (i) GGC (SEQ ID NO:178); (k) GGC-NH₂ (SEQ ID NO:179); (l) C-terminal gamma 1-linker, preferably DKTHTSPPCG (SEQ ID NO:180); (m) C-terminal gamma 3-linker, preferably PKPSTPPGSSGGAPGGCG (SEQ ID NO:181); (n) C-terminal glycine linkers, preferably GGGGCG (SEQ ID NO:182); (o) (G)_nC(G)_k with n=0-12 and k=0-5 (SEQ ID NO:183); (p) C-terminal glycine-serine linkers, preferably (SGGGG)_n n=1-3 (SEQ ID NO:184) with one further cysteine; (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:185). In a further preferred embodiment the linker is added to the N-terminus of the IL-1 molecule. In another preferred embodiment of the invention, the linker is added to the C-terminus of IL-1 molecule.

[00112] Preferred linkers according to this invention are glycine linkers (G)_n further containing a cysteine residue as second attachment site, such as N-terminal glycine linker (GCGGGG, SEQ ID NO:174) and C-terminal glycine linker (GGGGCG, SEQ ID NO:182). Further preferred embodiments are C-terminal glycine-lysine linker (GGKKGC, SEQ ID NO:186) and N-terminal glycine-lysine linker (CGKKGG, SEQ ID NO:187), GGCG (SEQ ID NO:188) and GGC (SEQ ID NO:178) or GGC-NH₂ (SEQ ID NO:179, "NH₂" stands for amidation) linkers at the C-terminus of the peptide or CGG (SEQ ID NO:171) at its N-terminus. In general, glycine residues will be inserted between bulky amino acids and the cysteine to be used as second attachment site, to avoid potential steric hindrance of the bulkier amino acid in the coupling reaction.

[00113] Linking of the IL-1 molecule to the VLP by using a hetero-bifunctional cross-linker according to the preferred methods described above, allows coupling of the IL-1 molecule to the VLP in an oriented fashion. Other methods of linking the IL-1 molecule to the VLP

include methods wherein the IL-1 molecule is cross-linked to the VLP, using the carbodiimide EDC, and NHS. The IL-1 molecule may also be first thiolated through reaction, for example with SATA, SATP or iminothiolane. The IL-1 molecule, after deprotection if required, may then be coupled to the VLP as follows. After separation of the excess thiolation reagent, the IL-1 molecule is reacted with the 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 IL-1 molecule can react, such as described above. Optionally, low amounts of a reducing agent are included in the reaction mixture. In further methods, the IL-1 molecule is attached to the VLP, using a homo-bifunctional cross-linker such as glutaraldehyde, DSG, BM[PEO]₄, BS₃, (Pierce) or other known homo-bifunctional cross-linkers with functional groups reactive towards amine groups or carboxyl groups of the VLP.

[00114] In other embodiments of the present invention, the composition comprises or alternatively consists essentially of a virus-like particle linked to IL-1 molecule via chemical interactions, wherein at least one of these interactions is not a covalent bond.

[00115] Linking of the VLP to the IL-1 molecule can be effected by biotinylating the VLP and expressing the IL-1 molecule as a streptavidin-fusion protein.

[00116] One or several antigen molecules, i.e. IL-1 molecules, can be attached to one subunit of the VLP, preferably of RNA bacteriophage coat proteins, preferably through the exposed lysine residues of the coat proteins of RNA bacteriophage VLP, if sterically allowable. A specific feature of the VLPs of RNA bacteriophage and in particular of the Q β coat protein VLP is thus the possibility to couple several antigens per subunit. This allows for the generation of a dense antigen array.

[00117] In very preferred embodiments of the invention, the IL-1 molecule is linked via a cysteine residue, having been added to either the N-terminus or the C-terminus of, or a natural cysteine residue within an IL-1 molecule, to lysine residues of coat proteins of the VLPs of RNA bacteriophage, and in particular to the coat protein of Q β .

[00118] As described above, four lysine residues are exposed on the surface of the VLP of Q β coat protein. Typically and preferably these residues are derivatized upon reaction with a cross-linker molecule. In the instance where not all of the exposed lysine residues can be coupled to an antigen, the lysine residues which have reacted with the cross-linker are left with a cross-linker molecule attached to the ϵ -amino group after the derivatization step. This leads to disappearance of one or several positive charges, which may be detrimental to the solubility and stability of the VLP. By replacing some of the lysine residues with arginines, as in the disclosed Q β coat protein mutants, we prevent the excessive disappearance of positive

charges since the arginine residues do not react with the preferred cross-linkers. Moreover, replacement of lysine residues by arginine residues may lead to more defined antigen arrays, as fewer sites are available for reaction to the antigen.

[00119] Accordingly, exposed lysine residues were replaced by arginines in the following Q β coat protein mutants: Q β -240 (Lys13-Arg; SEQ ID NO:16), Q β -250 (Lys 2-Arg, Lys13-Arg; SEQ ID NO:18), Q β -259 (Lys 2-Arg, Lys16-Arg; SEQ ID NO:20) and Q β -251; (Lys16-Arg, SEQ ID NO:19). In a further embodiment, we disclose a Q β mutant coat protein with one additional lysine residue Q β -243 (Asn 10-Lys; SEQ ID NO:17), suitable for obtaining even higher density arrays of antigens.

[00120] In one preferred embodiment of the invention, the VLP of an RNA bacteriophage is recombinantly produced by a host and wherein said VLP is essentially free of host RNA, preferably host nucleic acids. In one further preferred embodiment, the composition further comprises at least one polyanionic macromolecule bound to, preferably packaged in or enclosed in, the VLP. In a still further preferred embodiment, the polyanionic macromolecule is polyglutamic acid and/or polyaspartic acid.

[00121] In another preferred embodiment, the composition further comprises at least one immunostimulatory substance bound to, preferably packaged in or enclosed in, the VLP. In a still further preferred embodiment, the immunostimulatory substance is a nucleic acid, preferably DNA, most preferably an unmethylated CpG containing oligonucleotide.

[00122] Essentially free of host RNA, preferably host nucleic acids: The term “essentially free of host RNA, preferably host nucleic acids” as used herein, refers to the amount of host RNA, preferably host nucleic acids, comprised by the VLP, which amount typically and preferably is less than 30 μ g, preferably less than 20 μ g, more preferably less than 10 μ g, even more preferably less than 8 μ g, even more preferably less than 6 μ g, even more preferably less than 4 μ g, most preferably less than 2 μ g, per mg of the VLP. Host, as used within the afore-mentioned context, refers to the host in which the VLP is recombinantly produced. Conventional methods of determining the amount of RNA, preferably nucleic acids, are known to the skilled person in the art. The typical and preferred method to determine the amount of RNA, preferably nucleic acids, in accordance with the present invention is described in Example 17 of WO2006/037787A2. Identical, similar or analogous conditions are, typically and preferably, used for the determination of the amount of RNA, preferably nucleic acids, for inventive compositions comprising VLPs other than Q β . The modifications of the conditions eventually needed are within the knowledge of the skilled person in the art. The numeric value of the amounts determined should typically and

preferably be understood as comprising values having a deviation of $\pm 10\%$, preferably having a deviation of $\pm 5\%$, of the indicated numeric value.

[00123] Polyanionic macromolecule: The term “polyanionic macromolecule”, as used herein, refers to a molecule of high relative molecular mass which comprises repetitive groups of negative charge, the structure of which essentially comprises the multiple repetition of units derived, actually or conceptually, from molecules of low relative molecular mass. A polyanionic macromolecule should have a molecular weight of at least 2000 Dalton, more preferably of at least 3000 Dalton and even more preferably of at least 5000 Dalton. The term “polyanionic macromolecule” as used herein, typically and preferably refers to a molecule that is not capable of activating toll-like receptors. Thus, the term “polyanionic macromolecule” typically and preferably excludes Toll-like receptors ligands, and even more preferably furthermore excludes immunostimulatory substances such as Toll-like receptors ligands, immunostimulatory nucleic acids, and lipopolysacchrides (LPS). More preferably the term “polyanionic macromolecule” as used herein, refers to a molecule that is not capable of inducing cytokine production. Even more preferably the term “polyanionic macromolecule” excludes immunostimulatory substances. The term “immunostimulatory substance”, as used herein, refers to a molecule that is capable of inducing and/or enhancing immune response specifically against the antigen comprised in the present invention.

[00124] Host RNA, preferably host nucleic acids: The term “host RNA, preferably host nucleic acids” or the term “host RNA, preferably host nucleic acids, with secondary structure”, as used herein, refers to the RNA, or preferably nucleic acids, that are originally synthesized by the host. The RNA, preferably nucleic acids, may, however, undergo chemical and/or physical changes during the procedure of reducing or eliminating the amount of RNA, preferably nucleic acids, typically and preferably by way of the inventive methods, for example, the size of the RNA, preferably nucleic acids, may be shortened or the secondary structure thereof may be altered. However, even such resulting RNA or nucleic acids is still considered as host RNA, or host nucleic acids.

[00125] Methods to determine the amount of RNA and to reduce the amount of RNA comprised by the VLP have disclosed in US provisional application filed by the same assignee on October 5, 2004 and thus the entire application is incorporated herein by way of reference. Reducing or eliminating the amount of host RNA, preferably host nucleic, minimizes or reduces unwanted T cell responses, such as inflammatory T cell response and cytotoxic T cell response, and other unwanted side effects, such as fever, while maintaining strong antibody response specifically against IL-1.

[00126] In one preferred embodiment, this invention provides a method of preparing the inventive compositions and VLP of an RNA-bacteriophage the invention, wherein said VLP is recombinantly produced by a host and wherein said VLP is essentially free of host RNA, preferably host nucleic acids, comprising the steps of: a) recombinantly producing a virus-like particle (VLP) with at least one first attachment site by a host, wherein said VLP comprises coat proteins, variants or fragments thereof, of a RNA-bacteriophage; b) disassembling said virus-like particle to said coat proteins, variants or fragments thereof, of said RNA-bacteriophage; c) purifying said coat proteins, variants or fragments thereof; d) reassembling said purified coat proteins, variants or fragments thereof, of said RNA-bacteriophage to a virus-like particle, wherein said virus-like particle is essentially free of host RNA, preferably host nucleic acids; and e) linking at least one antigen of the invention with at least one second attachment site to said VLP obtained from step d). In a further preferred embodiment, the reassembling of said purified coat proteins, variants or fragments thereof, is effected in the presence of at least one polyanionic macromolecule.

[00127] In one aspect, the invention provides a vaccine comprising the composition of the invention. In one preferred embodiment, the IL-1 molecule which is linked to the VLP in the vaccine composition may be of animal, preferably mammal or human origin. In preferred embodiments, the IL-1 of the invention is of human, bovine, dog, cat, mouse, rat, pig or horse origin.

[00128] In one preferred embodiment, the vaccine composition further comprises at least one adjuvant. The administration of the at least one adjuvant may hereby occur prior to, contemporaneously or after the administration of the inventive composition. The term "adjuvant" as used herein refers to non-specific stimulators of the immune response or substances that allow generation of a depot in the host which when combined with the vaccine and pharmaceutical composition, respectively, of the present invention may provide for an even more enhanced immune response.

[00129] In another preferred embodiment, the vaccine composition is devoid of adjuvant.

[00130] An advantageous feature of the present invention is the high immunogenicity of the composition, even in the absence of adjuvants. The absence of an adjuvant, furthermore, minimizes the occurrence of unwanted inflammatory T-cell responses representing a safety concern in the vaccination against self antigens. Thus, the administration of the vaccine of the invention to a patient will preferably occur without administering at least one adjuvant to the same patient prior to, contemporaneously or after the administration of the vaccine.

[00131] The invention further discloses a method of immunization comprising administering the vaccine of the present invention to an animal or a human. The animal is

preferably a mammal, such as cat, sheep, pig, horse, bovine, dog, rat, mouse and particularly human. The vaccine may be administered to an animal or a human by various methods known in the art, but will normally be administered by injection, infusion, inhalation, oral administration, or other suitable physical methods. The conjugates may alternatively be administered intramuscularly, intravenously, transmucosally, transdermally, intranasally, intraperitoneally or subcutaneously. Components of conjugates 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.

[00132] Vaccines of the invention are said to be "pharmacologically acceptable" if their administration can be tolerated by a recipient individual. Further, the vaccines of the invention will be administered in a "therapeutically effective amount" (i.e., an amount that produces a desired physiological effect). The nature or type of immune response is not a limiting factor of this disclosure. Without the intention to limit the present invention by the following mechanistic explanation, the inventive vaccine might induce antibodies which bind to IL-1 and thus reducing its concentration and/or interfering with its physiological or pathological function.

[00133] In one aspect, the invention provides a pharmaceutical composition comprising the composition as taught in the present invention and an acceptable pharmaceutical carrier. When vaccine of the invention is administered to an individual, it may be in a form which contains salts, buffers, adjuvants, or other substances which are desirable for improving the efficacy of the conjugate. Examples of materials suitable for use in preparation of pharmaceutical compositions are provided in numerous sources including Remington's Pharmaceutical Sciences (Osol, A, ed., Mack Publishing Co., (1990)).

[00134] The invention teaches a process for producing the composition of the invention comprising the steps of: (a) providing a VLP with at least one first attachment site; (b) providing a IL-1 molecule with at least one second attachment site, and (c) combining said VLP and said IL-1 molecule to produce a composition, wherein said IL-1 molecule and said VLP are linked through the first and the second attachment sites.

