

MODIFIED VIRUS-LIKE PARTICLES OF CMV

The present invention relates to virus-like particles of plant virus Cucumber Mosaic Virus (CMV), and in particular to modified VLPs of CMV comprising Th cell epitopes, in particular universal Th cell epitopes. Furthermore, these modified VLPs serve as, preferably, vaccine platform, for generating immune responses, in particular antibody responses, against antigens linked to said modified VLPs. The presence of the Th cell epitopes, in particular universal Th cell epitopes, led to a further increase in the generated immune response.

RELATED ART

Over the last three decades, virus-like particles (VLPs) have evolved to become an accepted technology, in particular in the field of vaccine development (Zeltins A, Mol Biotechnol (2013) 53:92-107). The growing interest in these VLPs was particularly inspired by the successful development and introduction of the hepatitis B virus (HBV) surface antigen and the human papilloma virus (HPV) capsid protein L1 as commercial vaccines against hepatitis B and HPV-induced cervical cancer, respectively. Moreover, virus-like particles have been described as immunological carriers which are capable of inducing strong immune responses against conjugated antigens (Jennings GT and Bachmann MF, Annu Rev Pharmacol Toxicol (2009) 49:303-26, Jennings GT and Bachmann MF, Biol Chem (2008) 389:521-536, WO2002/056905, WO2003/024481). The latter led to the development of several VLP-based vaccine candidates that have entered different stages of clinical investigations with the aim to develop VLP-based vaccines for medical and veterinary purposes in the near future (Liu F, et al., Research in Veterinary Science (2012) 93:553-559; Roldao A., et al., Expert Review of Vaccines (2010) 9:1149-1176).

The VLPs developed so far originated from microbial, plant, insect or mammalian viruses. Beside the above referenced VLP systems, VLPs derived from plant viruses have recently attracted attention, mainly due to the role of plants as an economical and speedy alternative platform for producing VLP vaccines in the light of their ability to provide distinctive posttranslational modifications, cost-effectiveness, production speed and scalability (Chen Q and Lai H, Human Vaccines & Immunotherapeutics (2013) 9:26-49; Zeltins A, Mol Biotechnol (2013) 53:92-107).

Cucumber Mosaic Virus (CMV, family, *Bromoviridae*, genus, *Cucumovirus*) is a linear positive-sense isodiametric plant virus with an extremely wide host range. The virus genome consists of three single-stranded RNAs (RNA1, RNA2 and RNA3), the coat protein (CP) gene being present both in the genomic RNA3 (about 2200 nt) and in the subgenomic RNA4 (about 5 nt). The capsid comprises 180 copies of a single protein species of about 26 kDa. There are a plurality of different strains known from CMV associated with variable symptoms related to the host plant such as CMV-B strain, CMV-C strains CMV-D strain, CMV-L strains, CMV-S strain, CMV-T strain, CMV-WL-strain, CMV-V strains, CMV-Fny strain, CMV-Ix strain, CMV-Q strain, CMV-R strain or the like (Carrère I, et al., Arch Virol (1999) 144:1846-1857; Edwards 10 MC, et al., Phytopathology 81983) 73:1117-1120; www.dpvweb.net).

Recently, chimeric forms of CMV have been engineered to function as a presentation system and to express on their outer surface epitopes derived from the hepatitis C virus (HCV). In detail, a CMV pseudo-recombinant form CMV-D/S has been engineered to carry genomic RNA3 from the CMV-S strain and RNA1 and RNA2 from the CMV-D strain. This system 15 developed virus symptoms such as mild mosaic and vein clearing in *Xanthi* tobacco plants after inoculation. The CP gene was then engineered in different positions, to encode a Hepatitis C virus (HCV) epitope. The selected peptide was the so-called R9 mimotope, a synthetic peptide derived from many hypervariable region 1 (HVR1) sequences of the HCV envelope protein E2. Serum samples from patients with chronic hepatitis C displayed a significant immunoreactivity 20 to crude plant extracts infected with one of the selected engineered pseudo-recombinant chimeric forms of CMV. It has, therefore, been suggested that such systems may be suitable carriers enabling the development of promising oral immunization strategies. The latter would be in line with the concept of plants as possible bioreactors of the so-called nutraceuticals since they actively replicate in plants and, thus, are conceivable to be used as edible vaccines because 25 celery, lettuce, cucumber, tomato, carrot, pepper and banana are hosts of CMV. (Natilla A, et al., Arch Virol (2004) 149:137-154; Piazzolla G, et al., J Clin Immunol (2005) 25:142-152; Nuzzaci M, et al., Arch Virol (2007) 152:915-928; Nuzzaci M, et al., Journal of Virological Methods (2009) 155:118-121; Nuzzaci M, et al., Journal of Virological Methods (2010) 165:211-215; Piazzolla G, et al., J Clin Immunol (2012) 32:866-876).

30 The insertion of the R9 mimotopes has been effected at different locations within the CP gene of CMV-S RNA3 (AF063610, www.dpvweb.net). For the insertion of one single R9 mimotope within said CP gene, the R9 mimotope nucleotide sequence was inserted in positions 253, 475, 529 of said CP gene, whereas for the insertion of two R9 mimotopes, the R9 mimotope nucleotide sequence was inserted in position 392 and 529. The final products were CMV

chimeric particles carrying on their outer surface 180 or 360 copies of the R9 mimotope for each virus particle. (Natilla A, et al., Arch Virol (2004) 149:137–154; Piazzolla G, et al., J Clin Immunol (2005) 25:142-152; Nuzzaci M, et al., Arch Virol (2007) 152:915-928; Nuzzaci M, et al., Journal of Virological Methods (2009) 155:118–121; Nuzzaci M, et al., Journal of Virological Methods (2010) 165:211–215; Piazzolla G, et al., J Clin Immunol (2012) 32:866–876).

It has been stated that the selection of the insertion points of the R9 mimotope into the CMV-S RNA3 was based on taking several some essential factors into account: i) the need to protect the N-terminal region of the CMV coat protein (containing a high concentration of basic amino acids, known as an internal R-domain, involved in protein-RNA interactions stabilizing CMV (Wikoff WR, et al., Virology (1997) 232: 91-97)) characterized by an unusual N-terminal helix with an additional stabilizing role in the capsid (Smith TJ, et al., J Virol (2000) 74: 7578–7686)); ii) the surface location of the foreign epitope to increase the chance of its putative immunogenic capability; iii) the availability of mutagenesis routes able to produce the modified clones. On the basis of these considerations it has been focused to work on the aa range 70–192 and the corresponding nucleotide regions as indicated above. The so prepared chimeric CMVs retained their ability to spread systemically in the host plant, which is an irreplaceable goal in the building of a potential plant virus carrier for foreign gene expression in plants. ELISA and IEM tests demonstrated that the R9 mimotope was exposed as planned in the right position (Natilla A, et al., Arch Virol (2004) 149:137–154).

Furthermore, a cucumber mosaic virus based expression system for the production of porcine circovirus specific vaccines has recently been described (Gellert A, et al., PLoS ONE (2012) 7(12): e52688). In detail, porcine circovirus type 2 (PCV2) capsid protein epitopes were integrated into the plant virus coat protein of cucumber mosaic virus (CMV)-R strain after amino acid position 131. The recombinants were tested for infectivity and stability on different *Nicotiana* species and stable recombinant virus particles were purified. The particles were tested for their ability to bind to PCV induced porcine antibodies and used for specific antibody induction in mice and pigs. The results showed that PCV epitopes expressed on the CMV surface were recognized by the porcine antibodies and they were also able to induce PCV specific antibody response. Challenge experiment with PCV2 carried out in immunized pigs showed partial protection against the infection.

The PCV2 capsid protein epitopes were integrated in the coat protein of cucumber mosaic virus (CMV) at the three stable epitope insertion points of CMV reported to date (Nuzzaci M, et al., Arch Virol (2007) 152:915-928; Vitti A, et al., J Virol Methods (2010)

169:332-340). The built-in epitopes at these points did not block the propagation of CMV and the long-distance movement in plants. Two of them are displayed on the external surface of the virion while the third protrudes towards the inside of the virion. There is an electrostatic limitation to express epitopes facing the inside of the virion as only the expression of epitopes with positive charge, that do not interfere with the RNA-binding of the inner surface of the CMV capsid can successfully be attempted. In detail, the first insertion point is located at position of aa 83–84 and at the end of the $\beta\text{B}\beta\text{C}$ loop of the CMV CP. The built-in epitopes are expressed on the surface of CMV virions. The second insertion point is located at position of aa 131–132 and in the middle of the $\beta\text{E}-\alpha\text{EF}$ loop of the CMV CP. The built-in epitopes are also expressed on the surface of CMV virions. The third insertion point is located at position of aa 176–177 and in the middle of the $\beta\text{G}-\beta\text{H}$ loop. In this latter case the inserted epitopes are expressed on the inner surface of CMV virions.

Furthermore, the generation of chimeric *virus-like particles (VLPs)* based on CMV has been described and its use as an animal vaccine suggested (Natilla, A, et al., Arch Virol (2006) 151:1373-1386; Natilla, A, et al., Protein Expression and Purification (2008) 59:117-121). However, the expression of the viral capsid proteins (CPs) of CMV by the widely used traditional *E. coli* expression system led only to insoluble inclusion bodies or a very low quantity of soluble proteins (Xu Y, et al., Chem Commun (2008) 49-51). On the other hand, Potato virus X (PVX)-expressed coat protein (CP) of CMV-Fny formed VLPs, which served as carriers for surface display of different neutralizing epitopes of Newcastle disease virus (NDV), an economically important pathogen of poultry. For this purpose, epitopes from fusion (F), hemagglutinin-neuraminidase (NH) protein and the tandem F-NH peptide were genetically fused into the internal $\beta\text{H}-\beta\text{I}$ (motif 5) loop of the Fny-CMV CP (corresponding to amino acids 194–199 thereof) and expressed via the PVX vectors in *Nicotiana benthamiana* plants. The resulting chimeric CMV VLPs are morphologically indistinguishable from wild type CMV particles. Moreover, chickens immunized with purified NH-CMV VLPs developed antigen-specific antibody responses, however, the so immunized chickens were not protected against experimental challenge with NDV (Natilla, A, et al., Arch Virol (2006) 151:1373-1386; Natilla, A, et al., Protein Expression and Purification (2008) 59:117-121; Chen Q and Lai H, Human Vaccines & Immunotherapeutics (2013) 9: 26-49).

As indicated, the expression of the viral capsid proteins (CPs) of CMV in *E. coli* led only to insoluble inclusion bodies or a very low quantity of soluble proteins (Xu Y, et al., Chem Commun (2008) 49-51). On the other hand, Xu et al were able to *in vitro* assembly genetically recombinant CPs of CMV into biological nanotubes triggered by double-stranded DNAs of

different lengths (Xu Y, et al., Chem Commun (2008) 49-51). For this purpose, however, Xu et al had to produce pure soluble CMV CPs. This was achieved by a recombination procedure after the expression of the CPs in *E.coli*. The procedure comprises the separation and purification of the inclusion bodies formed after the expression of the full-length CP gene of 218 amino acid residues (ID AB008777, www.dpvweb.net), followed by solubilization of the inclusion bodies via denaturation and, finally by applying a refolding procedure to the denatured protein.

Even though progress has been made in the course of the development of VLP based vaccines, there is still a need for further distinct VLP systems. In particular, vaccines induce variable antibody responses in immunized subjects and individuals and antibody responses often span a range of more than 100-fold variation. In addition, some vaccines, such as the vaccine against Hepatitis B, suffer from a certain number of non-responders. Non-responsiveness is known to be associated with certain MHC class II molecules and it is believed that a failure to induce good T helper (Th) cell responses is responsible for the poor antibody responses seen in these individuals (Goncalves L, et al., Virology (2004) 326:20-28). Furthermore, elderly and old people mount poor antibody responses in general and poor Th cell responses are again thought to be the cause of the inefficient antibody responses. Therefore, vaccines inducing good Th cell responses in essentially all subjects and individuals are an important goal in the field of vaccine development.

SUMMARY OF THE INVENTION

We have surprisingly found that coat proteins of cucumber mosaic virus (CMV) can be modified to incorporate T helper (Th) cell epitopes, and preferably to incorporate universal Th cell epitopes. The latter is in particular surprising since these strong Th cell epitopes are known to cause aggregation and, thus, would be expected to hinder self-assembly upon expression leading to VLPs. Moreover, not only provides the present invention modified CMV VLPs comprising Th cell epitopes, preferably universal Th cell epitopes, but furthermore the inventive modified CMV VLPs as well as the wild-type CMV VLP have been obtained by expression in *E.coli*, which again is surprising as previous attempts to produce CMV VLPs in *E. coli* resulted in aggregates rather than VLPs. In addition, the inventive VLPs serve as a carrier platform, in particular a vaccine platform, wherein antigens to which immune responses are desired to be generated are linked to the inventive VLPs. The introduced Th cell epitopes incorporated within the inventive modified VLPs of CMV further increased the immunogenicity of the VLPs and led

to an increase of the overall immune responses generated by the inventive compositions, in particular to increased antibody responses.

Thus in a first aspect, the present invention provides for a modified virus-like particle (VLP) of cucumber mosaic virus (CMV), wherein said modified VLP of CMV comprises, essentially consists of, or alternatively consists of, at least one modified CMV polypeptide, wherein said modified CMV polypeptide comprises, or preferably consists of, (a) a CMV polypeptide, and (b) a T helper cell epitope; and wherein said CMV polypeptide comprises, or preferably consists of, (i) an amino acid sequence of a coat protein of CMV; or (ii) a mutated amino acid sequence, wherein the amino acid sequence to be mutated is an amino acid sequence of a coat protein of CMV, and wherein said mutated amino acid sequence and said coat protein of CMV show a sequence identity of at least 90 %, preferably of at least 95%, further preferably of at least 98% and again more preferably of at least 99%.

In another aspect, the present invention provides for a modified virus-like particle (VLP) of cucumber mosaic virus (CMV), wherein said modified VLP of CMV comprises, essentially consists of, or alternatively consists of, at least one modified CMV polypeptide, wherein said modified CMV polypeptide comprises, or preferably consists of, (a) a CMV polypeptide, and (b) a T helper cell epitope; and wherein said CMV polypeptide comprises, or preferably consists of, (i) an amino acid sequence of a coat protein of CMV; or (ii) a mutated amino acid sequence, wherein the amino acid sequence to be mutated is an amino acid sequence of a coat protein of CMV, and wherein said mutated amino acid sequence and said coat protein of CMV show a sequence identity of at least 90 %, preferably of at least 95%, further preferably of at least 98% and again more preferably of at least 99%; wherein said T helper cell epitope (i) is fused to the N-terminus of said CMV polypeptide; (ii) is fused to the C-terminus of said CMV polypeptide; (iii) replaces a region of consecutive amino acids of said CMV polypeptide, wherein the sequence identity between said replaced region of consecutive amino acids of said CMV polypeptide and the T helper cell epitope is at least 15%, preferably at least 20%; or (iv) replaces a N-terminal region of said CMV polypeptide, and wherein said replaced N-terminal region of said CMV polypeptide consists of 5 to 15 consecutive amino acids.

In another aspect, the present invention provides for a virus-like particle (VLP) of cucumber mosaic virus (CMV), wherein said VLP of CMV comprises, essentially consists of, or alternatively consists of, at least one CMV polypeptide comprising or preferably consisting of: (i) an amino acid sequence of a coat protein of CMV; or (ii) a mutated amino acid sequence, wherein the amino acid sequence to be mutated is an amino acid sequence of a coat protein of CMV, and wherein said mutated amino acid sequence and said a coat protein of CMV show a

sequence identity of at least 90 %, preferably of at least 95%, further preferably of at least 98% and again more preferably of at least 99%; and wherein said VLP of CMV is obtained by expression of said CMV polypeptide in *E.coli.*, and wherein preferably said expression is effected at temperatures of between 10°C to 25°C, preferably at a temperature of 20°C.

5 In another aspect, the present invention provides for a composition comprising a modified virus-like particle. Furthermore, these modified VLPs serve as, preferably, vaccine platform, for generating immune responses, in particular antibody responses, against antigens linked to said modified VLPs. The presence of the Th cell epitopes, in particular universal Th cell epitopes, led to a further increase in the generated immune response.

10 Further aspects and preferred embodiments thereof are described herein in more detail.

BRIEF DESCRIPTION OF FIGURES

FIG. 1 pET-CMVwt plasmid map. The relative positions of all relevant genes and restriction enzyme sites are denoted.

15 FIG. 2 SDS/PAGE analysis of expression and sucrose gradient purification of CMVwt protein. M - protein size marker; S - soluble proteins after ultrasonication; P - insoluble proteins after ultrasonication; Ps - soluble proteins in ammonium sulfate pellet (sample for sucrose gradient ultracentrifugation); Pp - insoluble proteins in ammonium sulfate pellet; 1, 2, 3, 4, 5, 6 - sucrose gradient fractions from the bottom of 35 ml gradient tube.

20 FIG. 3A Dynamic light scattering of purified CMVwt VLPs. The size of particles was detected by using Zetasizer Nano ZS (Malvern Instruments Ltd., United Kingdom).

25 FIG. 3B Electron-microscopy analysis of purified CMVwt VLPs. For the morphological analysis of VLPs the JEM-1230 electron microscope (Jeol Ltd., Tokyo, Japan) was used.

30 FIG. 4 Mass spectrometric analysis of purified CMV-derived VLPs. Matrix-assisted laser desorption/ionization (MALDI)-TOF MS analysis was carried out on an Autoflex MS (Bruker Daltonik, Germany). The protein molecular mass (MM) calibration standard II (22.3–66.5 kDa; Bruker Daltonik) was used for mass determination.

FIG. 4A CMVwild-type ("wt"); theoretical MM=24069; found MM=24058

FIG. 4B CMV-Npadr; theoretical MM=24161 (without first Met); found MM=24160

FIG. 4C CMV-Ntt830; theoretical MM=24483 (without first Met); found MM=24477

FIG. 5A Dynamic light scattering of purified CMV-Ntt830 VLPs. The size of particles was detected by using Zetasizer Nano ZS (Malvern Instruments Ltd., United Kingdom).

5 FIG. 5B Electron-microscopy analysis of purified CMV-Ntt830 VLPs. For the morphological analysis of VLPs the JEM-1230 electron microscope (Jeol Ltd., Tokyo, Japan) was used.

FIG. 6A Dynamic light scattering of purified CMV-Npadr VLPs. The size of particles was detected by using Zetasizer Nano ZS (Malvern Instruments Ltd., United Kingdom).

10 FIG. 6B Electron-microscopy analysis of purified CMV-Npadr VLPs. For the morphological analysis of VLPs the JEM-1230 electron microscope (Jeol Ltd., Tokyo, Japan) was used.

FIG. 7A The antibody response was measured after immunization of CMV wild type VLP. Groups of mice were immunized with either 10 µg, 1 µg or 0.1 µg of CMV-wt VLP on day 0 and 7. The specific total IgG titer was measured on day 0 (pre-immune), 7, 14 and 28. Mice per group = 4.

FIG. 7B The antibody response was measured after immunization of CMV-Npadr VLP. Groups of mice were immunized with either 10 µg, 1 µg or 0.1 µg of CMV-Npadr VLP on day 0 and 7. The specific total IgG titer was measured on day 0 (pre-immune), 7, 14 and 28. Mice per group = 4.

FIG. 7C The antibody response was measured after immunization of CMV-Ntt830 VLP. Groups of mice were immunized with either 10 µg, 1 µg or 0.1 µg of CMV-Ntt830 VLP on day 0 and 7. The specific total IgG titer was measured on day 0 (pre-immune), 7, 14 and 28. Mice per group = 4.

FIG. 8A The humoral response against the allergen Fel D1 was detected in mice. The major cat allergen Fel D1 was coupled to CMV Npadr. Mice were immunized with 5 µg of Fel D1-VLP on day 0 and 7. Fel D1 specific antibodies were measured at indicated time points. Mice per group = 5.

FIG. 8B The humoral response against the allergen Fel D1 was detected in mice. The major cat allergen Fel D1 was coupled to CMV Ntt830. Mice were immunized with 5 µg of Fel D1-VLP on day 0 and 7. Fel D1 specific antibodies were measured at indicated time points. Mice per group = 5

FIG. 9 The humoral response against α -synuclein derived peptide is shown. Mice were immunized on day 0 and 14 with 20 μ g α -synuclein derived peptide coupled to CMV Ntt830. The peptide specific antibody titer was measured at indicated time points. Number of mice = 4.

FIG. 10 The antibody response against the self protein IL17 was detected in mice. The cytokine IL17 was coupled to CMV Ntt830. Mice were immunized with 20 μ g of IL17-CMV Ntt830 on day 0 and 14. IL17 specific antibodies were measured at indicated time points. Number of mice = 3.

FIG. 11A SDS/PAGE analysis of expression and purification of F12H6GGC protein from *E.coli* C2566 cells, using PrepEase kit (USB). M - protein size marker; S - soluble protein fraction; P - cell debris; F - Flow through from Ni-IDA column (unbound proteins); W1, W2 - Wash fractions (2x2ml 1xLEW buffer) W3, W4 Wash fractions (2x2ml 1xLEW + 10 mM imidazole); E1, E2 - Elution fractions (2x1.5ml E buffer 250 mM imidazole).

FIG. 11B Mass spectrometric analysis of purified F12H6GGC. The calculated average mass of the F12H6GGC corresponds to 20089.8 Da. The observed mass of 20105.3 corresponds to F12H6GGC where one sulfur of a methionine is oxidized to a sulfoxide group.

FIG. 11C Coomassie Blue stained SDS-PAGE analysis of purification of F12GGC. (A) AmS - dissolved precipitate after 50% $(\text{NH}_4)_2\text{SO}_4$. Various fractions from the DEAE column procedure and subsequent purification by MonoQ: FT – flow through, A4-A7 - fractions eluted by increasing NaCl gradient (B) Final purification by Butyl HP column (HIC).

FIG. 12 A sandwich ELISA supplied from Indoor Biotechnologies using mAbs raised against the natural Fel d1 is shown. The mAbs recognize F12H6GGC and natural Fel d1 equally well.

FIG. 13A Basophil activation test (BAT) for natural Fel d1.

FIG. 13B Basophil activation test (BAT) for F12H6GGC. F12H6GGC and natural Fel d1 induce similar activation levels of basophils in blood from cat allergic patients indicated by the up-regulation of CD63 on CCR3+ basophils.

