

USE OF INTERLEUKIN-1 CONJUGATES IN THE TREATMENT OF DIABETES

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, pharmaceutical compositions and vaccines for the treatment, amelioration and / or prophylaxis of diabetes, preferably of type II diabetes. The compositions, pharmaceutical compositions and vaccines of the invention comprise a core particle and an antigen, wherein said antigen comprises an interleukin-1 (IL-1) molecule. When administered to an animal, preferably to a human, said compositions, pharmaceutical compositions, and vaccines induce efficient immune responses, in particular antibody responses, wherein typically and preferably said antibody responses are directed against IL-1. Thus, the invention provides methods of treating, ameliorating or preventing diabetes, preferably type II diabetes, by way of active immunization against IL-1.

RELATED ART

[0002] Type 2 diabetes is a chronic metabolic disorder characterized by the presence of hyperglycemia due to defective insulin secretion, insulin action or a combination of both. Although the mechanisms of pancreatic β -cell failure in type 2 diabetes are not fully elucidated, stress and inflammatory pathways have been implicated. Metabolic stress caused by repetitive glucose excursions, dyslipidemia and adipokines can induce an inflammatory response in the pancreas characterized by local cytokine secretion, islet immune-cell infiltration, β -cell apoptosis, amyloid deposits and fibrosis. IL-1 β has emerged as a master cytokine, which regulates islet chemokine production and causes impaired insulin production and β -cell death. Blockade of IL-1 signalling by administration of recombinant IL-1 receptor antagonist or neutralizing monoclonal antibodies has been shown to improve glycemic control in animal models of type 2 diabetes (Sauter et al., 2008, Osborn et al., 2008). Furthermore, treatment of type 2 diabetes patients with recombinant human IL-1 receptor antagonist (Anakinra) resulted in a decrease of glycated haemoglobin levels (a reliable readout for long term glycemia) and improved β -cell function (Larsen et al., 2007).

SUMMARY

[0002a] In a first aspect, there is provided the use of 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 comprises 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, for the manufacture of a medicament for the treatment of diabetes in an animal.

[0002b] In a second aspect, there is provided the use of a vaccine comprising the composition as defined above for the manufacture of a medicament for the treatment of diabetes in an animal.

[0002c] In a third aspect, there is provided the use of a pharmaceutical composition comprising

- (a) the composition as defined above or the vaccine as defined above; and
- (b) a pharmaceutically acceptable carrier

for the manufacture of a medicament for the treatment of type II diabetes in a human.

[0003] We have found that the disclosed compositions and vaccines, respectively, comprising at least one IL-1 molecule, preferably a IL-1 mutein, 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 active immunization with a composition of the disclosure resulted in the amelioration of the diet-induced diabetic phenotype in a mouse model (cf. Surwit et al., DIABETES, Vol. 37, 1988, 1163-1167) of diabetes. This observation was made for different IL-1 molecules including IL-1 alpha molecules (cf. Example 9) as well as IL-1 beta molecules (cf. Examples 12 and 13).

[0004] In one embodiment, the disclosure therefore provides a composition for the treatment, amelioration or prophylaxis of diabetes, preferably of type II diabetes, wherein said composition comprises: (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, preferably a virus-like particle; and (b) at least one antigen with at least one second attachment site, wherein the at least one antigen comprises or consists of or 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 linked, preferably covalently linked, through the at least one first and the at least one second attachment site.

[0005] In a further embodiment, the disclosure provides 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 comprises consists of or 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. In a preferred embodiment said at least one antigen with at least one second attachment site comprises or preferably consists of (i) an IL-1 molecule; and (ii) a linker.

[0006] In a further preferred embodiment wherein said first attachment site is linked to said second attachment site via at least one covalent bond, wherein preferably said at least one covalent bond is a non-peptide bond.

[0007] In a further preferred embodiment said at least one antigen with at least one second attachment site comprises or preferably consists of: (i) an IL-1 beta molecule, wherein said IL-1 beta molecule is SEQ ID NO:165 or SEQ ID NO:136, preferably SEQ ID NO:136; and (ii) a linker, wherein said linker comprises said second attachment site, and wherein preferably said linker comprises or preferably consists of GGC (SEQ ID NO:178) or GGCG (SEQ ID NO:188), preferably GGCG (SEQ ID NO:188); wherein further preferably said linker is covalently bound to the C-terminus of said IL-1 beta molecule by way of a peptide bond.

[0008] In a further preferred embodiment said at least one antigen with at least one second attachment site consists of (i) an IL-1 alpha molecule, wherein said IL-1 alpha molecule is SEQ ID NO:203 or SEQ ID NO:210, preferably SEQ ID NO:203; and (ii) a linker, wherein

said linker comprises said second attachment site, and wherein preferably said linker comprises or preferably consists of GGC (SEQ ID NO:178) or GGCG (SEQ ID NO:188), preferably GGCG (SEQ ID NO:188); wherein further preferably said linker is covalently bound to the C-terminus of said IL-1 alpha molecule by way of a peptide bond.

[0009] In a further preferred embodiment said virus-like particle is a virus-like particle of an RNA bacteriophage, wherein preferably said RNA bacteriophage is bacteriophage Q β .

[0010] 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 antigen to said virus-like particle, wherein said only one second attachment site that associates with said first attachment site is a sulfhydryl group, and wherein said antigen and said virus-like particle interact through said association to form an ordered and repetitive antigen array.

[0011] In a further embodiment, the disclosure provides a vaccine for the treatment of diabetes, preferably of type II diabetes, said vaccine comprising, or alternatively consisting of the composition of the disclosure, preferably in an effective amount.

[0012] In a further embodiment, the disclosure provides a pharmaceutical composition for the treatment of diabetes, preferably of type II diabetes, said pharmaceutical composition comprising: (a) the composition of the disclosure or the vaccine of the disclosure; and (b) a pharmaceutically acceptable carrier.

[0013] In a further embodiment, the disclosure provides a method of treating diabetes, preferably type II diabetes, said method comprising administering an immunologically effective amount of the composition of the disclosure, of the vaccine of the disclosure, and/or of the pharmaceutical composition of the disclosure to an animal, preferably to a human.

[0014] In a further embodiment, the disclosure provides the use of the composition of the disclosure, of the vaccine of the disclosure, and/or of the pharmaceutical composition of the disclosure for the manufacture of a medicament for treatment of diabetes, preferably of type II diabetes, in an animal, preferably in a human.

DETAILED DESCRIPTION OF THE INVENTION

[0015] 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.

[0016] 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 or with the pharmaceutical composition, respectively, may provide

for an even more enhanced immune response. Preferred adjuvants are complete and incomplete Freund's adjuvant, aluminum containing adjuvant, preferably aluminum hydroxide, most preferably alum, 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 Virosomal 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.

[0017] Antigen: As used herein, the term "antigen" refers to a molecule capable of being bound by an antibody or a T-cell receptor (TCR) if presented by MHC molecules. The term "antigen", as used herein, also encompasses T-cell epitopes. 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. The term "antigen" as used herein preferably refers to the IL-1 molecule, the IL-1 protein, IL-1 mature fragment, the IL-1 fragment, the IL-1 peptide and the IL-1 mutein, most preferably "antigen" refers to the IL-1 mutein. If not indicated otherwise, the term "antigen" as used herein does not refer to the virus particle or to the virus-like particle.

[0018] Epitope: "epitope" refers to continuous or discontinuous portions of a polypeptide which can be bound immunospecifically by an antibody or by a T-cell receptor within the context of an MHC molecule. Immunospecific binding excludes non-specific binding but does not necessarily exclude cross-reactivity. An epitope typically comprises 5-10 amino acids in a spatial conformation which is unique to the epitope.

[0019] 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).

[0020] 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.

[0021] 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.

[0022] Attachment Site, First: As used herein, the phrase "first attachment site" refers to an element which is naturally occurring with the VLP, preferably with the VLP of an RNA bacteriophage, or which is artificially added to the VLP, preferably to the VLP of an RNA bacteriophage, 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 guanidinyll group, histidinyll 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. In a preferred embodiment said first attachment site is the amino group of a lysine residue, wherein preferably said lysine residue is a lysine residue which is naturally occurring with said VLP, preferably with said VLP of an RNA bacteriophage. The first attachment site is located, typically on the surface, and preferably on the outer surface of

the VLP, preferably of the VLP of an RNA bacteriophage, most preferably of RNA bacteriophage Q β . Multiple first attachment sites are present on the surface, preferably on the outer surface of the virus-like particle, preferably of the VLP of an RNA bacteriophage, most preferably of the VLP of RNA bacteriophage Q β , typically and preferably 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. In a preferred embodiment the first attachment site is associated with said VLP through at least one covalent bond, preferably through at least one peptide bond, wherein said VLP is a VLP of an RNA bacteriophage, preferably of RNA bacteriophage Q β . In a further preferred embodiment said first attachment site is the amino group of a lysine residue, wherein said lysine residue is a lysine residue of a coat protein, preferably of a coat protein of an RNA bacteriophage, most preferably of RNA bacteriophage Q β . In a further preferred embodiment said first attachment site is an amino group of a lysine residue of a coat protein of an RNA bacteriophage, wherein preferably said coat protein comprises or preferably consists of the amino acid sequence of SEQ ID NO:3. In a further preferred embodiment said first attachment site is a lysine residue, wherein preferably said lysine residue is a lysine residue of a coat protein, preferably of a coat protein of an RNA bacteriophage, most preferably of RNA bacteriophage Q β . In a further preferred embodiment said first attachment site is a lysine residue of the coat protein of RNA bacteriophage Q β .

[0023] 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 a sulfhydryl group. In a further preferred embodiment said second attachment site is a sulfhydryl group, preferably a sulfhydryl group of acysteine residue. 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, preferably through a linker, wherein further preferably said linker comprises or alternatively consists of a cysteine. Very preferably said linker is fused to the IL-1 molecule by way of a peptide bond.

[0024] Coat protein: The term "coat protein" refers to a viral protein, preferably a subunit of a natural capsid of a virus, preferably of an RNA bacteriophage, which is capable of being incorporated into a virus capsid or a VLP. Coat proteins are also known as capsid proteins.

[0025] 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. Thus, in a preferred embodiment said least one first attachment site and said at least one second attachment site are covalently linked via least one, preferably exactly one, heterobifunctional cross-linker, wherein preferably said first attachment site is the amino group of a lysine residue and wherein further preferably said second attachment site is the sulfhydryl group of a cysteine residue. 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 bounds, 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 bounds, preferably by genetic fusion.

[0026] Linker: A "linker", as used herein, either associates the second attachment site with the IL-1 molecule or comprises, essentially consists of, or consists of the second attachment site. Preferably, a "linker", as used herein, comprises the second attachment site, typically and preferably - but not necessarily - as one amino acid residue, preferably as a cysteine residue. In a preferred embodiment said linker is an amino acid linker. In a very preferred embodiment said linker consists of exactly one cysteine residue. In a further preferred embodiment said linker comprises or consists of exactly one cysteine residue and said second attachment site is the sulfhydryl group of said exactly one cysteine residue. 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).

[0027] Amino acid linker: The term "amino acid linker" refers to a linker comprising least one amino acid residue. Generally the term "amino acid linker" does not imply that such linker would consists exclusively of amino acid residues. However, in a preferred embodiment said amino acid linker consists exclusively of amino acid residues. 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, most preferably all-L. 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.

[0028] Ordered and repetitive antigen array: As used herein, the term "ordered and repetitive antigen array" generally refers to a repeating pattern of antigen or to a structure which is 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 spacings 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.

[0029] IL-1 molecule: The term "IL-1 molecule" or shortly "IL-1", as used herein, refers to any polypeptide, wherein the amino acid sequence of said polypeptide shows 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, wherein the amino acid sequence of said polypeptide shows 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.

[0030] 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, wherein the amino acid sequence of said polypeptide shows 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).

[0031] 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, wherein the amino acid sequence of said polypeptide shows 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).

[0032] IL-1 protein: The term "IL-1 protein", as used herein, refers to a naturally occurring protein, wherein the amino acid sequence of said naturally occurring protein shows 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 the amino acid sequence of said naturally occurring protein shows 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, wherein the amino acid sequence of said polypeptide shows 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, wherein the amino acid sequence of said polypeptide shows 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, wherein the amino acid sequence of said polypeptide shows 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.

[0033] 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.

[0034] 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.

[0035] 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.

[0036] 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.

[0037] 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.

[0038] 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. Very preferred IL-1 beta muteins are IL-1 beta muteins derived from IL-1 beta mature fragments, preferably from human IL-1 β ₁₁₇₋₂₆₉ (SEQ ID NO:64). Very preferred IL-1 alpha muteins are derived from IL-1 alpha mature fragments, preferably from human IL-1 α ₁₁₉₋₂₇₁ (SEQ ID NO:63).

[0039] 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, wherein further preferably said biological activity is determined by the capacity of said IL-1 mutein to induce IL-6 in human PBMCs, wherein most preferably said biological activity is determined essentially as described in Example 8 B.

[0040] In preferred IL-1 beta 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 beta molecule it is derived from, wherein preferably said IL-1 beta molecule is an IL-1 beta mature fragment, preferably human IL-1 β ₁₁₇₋₂₆₉ (SEQ ID NO:64), and wherein further preferably said biological activity is determined by the capacity of said IL-1 beta mutein to induce IL-6 in human PBMCs, wherein most preferably said biological activity is determined essentially as described in Example 8 B.

[0041] In preferred IL-1 alpha 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 alpha molecule it is derived from, wherein preferably said IL-1 alpha molecule is an IL-1 alpha mature fragment, preferably human IL-1 α ₁₁₉₋₂₇₁ (SEQ ID NO:63), and wherein further preferably said biological activity is determined by the capacity of said IL-1 alpha mutein to induce IL-6 in human PBMCs, wherein most preferably said biological activity is determined essentially as described in Example 11.

[0042] 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, wherein preferably said biological activity is determined essentially as described in Examples 8 B or 11.

[0043] Further preferably, but not necessarily, IL-1 muteins are capable of specifically binding an IL-1 receptor.

[0044] Compositions comprising a preferred IL-1 mutein as the sole antigen induce a titer of antibodies capable of specifically binding the IL-1 molecule said IL-1 mutein is derived from, wherein said titer is at least 20 %, preferably at least 40 %, still more preferably at least 60 %, still more preferably at least 80 % and most preferably at least 100 % of the titer obtained with a composition comprising the IL-1 molecule said IL-1 mutein is derived from as the sole antigen, wherein preferably said titer is determined essentially as described in Example 9 D.

[0045] When introduced into an animal, compositions comprising a preferred IL-1 beta mutein as the sole antigen induce a titer of antibodies capable of specifically binding the IL-1 beta molecule said IL-1 beta mutein is derived from, wherein preferably said IL-1 beta molecule is an IL-1 beta mature fragment, most preferably human IL-1 β ₁₁₇₋₂₆₉ (SEQ ID NO:64), wherein said titer is at least 20 %, preferably at least 40 %, still more preferably at least 60 %, still more preferably at least 80 % and most preferably at least 100 % of the titer obtained with a composition comprising the IL-1 beta molecule said IL-1 beta mutein is derived from, preferably said IL-1 beta mature fragment, most preferably said human IL-1 β ₁₁₇₋₂₆₉ (SEQ ID NO:64) as the sole antigen, wherein further preferably said titer is determined essentially as described in Example 9 D.

[0046] When introduced into an animal, compositions comprising a preferred IL-1 alpha mutein as the sole antigen induce a titer of antibodies capable of specifically binding the IL-1 alpha molecule said IL-1 alpha mutein is derived from, wherein preferably said IL-1 alpha molecule is an IL-1 alpha mature fragment, most preferably human IL-1 α ₁₁₉₋₂₇₁ (SEQ ID NO:63), wherein said titer is at least 20 %, preferably at least 40 %, still more preferably at least 60 %, still more preferably at least 80 % and most preferably at least 100 % of the titer obtained with a composition comprising the IL-1 alpha molecule said IL-1 alpha mutein is derived from, preferably said IL-1 alpha mature fragment, most preferably said human IL-1 α ₁₁₉₋₂₇₁ (SEQ ID NO:63) as the sole antigen, wherein further preferably said titer is determined essentially as described in Example 9 D.

[0047] A very preferred IL-1 mutein is an IL-1 mutein, wherein 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, wherein further preferably said biological activity is determined by the capacity of said

IL-1 mutein to induce IL-6 in human PBMCs, wherein most preferably said biological activity is determined essentially as described in Example 8 B, and wherein additionally compositions comprising said very preferred IL-1 mutein as the sole antigen induce a titer of antibodies capable of specifically binding the IL-1 molecule said very preferred IL-1 mutein is derived from, wherein said titer is at least 20 %, preferably at least 40 %, still more preferably at least 60 %, still more preferably at least 80 % and most preferably at least 100 % of the titer obtained with a composition comprising the IL-1 molecule said very preferred IL-1 mutein is derived from as the sole antigen, wherein preferably said titer is determined essentially as described in Example 9 D.

[0048] 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.

[0049] IL-1 muteins useful in the context of the invention 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.

[0050] Preferred IL-1 muteins comprise or preferably consist of a polypeptide, wherein the amino acid sequence of said polypeptide 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, wherein the amino acid sequence of said polypeptide 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).

[0051] Further preferred IL-1 muteins comprise or more preferably consist of a polypeptide, wherein the amino acid sequence of said polypeptide differs from an amino acid sequence selected from SEQ ID NO:36 to SEQ ID NO:48 and 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, wherein the amino acid sequence of said polypeptide differs from an 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) any one 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).

[0052] Further preferred IL-1 muteins are IL-1 alpha muteins, wherein said IL-1 alpha muteins comprise or more preferably consist of a polypeptide, wherein the amino acid sequence of said polypeptide differs from an amino acid sequence selected from 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, wherein the amino acid sequence of said polypeptide differs from an amino acid sequence selected from 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).

[0053] Very preferred IL-1 alpha muteins comprise or preferably consist of a polypeptide, wherein the amino acid sequence of said polypeptide 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). Still more preferred IL-1 alpha muteins comprise or preferably consist of a polypeptide, wherein the amino acid sequence of said polypeptide is selected from the group consisting of SEQ ID NO:210 to SEQ ID NO:218.

[0054] Further preferred IL-1 muteins are IL-1 beta muteins, wherein said IL-1 beta muteins comprise or more preferably consist of a polypeptide, wherein the amino acid sequence of said polypeptide differs from an amino acid sequence selected from 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, wherein the amino acid sequence of said polypeptide differs from an amino acid sequence selected from 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, wherein the amino acid sequence of said polypeptide 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, wherein the amino acid sequence of said polypeptide is selected from SEQ ID NO:131 to SEQ ID NO:140 and SEQ ID NO:205 to SEQ ID NO:209.

[0055] "derived from": in the context of the invention, the expression an amino acid sequence which is "derived from" another amino acid sequence means that said amino acid

sequence is essentially identical with the amino acid sequence it is derived from, with the exception of certain mutations, wherein said mutations are selected from the group consisting of (i) amino acid exchanges, (ii) deletions, (iii) insertions, and (iv) any combination of (i) to (iii), wherein preferably said mutations are selected from (i) amino acid exchanges and (ii) deletions. In particular, a mutated amino acid sequence derived from a wild type amino acid sequence preferably differs from said wild type amino acid sequence 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) exchanged by another amino acid, (ii) deleted from said wild type amino acid, (iii) inserted into said wild type sequence, and (iv) any combination of (i) to (iii), wherein most preferably said amino acid residue(s) are (i) exchanged by another amino acid, or (ii) deleted from said wild type amino acid. Deletions of more than one amino acid residue preferably occur as a deletion of a consecutive stretch of amino acid residues of said wild type amino acid sequence. A mutated amino acid sequence which is derived from a wild type amino acid sequence preferably has at least 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, and most preferably at least 99 % sequence identity with said wild type amino acid sequence.

[0056] Similarly, the expression "mutein derived from a IL-1 molecule" refers to a mutein, wherein said mutein comprises or preferably consists of a polypeptide, wherein the amino acid sequence of said polypeptide is essentially identical to that of the IL-1 molecule it is derived from, with the exception of certain mutations, wherein said mutations are selected from the group consisting of (i) amino acid exchanges, (ii) deletions, (iii) insertions, and (iv) any combination of (i) to (iii), wherein preferably said mutations are selected from (i) amino acid exchanges and (ii) deletions. In particular, an IL-1 mutein derived from an IL-1 molecule differs from said IL-1 molecule 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) exchanged by another amino acid, (ii) deleted from said wild type amino acid, (iii) inserted into said wild type sequence, and (iv) any combination of (i) to (iii), wherein most preferably said amino acid residue(s) are (i) exchanged by another amino acid, or (ii) deleted from said wild type amino acid. Deletions of more than one amino acid residue preferably occur as a deletion of a consecutive stretch of amino acid residues of the IL-1 molecule said IL-1 mutein is derived from. A mutein derived from a wild type amino acid sequence preferably has at least 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, and most preferably at least 99 % sequence identity with the IL-1 molecule said IL-1 mutein is derived from.

[0057] Amino acid exchange: the expression amino acid exchange refers to the exchange of an amino acid residue in a certain position of an amino acid sequence by any other amino acid residue.

[0058] 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)). Very preferably, the term biological activity of an IL-1 molecule or an IL-1 mutein refers to the capacity of an IL-1 composition comprising said IL-1 molecule or said IL-1 mutein to induce IL-6 in human PBMCs, wherein preferably said IL-1 molecule or said IL-1 mutein is the sole antigen in said IL-1 composition, and wherein most preferably said biological activity is determined essentially as described in Example 8 B.

[0059] Packaged: The term "packaged" as used herein refers to the state of a polyanionic macromolecule or of an 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. In a preferred embodiment the term "packaged" refers to the enclosure, or partial enclosure, of a polyanionic macromolecule by the VLP. 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 into the VLP, most preferably in a non-covalent manner. Methods for packaging polyanionic macromolecules such as polyglutamic acid into VLPs, and in particular into VLPs of RNA bacteriophages, are disclosed in WO2006/037787. Reference is made in particular to Example 4 of WO2006/037787. Methods for packaging immunostimulatory substances, preferably immunostimulatory nucleic acids, and most preferably unmethylated CpG-containing oligonucleotides into a VLP are described in WO2003/024481A2. In case said immunostimulatory substances is nucleic acid, preferably a DNA, and most preferably an

unmethylated CpG-containing oligonucleotide, 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.