[00135] In a further preferred embodiment, the step of providing a VLP with at least one first attachment site comprises further steps: (a) disassembling said virus-like particle to said coat proteins, mutants or fragments thereof, of said RNA-bacteriophage; (b) purifying said coat proteins, mutants or fragments thereof; (c) reassembling said purified coat proteins, mutants or fragments thereof, of said RNA-bacteriophage to a virus-like particle, wherein said

virus-like particle is essentially free of host RNA, preferably host nucleic acids. In a still further preferred embodiment, the reassembling of said purified coat proteins is effected in the presence of at least one polyanionic macromolecule.

[00136] The invention provides a method of using the compositions of the invention for treating and/or attenuating diseases or conditions in which IL-1 exerts an important pathological function in an animal or in human.

[00137] The invention further provides for use of the compositions of the invention or the vaccine of the invention or the pharmaceutical composition of the invention for the manufacture of a medicament for treatment of a disease in an animal, preferably dog, cat horse or human, most preferably human, wherein said disease is preferably selected from the group consisting of: (a) vascular diseases, preferably coronary artery disease, atherosclerosis and vasculitis, most preferably atherosclerosis; (b) inherited IL-1-dependent inflammatory diseases, preferably Familial Mediterranean Fever (FMF), Familial Cold Autoinflammatory Syndrome (FCAS) Neonatal Onset Multisystem Inflammatory Disease (NOMID) and Muckle Wells Syndrome, most preferably Familial Mediterranean Fever (FMF); (c) chronic autoimmune inflammatory diseases, preferably rheumatoid arthritis, systemic onset juvenile idiopathic arthritis, adult onset Still's disease, psoriasis, Crohn's disease and ulcerative colitis, most preferably rheumatoid arthritis; (d) bone and cartilage degenerative diseases, preferably gout, osteoporosis and osteoarthritis, most preferably osteoarthritis; (e) allergic diseases, preferably contact hypersensitivity, type 1 hypersensitivity and allergy, most preferably allergy; and (f) neurological diseases, preferably Alzheimer's disease, epilepsy, Parkinson's disease and multiple sclerosis, most preferably multiple sclerosis.

[00138] The invention further provides for use of the compositions of the invention or the vaccine of the invention or the pharmaceutical composition of the invention for the manufacture of a medicament for treatment of a disease in an animal, preferably dog, cat horse or human, most preferably human, wherein said disease is a vascular disease, preferably coronary artery disease, atherosclerosis and vasculitis, most preferably atherosclerosis, and wherein said at least one antigen comprised by said composition, said vaccine or said pharmaceutical composition is an IL-1 alpha molecule of the invention, preferably an IL-1 alpha mature fragment, most preferably SEQ ID NO:63 or a mutein thereof.

[00139] The invention further provides for use of the compositions of the invention or the vaccine of the invention or the pharmaceutical composition of the invention for the manufacture of a medicament for treatment of a disease in an animal, preferably dog, cat horse or human, most preferably human, wherein said disease is selected from the group consisting of: (a) inherited IL-1-dependent inflammatory diseases, preferably Familial

Mediterranean Fever (FMF), Familial Cold Autoinflammatory Syndrome (FCAS) Neonatal Onset Multisystem Inflammatory Disease (NOMID) and Muckle Wells Syndrome, most preferably Familial Mediterranean Fever (FMF); (b) chronic autoimmune inflammatory diseases, preferably rheumatoid arthritis, systemic onset juvenile idiopathic arthritis, adult onset Still's disease, psoriasis, Crohn's disease and ulcerative colitis, most preferably rheumatoid arthritis; (c) bone and cartilage degenerative diseases, preferably gout, osteoporosis and osteoarthritis, most preferably osteoarthritis; (d) allergic diseases, preferably contact hypersensitivity, type 1 hypersensitivity and allergy, most preferably allergy; and (e) neurological diseases, preferably Alzheimer's disease, epilepsy, Parkinson's disease and multiple sclerosis, most preferably multiple sclerosis, and wherein said at least one antigen comprised by said composition, said vaccine or said pharmaceutical composition is an IL-1 beta molecule, preferably an IL-1 beta mature fragment, most preferably SEQ ID NO:64 or a mutein thereof.

[00140] The invention further provides for use of the compositions of the invention or the vaccine of the invention or the pharmaceutical composition of the invention for the manufacture of a medicament for treatment of a disease in an animal, preferably dog, cat horse or human, most preferably human, wherein said disease is an inherited IL-1-dependent inflammatory diseases, preferably Familial Mediterranean Fever (FMF); and wherein said at least one antigen comprised by said composition, said vaccine or said pharmaceutical composition is an IL-1 beta molecule, preferably an IL-1 beta mature fragment, most preferably SEQ ID NO:64 or a mutein thereof.

[00141] The invention further provides for use of the compositions of the invention or the vaccine of the invention or the pharmaceutical composition of the invention for the manufacture of a medicament for treatment of a disease in an animal, preferably human, wherein said disease is a vascular disease, preferably atherosclerosis.

[00142] The invention further provides for use of the compositions of the invention or the vaccine of the invention or the pharmaceutical composition of the invention for the manufacture of a medicament for treatment of a disease in an animal, preferably human, wherein said disease is an inherited IL-1-dependent inflammatory diseases, preferably familial mediterranean fever (FMF).

[00143] The invention further provides for use of the compositions of the invention or the vaccine of the invention or the pharmaceutical composition of the invention for the manufacture of a medicament for treatment of a disease in an animal, preferably human, wherein said disease is a chronic autoimmune inflammatory diseases, preferably rheumatoid arthritis.

[00144] The invention further provides for use of the compositions of the invention or the vaccine of the invention or the pharmaceutical composition of the invention for the manufacture of a medicament for treatment of a disease in an animal, preferably human, wherein said disease is a bone and cartilage degenerative diseases, preferably osteoarthritis.

[00145] The invention further provides for use of the compositions of the invention or the vaccine of the invention or the pharmaceutical composition of the invention for the manufacture of a medicament for treatment of a disease in an animal, preferably human, wherein said disease is a neurological disease, preferably multiple sclerosis.

[00146] The invention further provides a method of treating a disease, the method comprising administering the composition of the invention, the vaccine of the invention or the pharmaceutical composition of the invention to an animal, preferably dog, cat horse or human, most preferably human, wherein said disease is preferably selected from the group consisting of: (a) vascular diseases, preferably coronary artery disease, atherosclerosis and vasculitis, most preferably atherosclerosis; (b) inherited IL-1-dependent inflammatory diseases, preferably Familial Mediterranean Fever (FMF), Familial Cold autoinflammatory Syndrome (FCAS) Neonatal Onset Multisystem Inflammatory Disease (NOMID) and Muckle Wells Syndrome, most preferably Familial Mediterranean Fever (FMF); (c) chronic autoimmune inflammatory diseases, preferably rheumatoid arthritis, systemic onset juvenile idiopathic arthritis, adult onset Still's disease, psoriasis, Crohn's disease and ulcerative colitis, most preferably rheumatoid arthritis; (d) bone and cartilage degenerative diseases, preferably gout, osteoporosis and osteoarthritis, most preferably osteoarthritis; (e) allergic diseases, preferably contact hypersensitivity, type 1 hypersensitivity and allergy, most preferably allergy; and (f) neurological diseases, preferably Alzheimer's disease, epilepsy, Parkinson's disease and multiple sclerosis, preferably multiple sclerosis.

[00147] The invention further provides a method of treating a disease, the method comprising administering the composition of the invention, the vaccine of the invention or the pharmaceutical composition of the invention to an animal, preferably dog, cat horse or human, most preferably human, wherein said disease is a vascular diseases, preferably coronary artery disease, atherosclerosis and vasculitis, most preferably atherosclerosis, and wherein said at least one antigen comprised by said composition, said vaccine or said pharmaceutical composition is an IL-1 alpha molecule, preferably an IL-1 alpha mature fragment, most preferably SEQ ID NO:63 or a mutein thereof.

[00148] The invention further provides a method of treating a disease, the method comprising administering the composition of the invention, the vaccine of the invention or the pharmaceutical composition of the invention to an animal, preferably dog, cat horse or

human, most preferably human, wherein said disease is preferably selected from the group consisting of: (a) inherited IL-1-dependent inflammatory diseases, preferably Familial Mediterranean Fever (FMF), Familial Cold Autoinflammatory Syndrome (FCAS) Neonatal Onset Multisystem Inflammatory Disease (NOMID) and Muckle Wells Syndrome, most preferably Familial Mediterranean Fever (FMF); (b) chronic autoimmune inflammatory diseases, preferably rheumatoid arthritis, systemic onset juvenile idiopathic arthritis, adult onset Still's disease, psoriasis, Crohn's disease and ulcerative colitis, most preferably rheumatoid arthritis; (c) bone and cartilage degenerative diseases, preferably gout, osteoporosis and osteoarthritis, most preferably osteoarthritis; (d) allergic diseases, preferably contact hypersensitivity, type 1 hypersensitivity and allergy, most preferably allergy; and (e) neurological diseases, preferably Alzheimer's disease, epilepsy, Parkinson's disease and multiple sclerosis, most preferably multiple sclerosis, and wherein said at least one antigen comprised by said composition, said vaccine or said pharmaceutical composition is an IL-1 beta molecule, preferably an IL-1 beta mature fragment, most preferably SEQ ID NO:64 or a mutein thereof.

[00149] The invention further provides a method of treating a disease, the method comprising administering the composition of the invention, the vaccine of the invention or the pharmaceutical composition of the invention to an animal, preferably dog, cat horse or human, most preferably human, wherein said disease is an inherited IL-1-dependent inflammatory diseases, preferably Familial Mediterranean Fever (FMF); and wherein said at least one antigen comprised by said composition, said vaccine or said pharmaceutical composition is an IL-1 beta molecule, preferably an IL-1 beta mature fragment, most preferably SEQ ID NO:64 or a mutein thereof.

[00150] The invention further provides a method of treating a disease, the method comprising administering the composition of the invention, the vaccine of the invention or the pharmaceutical composition of the invention to an animal, preferably human, wherein said disease is a vascular disease, preferably atherosclerosis.

[00151] The invention further provides a method of treating a disease, the method comprising administering the composition of the invention, the vaccine of the invention or the pharmaceutical composition of the invention to an animal, preferably human, wherein said disease is an inherited IL-1-dependent inflammatory diseases, preferably familial mediterranean fever (FMF).

[00152] The invention further provides a method of treating a disease, the method comprising administering the composition of the invention, the vaccine of the invention or the

pharmaceutical composition of the invention to an animal, preferably human, wherein said disease is a chronic autoimmune inflammatory diseases, preferably rheumatoid arthritis.

[00153] The invention further provides a method of treating a disease, the method comprising administering the composition of the invention, the vaccine of the invention or the pharmaceutical composition of the invention to an animal, preferably human, wherein said disease is a bone and cartilage degenerative diseases, preferably osteoarthritis.

All references cited herein are incorporated entirely by reference.

EXAMPLES

EXAMPLE 1

Cloning, expression and purification of murine IL1 α ₁₁₇₋₂₇₀ and IL-1 β ₁₁₉₋₂₆₉

[00154] The nucleotide sequence encoding amino acids 117-270 of murine IL-1 α was amplified by PCR from a cDNA library of TNF α -activated murine macrophages using oligonucleotides IL1 α 1 (5'-ATATATGCTAGCCCCCTTACACCTACCAGAGTGATTTG-3'; SEQ ID NO:24) and IL1 α 2 (5'-ATATATCTCGAGTGATATCTGGAAGTCTGTCATAGAG-3'; SEQ ID NO:25). Using the same cDNA library, the nucleotide sequence encoding amino acids 119-269 of the murine IL-1 β precursor was amplified with oligonucleotides IL1 β 1 (5'-ATATATGCTAGCCCCCATAGACAGCTGCACTACAGG-3'; SEQ ID NO:26) and IL1 β 2 (5'-ATATATCTCGAGGGAAGACACAGATTCCATGGTGAAG-3'; SEQ ID NO: 27). Both DNA fragments were digested with *NheI* and *XhoI*, and cloned into the expression vector pModEC1 (SEQ ID NO:29)

[00155] The vector pModEC1 (SEQ ID NO:29) is a derivative of pET22b(+) (Novagen Inc.), and was constructed in two steps. In a first step the multiple cloning site of pET22b(+) was changed by replacing the original sequence between the *NdeI* and *XhoI* sites with the annealed oligos primerMCS-1F (5'-TATGGATCCGGCTAGCGCTCGAGGGTTTAACGGCGGCCGCAT-3'; SEQ ID NO:30) and primerMCS-1R (5'-TCGAATGCGGCCGCCGTTTAAACCCTCGAGCGCTAGCCGGATCCA-3'; SEQ ID NO:31) (annealing in 15 mM TrisHCl pH 8 buffer). The resulting plasmid was termed pMod00, and had *NdeI*, *BamHI*, *NheI*, *XhoI*, *PmeI* and *NotI* restriction sites in its multiple cloning site. The annealed pair of oligos Bamhis6-EK-Nhe-F (5'-GATCCACACCACCACCACCACCGGTTCTGGTGACGACGATGACAAAGCGCTAGCCC-3'; SEQ ID NO:32) and Bamhis6-EKNhe-R (5'-TCGAGGGCTAGCGCTTTGTCATCGTCGTCACCAGAACCGTGGTGGTGGTGGTGGTGTG-3'; SEQ ID NO:33) and the annealed pair of oligo1F-C-glycine-linker (5'-TCGAGGGTGGTGGTGGTGGTTGCGGTTAATAAGTTTAAACGC-3'; SEQ ID NO:34) and oligo1R-C-glycine-linker (5'-GGCCGCGTTTAAACTTATTAACCGCAACCACCACCACCACCC-3'; SEQ ID NO:35) were ligated together into the *BamHI*-*NotI* digested pMod00 plasmid to obtain pModEC1, which encodes an N-terminal hexahistidine tag, an enterokinase cleavage site and a C-terminal glycine linker containing one cysteine residue.