FIG. 14 Antibody response of mice which received 10 μ g of either Fel d1-CMV VLPs (Fel d1-CMV-Ntt830-VLP or Fel d1-CMV-Npadr-VLP) or CMV-VLPs (CMV-Ntt830-VLP or CMV-Npadr-VLP) simply mixed with Fel d1 fusion protein F12H6GGC on day 0 and day 14. Serum was collected on day 0, 14

and 21 and analyzed by ELISA for natural Fel d1 specific IgG-antibodies.
N=3.

DETAILED DESCRIPTION OF THE INVENTION

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

Virus-like particle (VLP): The term "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
10 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. A virus-like particle in accordance with the invention is non-replicative and non-infectious since it lacks all or part of the viral genome or genome function. A virus-like particle in accordance with the
15 invention may contain nucleic acid distinct from their genome. Recombinantly produced virus-like particles typically contain host cell derived RNA. A typical and preferred embodiment of a virus-like particle in accordance with the present invention is a viral capsid composed of polypeptides of the invention. A virus-like particle is typically a macromolecular assembly composed of viral coat protein which typically comprises 60, 120, 180, 240, 300, 360, or more
20 than 360 protein subunits per virus-like particle. 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. One feature of a virus-like particle is its highly ordered and repetitive arrangement of its subunits.

Virus-like particle of CMV: The terms "virus-like particle of CMV "or CMV VLPs
25 refer to a virus-like particle comprising, or preferably consisting essentially of, or preferably consisting of at least one CMV polypeptide. Preferably, a virus-like particle of CMV comprises said CMV polypeptide as the major, and even more preferably as the sole protein component of the capsid structure. Typically and preferably, virus-like particles of CMV resemble the structure of the capsid of CMV. Virus-like particles of CMV are non-replicative and/or non-infectious,
30 and lack at least the gene or genes encoding for the replication machinery of the CMV, and typically also lack the gene or genes encoding the protein or proteins responsible for viral attachment to or entry into the host. This definition includes also virus-like particles in which the aforementioned gene or genes are still present but inactive. Preferred methods to render a virus-

like particle of CMV non replicative and/or non-infectious is by physical or chemical inactivation, such as UV irradiation, formaldehyde treatment. Preferably, VLPs of CMV lack the gene or genes encoding for the replication machinery of the CMV, and also lack the gene or genes encoding the protein or proteins responsible for viral attachment to or entry into the host.

5 Again more preferably, non-replicative and/or non-infectious virus-like particles are obtained by recombinant gene technology. Recombinantly produced virus-like particles of CMV according to the invention typically and preferably do not comprise the viral genome. Virus-like particles comprising more than one species of polypeptides, often referred to as mosaic VLPs are also encompassed by the invention. Thus, in one embodiment, the virus-like particle according to the
10 invention comprises at least two different species of polypeptides, wherein at least one of said species of polypeptides is a CMV polypeptide. Preferably, a VLP of CMV is a macromolecular assembly composed of CMV coat protein which typically comprises 180 coat protein subunits per VLP. Typically and preferably, a VLP of CMV as used herein, comprises, essentially consists of, or alternatively consists of, at least one CMV polypeptide comprising or preferably
15 consisting of (i) an amino acid sequence of a coat protein of CMV; or (ii) a mutated amino acid sequence, wherein the amino acid sequence to be mutated is an amino acid sequence of a coat protein of CMV, and wherein said mutated amino acid sequence and said amino acid sequence to be mutated show a sequence identity of at least 90 %, preferably of at least 95%, further preferably of at least 98% and again more preferably of at least 99%.

20 Polypeptide: The term “polypeptide” as used herein refers to a polymer composed of amino acid monomers which are linearly linked by peptide bonds (also known as amide bonds). The term polypeptide refers to a consecutive chain of amino acids and does not refer to a specific length of the product. Thus, peptides, and proteins are included within the definition of polypeptide.

25 Cucumber Mosaic Virus (CMV) polypeptide: The term “cucumber mosaic virus (CMV) polypeptide” as used herein refers to a polypeptide comprising or preferably consisting of: (i) an amino acid sequence of a coat protein of cucumber mosaic virus (CMV), or (ii) a mutated amino acid sequence, wherein the amino acid sequence to be mutated is an amino acid sequence of a coat protein of CMV, and wherein said mutated amino acid sequence and said amino acid
30 sequence to be mutated, i.e. said coat protein of CMV, show a sequence identity of at least 90 %, preferably of at least 95%, further preferably of at least 98% and again more preferably of at least 99%. Typically and preferably, the CMV polypeptide is capable of forming a virus-like particle of CMV upon expression by self-assembly.

Coat protein (CP) of cucumber mosaic virus (CMV): The term “coat protein (CP) of cucumber mosaic virus (CMV)”, as used herein, refers to a coat protein of the cucumber mosaic virus which occurs in nature. Due to extremely wide host range of the cucumber mosaic virus, a lot of different strains and isolates of CMV are known and the sequences of the coat proteins of said strains and isolates have been determined and are, thus, known to the skilled person in the art as well. The sequences of said coat proteins (CPs) of CMV are described in and retrievable from the known databases such as Genbank, www.dpvweb.net, or www.ncbi.nlm.nih.gov/protein/. Examples of described CPs of CMV are CAD92034.1, CDO33961.2, AHZ89404.1, CDO33963.1, ACN60036.1, CAC18660.1, CAC18661.1, CAC18658.1, CAC18657.1, CAC18659.1, AFS64321.1, AFS64320.1, ABG76792.1, CAI68015.1, AAQ22992.1, AEM36051.1, AEM36048.1, ADA77134.1, AIS22690.1, AGT15706.1, AFR44555.1, AHY22574.1, ACM89097.1, AAB07137.1, AAD17927.1, AAM81371.1, AAM81365.1, AAM81374.1, AAM81372.1, AAM81368.1, ABC18318.1, AAX70966.1, AAU14864.1, AHJ11230.1, AHJ11229.1, AHJ11228.1, O40980.1, CAB43510.1, Q66143.1, CAA77065.1, CAB77390.1, CAC18666.1, NP_040777.1, P16489.1, AGV39216.1, AGW51600.1, AGN56074.1, AGN56050.1, AGG16159.1, AGG16145.1, AGG16143.1, AFZ99011.1, 1407131A, CAA71834.1, CAA07411.1, CAC18665.1, Q66135.1, 1F15_A, O40983.1, Q00259.1, AER25350.1, AER25348.1, ADN84922.1, ABU95611.1, BAF93916.1, BAF93914.1, AAY46239.1, AAY46233.1, AAY46235.1, AAY46231.1, AAY46230.1, AAY46237.1, BAF45130.1, ABM46611.1, ABC00925.1, AAY42625.1, CAH25533.1, CAH25541.1, CAH25539.1, AAV63980.1, AAQ89596.1, AAQ89571.1, AAQ89570.1, AAQ83697.1, AAQ83689.1, AAK52423.1, CCN27449.1, CEF39516.1, CAE51926.1, CAC13146.1, BAM15840.1, AEK33396.1, ABY21417.1, ABM89156.1, CAI84629.1, AGA20617.1, AAO17725.1, CAD42338.1, ABO18585.1, CDF77337.1, CAG25710.1, CAG25430.1, CAE30336.1, BAF45378.1, AAD17925.1, AGV39211.1, BAA07858.1, Q66141.1, O40981.1, ACB87210.1, CAI77626.1, CAH25521.1, ABG89138.1, AGT15712.1, AGT15703.1, AII01134.1, AIC76579.1, AIC76576.1, AIC76574.1, AIC76573.1, AIB09173.1, AIB09172.1, ADF81043.1, ACQ99349.1, ACQ99348.1, CBF03403.1, CBF03405.1, CAX45872.1, CAX45871.1, ACH48048.1, AAG01451.1, AAA46409.1, AAD45249.1, AAG25053.1, AAA46410.1, AAA46411.1, AAA46412.1, ABB89052.1, AAY19285.1, AAW21981.1, AAW21983.1, AAA74483.1, AHL30195.1, BAA95601.1, Q00261.1, CAB89799.1, AGZ63887.1, BAN67666.1, AFZ62498.1, AFZ62495.1, AFZ62494.1, AGN56098.1, AGN56028.1, AGG16155.1, AGG16151.1, ACS83814.1, ACS83810.1, ACS83800.1, ACS83791.1, CCM80413.1, CCM80411.1, CCM80409.1, AFX68432.1,

AFX68428.1, CAI39235.1, CAJ20021.1, CAE51924.1, AAS57946.1, Q66154.1, Q00260.1, CAA61802.1, P21368.1, BAB11693.1, AFV99523.1, AFV99515.1, AER25351.1, AFM56037.1, AFJ92022.1, AFH88687.1, AFC40207.1, BAL63166.1, AFA53169.1, AFA53167.1, BAL61194.1, AEU12505.1, AER35119.1, AEK86513.1, AEK69525.1, AEK69524.1, 5 AEK33395.1, AEK33393.1, AEG79911.1, ADZ54767.1, ADZ54111.1, ADX86746.1, ADN84923.1, ACR14828.1, ACR14827.1, ABU62578.1, ACA13281.1, ABZ80826.1, ABY47903.1, ABY21424.1, ABY21420.1, ABY21419.1, ABY21418.1, ABY21413.1, ABX38992.1, ABV55389.1, ABV49618.1, ABO18588.1, ABO18589.1, ABN72590.1, ABN72591.1, ABN12319.1, ABN12316.1, BAF44939.1, ABM46612.1, CAJ15158.1, 10 ABC00922.1, ABC00921.1, ABC00928.1, ABC00923.1, ABI94145.1, ABI94151.1, ABI94129.1, ABI94150.1, BAF33384.1, ABI93181.1, ABE68905.1, ABC00927.1, AAS48545.1, AAR89470.1, AAR23529.1, AAQ82698.1, AAM95241.1, AAM97677.1, AAK52977.1, AAW71967.1, ABJ55780.1, CAH17693.1, CAH17700.1, CAH17692.1, Q66140.1, ABM63376.1, AGT15704.1, CAB41491.1, AAR89478.1, CCJ09634.1, 15 AEK69527.1, ACS83801.1, AIC76582.1, AHJ58884.1, AHJ11231.1, AGJ94707.1, ACS83798.1, ACS83793.1, ABK81652.1, AAS57945.1, AAN04483.1, ADO66659.1, AAN17777.1, ADG26754.1, AFV99520.1, ABD73006.1, ACE07024.1, ABD64220.1, ABD72575.1, ABP87978.1, ABN13961.1, AAY85627.1, AGG16139.1, ACS83811.1, CAH17699.1, CAH17694.1, AFV69240.1, ACB56602.1, ADJ10637.1, ABM46614.1, 20 AAY45749.1, AAA74484.1, CAH17697.1, ADA63484.1, ADA63482.1, ADA63480.1, Q83251.1, AIC76584.1, AIC76583.1, AAK27169.1, ABC00924.1, ACD62520.1, ABO18591.1, AAO62575.1, AAL48223.1, AIA99525.1, CAH17698.1, AAZ38725.1, AAF09246.2, CAH17701.1, ACB58305.1, AGG16158.1, CAH17695.1, AHJ58883.1, AEZ03836.1, BAB63959.1, ACD62519.1, BAF93912.1, AAZ78354.1, ACN38326.1, AFV99522.1, 25 ADZ54109.1, CAA46151.1, AGV39213.1. Further examples of CMV coat proteins are provided in SEQ ID NOs 1-3.

It is noteworthy that these strains and isolates have highly similar coat protein sequences at different protein domains, including the N-terminus of the coat protein. In particular, 98.1% of all completely sequenced CMV isolates share more than 85% sequence identity within the first 30 28 amino acids of their coat protein sequence, and still 79.5% of all completely sequenced CMV isolates share more than 90% sequence identity within the first 28 amino acids of their coat protein sequence.

Typically and preferably, the coat protein of CMV used for the present invention is capable of forming a virus-like particle of CMV upon expression by self-assembly. Preferably,

the coat protein of CMV used for the present invention is capable of forming a virus-like particle of CMV upon expression by self-assembly in *E.coli*.

SEQ ID NO. 1 corresponds to the amino acid sequence of the CP isolated and cloned from CMV-infected lily leaves collected from a private garden in Riga, Latvia. Thus, preferably, the term “coat protein of cucumber mosaic virus (CMV)”, as used herein, refers to an amino acid sequence of a coat protein of CMV, wherein said amino acid sequence comprises, or preferably consists of, SEQ ID NO:1 or an amino acid sequence having a sequence identity of at least 75%, preferably of at least 80%, more preferably of at least 85%, again further preferably of at least 90 %, again more preferably of at least 95%, still further preferably of at least 98% and still again further more preferably of at least 99% of SEQ ID NO:1.

In a further very preferred embodiment, said coat protein of CMV is an amino acid sequence of a coat protein of CMV, wherein said amino acid sequence comprises SEQ ID NO:21 being the consecutive amino acids 2-27 of SEQ ID NO:1, or an amino acid sequence of a coat protein of CMV comprising an amino acid sequence region, wherein said amino acid sequence region has a sequence identity of at least 75%, preferably of at least 80%, more preferably of at least 85%, again further preferably of at least 90 %, again more preferably of at least 95%, still further preferably of at least 98% and still again further more preferably of at least 99% with SEQ ID NO:21. This is in particular preferred since the Th cell epitope preferably replaces a N-terminal region of the CMV polypeptide of the invention. A high sequence identity at the N-terminus of the CPs of the CMV to the one preferably used in the Examples, now ensures that proper assembly upon expression of the CPs can take place beside replacement of said N-terminal region by the typically aggregation-causing Th cell epitope.

In an again further very preferred embodiment, said coat protein of CMV is (a) an amino acid sequence of a coat protein of CMV, wherein said amino acid sequence comprises, or preferably consists of, SEQ ID NO:1 or (b) an amino acid sequence having a sequence identity of at least 75%, preferably of at least 80%, more preferably of at least 85%, again further preferably of at least 90 %, again more preferably of at least 95%, still further preferably of at least 98% and still again further more preferably of at least 99% of SEQ ID NO:1; and wherein said amino sequence as defined in (a) or (b) comprises SEQ ID NO:21 being the consecutive amino acids 2-27 of SEQ ID NO: 1; or wherein said amino sequence as defined in (a) or (b) comprises an amino acid sequence region, wherein said amino acid sequence region has a sequence identity of at least 75%, preferably of at least 80%, more preferably of at least 85%, again further preferably of at least 90 %, again more preferably of at least 95%, still further

preferably of at least 98% and still again further more preferably of at least 99% with SEQ ID NO:21.

Modified virus-like particle (VLP) of cucumber mosaic virus (CMV): The term “modified virus-like particle (VLP) of cucumber mosaic virus (CMV)” as used herein, refers to a VLP of CMV which is a modified one in such as it comprises, or preferably consists essentially of, or preferably consists of at least one modified CMV polypeptide, wherein said modified CMV polypeptide comprises, or preferably consists of, a CMV polypeptide, and a T helper cell epitope. Typically and preferably, said T helper cell epitope (i) is fused to the N-terminus of said CMV polypeptide, (ii) is fused to the C-terminus of said CMV polypeptide, (iii) replaces a region of consecutive amino acids of said CMV polypeptide, wherein the sequence identity between said replaced region of consecutive amino acids of said CMV polypeptide and the T helper cell epitope is at least 15%, preferably at least 20%, or (iv) replaces a N-terminal region of said CMV polypeptide, and wherein said replaced N-terminal region of said CMV polypeptide consists of 5 to 15 consecutive amino acids. Preferably, said T helper cell epitope replaces a N-terminal region of said CMV polypeptide, and wherein said replaced N-terminal region of said CMV polypeptide consists of 5 to 15 consecutive amino acids, preferably of 9 to 14 consecutive amino acids, more preferably of 11 to 13 consecutive amino acids, and most preferably of 11, 12 or 13 consecutive amino acids. Preferably said modified VLP of CMV of the present invention is a recombinant modified VLP of CMV.

Modified CMV polypeptide: The term “modified CMV polypeptide” as used herein refers to a CMV polypeptide modified in such as defined herein, that said modified CMV polypeptide comprises, or preferably consists of, a CMV polypeptide, and a T helper cell epitope. Typically, the modified CMV polypeptide is capable of forming a virus-like particle of CMV upon expression by self-assembly. Preferably, the modified CMV polypeptide is a recombinant modified CMV polypeptide and is capable of forming a virus-like particle of CMV upon expression by self-assembly in *E.coli*.

N-terminal region of the CMV polypeptide: The term “N-terminal region of the CMV polypeptide” as used herein, refers either to the N-terminus of said CMV polypeptide, and in particular to the N-terminus of a coat protein of CMV, or to the region of the N-terminus of said CMV polypeptide or said coat protein of CMV but starting with the second amino acid of the N-terminus of said CMV polypeptide or said coat protein of CMV if said CMV polypeptide or said coat protein comprises a N-terminal methionine residue. Preferably, in case said CMV polypeptide or said coat protein comprises a N-terminal methionine residue, from a practical point of view, the start-codon encoding methionine will usually be deleted and added to the N-

terminus of the Th cell epitope. Further preferably, one, two or three additional amino acids, preferably one amino acid, may be optionally inserted between the stating methionine and the Th cell epitope for cloning purposes. The term "N-terminal region of the mutated amino acid sequence of a CMV polypeptide or a CMV coat protein" as used herein, refers either to the N-terminus of said mutated amino acid sequence of said CMV polypeptide or said coat protein of CMV, or to the region of the N-terminus of said mutated amino acid sequence of said CMV polypeptide or said coat protein of CMV but starting with the second amino acid of the N-terminus of said mutated amino acid sequence of said CMV polypeptide or said coat protein of CMV if said mutated amino acid sequence comprises a N-terminal methionine residue. Preferably, in case said CMV polypeptide or said coat protein comprises a N-terminal methionine residue, from a practical point of view, the start-codon encoding methionine will usually be deleted and added to the N-terminus of the Th cell epitope. Further preferably, one, two or three additional amino acids, preferably one amino acid, may be optionally inserted between the stating methionine and the Th cell epitope for cloning purposes.

Recombinant polypeptide: In the context of the invention the term "recombinant polypeptide" refers to a polypeptide which is obtained by a process which comprises at least one step of recombinant DNA technology. Typically and preferably, a recombinant polypeptide is produced in a prokaryotic expression system. It is apparent for the artisan that recombinantly produced polypeptides which are expressed in a prokaryotic expression system such as *E. coli* may comprise an N-terminal methionine residue. The N-terminal methionine residue is typically cleaved off the recombinant polypeptide in the expression host during the maturation of the recombinant polypeptide. However, the cleavage of the N-terminal methionine may be incomplete. Thus, a preparation of a recombinant polypeptide may comprise a mixture of otherwise identical polypeptides with and without an N-terminal methionine residue. Typically and preferably, a preparation of a recombinant polypeptide comprises less than 10 %, more preferably less than 5 %, and still more preferably less than 1 % recombinant polypeptide with an N-terminal methionine residue.

Recombinant CMV polypeptide: The term "recombinant CMV polypeptide" refers to a CMV polypeptide as defined above which is obtained by a process which comprises at least one step of recombinant DNA technology. Typically and preferably a preparation of a recombinant CMV polypeptide comprises less than 10 %, more preferably less than 5 %, and still more preferably less than 1 % recombinant CMV polypeptide with an N-terminal methionine residue. Consequently, a recombinant virus-like particle of the invention may comprise otherwise identical recombinant polypeptides with and without an N-terminal methionine residue.

Recombinant modified CMV polypeptide: The term "recombinant modified CMV polypeptide" refers to a modified CMV polypeptide as defined above which is obtained by a process which comprises at least one step of recombinant DNA technology. Typically and preferably a preparation of a recombinant modified CMV polypeptide comprises less than 10 %, more preferably less than 5 %, and still more preferably less than 1 % recombinant modified CMV polypeptide with an N-terminal methionine residue. Consequently, a recombinant virus-like particle of the invention may comprise otherwise identical recombinant polypeptides with and without an N-terminal methionine residue.

Recombinant virus-like particle: In the context of the invention the term "recombinant virus-like particle" refers to a virus-like particle (VLP) which is obtained by a process which comprises at least one step of recombinant DNA technology. Typically and preferably, a recombinant virus-like particle comprises at least one recombinant polypeptide, preferably a recombinant CMV polypeptide or recombinant modified CMV polypeptide. Most preferably, a recombinant virus-like particle is composed of or consists of recombinant CMV polypeptides or recombinant modified CMV polypeptides. As a consequence, if in the context of the present invention the definition of inventive recombinant VLPs are effected with reference to specific amino acid sequences comprising a N-terminal methionine residue the scope of these inventive recombinant VLPs encompass the VLPs formed by said specific amino acid sequences without said N-terminal methionine residue but as well, even though typically in a minor amount as indicated herein, the VLPs formed by said specific amino acid sequences with said N-terminal methionine. Furthermore, it is within the scope of the present invention that if the definition of inventive recombinant VLPs are effected with reference to specific amino acid sequences comprising a N-terminal methionine residue VLPs are encompassed comprising both amino acid sequences comprising still said N-terminal methionine residue and amino acid sequences lacking the N-terminal methionine residue.

Mutated amino acid sequence: The term "mutated amino acid sequence" refers to an amino acid sequence which is obtained by introducing a defined set of mutations into an amino acid sequence to be mutated. In the context of the invention, said amino acid sequence to be mutated typically and preferably is an amino acid sequence of a coat protein of CMV. Thus, a mutated amino acid sequence differs from an amino acid sequence of a coat protein of CMV in at least one amino acid residue, wherein said mutated amino acid sequence and said amino acid sequence to be mutated show a sequence identity of at least 90 %. Typically and preferably said mutated amino acid sequence and said amino acid sequence to be mutated show a sequence identity of at least 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, or 99 %. Preferably, said

mutated amino acid sequence and said sequence to be mutated differ in at most 11, 10, 9, 8, 7, 6, 4, 3, 2, or 1 amino acid residues, wherein further preferably said difference is selected from insertion, deletion and amino acid exchange. Preferably, the mutated amino acid sequence differs from an amino acid sequence of a coat protein of CMV in least one amino acid, wherein
5 preferably said difference is an amino acid exchange.

Position corresponding to residues...: The position on an amino acid sequence, which is corresponding to given residues of another amino acid sequence can be identified by sequence alignment, typically and preferably by using the BLASTP algorithm, most preferably using the standard settings. Typical and preferred standard settings are: expect threshold: 10; word size: 3;
10 max matches in a query range: 0; matrix: BLOSUM62; gap costs: existence 11, extension 1; compositional adjustments: conditional compositional score matrix adjustment.