[0060] 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. The term "polyanionic macromolecule" as used herein refers to a molecule that is not capable of activating toll-like receptors. Thus, the term "polyanionic macromolecule" excludes Toll-like receptors ligands, and excludes substances capable of inducing and/or enhancing an immune response, such as Toll-like receptors ligands, nucleic acids capable of inducing and/or enhancing an immune response, and lipopolysacchrides (LPS). More preferably the term "polyanionic macromolecule" as used herein, refers to a molecule that is not capable of inducing cytokine production. Preferably, polyanionic macromolecules are polyanionic polypeptides or anionic dextrans. In a preferred embodiment said polyanionic macromolecules are polyanionic polypeptides, wherein preferably said polyanionic polypeptides are selected from a group consisting of: (a) polyglutamic acid; (b) polyaspartic acid; (c) poly(GluAsp) and (d) any chemical modifications of (a) to (c). Examples for chemical modifications include, but are not limited to glycosylations, acetylations, and phosphorylations. In a further preferred embodiment said polyanionic macromolecules are anionic dextrans selected from a group consisting of: (a) dextran sulfate; (b) carboxymethyl dextran; (c) sulfopropyl dextran; (d) methyl sulfonate dextran; and (e) dextrane phosphate.

[0061] Polyaspartic acid: The term "polyaspartic acid" as used herein, refers to a polypeptide comprising at least 50 %, preferably at least 70 %, more preferably at least 90 %, more preferably at least 95 %, more preferably at least 99 %, more preferably 100 %, aspartic acid residues out of the total number of amino acid residues comprised by said polypeptide. The aspartic acid residues of said polypeptide are hereby either all-L, all-D, or mixtures of L- and D-aspartic acid. Most preferably said polypeptide only comprises L-aspartic acid residues.

[0062] Polyglutamic acid: The term "polyglutamic acid", as used herein, refers to a polypeptide comprising at least 50 %, preferably at least 70 %, more preferably at least 90 %, more preferably at least 95 %, more preferably at least 99 %, and most preferably 100 % glutamic acid residues out of the total number of amino acid residues comprised by said polypeptide. The glutamic acid residues of said polypeptide are hereby either all-L, all-D, or

mixtures of L- and D-glutamic acid. Most preferably said polypeptide only comprises L-glutamic acid residues.

[0063] Poly (GluAsp): The term "Poly (GluAsp)" as used herein, refers to a polypeptide comprising at least 50 %, preferably at least 70 %, more preferably at least 90 %, still more preferably at least 95 %, still more preferably at least 99 %, and most preferably 100 % glutamic acid residues and aspartic acid residues, out of the total number of amino acid residues comprised by said polypeptide. The glutamic acid molecules and the aspartic acid molecules are hereby either all-L or all-D or mixtures thereof. Most preferably said polypeptide only comprises L-glutamic acid residues and L-aspartic acid residues.

[0064] 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.

[0065] 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.

[0066] 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.

[0067] 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.).

[0068] 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.

[0069] 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, a virus-like particle of an RNA bacteriophage is resembling the structure of an RNA bacteriophage. Furthermore, a virus-like particle of an RNA bacteriophage is non replicative and/or non-infectious. Typically and preferably a virus-like particle of an RNA bacteriophage is lacking at least one of the genes, preferably all genes, encoding the replication machinery of the RNA bacteriophage. Further preferably a virus-like particle of an RNA bacteriophage is also lacking the gene or genes encoding the protein or proteins responsible for viral attachment to or entry into the host. This definition, however, also encompasses virus-like particles of RNA bacteriophages, in which the aforementioned gene or genes are still present but inactive, and, therefore, also are 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.

[0070] 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.

[0071] diabetes: The term diabetes refers to any type of diabetes mellitus. Preferably diabetes refers to type I diabetes and/or type II diabetes. Most preferably diabetes refers to type II diabetes.

[0072] The compositions described herein are capable of inducing or enhancing an immune responses against IL-1 in an animal or in human. It has surprisingly been found that immunization with an IL-1 molecule, i.e. with an IL-1 alpha molecule or with an IL-1 beta molecule, or with a combination of both, resulted in a clear amelioration of the diet-induced diabetic phenotype in male C57BL/6 mice.

[0073] The invention therefore provides a composition for the treatment, amelioration or prophylaxis of diabetes, preferably of type II diabetes, wherein said composition comprises: (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, preferably a virus-like particle; and (b) at least one antigen with at least one second attachment site, wherein the at least one antigen comprises or consists of 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.

[0074] In a preferred embodiment, said composition 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 comprises 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. In a further preferred embodiment said at least one antigen with at least one second attachment site comprises or preferably consists of (i) an IL-1 molecule; and (ii) a linker, wherein preferably said linker comprises or preferably consists of said second attachment site.

[0075] 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.

[0076] 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.

[0077] Virus particles or virus-like particles can be produced and purified from virus-infected cell cultures. The resulting virus particles or virus-like particles 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.

[0078] In one preferred embodiment, the core particle is a virus particle, wherein preferably said virus particle is a bacteriophage, and wherein further preferably said bacteriophage is an RNA bacteriophage, and wherein still further preferably said RNA bacteriophage is an RNA bacteriophage selected from Q β , ϕ r, GA or AP205.

[0079] 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 the artisan.

[0080] 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, preferably BKV; (n) Tobacco mosaic virus; and (o) Flock House Virus.

[0081] A VLP comprising more than one species of recombinant protein 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 species, preferably of two different recombinant proteins, most preferably of two different recombinant capsid proteins, mutants or fragments thereof.

[0082] 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 at least 70 %, preferably at least 80 %, more preferably at least 90 %, even more preferably at least 95 % of the length of the

wild-type recombinant protein, or coat protein, respectively, and which preferably retains the capability of forming a 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.

[0083] 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 at least 90 %, more preferably at least 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.

[0084] 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 wherein preferably said amino acid sequence retains the ability to assemble into a VLP.

[0085] In one preferred embodiment, the virus-like particle is a virus like particle 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. 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.

[0086] 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.

[0087] In one preferred embodiment of the invention, the virus-like particle 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; (m) bacteriophage PRR1, and (n) bacteriophage AP205.

[0088] In one preferred embodiment of the invention, the virus-like particle comprises coat proteins, mutants or fragments thereof, of RNA bacteriophages, wherein said coat proteins comprise or preferably consists of an 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).

[0089] 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. In one very preferred embodiment, the VLP comprises or alternatively consists of two different coat proteins of an RNA bacteriophage, wherein said two different coat proteins comprise or preferably consist of the amino acid sequence of CP Q β (SEQ ID NO: 3) and CP Q β A1 (SEQ ID NO:4); or of the amino acid sequence of CP SP (SEQ ID NO:8) and CP SP A1 (SEQ ID NO:9).

[0090] In preferred embodiments of the present invention, the virus-like particle comprises, or alternatively consists essentially of, or alternatively consists of recombinant coat proteins, mutants or fragments thereof, of an RNA bacteriophage, wherein preferably said RNA bacteriophage is selected from bacteriophage Q β , bacteriophage fr, bacteriophage AP205, and bacteriophage GA.

[0091] In one preferred embodiment, the VLP is a VLP of RNA bacteriophage Q β . The capsid or virus-like particle of Q β shows 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.

[0092] Further preferred virus-like particles of RNA bacteriophages, in particular of RNA bacteriophage Q β and RNA bacteriophage ϕ r, are disclosed in WO 02/056905.

In particular, Example 18 of WO 02/056905 provides a detailed description of the preparation of VLPs of RNA bacteriophage Q β .

[0093] In another preferred embodiment, the VLP 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 or , AP205 coat protein with the substitution of asparagine to aspartic acid at amino acid 14 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 thereof. AP205 VLPs are highly immunogenic, and can be linked with the antigen to typically and preferably generate vaccine constructs displaying the IL-1 molecule oriented in a repetitive manner.

[0094] In one preferred embodiment, the VLP comprises, essentially consists of, or alternatively consists of a mutant coat protein of a virus, preferably of 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 comprises, essentially consists of, or alternatively consists of a mutant coat protein of a virus, preferably an RNA bacteriophage, wherein said 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 molecules per subunits of the VLP, preferably of the VLP of an RNA bacteriophage, in particular, to match and tailor the requirements of the vaccine.

[0095] 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.

[0096] 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. In a further preferred embodiment said first attachment site is an amino group of a lysine residue of SEQ ID NO:3. In a further preferred embodiment said first attachment site is the amino group of any one of the lysine residues in positions 2, 13, 16, 46, 60, 63, and 67 of SEQ ID NO:3. In a further preferred embodiment said first attachment site is the amino group of any one of the lysine residues of the coat protein, preferably of SEQ ID NO:3, which are exposed to the exterior of the capsid.

[0097] 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.

[0098] 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 of 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.

[0099] Further RNA bacteriophage coat proteins have also been shown to self-assemble upon expression in a bacterial host (Kastelein, R.A. et al., Gene 23:245-254 (1983), Kozlovskaya, T.M. et al., Dokl. Akad. Nauk SSSR 287:452-455 (1986), Adhin, M.R. 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, C.Z., 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.

[00100] 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 comprises or consists of 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, wherein the amino acid sequence of said polypeptide shows 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.

[00101] In a further preferred embodiment said antigen comprises or consists of 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. In a very preferred embodiment, the IL-1 molecule is a human IL-1 molecule. Further preferably said IL-1 molecule comprises or preferably consists of a polypeptide, wherein the amino acid sequence of said polypeptide shows 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.

[00102] In a further preferred embodiment said IL-1 molecule is derived from rat or mouse, preferably mouse, wherein said IL-1 molecule preferably comprises or even more preferably consists of a polypeptide, wherein the amino acid sequence of said polypeptide shows 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.

[00103] In a further preferred embodiment said 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, wherein the amino acid sequence of said polypeptide shows 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, wherein the amino acid sequence of said polypeptide shows 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.

[00104] 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, wherein the amino acid sequence of said polypeptide shows 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, wherein the amino acid sequence of said polypeptide shows 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.

[00105] 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.

[00106] 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, wherein the amino acid sequence of said polypeptide shows 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.

[00107] 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, wherein the amino acid sequence of said polypeptide shows 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, wherein the amino acid sequence of said polypeptide shows 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 SEQ ID NO:36.

[00108] 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, wherein the amino acid sequence of said polypeptide shows 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, wherein the amino acid sequence of said polypeptide shows 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 SEQ ID NO:49.

[00109] 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, wherein the amino acid sequence of said polypeptide shows 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.

[00110] 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, wherein the amino acid sequence of said polypeptide shows 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.

[00111] 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, wherein the amino acid sequence of said polypeptide shows 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.

[00112] 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, wherein the amino acid sequence of said polypeptide shows 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.

[00113] 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 preferably 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, wherein the amino acid sequence of said polypeptide 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(s).

[00114] In one preferred embodiment, said IL-1 mutein comprises at least one, preferably one, mutated amino acid sequence derived from a wild type amino acid sequence, wherein said wild type amino acid sequence is an IL-1 beta amino acid sequence selected from the group consisting of: (1) position 3 to 11 of SEQ ID NO:64; (2) position 46 to 56 of SEQ ID NO:64; (3) position 88 to 109 of SEQ ID NO:64; and (4) position 143 to 153 of SEQ ID NO:64; or wherein said wild type amino acid sequence is an IL-1 alpha amino acid sequence

selected from the group consisting of: (5) position 9 to 20 of SEQ ID NO:63; (6) position 52 to 62 of SEQ ID NO:63; (7) position 94 to 113 of SEQ ID NO:63; and (8) position 143 to 153 of SEQ ID NO:63; and wherein said at least one mutated amino acid sequence is characterized by an amino acid exchange in one to four positions, preferably in one, two or three positions, more preferably in one or two positions, as compared to said wild type amino acid sequence it is derived from; or wherein said at least one mutated amino acid sequence is characterized by a deletion of one to four consecutive amino acids of said wild type amino acid sequence it is derived from.

[00115] In a further preferred embodiment said IL-1 mutein comprises at most one mutated amino acid sequence derived from each of said IL-1 beta amino acid sequences (1) to (4); or wherein said IL-1 mutein comprises at most one mutated amino acid sequence derived from each of said IL-1 alpha amino acid sequences (5) to (8).

[00116] In a very preferred embodiment said IL-1 mutein comprises exactly one of said at least one mutated amino acid sequence, wherein preferably said exactly one mutated amino acid sequence is derived from a wild type amino acid sequence, wherein said wild type amino acid sequence is position 143 to 153 of SEQ ID NO:64 or position 143 to 153 of SEQ ID NO:63.

[00117] In a further preferred embodiment said at least one mutated amino acid sequence is characterized by a deletion of one to three, preferably of one to two, consecutive amino acids of said wild type amino acid sequence it is derived from.

[00118] In a further preferred embodiment said at least one mutated amino acid sequence is characterized by a deletion of exactly one amino acid of said wild type amino acid sequence it is derived from.

[00119] In a further preferred embodiment said at least one mutated amino acid sequence is derived from a wild type amino acid sequence, wherein said wild type amino acid sequence is position 143 to 153 of SEQ ID NO:64 or position 143 to 153 of SEQ ID NO:63. Most preferably said at least one mutated amino acid sequence is derived from position 143 to 153 of SEQ ID NO:64.

[00120] In a further preferred embodiment said at least one mutated amino acid sequence is derived from a wild type amino acid sequence, wherein said wild type amino acid sequence is position 46 to 56 of SEQ ID NO:64 or position 52 to 62 of SEQ ID NO:63, wherein preferably said at least one mutated amino acid sequence is characterized by a deletion of one to four, preferably of two to three, consecutive amino acids of said wild type amino acid sequence it is derived from. In a very preferred embodiment said IL-1 mutein comprises or preferably consists of a polypeptide, wherein the amino acid sequence of said polypeptide is

SEQ ID NO:137 or SEQ ID NO:138.

[00121] In a further preferred embodiment said at least one mutated amino acid sequence is derived from a wild type amino acid sequence, wherein said wild type amino acid sequence is position 88 to 109 of SEQ ID NO:64 or position 94-113 of SEQ ID NO:63, wherein said at least one mutated amino acid sequence is characterized by the deletion of one to four, preferably of one to three, more preferably of one to two consecutive amino acids of said wild type amino acid sequence it is derived from.

[00122] In a further preferred embodiment said at least one mutated amino acid sequence is characterized by an amino acid exchange in one or two positions, preferably in exactly one position, as compared to said wild type amino acid sequence it is derived from.

[00123] In a further preferred embodiment said wild type amino acid sequence is position 143 to 153 of SEQ ID NO:64 or position 143 to 153 of SEQ ID NO:63 and said at least one mutated amino acid sequence is characterized by an amino acid exchange in one or two positions, preferably in exactly one position, as compared to said wild type amino acid sequence, wherein further preferably said exactly one position is position 145 of SEQ ID NO:64 or position 145 of SEQ ID NO:63, wherein still further preferably said amino acid exchange is an exchange of aspartic acid (D) to an amino acid selected from the group consisting of lysine (K), tyrosine (Y), phenylalanine (F), asparagine (N) and arginine (R).

[00124] In a very preferred embodiment said amino acid exchange is an exchange of aspartic acid (D) to lysine (K).

[00125] In a further preferred embodiment said wild type amino acid sequence is position 143 to 153 of SEQ ID NO:64 or position 143 to 153 of SEQ ID NO:63 and said at least one mutated amino acid sequence is characterized by an amino acid exchange in exactly one position as compared to said wild type amino acid sequence, wherein further preferably said exactly one position is position 146 of SEQ ID NO:64 or position 146 of SEQ ID NO:63, wherein still further preferably said amino acid exchange is an exchange of phenylalanine (F) to an amino acid selected from the group consisting of asparagine (N), glutamine (Q), and serine (S).

[00126] 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, most preferably said IL-1 mutein is SEQ ID NO:136.

[00127] In a further preferred embodiment said IL-1 mutein is an IL-1 beta mutein, wherein preferably said IL-1 beta mutein comprises or preferably consists of a polypeptide, wherein the amino acid sequence of said polypeptide differs from the amino acid sequence of SEQ ID

NO:64 in 1 to 10, 1 to 9, 1 to 8, 1 to 7, 1 to 6, 1 to 5, 1 to 4, 1 to 3, or 1 to 2 amino acid residues. Most preferably, said amino acid sequence differs from the amino acid sequence of SEQ ID NO:64 in exactly 1 amino acid residue. In a very preferred embodiment, said IL-1 beta mutein comprises or preferably consists of a polypeptide, wherein the amino acid sequence of said polypeptide is selected from SEQ ID NO:131 to SEQ ID NO:140 and SEQ ID NO:205 to SEQ ID NO:209, wherein most preferably said IL-1 beta mutein comprises or preferably consists of a polypeptide, wherein the amino acid sequence of said polypeptide is SEQ ID NO:136.

[00128] In a very preferred embodiment, said IL-1 molecule, and preferably said IL-1 beta mutein, comprises or preferably consists of SEQ ID NO:136.

[00129] In a further preferred embodiment said IL-1 mutein is an IL-1 alpha mutein, wherein preferably said IL-1 alpha mutein comprises or preferably consists of a polypeptide, wherein the amino acid sequence of said polypeptide differs from the amino acid sequence of SEQ ID NO:63 in 1 to 10, 1 to 9, 1 to 8, 1 to 7, 1 to 6, 1 to 5, 1 to 4, 1 to 3, or 1 to 2 amino acid residues. Most preferably said amino acid sequence differs from the amino acid sequence of SEQ ID NO:63 in exactly 1 amino acid residue. In a very preferred embodiment said IL-1 alpha mutein comprise or preferably consist of a polypeptide, wherein the amino acid sequence of said polypeptide is selected from the group consisting of SEQ ID NO:210 to SEQ ID NO:218, wherein most preferably said IL-1 alpha mutein comprises or preferably consists of a polypeptide, wherein the amino acid sequence of said polypeptide is SEQ ID NO:210.

[00130] In a very preferred embodiment, said IL-1 molecule, and preferably said IL-1 alpha mutein comprises or preferably consists of SEQ ID NO:210.

[00131] Further disclosed is a method of producing the compositions of the invention comprising (a) providing a VLP with at least one first attachment site; (b) providing at least one antigen with at least one second attachment site, wherein said antigen comprises or consists of an IL-1 molecule, preferably an IL-1 protein, an IL-1 fragment, preferably an IL-1 mature fragment, an IL-1 peptide or an IL-1 mutein; and (c) combining said VLP and said at least one antigen with at least one second attachment site 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, comprising said IL-1 molecule, said IL-1 protein, said IL-1 fragment, preferably an IL-1 mature fragment, said IL-1 peptide or said 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.

[00132] In one preferred embodiment of the invention, the VLP with at least one first attachment site is linked to the antigen with at least one second attachment site via at least one peptide bond. A gene encoding an IL-1 molecule, preferably an IL-1 mutein, 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 molecule into a mutant coat protein where part of the coat protein sequence has been deleted. Such constructs 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.

[00133] 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.

[00134] In other embodiments, the at least one IL-1 molecule, preferably the IL-1 mutein can be fused to a number of other viral coat proteins, for example 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 molecule 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 papilloma virus, 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 of a virus, or to mutants or fragments thereof, have been disclosed in WO 2004/009124 page 62 line 20 to page 68 line 17.

[00135] 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.

[00136] 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).

[00137] Therefore, in a further very preferred embodiment of the present invention, the association or linkage of the VLP and the at least one antigen comprising or consisting of the IL-1 molecule, does not comprise a disulfide bond. In a further preferred embodiment 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 antigen does not comprise a sulphur-sulphur bond. Further preferably, 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.

[00138] In a further preferred embodiment said at least one first attachment comprises an amino group and said second attachment comprises a sulfhydryl group.

[00139] In a further preferred embodiment, said first attachment is an amino group and said second attachment site is a sulfhydryl group. In a still further preferred embodiment, said first attachment is an amino group of a lysine residue, and said second attachment site is a sulfhydryl group of a cysteine residue.

[00140] 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 antigen to said core particle, preferably to said virus-like particle, wherein said only one second attachment site that associates with said first attachment site is a sulfhydryl group, and wherein said antigen and said core particle, preferably said virus-like particle, interact through said association to form an ordered and repetitive antigen array, and wherein further preferably said first attachment site is an amino group of a lysine residue.

[00141] In a further preferred embodiment said virus-like particle comprises, essentially

consists of, or alternatively consists of, recombinant coat proteins, mutants or fragments thereof, of a virus, preferably of an RNA bacteriophage, wherein said at least one antigen is fused to the N- or the C- terminus of said recombinant coat proteins, mutants or fragments thereof.

[00142] In a further preferred embodiment said virus-like particle comprises, essentially consists of, or alternatively consists of, recombinant coat proteins, mutants or fragments thereof, of an RNA bacteriophage, wherein preferably said RNA bacteriophage is selected from the group consisting of: (a) bacteriophage AP205; (b) bacteriophage fr; and (c) bacteriophage GA; and wherein said at least one antigen is fused to the N- or the C- terminus, preferably to the C-terminus, of said recombinant coat proteins, mutants or fragments thereof, and wherein further preferably said at least one antigen comprises or preferably consists of a polypeptide, wherein the amino acid sequence of said polypeptide is SEQ ID NO:136 or SEQ ID NO:210, preferably SEQ ID NO:136.

[00143] In a further 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, mutant or fragments thereof, of RNA bacteriophage AP205.