[00156] The cloning of the above mentioned fragments into pModEC1 gave rise to plasmids pModEC1-His-EK-mIL1 α ₁₁₇₋₂₇₀ and pModEC1-His-EK-mIL1 β ₁₁₉₋₂₆₉, respectively. These

plasmids encode fusion proteins consisting of an N-terminal His-tag, an enterokinase cleavage site, the mature murine IL-1 α or IL-1 β , respectively, and a C-terminal cysteine-containing linker (GGGGGCG, SEQ ID NO:28). For expression, *Escherichia coli* BL21 cells harbouring either plasmid were grown at 37°C to an OD at 600 nm of 1.0 and then induced by addition of isopropyl- β -D-thiogalactopyranoside at a concentration of 1 mM. Bacteria were grown for 4 more hours at 37°C, harvested by centrifugation and resuspended in 80 ml lysis buffer (10 mM Na₂HPO₄, 30 mM NaCl, pH 7.0). Cells were then disrupted by sonication and cellular DNA and RNA were digested by 30 min incubation at room temperature with 64 μ l 2 M MgCl₂ and 10 μ l Benzonase. Cellular debris was removed by centrifugation (SS34 rotor, 20000 rpm, 4 °C, 60 min), and the cleared lysate was applied to a Ni²⁺-NTA agarose column (Qiagen, Hilden, Germany). After extensive washing of the column with washing buffer (50 mM NaH₂PO₄, 300 mM NaCl, 20 mM Imidazol, pH 8.0) the proteins were eluted with elution buffer (50 mM NaH₂PO₄, 300 mM NaCl, 200 mM Imidazol, pH 8.0). Purified proteins were dialysed against PBS pH 7.2, flash-frozen in liquid nitrogen and stored at -80°C until further use.

EXAMPLE 2

A. Coupling of mouse IL-1 β ₁₁₉₋₂₆₉ to Q β virus-like particles

[00157] A solution containing 1.3 mg/ml of the purified murine IL-1 β ₁₁₉₋₂₆₉ protein from EXAMPLE 1 (SEQ ID NO:66) in PBS pH 7.2 was incubated for 60 min at room temperature with an equimolar amount of TCEP for reduction of the C-terminal cysteine residue.

[00158] A solution of 6 ml of 2 mg/ml Q β capsid protein in PBS pH 7.2 was then reacted for 60 min at room temperature with 131 μ l of a SMPH solution (65 mM in DMSO). The reaction solution was dialysed at 4 °C against three 3 l changes of 20 mM HEPES, 150 mM NaCl pH 7.2 over 24 hours. Seventy-five μ l of the derivatized and dialyzed Q β solution was mixed with 117 μ l H₂O and 308 μ l of the purified and pre-reduced mouse IL-1 β ₁₁₉₋₂₆₉ protein and incubated over night at 15°C for chemical crosslinking. Uncoupled protein was removed by tangential flow filtration against PBS using cellulose ester membranes with a molecular weight cutoff of 300.000 Da.

[00159] Coupled products were analyzed on a 12 % SDS-polyacrylamide gel under reducing conditions. The Coomassie stained gel is shown in Fig. 1. Several bands of increased molecular weight with respect to the Q β capsid monomer are visible, clearly demonstrating the successful cross-linking of the mouse IL-1 β ₁₁₉₋₂₆₉ protein to the Q β capsid.

B. Immunization of mice with mouse IL-1 β ₁₁₉₋₂₆₉ protein coupled to Q β capsid (Q β -mIL-1 β ₁₁₉₋₂₆₉)

[00160] Five female balb/c mice were immunized with Q β -mIL-1 β ₁₁₉₋₂₆₉ (SEQ ID NO:66). Fifty μ g of total protein were diluted in PBS to 200 μ l and injected subcutaneously (100 μ l on two ventral sides) on day 0 and day 21. Mice were bled retroorbitally on day 0, 21, and 35, and sera were analyzed using mouse IL-1 β ₁₁₉₋₂₆₉-specific ELISA.

C. ELISA

[00161] ELISA plates were coated with mouse IL-1 β ₁₁₉₋₂₆₉ protein at a concentration of 1 μ g/ml. The plates were blocked and then incubated with serially diluted mouse sera from day 0, 21, and 35. Bound antibodies were detected with enzymatically labeled anti-mouse IgG antibody. Antibody titers of mouse sera were calculated as the average of those dilutions which led to half maximal optical density at 450 nm. The average anti-mouse IL-1 β ₁₁₉₋₂₆₉ titer was 1:22262 at day 21 and 1:309276 at day 35. This demonstrates that immunization with Q β coupled to the mouse IL-1 β ₁₁₉₋₂₆₉ protein could overcome immunological tolerance and produce high titer antibodies which recognize specifically IL-1 β ₁₁₉₋₂₆₉.

D. In vitro neutralization of IL-1 β

[00162] Sera of mice immunized with Q β -mIL-1 β ₁₁₉₋₂₆₉ (SEQ ID NO:66) were then tested for their ability to inhibit the binding of mouse IL-1 β protein to its receptor. ELISA plates were therefore coated with a recombinant mIL-1receptorI-hFc fusion protein at a concentration of 1 μ g/ml, and co-incubated with serial dilutions of sera from mice which had been immunized either with mouse IL-1 β ₁₁₉₋₂₆₉ coupled to Q β capsid or with mouse IL-1 α ₁₁₇₋₂₇₀ coupled to Q β capsid and 100 ng/ml of mouse IL-1 β ₁₁₉₋₂₆₉. Binding of IL-1 β ₁₁₉₋₂₆₉ to the immobilized mIL-1receptorI-hFc fusion protein was detected with a biotinylated anti-mouse IL-1 β antibody and horse radish peroxidase conjugated streptavidin. All sera from mice immunized against murine IL-1 β ₁₁₉₋₂₆₉ inhibited completely the binding of mouse IL-1 β ₁₁₉₋₂₆₉ to its receptor at concentrations of ≥ 0.4 %, whereas sera from mice immunized against mouse IL-1 α ₁₁₇₋₂₇₀ did not show any inhibitory effect even at the highest concentration used (3.3 %). These data demonstrate that immunization with mouse IL-1 β ₁₁₉₋₂₆₉ coupled to Q β capsid can yield antibodies which are able to neutralize the interaction of mouse IL-1 β ₁₁₉₋₂₆₉ and its receptor.

E. In vivo neutralization of IL-1 β

[00163] The in vivo neutralizing capacity of the antibodies raised by immunization with Q β -mIL-1 β ₁₁₉₋₂₆₉ was investigated next. Four female balb/c mice were therefore immunized twice at days 0 and 14 with Q β -mIL-1 β ₁₁₉₋₂₆₉ and four mice were immunized at the same time with Q β capsid alone. At day 21 all mice were injected intravenously with 1 μ g free IL-1 β ₁₁₉₋₂₆₉. As readout of the inflammatory activity of the injected IL-1 β ₁₁₉₋₂₆₉, serum samples were analysed 3 h after injection for the relative increase in the concentration of the pro-inflammatory cytokine IL-6. Q β -immunized mice showed an average increase in the serum IL-6 concentration of 1.01 ± 0.61 ng/ml, whereas mice immunized with Q β -mIL-1 β ₁₁₉₋₂₆₉ showed an average increase of only 0.11 ± 0.30 ng/ml ($p=0.04$). As a control on day 28 all mice were injected with 1 μ g mIL-1 α . Three hours after injection mice immunized with Q β carrier alone showed an average increase in serum IL-6 concentrations of 40.24 ± 8.06 ng/ml, while mice immunized with Q β -mIL-1 β ₁₁₉₋₂₆₉ showed an increase of 57.98 ± 29.92 ng/ml ($p=0.30$). These data indicate that the antibodies produced by immunization with Q β -mIL-1 β ₁₁₉₋₂₆₉ were able to neutralize specifically and efficiently the pro-inflammatory activity of IL-1 β .

F. Efficacy of Q β -mIL-1 β ₁₁₉₋₂₆₉ in a mouse model of rheumatoid arthritis

[00164] The efficacy of Q β -mIL-1 β ₁₁₉₋₂₆₉ immunization was tested in the murine collagen-induced arthritis model (CIA). This model reflects most of the immunological and histological aspects of human rheumatoid arthritis and is therefore routinely used to assay the efficacy of anti-inflammatory agents. Male DBA/1 mice were immunized subcutaneously three times (days 0, 14 and 28) with 50 μ g of either Q β -mIL-1 β ₁₁₉₋₂₆₉ ($n=8$) or Q β alone ($n=8$), and then injected intradermally at day 42 with 200 μ g bovine type II collagen mixed with complete Freund's adjuvant. After a booster injection of 200 μ g bovine type II collagen mixed with incomplete Freund's adjuvant at day 63 mice were examined on a daily basis for the development of arthritis symptoms.

[00165] A clinical score ranging from 0 to 3 was assigned to each limb according to the degree of reddening and swelling observed, and ankle thickness of all hind limbs was measured. The clinical score was assigned over 3 consecutive weeks to each limb according to the following definitions: 0 normal, 1 mild erythema and/or swelling of digits/paw, 2 erythema and swelling extending over whole paw/joint, 3 strong swelling, deformation of paw/joint, stiffness. Cumulative clinical scores of individual mice were calculated as the sum

of clinical scores of all four limbs, resulting in a possible maximal cumulative score per mouse of 12.

[00166] Two weeks after the second collagen injection Q β -immunized mice showed an average cumulative clinical score of 4.44, while Q β -mIL-1 β ₁₁₉₋₂₆₉-immunized mice showed an average score of only 1.06. Moreover, the average increase in hind ankle thickness was 18 % for Q β -immunized mice and only 1 % for mice which had been immunized with Q β -mIL-1 β ₁₁₉₋₂₆₉. As an additional readout of the inflammatory reaction, serum levels of IL-6 were determined 1 week after the second collagen injection. Q β -immunized mice had an average IL-6 serum concentration of 1.92 ± 0.36 while Q β -mIL-1 β ₁₁₉₋₂₆₉-immunized mice had an average IL-6 concentration of only 0.79 ± 0.16 ($p=0.01$). Taken together, these data show that immunization with Q β -mIL-1 β ₁₁₉₋₂₆₉ strongly protects mice from inflammation and clinical signs of arthritis in the CIA model.

EXAMPLE 3

A. Coupling of mouse IL-1 α ₁₁₇₋₂₇₀ to Q β virus-like particles

[00167] A solution containing 1.8 mg/ml of the purified IL-1 α ₁₁₇₋₂₇₀ protein from EXAMPLE 1 (SEQ ID NO:65) in PBS pH 7.2 was incubated for 60 min at room temperature with an equimolar amount of TCEP for reduction of the C-terminal cysteine residue.

[00168] A solution of 6 ml of 2 mg/ml Q β capsid protein in PBS pH 7.2 was then reacted for 60 minutes at room temperature with 131 μ l of a SMPH solution (65 mM in DMSO). The reaction solution was dialyzed at 4 °C against three 3 l changes of 20 mM HEPES, 150 mM NaCl pH 7.2 over 24 hours. Seventy-five μ l of the derivatized and dialyzed Q β solution was mixed with 192 μ l H₂O and 233 μ l of the purified and pre-reduced mouse IL-1 α ₁₁₇₋₂₇₀ protein and incubated over night at 15°C for chemical crosslinking. Uncoupled protein was removed by tangential flow filtration against PBS using cellulose ester membranes with a molecular weight cutoff of 300.000 Da.

[00169] Coupled products were analyzed on a 12 % SDS-polyacrylamide gel under reducing conditions. The Coomassie stained gel is shown in Fig. 2. Several bands of increased molecular weight with respect to the Q β capsid monomer are visible, clearly demonstrating the successful cross-linking of the mouse IL-1 α ₁₁₇₋₂₇₀ protein to the Q β capsid.

**B. Immunization of mice with mouse IL-1 α ₁₁₇₋₂₇₀ protein coupled
to Q β capsid (Q β -mIL-1 α ₁₁₇₋₂₇₀)**

[00170] Five female balb/c mice were immunized with Q β -mIL-1 α ₁₁₇₋₂₇₀. Fifty μ g of total protein were diluted in PBS to 200 μ l and injected subcutaneously (100 μ l on two ventral sides) on day 0 and day 21. Mice were bled retroorbitally on day 0, 21, and 35, and sera were analyzed using mouse IL-1 α ₁₁₇₋₂₇₀-specific ELISA.