Sequence identity: The sequence identity of two given amino acid sequences is determined based on an alignment of both sequences. Algorithms for the determination of sequence identity are available to the artisan. Preferably, the sequence identity of two amino acid
15 sequences is determined using publicly available computer homology programs such as the "BLAST" program (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) or the "CLUSTALW" (<http://www.genome.jp/tools/clustalw/>), and hereby preferably by the "BLAST" program provided on the NCBI homepage at <http://blast.ncbi.nlm.nih.gov/Blast.cgi>, using the default settings provided therein. Typical and preferred standard settings are: expect threshold: 10; word
20 size: 3; max matches in a query range: 0; matrix: BLOSUM62; gap costs: existence 11, extension 1; compositional adjustments: conditional compositional score matrix adjustment.

Amino acid exchange: The term amino acid exchange refers to the exchange of a given amino acid residue in an amino acid sequence by any other amino acid residue having a different chemical structure, preferably by another proteinogenic amino acid residue. Thus, in contrast to
25 insertion or deletion of an amino acid, the amino acid exchange does not change the total number of amino acids of said amino acid sequence. Very preferred in the context of the invention is the exchange of an amino acid residue of said amino acid sequence to be mutated by a lysine residue or by a cysteine residue.

Epitope: The term epitope refers to continuous or discontinuous portions of an antigen,
30 preferably a polypeptide, wherein said portions can be specifically bound by an antibody or by a T-cell receptor within the context of an MHC molecule. With respect to antibodies, specific binding excludes non-specific binding but does not necessarily exclude cross-reactivity. An epitope typically comprise 5-20 amino acids in a spatial conformation which is unique to the antigenic site.

T helper (Th) cell epitope: The term “T helper (Th) cell epitope” as used herein refers to an epitope that is capable of recognition by a helper Th cell.

Universal Th cell epitope: The term “universal Th cell epitope” as used herein refers to a Th cell epitope that is capable of binding to at least one, preferably more than one MHC class II molecules. The simplest way to determine whether a peptide sequence is a universal Th cell epitope is to measure the ability of the peptide to bind to individual MHC class II molecules. This may be measured by the ability of the peptide to compete with the binding of a known Th cell epitope peptide to the MHC class II molecule. A representative selection of HLA-DR molecules are described in e.g. Alexander J, et al., *Immunity* (1994) 1:751-761. Affinities of Th cell epitopes for MHC class II molecules should be at least 10^{-5} M. An alternative, more tedious but also more relevant way to determine the “universality” of a Th cell epitope is the demonstration that a larger fraction of people (>30%) generate a measurable T cell response upon immunization and boosting one months later with a protein containing the Th cell epitope formulated in IFA. A representative collection of MHC class II molecules present in different individuals is given in Panina-Bordignon P, et al., *Eur J Immunol* (1989) 19:2237-2242. As a consequence, the term “universal Th cell epitope” as used herein preferably refers to a Th cell epitope that generates a measurable T cell response upon immunization and boosting (one months later with a protein containing the Th cell epitope formulated in IFA) in more than 30% of a selected group of individuals as described in Panina-Bordignon P, et al., *Eur J Immunol* (1989) 19:2237-2242. Moreover, and again further preferred, the term “universal Th cell epitope” as used herein preferably refers to a Th cell epitope that is capable of binding to at least one, preferably to at least two, and even more preferably to at least three DR alleles selected from of DR1, DR2w2b, DR3, DR4w4, DR4w14, DR5, DR7, DR52a, DRw53, DR2w2a; and preferably selected from DR1, DR2w2b, DR4w4, DR4w14, DR5, DR7, DRw53, DR2w2a, with an affinity at least 500nM (as described in Alexander J, et al., *Immunity* (1994) 1:751-761 and references cited herein); a preferred binding assay to evaluate said affinities is the one described by Sette A, et al., *J Immunol* (1989) 142:35-40. In an even again more preferable manner, the term “universal Th cell epitope” as used herein refers to a Th cell epitope that is capable of binding to at least one, preferably to at least two, and even more preferably to at least three DR alleles selected from DR1, DR2w2b, DR4w4, DR4w14, DR5, DR7, DRw53, DR2w2a, with an affinity at least 500nM (as described in Alexander J, et al., *Immunity* (1994) 1:751-761 and references cited herein); a preferred binding assay to evaluate said affinities is the one described by Sette A, et al., *J Immunol* (1989) 142:35-40.

Universal Th cell epitopes are described, and known to the skilled person in the art, such as by Alexander J, et al., *Immunity* (1994) 1:751-761, Panina-Bordignon P, et al., *Eur J Immunol* (1989) 19:2237-2242, Calvo-Calle JM, et al., *J Immunol* (1997) 159:1362-1373, and Valmori D, et al., *J Immunol* (1992) 149:717-721.

5 In a preferred embodiment of the present invention, the Th cell epitope is selected from HA 307-319 (SEQ ID NO:26), HBV_{nc} 50-69 (SEQ ID NO:27), TT 830-843 (SEQ ID NO:4), CS 378-398 (SEQ ID NO:28), MT 17-31 (SEQ ID NO:29), TT 947-967 (SEQ ID NO:30) and PADRE (SEQ ID NO:5). In another preferred embodiment of the present invention, the universal Th cell epitope is selected from HA 307-319 (SEQ ID NO:26), HBV_{nc} 50-69 (SEQ ID
10 NO:27), TT 830-843 (SEQ ID NO:4), CS 378-398 (SEQ ID NO:28), MT 17-31 (SEQ ID NO:29), TT 947-967 (SEQ ID NO:30) and PADRE (SEQ ID NO:5). In a very preferred of the present invention, the Th cell epitope is TT 830-843 (SEQ ID NO:4) or PADRE (SEQ ID NO:5). In another very preferred embodiment of the present invention, the universal Th cell epitope is TT 830-843 (SEQ ID NO:4) or PADRE (SEQ ID NO:5). Therefore, in a very preferred
15 embodiment, said Th cell epitope is TT 830-843 (SEQ ID NO:4). In another very preferred embodiment of the present invention, said Th cell epitope is PADRE (SEQ ID NO:5). In another very preferred embodiment, said universal Th cell epitope is TT 830-843 (SEQ ID NO:4). In another very preferred embodiment of the present invention, said universal Th cell epitope is PADRE (SEQ ID NO:5). The epitopes of SEQ ID NO:4 and SEQ ID NO:5 are known and even
20 applications thereof have been described (Alexander J, et al., *Immunity* (1994) 1:751-761; Panina-Bordignon P, et al., *Eur J Immunol* (1989) 19:2237-2242; Valmori D, et al., *J Immunol* (1992) 149:717-721; Lanzavecchia A, et al. *Eur J Immunol* (1983) 13:733-738; Jemon K, et al., *PLoS ONE* (2013) 8(6): e66866; Jagu S, et al., *PLoS ONE* (2012) 8(1): e55538.

Coupling efficiency: The coupling efficiency of a virus-like particle with a specific
25 antigen is determined by SDS-PAGE of the coupling reactions. The intensities of Coomassie Blue-stained bands corresponding to components of the coupling reaction are determined by densitometry and used to calculate coupling efficiency. Coupling efficiency is defined as the ratio of VLP polypeptide coupled to said antigen to the total amount of VLP polypeptide.

Adjuvant: The term "adjuvant" as used herein refers to non-specific stimulators of the
30 immune response or substances that allow generation of a depot in the host which when combined with the vaccine and pharmaceutical composition, respectively, of the present invention may provide for an even more enhanced immune response. Preferred adjuvants are complete and incomplete Freund's adjuvant, aluminum containing adjuvant, preferably aluminum hydroxide, and modified muramyldipeptide. Further preferred adjuvants are mineral

gels such as aluminum hydroxide, surface active substances such as lyso lecithin, 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 may also comprise mixtures of these substances. Virus-like particles 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 inventive virus-like particle. Rather "adjuvant" relates to an additional, distinct component of the inventive compositions, vaccines or pharmaceutical compositions.

Antigen: As used herein, the term "antigen" refers to a molecule capable of being bound by an antibody or a T-cell receptor (TCR) if presented by MHC molecules. The term "antigen", as used herein, also refers to T-cell epitopes. An antigen is additionally capable of being recognized by the immune system and/or being capable of inducing a humoral immune response and/or cellular immune response leading to the activation of B- and/or T- lymphocytes. This may, however, require that, at least in certain cases, the antigen contains or is linked to a Th cell epitope and is given in adjuvant. An antigen can have one or more epitopes (B- and T-epitopes). The specific reaction referred to above is meant to indicate that the antigen will preferably react, typically in a highly selective manner, with its corresponding antibody or TCR and not with the multitude of other antibodies or TCRs which may be evoked by other antigens. Antigens as used herein may also be mixtures of several individual antigens. The polypeptide of the invention which is forming the inventive virus-like particles, may comprise the antigen. In particular, the inventive polypeptide may be a fusion product comprising the antigen. However, the term antigen does not encompass said mutated amino acid sequence which is comprised by said polypeptide. Typically and preferably, the term "antigen" as used herein does not encompass the virus-like particle according to the invention. Typically and preferably the term "antigen" rather refers to an additional component of the compositions, vaccines and pharmaceutical compositions of the invention having antigenic properties, wherein the antigen may be associated, bound, mixed with or linked to the virus- like particle by any means described herein.

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, carbon-phosphorus bonds, carbon-sulfur bonds such as thioether, or imide bonds.

Attachment Site, First: As used herein, the phrase "first attachment site" refers to an element which is naturally occurring with the virus-like particle or which is artificially added to the virus-like particle, 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 residue, preferably of a lysine residue. The first attachment site is typically located on the surface, and preferably on the outer surface of the VLP. Multiple first attachment sites are present on the surface, preferably on the outer surface of the VLP, typically in a repetitive configuration. In a preferred embodiment the first attachment site is associated with the VLP, through at least one covalent bond, preferably through at least one peptide bond. In a further preferred embodiment the first attachment site is naturally occurring with the VLP. Alternatively, in a preferred embodiment the first attachment site is artificially added to the VLP. In a very preferred embodiment said first attachment site is the amino group of a lysine residue of the amino acid sequence of said VLP polypeptide.

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 antigen and to which the first attachment site may be linked. The second attachment site of the antigen 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, preferably the sulfhydryl group of the amino acid cysteine most preferably the sulfhydryl group of a cysteine residue. The term "antigen with at least one second attachment site" refers, therefore, to a construct comprising the antigen and at least one second attachment site. However, in particular for a second attachment site, which is not naturally occurring within the antigen, such a construct typically and preferably further comprises a "linker". In another preferred embodiment the second attachment site is

associated with the antigen 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 antigen. In another further preferred embodiment, the second attachment site is artificially added to the antigen through a linker, wherein said linker comprises or alternatively consists of a cysteine. Preferably, the linker is fused to the antigen by a peptide bond.

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, carbon- phosphorus bonds, carbon-sulfur bonds such as thioether, or imide bonds. In certain preferred embodiments the first attachment site and the second attachment site are linked through at least one covalent bond, preferably through at least one non-peptide bond, and even more preferably through exclusively non-peptide bond(s). The term "linked" as used herein, however, shall not only refer to a direct linkage of the at least one first attachment site and the at least one second attachment site but also, alternatively and preferably, an indirect linkage of the at least one first attachment site and the at least one second attachment site through intermediate molecule(s), and hereby typically and preferably by using at least one, preferably one, heterobifunctional cross-linker. In other preferred embodiments the first attachment site and the second attachment site are linked through at least one covalent bond, preferably through at least one peptide bond, and even more preferably through exclusively peptide bond(s).

Linker: A "linker", as used herein, either associates the second attachment site with the antigen or already comprises, essentially consists of, or consists of the second attachment site. Preferably, a "linker", as used herein, already comprises the second attachment site, typically and preferably - but not necessarily - as one amino acid residue, preferably as a cysteine residue. A preferred linkers are an amino acid linkers, i.e. linkers containing at least one amino acid residue. The term amino acid linker does not imply that such a linker consists exclusively of amino acid residues. However, a linker consisting exclusively of amino acid residues is a preferred embodiment of the invention. The amino acid residues of the linker are, preferably, composed of naturally occurring amino acids or unnatural amino acids known in the art, all-L or all-D or mixtures thereof. Further preferred embodiments of a linker in accordance with this invention are molecules comprising a sulfhydryl group or a cysteine residue and such molecules are, therefore, also encompassed within this invention. Further linkers useful for the present invention are

molecules comprising a C1-C6 alkyl-, a cycloalkyl such as a cyclopentyl or cyclohexyl, a cycloalkenyl, aryl or heteroaryl moiety. Moreover, linkers comprising preferably a C1-C6 alkyl-, cycloalkyl- (C5, C6), aryl- or heteroaryl- moiety and additional amino acid(s) can also be used as linkers for the present invention and shall be encompassed within the scope of the invention.

5 Association of the linker with the antigen is preferably by way of at least one covalent bond, more preferably by way of at least one peptide bond.

Ordered and repetitive antigen array: As used herein, the term "ordered and repetitive antigen array" refers to a repeating pattern of antigen which typically and preferably is characterized by a high order of uniformity in spacial arrangement of the antigens with respect to
10 the VLP. 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 inventive VLP, are typical and preferred examples of suitable ordered and repetitive antigen arrays which, moreover, possess strictly repetitive paracrystalline orders of antigens, preferably with spacing of 1 to 30 nanometers, preferably 2 to 15 nanometers, even more preferably 2 to 10 nanometers, even again
15 more preferably 2 to 8 nanometers, and further more preferably 1.6 to 7 nanometers.

Fel d1 protein: The term "Fel d1 protein", as used herein, refers to a protein comprising or alternatively consisting of chain 1 of Fel d1 and chain 2 of Fel d1. Preferably chain 1 of Fel d1 and chain 2 of Fel d1 are linked covalently. In one preferred embodiment, the chain 1 of Fel d1 and chain 2 of Fel d1 are linked via at least one disulfide bond. In another preferred embodiment,
20 the chain 1 and chain 2 are fused either directly or via a spacer, in which case said Fel d1 protein further comprises or alternatively consists of a spacer. Preferably the Fel d1 protein, as defined herein, consists of at most 300, even more preferably at most 200 amino acids in total. Typically and preferably, Fel d1 protein, according to the invention, is capable of inducing *in vivo* the production of antibody specifically binding to either the naturally occurring Fel d1 or the
25 recombinant Fel d1 as produced according to EXAMPLE 8 of the present invention.

Chain 1 of Fel d1: The term "chain 1 of Fel d1", as used herein, refers to a polypeptide comprising or alternatively consisting of an amino acid sequence as of SEQ ID NO:37 or a homologous sequence thereof. The term "homologous sequence of SEQ ID NO:37", as used herein, refers to a polypeptide that has an identity to SEQ ID NO:37 which is greater than 80%,
30 more preferably greater than 90%, and even more preferably greater than 95%. The term "chain 1 of Fel d1", as used herein, should also refer to a polypeptide encompassing at least one post-translational modification, including but not limited to at least one glycosylation, of chain 1 of Fel d1, as defined herein. Preferably the chain 1 of Fel d1, as defined herein, consists of at most 130, even more preferably at most 100 amino acids in total.

Chain 2 of Fel d1: The term "chain 2 of Fel d1", as used herein, refers to a polypeptide comprising or alternatively consisting of an amino acid sequence as of SEQ ID NO:38, SEQ ID NO:39 or SEQ ID NO:40, or a homologous sequence thereof. The term "homologous sequence of SEQ ID NO:38, SEQ ID NO:39 or SEQ ID NO:40, as used herein, refers to a polypeptide that has an identity to SEQ ID NO:38, SEQ ID NO:39 or SEQ ID NO:40 which is greater than 80%, more preferably greater than 90%, and even more preferably greater than 95%. The term "chain 2 of Fel d1", as used herein, should also refer to a polypeptide encompassing at least one post-translational modification, including but not limited to at least one glycosylation, of chain 2 of Fel d1, as defined herein. Preferably the chain 2 of Fel d1, as defined herein, consists of at most 150, even more preferably at most 130, still more preferably at most 100 amino acids in total.

Immunostimulatory substance: As used herein, the term "immuno stimulatory substance" refers to a substance capable of inducing and/or enhancing an immune response. Immunostimulatory substances, as used herein, include, but are not limited to, toll-like receptor activating substances and substances inducing cytokine secretion. Toll-like receptor activating substances include, but are not limited to, immuno stimulatory nucleic acids, peptideoglycans, lipopolysaccharides, lipoteichonic acids, imidazoquinoline compounds, flagellins, lipoproteins, and immuno stimulatory organic substances such as taxol.

Immunostimulatory nucleic acid: As used herein, the term immuno stimulatory nucleic acid refers to a nucleic acid capable of inducing and/or enhancing an immune response. Immunostimulatory nucleic acids comprise ribonucleic acids and in particular desoxyribonucleic acids, wherein both, ribonucleic acids and desoxyribonucleic acids may be either double stranded or single stranded. Preferred ISS-NA are desoxyribonucleic acids, wherein further preferably said desoxyribonucleic acids are single stranded. Preferably, immunostimulatory nucleic acids contain at least one CpG motif comprising an unmethylated C. Very preferred immunostimulatory nucleic acids comprise at least one CpG motif, wherein said at least one CpG motif comprises or preferably consist of at least one, preferably one, CG dinucleotide, wherein the C is unmethylated. Preferably, but not necessarily, said CG dinucleotide is part of a palindromic sequence. The term immunostimulatory nucleic acid also refers to nucleic acids that contain modified bases, preferably 4-bromo-cytosine. Specifically preferred in the context of the invention are ISS-NA which are capable of stimulating IFN- alpha production in dendritic cells. Immunostimulatory nucleic acids useful for the purpose of the invention are described, for example, in WO2007/068747A1.

Oligonucleotide: As used herein, the term "oligonucleotide" refers to a nucleic acid sequence comprising 2 or more nucleotides, preferably about 6 to about 200 nucleotides, and

more preferably 20 to about 100 nucleotides, and most preferably 20 to 40 nucleotides. Very preferably, oligonucleotides comprise about 30 nucleotides, more preferably oligonucleotides comprise exactly 30 nucleotides, and most preferably oligonucleotides consist of exactly 30 nucleotides. Oligonucleotides are polyribonucleotides or polydeoxribonucleotides and are preferably selected from (a) unmodified RNA or DNA, and (b) modified RNA or DNA. The modification may comprise the backbone or nucleotide analogues. Oligonucleotides are preferably selected from the group consisting of (a) single- and double-stranded DNA, (b) DNA that is a mixture of single- and double-stranded regions, (c) single- and double-stranded RNA, (d) RNA that is mixture of single- and double-stranded regions, and (e) hybrid molecules comprising DNA and RNA that are single-stranded or, more preferably, double- stranded or a mixture of single- and double-stranded regions. Preferred nucleotide modifications/analogues are selected from the group consisting of (a) peptide nucleic acid, (b) inosin, (c) tritylated bases, (d) phosphorothioates, (e) alkylphosphorothioates, (f) 5-nitroindole desoxyribofliranosyl, (g) 5-methyldeoxycytosine, and (h) 5,6-dihydro-5,6- dihydroxydesoxythymidine. Phosphothioated nucleotides are protected against degradation in a cell or an organism and are therefore preferred nucleotide modifications. Unmodified oligonucleotides consisting exclusively of phosphodiester bound nucleotides, typically are more active than modified nucleotides and are therefore generally preferred in the context of the invention. Most preferred are oligonucleotides consisting exclusively of phosphodiester bound deoxinucleo tides, wherein further preferably said oligonucleotides are single stranded. Further preferred are oligonucleotides capable of stimulating IFN-alpha production in cells, preferably in dendritic cells. Very preferred oligonucleotides capable of stimulating IFN-alpha production in cells are selected from A-type CpGs and C-type CpGs. Further preferred are RNA-molecules without a Cap.

CpG motif: As used herein, the term "CpG motif refers to a pattern of nucleotides that includes an unmethylated central CpG, i.e. the unmethylated CpG dinucleotide, in which the C is unmethylated, surrounded by at least one base, preferably one or two nucleotides, flanking (on the 3' and the 5' side of) the central CpG. Typically and preferably, the CpG motif as used herein, comprises or alternatively consists of the unmethylated CpG dinucleotide and two nucleotides on its 5 ' and 3 ' ends. Without being bound by theory, the bases flanking the CpG confer a significant part of the activity to the CpG oligonucleotide.

Unmethylated CpG-containing oligonucleotide: As used herein, the term "unmethylated CpG-containing oligonucleotide" or "CpG" refers to an oligonucleotide, preferably to an oligodesoxynucleotide, containing at least one CpG motif. Thus, a CpG contains at least one unmethylated cytosine, guanine dinucleotide. Preferred CpGs stimulate/activate, e.g. have a

mitogenic effect on, or induce or increase cytokine expression by, a vertebrate bone marrow derived cell. For example, CpGs can be useful in activating B cells, NK cells and antigen-presenting cells, such as dendritic cells, monocytes and macrophages. Preferably, CpG relates to an oligodesoxynucleotide, preferably to a single stranded oligodesoxynucleotide, containing an unmethylated cytosine followed 3' by a guanosine, wherein said unmethylated cytosine and said guanosine are linked by a phosphate bond, wherein preferably said phosphate bound is a phosphodiester bound or a phosphothioate bound, and wherein further preferably said phosphate bond is a phosphodiester bound. CpGs can include nucleotide analogs such as analogs containing phosphorothio ester bonds and can be double-stranded or single-stranded. Generally, double-stranded molecules are more stable in vivo, while single-stranded molecules have increased immune activity. Preferably, as used herein, a CpG is an oligonucleotide that is at least about ten nucleotides in length and comprises at least one CpG motif, wherein further preferably said CpG is 10 to 60, more preferably 15 to 50, still more preferably 20 to 40, still more preferably about 30, and most preferably exactly 30 nucleotides in length. A CpG may consist of methylated and/or unmethylated nucleotides, wherein said at least one CpG motif comprises at least one CG dinucleotide wherein the C is unmethylated. The CpG may also comprise methylated and unmethylated sequence stretches, wherein said at least one CpG motif comprises at least one CG dinucleotide wherein the C is unmethylated. Very preferably, CpG relates to a single stranded oligodesoxynucleotide containing an unmethylated cytosine followed 3' by a guanosine, wherein said unmethylated cytosine and said guanosine are linked by a phosphodiester bound. The CpGs can include nucleotide analogs such as analogs containing phosphorothioester bonds and can be double-stranded or single-stranded. Generally, phosphodiester CpGs are A-type CpGs as indicated below, while phosphothioester stabilized CpGs are B-type CpGs. Preferred CpG oligonucleotides in the context of the invention are A-type CpGs.