[00144] In a very preferred embodiment said virus-like particle comprises, essentially consists of, or alternatively consists of, recombinant coat proteins, mutants or fragments thereof, of bacteriophage AP205, wherein said at least one antigen is fused to the C- terminus of said recombinant coat proteins, mutants or fragments thereof, and wherein said at least one antigen comprises or preferably consists of a polypeptide, wherein the amino acid sequence of said polypeptide is SEQ ID NO:136 or SEQ ID NO:210, preferably SEQ ID NO:136.

[00145] VLPs comprising fusion proteins of coat protein of bacteriophage AP205 with an antigen are generally disclosed in WO2006/032674A1.

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.

[00146] 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.

[00147] 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 *trpT*176 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.

[00148] 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.

[00149] 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 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.

[00150] 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.

[00151] In one preferred embodiment of the invention, the antigen 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. Most preferably, said hetero-bifunctional cross-linker is succinimidyl-6-[β -maleimidopropionamido]hexanoate (SMPH). 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).

[00152] In a preferred embodiment, the composition of the invention further comprises a linker. In a further preferred embodiment said at least one antigen with said at least one second attachment site further comprises a linker, wherein said linker comprises said second attachment site, and wherein said linker is associated to said antigen by way of one peptide bond, and wherein preferably said linker is a cysteine. 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 residue 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.

[00153] 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.

[00154] 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. In a further preferred embodiment said linker further comprises a His-tag. A very preferred linker comprises or preferably consists of LEHHHHHHGGC (SEQ ID NO:201) or LEHHHHHHGGCG (SEQ ID NO:219), preferably the linker consists of LEHHHHHHGGCG (SEQ ID NO:219).

[00155] Linking of the antigen with at least one second attachment site 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 antigen with at least one second attachment site 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 antigen with at least one second attachment site after deprotection if required, may then be coupled to the VLP as follows. After separation of the excess thiolation reagent, the antigen with at least one second attachment site 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 antigen with at least one second attachment site can

react, such as described above. Optionally, low amounts of a reducing agent are included in the reaction mixture. In further methods, the antigen with at least one second attachment site 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.

[00156] In other embodiments of the present invention, the composition comprises or alternatively consists essentially of a virus-like particle linked to antigen with at least one second attachment site via chemical interactions, wherein at least one of these interactions is not a covalent bond.

[00157] Linking of the VLP to the antigen with at least one second attachment site can be effected by biotinylating the VLP and expressing the IL-1 molecule as a streptavidin-fusion protein.

[00158] 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.

[00159] In very preferred embodiments of the invention, the antigen with at least one second attachment site 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 .

[00160] 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.

[00161] 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.

[00162] 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.

[00163] 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.

[00164] 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.

[00165] 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.

[00166] 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.

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.

[00167] 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.

[00168] In one aspect, the invention provides a vaccine for the treatment, amelioration and/or prevention of diabetes, preferably of type II diabetes, said vaccine comprising the composition of the invention, preferably in an effective amount. Thus, the invention provides a vaccine for the treatment, amelioration and/or prevention of diabetes, preferably of type II diabetes, said vaccine comprising a composition, preferably in an effective amount, 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 comprises or consists of 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.

[00169] An effective amount of a composition of the invention is an amount which is capable of inducing an immune response in the treated subject, preferably in a human, and which preferably results in a therapeutic or prophylactic effect in diabetes, preferably in type II diabetes.

[00170] In a preferred embodiment, said vaccine comprises (i) a first composition, preferably in an effective amount, wherein said first composition is a composition according to the invention wherein the IL-1 molecule comprised by said first composition is an IL-1 beta molecule, preferably SEQ ID NO:136 or SEQ ID NO:165; and (ii) a second composition, preferably in an effective amount, wherein said second composition is a composition according to the invention wherein the IL-1 molecule comprised by said second composition is an IL-1 alpha molecule, preferably SEQ ID NO:203 or SEQ ID NO:210.

[00171] In one preferred embodiment, the IL-1 molecule which is linked to the VLP in the composition contained in the vaccine 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.

[00172] In one embodiment, the vaccine further comprises at least one adjuvant.

[00173] An advantageous feature of the present invention is the high immunogenicity of the composition, even in the absence of adjuvants. Therefore, in a preferred embodiment, the vaccine composition is devoid of adjuvant. 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, simultaneously or after the administration of the vaccine.

[00174] However, when an adjuvant is administered, the administration of the at least one adjuvant may hereby occur prior to, simultaneously or after the administration of the inventive composition or of the vaccine.

[00175] When the composition and/or the 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 vaccines or pharmaceutical compositions are provided in numerous sources including Remington's Pharmaceutical Sciences (Osol, A, ed., Mack Publishing Co., (1990)). This includes 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.

[00176] The vaccines of the invention are said to be "pharmaceutically acceptable" if their administration can be tolerated by a recipient individual, preferably by a human. Further, the vaccines of the invention are 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 reduce its concentration and/or interfering with its physiological or pathological function.

[00177] The invention therefore provides a pharmaceutical composition for the treatment, amelioration and/or prevention of diabetes, preferably of type II diabetes, said pharmaceutical composition comprising (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 comprises or consists of 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; and (2) a pharmaceutically acceptable carrier.

[00178] The invention further provides a pharmaceutical composition for the treatment, amelioration and/or prevention of diabetes, preferably of type II diabetes, said pharmaceutical composition comprising (1) a vaccine, said vaccine comprising 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 comprises or consists of 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; and (2) a pharmaceutically acceptable carrier.

[00179] The invention further provides a method for the treatment, amelioration and / or prevention of diabetes, preferably of type II diabetes, said method comprising administering a composition, a vaccine or a pharmaceutical composition of the invention to an animal, preferably to a human. The invention further provides a method for the treatment, amelioration and / or prevention of diabetes, preferably of type II diabetes, said method comprising administering to an animal, preferably to a human, (i) a first composition, preferably in an effective amount, wherein said first composition is a composition according to the invention wherein the IL-1 molecule comprised by said first composition is an IL-1 beta molecule, preferably SEQ ID NO:136 or SEQ ID NO:165; and (ii) a second composition, preferably in an effective amount, wherein said second composition is a composition according to the invention wherein the IL-1 molecule comprised by said second composition

is an IL-1 alpha molecule, preferably SEQ ID NO:203 or SEQ ID NO:210.

[00180] Thus, the invention provides a method for the treatment, amelioration and / or prevention of diabetes, preferably of type II diabetes, said method comprising administering a composition to an animal, preferably to a human, said 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 comprises or consists of 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.

[00181] The invention further provides a method for the treatment, amelioration and / or prevention of diabetes, preferably of type II diabetes, said method comprising administering a vaccine to an animal, preferably to a human, said vaccine comprising 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 comprises or consists of 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.

[00182] The invention further provides a method for the treatment, amelioration and / or prevention of diabetes, preferably of type II diabetes, said method comprising administering a pharmaceutical composition to an animal, preferably to a human, said pharmaceutical composition comprising (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 comprises or consists of 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; and (2) a pharmaceutically acceptable carrier.

[00183] The invention further provides a method for the treatment, amelioration and / or prevention of diabetes, preferably of type II diabetes, said method comprising administering a pharmaceutical composition to an animal, preferably to a human, said pharmaceutical composition comprising (1) a vaccine, said vaccine comprising 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 comprises or consists of 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; and (2) a pharmaceutically acceptable carrier.

[00184] With respect to the methods of the invention, said composition, said vaccine and/or said pharmaceutical composition is administered to said animal, preferably to said human, in an immunologically effective amount.

[00185] In further preferred embodiments said animal is a mammal, preferably selected from

cat, sheep, pig, horse, cattle, dog, rat, mouse, and, most preferably, human.

[00186] In one embodiment, the compositions, vaccines and/or pharmaceutical compositions are administered to said animal, preferably to said human by injection, infusion, inhalation, oral administration, or other suitable physical methods. In a preferred embodiment, the compositions, vaccines and/or pharmaceutical compositions are administered to said animal, preferably to said human, intramuscularly, intravenously, transmucosally, transdermally, intranasally, intraperitoneally, subcutaneously, or directly into the lymph node.

[00187] A further aspect of the invention is the use of the compositions, the vaccines and/or of the pharmaceutical compositions described herein for the treatment, amelioration and/or prevention of diabetes, preferably of type II diabetes. In more detail, the invention provides for the use of a composition for the treatment, amelioration and/or prevention of diabetes, preferably of type II diabetes, said 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 comprises or consists of 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.

[00188] A further aspect of the invention is the use of the compositions, the vaccines and/or of the pharmaceutical compositions described herein for the manufacture of a medicament for the treatment, amelioration and/or prevention of diabetes, preferably of type II diabetes. In more detail, the invention provides for the use of a composition for the manufacture of a medicament for the treatment, amelioration and/or prevention of diabetes, preferably of type II diabetes, said 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 comprises or consists of 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.

[00189] It is to be understood that all technical features and embodiments described herein, in particular those described for the compositions of the invention, may be applied to all aspects of the invention, especially to the vaccine, pharmaceutical compositions, methods and uses, alone or in any possible combination. In this context it is explicitly underlined that the following embodiments of said at least one antigen with at least one second attachment site are specifically preferred.

[00190] In a further preferred embodiment said at least one antigen with at least one second attachment site comprises or preferably consists of (i) an IL-1 beta molecule, wherein said IL-1 beta molecule is selected from any one of SEQ ID NO:165, and SEQ ID NOs 131 to 140;

and (ii) a linker, wherein said linker comprises said second attachment site, and wherein preferably said linker comprises or preferably consists of GGC (SEQ ID NO:178) or GGCG (SEQ ID NO:188).

[00191] In a further preferred embodiment said at least one antigen with at least one second attachment site consists of (i) an IL-1 beta molecule, wherein said IL-1 beta molecule is SEQ ID NO:165 or SEQ ID NO:136, preferably SEQ ID NO:136; and (ii) a linker, wherein said linker comprises said second attachment site, and wherein said linker comprises or preferably consists of GGC (SEQ ID NO:178) or GGCG (SEQ ID NO:188), preferably GGCG (SEQ ID NO:188).

[00192] In a further preferred embodiment said at least one antigen with at least one second attachment site consists of (i) an IL-1 beta molecule, wherein said IL-1 beta molecule is SEQ ID NO:165 or SEQ ID NO:136, preferably SEQ ID NO:136; and (ii) a linker, wherein said linker comprises said second attachment site, and wherein said linker is covalently bound to the C-terminus of said IL-1 beta molecule by way of a peptide bond, and wherein said linker comprises or preferably consists of GGC (SEQ ID NO:178) or GGCG (SEQ ID NO:188), preferably of GGCG (SEQ ID NO:188).

[00193] In a further preferred embodiment said at least one antigen with at least one second attachment site consists of (i) an IL-1 beta molecule, wherein said IL-1 beta molecule is SEQ ID NO:165 or SEQ ID NO:136, preferably SEQ ID NO:136; and (ii) a linker, wherein said linker comprises said second attachment site, and wherein said linker is covalently bound to the C-terminus of said IL-1 beta molecule by way of a peptide bond, and wherein said linker consists of LEHHHHHHGGCG (SEQ ID NO:219).

[00194] In a further preferred embodiment said at least one antigen with at least one second attachment site is any one of SEQ ID NOs 220 to 223, preferably SEQ ID NO:220.

[00195] In a further preferred embodiment said at least one antigen with at least one second attachment site comprises or preferably consists of (i) an IL-1 alpha molecule, wherein said IL-1 alpha molecule is selected from any one of SEQ ID NOs 203 to 218; and (ii) a linker, wherein said linker comprises said second attachment site, and wherein preferably said linker comprises or preferably consists of GGC (SEQ ID NO:178) or GGCG (SEQ ID NO:188).

[00196] In a further preferred embodiment said at least one antigen with at least one second attachment site consists of (i) an IL-1 alpha molecule, wherein said IL-1 alpha molecule is SEQ ID NO:203 or SEQ ID NO:210, preferably SEQ ID NO:203; and (ii) a linker, wherein said linker comprises said second attachment site, and wherein said linker comprises or preferably consists of GGC (SEQ ID NO:178) or GGCG (SEQ ID NO:188), preferably GGCG (SEQ ID NO:188).

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[00197] In a further preferred embodiment said at least one antigen with at least one second attachment site consists of (i) an IL-1 alpha molecule, wherein said IL-1 alpha molecule is SEQ ID NO:203 or SEQ ID NO:210, preferably SEQ ID NO:203; and (ii) a linker, wherein said linker comprises said second attachment site, and wherein said linker is covalently bound to the C-terminus of said IL-1 alpha molecule by way of a peptide bond, and wherein said linker comprises or preferably consists of GGC (SEQ ID NO:178) or GGCG (SEQ ID NO:188), preferably of GGCG (SEQ ID NO:188).

[00198] In a further preferred embodiment said at least one antigen with at least one second attachment site is any one of SEQ ID NOs 224 or 225, preferably SEQ ID NO:224.

[00201] 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

[00202] 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.

[00203] 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.

[00204] 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)

[00205] 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

[00206] 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

[00207] 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

[00208] 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 .

EXAMPLE 3

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

[00209] 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.

[00210] 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.

[00211] 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)

[00212] 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

[00213] 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

[00214] 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

[00215] 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 .

EXAMPLE 4

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

[00216] 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 µg 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 µg bovine type II collagen mixed with incomplete Freund's adjuvant, and examined on a daily basis for the development of arthritis symptoms.

[00217] Four weeks after the second collagen injection, Q β -immunized control mice showed an average cumulative clinical score (as defined in EXAMPLE 2F of WO2008/037504A1) 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 1). Mice treated with 37.5 µg or 375 µg 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.

[00218] 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 µg or 375 µg 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.

[00219] 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 1: 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 5

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)

[00220] 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.

[00221] 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.

[00222] 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.

[00223] 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

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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 %.

[00224] 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

[00225] 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

[00226] 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

[00227] 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-1receptorl-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

[00228] 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 .

EXAMPLE 6

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)

[00229] 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 5. 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 µg/ml ampicillin and 10 µg/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)

[00230] 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

[00231] Four female C3H/HeJ mice were immunized with AP205_mIL-1 β ₁₁₉₋₂₆₉. Twentyfive µg of total protein were diluted in PBS to 200 µl and injected subcutaneously (100 µl 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 mIL-1 β ₁₁₉₋₂₆₉-specific ELISA.

D. ELISA

[00232] ELISA plates were coated with mouse IL-1 β ₁₁₉₋₂₆₉ protein at a concentration of 1 µg/ml. The plates were blocked and then incubated with serially diluted mouse sera from days 0, 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 lead to half maximal optical density at 450 nm. The average anti-mouse IL-1 β ₁₁₉₋₂₆₉ titer was 1:19000 on day 14, 1:58200 on day 28 and 1:104700 on day 35. This demonstrates that immunization with AP205_mIL-1 β ₁₁₉₋₂₆₉ could overcome immunological tolerance and produce high titer antibodies which recognize specifically mouse IL-1 β ₁₁₉₋₂₆₉.

E. In vitro neutralization of IL-1 β

[00233] 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

[00234] The in vivo neutralizing capacity of the antibodies raised by immunization with AP205_mIL-1 β ₁₁₉₋₂₆₉ were investigated next. Four female C3H/HeJ mice were therefore immunized three times on days 0, 14, and 28 with AP205_mIL-1 β ₁₁₉₋₂₆₉ 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 mIL-1 β ₁₁₉₋₂₆₉. As readout of the inflammatory activity of the injected mIL-1 β ₁₁₉₋₂₆₉, serum samples were 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 showed an increase of 0.28 ng/ml in serum IL-6 concentrations, whereas mice immunized with AP205_mIL-1 β ₁₁₉₋₂₆₉ showed no increase at all. These data indicate that the antibodies produced by immunization with AP205_mIL-1 β ₁₁₉₋₂₆₉ were able to neutralize specifically and efficiently the pro-inflammatory activity of IL-1 β .

H. Immunization of mice with AP205_hIL-1 β ₁₁₆₋₂₆₉

[00235] Four female C3H/HeJ mice were immunized with AP205_hIL-1 β ₁₁₆₋₂₆₉. 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 days 0, 14, and 28. Mice were bled retroorbitally on days 0, 14, 28 and 35, and sera were analyzed using human IL-1 β ₁₁₆₋₂₆₉-specific ELISA.

I. ELISA

[00236] ELISA plates were coated with human IL-1 β ₁₁₆₋₂₆₉ protein at a concentration of 1 μ g/ml. The plates were blocked and then incubated with serially diluted mouse sera from days 0, 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 lead to half maximal optical density at 450 nm. The average anti-human IL-1 β ₁₁₆₋₂₆₉ titer was 1:39600 on day 14, 1:58300 on day 28 and 1:65600 on day 35. This demonstrates that AP205_hIL-1 β ₁₁₆₋₂₆₉ induces high titers of hIL-1 β ₁₁₆₋₂₆₉-specific antibodies in mice.

EXAMPLE 7

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

[00237] 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(+).

[00238] 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'-TCGAGCACCACCACCACCACCGGTGGTTGCTAATAATAATTGATTAATAC-3'; SEQ ID NO:128) and 42T-2 (5'-CTAGGTATTAATCAATTATTATTAGCAACCACCGTGGTGGTGGTGGTGGTGC-3'; SEQ ID NO:129), resulting in plasmid pET-42T(+).

[00239] 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

[00240] 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 2 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 2: Overview over IL-1 muteins, expression vectors and oligonucleotides used for their construction.

Expression vector	mutain 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 CTCGGGGAC-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 TGAAGTGGG 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 ^{S0,S1})	hIL-1 β ₁₁₆₋₂₆₉ (Δ EE ^{S0,S1}) (SEQ ID NO:137)	EE-1 (5'- CATGTCCTTTGTACAAGGAAGTAATGACAAAATACC TGTG-3'; SEQ ID NO:153) EE-2 (5'- CACAGGTATTTGTCACTTCTCCTTGTACAAAGGAC 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'- CTTTGTACAAGGAGAAGAAAAAATACCTGTGGCCTT G-3'; SEQ ID NO:155) SND-2 (5'- CAAGGCCACAGGTATTTTTCTTCTCCTTGACAAAG -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 8

A. Biological activity of human IL-1 β ₁₁₆₋₂₆₉ and human IL-1 β ₁₁₆₋₂₆₉ muteins in mice

[00241] Three female C3H/HeJ mice per group were 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 7. Serum samples were withdrawn before and 3 h after injection and analysed for the relative increase in the concentration of the pro-inflammatory cytokine IL-6. As shown in Table 3, mice injected with the wild type human IL-1 β ₁₁₉₋₂₆₉ protein showed an increase of 2.38 ng/ml in serum IL-6 concentrations. With the exception of muteins hIL-1 β ₁₁₆₋₂₆₉ (D54R) and hIL-1 β ₁₁₆₋₂₆₉ (K63S/K65S), which induced similar serum IL-6 concentrations as wild type human IL-1 β ₁₁₉₋₂₆₉, all muteins tested induced lower amounts of IL-6, indicating reduced biological activity.

Table 3: Biological activity of human IL-1 β ₁₁₆₋₂₆₉ and human IL-1 β ₁₁₆₋₂₆₉ muteins in mice.

Protein/mutein	Average increase in serum IL-6 concentrations 3 h after injection in ng/ml ($\pm$ SD)
hIL-1 β ₁₁₆₋₂₆₉	2.38 $\pm$ 0.69
hIL-1 β ₁₁₆₋₂₆₉ (R4D)	0.16 $\pm$ 0.03
hIL-1 β ₁₁₆₋₂₆₉ (L6A)	1.03 $\pm$ 0.65
hIL-1 β ₁₁₆₋₂₆₉ (T9G)	0.82 $\pm$ 0.42
hIL-1 β ₁₁₆₋₂₆₉ (R11G)	0.34 $\pm$ 0.25
hIL-1 β ₁₁₆₋₂₆₉ (D54R)	3.25 $\pm$ 1.67
hIL-1 β ₁₁₆₋₂₆₉ (Δ EE ^{50,51})	1.10 $\pm$ 0.27
hIL-1 β ₁₁₆₋₂₆₉ (Δ SND ⁵²⁻⁵⁴)	0.13 $\pm$ 0.08
hIL-1 β ₁₁₆₋₂₆₉ (K63S/K65S)	2.22 $\pm$ 1.38
hIL-1 β ₁₁₆₋₂₆₉ (Q126A/E128A)	0.77 $\pm$ 0.55
hIL-1 β ₁₁₆₋₂₆₉ (D145K)	1.39 $\pm$ 0.26

B. Biological activity of human IL-1 β ₁₁₆₋₂₆₉ and human IL-1 β ₁₁₆₋₂₆₉ muteins in human PBMC

[00242] Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood of a healthy donor by Ficoll density gradient centrifugation. 5×10^5 cells per well were incubated with titrating amounts of either the wild type human IL-1 β ₁₁₉₋₂₆₉ protein or one of the human IL-1 β ₁₁₉₋₂₆₉ muteins of EXAMPLE 7. After over night incubation the amount of IL-6 in the cell culture supernatant was measured as readout of the biological activity. Table 4 shows that with the exception of muteins hIL-1 β ₁₁₆₋₂₆₉ (D54R) and hIL-1 β ₁₁₆₋₂₆₉ (K63S/K65S), much higher amounts of all mutants were necessary to induce the same IL-6 secretion as wild type human IL-1 β ₁₁₉₋₂₆₉, indicating a reduction in bioactivity. The factor by which biological activity was reduced ranged from 13 fold for mutein hIL-1 β ₁₁₆₋₂₆₉ (R11G) to 381 fold for mutein hIL-1 β ₁₁₆₋₂₆₉ (Δ SND⁵²⁻⁵⁴).

Table 4: Biological activity of human IL-1 β ₁₁₆₋₂₆₉ and human IL-1 β ₁₁₆₋₂₆₉ muteins in human PBMC.