C. ELISA

[00171] ELISA plates were coated with mouse IL-1 α ₁₁₇₋₂₇₀ protein at a concentration of 1 μ g/ml. The plates were blocked and then incubated with serially diluted mouse sera from day 0, 21, and 35. Bound antibodies were detected with enzymatically labeled anti-mouse IgG antibody. Antibody titers of mouse sera were calculated as the average of those dilutions which led to half maximal optical density at 450 nm. The average anti-mouse IL-1 α ₁₁₇₋₂₇₀ titer was 1:9252 at day 21 and 1:736912 at day 35. This demonstrates that immunization with Q β coupled to the mouse IL-1 α ₁₁₇₋₂₇₀ protein could overcome immunological tolerance and produce high titer antibodies which recognize specifically IL-1 α ₁₁₇₋₂₇₀.

D. In vitro neutralization of IL-1 α

[00172] Sera of mice immunized with Q β -mIL-1 α ₁₁₇₋₂₇₀ were then tested for their ability to inhibit the binding of mouse IL-1 α protein to its receptor. ELISA plates were therefore coated with a recombinant mIL-1receptorI-hFc fusion protein at a concentration of 1 μ g/ml, and co-incubated with serial dilutions of sera from mice which had been immunized either with mouse IL-1 α ₁₁₇₋₂₇₀ coupled to Q β capsid or with mouse IL-1 β ₁₁₉₋₂₆₉ coupled to Q β capsid and 5 ng/ml of mouse IL-1 α ₁₁₇₋₂₇₀. Binding of IL-1 α ₁₁₇₋₂₇₀ to the immobilized mIL-1receptorI-hFc fusion protein was detected with a biotinylated anti-mouse IL-1 α antibody and horse radish peroxidase conjugated streptavidin. All sera from mice immunized against murine IL-1 α ₁₁₇₋₂₇₀ inhibited completely the binding of mouse IL-1 α ₁₁₇₋₂₇₀ to its receptor at concentrations of $\geq 0.4\%$, whereas sera from mice immunized against mouse IL-1 β ₁₁₉₋₂₆₉ did not show a significant inhibitory effect even at the highest concentration used (3.3 %). These data demonstrate that immunization with mouse IL-1 α ₁₁₇₋₂₇₀ coupled to Q β capsid can yield antibodies which are able to neutralize specifically the interaction of mouse IL-1 α ₁₁₇₋₂₇₀ and its receptor.

E. In vivo neutralization of IL-1 α

[00173] The in vivo neutralizing capacity of the antibodies raised by immunization with Q β -mIL-1 α ₁₁₇₋₂₇₀ was investigated next. Four female balb/c mice were therefore immunized twice at days 0 and 14 with Q β -mIL-1 α ₁₁₇₋₂₇₀ and four mice were immunized at the same time with Q β capsid alone. At day 21 all mice were injected intravenously with 1 μ g free IL-1 α ₁₁₇₋₂₇₀. As readout of the inflammatory activity of the injected IL-1 α ₁₁₇₋₂₇₀, serum samples were analysed 3 h after injection for the relative increase in the concentration of the pro-inflammatory cytokine IL-6. Q β -immunized mice showed an average increase in the serum IL-6 concentration of 8.16 ± 2.33 ng/ml, whereas mice immunized with Q β -mIL-1 α ₁₁₇₋₂₇₀ showed an average increase of only 0.15 ± 0.27 ng/ml ($p=0.0005$). As a control on day 28 all mice were injected with 1 μ g mIL-1 β . Three hours after injection mice immunized with Q β carrier alone showed an average increase in serum IL-6 concentrations of 9.52 ± 7.33 ng/ml, while mice immunized with Q β -mIL-1 α ₁₁₇₋₂₇₀ showed an increase of 21.46 ± 27.36 ng/ml ($p=0.43$). These data indicate that the antibodies produced by immunization with Q β -mIL-1 α ₁₁₇₋₂₇₀ were able to neutralize specifically and efficiently the pro-inflammatory activity of IL-1 α .

F. Efficacy of Q β -mIL-1 α ₁₁₇₋₂₇₀ in a mouse model of rheumatoid arthritis

[00174] The efficacy of Q β -mIL-1 α ₁₁₇₋₂₇₀ immunization was tested in the murine collagen-induced arthritis model (CIA). This model reflects most of the immunological and histological aspects of human rheumatoid arthritis and is therefore routinely used to assay the efficacy of anti-inflammatory agents. Male DBA/1 mice were immunized subcutaneously three times (days 0, 14 and 28) with 50 μ g of either Q β -mIL-1 α ₁₁₇₋₂₇₀ ($n=8$) or Q β alone ($n=8$), and then injected intradermally at day 42 with 200 μ g bovine type II collagen mixed with complete Freund's adjuvant. After a booster injection of 200 μ g bovine type II collagen mixed with incomplete Freund's adjuvant at day 63 mice were examined on a daily basis for the development of arthritis symptoms. A clinical score as defined in EXAMPLE 2F was assigned to each limb according to the degree of reddening and swelling observed, and ankle thickness of all hind limbs was measured. Two weeks after the second collagen injection Q β -immunized mice showed an average cumulative clinical score of 4.44, while Q β -mIL-1 α ₁₁₇₋₂₇₀-immunized mice showed an average score of only 2.31. Moreover, the average increase in hind ankle thickness was 18 % for Q β -immunized mice and only 7 % for mice which had been immunized with Q β -mIL-1 α ₁₁₇₋₂₇₀. As an additional readout of the inflammatory reaction, serum levels of IL-6 were determined 1 week after the second collagen injection.

Q β -immunized mice had an average IL-6 serum concentration of 1.92 ± 0.36 while Q β -mIL-1 $\alpha_{117-270}$ -immunized mice had an average IL-6 concentration of only 0.94 ± 0.48 . Taken together, these data show that immunization with Q β -mIL-1 $\alpha_{117-270}$ protects mice from inflammation and clinical signs of arthritis in the CIA model.

EXAMPLE 4

Efficacy of Q β -mIL-1 $\alpha_{117-270}$ in a mouse model of atherosclerosis

[00175] Seven to eight weeks old male *Apoe*^{-/-} mice (The Jackson Laboratory, Bar Harbor ME) were injected subcutaneously with either 50 μ g Q β -mIL-1 $\alpha_{117-270}$ vaccine (n=13) or with 50 μ g Q β (n=12) on day 0, 14, 28, 56, 105 and 133 (5 animals, 3 in the Q β -mIL-1 $\alpha_{117-270}$ and 2 in the Q β groups were received their second boost on day 33). The mice were fed initially with a normal chow diet, which was replaced on day 21 by a western diet (20 % fat, 0.15 % cholesterol, Provimi Kliba AG, Switzerland). Mice were bled at regular intervals throughout the experiment and the antibody response against IL-1 α was measured in the sera. Sacrifice was on day 159, and the aorta were isolated and prepared essentially as described (Tangirala R.K. et al. (1995) *J. Lipid. Res.* 36: 2320-2328). In addition, hearts were removed and snap-frozen in liquid nitrogen for subsequent histologic preparation essentially as described by Paigen B. et al. (*Atherosclerosis* 1987;68:231-240) and Zhou X. et al. (*Arterioscler Thromb Vasc Biol* 2001;21:108-114). The animals were bled by cardiac puncture and perfused with cold PBS. The aorta was then exposed, as much as possible of the adventitia removed in situ, and the aorta finally sectioned 2 mm from the heart. The heart was sectioned in the middle, and the upper part was immediately frozen in Hank's balanced salt solution in a plastic tube in liquid nitrogen. Serial sections (7 μ m thickness) were cut in a cryostat through the origin of the aorta and harvested upon appearance of at least two valve cusps, until disappearance of the last valve cusps. Sections were fixed in formalin, stained with oil red O, and plaque load was evaluated in 4-7 sections (3 sections in one animal of the Q β group) per mouse by quantitative image analysis. An average plaque area was computed for each animal from the plaque area of each section used for the evaluation. An average group plaque area was computed for the Q β -mIL-1 $\alpha_{117-270}$ and Q β group respectively. Statistical analysis was performed with a *Student* t-test. $P < 0.05$ was considered statistically significant.

[00176] For the evaluation of atherosclerosis in the whole aorta, these were further cleaned from residual adventitia on a glass petri dish filled with cold PBS, and the arch was sectioned 5 mm down from the left sub-clavian artery. The aorta were cut longitudinally, pinned out on

a black wax surface and fixed overnight in 4 % formalin. They were then stained overnight in oil red O. The plaques were quantified with an imaging software (Motic Image Plus 2.0) on digital photographs. The plaque load was expressed as the sum of the surface of all plaques of the aorta taken up to the iliac bifurcation, divided by the total surface of the aorta measured up to the iliac bifurcation, in percentage. The difference in mean or median of the plaque load between the Q β -mIL-1 $\alpha_{117-270}$ and Q β group was analyzed.

[00177] The antibody response was measured in a classical ELISA, with recombinant IL-1alpha coated on the ELISA plate. Binding of specific antibodies was detected using a goat anti-mouse HRP conjugate. The titers against IL-1alpha were calculated as the reciprocal of the serum dilution giving half-maximal binding in the assay. Specificity of the response was assessed by measuring pre-immune serum. The pre-immune titer was below the lowest serum dilution used in the assay, and was assigned this lowest-serum dilution value. The results of the measurement of the antibody response in the Q β -mIL-1 $\alpha_{117-270}$ immunized animals are shown in Table 1, and clearly demonstrate that immunization against murine IL-1alpha coupled to Q β led to a strong and sustained specific antibody response against IL-1alpha, since nearly no titer was detectable in the preimmune (d0) sera. Furthermore, induction of an antibody response specific for IL-1alpha led to a reduction of 37 % in plaque area at the aortic origin in the Q β -mIL-1 $\alpha_{117-270}$ group compared to the Q β group ($292803 \pm 21272 \mu\text{m}^2$ vs. $464694 \pm 36545 \mu\text{m}^2$, $p=0.0005$). In addition, a reduction of 31 % in median plaque load in whole aortas prepared "en face" (5.7 vs. 8.3, $p=0.06$) was observed.

[00178] These data demonstrate that induction of anti-IL1alpha antibodies by the Q β -mIL-1 $\alpha_{117-270}$ vaccine inhibited the development of atherosclerosis and therefore that the Q β -mIL-1 $\alpha_{117-270}$ vaccine is an effective treatment for atherosclerosis. Furthermore, these data demonstrate that IL-1alpha is involved in the pathogenesis of atherosclerosis.

Table 1: Geometric mean anti-IL1alpha antibody titer in *Apoe*^{-/-} mice immunized with Q β -IL1alpha (geometric mean titer $\pm$ standard error of the mean)

	d0	d21	d28*	d56	d84	d105	d159
Geomean $\pm$	<1000	225400 $\pm$	167867 $\pm$	522864 $\pm$	712061 $\pm$	621687 $\pm$	805370 $\pm$
SEM	0	93385	121345	106887	144922	184389	155764

* For 5 animals, the values are from day 33.

EXAMPLE 5

**Protection from TNBS-induced inflammatory bowel disease by immunization
with Q β -mIL-1 α ₁₁₇₋₂₇₀ and/or Q β -mIL-1 β ₁₁₉₋₂₆₉**

[00179] Eight weeks old male SJL mice (5 per group) are injected subcutaneously three times at two week intervals with either 50 μ g of Q β -mIL-1 α ₁₁₇₋₂₇₀ or 50 μ g Q β -mIL-1 β ₁₁₉₋₂₆₉, or a mixture of 50 μ g each of Q β -mIL-1 α ₁₁₇₋₂₇₀ and Q β -mIL-1 β ₁₁₉₋₂₆₉. As a control 5 mice are injected at the same regimen with Q β VLPs alone. Two weeks after the last immunization, all mice are slightly anesthetized with Isofluran, and 1 mg of trinitrobenzenesulfonic acid (TNBS) in 100 μ l 50 % ethanol is administered intrarectally via a polyethylene catheter at a distance of 4 cm of the anus. Body weight is recorded daily as readout of disease progression, and 7 days after TNBS administration all mice are sacrificed. The colon of each mouse is removed, a specimen of colon located 2 cm proximal to the anus is fixed in PBS-buffered formalin, and the degree of inflammation is graded semi-quantitatively on hematoxylin- and eosin-stained colonic cross-sections according to Neurath M.F. et al. (JEM (1995), 182:1281-1290).

[00180] Immunization with either Q β -mIL-1 α ₁₁₇₋₂₇₀ or Q β -mIL-1 β ₁₁₉₋₂₆₉ alone, or with a combination of Q β -mIL-1 α ₁₁₇₋₂₇₀ and Q β -mIL-1 β ₁₁₉₋₂₆₉ reduces the TNBS-induced weight loss, as compared to Q β -immunized mice. Furthermore, histological examination of colonic cross-sections reveals, that Q β -mIL-1 α ₁₁₇₋₂₇₀ and/or Q β -mIL-1 β ₁₁₉₋₂₆₉-immunized mice display a markedly reduced infiltration of inflammatory cells into the colonic tissue when compared to Q β -immunized mice.