A-type CpG: As used herein, the term "A-type CpG" or "D-type CpG" refers to an oligodesoxynucleotide (ODN) comprising at least one CpG motif. A-type CpGs preferentially stimulate activation of T cells and the maturation of dendritic cells and are capable of stimulating IFN-alpha production. In A-type CpGs, the nucleotides of the at least one CpG motif are linked by at least one phosphodiester bond. A-type CpGs comprise at least one phosphodiester bond CpG motif which may be flanked at its 5' end and/or, preferably and, at its 3' end by phosphorothioate bound nucleotides. Preferably, the CpG motif, and hereby preferably the CG dinucleotide and its immediate flanking regions comprising at least one, preferably two nucleotides, are composed of phosphodiester nucleotides. Preferred A-type CpGs exclusively consist of phosphodiester (PO) bond nucleotides. Typically and preferably, the poly G motif

comprises or alternatively consists of at least one, preferably at least three, at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 Gs (guanosines), most preferably by at least 10 Gs. Preferably, the A-type CpG of the invention comprises or alternatively consists of a palindromic sequence.

Virus-like particle (VLP): The term virus-like particle 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 noninfectious 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. A virus-like particle in accordance with the invention is non-replicative and noninfectious since it lacks all or part of the viral genome or genome function. A virus-like particle in accordance with the invention may contain nucleic acid distinct from their genome. Recombinantly produced virus-like particles typically contain host cell derived RNA. A typical and preferred embodiment of a virus-like particle in accordance with the present invention is a viral capsid composed of polypeptides of the invention. A virus-like particle is a macro molecular assembly composed of viral coat protein which typically comprises 60, 120, 180, 240, 300, 360, or more than 360 protein subunits per virus-like particle. 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. One feature of a virus-like particle is its highly ordered and repetitive arrangement of its subunits. Virus-like particles comprising more than one species of polypeptides, often referred to as mosaic VLPs are also encompassed by the invention. Thus, in one embodiment, the virus-like particle according to the invention comprises at least two different species of polypeptides, wherein at least one of said species of polypeptides is a VLP-polypeptide containing a pan Th cell epitope.

Packaged: The term "packaged" as used herein refers to the state of a polyanionic macromolecule or immunostimulatory substances in relation to the VLP. The term "packaged" as used herein includes binding that may be covalent, e.g., by chemically coupling, or non-covalent, e.g., ionic interactions, hydrophobic interactions, hydrogen bonds, etc. The term also includes the enclosement, or partial enclosement, of a polyanionic macromolecule. Thus, the polyanionic macromolecule or immunostimulatory substances can be enclosed by the VLP without the existence of an actual binding, in particular of a covalent binding. In preferred embodiments, the at least one polyanionic macromolecule or immunostimulatory substances is packaged inside the VLP, most preferably in a non-covalent manner. In case said immunostimulatory substances is nucleic acid, preferably a DNA, the term packaged implies that

said nucleic acid is not accessible to nucleases hydrolysis, preferably not accessible to DNase hydrolysis (e.g. DNaseI or Benzonase), wherein preferably said accessibility is assayed as described in Examples 11-17 of WO2003/024481A2.

The present invention provides virus-like particles of plant virus Cucumber Mosaic Virus (CMV), and in particular modified VLPs of CMV comprising Th cell epitopes, in particular universal Th cell epitopes.

Thus in a first aspect, the present invention provides for a modified virus-like particle (VLP) of cucumber mosaic virus (CMV), wherein said modified VLP of CMV comprises, essentially consists of, or alternatively consists of, at least one modified CMV polypeptide, wherein said modified CMV polypeptide comprises, or preferably consists of, (a) a CMV polypeptide, and (b) a T helper cell epitope; and wherein said CMV polypeptide comprises, or preferably consists of, (i) an amino acid sequence of a coat protein of CMV; or (ii) a mutated amino acid sequence, wherein the amino acid sequence to be mutated is an amino acid sequence of a coat protein of CMV, and wherein said mutated amino acid sequence and said coat protein of CMV show a sequence identity of at least 90 %, preferably of at least 95%, further preferably of at least 98% and again more preferably of at least 99%.

In a preferred embodiment, said CMV polypeptide comprises, preferably consists of, an amino acid sequence of a coat protein of CMV. In an alternative embodiment, said CMV polypeptide comprises, preferably consists of a mutated amino acid sequence, wherein the amino acid sequence to be mutated is an amino acid sequence of a coat protein of CMV, and wherein said mutated amino acid sequence and said coat protein of CMV show a sequence identity of at least 90 %, preferably of at least 95%, further preferably of at least 98% and again more preferably of at least 99%; wherein preferably said mutated amino acid sequence and said amino acid sequence to be mutated differ in least one and in at most 11, 10, 9, 8, 7, 6, 5, 4, 3, or 2 amino acid residues, and wherein further preferably these differences are selected from (i) insertion, (ii) deletion, (iii) amino acid exchange, and (iv) any combination of (i) to (iii). Mutations may be introduced into an amino acid sequence to be mutated in order to modify certain features of the virus-like particles such as stability and/or coupling efficiency. Introducing cysteine residues may enhance stability of cysteine bridges are formed between subunits and introduction of lysines into the surface of VLPs may enhance coupling efficiencies.

In another preferred embodiment, said CMV polypeptide comprises, or preferably consists of, (i) (a) an amino acid sequence of a coat protein of CMV, wherein said amino acid sequence comprises, or preferably consists of, SEQ ID NO:1 or (b) an amino acid sequence having a sequence identity of at least 75%, preferably of at least 80%, more preferably of at least

85%, again further preferably of at least 90 %, again more preferably of at least 95%, still further preferably of at least 98% and still again further more preferably of at least 99% of SEQ ID NO:1; or (ii) a mutated amino acid sequence, wherein said amino acid sequence to be mutated is said amino acid sequence as defined in (i) of this claim, and wherein said mutated amino acid sequence and said amino acid sequence to be mutated show a sequence identity of at least 95%, preferably of at least 98%, and more preferably of at least 99%.

In a very preferred embodiment, said CMV polypeptide comprises, or preferably consists of, (a) an amino acid sequence of a coat protein of CMV, wherein said amino acid sequence comprises, or preferably consists of, SEQ ID NO:1 or (b) an amino acid sequence having a sequence identity of at least 75%, preferably of at least 80%, more preferably of at least 85%, again further preferably of at least 90 %, again more preferably of at least 95%, still further preferably of at least 98% and still again further more preferably of at least 99% of SEQ ID NO:1.

In another preferred embodiment, said CMV polypeptide comprises, or preferably consists of, (i) (a) an amino acid sequence of a coat protein of CMV, wherein said amino acid sequence comprises SEQ ID NO:21, or (b) an amino acid sequence of a coat protein of CMV comprising an amino acid sequence region, wherein said amino acid sequence region has a sequence identity of at least 75%, preferably of at least 80%, more preferably of at least 85%, again further preferably of at least 90 %, again more preferably of at least 95%, still further preferably of at least 98% and still again further more preferably of at least 99% with SEQ ID NO:21; or (ii) a mutated amino acid sequence, wherein said amino acid sequence to be mutated is said amino acid sequence as defined in (i) of this claim, and wherein said mutated amino acid sequence and said amino acid sequence to be mutated show a sequence identity of at least 95%, preferably of at least 98%, and more preferably of at least 99%.

In another very preferred embodiment, said CMV polypeptide comprises, or preferably consists of, (a) an amino acid sequence of a coat protein of CMV, wherein said amino acid sequence comprises SEQ ID NO:21, or (b) an amino acid sequence of a coat protein of CMV comprising an amino acid sequence region, wherein said amino acid sequence region has a sequence identity of at least 75%, preferably of at least 80%, more preferably of at least 85%, again further preferably of at least 90 %, again more preferably of at least 95%, still further preferably of at least 98% and still again further more preferably of at least 99% with SEQ ID NO:21.

In again another preferred embodiment, said CMV polypeptide comprises, or preferably consists of, (i) (a) an amino acid sequence of a coat protein of CMV, wherein said amino acid

sequence comprises, or preferably consists of, SEQ ID NO:1 or (b) an amino acid sequence having a sequence identity of at least 75%, preferably of at least 80%, more preferably of at least 85%, again further preferably of at least 90 %, again more preferably of at least 95%, still further preferably of at least 98% and still again further more preferably of at least 99% of SEQ ID NO:1; and wherein said amino sequence as defined in (a) or (b) in this claim comprises SEQ ID NO:21; or wherein said amino sequence as defined in (a) or (b) in this claim comprises an amino acid sequence region, wherein said amino acid sequence region has a sequence identity of at least 75%, preferably of at least 80%, more preferably of at least 85%, again further preferably of at least 90 %, again more preferably of at least 95%, still further preferably of at least 98% and still again further more preferably of at least 99% with SEQ ID NO:21; or (ii) a mutated amino acid sequence, wherein said amino acid sequence to be mutated is said amino acid sequence as defined in (i) of this claim, and wherein said mutated amino acid sequence and said amino acid sequence to be mutated show a sequence identity of at least 98% preferably of at least 99%.

In again another very preferred embodiment, said CMV polypeptide comprises, or preferably consists of, (a) an amino acid sequence of a coat protein of CMV, wherein said amino acid sequence comprises, or preferably consists of, SEQ ID NO:1 or (b) an amino acid sequence having a sequence identity of at least 90 % of SEQ ID NO:1; and wherein said amino sequence as defined in (a) or (b) in this claim comprises SEQ ID NO:21; or wherein said amino sequence as defined in (a) or (b) in this claim comprises an amino acid sequence region, wherein said amino acid sequence region has a sequence identity of at least 90% with SEQ ID NO:21.

Typically and preferably, said T helper cell epitope (i) is fused to the N-terminus of said CMV polypeptide, (ii) is fused to the C-terminus of said CMV polypeptide, (iii) replaces a region of consecutive amino acids of said CMV polypeptide, wherein the sequence identity between said replaced region of consecutive amino acids of said CMV polypeptide and the T helper cell epitope is at least 15%, preferably at least 20%, or (iv) replaces a N-terminal region of said CMV polypeptide, and wherein said replaced N-terminal region of said CMV polypeptide consists of 5 to 15 consecutive amino acids.

In a preferred embodiment, said T helper cell epitope replaces a N-terminal region of said CMV polypeptide, wherein preferably the number of amino acids of said N-terminal region replaced is equal to or lower than the number of amino acids of which said T helper cell epitope consists. In other words, said N-terminal region of said CMV polypeptide consists of a first number of amino acids and the Th cell epitope consists of a second number of amino acids, and

in a preferred embodiment, said first number of amino acids is equal to or lower than the second number of amino acids.

In a further preferred embodiment, said replaced N-terminal region of said CMV polypeptide consists of 5 to 15 consecutive amino acids, preferably of 9 to 14 consecutive amino acids, more preferably of 11 to 13 consecutive amino acids. In a very preferred embodiment, said N-terminal region of said CMV polypeptide corresponds to amino acids 2-12 of SEQ ID NO:1.

Thus, in a very preferred embodiment, said CMV polypeptide comprises, or preferably consists of, (i) (a) an amino acid sequence of a coat protein of CMV, wherein said amino acid sequence comprises, or preferably consists of, SEQ ID NO:1 or (b) an amino acid sequence having a sequence identity of at least 75%, preferably of at least 80%, more preferably of at least 85%, again further preferably of at least 90 %, again more preferably of at least 95%, still further preferably of at least 98% and still again further more preferably of at least 99% of SEQ ID NO:1; or (ii) a mutated amino acid sequence, wherein said amino acid sequence to be mutated is said amino acid sequence as defined in (i) of this claim, and wherein said mutated amino acid sequence and said amino acid sequence to be mutated show a sequence identity of at least 95%, preferably of at least 98%, and more preferably of at least 99%, and wherein said T helper cell epitope replaces a N-terminal region of said CMV polypeptide, wherein preferably the number of amino acids of said N-terminal region replaced is equal to or lower than the number of amino acids of which said T helper cell epitope consists.

Thus, in a very preferred embodiment, said CMV polypeptide comprises, or preferably consists of, (i) (a) an amino acid sequence of a coat protein of CMV, wherein said amino acid sequence comprises, or preferably consists of, SEQ ID NO:1 or (b) an amino acid sequence having a sequence identity of at least 75%, preferably of at least 80%, more preferably of at least 85%, again further preferably of at least 90 %, again more preferably of at least 95%, still further preferably of at least 98% and still again further more preferably of at least 99% of SEQ ID NO:1; or (ii) a mutated amino acid sequence, wherein said amino acid sequence to be mutated is said amino acid sequence as defined in (i) of this claim, and wherein said mutated amino acid sequence and said amino acid sequence to be mutated show a sequence identity of at least 95%, preferably of at least 98%, and more preferably of at least 99%, wherein said T helper cell epitope replaces a N-terminal region of said CMV polypeptide, and wherein said replaced N-terminal region of said CMV polypeptide consists of 5 to 15 consecutive amino acids, preferably of 9 to 14 consecutive amino acids, more preferably of 11 to 13 consecutive amino acids.

Thus, in a very preferred embodiment, said CMV polypeptide comprises, or preferably consists of, (i) (a) an amino acid sequence of a coat protein of CMV, wherein said amino acid

sequence comprises, or preferably consists of, SEQ ID NO:1 or (b) an amino acid sequence having a sequence identity of at least 75%, preferably of at least 80%, more preferably of at least 85%, again further preferably of at least 90 %, again more preferably of at least 95%, still further preferably of at least 98% and still again further more preferably of at least 99% of SEQ ID NO:1; or (ii) a mutated amino acid sequence, wherein said amino acid sequence to be mutated is said amino acid sequence as defined in (i) of this claim, and wherein said mutated amino acid sequence and said amino acid sequence to be mutated show a sequence identity of at least 95%, preferably of at least 98%, and more preferably of at least 99%, wherein said T helper cell epitope replaces a N-terminal region of said CMV polypeptide, and wherein said N-terminal region of said CMV polypeptide corresponds to amino acids 2-12 of SEQ ID NO:1.

Thus, in a very preferred embodiment, said CMV polypeptide comprises, or preferably consists of, (a) an amino acid sequence of a coat protein of CMV, wherein said amino acid sequence comprises, or preferably consists of, SEQ ID NO:1 or (b) an amino acid sequence having a sequence identity of at least 75%, preferably of at least 80%, more preferably of at least 85%, again further preferably of at least 90 %, again more preferably of at least 95%, still further preferably of at least 98% and still again further more preferably of at least 99% of SEQ ID NO:1, and wherein said T helper cell epitope replaces a N-terminal region of said CMV polypeptide, wherein preferably the number of amino acids of said N-terminal region replaced is equal to or lower than the number of amino acids of which said T helper cell epitope consists.

Thus, in a very preferred embodiment, said CMV polypeptide comprises, or preferably consists of, (a) an amino acid sequence of a coat protein of CMV, wherein said amino acid sequence comprises, or preferably consists of, SEQ ID NO:1 or (b) an amino acid sequence having a sequence identity of at least 75%, preferably of at least 80%, more preferably of at least 85%, again further preferably of at least 90 %, again more preferably of at least 95%, still further preferably of at least 98% and still again further more preferably of at least 99% of SEQ ID NO:1, wherein said T helper cell epitope replaces a N-terminal region of said CMV polypeptide, and wherein said replaced N-terminal region of said CMV polypeptide consists of 5 to 15 consecutive amino acids, preferably of 9 to 14 consecutive amino acids, more preferably of 11 to 13 consecutive amino acids.

Thus, in a very preferred embodiment, said CMV polypeptide comprises, or preferably consists of, (a) an amino acid sequence of a coat protein of CMV, wherein said amino acid sequence comprises, or preferably consists of, SEQ ID NO:1 or (b) an amino acid sequence having a sequence identity of at least 75%, preferably of at least 80%, more preferably of at least 85%, again further preferably of at least 90 %, again more preferably of at least 95%, still further

preferably of at least 98% and still again further more preferably of at least 99% of SEQ ID NO:1, wherein said T helper cell epitope replaces a N-terminal region of said CMV polypeptide, and wherein said N-terminal region of said CMV polypeptide corresponds to amino acids 2-12 of SEQ ID NO:1.

5 Thus, in a very preferred embodiment, said CMV polypeptide comprises, or preferably consists of, (a) an amino acid sequence of a coat protein of CMV, wherein said amino acid sequence comprises SEQ ID NO:21, or (b) an amino acid sequence of a coat protein of CMV comprising an amino acid sequence region, wherein said amino acid sequence region has a sequence identity of at least 75%, preferably of at least 80%, more preferably of at least 85%,
10 again further preferably of at least 90 %, again more preferably of at least 95%, still further preferably of at least 98% and still again further more preferably of at least 99% with SEQ ID NO:21, and wherein said T helper cell epitope replaces a N-terminal region of said CMV polypeptide, wherein preferably the number of amino acids of said N-terminal region replaced is equal to or lower than the number of amino acids of which said T helper cell epitope consists.

15 Thus, in a very preferred embodiment, said CMV polypeptide comprises, or preferably consists of, (a) an amino acid sequence of a coat protein of CMV, wherein said amino acid sequence comprises SEQ ID NO:21, or (b) an amino acid sequence of a coat protein of CMV comprising an amino acid sequence region, wherein said amino acid sequence region has a sequence identity of at least 75%, preferably of at least 80%, more preferably of at least 85%,
20 again further preferably of at least 90 %, again more preferably of at least 95%, still further preferably of at least 98% and still again further more preferably of at least 99% with SEQ ID NO:21, wherein said T helper cell epitope replaces a N-terminal region of said CMV polypeptide, and wherein said replaced N-terminal region of said CMV polypeptide consists of 5 to 15 consecutive amino acids, preferably of 9 to 14 consecutive amino acids, more preferably of
25 11 to 13 consecutive amino acids.

 Thus, in a very preferred embodiment, said CMV polypeptide comprises, or preferably consists of, (a) an amino acid sequence of a coat protein of CMV, wherein said amino acid sequence comprises SEQ ID NO:21, or (b) an amino acid sequence of a coat protein of CMV comprising an amino acid sequence region, wherein said amino acid sequence region has a
30 sequence identity of at least 75%, preferably of at least 80%, more preferably of at least 85%, again further preferably of at least 90 %, again more preferably of at least 95%, still further preferably of at least 98% and still again further more preferably of at least 99% with SEQ ID NO:21, wherein said T helper cell epitope replaces a N-terminal region of said CMV

polypeptide, and wherein said N-terminal region of said CMV polypeptide corresponds to amino acids 2-12 of SEQ ID NO:1.

Thus, in a very preferred embodiment, said CMV polypeptide comprises, or preferably consists of, (a) an amino acid sequence of a coat protein of CMV, wherein said amino acid sequence comprises, or preferably consists of, SEQ ID NO:1 or (b) an amino acid sequence having a sequence identity of at least 90 % of SEQ ID NO:1; and wherein said amino sequence as defined in (a) or (b) in this claim comprises SEQ ID NO:21; or wherein said amino sequence as defined in (a) or (b) in this claim comprises an amino acid sequence region, wherein said amino acid sequence region has a sequence identity of at least 90% with SEQ ID NO:21, and wherein said T helper cell epitope replaces a N-terminal region of said CMV polypeptide, wherein preferably the number of amino acids of said N-terminal region replaced is equal to or lower than the number of amino acids of which said T helper cell epitope consists.

Thus, in a very preferred embodiment, said CMV polypeptide comprises, or preferably consists of, (a) an amino acid sequence of a coat protein of CMV, wherein said amino acid sequence comprises, or preferably consists of, SEQ ID NO:1 or (b) an amino acid sequence having a sequence identity of at least 90 % of SEQ ID NO:1; and wherein said amino sequence as defined in (a) or (b) in this claim comprises SEQ ID NO:21; or wherein said amino sequence as defined in (a) or (b) in this claim comprises an amino acid sequence region, wherein said amino acid sequence region has a sequence identity of at least 90% with SEQ ID NO:21, wherein said T helper cell epitope replaces a N-terminal region of said CMV polypeptide, and wherein said replaced N-terminal region of said CMV polypeptide consists of 5 to 15 consecutive amino acids, preferably of 9 to 14 consecutive amino acids, more preferably of 11 to 13 consecutive amino acids.

Thus, in a very preferred embodiment, said CMV polypeptide comprises, or preferably consists of, (a) an amino acid sequence of a coat protein of CMV, wherein said amino acid sequence comprises, or preferably consists of, SEQ ID NO:1 or (b) an amino acid sequence having a sequence identity of at least 90 % of SEQ ID NO:1; and wherein said amino sequence as defined in (a) or (b) in this claim comprises SEQ ID NO:21; or wherein said amino sequence as defined in (a) or (b) in this claim comprises an amino acid sequence region, wherein said amino acid sequence region has a sequence identity of at least 90% with SEQ ID NO:21, wherein said T helper cell epitope replaces a N-terminal region of said CMV polypeptide, and wherein said N-terminal region of said CMV polypeptide corresponds to amino acids 2-12 of SEQ ID NO:1.

Very preferably, said T helper cell epitope is a universal T helper cell epitope, wherein preferably said T helper cell epitope consists of at most 20 amino acids.

In a further preferred embodiment, said Th cell epitope is a PADRE sequence. In again further preferred embodiment, said Th cell epitope, comprises, preferably consists of, the amino acid sequence of SEQ ID NO:5.

In another preferred embodiment, said T helper cell epitope is derived from a human vaccine. Preferably said Th cell epitope is derived from tetanus toxin. Again preferably, said Th cell epitope has, preferably consists of, the amino acid sequence of SEQ ID NO:4.

In a very preferred embodiment of the present invention, said CMV polypeptide comprises, or preferably consists of, an amino acid sequence of a coat protein of CMV, wherein said amino acid sequence comprises, or preferably consists of, SEQ ID NO:1 or an amino acid sequence having a sequence identity of at least 95 % of SEQ ID NO:1; and wherein said amino sequence comprises SEQ ID NO:21, and wherein said T helper cell epitope replaces the N-terminal region of said CMV polypeptide, and wherein said replaced N-terminal region of said CMV polypeptide consists of 11 to 13 consecutive amino acids, preferably of 11 consecutive amino acids, and wherein further preferably said N-terminal region of said CMV polypeptide corresponds to amino acids 2-12 of SEQ ID NO:1.

In another very preferred embodiment, said modified CMV polypeptide comprises, preferably consists of, an amino acid sequence of SEQ ID NO:6. In a further very preferred embodiment, said modified CMV polypeptide comprises, preferably consists of, an amino acid sequence of SEQ ID NO:7.