Protein/mutein	Protein / mutein concentration (in ng/ml) required to induce 600 pg/ml IL-6 from human PBMC	Fold reduction in bioactivity relative to wild type hIL-1 β ₁₁₆₋₂₆₉
hIL-1 β ₁₁₆₋₂₆₉	2	-/-
hIL-1 β ₁₁₆₋₂₆₉ (R4D)	333	146
hIL-1 β ₁₁₆₋₂₆₉ (L6A)	31	14
hIL-1 β ₁₁₆₋₂₆₉ (T9G)	79	34
hIL-1 β ₁₁₆₋₂₆₉ (R11G)	30	13
hIL-1 β ₁₁₆₋₂₆₉ (D54R)	5	2
hIL-1 β ₁₁₆₋₂₆₉ (Δ EE ^{50,51})	187	82
hIL-1 β ₁₁₆₋₂₆₉ (Δ SND ⁵²⁻⁵⁴)	872	381
hIL-1 β ₁₁₆₋₂₆₉ (K63S/K65S)	13	6
hIL-1 β ₁₁₆₋₂₆₉ (Q126A/E128A)	94	41
hIL-1 β ₁₁₆₋₂₆₉ (D145K)	386	169

EXAMPLE 9**A. Coupling of human IL-1 β ₁₁₆₋₂₆₉ and human IL-1 β ₁₁₆₋₂₆₉ muteins to Q β virus-like particles**

[00243] 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 7 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

[00244] 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

[00245] 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 5.

Table 5: 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

[00246] 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

[00247] 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 %.

[00248] The same sera were also tested for their ability to inhibit the hIL-1 β ₁₁₆₋₂₆₉-induced secretion of IL-6 from human cells. Human PBMCs were therefore prepared as described in EXAMPLE 8B and incubated with 10 ng/ml wild type hIL-1 β ₁₁₆₋₂₆₉, which had been premixed with titrating concentrations of the sera described above. After over night incubation the cell culture supernatants were analyzed for the presence of IL-6. The neutralizing capacity of the sera was expressed as those dilutions which lead to half maximal inhibition of IL-6 secretion. In order to allow a direct comparison to the neutralizing capacity of the serum raised against wild type hIL-1 β ₁₁₆₋₂₆₉, the neutralizing titers of all sera raised against hIL-1 β ₁₁₆₋₂₆₉ muteins were corrected for the respective ELISA titers measured against wild type hIL-1 β ₁₁₆₋₂₆₉ (see Table 5). As shown in Table 6 all sera raised against hIL-1 β ₁₁₆₋₂₆₉ muteins were able to inhibit the secretion of IL-6 induced by wild type hIL-1 β ₁₁₆₋₂₆₉. The neutralizing titers ranged from 1:113 for sera raised against Q β -hIL-1 β ₁₁₆₋₂₆₉ (R11G) to 1:4532 for sera raised against Q β -hIL-1 β ₁₁₆₋₂₆₉ (D54R).

Table 6: Neutralizing titer determined in sera of mice immunized with various IL-1 beta muteins.

Vaccine	Neutralizing titer (corrected for ELISA titer against wild type hIL-1 β ₁₁₆₋₂₆₉)
Q β -hIL-1 β ₁₁₆₋₂₆₉	3333
Q β -hIL-1 β ₁₁₆₋₂₆₉ (R4D)	2150
Q β -hIL-1 β ₁₁₆₋₂₆₉ (L6A)	2062
Q β -hIL-1 β ₁₁₆₋₂₆₉ (T9G)	1036
Q β -hIL-1 β ₁₁₆₋₂₆₉ (R11G)	113
Q β -hIL-1 β ₁₁₆₋₂₆₉ (D54R)	4532
Q β -hIL-1 β ₁₁₆₋₂₆₉ (Δ EE ^{50,51})	2871

Q β -hIL-1 β ₁₁₆₋₂₆₉ (Δ SND ⁵²⁻⁵⁴)	1109
Q β -hIL-1 β ₁₁₆₋₂₆₉ (K63S/K65S)	3432
Q β -hIL-1 β ₁₁₆₋₂₆₉ (Q126A/E128A)	1237
Q β -hIL-1 β ₁₁₆₋₂₆₉ (D145K)	2369

E. In vivo neutralization of IL-1 β

[00249] 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 10

A Cloning, expression and purification of mouse IL-1 α ₁₁₅₋₂₇₀ and mouse IL-1 α ₁₁₅₋₂₇₀ (D145K)

[00250] The nucleotide sequence encoding amino acids 115-270 of wild type murine IL-1 α was amplified by PCR from a library of TNF α -activated murine macrophages using oligonucleotides IL1 α 1C (5'-ATATATCATA TGTCTGCCCC TTACACCTAC CAGAGTG-3'; SEQ ID NO:196) and IL1 α 2 (5'-ATATATCTCG AGTGATATCTT GGAAGTCTGT CATAGAG-3'; SEQ ID NO:25). The DNA fragment was digested with NheI and XhoI, and cloned into the expression vector pET42T(+), giving rise to the expression plasmid pET42T-mIL-1 α ₁₁₅₋₂₇₀.

[00251] By site directed mutagenesis of the latter plasmid, an expression vector for the mutein mIL-1 α ₁₁₅₋₂₇₀ (D145K) was constructed. Using the oligonucleotide pair alphaD145K-1: (5'-GGACTGCCCTCTATGACAAAATTCCAGATATCACTCGAG-3'; SEQ ID NO:197)

alphaD145K-2 (5'-CTCGAGTGATATCTGGAATTTTGTGCATAGAGGGCAGTCC-3'; SEQ ID NO:198) and the Quik-Change® Site directed mutagenesis kit (Stratagene), the D 145K mutation was introduced. Expression and purification of wild type mouse IL-1 α ₁₁₅₋₂₇₀ and the mutein mouse IL-1 α ₁₁₅₋₂₇₀ (D145K) was performed as described in EXAMPLE 1.

B Cloning, expression and purification of human IL-1 α ₁₁₉₋₂₇₁ and human IL-1 α ₁₁₉₋₂₇₁ (D145K)

[00252] The nucleotide sequence encoding amino acids 119-271 of wild type human IL-1 α was amplified by PCR from a LPS-activated human B cell cDNA library using oligonucleotides HIL-3 (5'-ATATATCATATA TGCTGAGCAA TGTGAAATAC AACTTTATG-3'; SEQ ID NO:141) and HIL-4 (5'-ATATATCTCG AGCGCCTGGT TTTCCAGTAT CTGAAAG-3'; SEQ ID NO:142). The DNA fragment was digested with *NheI* and *XhoI*, and cloned into the expression vector pET42T(+), giving rise to the expression plasmid pET42T-hIL-1 α ₁₁₉₋₂₇₁.

[00253] By site directed mutagenesis of the latter plasmid, an expression vector for the mutein hIL-1 α ₁₁₉₋₂₇₁ (D145K) was constructed. Using the oligonucleotide pair halphaD145K-1 (5'-GGGCCACCCT CTATCACTAA ATTCAGATA CTGGAAAACC-3'; SEQ ID NO:199) and halphaD145K-2 (5'-GGTTTTCAG TATCTGAAAT TTAGTGATAG AGGGTGGCCC-3'; SEQ ID NO:200) and the Quik-Change® Site directed mutagenesis kit (Stratagene), the D145K mutation was introduced. Expression and purification of wild type human IL-1 α ₁₁₉₋₂₇₁ and the human IL-1 α ₁₁₉₋₂₇₁ (D145K) mutein was performed as described in EXAMPLE 1.

EXAMPLE 11

A. Biological activity of human IL-1 α ₁₁₉₋₂₇₁, human IL-1 α ₁₁₉₋₂₇₁ (D145K), mouse IL-1 α ₁₁₅₋₂₇₀, and mouse IL-1 α ₁₁₅₋₂₇₀ (D145K) in human PBMC

[00254] PBMC from a healthy donor (5x10⁵ cells per well) were incubated with titrating amounts of either the wild type human IL-1 α ₁₁₉₋₂₇₁ protein, the human IL-1 α ₁₁₉₋₂₇₁ (D145K) mutein, the wild type mouse IL-1 α ₁₁₅₋₂₇₀ protein, or the mouse IL-1 α ₁₁₅₋₂₇₀ (D145K) mutein. After over night incubation the amount of IL-6 in the cell culture supernatant was measured by Sandwich ELISA as readout of the biological activity of the different proteins. Table 9 shows that 21 fold higher amounts of the mouse IL-1 α ₁₁₅₋₂₇₀ (D145K) mutein were required to induce the same amount of IL-6 as the corresponding wild type mouse IL-1 α ₁₁₅₋₂₇₀ protein. In the case of the human IL-1 α ₁₁₉₋₂₇₁ (D145K) mutein 46-fold higher amounts than the wild type

human IL-1 α ₁₁₉₋₂₇₁ protein were required. This demonstrates that both the human IL-1 α ₁₁₉₋₂₇₁ (D145K) mutein and the mouse IL-1 α ₁₁₅₋₂₇₀ (D145K) mutein have reduced bioactivity in human cells as compared to their wild type counterparts.

Table 7: Biological activity of IL-1 α wild type proteins and muteins in human PBMC as determined by IL-6 induction.

Protein/mutein (expressed with SEQ ID NO:201 as C-terminal tag)	Protein/mutein concentration required to induce 600 pg/ml IL-6 from human PBMC (ng/ml)
mouse IL-1 α ₁₁₅₋₂₇₀ (SEQ ID NO:202)	4.7
mouse IL-1 α ₁₁₅₋₂₇₀ (D145K) (SEQ ID NO:204)	100
human IL-1 α ₁₁₉₋₂₇₁ (SEQ ID NO:203)	0.8
human IL-1 α ₁₁₉₋₂₇₁ (D145K) (SEQ ID NO:210)	37

B. Biological activity of human IL-1 α ₁₁₉₋₂₇₁, human IL-1 α ₁₁₉₋₂₇₁ (D145K), mouse IL-1 α ₁₁₅₋₂₇₀ protein, and mouse IL-1 α ₁₁₅₋₂₇₀ (D145K) in mice

[00255] Four female Balb/c mice per group were injected intravenously with 10 ng of either the wild type human IL-1 α ₁₁₉₋₂₇₁ protein, the human IL-1 α ₁₁₉₋₂₇₁ (D145K) mutein, the wild type mouse IL-1 α ₁₁₅₋₂₇₀ protein, or the mouse IL-1 α ₁₁₅₋₂₇₀ (D145K) mutein. Three hours after injection serum amyloid A (SAA) was measured in serum of injected mice as readout of the bioactivity of the respective protein. As shown in Table 8 the mouse IL-1 α ₁₁₅₋₂₇₀ (D145K) mutein induced 53 % less SAA than the corresponding wild type mouse IL-1 α ₁₁₅₋₂₇₀ protein ($p < 0.05$, Student *t*-test) and the human IL-1 α ₁₁₉₋₂₇₁ (D145K) mutein induced 67% less SAA than the corresponding wild type human IL-1 α ₁₁₉₋₂₇₁ protein ($p < 0.001$ Student *t*-test). This demonstrates that both the human IL-1 α ₁₁₉₋₂₇₁ (D145K) mutein and the mouse IL-1 α ₁₁₅₋₂₇₀ (D145K) mutein have reduced bioactivity in mice when compared to their wild type counterparts.

Table 8: Biological activity of IL-1 α wild type proteins and muteins in mice determined by SAA.

Protein/mutein	Serum SAA concentration ($\mu\text{g/ml}$) 3 h after protein/mutein injection ($\pm\text{SD}$)
mouse IL-1 α ₁₁₅₋₂₇₀ (SEQ ID NO:202)	115 $\pm$ 32
mouse IL-1 α ₁₁₅₋₂₇₀ (D145K) (SEQ ID NO:204)	55 $\pm$ 10
human IL-1 α ₁₁₉₋₂₇₁ (SEQ ID NO:203)	92 $\pm$ 20
human IL-1 α ₁₁₉₋₂₇₁ (D145K) (SEQ ID NO:210)	31 $\pm$ 2

Table 9: Mouse IL-1 beta and Mouse IL-1 alpha muteins corresponding to preferred human IL-1 beta muteins (SEQ ID NO:131 to 140 and SEQ ID NO:205 to 209) and human IL-1 alpha muteins (SEQ ID NO:210 to 218) are created according to this table and tested in the mouse model of rheumatoid arthritis.

Human hIL-1beta 116-269 muteins	Amino acid changes introduced in mouse IL-1beta 119-269 (SEQ ID NO:164) in order to obtain the corresponding mutation
R4D (SEQ ID NO:131)	Exchange arginine at position 3 to aspartate
L6A (SEQ ID NO:132)	Exchange leucine at position 5 to alanine
T9G (SEQ ID NO:133)	Exchange arginine at position 8 to glycine
R11G (SEQ ID NO:134)	Exchange arginine at position 10 to glycine
D54R (SEQ ID NO:135)	Exchange aspartate at position 53 to arginine
D145K (SEQ ID NO:136)	Exchange aspartate at position 143 to lysine
$\Delta\text{EE}50,51$ (SEQ ID NO:137)	Delete glutamate, proline at positions 49,50
$\Delta\text{SND}52-54$ (SEQ ID NO:138)	Delete serine, asparagine, aspartate at positions 51 to 53
K63S/K65S (SEQ ID NO:139)	Exchange lysines at positions 62 and 64 to serines
Q126A/E128A (SEQ ID NO:140)	Exchange glutamine at position 125 to alanine and glutamate at position 127 to alanine
K88N (SEQ ID NO:205)	Exchange lysine at position 87 to asparagine
R98Q (SEQ ID NO:206)	Exchange arginine at position 97 to glutamine
K103L (SEQ ID NO:207)	Exchange lysine at position 102 to leucine
$\Delta\text{KKK}92-94$ (SEQ ID NO:208)	Delete lysine, lysine, lysine at positions 91 to 93
L10N (SEQ ID NO:209)	Exchange leucine at position 9 to asparagine

Human hIL-1alpha 119-271 mutants	Amino acid changes introduced in mouse IL-1alpha 115-270 (SEQ ID NO:202) in order to obtain corresponding mutation
D145K (SEQ ID NO:210)	Exchange aspartate at position 153 to lysine
L18K (SEQ ID NO:211)	Exchange methionine at position 25 to lysine
F146N (SEQ ID NO:212)	Exchange phenylalanine at position 154 to asparagine
R10A (SEQ ID NO:213)	Exchange lysine at position 17 to alanine
I62A (SEQ ID NO:214)	Exchange tyrosine at position 70 to alanine
W107F (SEQ ID NO:215)	Exchange tryptophane at position 115 to phenylalanine
D20V (SEQ ID NO:216)	Exchange aspartate at position 27 to valine
Δ FIL16-18 (SEQ ID NO:217)	Delete phenylalanine, valine, methionine at positions 23 to 25
Δ ITGS96-99 (SEQ ID NO:218)	Delete isoleucine, threonine, glycine, serine at positions 104 to 107

EXAMPLE 12

Amelioration of diet-induced type II diabetes in male C57BL/6 mice (prophylactic setting)

[00256] Male C57BL/6 mice were immunized on days 0, 14, and 28 with 50 μ g of either Q β , Q β -mIL-1 α ₁₁₅₋₂₇₀, Q β -mIL-1 β ₁₁₉₋₂₆₉ or a mixture of 50 μ g Q β -mIL-1 α ₁₁₅₋₂₇₀ and 50 μ g Q β -mIL-1 β ($n=16$ per group). All mice were fed normal rodent chow (Provimi Kliba no. 3436: 18.5 % protein, 4.5 % fat, 4.5 % fiber, 6.5 % ash, 54 % carbohydrates) during the immunization period. On day 35 this diet was replaced by a high fat diet (Provimi Kliba no. 2127: 23.9 % protein, 35 % fat, 4.9 % fiber, 5 % ash, 23.2 % carbohydrates) for half of the mice of each group ($n=8$) while the other half ($n=8$) was kept on normal chow. Five months after the last immunization mice fed the high-fat diet were obese (average body weight > 45 g) and showed elevated fasting glucose levels (Table 10, 0').

[00257] In order to investigate on the diabetic phenotype of these mice an oral glucose tolerance test was performed by administering a dose of 2mg/g body weight of D-glucose intragastrically and determining blood glucose levels at regular intervals using the Accu-check blood glucose meter (Roche). Table 10 shows that Q β -immunized mice on normal chow showed an initial peak of 291.5 mg/dl in blood glucose levels after 15 minutes which was followed by a sharp drop and a complete return to pre-challenge levels within 90 minutes. The peak levels and kinetics of this response were essentially identical in all mouse groups that had been maintained on normal chow (Table 10). Q β -immunized mice on high fat diet on the other hand peaked at higher levels (367.9 mg/dl) and failed to show a significant decline until 60 min post-challenge; only thereafter blood glucose levels started to decrease, without however returning to baseline levels within the 2 hour observation period. This severe

impairment in glucose clearance indicates that obese Q β -immunized mice had developed a diabetic phenotype. Obese Q β -mIL-1 α -, Q β -mIL-1 β -, or double immunized mice showed an initial increase in blood glucose levels to ~350 mg/dl, which was immediately followed by a sustained decline, resulting in glucose levels that were consistently lower than in obese Q β -immunized control mice. When calculating the area under the curves resulting from the repeated glucose measurements shown in table 10, it becomes evident, that obese Q β -mIL-1 α -, Q β -mIL-1 β -, or double immunized mice manifested an improved glucose clearance with respect to obese Q β -immunized control mice (Table 11). Taken together these data show that immunization with Q β -mIL-1 α or Q β -mIL-1 β or a combination of both resulted in a clear amelioration of the diet-induced diabetic phenotype.

Table 10: Blood glucose levels (mg/dl; mean $\pm$ SEM) before and at different time points after intragastric administration of 2mg/g glucose. (Mice were fasted for a period of 5 hours before the experiment)

Mouse group	0'	15'	30'	45'	60'	90'	120'
Q β high fat diet	194.3 $\pm$ 8.0	342.4 $\pm$ 20.9	367.9 $\pm$ 28.3	356.9 $\pm$ 29.3	350.4 $\pm$ 31.6	298.5 $\pm$ 31.9	250.3 $\pm$ 23.3
Q β -mIL-1 α ₁₁₅₋₂₇₀ high fat diet	195.6 $\pm$ 4.5	356.6 $\pm$ 7.4	324.3 $\pm$ 21.4	320.6 $\pm$ 26.9	303.3 $\pm$ 26.6	234.9 $\pm$ 19.6	208.6 $\pm$ 13.2
Q β -mIL-1 β ₁₁₉₋₂₆₉ high fat diet	200.1 $\pm$ 11.6	341.9 $\pm$ 11.6	348.3 $\pm$ 27.6	313.3 $\pm$ 31.2	297.6 $\pm$ 27.3	256.9 $\pm$ 22.6	234.7 $\pm$ 19.0
Q β -mIL-1 α ₁₁₅₋₂₇₀ /Q β -mIL-1 β ₁₁₉₋₂₆₉ high fat diet	190.6 $\pm$ 10.4	337.9 $\pm$ 27.1	309.9 $\pm$ 44.2	284.3 $\pm$ 47.6	281.6 $\pm$ 45.8	240.6 $\pm$ 40.8	221.0 $\pm$ 37.3
Q β normal chow	154.4 $\pm$ 3.5	291.5 $\pm$ 14.7	210.0 $\pm$ 10.0	193.6 $\pm$ 7.2	183.1 $\pm$ 6.2	149.5 $\pm$ 4.7	134.0 $\pm$ 4.2
Q β -mIL-1 α ₁₁₅₋₂₇₀ normal chow	171.6 $\pm$ 4.8	297.4 $\pm$ 12.6	230.4 $\pm$ 10.9	203.0 $\pm$ 11.4	192.6 $\pm$ 11.7	158.0 $\pm$ 8.5	136.4 $\pm$ 6.2
Q β -mIL-1 β ₁₁₉₋₂₆₉ normal chow	158.0 $\pm$ 3.6	312.3 $\pm$ 6.8	229.0 $\pm$ 10.8	188.3 $\pm$ 4.4	172.6 $\pm$ 4.9	145.8 $\pm$ 7.6	134.0 $\pm$ 3.8
Q β -mIL-1 α ₁₁₅₋₂₇₀ /Q β -mIL-1 β ₁₁₉₋₂₆₉ normal chow	152.4 $\pm$ 4.4	283.6 $\pm$ 8.4	220.3 $\pm$ 11.3	188.5 $\pm$ 11.1	187.8 $\pm$ 9.0	149.1 $\pm$ 8.6	131.5 $\pm$ 6.9

Table 11: Glucose clearance in immunized mice. The area under the curve (AUC) resulting from the consecutive glucose measurements represented in Table 10 was calculated for each individual mouse. Group means of AUC are expressed with SEM.

Mouse group	Normal chow	High fat diet
Q β	4120 $\pm$ 460	14746 $\pm$ 2262
Q β -mIL-1 α ₁₁₅₋₂₇₀	6104 $\pm$ 2313	10184 $\pm$ 1800
Q β -mIL-1 β ₁₁₉₋₂₆₉	4276 $\pm$ 419	10459 $\pm$ 1699
Q β -mIL-1 α ₁₁₅₋₂₇₀ /Q β -mIL-1 β ₁₁₉₋₂₆₉	4464 $\pm$ 531	9500 $\pm$ 3382

EXAMPLE 13

Amelioration of diet-induced type II diabetes in male C57BL/6 mice

[00258] The DNA sequence encoding amino acids 119-269 of mouse IL-1 β was amplified by PCR from cDNA of TNF α -activated murine macrophages using oligonucleotides IL1BETA-3 (5'-ATATATGATATCCCCATTAGACAGCTGCACTACAGG-3'; SEQ ID NO:226) and IL1BETA-2 5'-ATATATCTCGAGGGAAGACACAGATTCCATGGTGAAG-3'; SEQ ID NO:227) and cloned into the vector pET42T (EXAMPLE 7). The resulting plasmid pET42T-mIL-1 β ₁₁₉₋₂₆₉ encodes the mature mouse IL-1 β protein fused to a hexahistidine tag and a cysteine containing linker at the C-terminus. Due to the introduction of the EcoRV restriction site the valine residue at the N-terminus of mouse IL-1 β is substituted by a short N-terminal extension consisting of three amino acids (MDI). By site directed mutagenesis of the plasmid pET42T-mIL-1 β ₁₁₆₋₂₆₉, an expression vector was constructed which encodes the murine version of the human IL-1 β mutein hIL-1 β ₁₁₆₋₂₆₉ (D145K) (SEQ ID NO:136) with the C-terminal tag of SEQ ID NO:201, namely mIL-1 β ₁₁₆₋₂₆₉ (D145K) (SEQ ID NO:228). The mutation was introduced using the Quik-Change® Site directed mutagenesis kit (Stratagene) and the oligonucleotides D143K-1 (5'-CAGTGGTCAG GACATAATTA AATTCACCAT GGAATCTGTGTC-3'; SEQ-ID:229) and D143K-2 (5'-GACACAGATT CCATGGTGAA TTTAATTATG TCCTGACCACTG-3'; SEQ ID NO:230). Expression and purification of the mutein mIL-1 β ₁₁₆₋₂₆₉ (D145K) was performed as described in EXAMPLE 1 and coupling to Q β was performed as described in EXAMPLE 2.