EXAMPLE 6

**Amelioration of Endotoxin-hypersensitivity in mice carrying a truncated version of the
MEFV gene by immunization with Q β -mIL-1 β ₁₁₉₋₂₆₉**

[00181] Familial Mediterranean Fever is a recessively inherited inflammatory disorder characterized by recurrent fever as well as peritonitis, serositis, arthritis and skin rashes. Affected individuals carry a missense mutation in the MEFV gene, leading to expression of a truncated pyrin protein. Mice carrying a similar mutation in the MEFV gene show an increased caspase-1 activity, leading to overproduction of mature IL-1 β and increased hypothermia and lethality after LPS administration. Eight weeks old homozygote pyrin-truncation mice (5 per group) are immunized three times at two weeks intervals with 50 μ g of Q β -mIL-1 β ₁₁₉₋₂₆₉ or 50 μ g of Q β VLPs alone. Two weeks after the last immunization all mice are injected intraperitoneally with a mixture 20 mg D-Galactosamine and 0.01 μ g/g LPS. Q β -

mIL-1 β ₁₁₉₋₂₆₉-immunized mice show a markedly reduced hypothermia and a reduced lethality in response to LPS administration, when compared to Q β -immunized controls.

EXAMPLE 7

Comparison of Q β -mIL-1 α ₁₁₇₋₂₇₀ and Q β -mIL-1 β ₁₁₉₋₂₆₉ immunization to Kineret® treatment in a mouse model of rheumatoid arthritis

[00182] Kineret® (Anakinra, Amgen) is a recombinant version of the human IL-1 receptor antagonist, which is approved for the treatment of human rheumatoid arthritis. In order to reach a clinical benefit, relatively high amounts (100 mg) have to be applied via subcutaneous injection on a daily basis. The collagen-induced arthritis model was used to compare the efficacy of Q β -mIL-1 α ₁₁₇₋₂₇₀ and Q β -mIL-1 β ₁₁₉₋₂₆₉ immunization with daily applications of different doses of Kineret®. Male DBA/1 mice were immunized subcutaneously three times (days 0, 14 and 28) with 50 μ g of either Q β -mIL-1 α ₁₁₇₋₂₇₀ (n=8), Q β -mIL-1 β ₁₁₉₋₂₆₉ (n=8) or Q β alone (n=32), and then injected intradermally on day 42 with 200 μ g bovine type II collagen mixed with complete Freund's adjuvant. From day 42 on, mice immunized with Q β -mIL-1 α ₁₁₇₋₂₇₀ and Q β -mIL-1 β ₁₁₉₋₂₆₉, and one group of Q β -immunized mice (n=8) received daily intraperitoneal injections of 200 μ l PBS, while three additional Q β -immunized groups received daily intraperitoneal injections of either 37.5 μ g (n=8), 375 μ g (n=8), or 3.75 mg (n=8) Kineret®. A daily injection of 37.5 μ g Kineret® per mouse corresponds roughly to a dose of 1.5 mg/kg, which is in the range of the recommended efficacious amount for humans (100 mg). All mice were boosted on day 63 by intradermal injection of 200 μ g bovine type II collagen mixed with incomplete Freund's adjuvant, and examined on a daily basis for the development of arthritis symptoms.

[00183] Four weeks after the second collagen injection, Q β -immunized control mice showed an average cumulative clinical score (as defined in EXAMPLE 2F) of 3.75, while Q β -mIL-1 α ₁₁₇₋₂₇₀- and Q β -mIL-1 β ₁₁₉₋₂₆₉-immunized mice showed average scores of only 0.81 and 1.44, respectively (see Table 2). Mice treated with 37.5 μ g or 375 μ g Kineret® reached an average score of 2.44 and 2.63, respectively, while mice treated with 3.75 mg Kineret® remained largely asymptomatic, reaching a maximal score of only 0.19.

[00184] As an additional readout of the inflammatory reaction, the hind ankle thickness of all animals was measured on a regular basis. Four weeks after the second collagen injection Q β -immunized control mice showed an average increase in hind ankle thickness of 16 %, while Q β -mIL-1 α ₁₁₇₋₂₇₀-immunized mice showed an increase of 2% and Q β -mIL-1 β ₁₁₉₋₂₆₉-immunized mice showed an increase of 6 %. Mice treated with either 37.5 μ g or 375 μ g

Kineret® showed an average increase of 13 % and 10 %, respectively, while mice treated with 3.75 mg Kineret® showed no increase in hind ankle thickness at all.

[00185] In conclusion we surprisingly found that three injections of either Q β -mIL-1 α ₁₁₇₋₂₇₀ or Q β -mIL-1 β ₁₁₉₋₂₆₉ protected mice better from the development of arthritis symptoms than daily injections of Kineret® in amounts corresponding to the human dose or even the ten-fold human dose. Only application of the 100-fold human dose of Kineret® showed an increased benefit with respect to Q β -mIL-1 α ₁₁₇₋₂₇₀ or Q β -mIL-1 β ₁₁₉₋₂₆₉ vaccination.

Table 2: clinical disease symptoms in collagen-induced arthritis model.

Treatment	average clinical score day 91	Average increase in hind ankle thickness (%) day 63-91
3x Q β s.c. + PBS i.p. (200 μ l/day)	3.75	16
3x Q β -mIL-1 α ₁₁₇₋₂₇₀ s.c. + PBS i.p. (200 μ l/day)	0.81	2
3x Q β -mIL-1 β ₁₁₉₋₂₆₉ s.c. + PBS i.p. (200 μ l/day)	1.44	6
3x Q β s.c. + Kineret® i.p. (37.5 μ g/day)	2.44	13
3x Q β s.c. + Kineret® i.p. (375 μ g/day)	2.63	10
3x Q β s.c. + Kineret® i.p. (3.75 mg/day)	0.19	0

EXAMPLE 8

A. Cloning, expression, and purification of virus-like particles consisting of AP205 coat protein genetically fused to mouse IL-1 α ₁₁₇₋₂₇₀ (AP205_mIL-1 α ₁₁₇₋₂₇₀)

[00186] Given the large size of interleukin-1 α and for steric reasons, an expression system producing so called mosaic particles, comprising AP205 coat proteins fused to interleukin-1 α as well as wt coat protein subunits was constructed. In this system, suppression of the stop codon yields the AP205-interleukin-1 α coat protein fusion, while proper termination yields the wt AP205 coat protein. Both proteins are produced simultaneously in the cell and assemble into a mosaic virus-like particle. Two intermediary plasmids, pAP590 and pAP592, encoding the AP205 coat protein gene terminated by the suppressor codons TAG (amber, pAP590) or TGA (opal, pAP592) were made. A linker sequence encoding the tripeptide Gly-Ser-Gly (SEQ ID NO:189) was added downstream and in frame of the coat protein gene. Kpn2I and HindIII sites were added for cloning sequences encoding foreign amino acid sequences at the C-terminus of the Gly-Ser-Gly amino acid linker, C-terminal to the AP205 coat protein. The resulting constructs were: AP590 (SEQ ID

NO:117): AP205 coat protein gene – amber codon – GSG(Kpn2I – HindIII); and AP592 (SEQ ID NO:118): AP205 coat protein gene – opal codon – GSG(Kpn2I – HindIII). For construction of plasmid pAP590, a PCR fragment obtained with oligonucleotides p1.44 (5'-NNCCATGGCAAATAAGCCAATGCAACCG-3'; SEQ ID NO:119) and pINC-36 (5'-GTAAGCTTAGATGCATTATCCGGA TCCCTAAGCAGTAGTATCAGACGATACG-3'; SEQ-ID NO:120) was digested with NcoI and HindIII, and cloned into vector pQb185, which had been digested with the same restriction enzymes. pQb185 is a vector derived from pGEM vector. Expression of the cloned genes in this vector is controlled by the *trp* promoter (Kozlovskaya, T. M. *et al.*, *Gene* 137:133-37 (1993)). Similarly, plasmid pAP592 was constructed by cloning a NcoI/HindIII-digested PCR fragment obtained with oligonucleotides p1.44 and pINC-40 (5'-GTAAGCTTAGATGCATTATCCGGATCCTCAAGCAGTAGTATCAGACGATACG-3'; SEQ-ID NO:121) into the same vector.

[00187] The sequence encoding amino acids 117-270 of murine IL-1 α was amplified by PCR from plasmid pModEC1-His-EK-mIL1 α ₁₁₇₋₂₇₀ (see EXAMPLE 1), using primers pINC-34 (5'-GGTCCGGAGCGCTAGCCCCTTACAC-3'; SEQ ID NO:122) and pINC-35 (5'-GTAAGCTTATGCATTATGATATCTGGAAGTCTGTCATAGA-3'; SEQ ID NO:123), which added Kpn2I and HindIII restriction sites to the 5' and 3' ends, respectively. The obtained DNA fragment was digested with Kpn2I and HindIII and cloned into both vector pAP590, creating plasmid pAP594 (amber suppression), and into vector pAP592, creating plasmid pAP596 (opal suppression), respectively.

[00188] For expression of mosaic AP205 VLPs displaying murine IL-1 α on their surface, *E.coli* JM109 cells containing plasmid pISM 579 or pISM 3001 were transformed with plasmid pAP594 or pAP596, respectively. Plasmid pISM579 was generated by excising the *trpT176* gene from pISM3001 with restriction endonuclease *EcoRI* and replacing it by an *EcoRI* fragment from plasmid pMY579 (gift of Michael Yarus) containing an amber t-RNA suppressor gene. This t-RNA suppressor gene is a mutant of *trpT175* (Raftery LA. *Et al.* (1984) *J. Bacteriol.* 158:849-859), and differs from *trpT* at three positions: G33, A24 and T35. Five milliliters of LB liquid medium containing 20 μ g/ml ampicillin and 10 μ g/ml kanamycin were inoculated with a single colony, and incubated at 37 °C for 16-24 h without shaking. The prepared inoculum was diluted 50 x with M9 medium containing 20 μ g/ml ampicillin and 10 μ g/ml Kanamycin and incubated at 37 °C overnight on a shaker. Cells were harvested by centrifugation.

[00189] Cells (1 g, transformed with plasmid pAP594 and containing pISM579) were lysed by ultrasonication in lysis buffer (20 mM Tris-HCl, 5mM EDTA, 150 mM NaCl, pH 7.8, 0.1 % Tween 20). The lysate was cleared by centrifugation, and the cell debris were washed

with lysis buffer. Pooled supernatant were loaded on a Sepharose CL-4B column eluted in TEN buffer (20 mM Tris-HCl, 5mM EDTA, 150 mM NaCl, pH 7.8). The presence of capsids in the cleared lysate and wash supernatant was confirmed by agarose gel electrophoresis (1 % TAE, ethidium bromide stained gel and UV detection). Two peaks eluted from the column as determined by SDS-PAGE or UV-spectrometric analysis of light scattering at 310 nm. Fractions of the second peak, containing the capsids, were pooled and loaded on a Sepharose CL-6B column. Peak fractions from the CL-6B column were pooled and concentrated using a centrifugal filter unit (Amicon Ultra 15 MWCO 30000, Millipore). The protein was purified further by one additional round of gel filtration on a CL-4B column, and the resulting peak fractions were pooled and concentrated on a centrifugal filter unit as above. The buffer was exchanged to 10 mM Hepes, pH 7.5, and glycerol was added to a final concentration of 50 %.

[00190] Purification of AP205_mIL-1 α ₁₁₇₋₂₇₀ from plasmid pAP596 was performed essentially as described for pAP594 above, with the inclusion of an additional sucrose gradient purification step after the last CL-4B column. The protein was layered on a gradient prepared with the following sucrose solutions: 9 ml 36 %, 3 ml 30 %, 6 ml 25 %, 8 ml 20 %, 6 ml 15 %, 6 ml 10 % and 3 ml 5% sucrose. Fractions were identified by UV spectroscopy, and pooled fractions containing the capsids were concentrated on a centrifugal filter unit as above, and the buffer exchanged to 10 mM Hepes, pH 7.5. Glycerol was finally added to a final concentration of 50 %.

B. Immunization of mice with AP205_mIL-1 α ₁₁₇₋₂₇₀

[00191] Four female balb/c mice were immunized with AP205_mIL-1 α ₁₁₇₋₂₇₀. Twentyfive μ g of total protein were diluted in PBS to 200 μ l and injected subcutaneously (100 μ l on two ventral sides) on day 0, day 14, and day 28. Mice were bled retroorbitally on days 0, 14, 28 and 35, and sera were analyzed using mouse IL-1 α ₁₁₇₋₂₇₀-specific ELISA.

C. ELISA

[00192] ELISA plates were coated with mouse IL-1 α ₁₁₇₋₂₇₀ protein at a concentration of 1 μ g/ml. The plates were blocked and then incubated with serially diluted mouse sera from days 14, 28 and 35. Bound antibodies were detected with enzymatically labeled anti-mouse IgG antibody. Antibody titers of mouse sera were calculated as the average of those dilutions which led to half maximal optical density at 450 nm. The average anti-mouse IL-1 α ₁₁₇₋₂₇₀ titer was 1:4412 at day 14, 1:27955 on day 28 and 1:34824 on day 35. This demonstrates that

immunization with AP205_mIL-1 α ₁₁₇₋₂₇₀ could overcome immunological tolerance and produce high titer antibodies which recognize specifically IL-1 α ₁₁₇₋₂₇₀.