The VLPs of the invention may be expressed in prokaryotic or eukaryotic expression systems. Preferred systems are *E.coli*, yeast, insect cells as well as mammalian cell lines. Very preferred said modified VLP of CMV or said VLP of CMV is obtained by expression of said modified CMV polypeptide or said CMV polypeptide in *E.coli*, and wherein preferably said expression is effected at temperatures of between 10°C to 25°C, preferably at a temperature of 20°C. As indicated above, recombinantly produced polypeptides may comprise an N-terminal methionine residue. In one embodiment said CMV polypeptide or modified CMV polypeptide therefore comprises an N-terminal methionine residue. However, typically and preferably said N-terminal methionine residue is cleaved off said CMV polypeptide or said modified CMV polypeptide.

Therefore, in another aspect, the present invention provides for a virus-like particle (VLP) of cucumber mosaic virus (CMV), wherein said VLP of CMV comprises, essentially consists of, or alternatively consists of, at least one CMV polypeptide comprising or preferably

consisting of: (i) an amino acid sequence of a coat protein of CMV; or (ii) a mutated amino acid sequence, wherein the amino acid sequence to be mutated is an amino acid sequence of a coat protein of CMV, and wherein said mutated amino acid sequence and said a coat protein of CMV show a sequence identity of at least 90 %, preferably of at least 95%, further preferably of at least 98% and again more preferably of at least 99%; and wherein said VLP of CMV is obtained by expression of said CMV polypeptide in *E.coli.*, and wherein preferably said expression is effected at temperatures of between 10°C to 25°C, preferably at a temperature of 20°C.

The invention encompasses compositions wherein said VLPs of the invention, comprise any one of the technical features as described herein, either alone or in any possible combination.

In a further aspect, the present invention provides for a composition comprising a modified virus-like particle. Furthermore, these modified VLPs serve as, preferably, vaccine platform, for generating immune responses, in particular antibody responses, against antigens linked to said modified VLPs. The presence of the Th cell epitopes, in particular universal Th cell epitopes, led to a further increase in the generated immune response.

Thus, in a further preferred embodiment said composition comprises (a) at least one modified virus- like particle of the invention, wherein said modified virus-like particle comprises at least one first attachment site; and (b) at least one antigen, wherein said antigen comprises at least one second attachment site; wherein (a) and (b) are linked through said at least one first and said at least one second attachment site. Methods for linking said modified virus-like particle and said antigen via said first and said second attachment site are described, for example, in WO2002/056905A2 and WO2004/084940A1.

In a further preferred embodiment, said first attachment site is linked to said second attachment site via at least one covalent bond. In a further preferred embodiment, said first attachment site and said second attachment site are linked via at least one covalent peptide-bond

In a further preferred embodiment, said covalent bond is a non-peptide bond. Thus, in again another preferred embodiment, said first attachment site and said second attachment site are linked via at least one covalent non-peptide-bond. In a further preferred embodiment said first attachment site is an amino group, preferably an amino group of a lysine.

Attachment between modified virus-like particles and antigens 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) J. Biol. Chem. 257: 286-288). Therefore, in a further very preferred embodiment of the present invention, the association or linkage of the modified VLP and the at least one antigen does not comprise a disulfide bond. Further preferred hereby, the at least one second attachment

comprise, or preferably is, a sulfhydryl group. Moreover, the association or linkage of the modified VLP and the at least one antigen does preferably not comprise a sulphur-sulphur bond. Further preferred hereby, the at least one second attachment comprise, or preferably is, a sulfhydryl group. In a further very preferred embodiment, said at least one first attachment site is not or does not comprise a sulfhydryl group. In again a further very preferred embodiment, said at least one first attachment site is not or does not comprise a sulfhydryl group of a cysteine. In a further preferred embodiment said second attachment site is a sulfhydryl group, preferably a sulfhydryl group of a cysteine.

In a very preferred embodiment, 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 residue or a sulfhydryl group that has been chemically attached to the antigen. 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 modified 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 modified virus-like particle interact through said association to form an ordered and repetitive antigen array.

In one preferred embodiment of the invention, the antigen is linked to the modified 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 modified 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 antigen, 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, Sulfo-KMUS SVSB, SIA, and other cross-linkers available for example from the Pierce Chemical Company, and having one functional group reactive towards amino groups and one functional group reactive towards sulfhydryl groups. The above mentioned cross-linkers all lead to formation of an amide bond after reaction with the amino group and a thioether linkage with the sulfhydryl groups. Another class of cross-linkers suitable in the practice of the invention is characterized by the introduction of a disulfide linkage between the antigen and the modified VLP upon coupling.

Preferred cross-linkers belonging to this class include, for example, SPDP and Sulfo-LC-SPDP (Pierce).

Linking of the antigen to the modified VLP by using a hetero-bifunctional cross-linker according to the preferred methods described above, allows coupling of the antigen to the modified VLP in an oriented fashion. Other methods of linking the antigen to the modified VLP include methods wherein the antigen is cross-linked to the modified VLP, using the carbodiimide EDC, and NHS. The antigen may also be first thiolated through reaction, for example with SATA, SATP or iminothiolane. The antigen, after deprotection if required, may then be coupled to the modified VLP as follows. After separation of the excess thiolation reagent, the antigen is reacted with the modified 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 can react, such as described above. Optionally, low amounts of a reducing agent are included in the reaction mixture. In further methods, the antigen is attached to the modified 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 modified VLP.

In very preferred embodiments of the invention, the antigen 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 the antigen, to lysine residues of the modified virus-like particle. In a preferred embodiment, the composition of the invention further comprises a linker, wherein said linker associates said antigen with said second attachment site, and wherein preferably said linker comprises or alternatively consists of said second attachment site.

Engineering of a second attachment site onto the antigen is achieved by the association of a linker, preferably containing at least one amino acid suitable as second attachment site according to the disclosures of this invention. Therefore, in a preferred embodiment of the present invention, a linker is associated to the antigen 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.

The selection of a linker will be dependent on the nature of the antigen, on its biochemical properties, such as π , 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. In a further preferred embodiment said linker comprises or alternatively consists of said second attachment site.

In a further preferred embodiment said linker is an amino acid linker, and wherein preferably said amino acid linker is selected from the group consisting of: (a) CGG; (b) N-terminal gamma 1 -linker; (c) N-terminal gamma 3 -linker; (d) Ig hinge regions; (e) N-terminal glycine linkers; (f) (G)kC(G)n with n=0-12 and k=0-5; (g) N-terminal glycine-serine linkers (h) (G)kC(G)m(S)l(GGGGS)n with n=0-3, k=0-5, m=0-10, l=0-2; (i) GGC; (j) GGC-NH₂; (k) C-terminal gamma 1 -linker; (l) C-terminal gamma 3-linker; (m) C-terminal glycine linkers; (n) (G)nC(G)k with n=0-12 and k=0-5; (o) C-terminal glycine-serine linkers; and (p) (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. 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 the linker is added to the N-terminus of the antigen. In another preferred embodiment of the invention, the linker is added to the C- terminus of antigen. In other embodiments of the present invention, the composition comprises or alternatively consists essentially of a virus-like particle linked to the antigen via chemical interactions, wherein at least one of these interactions is not a covalent bond. Linking of the modified VLP to the antigen can be effected by biotinylating the modified VLP and expressing the antigen as a streptavidin- fusion protein. In other embodiments of the present invention, the sulhydryl groups used for chemical coupling to lysines are attached to the modified VLPs or the antigen by means of chemical modification.

In a further preferred embodiment said composition further comprises at least one immuno- stimulatory substance. In a very preferred embodiment, said immunostimulatory substance is packaged into the modified VLPs of the invention. In another preferred embodiment, the immunostimulatory substance is mixed with the modified VLPs of the invention. Immunostimulatory substances useful for the invention are generally known in the art and are disclosed, inter alia, in WO2003/024481A2.

In another embodiment of the present invention, said immunostimulatory substance consists of DNA or RNA of non-eukaryotic origin. In a further preferred embodiment said immunostimulatory substance is selected from the group consisting of: (a) immunostimulatory

nucleic acid; (b) peptidoglycan; (c) lipopolysaccharide; (d) lipoteichonic acid; (e) imidazoquinoline compound; (f) flagelline; (g) lipoprotein; and (h) any mixtures of at least one substance of (a) to (g). In a further preferred embodiment said immunostimulatory substance is an immunostimulatory nucleic acid, wherein said immunostimulatory nucleic acid is selected from the group consisting of: (a) ribonucleic acids; (b) deoxyribonucleic acids; (c) chimeric nucleic acids; and (d) any mixture of (a), (b) and/or (c). In a further preferred embodiment said immunostimulatory nucleic acid is a ribonucleic acid, and wherein said ribonucleic acid is bacteria derived RNA. In a further preferred embodiment said immunostimulatory nucleic acid is poly-(LC) or a derivative thereof. In a further preferred embodiment said immunostimulatory nucleic acid is a deoxyribonucleic acid, wherein said deoxyribonucleic acid is an unmethylated CpG-containing oligonucleotide.

In a very preferred embodiment said immunostimulatory substance is an unmethylated CpG-containing oligonucleotide. In a further preferred embodiment said unmethylated CpG-containing oligonucleotide is an A-type CpG. In a further preferred embodiment said A-type CpG comprises the sequence GACGATCGTC (SEQ ID NO: 31). In a further preferred embodiment said palindromic sequence is flanked at its 5'-terminus and at its 3'-terminus by guanosine entities. In a further preferred embodiment said palindromic sequence is flanked at its 5'-terminus by at least 3 and at most 15 guanosine entities, and wherein said palindromic sequence is flanked at its 3'-terminus by at least 3 and at most 15 guanosine entities.

In another preferred embodiment, said immunostimulatory substance is an unmethylated CpG-containing oligonucleotide, and wherein preferably said unmethylated CpG-containing oligonucleotide comprises a palindromic sequence, and wherein further preferably the CpG motif of said unmethylated CpG-containing oligonucleotide is part of a palindromic sequence, and wherein again further preferably said palindromic sequence is GACGATCGTC (SEQ ID NO: 31).

In a further preferred embodiment, said immunostimulatory nucleic acid is an unmethylated CpG containing oligonucleotide consisting of the sequence GGGGGGGGGGACGATCGTCGGGGGGGGG (SEQ ID NO:36), wherein said unmethylated CpG-containing oligonucleotide consists exclusively of phosphodiester bound nucleotides.

Antigens which are useful for the purpose of the invention are disclosed for example in WO2002/056905A2, WO2004/007538A2, WO2006/037787A2, WO2004/084940A1, and WO2006/032674A1. In one embodiment said antigen is derived from a source selected from the group consisting of: (a) viruses; (b) bacteria; (c) parasites; (d) tumors; (e) self- molecules; (f)

non-peptidic hapten molecules (g) allergens; (h) hormones; (i) cytokines; (k) chemokines; (l) biologically active peptides. In another preferred embodiment, said antigen is a tumor antigen, a self antigen, a polypeptide of a pathogen, an allergen or a hapten. In a further very preferred embodiment, said antigen is calcitonin gene-related peptide (CGRP).

5 In a further preferred embodiment said antigen is a tumor antigen, wherein preferably said tumor antigen is selected from the group consisting of: (a) a polypeptide of breast cancer cells; (b) a polypeptide of kidney cancer cells; (c) a polypeptide of prostate cancer cells; (d) a polypeptide of skin cancer cells; (e) a polypeptide of brain cancer cells; and (f) a polypeptide of leukemia cells.

10 In a further preferred embodiment said antigen is a tumor antigen selected from the group consisting of: (a) Her2; (b) gangliosid GD2; (c) EGF-R; (d) carcino embryonic antigen (CEA); (e) CD52; (f) CD21; (g) human melanoma gp100; (h) human melanoma melanA/MART-1; (i) Human melanoma melanA/MART-1 analogue; (j) tyrosinase; (k) NA17-A nt; (l) MAGE3; (m) p53 protein; and (n) antigenic fragments of any of the tumor antigens of (a) to (m).

15 In a further preferred embodiment said antigen is a polypeptide selected from the group consisting of: (a) IgE, (b) IL-6 (c) receptor activator of nuclear factor κ B ligand (RANKL); (d) vascular endothelial growth factor (VEGF); (e) vascular endothelial growth factor receptor (VEGF-R); hepatocyte growth factor (HGF) (f) interleukin-1 α ; (g) interleukin-1 β ; (h) interleukin-5; (i) interleukin-8; (j) interleukin-13; (k) interleukin-15; (l) interleukin-17 (IL-17);
 20 (m) IL-23; (n) Ghrelin; (o) angiotensin; (p) chemokine (C-C motif) (CCL21); (q) chemokine (C-X motif) (CXCL 12); (r) stromal cell derived factor 1 (SDF-1); (s) macrophage colony stimulating factor (M-CSF); (t) monocyte chemotactic protein 1 (MCP-1); (u) endoglin; (v) resistin; (w) gonadotropin releasing hormone (GnRH); (x) growth hormone releasing (GHRH); (y) luteinizing hormone releasing hormone (LHRH); (z) thyrotropin releasing hormone (TRH);
 25 (aa) macrophage migration inhibitory factor (MIF); (bb) glucose-dependent insulinotropic peptide (GIP); (cc) eotaxin; (dd) bradykinin; (ee) Des-Arg bradykinin; (ff) B-lymphocyte chemoattractant (BLC); (gg) macrophage colony stimulating factor M-CSF; (hh) tumor necrosis factor α (TNF α); (ii) amyloid beta peptide (A β 1-42); (jj) amyloid beta peptide (A β 1-6); (kk) human IgE; (ll) CCR5 extracellular domain; (mm) CXCR4 extracellular domain; (nn) Gastrin;
 30 (oo) CETP; (pp) C5a; (qq) epidermal growth factor receptor (EGF-R); (rr) CGRP; (ss) α -synuclein; (tt) calcitonin gene-related peptide (CGRP) (uu) Amylin or (vv) a fragment of any one of the polypeptides (a) to (uu); and (xx) an antigenic mutant or fragment of any one of the polypeptides (a) to (uu).

In a further preferred embodiment said antigen is a self antigen, wherein said self antigen is a polypeptide selected from the group consisting of: (a) IgE, (b) IL-6 (c) receptor activator of nuclear factor κ B ligand (RANKL); (d) vascular endothelial growth factor (VEGF); (e) vascular endothelial growth factor receptor (VEGF-R); hepatocyte growth factor (HGF) (f) interleukin-1 α ; (g) interleukin-1 β ; (h) interleukin-5; (i) interleukin-8; (j) interleukin-13; (k) interleukin-15; (l) interleukin-17 (IL-17); (m) IL-23; (n) Ghrelin; (o) angiotensin; (p) chemokine (C-C motif) (CCL21); (q) chemokine (C-X motif) (CXCL12); (r) stromal cell derived factor 1 (SDF-1); (s) macrophage colony stimulating factor (M-CSF); (t) monocyte chemotactic protein 1 (MCP-1); (u) endoglin; (v) resistin; (w) gonadotropin releasing hormone (GnRH); (x) growth hormone releasing (GHRH); (y) luteinizing hormone releasing hormone (LHRH); (z) thyrotropin releasing hormone (TRH); (aa) macrophage migration inhibitory factor (MIF); (bb) glucose-dependent insulinotropic peptide (GIP); (cc) eotaxin; (dd) bradykinin; (ee) Des-Arg bradykinin; (ff) B-lymphocyte chemoattractant (BLC); (gg) macrophage colony stimulating factor M-CSF; (hh) tumor necrosis factor α (TNF α); (ii) amyloid beta peptide (A β 1-42); (jj) amyloid beta peptide (A β 1-6); (kk) human IgE; (ll) CCR5 extracellular domain; (mm) CXCR4 extracellular domain; (nn) Gastrin; (oo) CETP; (pp) C5a; (qq) epidermal growth factor receptor (EGF-R); (rr) CGRP; (ss) α -synuclein; (tt) calcitonin gene-related peptide (CGRP) (uu) Amylin or (vv) a fragment of any one of the polypeptides (a) to (uu); and (xx) an antigenic mutant or fragment of any one of the polypeptides (a) to (uu).

In a very preferred embodiment, said antigen is interleukin 17 (IL-17). Interleukin 17 is a T cell-derived cytokine that induces the release of pro-inflammatory mediators in a wide range of cell types. Aberrant Th17 responses and overexpression of IL-17 have been implicated in a number of autoimmune disorders including rheumatoid arthritis and multiple sclerosis. Molecules blocking IL-17 such as IL-17-specific monoclonal antibodies have proved to be effective in ameliorating disease in animal models. Moreover, active immunization targeting IL-17 has recently been suggested using virus-like particles conjugated with recombinant IL-17 (Röhn TA, et al., Eur J Immunol (2006) 36: 1-11). Immunization with IL-17-VLP induced high levels of anti-IL-17 antibodies thereby overcoming natural tolerance, even in the absence of added adjuvant. Mice immunized with IL-17-VLP had lower incidence of disease, slower progression to disease and reduced scores of disease severity in both collagen-induced arthritis and experimental autoimmune encephalomyelitis.

Thus, in a further very preferred embodiment, said antigen comprises, or preferably consists of SEQ ID NO:42. Furthermore, the inventive compositions comprising the modified VLPs are used in a method of treating an inflammatory disease, preferably a chronic

inflammatory disease in an animal or human. Preferably, said inflammatory disease is selected from RA, MS, Psoriasis, asthma, Crohns, Colitis, COPD, diabetes, neurodermatitis (allergic dermatitis), again preferably wherein said inflammatory disease MS. Further preferably said antigen of said inventive compositions comprises, or preferably consists of SEQ ID NO:42.

5 In another very preferred embodiment, said antigen is IL-5. In again a further very preferred embodiment, said antigen comprises, or preferably consists of SEQ ID NO:41. Furthermore, the inventive compositions comprising the modified VLPs are used in a method of treating an inflammatory disease, preferably a chronic inflammatory disease in an animal or human. Preferably, said inflammatory disease is selected from RA, MS, Psoriasis, asthma,
10 Crohns, Colitis, COPD, diabetes, neurodermatitis (allergic dermatitis). Further preferably said antigen of said inventive compositions comprises, or preferably consists of SEQ ID NO:41.

In another very preferred embodiment, said antigen is canine IL-5. In again a further very preferred embodiment, said antigen comprises, or preferably consists of SEQ ID NO:61 or an amino acid sequence having a sequence identity of at least 90%, preferably of at least 95%, with
15 SEQ ID NO:61. Preferably, said antigen comprises, or preferably consists of SEQ ID NO:61. In another preferred embodiment, said antigen of said inventive composition comprises, or preferably consists of SEQ ID NO:61. Further preferably, the inventive composition comprises (i) a modified virus-like particle (VLP) of cucumber mosaic virus (CMV) with at least one first attachment site, wherein said modified VLP of CMV comprises, essentially consists of, or
20 alternatively consists of, at least one modified CMV polypeptide, wherein said modified CMV polypeptide comprises, or preferably consists of, (a) a CMV polypeptide, and (b) a T helper cell epitope; and wherein said modified CMV polypeptide comprises, preferably consists of, an amino acid sequence of SEQ ID NO:6 or SEQ ID NO:7; (ii) at least one antigen with at least one second attachment site, and wherein said antigen is canine IL-5; and wherein said VLP of CMV
25 and said canine IL-5 are linked through said at least one first and said at least one second attachment site, and wherein said canine IL-5 comprises, or preferably consists of SEQ ID NO:61 or an amino acid sequence having a sequence identity of at least 90%, preferably of at least 95% with SEQ ID NO:61, and again preferably wherein said canine IL-5 comprises, or preferably consists of SEQ ID NO:61.

30 In another very preferred embodiment, said antigen is IL-4. In again a further very preferred embodiment, said antigen comprises, or preferably consists of SEQ ID NO:45.

In another very preferred embodiment, said antigen is canine IL-4. In again a further very preferred embodiment, said antigen comprises, or preferably consists of SEQ ID NO:62 or an amino acid sequence having a sequence identity of at least 90%, preferably of at least 95%, with

SEQ ID NO:62. Preferably, said antigen comprises, or preferably consists of SEQ ID NO:62. In another preferred embodiment, said antigen of said inventive composition comprises, or preferably consists of SEQ ID NO:62. Further preferably, the inventive composition comprises (i) a modified virus-like particle (VLP) of cucumber mosaic virus (CMV) with at least one first attachment site, wherein said modified VLP of CMV comprises, essentially consists of, or alternatively consists of, at least one modified CMV polypeptide, wherein said modified CMV polypeptide comprises, or preferably consists of, (a) a CMV polypeptide, and (b) a T helper cell epitope; and wherein said modified CMV polypeptide comprises, preferably consists of, an amino acid sequence of SEQ ID NO:6 or SEQ ID NO:7; (ii) at least one antigen with at least one second attachment site, and wherein said antigen is canine IL-4; and wherein said VLP of CMV and said canine IL-4 are linked through said at least one first and said at least one second attachment site, and wherein said canine IL-4 comprises, or preferably consists of SEQ ID NO:62 or an amino acid sequence having a sequence identity of at least 90%, preferably of at least 95% with SEQ ID NO:62, and again preferably wherein said canine IL-4 comprises, or preferably consists of SEQ ID NO:62.

In another very preferred embodiment, said antigen is IL-13. In again a further very preferred embodiment, said antigen comprises, or preferably consists of SEQ ID NO:46.

In another very preferred embodiment, said antigen is canine IL-13. In again a further very preferred embodiment, said antigen comprises, or preferably consists of SEQ ID NO:63 or an amino acid sequence having a sequence identity of at least 90%, preferably of at least 95%, with SEQ ID NO:63. Preferably, said antigen comprises, or preferably consists of SEQ ID NO:63. In another preferred embodiment, said antigen of said inventive composition comprises, or preferably consists of SEQ ID NO:63. Further preferably, the inventive composition comprises (i) a modified virus-like particle (VLP) of cucumber mosaic virus (CMV) with at least one first attachment site, wherein said modified VLP of CMV comprises, essentially consists of, or alternatively consists of, at least one modified CMV polypeptide, wherein said modified CMV polypeptide comprises, or preferably consists of, (a) a CMV polypeptide, and (b) a T helper cell epitope; and wherein said modified CMV polypeptide comprises, preferably consists of, an amino acid sequence of SEQ ID NO:6 or SEQ ID NO:7; (ii) at least one antigen with at least one second attachment site, and wherein said antigen is canine IL-13; and wherein said VLP of CMV and said canine IL-13 are linked through said at least one first and said at least one second attachment site, and wherein said canine IL-13 comprises, or preferably consists of SEQ ID NO:63 or an amino acid sequence having a sequence identity of at least 90%, preferably of at

least 95% with SEQ ID NO:63, and again preferably wherein said canine IL-13 comprises, or preferably consists of SEQ ID NO:63.

In a further very preferred embodiment, said antigen is TNF α . In a further very preferred embodiment, said antigen is IL-1 α .