[00259] Groups of male C57BL/6 mice (8 weeks of age, n=8) were immunized subcutaneously on days 0, 14, 28, 42, and 147 with 50 μ g of either Q β or Q β -mIL-1 β ₁₁₉₋₂₆₉ (D145K). Starting with day 0, one half of the mice (n = 16) was put on a high fat diet (Provimi Kliba no. 2127: 23.9 % protein, 35 % fat, 4.9 % fiber, 5 % ash, 23.2 % carbohydrates), while the other half (n = 16) was maintained on normal diet (Provimi Kliba

no. 3436: 18.5 % protein, 4.5 % fat, 4.5 % fiber, 6.5 % ash, 54 % carbohydrates) throughout the experiment. After eight months mice fed the high-fat diet were obese (average body weight > 45 g) and showed elevated fasting glucose levels (Table 12).

[00260] In order to investigate on the diabetic phenotype of the mice on high fat diet an oral glucose tolerance test was performed by administering a dose of 2 mg / g body weight of D-glucose intragastrically and determining blood glucose levels at regular intervals using the Accu-check blood glucose meter (Roche). Table 13 shows that Q β -immunized mice on high fat diet peaked at 318.5 mg/dl 30 minutes after injection and failed to show a significant decline until 60 min post-challenge; only thereafter blood glucose levels started to decrease, without however returning to baseline levels within the 2 hour observation period. This severe impairment in glucose clearance indicates that obese Q β -immunized mice had developed a diabetic phenotype. Obese Q β -mIL-1 β 119-269(D145K)-immunized mice showed an initial increase in blood glucose levels to 318.6 mg/dl, which was immediately followed by a sustained decline, resulting in glucose levels that were consistently lower than in obese Q β -immunized control mice. Two hours after challenge blood glucose levels had returned to pre-challenge levels in these mice. When calculating the area under the curves resulting from the repeated glucose measurements shown in Table 13, it becomes evident, that obese Q β -mIL-1 β 119-269 (D145K)-immunized mice manifested an improved glucose clearance with respect to obese Q β -immunized control mice (Table 14). Taken together these data show that immunization with Q β -mIL-1 β 119-269 (D145K) resulted in a clear amelioration of the diet-induced diabetic phenotype.

Table 12: Average body weights and fasting blood glucose levels after 5 hours fasting (means $\pm$ SEM).

	Average body weight (g)	Fasting blood glucose levels (mg/dl)
Q β high fat diet	47.16 $\pm$ 2.24	185.9 $\pm$ 6.3
Q β -mIL-1 β 119-269(D145K) high fat diet	51.08 $\pm$ 1.23	194.0 $\pm$ 4.2
Q β normal chow	36.95 $\pm$ 0.97	148.4 $\pm$ 6.5
Q β -mIL-1 β 119-269(D145K) normal chow	36.23 $\pm$ 1.30	147.0 $\pm$ 3.1

Table 13: Blood glucose levels (mg/dl; mean $\pm$ SEM) before and at different time points after intragastric administration of 2mg/g glucose. Mice were fasted for a period of 5 hours before the experiment.

Mouse group	0'	15'	30'	45'	60'	90'	120'
Q β	185.9 $\pm$	315.1 $\pm$	318.5 $\pm$	305.5 $\pm$	316.8 $\pm$	238.4 $\pm$	220.1 $\pm$
high fat diet	6.3	15.5	23.1	24.4	33.5	21.8	15.3
Q β -mIL-1 β ₁₁₉₋₂₆₉ (D145K)	194.0 $\pm$	318.6 $\pm$	290.0 $\pm$	278.6 $\pm$	280.1 $\pm$	218.4 $\pm$	199.4 $\pm$
high fat diet	4.2	18.3	15.7	12.5	10.4	7.8	7.5

Table 14: Glucose clearance in immunized mice. The area under the curve (AUC) resulting from the consecutive glucose measurements represented in Table 2 was calculated for each individual mouse. Group means of AUC are expressed with SEM. Peaks below baseline were excluded from the analysis.

Mouse group	AUC
Q β high fat	11060 $\pm$ 1895
Q β -mIL-1 β ₁₁₉₋₂₆₉ (D145K) high fat	7375 $\pm$ 539

CLAIMS

1. Use of 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 comprises 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, for the manufacture of a medicament for the treatment of diabetes in an animal.
2. The use of claim 1, wherein said diabetes is type II diabetes.
3. The use of claim 1 or 2, wherein said animal is a human.
4. The use of any one of claims 1 to 3, wherein said IL-1 molecule is selected from the group consisting of:
 - (a) IL-1 mutein;
 - (b) IL-1 protein;
 - (c) IL-1 mature fragment;
 - (d) IL-1 fragment; and
 - (e) IL-1 peptide.
5. The use of any one of claims 1 to 4, wherein said IL-1 molecule is an IL-1 beta molecule comprising 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 95 % identical with any one of SEQ ID NO:64, SEQ ID NO:165, or SEQ ID NO:164.

6. The use of any one of claims 1 to 4, wherein said IL-1 molecule is an IL-1 beta molecule comprising 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 99 % identical with any one of SEQ ID NO:64, SEQ ID NO:165, or SEQ ID NO:164.
7. The use of any one of claims 1 to 4, wherein said IL-1 molecule is an IL-1 beta molecule 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 95 % identical with any one of SEQ ID NO:64, SEQ ID NO:165, or SEQ ID NO:164.
8. The use of any one of claims 1 to 4, wherein said IL-1 molecule is an IL-1 beta molecule 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 99 % identical with any one of SEQ ID NO:64, SEQ ID NO:165, or SEQ ID NO:164.
9. The use of any one of claims 1 to 8, wherein said IL-1 molecule is an IL-1 beta mutein.

10. The use of claim 9, wherein said IL-1 beta mutein comprises a polypeptide, wherein the amino acid sequence of said polypeptide differs from the amino acid sequence of SEQ ID NO:64 in 1 to 4 amino acid residues.
11. The use of claim 9, wherein said IL-1 beta mutein consists of a polypeptide, wherein the amino acid sequence of said polypeptide differs from the amino acid sequence of SEQ ID NO:64 in 1 to 4 amino acid residues.
12. The use of claim 9, wherein said IL-1 beta mutein comprises a polypeptide, wherein the amino acid sequence of said polypeptide is selected from SEQ ID NO:131 to SEQ ID NO:140 and SEQ ID NO:205 to SEQ ID NO:209.
13. The use of claim 9, wherein said IL-1 beta mutein consists of a polypeptide, wherein the amino acid sequence of said polypeptide is selected from SEQ ID NO:131 to SEQ ID NO:140 and SEQ ID NO:205 to SEQ ID NO:209.
14. The use of claim 9, wherein said IL-1 beta mutein comprises a polypeptide, wherein the amino acid sequence of said polypeptide is SEQ ID NO:136.
15. The use of claim 9, wherein said IL-1 beta mutein consists of a polypeptide, wherein the amino acid sequence of said polypeptide is SEQ ID NO:136.
16. The use of any one of claims 1 to 15, wherein said at least one antigen with at least one second attachment site comprises:
 - (i) an IL-1 beta molecule, wherein said IL-1 beta molecule is SEQ ID NO:165 or

SEQ ID NO:136; and

- (ii) a linker, wherein said linker comprises said second attachment site, and wherein said linker comprises or preferably consists of GGC (SEQ ID NO:178) or GGCG (SEQ ID NO:188)

17. The use of any one of claims 1 to 15, wherein said at least one antigen with at least one second attachment site consists of:
 - (i) an IL-1 beta molecule, wherein said IL-1 beta molecule is SEQ ID NO:165 or SEQ ID NO:136; and
 - (ii) a linker, wherein said linker comprises said second attachment site, and wherein said linker comprises GGC (SEQ ID NO:178) or GGCG (SEQ ID NO:188).
18. The use of any one of claims 16 or 17, wherein said IL-1 beta molecule is SEQ ID NO:136.
19. The use of any one of claims 16 to 18, wherein said linker comprises GGCG (SEQ ID NO:188).
20. The use of any one of claims 16 to 18, wherein said linker consists of GGCG (SEQ ID NO:188).
21. The use of any one of claims 16 to 20, wherein said linker is covalently bound to the C-terminus of said IL-1 beta molecule by way of a peptide bond.
22. The use of any one of claims 1 to 21, wherein said at least one antigen with at least one second attachment site is any one of SEQ ID NOs 220 to 223.

23. The use of any one of claims 1 to 21, wherein said at least one antigen with at least one second attachment site is SEQ ID NO:220.
24. The use of any one of claims 1 to 4, wherein said IL-1 molecule is an IL-1 alpha molecule 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) human IL-1 alpha 119-271 (SEQ ID NO:203);
 - (c) mouse IL-1 alpha 117-270 (SEQ ID NO:163); 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:63, SEQ ID NO:163, or SEQ ID NO:203.
25. The use of any one of claims 1 to 4 or 24, wherein said IL-1 molecule is an IL-1 alpha mutein, wherein preferably said IL-1 alpha mutein comprises or preferably consists of a polypeptide, wherein the amino acid sequence of said polypeptide differs from the amino acid sequence of SEQ ID NO:63 in 1 to 4 amino acid residues, and wherein further preferably said IL-1 alpha mutein comprises or preferably consists of a polypeptide, wherein the amino acid sequence of said polypeptide is selected from SEQ ID NO:210 to SEQ ID NO:218, and wherein still further preferably said IL-1 alpha mutein comprises or preferably consists of a polypeptide, wherein the amino acid sequence of said polypeptide is SEQ ID NO:210.
26. The use of any one of claims 1 to 4, 24 or 25, wherein said at least one antigen with at least one second attachment site consists of
 - (i) an IL-1 alpha molecule, wherein said IL-1 alpha molecule is SEQ ID NO:203 or

SEQ ID NO:210, preferably SEQ ID NO:203; and

- (ii) a linker, wherein said linker comprises said second attachment site, and wherein said linker comprises or preferably consists of GGC (SEQ ID NO:178) or GGCG (SEQ ID NO:188), preferably GGCG (SEQ ID NO:188);

wherein preferably said linker is covalently bound to the C-terminus of said IL-1 alpha molecule by way of a peptide bond.

- 27. The use of any one of claims 1 to 4, or 24 to 26, wherein said at least one antigen with at least one second attachment site is any one of SEQ ID NOs 224 or 225, and wherein preferably said at least one antigen with at least one second attachment site is SEQ ID NO:224.
- 28. The use of any one of the preceding claims, wherein said virus-like particle is a virus-like particle of an RNA bacteriophage.
- 29. The use of claim 28 wherein said RNA bacteriophage is selected from the group consisting of:
 - (a) bacteriophage Q β ;
 - (b) bacteriophage AP205;
 - (c) bacteriophage fr; and
 - (d) bacteriophage GA.
- 30. The use of claim 28, wherein said RNA bacteriophage is bacteriophage Q β .
- 31. The use of any one of the preceding claims, wherein said virus-like particle comprises recombinant coat proteins, mutants or fragments thereof, of an RNA bacteriophage.

32. The use of any one of the claims 1 to 30, wherein said virus-like particle comprises recombinant coat proteins of an RNA bacteriophage.
33. The use of any one of the claims 1 to 30, wherein said virus-like particle essentially consists of recombinant coat proteins of an RNA bacteriophage.
34. The use of any one of the claims 1 to 30, wherein said virus-like particle consists of recombinant coat proteins of an RNA bacteriophage.
35. The use of any one of the preceding claims, wherein said RNA bacteriophage is selected from the group consisting of:
 - (a) bacteriophage Q β ;
 - (b) bacteriophage AP205;
 - (c) bacteriophage fr; and
 - (d) bacteriophage GA.
36. The use of any one of the preceding claims, wherein said recombinant coat proteins comprise SEQ ID NO:3.
37. The use of any one of the preceding claims, wherein said recombinant coat proteins consist of SEQ ID NO:3.
38. The use of any one of the preceding claims, wherein said first attachment site is linked to said second attachment site via at least one covalent bond.

39. The use of claim 38, wherein said at least one covalent bond is a non-peptide bond.
40. The use of any one of the preceding claims, wherein said first attachment site comprises, or preferably is, an amino group, preferably an amino group of a lysine.
41. The use of any one of the preceding claims, wherein said second attachment site comprises, or preferably is, a sulfhydryl group, preferably a sulfhydryl group of a cysteine.
42. The use of any one of the preceding claims, wherein said first attachment is an amino group and wherein said second attachment site is a sulfhydryl group.
43. The use of any of the preceding claims, wherein said first attachment is an amino group of a lysine, and said second attachment site is a sulfhydryl group of a cysteine.
44. The use of any of the preceding claims, wherein said first attachment site is not a sulfhydryl group, or wherein said linkage of said virus-like particle and said at least one antigen, does not comprise a disulfide bond.
45. The use of any of the preceding claims, 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 antigen to said virus-like particle, wherein said only one second attachment site that associates with said first attachment site is a sulfhydryl group, and wherein said antigen and said virus-like particle interact through said association to form an ordered and repetitive antigen array.
46. The use of any of claims 1 to 37, wherein said first attachment site is linked to said second attachment site via at least one covalent bond, wherein said covalent bond is a peptide bond; and wherein preferably said virus-like particle comprises,

essentially consists of, or alternatively consists of, recombinant coat proteins, mutants or fragments thereof, of an RNA bacteriophage, and wherein said at least one antigen is fused to the N- or the C- terminus of said recombinant coat proteins, mutants or fragments thereof.

47. The use of claim 46, wherein said RNA bacteriophage is selected from the group consisting of:
 - (a) bacteriophage AP205;

- (b) bacteriophage fr; and
 - (c) bacteriophage GA,
- and wherein preferably said RNA bacteriophage is bacteriophage AP205.

- 48. The use of any of the preceding claim, wherein said at least one antigen with said at least one second attachment site further comprises a linker, wherein said linker comprises said second attachment site, and wherein said linker is associated to said antigen by way of one peptide bond.
- 49. Use of a vaccine comprising the composition of any one of the preceding claims for the manufacture of a medicament for the treatment of diabetes in an animal.
- 50. The use of claim 49, wherein said diabetes is type II diabetes.
- 51. The use of claim 49 or 50, wherein said animal is a human.
- 52. The use of any one of claims 49 to 51, wherein said vaccine consists of the composition of any one of claims 1 to 48.
- 53. The use of any one of claims 49 to 52, wherein said composition is present in an effective amount.
- 54. The use of any one of claims 49 to 53, comprising
 - (i) a first composition, preferably in an effective amount, wherein said first composition is a composition according to any one of claims 1 to 48, and wherein the IL-1 molecule comprised by said first composition is an IL-1 beta molecule, preferably SEQ ID NO:136 or SEQ ID NO:165; and

- (ii) a second composition, preferably in an effective amount, wherein said second composition is a composition according to any one of claims 1 to 48, and wherein the IL-1 molecule comprised by said second composition is an IL-1 alpha molecule, preferably SEQ ID NO:203 or SEQ ID NO:210.

55. The use of any one of claims 49 to 54, wherein said vaccine is devoid of an adjuvant.

56. Use of a pharmaceutical composition comprising

- (a) the composition of any of claims 1 to 48 or the vaccine of any one of claims 49 to 55; and

- (b) a pharmaceutically acceptable carrier

for the manufacture of a medicament for the treatment of type II diabetes in a human.

SEQUENCE LISTING

SEQ ID NO:1 HBC

MDIDPYKEFG	ATVELLSFLP	SDFFPSSVRDL	LDTASALYRE	ALESPEHCSP	HHTALRQAIL	60
CWGELMTLAT	WVGNNLEDEA	SRDLVVNYVN	TNMGLKIRQL	LWFHISCLTF	GRETVLEYLV	120
SFGVWIRTPP	AYRPPNAPIL	STLPETTIVR	RRDRCSRPRR	RTPSPRRRRS	QSPRRRRRSQS	180
RESQC						185

SEQ ID NO:2 HBC mutant

MDIDPYKEFG	ATVELLSFLP	SDFFPSSVRDL	LDTAAALYRD	ALESPEHCSP	HHTALRQAIL	60
CWGDIMTLAT	WVGNNLEDEG	KGGSRLVVS	YVNTNVGLKF	RQLLWFHISC	LTFGRETIVLE	120
YLVSEGVWIR	TPPAYRPPNA	PILSTLPETT	VVRRDRGRS	PRRRTSPRRR	RRSQSPRRRR	180
SQSRESQC						188

SEQ ID NO:3 Qbeta CP

AKLETVTLGN	IGKDGKQTLV	LNPRGVNPTN	GVASLSQAGA	VPALRKRVT	SVSQPSRNRK	60
NYKVQVKIQN	PTACTANGSC	DPSVTRQAYA	DVTFSFTQYS	TDEERAFVRT	ELAALLASPL	120
LIDAIDQLNP	AY					132

SEQ ID NO:4 Qbeta A1

MAKLETVTLG	NIGKDGKQTL	VLNPRGVNPT	NGVASLSQAG	AVPALEKRVT	VSVSQPSRNR	60
KNYKVQVKIQ	NPTACTANGS	CDPSVTRQAY	ADVTFSFTQY	STDEERAFVR	TELAALLASP	120
LLIDAIDQLN	PAYWTLIIAG	GGSGSKPDV	IPDPIDPPP	GTGKYTCPFA	IWSLEEVYEP	180
PTKNRPWPIY	NAVELQPREF	DVALKDLLGN	TKWRDWDSSL	SYTTFRGCRG	NGYIDLDATY	240
LATDQAMRDQ	KYDIREGKKP	GAFGNIERFI	YLKSIINAYCS	LSDIAAYHAD	GVIVGFWRDP	300
SSGGAIPFDF	TKFDKTKCFI	QAVIVVPRA				329

SEQ ID NO:5 R17

ASNFTQFVLV	NDGGTGNVTV	APSNFANGVA	EWISSNSRSQ	AYKVTCSVRQ	SSAQNRKYTI	60
KVEVPKVATQ	TVGGVELPVA	AWRSYLMEL	TIPIFATNSD	CELIVKAMQG	LLKDGNIPIPS	120
ATAANSIGIY						129

SEQ ID NO:6 fr

MASNFEFVLV	VDNGGTGDKV	VAPSNFANGV	AZWISSNSRS	QAYKVTCSVR	QSSANNRKYT	60
VKVEVPKVAT	QVGGVELPV	AAWRSYMNME	LTIPVFATND	DCALIVKALQ	GTFTKGNPIA	120
TAIAANSIGIY						130

SEQ ID NO:7 GA

MATLRSFVLV	DNGGTGNVTV	VPVSNANGVA	EWSNNSRSQ	AYRVTASYRA	SGADKRKYAI	60
KLEVPKIVTQ	VVNGVELPGS	AWKAYASIDL	TIPIFAATDD	VTVISKSLAG	LFKVGNIPIAE	120
AISSQSGFYA						130

SEQ ID NO:8 SP

MAKLNQVTLS	KIGKNGDQTL	TLTPRGVNPT	NGVASLSEAG	AVPALEKRVT	VSVAQPSRNR	60
KNFKVQIKLQ	NPTACTRDAC	DPSVTRSAFA	DVTLSFTSYS	TDEERALIRT	ELAALLADPL	120
IVDAIDNLNP	AY					132

SEQ ID NO:9 SP A1

AKLNQVTLSK	ICKNGDQTLT	LTPRGVNPTN	GVASLSEAGA	VPALRKRVT	SVAQPSRNRK	60
NFKVQIKLQN	PTACTRDACD	PSVTRSAFAD	VTLSFTSYST	DEERALIRTE	LAALLADPLI	120
VDAIDNLNPA	YWAALLVASS	GGGDNPSDPD	VPVVPDVKPP	DGTGRYKCPF	ACYRLGSIYE	180
VGKEGSPDIY	ERGDEVSVTF	DYALEDFLGN	TNWRNWDQRL	SDYDIANRRR	CRNGYIDLD	240
ATAMQSDDFV	LSGRYGVKRV	KFPGAFGSIK	YLLNIQGDW	LDLSEVTAYR	SYGMVIGFWT	300
DSKSPQLPTD	FTQNSANCP	VQTVIITPS				329

SEQ ID NO:10 MS2

MASNFTQFVL	VDNGGTGDKV	VAPSNFANGV	AZWISSNSRS	QAYKVTCSVR	QSSAQNRKYT	60
IKVEVPKVAT	QTVGGVELPV	AAWRSYLNME	LTIPVFATNS	DCELIVKAMQ	GLLKDGNIPI	120
SAIAANSIGIY						130

SEQ ID NO:11 M11

MAKLQAITLS	GIGKNGDVTL	DLNPRGVNPT	NGVAALSEAG	AVPALEKRVT	ISVSQPSRNR	60
KNYKVQVKIQ	NPTSCTASGT	CDPSVTRSAY	SDVTFSFTQY	STVEERALVR	TELQALLADP	120
MLVNAIDNLN	PAY					133