D. In vitro neutralization of IL-1 α

[00193] Sera of mice immunized with AP205_mIL-1 α ₁₁₇₋₂₇₀ were tested for their ability to inhibit the binding of mouse IL-1 α protein to its receptor. ELISA plates were therefore coated with a recombinant mIL-1receptorI-hFc fusion protein at a concentration of 1 μ g/ml, and co-incubated with serial dilutions of sera from mice which had been immunized either with AP205_mIL-1 α ₁₁₇₋₂₇₀ or with AP205 alone and 100 ng/ml of mouse IL-1 α ₁₁₇₋₂₇₀. Binding of mIL-1 α ₁₁₇₋₂₇₀ to the immobilized mIL-1receptorI-hFc fusion protein was detected with a biotinylated anti-mouse IL-1 α antibody and horse radish peroxidase conjugated streptavidin. All sera from mice immunized AP205_mIL-1 α ₁₁₇₋₂₇₀ inhibited completely the binding of mouse IL-1 α ₁₁₇₋₂₇₀ to its receptor at concentrations of ≥ 3.3 %, whereas sera from mice immunized with AP205 did not show a significant inhibitory effect at any concentration used. These data demonstrate that immunization with AP205_mIL-1 α ₁₁₇₋₂₇₀ can yield antibodies which are able to neutralize specifically the interaction of mouse IL-1 α ₁₁₇₋₂₇₀ with its receptor.

E. In vivo neutralization of IL-1 α

[00194] The in vivo neutralizing capacity of the antibodies raised by immunization with AP205_mIL-1 α ₁₁₇₋₂₇₀ was investigated next. Four female balb/c mice were therefore immunized three times on days 0, 14, and 28 with AP205_mIL-1 α ₁₁₇₋₂₇₀ and four mice were immunized at the same time with AP205 alone. On day 42 all mice were injected intravenously with 1 μ g of free murine IL-1 α ₁₁₇₋₂₇₀. As readout of the inflammatory activity of the injected IL-1 α ₁₁₇₋₂₇₀, serum samples were withdrawn before and 3 h after injection and analyzed for the relative increase in the concentration of the pro-inflammatory cytokine IL-6. AP205-immunized mice showed an average increase in the serum IL-6 concentration of 12.92 ± 3.95 ng/ml, whereas mice immunized with AP205_mIL-1 α ₁₁₇₋₂₇₀ showed an average increase of only 0.06 ± 0.05 ng/ml ($p < 0.01$). These data indicate that the antibodies produced by immunization with AP205_mIL-1 α ₁₁₇₋₂₇₀ were able to neutralize specifically and efficiently the pro-inflammatory activity of IL-1 α .

F. Efficacy of AP205_mIL-1 α ₁₁₇₋₂₇₀ in a mouse model of rheumatoid arthritis

[00195] The efficacy of AP205_mIL-1 α ₁₁₇₋₂₇₀-immunization was tested in the murine collagen-induced arthritis model (CIA). Male DBA/1 mice were immunized subcutaneously three times (days 0, 14 and 28) with 50 μ g of either AP205_mIL-1 α ₁₁₇₋₂₇₀ (n=8) or AP205 alone (n=8), and then injected intradermally on day 42 with 200 μ g bovine type II collagen mixed with complete Freund's adjuvant. After a booster injection of 200 μ g bovine type II collagen mixed with incomplete Freund's adjuvant on day 63, mice were examined on a daily basis for the development of arthritis symptoms. A clinical score ranging from 0 to 3 was assigned to each limb according to the degree of reddening and swelling observed, and ankle thickness of all hind limbs was measured. Four weeks after the second collagen injection Q β -immunized mice showed an average cumulative clinical score of 5.81, while AP205_mIL-1 α ₁₁₇₋₂₇₀-immunized mice showed an average score of only 2.06. Moreover, the average increase in hind ankle thickness was 19 % for AP205-immunized mice and only 9 % for mice which had been immunized with AP205_mIL-1 α ₁₁₇₋₂₇₀. Taken together, these data show that immunization with AP205_mIL-1 α ₁₁₇₋₂₇₀ strongly protects mice from inflammation and clinical signs of arthritis in the CIA model.

EXAMPLE 9

A. Cloning and expression of virus-like particles consisting of AP205 coat protein genetically fused to mouse IL-1 β ₁₁₉₋₂₆₉ (AP205_mIL-1 β ₁₁₉₋₂₆₉)

[00196] Cloning, expression and purification of virus-like particles consisting of AP205 coat protein genetically fused to mouse IL-1 β ₁₁₉₋₂₆₉ is carried out essentially as described for AP205_mIL-1 α ₁₁₇₋₂₇₀ in EXAMPLE 8. The sequence of murine interleukin 1 beta was amplified from plasmid pModEC1-His-EK-mIL1 β ₁₁₉₋₂₆₉ coding for murine interleukin 1 beta using primers pINC-75 (5'-GATCCGGAGGTGGTGTCCCCATTAGACAGCT-3', SEQ ID NO:192) and pINC-77 (5'-GTAAGCTTAGGAAGACACAGATTCCAT-3', SEQ ID NO:193). These primers amplify a murine interleukin-1 beta gene with 5' Kpn2I and 3' Hind III sites, and encoding additionally the amino acid sequence Gly-Gly at the N-terminus of murine interleukin 1beta. The obtained mur-IL-1 β fragment was digested with *Kpn2I* and *HindIII* and cloned in the same restriction sites into vector pAP590 (amber suppression) creating plasmid pAP630. *E.coli* JM109 containing plasmid pISM 579, providing amber suppression, was transformed with plasmid pAP630. 5 ml of LB liquid medium with 20 μ g/ml ampicillin and 10 μ g/ml kanamycin were inoculated with a single colony, and incubated at 37 °C for 16-24 h without shaking. The prepared inoculum was diluted 50x with M9 medium

containing 20 µg/ml ampicillin and 10 µg/ml kanamycin and incubated at 37 °C overnight on a shaker. Cells were harvested by centrifugation.

B. Cloning and expression of virus-like particles consisting of AP205 coat protein genetically fused to human IL-1 β ₁₁₆₋₂₆₉ (AP205_hIL-1 β ₁₁₆₋₂₆₉)

[00197] The sequence of human interleukin 1 beta was amplified from plasmid pET42T-hIL-1 β ₁₁₆₋₂₆₉ coding for human interleukin 1 beta using primers pINC-74 (5'- GA TCC GGA GGT GGT GCC CCT GTA CGA TCA CTG AAC TG -3', SEQ ID NO:194) and pINC-76 (5'- GTATGCATTAGGAAGACACAAATTGCATGGTGAAGTC-3, SEQ ID NO:195), introducing a 5' *Kpn2I* and 3' *Mph1103I* site, respectively. The obtained human-IL-1 β fragment was digested with *Kpn2I* and *Mph1103I* and cloned in the same restriction sites into vector pAP590 (amber suppression) creating plasmid pAP649. *E.coli* JM109 containing plasmid pISM 579 (providing amber suppression), was transformed with plasmid pAP649. 5 ml of LB liquid medium with 20 µg/ml ampicillin and 10 µg/ml canamicin were inoculated with a single colony, and incubated at 37 °C for 16-24 h without shaking. The prepared inoculum was diluted 50x with M9 medium containing 20 µg/ml ampicillin and 10 µg/ml kanamycin and incubated at 37 °C overnight on a shaker. Cells were harvested by centrifugation.

C. Immunization of mice with AP205_mIL-1 β ₁₁₉₋₂₆₉

[00198] Four female balb/c mice are immunized with AP205_mIL-1 β ₁₁₉₋₂₆₉. Twentyfive µg of total protein are diluted in PBS to 200 µl and injected subcutaneously (100 µl on two ventral sides) on day 0, day 14, and day 28. Mice are bled retroorbitally on days 0, 14, 28 and 35, and sera are analyzed using mouse mIL-1 β ₁₁₉₋₂₆₉-specific ELISA.

D. ELISA

[00199] ELISA plates are coated with mouse IL-1 β ₁₁₉₋₂₆₉ protein at a concentration of 1 µg/ml. The plates are blocked and then incubated with serially diluted mouse sera from days 0, 14, 28, and 35. Bound antibodies are detected with enzymatically labeled anti-mouse IgG antibody. Antibody titers of mouse sera are calculated as the average of those dilutions which lead to half maximal optical density at 450 nm. Immunization with AP205_mIL-1 β ₁₁₉₋₂₆₉ yields a high specific anti-mouse IL-1 β ₁₁₉₋₂₆₉ titer. This demonstrates that immunization with AP205_mIL-1 β ₁₁₉₋₂₆₉ can overcome immunological tolerance and produce high titer antibodies which recognize specifically IL-1 β ₁₁₉₋₂₆₉.

E. In vitro neutralization of IL-1 β

[00200] Sera of mice immunized with AP205_mIL-1 β ₁₁₉₋₂₆₉ are then tested for their ability to inhibit the binding of mouse IL-1 β protein to its receptor. ELISA plates are therefore coated with a recombinant mIL-1receptorI-hFc fusion protein at a concentration of 1 μ g/ml, and co-incubated with serial dilutions of sera from mice immunized either with AP205_mIL-1 β ₁₁₉₋₂₆₉ or with AP205 alone, and 100 ng/ml of mouse IL-1 β ₁₁₉₋₂₆₉. Binding of IL-1 β ₁₁₉₋₂₆₉ to the immobilized mIL-1receptorI-hFc fusion protein is detected with a biotinylated anti-mouse IL-1 β antibody and horse radish peroxidase conjugated streptavidin. All sera from mice immunized with AP205_mIL-1 β ₁₁₉₋₂₆₉ strongly inhibit the binding of mouse IL-1 β ₁₁₉₋₂₆₉ to its receptor, whereas sera from mice immunized with AP205 alone do not show any inhibitory effect. These data demonstrate that immunization with AP205_mIL-1 β ₁₁₉₋₂₆₉ can yield antibodies which are able to neutralize the interaction of mouse IL-1 β ₁₁₉₋₂₆₉ and its receptor.

F. In vivo neutralization of IL-1 β

[00201] The in vivo neutralizing capacity of the antibodies raised by immunization with AP205_mIL-1 β ₁₁₉₋₂₆₉ is investigated next. Four female balb/c mice are therefore immunized three times on days 0, 14, and 28 with AP205_mIL-1 β ₁₁₉₋₂₆₉ and four mice are immunized at the same time with AP205 alone. On day 35 all mice are injected intravenously with 1 μ g of free mIL-1 β ₁₁₉₋₂₆₉. As readout of the inflammatory activity of the injected mIL-1 β ₁₁₉₋₂₆₉, serum samples are withdrawn before and 3 h after injection and analysed for the relative increase in the concentration of the pro-inflammatory cytokine IL-6. AP205-immunized mice show a strong increase in serum IL-6 concentrations, whereas mice immunized with AP205_mIL-1 β ₁₁₉₋₂₆₉ show only a very mild increase. These data indicate that the antibodies produced by immunization with AP205_mIL-1 β ₁₁₉₋₂₆₉ are able to neutralize specifically and efficiently the pro-inflammatory activity of IL-1 β .

G. Efficacy of AP205_mIL-1 β ₁₁₉₋₂₆₉ in a mouse model of rheumatoid arthritis

[00202] The efficacy of AP205_mIL-1 β ₁₁₉₋₂₆₉-immunization is tested in the murine collagen-induced arthritis model (CIA). Male DBA/1 mice are immunized subcutaneously three times (days 0, 14 and 28) with 50 μ g of either AP205_mIL-1 β ₁₁₉₋₂₆₉ (n=8) or AP205 alone (n=8), and then injected intradermally on day 42 with 200 μ g bovine type II collagen mixed with complete Freund's adjuvant. After a booster injection of 200 μ g bovine type II collagen

mixed with incomplete Freund's adjuvant on day 63 mice are examined on a daily basis for the development of arthritis symptoms. A clinical score ranging from 0 to 3 is assigned to each limb according to the degree of reddening and swelling observed, and ankle thickness of all hind limbs is measured. Four weeks after the second collagen injection AP205_mIL-1 β ₁₁₉₋₂₆₉-immunized mice show a strongly reduced average clinical score when compared to AP205-immunized mice. Moreover, AP205_mIL-1 β ₁₁₉₋₂₆₉-immunized mice display only a minor increase in hind ankle thickness, while AP205-immunized mice show a strong increase in hind ankle thickness. Taken together, these data show that immunization with AP205_mIL-1 β ₁₁₉₋₂₆₉ strongly protects mice from inflammation and clinical signs of arthritis in the CIA model.

EXAMPLE 10

A Cloning, expression and purification of human IL-1 β ₁₁₆₋₂₆₉

[00203] The nucleotide sequence encoding amino acids 116-269 of human IL-1 β (hIL-1 β ₁₁₆₋₂₆₉) was amplified by PCR from a cDNA library of human liver tissue using oligonucleotides HIL-1 (5'-ATATATGATATCCCTGTACGATCACTGAACTGCACG-3'; SEQ ID NO:124) and HIL-2 (5'-ATATATCTCGAGGGAAGACA CAAATTGCATGGTGAAG-3'; SEQ ID NO:125), digested with XhoI and EcoRV and cloned into the expression vector pET42T(+).