5 In a further very preferred embodiment, said antigen is IL-1 β . In again a further very preferred embodiment, said antigen comprises, or preferably consists of SEQ ID NO:60.

In again a further very preferred embodiment, said antigen is a peptide derived from A β -1-42. In again a further very preferred embodiment, said antigen is IgE or a peptide or domain comprised in IgE.

10 In yet another very preferred embodiment, said antigen is human, feline or canine IL-31. In another very preferred embodiment, said antigen comprises, or preferably consists of SEQ ID NO:43 or SEQ ID NO:44. In another very preferred embodiment, said antigen is canine IL-31. In again a further very preferred embodiment, said antigen comprises, or preferably consists of SEQ ID NO:43 or an amino acid sequence having a sequence identity of at least 90%, preferably
15 of at least 95%, with SEQ ID NO:43. Preferably, said antigen comprises, or preferably consists of SEQ ID NO:43. In another preferred embodiment, said antigen of said inventive compositions comprises, or preferably consists of SEQ ID NO:43. Further preferably, the inventive composition comprises (i) a modified virus-like particle (VLP) of cucumber mosaic virus (CMV) with at least one first attachment site, wherein said modified VLP of CMV comprises, essentially
20 consists of, or alternatively consists of, at least one modified CMV polypeptide, wherein said modified CMV polypeptide comprises, or preferably consists of, (a) a CMV polypeptide, and (b) a T helper cell epitope; and wherein said modified CMV polypeptide comprises, preferably consists of, an amino acid sequence of SEQ ID NO:6 or SEQ ID NO:7; (ii) at least one antigen with at least one second attachment site, and wherein said antigen is canine IL-31; and wherein
25 said VLP of CMV and said canine IL-31 are linked through said at least one first and said at least one second attachment site, and wherein said canine IL-31 comprises, or preferably consists of SEQ ID NO:43 or an amino acid sequence having a sequence identity of at least 90%, preferably of at least 95% with SEQ ID NO:43, and again preferably wherein said canine IL-31 comprises, or preferably consists of SEQ ID NO:43.

30 In another very preferred embodiment, said antigen is α -synuclein or a peptide derived from α -synuclein, and wherein preferably said peptide consists of 6 to 14 amino acids, and wherein further preferably said antigen is a peptide derived from α -synuclein selected from any one of SEQ ID NO:32, SEQ ID NO:33 and SEQ ID NO:34. Further preferred peptides derived

from α -synuclein are disclosed in WO 2011/020133, which is incorporated herein by way of reference.

Alpha-synuclein (α -Syn), a small protein with multiple physiological and pathological functions, is one of the dominant proteins found in Lewy Bodies, a pathological hallmark of
5 Lewy body disorders, including Parkinson's disease (PD). More recently, α -Syn has been found in body fluids, including blood and cerebrospinal fluid, and is likely produced by both peripheral tissues and the central nervous system. Exchange of α -Syn between the brain and peripheral tissues could have important pathophysiologic and therapeutic implications (Gardai SJ et al., PLoS ONE (2013) 8(8): e71634). The evidence implicating alpha-synuclein (a-syn) in the
10 pathogenesis of Parkinson's Disease (PD) is overwhelming. However, there is not a clear consensus on the manner in which a-syn leads to pathology in PD and other synucleinopathies.

Alpha-synuclein is a major component of Lewy bodies (LBs), and descriptions of a-syn overexpression leading to aggregation are abundant. Human genetic data have demonstrated that missense mutations and multiplications in the a-syn gene cause familial PD. In the case of gene
15 multiplication, increased levels of a-syn protein are presumed to result in a dominant gain-of-function that leads to pathology. While increased levels of a-syn may lead to aggregation and toxicity, research over the past few years has also revealed that elevated a-syn can interfere with the creation, localization, and/or maintenance of vesicle pools (Gardai SJ et al., PLoS ONE (2013) 8(8): e71634; and references cited therein.

20 In again a further very preferred embodiment, said antigen is Amylin. In a very preferred embodiment, said antigen is is angiotensin I or a peptide derived from angiotensin I. In another very preferred embodiment, said antigen is angiotensin II or a peptide derived from angiotensin II. In a further very preferred embodiment, said antigen is GnRH. In a further very preferred embodiment, said antigen is eotaxin.

25 In a further preferred embodiment said antigen is a polypeptide of a parasite, wherein preferably said pathogen is selected from the group consisting of: (a) Toxoplasma spp.; (b) Plasmodium falciparum; (c) Plasmodium vivax; (d) Plasmodium ovale; (e) Plasmodium malariae; (f) Leishmania; (g) Schistosoma and (h) Nematodes. Preferably, said antigen antigen is derived from Plasmodium falciparum or Plasmodium Vivax.

30 In a further preferred embodiment, said antigen is a polypeptide of a bacterium, wherein preferably said bacterium is selected from the group consisting of: (a) Chlamydia (b) Streptococcus; (c) Pneumococcus; (d) Staphylococcus; (e) Salmonella; (f) Mycobacteria; (g) Clostridia (h) Vibrio (i) Yersinia (k) Meningococcus (l) Borelia.

In a further preferred embodiment said antigen is a viral antigen, wherein preferably said viral antigen is a polypeptide selected from the group consisting of: (a) HIV and other retroviruses; (b) influenza virus, preferably influenza A M2 extracellular domain or HA or HA globular domain; (c) a polypeptide of Hepatitis B virus, preferably preS1; (d) Hepatitis C virus; 5 (e) HPV, preferably HPV16E7 (f) RSV, (g) SARS and other Coronaviruses, (h) Dengue and other Flaviviruses, such as West Nile Virus and Hand Foot and Mouth Disease Virus, (i) Chikungunya and other Alphaviruses. (k) CMV and other Herpesviruses, (l) Rotavirus. In a further very preferred embodiment, said antigen is the derived from RSV.

10 In a preferred embodiment, said antigen is the extracellular domain of Influenza A virus M2 protein, or an antigenic fragment thereof. In a very preferred embodiment said antigen comprises or preferably consists of the extracellular domain of the Influenza A virus M2 protein, wherein preferably said extracellular domain of the Influenza A virus M2 protein is SEQ ID NO:24. In another preferred embodiment, said antigen is the globular domain of Influenza virus.

15 In a further preferred embodiment said antigen is an allergen, wherein preferably said allergen is derived from the group consisting of: (a) pollen extract; (b) dust extract; (c) dust mite extract; (d) fungal extract; (e) mammalian epidermal extract; (f) feather extract; (g) insect extract; (h) food extract; (i) hair extract; (j) saliva extract; and (k) serum extract. In a further preferred embodiment said antigen is an allergen, wherein said allergen is selected from the group consisting of: (a) trees; (b) grasses; (c) house dust; (d) house dust mite; (e) aspergillus; (f) 20 animal hair; (g) animal feather; (h) bee venom; (i) animal products; (j) plant products; (k) animal dander; (l) peanut allergens.

In a further preferred embodiment said antigen is a recombinant allergen, wherein said allergen is selected from the group consisting of: (a) bee venom phospholipase A2; (b) ragweed pollen Amb a 1; (c) birch pollen Bet v I; (d) white faced hornet venom 5 DoI m V; (e) house dust 25 mite Der p 1; (f) house dust mite Der f 2; (g) house dust mite Der p 2; (h) dust mite Lep d; (i) fungus allergen Alt a 1; (j) fungus allergen Asp f 1; (k) fungus allergen Asp f 16; (l) peanut allergens (m) cat allergen Fel d1; (n) Canine allergens Can f1, Can f2 (o) peanut-derived allergens; or (p) Japanese cedar allergen Cry J2 . In another very preferred embodiment of the present invention, said antigen dog allergen Can f1 or Can f2.

30 In a further very preferred embodiment, said antigen is a peanut allergen. Preferably, said antigen is a peanut allergen comprising an amino acid sequence selected from SEQ ID NO:57, SEQ ID NO:64 or SEQ ID NO:65.

In a further very preferred embodiment, said antigen is an allergen derived from Japanese Cedar Cry J 2. Preferably, said antigen is derived from Japanese Cedar Cry J 2 and comprises the amino acid sequence of SEQ ID NO:55.

5 In a further very preferred embodiment, said antigen is an allergen derived from ragweed pollen Amb a 1. Preferably, said antigen is derived from ragweed pollen Amb a 1 and comprises the amino acid sequence of SEQ ID NO:54.

10 In a very preferred embodiment of the present invention, said antigen cat allergen Fel d1. The domestic cat (*Felis domesticus*) is an important source of indoor allergens (Lau, S., et al. (2000) Lancet 356, 1392-1397). Indeed, cats are found in about 25% of households in Western countries and allergy to cats is found in a large part of the population. The severity of symptoms range from relatively mild rhinitis and conjunctivitis to potentially life-threatening asthmatic exacerbation. Although patients are occasionally sensitized to several different molecules in cat dander and pelts, the major allergen is Fel d1. The importance of this allergen has been emphasised in numerous studies. In fact more than 80% of cat allergic patients exhibit IgE
15 antibodies to this potent allergen (van Ree, R., et al. (1999) J. Allergy Clin Immunol 104, 1223-1230).

Fel d1 is a 35-39 kDa acidic glycoprotein containing 10-20% N-linked carbohydrates and is found in the pelt, saliva and lachrymal glands of cats. It is formed by two non-covalently linked heterodimers. Each heterodimer consists of one 70 residue peptide (known as "chain 1")
20 and one 78, 85, 90 or 92 residue peptide (known as "chain 2") which are encoded by separate genes (see Duffort, O. A., et al. (1991) Mol Immunol 28, 301-309; Morgenstern, J. P., et al; (1991) Proc Natl Acad Sci USA 88, 9690-9694 and Griffith, I. J., et al. (1992) Gene 113, 263-268).

Several recombinant constructs of Fel d1 have been described (Vailes LD, et al., J
25 Allergy Clin Immunol (2002) 110:757-762; Grönlund H, et al., J Biol Chem (2003) 278:40144-40151; 2003Schmitz N, et al., J Exp Med (2009) 206:1941-1955; WO2006/097530).

Thus, in a further very preferred embodiment, said antigen is a rFel d1. In a further very preferred embodiment, said antigen is a Fel d1 protein, wherein said Fel d1 protein is a fusion protein comprising chain 1 of Fel d1 and chain 2 of Fel d1, wherein said chain 2 of Fel d1 is
30 fused via its C-terminus to the N-terminus of said chain 1 of Fel d1 either directly via one peptide bond or via a spacer, wherein said spacer consists of an amino acid sequence having 1-20 amino acid residues, wherein preferably said spacer consists of an amino acid sequence having 10-20 amino acid residues. Very preferably, said spacer consists of an amino acid sequence of 15 amino acid residues, and further preferably said spacer has the amino acid

sequence of SEQ ID NO:47. In a further very preferred embodiment, said antigen is a Fel d1 protein, wherein said Fel d1 protein is a fusion protein comprising chain 1 of Fel d1 and chain 2 of Fel d1, wherein said chain 1 of Fel d1 is fused via its C-terminus to the N-terminus of said chain 2 of Fel d1 either directly via one peptide bond or via a spacer, wherein said spacer
5 consists of an amino acid sequence having 1-20 amino acid residues, wherein preferably said spacer consists of an amino acid sequence having 10-20 amino acid residues. Preferably, said chain 1 of Fel d1 comprises a sequence of SEQ ID NO:37 or a homologue sequence thereof, wherein said homologue sequence has an identity to SEQ ID NO:37 of greater than 90%, or even more preferably greater than 95%. Further preferably, said chain 2 of Fel d1 comprises a
10 sequence of SEQ ID NO:38, SEQ ID NO:39 or SEQ ID NO:40, or a homologue sequence thereof, wherein said homologue sequence has an identity to SEQ ID NO:38, SEQ ID NO:39 or SEQ ID NO:40 of greater than 90%, and even more preferably greater than 95%.

In a very preferred embodiment, said antigen comprises, preferably consists of, SEQ ID NO:17. In another very preferred embodiment, said antigen comprises, preferably consists of,
15 SEQ ID NO:18. In another very preferred embodiment, said antigen comprises, preferably consists of, SEQ ID NO:52. In a very preferred embodiment said antigen is a Fel d1 protein comprising an amino acid sequence selected from: (a) SEQ ID NO:17; (b) SEQ ID NO:18; (c) SEQ ID NO:50; (d) SEQ ID NO:56; (e) SEQ ID NO:59; or SEQ ID NO:52.

In a further very preferred embodiment, said Fel d1 protein comprises an amino acid
20 sequence selected from: (a) SEQ ID NO:50; (b) SEQ ID NO:56 (c) SEQ ID NO:59; or (d) SEQ ID NO:52. In a further very preferred embodiment, said Fel d1 protein comprises an amino acid sequence selected from: (a) SEQ ID NO:50; (b) SEQ ID NO:56 or (c) SEQ ID NO:59.

In another very preferred embodiment, said Fel d1 protein comprises, preferably consists of, an amino acid sequence of SEQ ID NO:52. In another very preferred embodiment, said Fel
25 d1 protein comprises, preferably consists of, an amino acid sequence of SEQ ID NO:59. In another very preferred embodiment, said Fel d1 protein comprises, preferably consists of, an amino acid sequence of SEQ ID NO:50. In another very preferred embodiment, said Fel d1 protein comprises, preferably consists of, an amino acid sequence of SEQ ID NO:56.

In a very preferred embodiment, the inventive composition comprises (i) a modified virus-
30 like particle (VLP) of cucumber mosaic virus (CMV) with at least one first attachment site, wherein said modified VLP of CMV comprises, essentially consists of, or alternatively consists of, at least one modified CMV polypeptide, wherein said modified CMV polypeptide comprises, or preferably consists of, (a) a CMV polypeptide, and (b) a T helper cell epitope; and wherein said modified CMV polypeptide comprises, preferably consists of, an amino acid sequence of

SEQ ID NO:6 or SEQ ID NO:7; (ii) at least one antigen with at least one second attachment site, and wherein said antigen is a Fel d1 protein; and wherein said VLP of CMV and said Fel d1 protein are linked through said at least one first and said at least one second attachment site, and wherein said Fel d1 protein comprises an amino acid sequence selected from (a) SEQ ID NO:50; (b) SEQ ID NO:56 (c) SEQ ID NO:59; or (d) SEQ ID NO:52, and wherein preferably said Fel d1 protein comprises an amino acid sequence selected from: (a) SEQ ID NO:50; (b) SEQ ID NO:56 or (c) SEQ ID NO:59.

In a further preferred embodiment said antigen is an animal venom. In a preferred embodiment, the antigen is inactivated snake venom. In a further preferred embodiment said antigen is a hapten. In a further preferred embodiment said hapten is a drug, wherein preferably said drug is selected from the group consisting of: (a) codeine; (b) fentanyl; (c) heroin; (d) morphine; (e) amphetamine; (f) cocaine; (g) methylenedioxymethamphetamine; (h) methamphetamine; (i) methylphenidate; (j) nicotine; (k) LSD; (l) mescaline; (m) psilocybin; (n) tetrahydrocannabinol; and (o) nicotine

In a further preferred embodiment said hapten is a hormone, wherein preferably said hormone is selected from the group consisting of: (a) progesterone; (b) estrogen; (c) testosterone; (d) follicle stimulating hormone; (e) melanin stimulating hormone; (f) adrenalin; and (g) noradrenalin. In a further preferred embodiment said hapten is a toxin, wherein preferably said toxin is selected from the group consisting of: (a) aflatoxin; (b) ciguatera toxin; (c) tetrodotoxin; and (d) antibiotics. In a further preferred embodiment said hapten is a pathogen-derived carbohydrate.

In a further aspect the invention provides a vaccine comprising or alternatively consisting of the modified virus-like particle of the invention or of the composition of the invention. Encompassed are vaccines wherein said modified VLPs, and/or said composition comprise any one of the technical features disclosed herein, either alone or in any possible combination. In one embodiment the vaccine further comprises an adjuvant. In a further embodiment the vaccine is devoid of an adjuvant. In a preferred embodiment said vaccine comprises an effective amount of the composition of the invention. An "effective amount" refers to an amount which needs to be administered to a subject in order to achieve a detectable physiological effect.

In a further aspect, the invention relates to a pharmaceutical composition comprising: (a) a modified VLP of the invention, a composition of the invention, or a vaccine of the invention; and (b) a pharmaceutically acceptable carrier, diluent and/or excipient. Said diluent 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. Pharmaceutical compositions of the invention may be in a form which contain salts, buffers, adjuvants, or other substances which are desirable for improving the efficacy of the conjugate. Examples of materials suitable for use in preparation of pharmaceutical compositions are provided in numerous sources including Remington's Pharmaceutical Sciences (Osol, A, ed., Mack Publishing Co., (1990)). In one embodiment said pharmaceutical composition comprises an effective amount of the vaccine of the invention. An "effective amount" refers to an amount which needs to be administered to a subject in order to achieve an detectable physiological effect.

A further aspect of the invention is a method of immunization comprising administering a modified VLP of the invention, a composition of the invention, a vaccine of the invention, or a pharmaceutical composition of the invention to an animal or a human. In a preferred embodiment said method comprises administering a composition of the invention, a vaccine of the invention, or a pharmaceutical composition of the invention to an animal or a human.

A further aspect of the invention is a method of treating or preventing a disease, disorder or physiological condition in an animal said method comprising administering a modified VLP of the invention, a composition of the invention, a vaccine of the invention, or a pharmaceutical composition of the invention to said animal, wherein preferably said animal can be a human. In a further preferred embodiment said modified VLP, said composition, said vaccine, or said pharmaceutical composition is administered to said animal subcutaneously, intravenously, intradermally, intranasally, orally, intranodal or transdermally.

EXAMPLES

EXAMPLE 1

Isolation and Cloning of a Coat Protein (CP) of Cucumber Mosaic Virus (CMV)

The total RNA from CMV-infected lily leaves collected from a private garden in Riga, Latvia, was isolated using TRI reagent (Sigma, Saint Louis, USA) in accordance with manufacturer's recommendations. For cDNA synthesis, OneStep RT-PCR kit (Qiagen, Venlo, Netherlands) was used. For amplification of the CMV CP gene, the primer sequences were chosen following analysis of CMV sequences from GenBank: CMcpF

(CACCATGGACAAATCTGAA TCAACCAGTGCTGGT) (SEQ ID NO: 8) and CMcpR (CAAAGCTTATCAAACTGGGAGCA CCCCAGATGTGGGA) (SEQ ID NO:9); NcoI and HindIII sites underlined. The corresponding PCR products were cloned into the pTZ57R/T vector (Fermentas, Vilnius, Lithuania). *E. coli* XL1-Blue cells were used as a host for cloning and plasmid amplification. To avoid RT-PCR errors, several CP gene-containing pTZ57 plasmid clones were sequenced using a BigDye cycle sequencing kit and an ABI Prism 3100 Genetic analyzer (Applied Biosystems, Carlsbad, USA). After sequencing, the cDNA of CMV CP gene without sequence errors (SEQ ID NO: 10) coding for CMV coat protein of SEQ ID NO:1 was then subcloned into the NcoI/HindIII sites of the pET28a(+) expression vector (Novagen, San Diego, USA), resulting in the expression plasmid pET-CMVwt (FIG.1).

EXAMPLE 2

Expression of CP of SEQ ID NO:1 in *E.coli* leading to VLPs of CMV

To obtain CMV VLPs, *E. coli* C2566 cells (New England Biolabs, Ipswich, USA) were transformed with the CMV CP gene-containing plasmid pET-CMVwt. After selection of clones with the highest expression levels of target protein, *E. coli* cultures were grown in 2xTY medium containing kanamycin (25 mg/l) on a rotary shaker (200 rev/min; Infors, Bottmingen, Switzerland) at 30°C to an OD600 of 0.8–1.0. Then, the cells were induced with 0.2 mM IPTG, and the medium was supplemented with 5 mM MgCl₂. Incubation was continued on the rotary shaker at 20°C for 18 h. The resulting biomass was collected by low-speed centrifugation and was frozen at -20°C. After thawing on ice, the cells were suspended in the buffer containing 50 mM sodium citrate, 5 mM sodium borate, 5 mM EDTA, 5 mM mercaptoethanol (pH 9.0, buffer A) and were disrupted by ultrasonic treatment. Insoluble proteins and cell debris were removed by centrifugation (13,000 rpm, 30 min at 5°C). The soluble CMV CP protein in clarified lysate was pelleted using saturated ammonium sulfate (1:1, vol/vol) overnight at +4°C. Precipitated proteins were solubilized in the same buffer A (without mercaptoethanol) for 4 h at +4°C. Insoluble proteins were removed by low speed centrifugation (13,000 rpm, 15 min at 4°C). Soluble CMV CP-containing protein solution was separated from the cellular proteins by ultracentrifugation (SW28 rotor, Beckman, Palo Alto, USA; at 25,000 rpm, 6 h, 5°C) in a sucrose gradient (20–60% sucrose in buffer A, without mercaptoethanol, supplemented with 0.5% Triton X-100). The gradient was divided into six fractions, starting at the bottom of the gradient, and the fractions were analyzed by SDS-PAGE (FIG. 2). Fractions No.2 and No.3 containing recombinant CMV CP were combined and were dialyzed against 200 volumes of the

buffer (5 mM sodium borate, 2 mM EDTA, pH 9.0) to remove the sucrose and Triton X-100. After dialysis, CMV CP solution was sterilized by filtration through the 0.2 μ filter. Next, CMV CP was concentrated using Type70 rotor (Beckman, Palo Alto, USA) ultracentrifugation through the 20% sucrose “cushion” under sterile conditions (50 000 rpm, 4 h, +5°C). The concentration of purified CMVwt was estimated using the QuBit fluorometer in accordance with manufacturer’s recommendations (Invitrogen, Eugene, USA). Concentrated VLP solutions (approx. 3 mg/ml) were stored at +4°C in 5 mM sodium borate, 2 mM EDTA, buffer (pH 9.0). All steps involved in the expression and purification of VLP were monitored by SDS-PAGE using 12.5% gels.

As shown in FIG. 2, CMV coat protein can be successfully expressed in *E.coli* cells and significant part obtained can be in soluble fraction. Moreover, these proteins are found directly in *E.coli* cell extracts in the form of isometric VLPs, as demonstrated by sucrose gradient analysis (FIG. 3A), dynamic light scattering and electron-microscopy analysis (FIG. 3B).

EXAMPLE 3

Cloning of a modified Coat Protein of CMV containing an tetanus toxoid epitope (CMV-Ntt830)

To replace the original amino acids at the N-terminus of CMV CP of SEQ ID NO:1 against foreign epitope sequences, as a template for PCR amplification and mutagenesis the pET-CMVwt plasmid was used. Internally in CMVwt gene located SalI site (FIG.1) was used for cloning corresponding PCR products.