SEQ ID NO:12 MX1

MAKLQAITLS	GIGKNGDVTL	NLNPRGVNPT	NGVAALSEAG	AVPALEKRVT	ISVSQPSRNR	60
KNYKVQVKIQ	NPTSCTASGT	CDPSVTRSAY	ADVTFSFTQY	STDEERALVR	TELKALLADP	120
MLIDAIDNLN	PAY					133

SEQ ID NO:13 NL95

MAKLNKVTLT	GIGKAGNQTL	TLTPRGVNPT	NGVASLSEAG	AVPALEKRV	VSVAQPSRNR	60
KNYKVQIKLQ	NPTACTKDAC	DPSVTRSGSR	DVTLSTTSYS	TERERALIRT	ELAALLKDDL	120
IVDAIDNLNP	AYWAALLAAS	PGGGNNPYPG	VPDSPNVKPP	GGTGTYRCP	ACYRRGELIT	180
EAKDGACALY	ACGSEALVEF	EYALEDFLGN	EFWRNWDGRL	SKYDIETHRR	CRGNGYVDLD	240
ASVMQSDDEYV	LSGAYDVVKM	QPPGTEDSPR	YYLHLMGDIY	VDLAEVTAIR	SYGMVIGFWT	300
DSKSPQLPTD	FTRFNHNCP	VQTVIVIPSL				330

SEQ ID NO:14 f2

ASNFTQFVLV	NDGGTGNVT	APSNFANGVA	EWISSNSRSQ	AYKVTCSVRQ	SSAQNRYTI	60
KVEVPKVATQ	TVGGVELPVA	AWRSYLNLEL	TIPIFATNSD	CELIVKAMQG	LLKDGNIPI	120
AIAANSGLY						129

SEQ ID NO:15 PP7

MSKTIVLSVG	EATRTLTEIQ	STADRQIFEE	KVGPLVGRLR	LTASLRQNGA	KTAYRVNLKL	60
DQADVVDCT	SVCGELPKVR	YTQVWSDVT	IVANSTEASR	KSLYDLTKSL	VATSQVEDLV	120
VNLVPLGR						128

SEQ ID NO:16 Qbeta-240

AKLETVTLGN	IGRDGKQTLV	LNPRGVNPTN	GVASLSQAGA	VPALAKRVTV	SVSQPSRNRK	60
NYKVQVKIQN	PTACTANGSC	DPSVTRQKYA	DVTFSTQYS	TDEERAFVRT	ELAALLASPL	120
LIDAIDQLNP	AY					132

SEQ ID NO:17 Qbeta-243

AKLETVTLGN	IGRDGKQTLV	LNPRGVNPTN	GVASLSQAGA	VPALAKRVTV	SVSQPSRNRK	60
NYKVQVKIQN	PTACTANGSC	DPSVTRQKYA	DVTFSTQYS	TDEERAFVRT	ELAALLASPL	120
LIDAIDQLNP	AY					132

SEQ ID NO:18 Qbeta-250

ARLETVTLGN	IGRDGKQTLV	LNPRGVNPTN	GVASLSQAGA	VPALAKRVTV	SVSQPSRNRK	60
NYKVQVKIQN	PTACTANGSC	DPSVTRQKYA	DVTFSTQYS	TDEERAFVRT	ELAALLASPL	120
LIDAIDQLNP	AY					132

SEQ ID NO:19 Qbeta-251

ARLETVTLGN	IGRDGKQTLV	LNPRGVNPTN	GVASLSQAGA	VPALAKRVTV	SVSQPSRNRK	60
NYKVQVKIQN	PTACTANGSC	DPSVTRQKYA	DVTFSTQYS	TDEERAFVRT	ELAALLASPL	120
LIDAIDQLNP	AY					132

SEQ ID NO:20 Qbeta-259

ARLETVTLGN	IGRDGKQTLV	LNPRGVNPTN	GVASLSQAGA	VPALAKRVTV	SVSQPSRNRK	60
NYKVQVKIQN	PTACTANGSC	DPSVTRQKYA	DVTFSTQYS	TDEERAFVRT	ELAALLASPL	120
LIDAIDQLNP	AY					132

SEQ ID NO:21 AP205

MANKPMQPI	TANKIIVSD	PTLSTTFSA	SLLRQVRKVG	IAELNNVSGQ	YVSVYKRPAP	60
KPEGCADACV	IMPENQSR	TVISGSAENL	ATLKAEWETH	KRNVDTLFAS	GNAGLGFLDP	120
TAAIVSSDTT	A					131

SEQ ID NO:22 AP205 P5T

MANKPMQPI	TANKIIVSD	PTLSTTFSA	SLLRQVRKVG	IAELNNVSGQ	YVSVYKRPAP	60
KPEGCADACV	IMPENQSR	TVISGSAENL	ATLKAEWETH	KRNVDTLFAS	GNAGLGFLDP	120
TAAIVSSDTT	A					131

SEQ ID NO:23 AP205 N14D

MANKPMQPI	STADKIVSD	PTLSTTFSA	SLLRQVRKVG	IAELNNVSGQ	YVSVYKRPAP	60
KPEGCADACV	IMPENQSR	TVISGSAENL	ATLKAEWETH	KRNVDTLFAS	GNAGLGFLDP	120
TAAIVSSDTT	A					131

SEQ ID NO:24 IL1alpha1

atatatgcta	gccccctaca	cctaccagag	tgatttg			37
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SEQ ID NO:25 IL1alpha2

atatatctcg	agtgatatct	ggaagtctgt	catagag			37
------------	------------	------------	---------	--	--	----

SEQ ID NO:26 ILbeta1

atatatgcta	gccccctatta	gacagctgca	ctacagg			37
------------	-------------	------------	---------	--	--	----

SEQ ID NO:27 ILbeta2

atatatctcg	agggaagaca	cagattccat	ggtgaag			37
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SEQ ID NO:28 cystein containing linker

GGGGGCG

7

SEQ ID NO:29 pModEC1

acatcgtata	acgtttactgg	tttcacattc	accacccctga	attgactctc	ttccggggcgc	60
tatcatgcc	taccgcgaaa	ggttttgocg	cattcgatgg	tgctccgggat	ctcgacgctc	120
tccttatgc	gactcctgca	ttaggaagca	gccagtagt	aggttgaggc	cgttgagcac	180
cgccgcgca	aggaatggtg	catgcaagga	gatggcgccc	aacagtcccc	cggccacggg	240
gcttgccacc	atacccaagc	cgaacaagc	gctcatgagc	cogaagtggc	gagcccgatc	300
ttcccatcg	gtgatgtcgg	cgatataggg	gccagcaacc	gcacctgtgg	cgcgggtgat	360
gcgggcccag	atgcgtccgg	cgtagaggat	cgagatctcg	alcccgcgaa	attaatacga	420
ctcactatag	gggaattgtg	agcggataac	aattccccctc	tagaaataat	tttgtttaac	480
tttaagaagg	agatatacat	atggatccac	accaccacca	ccaccacggg	tctggtgacg	540
acgatgacaa	agcgtatagc	ctcaggggtg	gtggtggtgg	ttgcggttaa	taagtttaaa	600
cgcgcccgca	tgaccaccca	ccaccaccac	tgagatccgg	ctgctaacaa	agcccgaag	660
gaagctgagt	tggctgctgc	caccgctgag	caataactag	cataaccctt	tggggcctct	720
aaacgggtct	tgaggggttt	tttgctgaaa	ggaggaacta	tatccggatt	ggcgaatggg	780
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caatgatgag	caacttttaa	gttctgctat	gtggcgcggt	attatcccg	attgacgcgc	1620
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taaccatgag	tgataaacct	gcggccaact	tacttctgac	aacgatcgga	ggaccgaagg	1800
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						4200

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ggcatactct ggc 5473

```

SEQ ID NO:30 primerMCS-1F

```
tatggatccg gctagcgctc gagggtttaa acggcgcccg cat 43
```

SEQ ID NO:31 primerMCS-1R

```
tcgaatgcgg ccgcggttta aaccctcgag cgctagccgg atcca 45
```

SEQ ID NO:32 Bamhis6-EK-Nhe-F

```
gatccacacc accaccacca ccacggttct ggtgacgacg atgacaaagc gctagccc 58
```

SEQ ID NO:33 Bamhis6-EK-Nhe-R

```
tcgagggeta ggcctttgtc atcgctgtca ccagaaccgt ggtgggtgtg gtggtgtg 58
```

SEQ ID NO:34 oligo1F-C-glycine-linker

```
tcgagggtgg tgggtgtgtg tgccgttaat aagtttaaac gc 42
```

SEQ ID NO:35 oligo1R-C-glycine-linker

```
ggccgcggtt aaacttatta accgcaacca ccaccaccac cc 42
```

SEQ ID NO:36 HIL-1 alpha

```

MAKVPDMFED LKNCYSENEE DSSSIDHLSL NQKSFYHVSY GPLHEGCMQ SVSLSISETS 60
KTSKLTFKES MVVVATNGKV LKKRRLSLSQ SITDDLEAI ANDSEEEIIR PRSSPFSFSL 120
NVKYNFMRII KYEFILNDAL NQSIIRANDQ YLTAAALHNL DEAVKFDMDA YKSSKDDAKI 180
TVILRISKTO LYVTAQDEDEQ PVLLKEMPEI PKTITGSETN LFFWETHGT KNYFTSVAHP 240
NLFIATKQDY WVCLAGGPPS ITDFQILENQ A 271

```

SEQ ID NO:37 IL-1 alpha Macaca

```

MAKVPDMFED LKNCYSENEE DSSSIDHLSL NQKSFYDVSY GPLHEGCMQ SVSLSISEIS 60
KTSKLTFKQS MVVVSTNGKV LKKRRLSLSQ SITDNNLEAI ANDSEEEIIR PRSAPFSFSL 120
NMTYHFIRII KHEFILNDAL NQTIIRANDQ HLTAAAIHNL DEAVKFDMDA YTSSKDDTKV 180
PVILRISKTO LYVSAQDEDEQ PVLLKEMPEI NKTITGSETN LFFWETHGT KNYFISVAHP 240
NLFIATKHON WVCLAKGLPS ITDFQILENQ A 271

```

SEQ ID NO:38 IL-1 alpha Equus

```

MAKVPDLFED LKNCYSENEE YSSEIDHLSL TQKSFYDASY DPLPEDCMDT FMSLTSETS 60
KTSKLNFKES VVLVAANGKI LKKRRLSLNQ FITDDLEAI ANDPEEGIIR PRSVHYNFQS 120
NTKYNFMRII NHQCTLDAL NQSVIRDTSG QYLATAALNN LDDAVKFDMDG AYTSSEDSQL 180
PVTLRISKTR LFVSAQNEDE PVLLKEMPDT PKTIKDETNL LFFWERHGSK NYFKSVAHPK 240
LFIATKQGKL VHMARGQPSI TDFQILDNQF 270

```

SEQ ID NO:39 IL-1 alpha Ovis

```

MAKVPDLFED LKNCYSENEE YSSEIDHLSL NQKSFYDASY EPLREDEMKN FMSLDTSETS 60
KTSRLSFKEN VVMVTANGKI LKKRRLSLNQ FITDDLEAI ANDTEEEIIR PRSAHYSFQS 120
NVKYNFMRII HQECILNDAL NQSIIRDMG PYLTAATLNN LEEAVKFDMDV AYVSEEDSQL 180
PVTLRISKTO LFVSAQNEDE PVLLKEMPET PKIIKDETNL LFFWEKBSM DYFKSVAHPK 240
LFIATKQEKI VHMASGPPSI TDFQILEK 268

```

SEQ ID NO:40 IL-1 alpha Felis