[00204] Plasmid pET-42T(+) was constructed by replacing the whole region between the T7 promoter and the T7 terminator of pET-42a(+) (Novagen) in two steps by new linker sequences, which facilitate the expression of a protein of interest as a fusion with a C-terminal tag (SEQ ID NO:190) comprising a His-tag and a cysteine containing linker. In a first step plasmid pET-42a(+) was digested with the restriction enzymes NdeI and AvrII, liberating a 958 bp fragment between the T7 promoter and T7 terminator composed of a GST-tag, S-tag, two His-tags and the multiple cloning site. The residual 4972 bp fragment containing the vector backbone of pET-42a(+) was isolated and ligated to the annealed complementary oligonucleotides 42-1 (5'-TATGGATATCGAATTCAAGCTTCTGCAGCTGCTCGAGTAA TTGATTAC-3'; SEQ ID NO:126) and 42-2 (5'-CTAGGTAATC AATTACTCGA GCAGCTGCAGAAGCTTGAATTCGATATCCA-3'; SEQ-ID NO:127), giving rise to plasmid pET-42S(+). In the second step plasmid pET-42S(+) was linearized by digestion with restriction enzymes XhoI and AvrII, and ligated to the complementary annealed oligonucleotides 42T-1 (5'-TCGAGCACCACCACCACCACCGGTGGTT GCTAATAATAATTGATTAATAC-3'; SEQ ID NO:128) and 42T-2 (5'-CTAGGTATTAATCAATTATTATTAGCAACCACCGTGGTGGTGGTGGTGGTGC-3'; SEQ ID NO:129), resulting in plasmid pET-42T(+).

[00205] The cloning of the above mentioned fragment hIL-1 β ₁₁₆₋₂₆₉ into pET-42T(+) gave rise to plasmid pET42T-hIL-1 β ₁₁₆₋₂₆₉. This plasmid encodes a fusion protein corresponding to the mature human IL-1 β and a His-tag and a C-terminal cysteine-containing linker (GGC, SEQ ID NO:178). Thus, the fusion protein consists of SEQ ID NO:190 C-terminally fused to SEQ ID NO:165. The original alanine residue at position 117 of human IL-1 β was changed to isoleucine in this fusion protein. Expression and purification of the human IL-1 β ₁₁₆₋₂₆₉ protein was performed essentially as described for the murine mIL1 β ₁₁₉₋₂₆₉ protein in EXAMPLE 1.

B Cloning, expression and purification of human IL-1 β ₁₁₆₋₂₆₉ muteins

[00206] By site directed mutagenesis of the plasmid pET42T-hIL-1 β ₁₁₆₋₂₆₉, expression vectors for ten different mutant human IL-1 β ₁₁₆₋₂₆₉ fusion proteins were constructed. To this aim the Quik-Change® Site directed mutagenesis kit (Stratagene) was used according to the manufacturer's instructions. The expression vectors for these mutant IL-1 β ₁₁₉₋₂₆₉ proteins are listed in Table 3 together with the oligonucleotide pairs used for their construction. Expression and purification of the different human IL-1 β ₁₁₆₋₂₆₉ muteins was performed as described in EXAMPLE 1.

Table 3: Overview over IL-1 muteins, expression vectors and oligonucleotides used for their construction.

Expression vector	mutein sequence (without purification tag)	Oligonucleotide pair
pET42T-hIL-1 β ₁₁₆₋₂₆₉ (R4D)	hIL-1 β ₁₁₆₋₂₆₉ (R4D) (SEQ-ID NO:131)	R4D-1 (5'-CATATGGATA TCCCTGTAGA CTCACTGAAC TGCACGCTC-3'; SEQ-ID NO:143); R4D-2 (5'-GAGCGTGCAG TTCAGTGAGT CTACAGGGAT ATCCATATG-3'; SEQ-ID NO:144)
pET42T-hIL-1 β ₁₁₆₋₂₆₉ (L6A)	hIL-1 β ₁₁₆₋₂₆₉ (L6A) (SEQ-ID NO:132)	L6A-1 (5'-GATATCCCTG TACGATCAGC TAACTGCACG CTCCGGGAC-3'; SEQ-ID NO:145); L6A-2 (5'-GTCCCGGAGC GTGCAGTTAG CTGATCGTAC AGGGATATC-3'; SEQ-ID NO:146)
pET42T-hIL-1 β ₁₁₆₋₂₆₉ (T9G)	hIL-1 β ₁₁₆₋₂₆₉ (T9G) (SEQ-ID NO:133)	T9G-1 (5'-GTACGATCAC TGAAGTCCGG TCTCCGGGAC TCACAGC-3'; SEQ-ID NO:147) T9G-2 (5'-GCTGTGAGTC CCGGAGACCG CAGTTCAGTG ATCGTAC-3'; SEQ-ID NO:148)
pET42T-hIL-1 β ₁₁₆₋₂₆₉ (R11G)	hIL-1 β ₁₁₆₋₂₆₉ (R11G) (SEQ-ID NO:134)	R11G-1 (5'-GAACTGCACG CTCGGGGACT CACAGC-3'; SEQ-ID NO:149) R11G-2 (5'-GCTGTGAGTC CCCGAGCGTG CAGTTC-3';

		SEQ-ID NO:150)
pET42T-hIL-1 β ₁₁₆₋₂₆₉ (D54R)	hIL-1 β ₁₁₆₋₂₆₉ (D54R) (SEQ-ID NO:135)	D54R-1 (5'- CAAGGAGAAGAAAGTAATCGCAAAATACCTGTGGC CTTG-3'; SEQ-ID NO:151 D54R-2 (5'- CAAGGCCACAGGTATTTTGCATTACTTTCTTCTCCT TG-3'; SEQ-ID NO:152)
pET42T-hIL-1 β ₁₁₆₋₂₆₉ (D145K)	hIL-1 β ₁₁₆₋₂₆₉ (D145K) (SEQ-ID NO:136)	D145K-1 (5'- GCGGCCAGGATATAACTAAATTCACCATGCAATTTG TGTC-3'; SEQ-ID NO:161) D145K-2 (5'- GACACAAATTGCATGGTGAATTTAGTTATATCCTGG CCGC-3'; SEQ-ID NO:162)
pET42T-hIL-1 β ₁₁₆₋₂₆₉ (Δ EE ^{50,51})	hIL-1 β ₁₁₆₋₂₆₉ (Δ EE ^{50,51}) (SEQ-ID NO:137)	EE-1 (5'- CATGTCCTTTGTACAAGGAAGTAATGACAAAATACC TGTG-3'; SEQ-ID NO:153) EE-2 (5'- CACAGGTATTTTGTCTTACTTCCTTGTACAAAGGAC ATG-3'; SEQ-ID NO:154)
pET42T-hIL-1 β ₁₁₆₋₂₆₉ (Δ SND ⁵²⁻⁵⁴)	hIL-1 β ₁₁₆₋₂₆₉ (Δ SND ⁵²⁻⁵⁴) (SEQ-ID NO:138)	SND-1 (5'- CTTTGTACAAGGAGAAGAAAAATACCTGTGGCCTT G-3'; SEQ-ID NO:155) SND-2 (5'- CAAGGCCACAGGTATTTTTTCTTCTCCTTGTACAAAG -3'; SEQ-ID NO:156)
pET42T-hIL-1 β ₁₁₆₋₂₆₉ (K63S/K65S)	hIL-1 β ₁₁₆₋₂₆₉ (K63S/K65S) (SEQ-ID NO:139)	K6365S-1 (5'- GTGGCCTTGGGCCTCAGCGAAAGCAATCTGTACCTG TCCTG-3'; SEQ-ID NO:157) K6365S-2 (5'- CAGGACAGGTACAGATTGCTTTCGCTGAGGCCCAAG GCCAC-3'; SEQ-ID NO:158)
pET42T-hIL-1 β ₁₁₆₋₂₆₉ (Q126A/E128A)	hIL-1 β ₁₁₆₋₂₆₉ (Q126A/E128A) (SEQ-ID NO:140)	QE-1 (5'- GTACATCAGCACCTCTGCAGCAGCAAACATGCCCGT CTTC-3'; SEQ-ID NO:159) QE-2 (5'- GAAGACGGGCATGTTTGCTGCTGCAGAGGTGCTGAT GTAC-3'; SEQ-ID NO:160)

EXAMPLE 11

Biological activity of human IL-1 β ₁₁₆₋₂₆₉ and human IL-1 β ₁₁₆₋₂₆₉ muteins

[00207] Three female C3H/HeJ mice per group are injected intravenously with 10 μ g of either the wild type human IL-1 β ₁₁₉₋₂₆₉ protein or one of the human IL-1 β ₁₁₉₋₂₆₉ protein muteins of EXAMPLE 10. Serum samples are withdrawn before and 3 h after injection and analysed for the relative increase in the concentration of the pro-inflammatory cytokine IL-6. Mice injected with the wild type human IL-1 β ₁₁₉₋₂₆₉ protein show a strong increase in serum IL-6 concentrations, whereas mice injected with either of the human IL-1 β ₁₁₉₋₂₆₉ mutein proteins show only a mild increase or no increase at all.

EXAMPLE 12

A. Coupling of human IL-1 β ₁₁₆₋₂₆₉ and human IL-1 β ₁₁₆₋₂₆₉ muteins to Q β virus-like particles

[00208] Chemical cross-linking of the wild type human IL-1 β ₁₁₉₋₂₆₉ protein and the human IL-1 β ₁₁₉₋₂₆₉ muteins of EXAMPLE 10 to Q β virus-like particles was performed essentially as described in EXAMPLE 2A.

B. Immunization of mice with human IL-1 β ₁₁₆₋₂₆₉ and human IL-1 β ₁₁₆₋₂₆₉ muteins coupled to Q β capsid

[00209] Four female balb/c mice per group were immunized with Q β coupled to either the wild type hIL-1 β ₁₁₆₋₂₆₉ protein or one of the hIL-1 β ₁₁₆₋₂₆₉ mutein proteins. Fifty μ g of total protein were diluted in PBS to 200 μ l and injected subcutaneously (100 μ l on two ventral sides) on day 0, 14 and 28. Mice were bled retroorbitally on day 35, and sera were analyzed using ELISAs specific for either for the respective human IL-1 β ₁₁₆₋₂₆₉ mutein used as immunogen, or the wild type human IL-1 β ₁₁₆₋₂₆₉ protein.

C ELISA

[00210] ELISA plates were coated either with the wild type hIL-1 β ₁₁₆₋₂₆₉ protein or the respective hIL-1 β ₁₁₆₋₂₆₉ mutein at a concentration of 1 μ g/ml. The plates were blocked and then incubated with serially diluted mouse sera from day 35. Bound antibodies were detected with enzymatically labeled anti-mouse IgG antibody. Antibody titers of mouse sera were calculated as the average of those dilutions which led to half maximal optical density at 450 nm, and are shown in Table 4.

Table 4: Anti- hIL-1 β ₁₁₆₋₂₆₉ (wild type and mutein)–specific IgG titers raised by immunization with Q β -hIL-1 β ₁₁₆₋₂₆₉ or Q β -hIL-1 β ₁₁₆₋₂₆₉ mutein vaccines.

Vaccine	Average anti-hIL-1 β ₁₁₆₋₂₆₉ wild type IgG titer ($\pm$ SD)	Average anti-hIL-1 β ₁₁₆₋₂₆₉ mutein IgG titer ($\pm$ SD)
Q β -hIL-1 β ₁₁₆₋₂₆₉	253325 $\pm$ 184813	-/-
Q β -hIL-1 β ₁₁₆₋₂₆₉ (R4D)	231879 $\pm$ 115475	160666 $\pm$ 79478
Q β -hIL-1 β ₁₁₆₋₂₆₉ (L6A)	120224 $\pm$ 7658	89377 $\pm$ 17965
Q β -hIL-1 β ₁₁₆₋₂₆₉ (T9G)	261249 $\pm$ 153716	224809 $\pm$ 131823
Q β -hIL-1 β ₁₁₆₋₂₆₉ (R11G)	278342 $\pm$ 50296	279290 $\pm$ 47232
Q β -hIL-1 β ₁₁₆₋₂₆₉ (D54R)	269807 $\pm$ 122351	206516 $\pm$ 90998
Q β -hIL-1 β ₁₁₆₋₂₆₉ (D145K)	78365 $\pm$ 26983	93241 $\pm$ 28856
Q β -hIL-1 β ₁₁₆₋₂₆₉ (Δ EE ^{50,51})	287625 $\pm$ 143835	229862 $\pm$ 140169
Q β -hIL-1 β ₁₁₆₋₂₆₉ (Δ SND ⁵²⁻⁵⁴)	68895 $\pm$ 14267	106116 $\pm$ 25295
Q β -hIL-1 β ₁₁₆₋₂₆₉ (K63S/K65S)	403712 $\pm$ 402594	244552 $\pm$ 173597
Q β -hIL-1 β ₁₁₆₋₂₆₉ (Q126A/E128A)	195165 $\pm$ 71436	170434 $\pm$ 86831

[00211] Q β -hIL-1 β ₁₁₆₋₂₆₉-immunization induced high titers of IgG antibodies against hIL-1 β ₁₁₆₋₂₆₉. Moreover, vaccination with either of the Q β -hIL-1 β ₁₁₆₋₂₆₉ mutein vaccines induced high IgG titers against both the respective hIL-1 β ₁₁₆₋₂₆₉ mutein used as immunogen, and the wild type hIL-1 β ₁₁₆₋₂₆₉ protein.

D. In vitro neutralization of human IL-1 β

[00212] Sera of mice immunized with Q β coupled to either wild type hIL-1 β ₁₁₆₋₂₆₉ protein or to one of the hIL-1 β ₁₁₆₋₂₆₉ muteins were tested for their ability to inhibit the binding of human IL-1 β protein to its receptor. ELISA plates were therefore coated with a recombinant human IL-1receptorI-hFc fusion protein at a concentration of 1 μ g/ml, and co-incubated with serial dilutions of the above mentioned sera and 100 ng/ml of hIL-1 β ₁₁₆₋₂₆₉ protein. Binding of hIL-1 β ₁₁₆₋₂₆₉ to the immobilized human IL-1receptorI-hFc fusion protein was detected with a biotinylated anti-human IL-1 β antibody and horse radish peroxidase conjugated streptavidin. All sera raised against Q β -hIL-1 β ₁₁₆₋₂₆₉ mutein vaccines completely inhibited the binding of 100 ng/ml wild type hIL-1 β ₁₁₆₋₂₆₉ to hIL-1RI at serum concentrations $\geq$ 3.3 %.