To introduce the tetanus toxoid epitope coding sequence in CMVwt gene, two step PCR mutagenesis was necessary. For the first step amplification following primers were used for PCR: pET-220 (AGCACCGCCGCCGCAAGGAA (SEQ ID NO: 11) –upstream from polylinker, the amplified region includes BglII site) and CMV-tt83-1R (ATTGAGTTGGCCTTAATATACT GGCCCATGGTATATCTCCTTCTTAAAGT) (SEQ ID NO:12). For the second round the PCR product from the first round was diluted 1:50 and re-amplified with primers pET-220 (SEQ ID NO: 11) and CMV-tt83Sal-R2 (GACGTCGACGCTCGGTAATCCCGATAAATTTGGAGTTG GCCTTAATATACTG) (SEQ ID NO:13). Resulting PCR product (cDNA of SEQ ID NO:14 coding for CMV-Ntt830 of SEQ ID NO: 6) was subcloned in BglII/SaLI sites of pET-CMVwt. The correct clone was identified by sequencing and designated as pET-CMV-Ntt830.

EXAMPLE 4

Expression of CMV-Ntt830 in *E.coli* leading to modified VLPs of CMV

The procedures for expression and purification of CMV-Ntt830 were essentially the same as for CMVwt and are described in Example 2. To demonstrate the presence of tetanus epitope in CMV VLPs, the mass spectrometric analysis of the purified CMV-Ntt830 VLPs was used. As shown in Fig. 4C, the major peak obtained corresponds to the theoretical molecular mass of the protein if the first methionine is removed during the protein synthesis in *E.coli* cells. Dynamic light scattering and electron microscopy analysis confirm the isometric particle morphology similar to CMVwt VLPs (FIG. 5).

EXAMPLE 5

Cloning of a modified Coat Protein of CMV containing a PADRE epitope (CMV-Npadr)

To introduce the PADRE epitope coding sequence in CMVwt gene, PCR mutagenesis was carried out using as the template for amplification and subcloning the pET-CMVwt plasmid (see also Example 3). For the amplification following primers were used: pET-220 (SEQ ID NO: 11) and CMV-padrSal-R (GACGTCGACGCGCGGCCGCTTGAGGGTCCACGCGGCCACAAATTT CGCCATGGT) (SEQ ID NO:15). The resulting PCR product (cDNA of SEQ ID NO:16 coding for CMV-Npadr of SEQ ID NO: 7) was again subcloned in BglII/SalI sites of pET-CMVwt. The correct clone was identified by sequencing and designated as pET-CMV-Npadr.

EXAMPLE 6

Expression of CMV-Npadr in *E.coli* leading to modified VLPs of CMV

The procedures for expression and purification of CMV-Npadr were essentially the same as for CMVwt and are described in Example 2. To demonstrate the presence of the PADRE epitope in CMV VLPs, the mass spectrometric analysis of the purified CMV-Npadr VLPs was used. As shown in Fig. 4B, the major peak obtained corresponds to the theoretical molecular mass of the protein if the first methionine is removed during the protein synthesis in *E.coli* cells. Dynamic light scattering and electron microscopy analysis confirm the isometric particle morphology, which is slightly different from CMVwt and CMV-Ntt830 VLPs (FIG. 6).

EXAMPLE 7

Immunization of mice with CMVwt, CMV-Ntt830 and CMV-Npadr

Groups of four female Balb/c mice were either immunized with CMVwt, CMV-Npadr VLP and CMV-Ntt830 VLP. VLPs were formulated in 150 mM PBS, pH 7.4 and 150 µl were injected i.v. on day 0 and day 7. CMVwt, CMV-Npadr VLP and CMV-Ntt830 VLP were injected in three amounts: 10, 1 or 0.1 µg. Mice were bled on days 0 (pre-immune), day 7, day 14, and day 25, and sera were analyzed using CMVwt VLPs, CMV-Npadr VLP and CMV-Ntt830 VLP – specific ELISA. All immune sera were tested against the respective VLPs used for immunization.

ELISA

The antibody response in mouse sera were analyzed at the indicated time. For determination of CMVwt, CMV-Npadr VLP and CMV-Ntt830 VLP specific antibody titers, ELISA plates (Nunc Immuno MaxiSorp, Rochester, NY) were coated overnight at 4°C with 100 µl of the corresponding VLP (1 µg/ml) in coating buffer (0.1 M Sodium hydrogen carbonate buffer, pH 9.6). In order to avoid unspecific binding the ELISA plates were blocked with 200 µl of 2% BSA in PBST and incubated for 2 hours at RT. The serum samples were diluted in 2% BSA/PBST. Pre-diluted sera were transferred onto the coated plates and further serial diluted to obtain antibody titers based on OD50 calculation. After 2 hours of incubation at RT, the ELISA plates were washed 5x with 200 µl of PBST. Binding of serum antibodies was detected by horse-radish peroxidase-conjugated goat anti-mouse IgG (Jackson ImmunoResearch). The detection antibody was diluted 1:1000 in 2% BSA/PBST and a volume of 100 µl per sample was transferred. The plates were incubated for 1 hour at RT. ELISA plates were washed as described before. Prior washing the substrate solution was prepared. To this end, 1 tablet (10 mg) of OPD (1,2-Phenylenediamine dihydrochloride) and 9 µl of 30% H₂O₂ was dissolved in 25 ml citric acid buffer (0.066 M Na₂HPO₄, 0.035 M citric acid, pH 5.0). A volume of 100 µl of the substrate solution was pipetted onto the plates and exactly incubated for 7 minutes at RT. To stop the reaction 50 µl of stop solution (5% H₂SO₄ in H₂O) was directly pipetted onto the plates. Absorbance readings at 450 nm of the 1, 2-Phenylenediamine dihydrochloride color reaction were analyzed. FIG. 7 shows antibodies specific for CMVwt (FIG. 7A), -CMV-Npadr (FIG. 7B) and CMV-Ntt880 (FIG. 7C) in a dose dependent manner.

EXAMPLE 8

Coupling of recombinant Fel d1 constructs to VLP CMV-Npadr and VLP CMV-Ntt830 and induction of immune responses

A covalent fusion of rFel d1 (SEQ ID NO:17) was generated as previously described (Schmitz N, et al., J Exp Med (2009) 206:1941-1955). In brief, a complementary DNA encoding a covalent dimer of chain 2 and chain 1, wherein the chain 2 of Fel d1 is fused via its C-terminus to the N-terminus of chain 1 of Fel d1 spaced by a 15aa-linker (GGGGS)₃ was obtained by PCR amplification using sets of overlapping DNA-primers (the construct is named "rFel-2-G3-1-construct"). This complementary DNA was cloned in frame into a modified version of pET-42a(+) (EMD), leading to the addition of the coding sequence for LEHHHHHHHGGC (SEQ ID NO:35) at the C terminus of the rFel-2-G3-1-construct. The added sequence contains a His tag for purification, followed by a GGC linker used for the coupling of this Fel d1 fusion protein to CMV-Npadr and CMV-Ntt830.

Analogously, another covalent fusion of rFel d1 (SEQ ID NO:18) is generated as previously described (WO2006/097530) corresponding to a rFel d1 fusion protein comprising chain 1 of Fel d1 and chain 2 of Fel d1, wherein the chain 1 of Fel d1 is fused via its C-terminus to the N-terminus of chain 2 of Fel d1 by a 15aa-linker (GGGGS)₃ ("rFel-1-G3-2-construct").

The virus like particles VLP CMV-Npadr and VLP CMV-Ntt830 in a volume of 2.5 ml with a concentration of 2 mg/ml (71 μ M) reacted with 27 μ l of 50 mM of the heterobifunctional chemical cross-linker succinimidyl-6-(b-maleimidopropionamide) hexanoate (SMPH) for 1 hour at room temperature (RT). The linker contains a NHS ester which reacts with the lysine on the surface of the VLP. The amount of SMPH refers to 7.5x molar excess regarding one VLP monomer, i.e. one CMV polypeptide. Non-reacted cross-linker was removed by dialysis against 150 mM PBS, pH 7.4. To produce the rFel d1-CMV-Npadr and rFel d1-CMV-Ntt830 compositions, a protein solution of rFel d1 with a concentration of 2.7 mg/ml referring to 150 μ M in a volume of 200 μ l was mixed with 5x molar excess of the reducing agent Tris-2-carboxyethyl-phosphine (TCEP) and incubated for 5 min at RT. The molar excess was related to the protein Fel d1 amount. TCEP reduces disulfide bridges between two cysteine residues of two protein molecules in order to assure their availability for the coupling reaction to derivatized VLPs. The pre-treated protein Fel d1 were reacted in a molar ratio of 1:1 with 7.5x SMPH derivatized VLP CMV-Npadr and VLP CMV-Ntt830 for 3 hours at RT while shaking. The reduced cysteine of the protein reacted with the maleimide of the crosslinker SMPH bound to the VLP. After covalent coupling, non-coupled rFel d1 was removed by gel filtration in 150 mM PBS, pH 7.4.

The compositions were analyzed by SDS-PAGE and immunoblotting to observe and to confirm coupling bands. For this purpose, samples of SMPH-derivatized VLP CMV-Npdr and CMV-Ntt830, the compositions rFel d1-CMV-Npdr and rFel d1-CMV-Ntt830 and Fel d1 protein were loaded in parallel on a gel. Coupling was observed by immunoblotting using an anti-penta His antibodies binding to the His tag of the protein Fel d1. Visible bands indicated Fel d1 protein as well as the SMPH-derivatized VLP CMV-Npdr, VLP CMV-Ntt830, and the compositions rFel d1-CMV-Npdr and rFel d1-CMV-Ntt830 all of which were easily distinguished by their size. In addition, a SDS-PAGE gel was stained with Coomassie blue and coupling was observed and confirmed.

Immunization of mice with rFel d1 coupled to CMV-Npdr and CMV-Ntt830

Groups of five female Balb/c mice were either immunized with CMV-Npdr VLP and CMV-Ntt830 VLP coupled via SMPH to rFel d1. An amount of 5 µg rFel d1-CMV-Npdr and rFel d1-CMV-Ntt830, respectively, were diluted in 150 mM PBS, pH 7.4 to 150 µl and injected intravenously on day 0 and day 7. Mice were bled on days 0 (pre-immune), day 7, day 14, and day 25, and sera were analyzed using Fel d1 specific ELISA.

ELISA

The antibody response in mouse sera were analyzed at the indicated time. Antibodies specific for Fel d1 were analyzed by coating ELISA plates with a concentration of 1 µg/ml in a volume of 100 µl in PBS (pH 7.2) at 4°C overnight. ELISA plates were washed 5x with 200 µl PBS containing 0.05% Tween20 (pH 7.2, PBST). In order to avoid unspecific binding the ELISA plates were blocked with 200 µl of 2% BSA in PBST and incubated for 2 hours at RT. The serum samples were diluted in 2% BSA/PBST. Pre-diluted sera were transferred onto the coated plates and further serial diluted to obtain antibody titers based on OD50 calculation. After 2 hours of incubation at RT, the ELISA plates were washed 5x with 200 µl of PBST. Binding of serum antibodies was detected by horse-radish peroxidase-conjugated goat anti-mouse IgG (Jackson ImmunoResearch). The detection antibody was diluted 1:1000 in 2% BSA/PBST and a volume of 100 µl per sample was transferred. The plates were incubated for 1 hour at RT. ELISA plates were washed as described before. Prior washing the substrate solution was prepared. To this end, 1 tablet (10 mg) of OPD (1,2-Phenylenediamine dihydrochloride) and 9 µl of 30% H₂O₂ was dissolved in 25 ml citric acid buffer (0.066 M Na₂HPO₄, 0.035 M citric acid, pH 5.0). A volume of 100 µl of the substrate solution was pipetted onto the plates and exactly incubated for 7 minutes at RT. To stop the reaction 50 µl of stop solution (5% H₂SO₄ in H₂O)

was directly pipetted onto the plates. Absorbance readings at 450 nm of the 1, 2-Phenylenediamine dihydrochloride color reaction were analyzed. FIG. 8 shows that antibodies specific for Fel d1 were induced in mice with the inventive compositions rFel d1-CMV-Npdr (FIG. 8A) and rFel d1-CMV-Ntt830 (FIG. 8B).

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EXAMPLE 9

Coupling of α -synuclein peptide to CMV-Ntt830 and induction of immune response

Coupling of the α -synuclein peptide of SEQ ID 22 to the VLPs formed from CMV-Ntt830 (SEQ ID NO: 6) was performed as follows. A solution of 1 ml of 1 mg/ml CMV-Ntt830 in 20 mM HEPES, 50 mM NaCl, pH 7.3 was reacted for 60 min at room temperature with 79.3 μ l of a SMPH solution (10 mM in DMSO). Also, a solution of 1 ml of 1 mg/ml CMV-Ntt830 in 20 mM HEPES, 50 mM NaCl, pH 7.3 was reacted for 60 min at room temperature with 79.3 μ l of a Sulfo-KMUS solution (10 mM in 20 mM HEPES, pH 7.4). The reactions were dialysed at 4°C against three times of 2 L of 20 mM HEPES, 50 mM NaCl, pH 7.3 for 2 h, 14 h and 2 h in a Slide-A-Lyzer dialysis cassette with a MWCO of 10 kDa. 800 μ l of the derivatized and dialyzed CMV-Ntt830 solutions were mixed with 21.5 μ l of α -synuclein peptide (22.32 mg/ml) and incubated for 3 h at RT for chemical cross-linking at 500 rpm on a shaking Eppendorf Thermomixer. The coupling reactions were cleared by centrifugation at 20000 x g for 10 min at 4°C. Derivatized CMV-Ntt830 coat protein and coupled products were analyzed by SDS-PAGE analysis under reducing conditions on a NuPAGE® 4-12 % Bis-Tris gel. Subsequently, a densitometric analysis of the Coomassie-stained gel was performed. Several bands showing increased molecular weight with respect to the CMV-Ntt830 coat monomer were visible, clearly demonstrating the successful cross-linking of the α -synuclein peptide to the CMV-Ntt830 (Coomassie-stained NuPAGE®, not shown). Coupling density was above 1.0 and coupling efficiency was around 60%. To proof the integrity of the CMV-Ntt830 VLP after derivatization and coupling, derivatized CMV-Ntt830 and coupled products were analyzed by 1% agarose gel electrophoresis in 1 x TAE followed by Coomassie- and ethidium bromide-stain. Protein- and nucleic acid bands of derivatized and coupled CMV-Ntt830 VLP, respectively, co-migrate and display a similar migration behavior as non-derivatized and non-coupled CMV-Ntt830. The co-migration between coat protein and nucleic acids clearly demonstrated that the CMV-Ntt830 VLPs were still intact and that no nucleic acid was released from the CMV-Ntt830 VLPs.

Immunization of mice with α -synuclein derived-peptide coupled to CMV-Ntt830 coat protein

Groups of four female C57B/6 mice were immunized with CMV-Ntt830 VLPs coupled via SMPH to α -synuclein peptide (SEQ ID NO: 22). An amount of 250 μ g of total protein were diluted in 150 mM PBS, pH 7.4 to 150 μ l and injected i.v. on day 0 and day 14. Mice were bled on days 0 (pre-immune), day 7, day 14, day 21, day 28 and day 34, and sera were analyzed using α -synuclein specific ELISA.

ELISA

The α -synuclein peptides were coupled to bovine RNase A using the chemical cross-linker sulfo-SPDP. ELISA plates were coated with α -synuclein peptide-coupled RNase preparations at a concentration of 5 μ g/ml. The plates were blocked and then incubated with serially diluted mouse sera. Bound antibodies were detected with enzymatically labeled anti-mouse IgG. As a control, pre-immune sera of the same mice were also tested. All mice made a clear and strong IgG response against the α -synuclein derived peptide seen in FIG. 9.

EXAMPLE 10

Coupling of IL-17A peptide to CMV-Ntt830 and induction of immune response

Coupling of murine IL17A protein (SEQ ID NO: 20) to CMV-Ntt830 coat protein (SEQ ID NO:6) was performed. The mIL-17 protein was covalently conjugated to CMV-Ntt830 by a two- step procedure. First, CMV-Ntt830 VLPs (2 mg/ml in 50 mM NaH₂PO₄, 10% glycerol, pH 7.4) were reacted at RT for 60 min with an equimolar amount of the heterobifunctional chemical cross- linker, succinimidyl-6-(β -maleimidopropionamido)hexanoate (SMPH). Unreacted cross-linker was removed by gel filtration with a PD-10 desalting column using the same buffer (50 mM NaH₂PO₄, 10% glycerol, pH 7.4). Prior to the conjugation step, purified mIL-17 protein was incubated for 5 min at RT with a 10-fold excess of tri(2-carboxyethyl)phosphine hydrochloride (TCEP-HCl) to reduce any cysteine residues in the linker. Then mIL-17 protein was covalently linked to the derivatized VLPs by reacting equimolar amounts of mIL-17 protein and VLP for 3 h at RT. The vaccine was analyzed by SDS-PAGE followed by Coomassie- staining and immunoblotting with anti-His antibodies. The intensities of Coomassie blue-stained bands corresponding to the various components of the coupling reaction are determined by densitometry and are used to calculate coupling efficiency. Monomeric, derivatized CMV-Ntt830 migrated as a discrete 28 kDa band while the CMV-Ntt830 -mIL-17 conjugate migrated

at 45 kDa (28 kDa CMV-Ntt830 monomer + 17 kDa mIL-17 protein). The analysis by SDS-PAGE and Coomassie-staining visualized several bands of increased molecular weight with respect to the CMV-Ntt830 coat protein indicating the successful cross-linking of the IL-17 protein to the CMV-Ntt830 VLP. Coupling efficiency was defined as the molar ratio of CMV-Ntt830 monomers coupled to mIL-17 (45 kDa band) to total CMV-Ntt830 monomers (sum of 28 and 45 kDa bands). Coupling efficiency was determined to be at least 15.3% equaling one molecule mIL-17 per 6.5 molecules CMV-Ntt830. The coupling efficiency calculated in this way is a minimum estimate of the degree of coupling, because it does not take into account CMV-Ntt830 monomers coupled to more than one mIL-17 molecule. Thus, proteins of interest such as IL-17A can be efficiently coupled to CMV-Ntt830 VLPs.

Immunization of mice with mouse IL17A protein coupled to CMV-Ntt830 VLP

A group of 3 female BALB/c mice were immunized with CMV-Ntt830 coat protein coupled to mouse IL17A protein. Twenty µg of total protein were diluted in PBS, pH 7.4 to 150 µl and injected intravenously on day 0, day 14. Mice were bled on days 0 (pre-immune), day 7, day 14, day 21, and day 34 and sera were analyzed using mouse IL17A-specific ELISA.

ELISA

ELISA plates were coated with mouse IL17A, at a concentration of 1 µg/ml. The plates were blocked and then incubated with serially diluted mouse sera from day 0, 4, 14, 21 and 34. 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. Mice immunized with CMV-Ntt830 VLP coupled to IL17A mounted a strong immune response against IL17 protein (FIG.10).

Detection of neutralizing antibodies

Sera of mice immunized with mouse IL17 coupled to CMV-Ntt830 as described above is then tested for their ability to inhibit the binding of mouse IL17A protein to IL-17 receptor. ELISA plates are therefore coated with mouse IL-17 receptor A protein at a concentration of 1 µg/ml. Serial dilutions of mouse sera from day 35 mouse were pre-incubated with 10 ng/ml biotinylated mouse IL-17A for one hour and then added to the IL-17 receptor A coated plates. Binding of IL-17 to the coated receptor is detected with horse radish peroxidase conjugated to streptavidin. Neutralizing antibody titers are calculated as the average of those serum dilutions which led to half maximal optical density at 450 nm.

Efficacy of CMV-Ntt830 -mIL17A by amelioration of experimental autoimmune encephalitis in a mouse model for multiple sclerosis

Female SJL mice are immunized as described above and are injected subcutaneously with 100 µg PLP peptide mixed with complete Freund's adjuvant one week after the last immunization. On the same day all mice are injected intraperitoneally with 400 ng of pertussis toxin. Mice are scored on a daily basis for development of neurological symptoms according to the following scheme: 0, no clinical disease; 0.5, end of tail limp; 1, tail completely limp; 1.5, limp tail and hind limb weakness (unsteady gait and poor grip of hind legs); 2, unilateral partial hind limb paralysis; 2.5, bilateral partial hind limb paralysis; 3, complete bilateral hind limb paralysis; 3.5, complete bilateral hind limb paralysis and unilateral front limb paralysis; 4, total paralysis of hind and front limbs. The average clinical scores are assessed for mice which are immunized with CMV-Ntt830 -mIL17A or CMV-Ntt830 as described above. CMV-Ntt830-mIL 17 A- immunized mice show significantly reduced clinical symptoms between days 53 and 61 compared to CMV-Ntt830 -immunized mice. This demonstrates that the anti-IL-17 antibodies generated by immunization with CMV-Ntt830 -mIL17A are able to improve the clinical symptoms in a mouse model of multiple sclerosis.

EXAMPLE 11

Coupling of murine IL-5 to CMV-Ntt830 and induction of immune response

IL-5 was expressed and purified as described (Zou Y, et al., Vaccine 28 (2010) 3192–3200). Coupling of mIL-5 (SEQ ID NO:23) to the VLP CMV-Ntt830 with the modified CP of SEQ ID NO:6 was performed as follows. In order to be coupled to IL-5 CMV-Ntt830 VLPs were first derivatized with 30-fold excess of a heterobifunctional chemical cross-linker, succinimidyl-6-(β-maleimidopropionamido)hexanoate (SMPH). The unbound SMPH was removed by dialysis against PBS. rIL-5 was reduced for 1 h with an equimolar amount of tri(2-carboxyethyl)phosphine hydrochloride (TCEP) in PBS (pH 8.0). Reduced rIL-5 (80 µM) was incubated for 4 h at 22°C with 40 µM of SMPH derivatized CMV-Ntt830. The reaction was dialysed 12 h against PBS pH 8.0.

Immunization of mice with IL-5 derived-peptides coupled to VLPs of CMV-Ntt830 modified coat protein

Groups of four female Balb/c mice were either immunized with CMV-Ntt830 VLPs coupled via SMPH to IL-5 (SEQ ID NO:23). 50 µg of total protein were diluted in 20 mM

HEPES, 50 mM NaCl, pH 7.3 to 200 µl and injected subcutaneously (100 µl on two ventral sides) on day 0 and day 14. Mice were bled on days 0 (pre-immune), day 14, and day 21, and sera were analyzed using IL-5 and CMV-Ntt830 – specific ELISA.