```
MAKVPDLFED LKNCYSENEE YSSEIDHLSL NQKSFYDASY DPLHEDCTDK FMSPTSETS 60
```

KTPQLTLKKS	VVMVAANGKI	LKKRRLSLNQ	FLTADDLEAI	ANEVEERIMK	PRSVAPNFYS	120
SEKYNQKII	KSQFILNDNL	SQSVIRKAGG	KYLAALALQN	LDDAVKFDMG	AYTSKEDSKL	180
PVTLRISKTR	LFVSAQNEDE	PVLLKEMPET	PKTIRDETNL	LFFWERHGSK	NYFKSVAHPK	240
LFIATQEEQL	VHMARGLPVS	TDFQILETQS				270

SEQ ID NO:41 IL-1 alpha Bos

MAKVPDLFED	LKNCYSENEE	YSSEIDHLSL	NQKSFYDASY	EPLREDQMKN	FMSLDTSETS	60
KTSKLSFKEN	VVMVAASGKI	LKKRRLSLNQ	FITDDDLAI	ANNTTEELIK	PRSAHYSFQS	120
NVKYNFMRVI	HQECILNDAL	NQSIIRDMSG	PYLTTATLNN	LEEAVKFDMV	AYVSEEDSQL	180
PVTLRISKTR	LFVSAQNEDE	PVLLKEMPET	PKIIKDETNL	LFFWEKHGSM	DYFKSVAHPK	240
LFIATQEKEL	VHMASGPPSI	TDFQILEK				268

SEQ ID NO:42 IL-1 alpha Sus

MAKVPDLFED	LKNCYSENEE	YSSDIDHLSL	NQKSFYDASY	EPLPGDGMK	FMPLSTSKTS	60
KTSRLNFKDS	VVMAAANGKI	LKKRRLSLNQ	FITDDDLAI	ANDTEELIK	PRSATYSFQS	120
NMKYNFMRVI	NHQCILNDAR	NQSIIRDPSG	QYLMAAVLNN	LDEAVKFDMA	AYTSNDDSQL	180
PVTLRISETR	LFVSAQNEDE	PVLLKELPET	PKTIKDETSL	LFFWEKHGSM	DYFKSAAHPK	240
LFIATQEKEL	VHMAPGLPSV	TDFQILENQS				270

SEQ ID NO:43 IL-1 alpha Oryctolagus

MAKVPDLFED	LKNCFSENEE	YSSAIDHLSL	NQKSFYDASY	EPLHEDCMNK	VVSLSTSETS	60
VSPNLTFQEN	VVAVTASGKI	LKKRRLSLNQ	PITDVLDTN	VSDPEEGIIK	PRSVPYTFQR	120
NMRYKYLRII	KQETLNDAL	NQSLVRDTS	QYLRAAPLQN	LGDVAVKFDMG	VYMTSKEDSI	180
LPVTLRISQT	PLFVSAQNEDE	EPVLLKEMPE	TPRIITDSES	DILFFWETQG	NKNYFKSAAN	240
PQLFIATKPE	HLVHMARGLP	SMTDFQIS				268

SEQ ID NO:44 IL-1 alpha Canis

MAKVPDLFED	LKNCYSENEE	YSSEIDHLSL	NQKSFYDMSC	DPLHEDCMNL	STSEISKTSQ	60
LTFKENVVVV	AANGKILKKR	RLSLSQFITD	DDLEGIANOT	EEVIMKPRSV	AYNFHNNEKY	120
NYTRITKSQF	TINDNINQST	VRQTGGNYLM	TAATQNLDDA	VKFDMGAYTS	FDSKLPVTLR	180
ISKTRLFVSA	QNEDEPVLLK	EMPETPKTIR	DETNLFFWE	RHGSKHYFKS	VAQPKLFIAT	240
QERKLVMAR	GQPSITDFRL	LETQP				265

SEQ ID NO:45 IL-1 alpha Mus

MAKVPDLFED	LKNCYSENEE	YSSAIDHLSL	NQKSFYDASY	GSLHETCTDQ	FVSLRTSETS	60
KMSNFTFKES	RVTVSATSSN	GKILKKRRLS	FSETFTEDDL	QSITHLEET	IQPRSAPTY	120
QSDLRKXLMK	LVRQKFVMDN	SLNQTIYQDV	DKHYLSTTWL	NDLQEVKFD	MYAYSSGGDD	180
SKYPVTLKIS	DSQLFVSAQG	EDQPVLLKEE	PETPKLITGS	ETDLIFFWKS	INSKNYFTSA	240
AYPELFIATK	EQSRVHLARG	LPSMTDFQIS				270

SEQ ID NO:46 IL-1 alpha Rattus

MAKVPDLFED	LKNCYSENEE	YSSAIDHLSL	NQKSFYDASY	GSLHENCTDK	FVSLRTSETS	60
KMSTFTFKES	RVVVSATSNK	GKILKKRRLS	FNQPTEDDL	EATAHLEET	IQPRSAHSGF	120
QNNLRKLIIR	IVKQEFIMND	SLNQNIYVDM	DRIHLKAASL	NDLQLEVKFD	MYAYSSGGDD	180
SKYPVTLKVS	NTQLFVSAQG	EDKPVLLKEI	PETPKLITGS	ETDLIFFWEK	INSKNYFTSA	240
APPELLIATK	EQSQVHLARG	LPSMIDFQIS				270

SEQ ID NO:47 IL-1 alpha Gorilla

KNCYSENEED	SSSIDHLSLN	QKSFYHVTYG	PLHEGCMQDS	VSLSISETSK	TSKLTFKESM	60
VVVATNGKVL	KKRRLSLSQS	ITDODLEAIA	NDSEEEIIPK	RSAPFSFLSN	VKYNFMRIIK	120
NEFILNDALN	QSIIRANDQY	LTAAALHNLD	EA			152

SEQ ID NO:48 hIL-1 alpha Cavia

FEDLKNCYSE	NEEYASAIH	LSLNQKSFYD	TNYDPLHENR	VDEPVSPNPY	ENSEESNFTL	60
EDSSSSSAVV	LTSABGEVLK	KRRLSLNQTM	SNEDLEAIAN	DSEEEIIEPW	SVPSYFQSNL	120
KFKYQRSIKK	GAVITDAMEQ	SLIRESNGQH	LKAMHVVDRK	HEVKFDIDGY	VSTATRIRPV	180
TLKISKTQLY	VCAQEEGQPV	LLKE				204

SEQ ID NO:49 hIL-1 beta

MAEVPDLASE	MMAYYSGNED	DLFFEADGPK	QMKCSFQDLB	LCPLDGGIQL	RISDHHYSKG	60
FRQAASVVVA	MDKLRRMLVP	CPQTFQBNDL	STFFPFIFEE	EPIFFDTWDN	EAYVHDAPVR	120
SLNCTLRDSQ	QKSLVMSGPY	ELKALHLQGG	DMEQQVVFSM	SVFQGEESND	KIPVALGLKE	180
KNLYLSCVLK	DDKPTLQLES	VDPKNYPKKK	MEKRFVFNKI	EINNKLFEFS	AQFPNWIIST	240
SQAENMPVFL	GGTKGGQDIT	DFTMQFVSS				269

SEQ ID NO:50 IL-1 beta Pan

MLVPCPQTFQ	ENDLSTFFPF	IFEEPIFFD	TWENEAYVHD	APVRSINCTL	RDSQKSLVM	60
SGPYELKALH	LQGDMEQQV	VFSMSFVQGE	ESNDKIPVAL	GLKEKNLYLS	CVLKDDKPTL	120
QLESVDPKNY	PKKMEKRFV	FNKIEINNKL	EFESAQFPNW	YISTSQAENM	PVFLGGTKGG	180
QDITDFTMQF	VSS					193

SEQ ID NO:51 IL-1 beta Macaca

MAEVPELASE	MMAYYSGNED	DLFFDVDPK	QMKCSFQDL	LCPLGGGIQL	QISHEHYNEG	60
FRQAVSVVVA	MEKLRKMLVP	CPQIFQDNDL	STLIPFIFEE	EPVFLDTRNN	DACVHDAPVR	120
SLBCTLRDAQ	LKSLVMSGPY	ELKALHLQGG	DLEQQVVFMS	SFVQGEESND	KIPVALGLKA	180
KNLYLSCVLK	DDKPTLQLES	VDPKNYPKKK	MEKRFVFNKI	EINNKEFES	AQFPNWIIST	240
SQAENMPVFL	GGTRGGQDIT	DFTMQFVSS				269

SEQ ID NO:52 IL-1 beta Oryctolagus

MATVPELTSE	MMAYHSGNEN	DLFFEADGPN	YMKSCFQDL	LCCPDEGIQL	RISCQPYNKS	60
FRQVLSVVVA	LEKLRQKAVP	CPQAFQDDGL	RTFFSLIFEE	BPVLCNTWDD	YSLECDAVRS	120
LHCRILQDAQ	KSLVLSGTYE	LKALHLNAEN	LNQQVVFMS	FVQGEESNDK	IPVALGLRGK	180
NLYLSCVMKD	DKPTLQLESV	DPNRYPKKKM	EKRPFVFNKI	IKDKLEFESA	QFPNWIISTS	240
QTEYMPVFLG	NNSGGQDLID	FSMEFVSS				268

SEQ ID NO:53 IL-1 beta Mus

MATVPELNCE	MPPFDSDEND	LFEEVDGPQK	MKGCFQTFDL	GCPDESIQLQ	ISQQHINKSF	60
RQAVSLIVAV	EKLWQLPVSE	PWTFQEDDMS	TFFSFIFEEE	PILCDSWDDD	DNLLVCDVPI	120
RQLHYRLRDE	QKSLVLSDP	YELKALHLNG	QNINQQVIFS	MSFVQGEPSN	DKIPVALGLK	180
GKNLYLSCVM	KDGTPTLQLE	SVDPKQYPKK	KMEKRFVFNK	TEVKSKEVEF	SAEFPNWIIS	240
TSQAEBKPVF	LGNNSGQDII	DFTMESVSS				269

SEQ ID NO:54 IL-1 beta Rattus

MATVPELNCE	IAAFDSEEND	LFEEADRPQK	IKDCFQALDL	GCPDESIQLQ	ISQQHLDKSF	60
RKAVSLIVAV	EKLWQLPMSC	PWSFQDEDDPS	TFFSFIFEEE	PVLCDSWDDD	DLVCDVPIR	120
QLBCRLRDEQ	QKCLVLSDFC	ELKALHLNGQ	NISQQVVFMS	SFVQGETSND	KIPVALGLKG	180
LNLYLSCVMK	DGTPTLQLES	VDPKQYPKKK	MEKRFVFNKI	EVKTKVEFES	AQFPNWIIST	240
SQAEBRPVFL	GNSNGRDIVD	FTMEPVSS				268

SEQ ID NO:55 IL-1 beta Equus

MAAVPETSOM	MTYCSCNEND	LFEEEDGPKQ	MKGSFQDLDL	SSMCNCGIQL	QFSHQLYNKT	60
VKHVSVIIVA	MEKLKKIPVP	CSQAFQDDDL	RSLFSVIFEE	EPICDNWDD	DYVCDAAVHS	120
VNCRLRDIYH	KSLVLSGACE	LQAVHLNGEN	TNQQVVFMS	FVQGEETDK	IPVALGLKEK	180
NLYLSCGMKD	GKPTLQLETV	DPNTYPKRKM	EKRPFVFNKME	IKGNVEFESA	MYPNWIISTS	240
QAEKKPVFLG	NTRGGRDITD	FIMEITSA				268

SEQ ID NO:56 IL-1 beta Felis

MAPVPELTSE	MMAYYSDEND	LFEEADGPEK	MKGSQNLSE	SFLGDEGIQL	QISHQPDNKS	60
LRAVSVIIVA	MEKLKKISFA	CSQPLQDEDL	KSLFCCIFEE	EPICDTWDD	CFVCDAAIOS	120
QDYTRDISQ	KSLVLSGSYE	LRALHLNGQN	MNQQVVFMS	FVHGEENSKK	IPVVLICIKKN	180
NLYLSCVMKD	GKPTLQLEML	DPKVPYPKKK	EKRPFVFNKTE	IKGNVEFESS	QFPNWIISTS	240
QAEEMPVFLG	NTKGGQDITD	FIMESAS				267

SEQ ID NO:57 IL-1 beta Sus

MATVPEPAKE	VMANNGDNNN	DLFFEADGPK	EMKCRQNL	LSPLGDGSIQ	LQISHQLCNE	60
SSRPVSVIV	AKKEEPMNPSS	QVVCDDDPKS	IFSSVFEEEP	IVLEKHANGF	LCDATPVQSV	120
DCKLQDKDER	ALVLGAPHEL	KALHLLKGD	KREVVFCMSF	VQGDSDDKI	PVTLGKIGKN	180
LYLSCVMKDD	TPTLQLEVD	PKSYPKRDME	KRFVFKTEI	KNRVEFESAL	YPNWIISTSQ	240
AEQKPVFLGN	SKGRQDITDF	TMEVLSF				267

SEQ ID NO:58 IL-1 beta Cavia

MAAVPELSSE	VTAYHSDENE	LFEEVDGPNK	MQYCFQDRDL	CSLDEGIKLQ	ISHQHFNKSF	60
RQTVSLIVAV	EKLAKKLAPC	TWAFQDDDLR	PLLPFIFEEE	PVCDTWDEE	YESDTPVPSR	120
NCTLBIDIQH	KRLVSDPCEL	KALHLNGDNL	NRQVVFMSF	VQGERSDNKM	PVALGLKGKN	180
LYLSCVMKDG	KPVQLLESVD	GKQYPKKKME	KRFVFNKITS	KSTVEFESAQ	FPNWIISTSQ	240
AEHKPVFLGN	NNGQDIIDFK	LELVSS				266

SEQ ID NO:59 IL-1 beta Bos

MATVPEPINE	MMAYYSDENE	LLFEADDPKQ	MKSCIOHLDL	GSMGDGNIQL	QISHQFYNKS	60
FRQVSVIIVA	MEKLRSAYA	HVFHDDDLRS	ILSFIFEEEP	VIFETSSDEF	LCDAPVQSIK	120
CKLQDREQKS	LVLASPCVLK	ALHLLSQEMN	REVVFMSFV	QGEERDNKIP	VALGIRDKNL	180
YLSCVKKGDT	PTLQLEEDVP	KVYPKRNMEK	RFVFKTEIK	NTVEFESVLY	PNWIISTSQI	240
EERPVELGHF	RGGQDITDFR	METLSP				266

SEQ ID NO:60 IL-1 beta Ovis

MATVPEPINE	VMAYYSDENE	LLFEVDGPKQ	MKSCTQHLDL	GSMGDGNIQL	QISHQLYNKS	60
FRQVSVIIVA	MEKLRSRAYE	HVFRDDDLRS	ILSFIFEEEP	VIFETSSDEL	LCDAAVQSVK	120
CKLQDREQKS	LVLDSPCVLK	ALHLLSQEMS	REVVFMSFV	QGEERDNKIP	VALGIRDKNL	180
YLSCVKKGDT	PTLQLEEDVP	KVYPKRNMEK	RFVFKTEIK	NTVEFESVLY	PNWIISTSQI	240
BEKPVFLGRF	RGGQDITDFR	METLSP				266

SEQ ID NO:61 IL-1 beta Gallus

MAFVPDLQVLE	ESSSLSEETF	YGPSCICLQK	KPRLDSEHTT	VDVQVTVRKG	RGARSFRRAA	60
VLVVAMTKLL	RRPRSDFAD	SDLSALLEEV	FEPVTFQRL	SSYAGAPAFR	YTRSQSFDIF	120
DINQKCFVLE	SPTQLVALHL	QGPSSSQKVR	LNIALYRPRG	PRGSAGTGQM	PVALGIKGYK	180
LYMSCVMSGT	EPTLQLEED	VMRDIDSVEL	TRFIFYRLDS	PTEGTTRFES	AAFPGWFICT	240
SLQPRQPVGI	TNQPDQVNIA	TYKLGR				267

SEQ ID NO:62 IL-1 beta Canis

GPGGGSNVKCC	CQDLNHSSLV	DEGIQLQVSH	QLCNKSLRHF	VSVIVALEKL	KKPCPQVLQE	60
DDLKSIFCYI	FEEPIICTT	DADNFMSDAA	MQSVDCQLQD	ISHKYLVLNS	SYELRALHLN	120
GENVNKA						127

SEQ ID NO:63 hIL-1 alpha 119-271

LSNVKYNFMR	IIKYEFLND	ALNQSIIRAN	DQYLTAALH	NLDEAVKFDM	GAYKSSKDDA	60
KITVILRISK	TQLYVTAQDE	DQPVLLKEMP	EIPKTTITGSE	TNLLFFWETH	GTKNYFTSVA	120
HPNLFIAATKQ	DYWVCLAGGP	PSITDFQILE	NQA			153

SEQ ID NO:64 hIL-1 beta 117-269

APVRSNLCTL	RDSQQKSLVM	SGPYELKALH	LQGQDMEQQV	VFSMSFVQGE	ESNDKIPVAL	60
GLKEKNLYLS	CVLKDDKPTL	QLESVDPKNY	PKKKMEKRFV	FNKIEINNKL	EFESAQFPNW	120
YISTSQAENM	PVFLGGTKGG	QDITDFTMQF	VSS			153

SEQ ID NO:65 mIL-1alpha117-270

MDPBBBBHHG	SGDDDDKALA	PYTYQSDLR	KLMKLVQRKF	VMNDSLNTI	YQDVDPKHYS	60
TTWLNDLQGE	VKFDMYAYSS	GGDDSKYPVT	LKISDSQLFV	SAQGEDQPV	LKELPETPKL	120
ITGSETDLIF	FWKSINSKNY	FTSAAYPELF	IATKEQSRVH	LARGLPSTMD	FQISLEGGGG	180
GCG						183

SEQ ID NO:66 mIL-1beta119-269

MDPBBBBHHG	SGDDDDKALA	PIRQLHYRLR	DEQKSLVLS	DPYELKALHL	NGQNINQQVI	60
FMSMSFVQGE	SNDKIPVALG	LKGKNLYLSC	VMKGTPTLQ	LESVDPKQYP	KKKMEKRFV	120
NKIEVRSKVE	FESAEPFNWY	ISTSQAERKP	VFLGNNSGQD	IIDFTMESVS	SLEGGGGGCG	180

SEQ ID NO:67 hIL-1 alpha 009-020

MRIIKYEFL ND						12
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SEQ ID NO:68 hIL-1 alpha 010-015

RIIKYE						6
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SEQ ID NO:69 hIL-1 alpha 014-023

YEFILNDALN						10
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SEQ ID NO:70 hIL-1 alpha 019-030

NDALNQSIIR AN						12
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SEQ ID NO:71 hIL-1 alpha 035-044

TAAALHNLDE						10
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SEQ ID NO:72 hIL-1 alpha 035-046

TAAALHNLDE AV						12
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SEQ ID NO:73 hIL-1 alpha 045-054

AVKFDMGAYK						10
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SEQ ID NO:74 hIL-1 alpha 052-062

AYKSSKDDAK I						11
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SEQ ID NO:75 hIL-1 alpha 055-084

SSKDDAKITV	ILRISKTLQY	VTAQDEDPV	L			31
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SEQ ID NO:76 hIL-1 alpha 063-075

VILRISKTLQ	YVT					13
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SEQ ID NO:77 hIL-1 alpha 063-084

VILRISKTLQ	YVTAQDEDP	VL				22
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SEQ ID NO:78 hIL-1 alpha 069-089

TQLYVTAQDE	DQPVLLKEMP					20
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SEQ ID NO:79 hIL-1 alpha 082-102

PVLLKEMPEI PKTITGSETN L	21
SEQ ID NO:80 hIL-1 alpha 087-100 EMPEIPKTIT GSET	14
SEQ ID NO:81 hIL-1 alpha 093-104 KTITGSETNL LF	12
SEQ ID NO:82 hIL-1 alpha 093-113 PKTITGSETN LLFFWETHGT K	21
SEQ ID NO:83 hIL-1 alpha 097-106 GSETNLLFFW	10
SEQ ID NO:84 hIL-1 alpha 123-134 LFIATKQDYW VC	12
SEQ ID NO:85 hIL-1 alpha 124-136 FIATKQDYWV CLA	13
SEQ ID NO:86 hIL-1 alpha 125-142 IATKQDYWVC LAGGPPSI	18
SEQ ID NO:87 hIL-1 alpha 129-133 KQDYW	5
SEQ ID NO:88 hIL-1 alpha 147-153 QILENQA	7
SEQ ID NO:89 hIL-1 beta 001-006 APVRSI	6
SEQ ID NO:90 hIL-1 beta 001-012 APVRSINCTL RD	12
SEQ ID NO:91 hIL-1 beta 006-015 LNCTLRDSQQ	10
SEQ ID NO:92 hIL-1 beta 011-022 RDSQQKSLVM SG	12
SEQ ID NO:93 hIL-1 beta 027-038 KALHLQGQDM EQ	12
SEQ ID NO:94 hIL-1 beta 027-040 KALHLQGQDM EQQV	14
SEQ ID NO:95 hIL-1 beta 039-048 QVVFMSFVQ	10
SEQ ID NO:96 hIL-1 beta 046-056 FVQGEESNDK I	11
SEQ ID NO:97 hIL-1 beta 049-080 GEESNDKIPV ALGLKEKNLY LSCVLKDDKP TL	32
SEQ ID NO:98 hIL-1 beta 058-070 VALGLKEKNL YLS	13
SEQ ID NO:99 hIL-1 beta 058-080 VALGLKEKNL YLSCVLKDDK PTL	23
SEQ ID NO:100 hIL-1 beta 066-085 KNLYLSCVLK DDKPTLQLES V	21
SEQ ID NO:101 hIL-1 beta 078-097 PTLQLESVDP KNPYKKKMEK	20

SEQ ID NO:102 hIL-1 beta 083-095 ESVDPKNYPK KKM	13
SEQ ID NO:103 hIL-1 beta 088-099 KNYPKKKMEK RF	12
SEQ ID NO:104 hIL-1 beta 092-101 KKKMEKRFFV	10
SEQ ID NO:105 hIL-1 beta 092-109 KKKMEKRFFV NKIEINNK	18
SEQ ID NO:106 hIL-1 beta 120-133 WYISTSQAEN MPVF	14
SEQ ID NO:107 hIL-1 beta 121-135 YISTSQAENM PVFLG	15
SEQ ID NO:108 hIL-1 beta 122-143 ISTSQAENMP VFLGGTKGGQ DI	22
SEQ ID NO:109 hIL-1 beta 126-131 QAENMP	6
SEQ ID NO:110 hIL-1 beta 147-152 TMQFVSS	7
SEQ ID NO:111 mIL-1 beta 001-012 VPIRQLBYRL RD	12
SEQ ID NO:112 mIL-1 beta 027-040 KALHLNGQNI NQQV	14
SEQ ID NO:113 mIL-1 beta 121-135 YISTSQAENK PVFLG	15
SEQ ID NO:114 rIL-1 beta 066-085 NLYLSCVMKD GTPTLQLESV	20
SEQ ID NO:115 rIL-1 beta 078-097 PTLQLESVDP KQYPKKKMEK	20
SEQ ID NO:116 rIL-1 beta 122-143 ISTSQAENHP VFLGNSNGRD IV	22
SEQ ID NO:117 AP590 atggcaaata agccaatgca accgatcaca tctacagcaa ataaaattgt gtggtcggat ccaactcggt tatcaactac attttcagca agtctgttac gccaacgtgt taaagttggt atagcogaac tgaataatgt ttcaggtcaa tatgtatctg tttataagcg tcttgcacct aaaccggaag gttgtgcaga tgcctgtgtc attatgccga atgaaaacca atccattcgc acagtgatgt cagggtcagc cgaaaacttg gctacottaa aagcagaatg ggaaactcac aaacgtaacg ttgacacact cttcgcgagc ggcaacgccg gtttgggttt ccttgacct actgcggcta tcgtatcgtc tgatactact gcttaggat caggataatg catctaagct t	60 120 180 240 300 360 420 421
SEQ ID NO:118 AP592 atggcaaata agccaatgca accgatcaca tctacagcaa ataaaattgt gtggtcggat ccaactcggt tatcaactac attttcagca agtctgttac gccaacgtgt taaagttggt atagcogaac tgaataatgt ttcaggtcaa tatgtatctg tttataagcg tcttgcacct aaaccggaag gttgtgcaga tgcctgtgtc attatgccga atgaaaacca atccattcgc acagtgatgt cagggtcagc cgaaaacttg gctacottaa aagcagaatg ggaaactcac aaacgtaacg ttgacacact cttcgcgagc ggcaacgccg gtttgggttt ccttgacct actgcggcta tcgtatcgtc tgatactact gcttaggat caggataatg catctaagct t	60 120 180 240 300 360 420 421
SEQ ID NO:119 p1.44 nnccatggca aataagccaa tgcaaccg	28

SEQ ID NO:120 pINC-36
gtaagcttag atgcattatc cggatcccta agcagtagta tcagacgata cg 52

SEQ ID NO:121 pINC-40
gtaagcttag atgcattatc cggatcccta agcagtagta tcagacgata cg 52

SEQ ID NO:122 pINC-34
gggccggagc gctagccctt tacac 25

SEQ ID NO:123 pINC-35
gtaagcttat gcattatgat atctggaagt ctgtcataga 40

SEQ ID NO:124 HIL-1
atatatgata tccctgtacg atcactgaac tgcacg 36

SEQ ID NO:125 HIL-2
atatatctcg agggaagaca caaattgcat ggtgaag 37

SEQ ID NO:126 42-1
tatggatata gaattcaagc ttctgcagct gctcagagtaa ttgattac 48

SEQ ID NO:127 42-2
ctaggaatac aattactcga gcagctgcag aagcttgaat togatatcca 50

SEQ ID NO:128 42T-1
tcgagcacca ccaccaccac caccggtggtt gctaataata attgattaat ac 52

SEQ ID NO:129 42T-2
ctaggtatta atcaattatt attagcaacc accgtggtgg tgggtggtgg gc 52

SEQ ID NO:130 hIL-1 beta 116-269
MDIPVRS LNC TLRDSQQKSL VMSGPYELKA LHLQGQDMEQ QVVFSMSFVQ GEESNDKIPV 60
ALGLKEKNLY LSCVLKDDKP TLQLESVDPK NYPKKKMEKR FVFNKIEINN KLEFESAQFP 120
NWIYSTSQAE NMPVFLGGTK GGQDITDFTM QFVSSLEHHN HHHGGC 166

SEQ ID NO:131 hIL-1 beta 116-269(R4D)
MDIPVRS LNC TLRDSQQKSL VMSGPYELKA LHLQGQDMEQ QVVFSMSFVQ GEESNDKIPV 60
ALGLKEKNLY LSCVLKDDKP TLQLESVDPK NYPKKKMEKR FVFNKIEINN KLEFESAQFP 120
NWIYSTSQAE NMPVFLGGTK GGQDITDFTM QFVSS 155

SEQ ID NO:132 hIL-1 beta 116-269(L6A)
MDIPVRS LNC TLRDSQQKSL VMSGPYELKA LHLQGQDMEQ QVVFSMSFVQ GEESNDKIPV 60