E. In vivo neutralization of IL-1 β

[00213] The in vivo neutralizing capacity of the antibodies raised by immunization with Q β coupled to either wild type hIL-1 β ₁₁₆₋₂₆₉ protein or to one of the hIL-1 β ₁₁₆₋₂₆₉ muteins is investigated. Three female C3H/HeJ mice per group are therefore immunized three times on days 0, 14, and 28 with 50 μ g of either vaccine. On day 35 all immunized mice are injected intravenously with 1 μ g of free wild type hIL-1 β ₁₁₆₋₂₆₉. As a control three naive mice are injected at the same time with the same amount of wild type hIL-1 β ₁₁₆₋₂₆₉. As readout of the inflammatory activity of the injected hIL-1 β ₁₁₆₋₂₆₉, serum samples are withdrawn immediately before and 3 h after injection and analysed for the relative increase in the concentration of the pro-inflammatory cytokine IL-6. Whereas naive mice show a strong increase in serum IL-6 concentrations 3h after injection of hIL-1 β ₁₁₆₋₂₆₉, all mice immunized with Q β coupled to the wild type hIL-1 β ₁₁₆₋₂₆₉ protein or to one of the hIL-1 β ₁₁₆₋₂₆₉ muteins do not show any increase in serum IL-6, indicating that the injected hIL-1 β ₁₁₆₋₂₆₉ is efficiently neutralized by the antibodies induced by the vaccines.

EXAMPLE 13

Amelioration of MSU-induced inflammation by immunization with Q β -mIL-1 β ₁₁₉₋₂₆₉

[00214] Gout is a painful inflammatory disorder caused by the precipitation of monosodium urate (MSU) crystals in joints and periarticular tissues. MSU crystals have been shown to activate the so called NALP3 inflammasome, resulting in the production of active IL-1 β , which is mainly responsible for initiating and promoting the inflammatory response characteristic of the disease. C57BL/6 mice (5 per group) are immunized subcutaneously three times at two weeks intervals with 50 μ g Q β -mIL-1 β ₁₁₉₋₂₆₉ or 50 μ g of Q β VLPs alone. One week after the last immunization all mice are challenged intraperitoneally with 1.5 mg MSU crystals. Six hours after the challenge mice are sacrificed and neutrophil numbers as well as the concentrations of the neutrophil chemoattractants KC and MIP-2 are measured in peritoneal exsudates. Q β -mIL-1 β ₁₁₉₋₂₆₉-immunized mice show markedly reduced neutrophilia and MIP-2 and KC concentrations, when compared to Q β -immunized controls.

EXAMPLE 14

Amelioration of experimental autoimmune encephalitis by immunization with Q β -mIL-1 β ₁₁₉₋₂₆₉

[00215] In a mouse model for multiple sclerosis, C57BL/6 mice (8 per group) are immunized subcutaneously three times at two weeks intervals with 50 μ g Q β -mIL-1 β ₁₁₉₋₂₆₉ or

50 µg of Qβ VLPs alone. One week after the last immunization all mice are injected subcutaneously with 100 µg MOG peptide (MEVGWYRSPFSRVVHLYRNGK, SEQ ID NO:191) mixed with complete Freund's adjuvant. On the same day and two days later all mice are injected intraperitoneally with 400 ng of pertussis toxin. Mice are scored on a daily basis for development of neurological symptoms according to the following scheme: 0, no clinical disease; 0.5, end of tail limp; 1, tail completely limp; 1.5, limp tail and hind limb weakness (unsteady gait and poor grip of hind legs); 2, unilateral partial hind limb paralysis; 2.5, bilateral partial hind limb paralysis; 3, complete bilateral hind limb paralysis; 3.5, complete bilateral hind limb paralysis and unilateral front limb paralysis; 4, total paralysis of hind and front limbs. Qβ-mIL-1β₁₁₉₋₂₆₉-immunized mice show clearly reduced clinical symptoms when compared to Qβ-immunized mice.

WHAT IS CLAIMED IS:

1. A composition comprising:
 - (a) a virus-like particle (VLP) with at least one first attachment site; and
 - (b) at least one antigen with at least one second attachment site;wherein said at least one antigen is an IL-1 molecule and wherein (a) and (b) are linked through said at least one first and said at least one second attachment site.
2. The composition of claim 1, wherein said IL-1 molecule is selected from the group consisting of:
 - (a) IL-1 protein;
 - (b) IL-1 mature fragment;
 - (c) IL-1 fragment
 - (d) IL-1 peptide; and
 - (e) IL-1 mutein.
3. The composition of claim 2, wherein said IL-1 molecule is derived from a human.
4. The composition of any one of claims 1 to 3, wherein said IL-1 molecule is an IL-1 beta molecule.
5. The composition of any one of claims 1 to 3, wherein said IL-1 molecule is an IL-1 alpha molecule.
6. The composition of claim 4, wherein said IL-1 beta molecule is selected from the group consisting of:
 - (a) IL-1 beta protein;
 - (b) IL-1 beta mature fragment;
 - (c) IL-1 beta fragment
 - (d) IL-1 beta peptide; and
 - (e) IL-1 beta mutein.
7. The composition of claim 5, wherein said IL-1 alpha molecule is selected from the group consisting of:
 - (a) IL-1 alpha protein;

- (b) IL-1 alpha mature fragment;
 - (c) IL-1 alpha fragment
 - (d) IL-1 alpha peptide; and
 - (e) IL-1 alpha mutein.
8. The composition of any one of claims 1 to 4, wherein said IL-1 molecule is an IL-1 beta protein comprising or preferably consisting of an amino acid sequence selected from the group consisting of:
- (a) human IL-1 beta (SEQ ID NO:49);
 - (b) any one of SEQ ID NO:50 to SEQ ID NO:62; and
 - (c) an amino acid sequence which is at least 80 %, or preferably at least 90 %, more preferably at least 95 %, or most preferably at least 99 % identical with any one of SEQ ID NO:49 to SEQ ID NO:62.
9. The composition of any one of claims 1 to 4, wherein said IL-1 molecule is an IL-1 beta mature fragment comprising or preferably consisting of an amino acid sequence selected from the group consisting of:
- (a) human IL-1 beta 117-269 (SEQ ID NO:64);
 - (b) human IL-1 beta 116-269 (SEQ ID NO:165);
 - (c) mouse IL-1 beta 119-269s (SEQ ID NO:164); and
 - (d) an amino acid sequence which is at least 80 %, or preferably at least 90 %, more preferably at least 95 %, or most preferably at least 99 % identical with any one of SEQ ID NO:64, SEQ ID NO:165, and SEQ ID NO:164.
10. The composition of any one of claims 1 to 4, wherein said IL-1 molecule is an IL-1 beta peptide comprising or preferably consisting of an amino acid sequence selected from the group consisting of:
- (a) any one of SEQ ID NO:89 to SEQ ID NO:116; and
 - (b) an amino acid sequence which is at least 80 %, or preferably at least 90 %, more preferably at least 95 %, or most preferably at least 99 % identical with any one of SEQ ID NO:89 to SEQ ID NO:116.
11. The composition of any one of claims 1 to 3 or 5, wherein said IL-1 molecule is an IL-1 alpha protein comprising or preferably consisting of an amino acid sequence selected from the group consisting of:

- (a) human IL-1 alpha (SEQ ID NO:36);
 - (b) any one of SEQ ID NO:37 to SEQ ID NO:48; and
 - (c) an amino acid sequence which is at least 80 %, or preferably at least 90 %, more preferably at least 95 %, or most preferably at least 99 % identical with any one of SEQ ID NO:36 to SEQ ID NO:48.
12. The composition of one of claims 1 to 3 or 5, wherein said IL-1 molecule is an IL-1 alpha mature fragment comprising or preferably consisting of an amino acid sequence selected from the group consisting of:
- (a) human IL-1 alpha 119-271 (SEQ ID NO:63);
 - (b) mouse IL-1 alpha 117-270s (SEQ ID NO:163); and
 - (c) an amino acid sequence which is at least 80 %, or preferably at least 90 %, more preferably at least 95 %, or most preferably at least 99 % identical with any one of SEQ ID NO:63 or SEQ ID NO:163.
13. The composition of one of claims 1 to 3 or 5, wherein said IL-1 molecule is an IL-1 alpha peptide comprising or preferably consisting of an amino acid sequence selected from the group consisting of:
- (a) any one of SEQ ID NO:67 to SEQ ID NO:88; and
 - (b) an amino acid sequence which is at least 80 %, or preferably at least 90 %, more preferably at least 95 %, or most preferably at least 99 % identical with any one of SEQ ID NO:67 to SEQ ID NO:88.
14. The composition of any of claims 1 to 13, wherein said VLP comprises, or alternatively consists of, recombinant coat proteins, mutants or fragments thereof, of an RNA bacteriophage.
15. The composition of any one of claims 1 to 14, wherein said VLP is a VLP of an RNA bacteriophage.
16. The composition of any one of claims 14 or 15, wherein said RNA bacteriophage is an RNA bacteriophage selected from Q β and AP205.

17. The composition of any of claims 1 to 16, wherein said first attachment site is linked to said second attachment site via at least one covalent bond, wherein preferably said covalent bond is a non-peptide bond.
18. The composition of any of claims 1 to 17, wherein said first attachment site comprises, or preferably is, an amino group, preferably an amino group of a lysine.
19. The composition of any of claims 1 to 18, wherein said second attachment site comprises, or preferably is, a sulfhydryl group, preferably a sulfhydryl group of a cysteine.
20. The composition of any of claims 1 to 17, wherein said first attachment site is not a sulfhydryl group.
21. The composition of any of claims 1 to 17 and 20, wherein said linkage of said VLP and said at least one antigen, does not comprise a disulfide bond
22. The composition of any of claims 1 to 19, wherein only one of said second attachment sites associates with said first attachment site through at least one non-peptide covalent bond leading to a single and uniform type of binding of said IL-1 molecule to said core particle, wherein said only one second attachment site that associates with said first attachment site is a sulfhydryl group, and wherein said IL-1 molecule and said core particle interact through said association to form an ordered and repetitive antigen array.
23. The composition of any one of claims 1 to 16, wherein said antigen is fused to the N- or the C- terminus of the coat protein, mutants or fragments thereof, of AP205.
24. The composition of any one of claims 1 to 22 further comprising a linker.
25. The composition of any one of claims 1 to 24, wherein said IL-1 molecule is SEQ ID NO:64 or a mutein derived thereof.
26. The composition of any one of claims 1 to 24, wherein said IL-1 molecule is SEQ ID NO:63 or a mutein derived thereof.

27. A vaccine comprising, or alternatively consisting of, the composition of any one of claims 1 to 26.
28. The vaccine of claim 27, wherein said vaccine is devoid of an adjuvant.
29. A method of immunization comprising administering the vaccine of any one of claims 27 or 28 to an animal or human.
30. A pharmaceutical composition comprising:
 - (a) the composition of any of claims 1 to 23 or the vaccine of any one of claims 27 or 28; and
 - (b) an acceptable pharmaceutical carrier.
31. A method of producing the composition of any of claims 1 to 26, or the vaccine of any one of claims 27 or 28, comprising:
 - (a) providing a VLP with at least one first attachment site;
 - (b) providing at least one antigen, wherein said antigen is an IL-1 molecule, an IL-1 protein, an IL-1 mature fragment, an IL-1 peptide or an IL-1 mutein, with at least one second attachment site; and
 - (c) linking said VLP and said at least one antigen to produce said composition or said vaccine, wherein said at least one antigen and said VLP are linked through said at least one first and said at least one second attachment site.
32. Use of the composition of any one of claims 1 to 26, the vaccine of any one of claim 27 or 28 or the pharmaceutical composition of claim 30 for the manufacture of a medicament for treatment of a disease, wherein said disease is preferably selected from the group consisting of:
 - (a) vascular diseases;
 - (b) inherited IL-1-dependent inflammatory diseases;
 - (c) chronic autoimmune inflammatory diseases;
 - (d) bone and cartilage degenerative diseases;
 - (e) allergic diseases; and
 - (f) neurological disease;in an animal, preferably in a human.

33. Use according to claim 32, wherein said disease is a vascular disease, wherein said vascular disease is atherosclerosis.
34. Use according to claim 32, wherein said disease is an inherited IL-1-dependent inflammatory disease, wherein said inherited IL-1-dependent inflammatory disease is familial mediterranean fever (FMF).
35. Use according to claim 32, wherein said disease is a chronic autoimmune inflammatory diseases, wherein said chronic autoimmune inflammatory diseases is systemic onset juvenile idiopathic arthritis or rheumatoid arthritis.
36. Use according to claim 32, wherein said disease is a bone and cartilage degenerative diseases, wherein said bone and cartilage degenerative diseases is gout or osteoarthritis.
37. Use according to claim 32, wherein said disease is a neurological disease, wherein said a neurological disease is multiple sclerosis.