ALGLKEKNLY LSCVLKDDKP TLQLESVDPK NYPKKKMEKR FVFNKIEINN KLEFESAQFP 120
NWIYSTSQAE NMPVFLGGTK GGQDITDFTM QFVSS 155

SEQ ID NO:133 hIL-1 beta 116-269(T9G)
MDIPVRS LNC TLRDSQQKSL VMSGPYELKA LHLQGQDMEQ QVVFSMSFVQ GEESNDKIPV 60
ALGLKEKNLY LSCVLKDDKP TLQLESVDPK NYPKKKMEKR FVFNKIEINN KLEFESAQFP 120
NWIYSTSQAE NMPVFLGGTK GGQDITDFTM QFVSS 155

SEQ ID NO:134 hIL-1 beta 116-269(R11G)
MDIPVRS LNC TLRDSQQKSL VMSGPYELKA LHLQGQDMEQ QVVFSMSFVQ GEESNDKIPV 60
ALGLKEKNLY LSCVLKDDKP TLQLESVDPK NYPKKKMEKR FVFNKIEINN KLEFESAQFP 120
NWIYSTSQAE NMPVFLGGTK GGQDITDFTM QFVSS 155

SEQ ID NO:135 hIL-1 beta 116-269(D54R)
MDIPVRS LNC TLRDSQQKSL VMSGPYELKA LHLQGQDMEQ QVVFSMSFVQ GEESNRKIPV 60
ALGLKEKNLY LSCVLKDDKP TLQLESVDPK NYPKKKMEKR FVFNKIEINN KLEFESAQFP 120
NWIYSTSQAE NMPVFLGGTK GGQDITDFTM QFVSS 155

SEQ ID NO:136 hIL-1 beta 116-269(D145K)
MDIPVRS LNC TLRDSQQKSL VMSGPYELKA LHLQGQDMEQ QVVFSMSFVQ GEESNDKIPV 60
ALGLKEKNLY LSCVLKDDKP TLQLESVDPK NYPKKKMEKR FVFNKIEINN KLEFESAQFP 120
NWIYSTSQAE NMPVFLGGTK GGQDITDFTM QFVSS 155

SEQ ID NO:137 hIL-1 beta 116-269(DEE50,51)
MDIPVRS LNC TLRDSQQKSL VMSGPYELKA LHLQGQDMEQ QVVFSMSFVQ GSNDKIPVAL 60
GLKEKNLYLS CVLKDDKPTL QLESVDPKNY PKKKMEKRFV FNKIEINNKL EFESAQFPNW 120

YISTSQAENM PVFLGGTKGG QDITDFTMQF VSS	153
SEQ ID NO:138 hIL-1 beta 116-269(DSND52-54)	
MDIPVRS LNC TLRDSQQKSL VMSGPYELKA LHLQGQDMEQ QVVFSMSFVQ GEEKIPVALG	60
LKEKNLYLSC VLRDDKPTLQ LESVDPKNYP KKKMEKR FVF NKIEINN KLE FESAQFPNWY	120
ISTSQAENMP VFLGGTKGGQ DITDFTMQFV SS	152
SEQ ID NO:139 hIL-1 beta 116-269(K63S/K65S)	
MDIPVRS LNC TLRDSQQKSL VMSGPYELKA LHLQGQDMEQ QVVFSMSFVQ GEESNDKIPV	60
ALGLSES NLY LSCVLKDDKP TLQLESVDPK NYPKKKMEKR FVF NKIEINN KLEFESAQFP	120
NWYISTSQAE NMPVFLGGTK GGQDITDFTM QFVSS	155
SEQ ID NO:140 hIL-1 beta 116-269(Q126A/E128A)	
MDIPVRS LNC TLRDSQQKSL VMSGPYELKA LHLQGQDMEQ QVVFSMSFVQ GEESNDKIPV	60
ALGLKEKNLY LSCVLKDDKP TLQLESVDPK NYPKKKMEKR FVF NKIEINN KLEFESAQFP	120
NWYISTSAAA NMPVFLGGTK GGQDITDFTM QFVSS	155
SEQ ID NO:141 HIL-3	
atatatcata tgctgagcaa tglgaaatac aactttatg	39
SEQ ID NO:142 HIL-4	
atatatctcg agcgccctggt ttccagtat ctgaaag	37
SEQ ID NO:143 R4D-1	
catatggata tcctgtaga ctcaactgaac tgcaagctc	39
SEQ ID NO:144 R4D-2	
gagcgtgcag ttcagtgagt ctacagggat atccatag	39
SEQ ID NO:145 L6A-1	
gatatccctg tacgatcagc taactgcacg ctccgggac	39
SEQ ID NO:146 L6A-2	
gtcccgagc gtgcagttag ctgacgtac agggatatc	39
SEQ ID NO:147 T9G-1	
gtacgatcac tgaactgcgg tctccgggac tcacagc	37
SEQ ID NO:148 T9G-2	
gctgtgagtc ccggagaccg cagttcagtg atcgtag	37
SEQ ID NO:149 R11G-1	
gaactgcacg ctccgggact cacagc	26
SEQ ID NO:150 R11G-2	
gctgtgagtc ccgagcgtg cagttc	26
SEQ ID NO:151 D54R-1	
caaggagaag aaagtaatcg caaaatacct gtggccttg	39
SEQ ID NO:152 D54R-2	
caaggccaca ggtattttgc gattactttc ttctccttg	39
SEQ ID NO:153 EE-1	
catgtccttt gtacaaggaa gtaatgacaa aatacctgtg	40
SEQ ID NO:154 EE-2	
cacaggtatt ttgtcattac ttcottgtac aaaggacatg	40
SEQ ID NO:155 SND-1	
ctttgtacaa ggagaagaaa aaatacctgt ggccttg	37
SEQ ID NO:156 SND-2	
caaggccaca ggtatttttt cttctccttg taaaaag	37
SEQ ID NO:157 K6365S-1	
gtggccttgg gcctcagcga aagcaatctg tacctgtcct g	41

SEQ ID NO:158 K6365S-2	
caggacaggt acagattgct ttcgtgagg cccaaggcca c	41
SEQ ID NO:159 QE-1	
gtacatcagc acctctgcag cagcaaacat gcccgctcttc	40
SEQ ID NO:160 QE-2	
gaagacgggc atgtttgctg ctgcagaggt gctgatgtac	40
SEQ ID NO:161 D145K-1	
gcggccagga tataactaaa ttcaccatgc aatttgtgtc	40
SEQ ID NO:162 D145K-2	
gacacaaatt gcatggtgaa tttagttata ttcgtggccgc	40
SEQ ID NO:163 mIL-1 alpha 117-270 without tag	
PYTYQSDLRV KLMKLVQRKF VMNDSLQNTI YQDVVDKHYLS TTWLNLDLQOE VKFDMYAYSS	60
GGDDSKYPVT LKISDSQLFV SAQGEDQPVV LKELPETPKL ITGSETDLIF FWKSINSKNY	120
FTSAAYPELF IATKEQSRVH LARGLPSTMD FQIS	154
SEQ ID NO:164 mIL-1 beta 119-269 without tag	
PIRQLEHYRLR DEQQKSLVLS DPYELKALHL NGQINQQVI FMSFVQGEF SNDKIPVALG	60
LKGNLYLSC VMKDGTPTLQ LESVDPKQYP KKKMEKRFVF NKIEVKSKVE FESAEFPNWY	120
ISTSQAELHP VFLGNNSGQD IIDFTMESVS S	151
SEQ ID NO:165 hIL-1 beta 116-269 without tag	
MDIPVRSLLNC TLRDSQQKSL VMSGPYELKA LHLQGQDMEQ QVVFSMSFVQ GEESNDKIPV	60
ALGLKEKNLY LSCVLKDDKP TLQLESVDPK NYPKKKMEKR FVFNKIEINN KLEFESAQFP	120
NWYISTSQAE NMPVELGGTK GGQDITDFTM QFVSS	155
SEQ ID NO:166 interleukin 1 receptor, type I	
MKVLLRLICF IALLISSLEA DKCKEREKEKI ILVSSANEID VRPCPLNPNE HKGTITWYKD	60
DSKTPVSTEQ ASRIHQHKEK LWFVPAKVED SGHYVCVVRN SSYCLRIKIS AKFVENEPNL	120
CYNAQAIFKQ NLPVAGDGGI VCPYMEFFKN ENNELPKLQW YKDCPKLLLD NIHFSGVKDR	180
LIVMNVAEKH RGNYTCHASY TYLGKQYPIT RVIEFITLEE NKPTRPVIVS PANETMEVDL	240
GSQIQILCNV TQQLSDIAYV KWNQSVIDED DPVLGEDYYS VENPANKRRS TLITVLNISE	300
IESRFYKHPF TCFANKTHGI DAAYIQLIYP VTNFQKHMIG ICVTLTVIIV CSVFIYKIFK	360
IDIVLWYRDS CYDFLPIKAS DGKTYDAYIL YPKTVGEGST SDCDIFVFKV LPEVLEKQCG	420
YKLFYIGRDD YVGEDIVEVI NENVKRSRL IILVRETSS FSWLGGSSSE QIAMYNALVQ	480
DGKVVLEL EKIQDYKMP ESIKFLKQKH GAIRWSGDFT QGPQSAKTRF WKNVRYHMZV	540
QRRSPSSKHQ LLSPATKEKL QREAHVPLG	569
SEQ ID NO:167 interleukin 1 receptor, type II	
MLRLVVLVMG VSAFTLQPAH HTGAARSCRF RGRHYKREFR LEGEPVALRC PQVPYWLWAS	60
VSPRINTWH KNSARTVPG EETRMWAQD GALWLLPALQ EDSGTIVCTT RNASYCDKMS	120
IELRVFENTD AFLPFIQYQ IITLSTSGVL VCPDLSEFTR DKTDVKIQWY KDSLLDQKDN	180
EKFLSVRGTT HLLVHDVALE DAGYRCVLT FAHEGQQYNI TRSIELRIKK KKEETIPVII	240
SPLKTIASL GSRLTIPCKV FLGTGTPLT MLWWTANDTH IESAYPGGRV TEGPRQYSE	300
NNENYIEVPL IFDPVTREDL HMDFKCVVEN TLFQTLRTT VKEASSTFSW GIVLAPLSLA	360
FLVLGGIWMH RRCKHRTGKA DGLTVLWPHH QDFQSYPK	398
SEQ ID NO:168 interleukin 1 receptor, type I (mouse)	
MENMKVLLGL ICLMVPLLSL EIDVCTEYPN QIVLFLSVNE IDIRKCPDTP NKMHGDTIIV	60
YKNDKSTPIS ADRDSRIHQ NEHLWFPVAK VEDSGYYYCI VRNSTYCLKT KVTVTVLEND	120
PGLCYSTQAT FPQRLHIAGD GSVLCPYVSY FKDNNELPE VQWYKNCKPL LLDNVSEFGV	180
KDKLLVRNVA EHRGDYICR MSYTFRGKQY PVTRVIQFIT IDENKRDPRV ILSRNETIE	240
ADPGSMICLI CNVTGQFSDL VYWKWNGSEI EWNDPFLAED YQFVEHPSTK RXYTLITTLN	300
ISEVKSQFYR YPFIQVVKNT NIFESAHVQL IYPVPDFKNY LIGGFILTA TIVCCVCIYK	360
VFKVDIVLWY RDSGSGFLPS KASDGKTYDA YILYPTLGE GSFSDLDTFV FKLLPEVLEG	420
QFGYKLFYIG RDDYVGEDTI EVTNENVKKS RRLIILVLD MGGFSWLGQS SEEQIATYNA	480
LIQEGIKIVL LELEKIQDYE KMPDSIQFIK QKHGVICWSG DFQERPQSAK TRFWKNLRYQ	540
MPAQRRSPLS KHRLLTLDPV RDTKEKLPAH THLPLG	576
SEQ ID NO:169 interleukin 1 receptor, type II (mouse)	
MFILLVLVTG VSAFTTPTVV HTGKVSESPI TSEKPTVHGD NCQFRGREFK SELRLEGEPV	60
VLRCPLAPHS DISSSSHSFL TWSKLDSSQL IPRDEPRMWV KGNILWILPA VQDQSGTYIC	120
TFRNASHCEQ MSVELKVFNK TEASLPHVSY LQISALSTG LLVCPDLKEF ISSNADGKIQ	180
WYKGAILLDK GNKEFLSAGD PTRLLISNTS MDDAGYYRCV MTFTYNGQY NITRNIELRV	240
KGTTTEPIV IISPLETIPA SLGSRLIVPC KVFLGTGTSS NTIVWWLANS TFISAAYPRG	300
RVTEGLHHQY SENDENYVEV SLIFDPVIRE DLHTDFKQVA SNPRSSQSLH TTVKEVSSTF	360
SWSIALAPLS LIIILVVGAIW MRRRCKRRAK KTYGLTKLRT DNQDFPSSPN	410

SEQ ID NO:170 peptide GGKGG	5
SEQ ID NO:171 linker CGG CGG	3
SEQ ID NO:172 linker CGDKTHTSPP CGDKTHTSPP	10
SEQ ID NO:173 linker CGGPKPSTPPGSSGGAP CGGPKPSTPP GSSGGAP	17
SEQ ID NO:174 linker GCGGGG GCGGGG	6
SEQ ID NO:175 linker GCG GCG	3
SEQ ID NO:176 linker GGGGS GGGGS	5
SEQ ID NO:177 linker GCGSGGGGS GCGSGGGGS	9
SEQ ID NO:178 linker GGC GGC	3
SEQ ID NO:179 linker GGC-NH2 GGC	3
SEQ ID NO:180 linker DKTHTSPPCG	10
SEQ ID NO:181 linker PKPSTPPGSSGGAPGGCG PKPSTPPGSS GGAPGGCG	18
SEQ ID NO:182 linker GGGGCG GGGGCG	6
SEQ ID NO:183 linker GCG (2) GCG	3
SEQ ID NO:184 linker SGGGG SGGGG	5
SEQ ID NO:185 linker GSGGGGSGCG	10
SEQ ID NO:186 linker GGKKGC GGKKGC	6
SEQ ID NO:187 linker CGKKGG CGKKGG	6
SEQ ID NO:188 linker GGCG GGCG	4
SEQ ID NO:189 linker GSG GSG	3
SEQ ID NO:190 His-Tag with Linker LEHHHHHHGG C	11
SEQ ID NO:191 MOG peptide MEVGWYRSPF SRVHLYRNG K	21

SEQ ID NO:192 pINC-75
gatcgggagg tgggtgtcccc attagacagc t 31

SEQ ID NO:193 pINC-77
gtaagcttag gaagacacag attccat 27

SEQ ID NO:194 pINC-74
gatcgggagg tgggtgcccct gtacgatcac tgaactg 37

SEQ ID NO:195 pINC-76
gtatgcatta ggaagacaca aattgcatgg tgaagtc 37

SEQ ID NO:196 IL1a1C
atatacata tgtctgcccc ttacacctac cagagtg 37

SEQ ID NO:197 alphaD145K-1
ggactgcccct ctatgacaaa attccagata tcaactcgag 39

SEQ ID NO:198 alphaD145K-2
ctcgagtgat atctggaatt ttgtcataga gggcagtc 39

SEQ ID NO:199 halphad145K-1
gggccaccct ctatcactaa atttcagata ctggaaaacc 40

SEQ ID NO:200 halphad145K-2
gggtttccag tatctgaaat ttagtgatag aggggtggccc 40

SEQ ID NO:201 Histag-GGC
LEHHHHHHGG C 11

SEQ ID NO:202 mIL-1 alpha 115-270 without tag
MSAPYTYQSD LRYKLMKLVK QKFVMNDSL N QTIYQDVVDKH YLSTTWLNDL QQEVKFDMYA 60
YSSGGDDSKY PVTLKISDSQ LRVSAQGEDQ PVLLKELPET PKLITGSETD LIPFWKSINS 120
KNYFTSAAYP ELFIATKEQS RVHLARGLPS MTDFAQIS 157

SEQ ID NO:203 hIL-1 alpha 119-271 without tag
MLSNVKNYFM RIIKYEFLN DALNQSIIRA NDQVLTAAAL HNLDEAVKFD MGAYKSSKDD 60
AKITVILRIS KTQLYVTAQD EDQPVLLKEM PEIPKTIIGS ETNLLFFWET HGTKNYFTSV 120
AHPNLFIAIK QDYVWVCLAGG PPSITDFQIL ENQA 154

SEQ ID NO:204 mIL-1 alpha 115-270(D145K)
DSAPYTYQSD LRYKLMKLVK QKFVMNDSL N QTIYQDVVDKH YLSTTWLNDL QQEVKFDMYA 60
YSSGGDDSKY PVTLKISDSQ LRVSAQGEDQ PVLLKELPET PKLITGSETD LIPFWKSINS 120
KNYFTSAAYP ELFIATKEQS RVHLARGLPS MTKFAQIS 157

SEQ ID NO:205 hIL-1 beta 116-269(K88N)
MDIPVRS LNC TLRDSQQKSL VMSGPYELKA LHLQGQDMEQ QVVFMSMFVQ GEESNDKIPV 60
ALGLKEKNLY LSCVLKDDKP TLQLESVDPN NYPKKKMEKR FVFNKIEINN KLEFESAQFP 120
NWIYSTQAE NMPVFLGGTK GGQDITDFTM QFVSS 155

SEQ ID NO:206 hIL-1 beta 116-269(R98Q)
MDIPVRS LNC TLRDSQQKSL VMSGPYELKA LHLQGQDMEQ QVVFMSMFVQ GEESNDKIPV 60
ALGLKEKNLY LSCVLKDDKP TLQLESVDPK NYPKKKMEKR FVFNKIEINN KLEFESAQFP 120
NWIYSTQAE NMPVFLGGTK GGQDITDFTM QFVSS 155

SEQ ID NO:207 hIL-1 beta 116-269(K103L)
MDIPVRS LNC TLRDSQQKSL VMSGPYELKA LHLQGQDMEQ QVVFMSMFVQ GEESNDKIPV 60
ALGLKEKNLY LSCVLKDDKP TLQLESVDPK NYPKKKMEKR FVFNKIEINN KLEFESAQFP 120
NWIYSTQAE NMPVFLGGTK GGQDITDFTM QFVSS 155

SEQ ID NO:208 hIL-1 beta 116-269(Rkkk92-94)
MDIPVRS LNC TLRDSQQKSL VMSGPYELKA LHLQGQDMEQ QVVFMSMFVQ GEESNDKIPV 60
ALGLKEKNLY LSCVLKDDKP TLQLESVDPK NYPMEKRFFV NKIEINN KLEFESAQFP 120
ISTSQAE NMP VFLGGTKGGQ DITDFTMQFV SS 152

SEQ ID NO:209 hIL-1 beta 116-269(L10N)
MDIPVRS LNC TLRDSQQKSL VMSGPYELKA LHLQGQDMEQ QVVFMSMFVQ GEESNDKIPV 60
ALGLKEKNLY LSCVLKDDKP TLQLESVDPK NYPKKKMEKR FVFNKIEINN KLEFESAQFP 120
NWIYSTQAE NMPVFLGGTK GGQDITDFTM QFVSS 155

SEQ ID NO:210 hIL-1 alpha 119-271 (D145K)
 MLSNVKYNFM RIIKYEFILN DALNQSIIRA NDQYLTAAL HNLDEAVKFD MGAYKSSKDD 60
 AKITVILRIS KTQLYVTAQD EDQPVLLKEM PEIPKTITGS ETNLLFFWET HGTKNYFTSV 120
 AHPNLFIAATK QDYWVCLAGG PPSITKFQIL ENQA 154

SEQ ID NO:211 hIL-1 alpha 119-271 (L18K)
 MLSNVKYNFM RIIKYEFILN DALNQSIIRA NDQYLTAAL HNLDEAVKFD MGAYKSSKDD 60
 AKITVILRIS KTQLYVTAQD EDQPVLLKEM PEIPKTITGS ETNLLFFWET HGTKNYFTSV 120
 AHPNLFIAATK QDYWVCLAGG PPSITDFQIL ENQA 154

SEQ ID NO:212 hIL-1 alpha 119-271 (F146N)
 MLSNVKYNFM RIIKYEFILN DALNQSIIRA NDQYLTAAL HNLDEAVKFD MGAYKSSKDD 60
 AKITVILRIS KTQLYVTAQD EDQPVLLKEM PEIPKTITGS ETNLLFFWET HGTKNYFTSV 120
 AHPNLFIAATK QDYWVCLAGG PPSITDNQIL ENQA 154

SEQ ID NO:213 hIL-1 alpha 119-271 (R10A)
 MLSNVKYNFM AIIKYEFILN DALNQSIIRA NDQYLTAAL HNLDEAVKFD MGAYKSSKDD 60
 AKITVILRIS KTQLYVTAQD EDQPVLLKEM PEIPKTITGS ETNLLFFWET HGTKNYFTSV 120
 AHPNLFIAATK QDYWVCLAGG PPSITDFQIL ENQA 154

SEQ ID NO:214 hIL-1 alpha 119-271 (I62A)
 MLSNVKYNFM RIIKYEFILN DALNQSIIRA NDQYLTAAL HNLDEAVKFD MGAYKSSKDD 60
 AKITVILRIS KTQLYVTAQD EDQPVLLKEM PEIPKTITGS ETNLLFFWET HGTKNYFTSV 120
 AHPNLFIAATK QDYWVCLAGG PPSITDFQIL ENQA 154

SEQ ID NO:215 hIL-1 alpha 119-271 (W107F)
 MLSNVKYNFM RIIKYEFILN DALNQSIIRA NDQYLTAAL HNLDEAVKFD MGAYKSSKDD 60
 AKITVILRIS KTQLYVTAQD EDQPVLLKEM PEIPKTITGS ETNLLFFWET HGTKNYFTSV 120
 AHPNLFIAATK QDYWVCLAGG PPSITDFQIL ENQA 154

SEQ ID NO:216 hIL-1 alpha 119-271 (D20V)
 MLSNVKYNFM RIIKYEFILN DALNQSIIRA NDQYLTAAL HNLDEAVKFD MGAYKSSKDD 60
 AKITVILRIS KTQLYVTAQD EDQPVLLKEM PEIPKTITGS ETNLLFFWET HGTKNYFTSV 120
 AHPNLFIAATK QDYWVCLAGG PPSITDFQIL ENQA 154

SEQ ID NO:217 hIL-1 alpha 119-271 (rFIL16-18)
 RRSNVKYNFM RIIKYENDAL NQSIIRANDQ YLTAAALHNL DEAVKFDMDGA YKSSKDDAKI 60
 TVILRISKIQ LYVTAQDEQD PVLLKEMPEI PKTITGSETN LFFWETHGT KNYFTSVVHP 120
 NLFIAATKQDY WVCLAGGPPS ITDFQILENQ A 151

SEQ ID NO:218 hIL-1 alpha 119-271 (rITGS96-99)
 MLSNVKYNFM RIIKYEFILN DALNQSIIRA NDQYLTAAL HNLDEAVKFD MGAYKSSKDD 60
 AKITVILRIS KTQLYVTAQD EDQPVLLKEM PEIPKTETNL LFFWETHGT NYFTSVVHPN 120
 LFIATKQDYW VCLAGGPPSI TDFQILENQ 150

SEQ ID NO:219 Histag-GGC
 LEHHHHHHGG CG 12

SEQ ID NO:220 hIL-1 beta 116-269 (D145K) with LEHHHHHHGGCG-tag
 MDIPVRSNLC TLRDSQQKSL VMSGPYELKA LHLQGQDMEQ QVVFMSFVQ GEESNDKIPV 60
 ALGLKEKNLY LSCVLKDDKP TLQLESVDPK NYPKKKMEKR FVFNKIEINN KLEFESAQFP 120
 NWWISTSQAE NMPVFLGGTK GGQDITKFTM QFVSSLEHHH HHHGGCG 167

SEQ ID NO:221 hIL-1 beta 116-269 (D145K) with GGCG-tag
 MDIPVRSNLC TLRDSQQKSL VMSGPYELKA LHLQGQDMEQ QVVFMSFVQ GEESNDKIPV 60
 ALGLKEKNLY LSCVLKDDKP TLQLESVDPK NYPKKKMEKR FVFNKIEINN KLEFESAQFP 120
 NWWISTSQAE NMPVFLGGTK GGQDITKFTM QFVSSGGCG 159

SEQ ID NO:222 hIL-1 beta 116-269 (D145K) with GGC-tag
 MDIPVRSNLC TLRDSQQKSL VMSGPYELKA LHLQGQDMEQ QVVFMSFVQ GEESNDKIPV 60
 ALGLKEKNLY LSCVLKDDKP TLQLESVDPK NYPKKKMEKR FVFNKIEINN KLEFESAQFP 120
 NWWISTSQAE NMPVFLGGTK GGQDITKFTM QFVSSGGCG 158

SEQ ID NO:223 hIL-1 beta 116-269 with GGCG-tag
 MDIPVRSNLC TLRDSQQKSL VMSGPYELKA LHLQGQDMEQ QVVFMSFVQ GEESNDKIPV 60
 ALGLKEKNLY LSCVLKDDKP TLQLESVDPK NYPKKKMEKR FVFNKIEINN KLEFESAQFP 120
 NWWISTSQAE NMPVFLGGTK GGQDITDFTM QFVSSGGCG 159

SEQ ID NO:224 hIL-1 alpha 119-271 (SEQ ID NO:203) with GGCG-tag

MLSNVKYNFM RIIKYEFILN DALNQSIIRA NDQYLTAAL HNLDEAVKFD MGAYKSSKDD	60
AKITVILRIS KTQLYVTAQD EDQPVLLKEM PEIPKTITGS ETNLLFFWET HGTKNYFTSV	120
AHPNLFIAATK QDYWVCLAGG PPSITDFQIL ENQAGGCG	158

SEQ ID NO:225 hIL-1 alpha 119-271 (D145K) (SEQ ID NO:204) with GGCG-tag

MLSNVKYNFM RIIKYEFILN DALNQSIIRA NDQYLTAAL HNLDEAVKFD MGAYKSSKDD	60
AKITVILRIS KTQLYVTAQD EDQPVLLKEM PEIPKTITGS ETNLLFFWET HGTKNYFTSV	120
AHPNLFIAATK QDYWVCLAGG PPSITKFQIL ENQAGGCG	158

SEQ ID NO:226 IL1BETA-3

atatatgata tocccattag acagctgcac tacagg	36
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SEQ ID NO:227 IL1BETA-2

atatatctcg aggggaagaca cagattccat ggtgaag	37
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SEQ ID NO:228 mIL-1 beta 116-269 (D145K)

MDIPIRQLHY RLRDEQQKSL VLSDPYELKA LELNGQNINQ QVIFSMSEVQ GEPSNDKIPV	60
ALGLKGKNLY LSCVMKDGTP TLQLESVDPK QYPKKKMEKR FVFNKIEVKS KVEFESAEPF	120
NWYISTSQAE BKPVPLGNS GQDIKFTME SVSSLEHHHH HHGGC	165

SEQ ID NO:229 D143K-1

cagtgggtcag gacataatta aattcaccat ggaatctgtg tc	42
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SEQ ID NO:230 D143K-2

gacacagatt ccatgggtgaa tttaattatg tctgaccac tg	42
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