5 **ELISA**

ELISA plates were coated either with IL-5 at a concentration of 10 µg/ml or CMV-Ntt830 VLPs at a concentration of 2 µg/ml. The plates were blocked and then incubated with serially diluted mouse sera. Bound antibodies were detected with enzymatically labeled anti-mouse IgG. As a control, pre-immune sera of the same mice were also tested (data not shown).

10 All mice made a clear and strong IgG response against the peptides as well as CMV-Ntt830

OVA based model of allergic airway inflammation

To induce allergic airway inflammation, female BALB/c mice (5 per group) were injected (i.p.) with 10µg of OVA (Grade V, Sigma–Aldrich) mixed with 2 mg of alum (Aluminium Hydroxide Gel Adjuvant, Brenntag Biosector, Denmark). 10 days later, mice were

15 challenged daily with 100 µg of OVA by intranasal administration for 4 days. 24 hours after the last challenge, BAL and lungs were subjected to histology. Mice injected i.p. with OVA and alum but not challenged intranasally with OVA served as a negative control for disease induction in these experiments.

20 Using this model, mice were immunized twice (d-0, d-14) with 100 µg CMV-Ntt830 VLPs or CMV-Ntt830 VLPs coupled to IL-5. On day 21, mice were sensitized with OVA and on day 31 challenged intranasally with OVA. IL-5 immunized mice had dramatically reduced eosinophil counts bronchoalveolar fluid.

25 **EXAMPLE 12**

Coupling of M2 peptide to CMV-Ntt830 virus-like particles

A solution of 2 ml of 1 mg/ml CMV-Ntt830 VLPs with the modified CP of SEQ ID NO:6 in PBS / 10 % glycerol pH 7.2 was reacted for 60 min at room temperature with 42.6 µl of a SMPH solution (150 mM in DMSO). The reaction solution was dialysed at 4⁰C against two 2 l

30 changes of 20 mM HEPES/10 % glycerol pH 7.2 over 12 and 4 hours. 0.6 ml of the derivatized and dialyzed CMV-Ntt830 solution was mixed with 21.45 µl of a 10 mM DMSO solution of the influenza peptide M2 (SEQ ID NO:24) and incubated 4 h at room temperature for chemical crosslinking resulting in CMV-Ntt830 -M2 conjugate vaccine. Uncoupled peptide was removed

by dialysis against 20 mM HEPES / 10 % glycerol pH 7.2 for 12 h and 4 h. The coupled product was analyzed on a 12 % Bis-Tris-polyacrylamide gel under reducing conditions. Several bands of increased molecular weight with respect to the CMV-Ntt830 capsid monomer were visible, clearly demonstrating the successful cross-linking of the influenza M2 peptide to the CMV-Ntt830 capsid.

Immunization of mice with M2 peptide coupled to CMV-Ntt830 capsids (CMV-Ntt830 -M2)

Four female Balb/c mice per group were immunized with 40 µg of CMV-Ntt830 -M2 vaccine formulated in 200 µl PBS and injected subcutaneously on day 0 and day 20. Mice were bled on day 34 and sera were analyzed using M2-specific and CMV-Ntt830 -specific ELISA.

Detection of anti-M2 antibodies by ELISA

ELISA plates were coated with M2 peptide (SEQ ID NO:24) at a concentration of 10 µg/ml or with CMV-Ntt830 virus-like particles at a concentration of 10 µg/ml. The plates were blocked and then incubated with serially diluted mouse sera. Bound antibodies were detected with enzymatically labeled anti-mouse IgG antibodies. Antibody titers of mouse sera are defined as the reciprocals of the dilutions leading to 50 % of the OD measured at saturation (OD₅₀). The average anti-M2 antibody titers and anti- CMV-Ntt830 titers at day 34 of mice which were >1:10000. The data demonstrate that coupling of M2 peptide to CMV-Ntt830 capsids is strongly enhancing the immunogenicity of the M2 peptide as the M2 peptide alone is not immunogenic.

The efficacy of CMV-Ntt830 -M2 immunization was tested in a murine model of influenza infection. Mice were challenged with a lethal dose of 4xLD₅₀ of mouse adapted influenza A/PR/8/34 virus. The virus was diluted in PBS and administered (2 x 50 µl) via the nose under light anaesthesia with isofuran. Mice, which lost more than 30 % body weight or which showed a body temperature equal to or lower than 30°C, were euthanized. The survival of the mice in both groups were as follows: All mice immunized with CMV-Ntt830-M2 survived while all mice immunized with CMV-Ntt830 alone died. The result demonstrates that immunization of mice with M2 peptide coupled to CMV-Ntt830 induces M2 specific antibodies which protect mice against a lethal infection with influenza A/PR/8/34 virus.

EXAMPLE 13

Coupling of Aβ1-6 peptide to CMV-Ntt830 virus-like particles

A solution of 2 ml of 1 mg/ml CMV-Ntt830 VLPs with the modified CP of SEQ ID NO:6 in PBS / 10 % glycerol pH 7.2 was reacted for 60 min at room temperature with 42.6 µl of a SMPH solution (150 mM in DMSO). The reaction solution was dialysed at 4°C against two 2 l changes of 20 mM HEPES/10 % glycerol pH 7.2 over 12 and 4 hours. 0.6 ml of the derivatized and dialyzed CMV-Ntt830 solution was mixed with 21.45 µl of a 10 mM DMSO solution of Aβ1-6 (SEQ ID NO:25) and incubated 4 h at room temperature for chemical crosslinking resulting in CMV-Ntt830 - Aβ1-6 conjugate vaccine. Uncoupled peptide was removed by dialysis against 20 mM HEPES / 10 % glycerol pH 7.2 for 12 h and 4 h. The coupled product was analyzed on a 12 % Bis-Tris-polyacrylamide gel under reducing conditions. Several bands of increased molecular weight with respect to the CMV-Ntt830 capsid monomer were visible, clearly demonstrating the successful cross-linking Aβ1-6 peptide CMV-Ntt830.

Immunization of mice with Aβ1-6 peptide coupled to CMV-Ntt830 capsids (CMV-Ntt830 -Aβ1-6)

Four female Balb/c mice per group were immunized with 40 µg of CMV-tt830 - Aβ1-6 vaccine formulated in 200 µl PBS and injected subcutaneously on day 0 and day 20. Mice were bled on day 34 and sera were analyzed using Aβ1-6 -specific and CMV-Ntt830-specific ELISA.

Detection of anti- Aβ1-6 antibodies by ELISA

ELISA plates were coated with Aβ1-6 coupled to RNase at a concentration of 10 µg/ml or with CMV-Ntt830 virus-like particles at a concentration of 2 µg/ml. The plates were blocked and then incubated with serially diluted mouse sera. Bound antibodies were detected with enzymatically labeled anti-mouse IgG antibodies. Antibody titers of mouse sera are defined as the reciprocals of the dilutions leading to 50 % of the OD measured at saturation (OD50). The average anti-Aβ1-6 antibody titers and anti- CMV-Ntt830 titers at day 34 of mice which were >1:10000. The data demonstrate that coupling of Aβ1-6 peptide to CMV-Ntt830 capsids is strongly enhancing the immunogenicity of the Aβ1-6 peptide as the Aβ1-6 peptide alone is not immunogenic. Brain section of human Alzheimer patients were stained with murine sera and plaques became clearly visible.

EXAMPLE 14

Cloning of Fel d 1 fusion proteins

A Fel d1 fusion protein (named F12H6GGC) consisting of chain 1 of Fel d1 fused to the N-terminus of chain 2 of Fel d1 via a 15 amino acid sequence (GGGGS)₃ (SEQ ID NO:47) and incorporating a HHHHHHGGC sequence (SEQ ID NO:48) fused to the C-terminus of chain 2 of Fel d1 was produced by oligonucleotide directed gene synthesis. The corresponding
 5 oligonucleotide sequence has the sequence of SEQ ID NO:49, wherein the protein sequence of F12H6GGC has the sequence of SEQ ID NO:50:

MEICPAVKRDVDLFLTGTPDEYVEQVAQYKALPVVLENARILKNCVDAKMTEEDKENA
 LSVLDKIYTSPLCGGGSGGGSGGGSGVSKMAETCPIFYDVFFAVANGNELLLDLSTLK
 VNATEPERTAMKKIQDCYVENGLISRVL DGLVMTTISSSKDCMGEAVQNTVEDLKLNT
 10 LGRHHHHHHGGC

After synthesis of the gene, it was excised from its helper plasmid and subcloned in frame into NdeI/XhoI sites of the plasmid pET42a(+) (Novagen, USA) resulting in the expression vector pET42-F12H6GGC.

Fel d1 fusion proteins without a hexa-histidine sequence (named F12GGC) were produced
 15 by PCR mutagenesis using the plasmid pET42-F12H6GGC as a template. The oligonucleotide primers used in the PCRs to produce these fusion proteins were:

For F12GGC, the forward primer was Fel_BglF (SEQ ID NO:51) and the reverse primer was Fel-dHR (SEQ ID NO:53).

All PCR products were cut with restriction enzymes BglII/XhoI and subcloned back into
 20 vector pET42-F126HGGC at the same excision sites. After isolation of plasmid DNA, the introduced changes were confirmed using a BigDye cycle sequencing kit and an ABI Prism 3100 Genetic analyzer (Applied Biosystems, Carlsbad, USA). The resulting expression vectors was named as pET42-F12GGC. It correspondingly encodes the Fel d1 fusion protein F12GGC (SEQ ID NO: 56).

MEICPAVKRDVDLFLTGTPDEYVEQVAQYKALPVVLENARILKNCVDAKMTEED
 25 KENALSVLDKIYTSPLCGGGSGGGSGGGSGVSKMAETCPIFYDVFFAVANGNELLLDL
 SLTKVNATEPERTAMKKIQDCYVENGLISRVL DGLVMTTISSSKDCMGEAVQNTVEDLK
 LNTLGRGGC (SEQ ID NO:56)

The hexa-histidine sequence enables purification by metal chelate affinity chromatography
 30 and the C-terminal sequence comprising GGC or GGCG (SEQ ID NO:58) enables coupling of the Fel d1 fusion proteins to CMV-Ntt830 and CMV-Npadr.

EXAMPLE 15

Expression and purification of Fel d 1 fusion proteins.

Expression of Fel d1 fusion proteins in *E.coli*. The Fel d1-expression vectors pET42-F12H6GGC and pET42-F12GGC were transformed into *E. coli* C2566 cells (New England Biolabs, Ipswich, USA). Clones expressing the highest levels of target protein were selected and used in further experiments. Expression of the various recombinant Fel d1 fusion proteins was performed in the following way. Cultures of *E. coli* harboring expression plasmids were grown in 2xTY medium containing kanamycin (25 mg/l) on a rotary shaker (200 rev/min; Infors, Bottmingen, Switzerland) at 30°C to an OD600 of 0.8–1.0. Expression of the Fel d1 fusion protein genes was then induced by adding 0.2 mM IPTG. The medium was supplemented with 5 mM MgCl₂. Incubation was continued on a rotary shaker at 20°C for 18 h. The resulting biomass was collected by low-speed centrifugation and frozen at -20°C until purification.

Purification of hexa-histidine-tagged Fel d1 fusion proteins. For purification of F12H6GGC fusion proteins, the USB PrepEase Kit (Affymetrix, High Wycombe, UK) was used according to manufacturer's instructions. After thawing on ice, *E. coli* cells from 100 ml culture (approx. 0.75 g) were suspended in 1 x LEW buffer containing 5 mM DTT and then disrupted by sonication. Insoluble proteins and cell debris were removed by centrifugation (13,000 rpm, 30 min at 5°C). The clarified lysate was applied to a Ni-IDA column, washed twice with the same buffer (without DTT) and eluted with 2 x 1.5 ml of imidazole containing 1 x E buffer. The fractions containing Fel d1 were identified by SDS/PAGE (FIG. 11A) and twice dialyzed against 200 volumes of the buffer (20 mM sodium phosphate, 2 mM EDTA, pH 7.0). After dialysis, the protein concentration was estimated using a QuBit fluorometer in accordance with manufacturer's instructions (Invitrogen, Eugene, USA) or by UV spectrophotometric measurement at 280 nm. The identity of the purified proteins was confirmed by mass spectrometric analysis (FIG. 11B) and by Western blot using anti-His-tag antibodies (Novagen, Cat.No. 71840-3; data not shown).

Purification of Fel d1 fusion proteins without hexa-histidine tags. For purification of F12GGC fusion proteins, anion exchange and hydrophobic interaction chromatography were used. Three grams of IPTG induced *E. coli* were disrupted by sonication in 20 ml of lysis buffer LB (20 mM Tris/HCl pH 8.0, 50 mM NaCl, 5 mM DTT). After sonication the solution was centrifuged for 15 min at 15 000 g and the supernatant collected. Ammonium sulfate was added with constant stirring until 30% saturation was achieved then incubated for 5 min at RT. After

centrifugation, solid ammonium sulfate was added to the recovered supernatant until 50% saturation. After centrifugation, protein pellets were collected and dissolved in 2 ml of LB and excess salt removed with a 5 ml HiTrapTM Desalting Column (GE Healthcare Life Sciences) equilibrated with LB. The desalted protein eluate was loaded onto a 1 ml HiTrapTMCaptoTM DEAE column equilibrated with LB. Bound F12GGC was eluted with an increasing gradient of NaCl. Fractions containing Fel d1 fusion proteins were collected and pooled. The resulting solution was diluted with 4 volumes of 20 mM Tris/HCl pH 8.0, 5 mM DTT and loaded onto a MonoQ 5/50 GL column in LB and eluted with an increasing NaCl gradient. Fractions containing Fel d1 fusion proteins were collected and pooled. 5 M NaCl was added until a concentration of 2.5 M was reached and DTT added to the solution, to maintain a concentration of 5 mM. The Fel d1 containing solution was then loaded onto a 1 ml HiTrapTM Butyl HP column in 2.5 M NaCl, 5 mM DTT and eluted with a continuously decreasing NaCl concentration. Fractions containing the Fel d1 fusion proteins were collected and pooled. All purification steps were monitored by Coomassie-stained SDS/PAGE gels (FIG. 11C). The identity of purified proteins was confirmed by Western blot using polyclonal antibodies raised against recombinant Fel d1 (data not shown).

EXAMPLE 16

Authenticity of recombinant Fel d1 fusion protein(s)

Fel d1 fusion proteins are similarly recognized by Fel d1-specific monoclonal antibodies. The binding of the Fel d1 fusion protein F12H6GGC and natural Fel d1 (nFel d1) to Fel d1-specific monoclonal antibodies (mAb) was compared using a sandwich ELISA Fel d1 ELISA kit (6F9/3E4) from Indoor biotechnologies (Cardiff, UK). To this end, Nunc ELISA plates were coated with the anti-Fel d1 mAb 6F9 (at 1 microg/ml) at 4°C overnight. Plates were washed with PBS containing 0.05% Tween 20 (PBST) and blocked with Superblock (Invitrogen) for 2 h at room temperature (RT). Natural Fel d1 as well as F12H6GGC (1 µg/ml) were serially diluted 1:3 and incubated for 2 h at RT. Plates were washed with PBST and biotinylated anti-Fel d1 mAb 3E4 (at 1 µg/ml) was added and incubated for 1 h at RT. Detection utilized Streptavidin conjugated to horse radish peroxidase (HRPO). To this end, plates were washed with PBST then Streptavidin-Peroxidase (Sigma, 1:1000 dilution) was added to the plates for 30 min at RT. Detection was performed with OPD substrate solution and 5% H₂SO₄ as stop solution. The absorbance was measured using an ELISA reader (BioRad) at 450 nm.

Natural Fel d1 and F12H6GGC gave similar titers in the ELISA which demonstrates they were similarly recognized by Fel d1-specific mAbs thus confirming the authenticity of the recombinant Fel d1 F12H6GGC (FIG. 12).

Recombinant Fel d1 fusion proteins activate basophils in whole blood of cat allergic patients. Blood of cat allergic patients contain basophils which carry Fel d1-specific IgE antibodies on their surface, which upon allergen exposure crosslink the FcεRI and cause degranulation. To check the ability of recombinant Fel d1 to cause degranulation, whole blood from an Fel d1-allergic patients was collected and used in combination with recombinant Fel d1 fusion protein F12H6GGC in a Basophil Activation Test kit of Bühlmann Laboratories (Flow Cast®, FK CCR). This assay measures up-regulation of an exclusive degranulation marker CD63 on CCR3+ basophils. Briefly, 100 µl of stimulation buffer was mixed with 50 µl of EDTA-treated whole blood. In addition, 50 µl of various dilutions of natural Fel d1 or recombinant Fel d1 fusion protein F12H6GGC were added. Positive control solutions including a mAb against FcεRI as well as an unspecific cell activator (fMLP) were also tested in the assay. Staining dye (20 µl per sample), containing anti-CCR3 Ab labeled to PE and anti-CD63 Ab labeled to FITC, was added and incubated at 37°C for 25 min. Erythrocytes were subsequently lysed adding lysis buffer. After 10 min incubation, the samples were centrifuged at 500 x g for 5 min and washed with wash buffer (PBS containing 2% FCS). After a second centrifugation step, the cell pellets were suspended in 200 µl wash buffer and acquired using a flow cytometer (FACS Calibur). The samples were analyzed with Cell Quest Pro software. The percentage of the CD63 expression on CCR3+ basophils was analyzed.

Recombinant Fel d1 fusion proteins was found to readily trigger degranulation of basophils from cat allergic patients. Moreover, when compared to natural Fel d1, similar levels of degranulation were achieved thus demonstrating authenticity of the recombinantly produced Fel d1 fusion proteins. (FIG. 13A/FIG. 13B).

EXAMPLE 17

Coupling of Fel d1 fusion proteins to CMV-Ntt830 and CMV-VLPs

The Fel d1 fusion protein F12H6GGC was covalently linked to CMV-Ntt830 and CMV-Npdr VLPs using the heterobifunctional chemical cross-linker succinimidyl-6-[(β-maleimidopropionamido) hexanoate] (SMPH) in the following way.

CMV-Ntt830 and CMV-Npdr virus-like particles stored in 5 mM Na-borate, 2 mM EDTA buffer, pH 9.0, were subject to buffer exchange with 20 mM Na-phosphate containing

5 Fel d1 fusion protein F12H6GGC was treated with 10 x molar excess TCEP (Thermo Fisher). Derivatized CMV-Ntt830 and CMV-Npadr-VLPs were reacted with 1x or 2x molar excess of recombinant Fel d1 fusion protein F12H6GGC for 3 h at 23°C. The coupling reaction was analyzed by reducing SDS-PAGE (NuPAGE® 4-12 % Bis-Tris gel) stained with Coomassie Blue. Protein bands with masses of approximately 44.5 kDa and 69 kDa were evident after the chemical conjugation reaction (data not shown). These bands correspond to the CMV coat protein (24.5 kDa) covalently linked with the Fel d1 fusion protein F12H6GGC (20 kDa) and two CMV coat protein molecules covalently linked with (49 kDa) one Fel d1 fusion protein F12H6GGC respectively indicating the formation of Fel d1-CMV VLPs.

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demonstrating the immune-enhancing effect of chemical conjugation of the Fel d1 fusion proteins to the VLP (FIG. 14).

EXAMPLE 19

5 **Coupling of human IL-1 β to CMV-Ntt830 and CMV-VLPs**

The human IL-1 β mature protein (SEQ ID NO:60) was covalently linked to CMV-Ntt830 and CMV-Npadr VLPs using the heterobifunctional chemical cross-linker succinimidyl-6-[(β -maleimidopropionamido) hexanoate] (SMPH) in the following way.

10 CMV-Ntt830 and CMV-Npadr virus-like particles stored in 5 mM Na-borate, 2 mM EDTA buffer, pH 9.0, were subject to buffer exchange with 20 mM Na-phosphate containing 30% sucrose and 2 mM EDTA using PD10 columns (GE Healthcare) A solution of CMV-Npadr or CMV-Ntt830 VLPs reacted for 60 min at RT with 7.5 x molar excess of heterobifunctional crosslinker SMPH. Unreacted SMPH was removed with PD10 columns in 20 mM Na-phosphate containing 30% sucrose and 2 mM EDTA.

15 The IL-1 β protein was treated with 5x molar excess TCEP (Thermo Fisher). Derivatized CMV-Ntt830 and CMV-Npadr-VLPs were reacted with 1x or 2x molar excess of human IL1 β for 3 h at 23°C. The coupling reaction was analyzed by reducing SDS-PAGE (NuPAGE® 4-12 % Bis-Tris gel) stained with Coomassie Blue. Protein bands with masses of approximately 41.5 kDa and 66 kDa were evident after the chemical conjugation reaction (data not shown). These
20 bands correspond to the CMV coat protein (24.5 kDa) covalently linked with the IL1 β protein (17 kDa) and two CMV coat protein molecules (49 kDa) covalently linked with one IL1 β protein respectively indicating the formation of IL1 β -CMV VLPs.

EXAMPLE 20

25 **Coupling of canine IL-5 to CMV-Ntt830 and induction of immune response**

Canine IL-5 (cIL-5) (SEQ ID NO:61) is modified to contain a free Cys expressed analogously as described for murine IL-5 in Zou Y, et al., Vaccine 28 (2010) 3192–3200. Coupling of the so modified cIL-5 to the VLP CMV-Ntt830 is performed as follows. In order to be coupled to cIL-5 CMV-Ntt830 VLPs are first derivatized with 30-fold excess of a
30 heterobifunctional chemical cross-liker, succinimidyl-6-(β -maleimidopropionamido)hexanoate (SMPH). The unbound SMPH is removed by dialysis against PBS. cIL-5 was reduced for 1 h with an equimolar amount of tri(2-carboxyethyl)phosphine hydrochloride (TCEP) in PBS (pH

8.0). Reduced cIL-5 (80 μ M) was incubated for 4 h at 22°C with 40 μ M of SMPH derivatized CMV-Ntt830. The reaction was dialysed 12 h against PBS pH 8.0.

Immunization of dogs with cIL-5 coupled to VLPs of CMV-Ntt830

Groups of four female outbreed dogs are immunized with CMV-Ntt830 VLPs coupled via SMPH to cIL-5 as described above. 50 μ g of the composition are diluted in 20 mM HEPES, 50 mM NaCl, pH 7.3 to 500 μ l and injected subcutaneously on day 0 and day 14. Dogs are bled on days 0 (pre-immune), day 14, and day 21, and sera are analyzed using cIL-5 and CMV-Ntt830 – specific ELISA.

ELISA

ELISA plates are coated either with cIL-5 at a concentration of 10 μ g/ml or CMV-Ntt830 VLPs at a concentration of 2 μ g/ml. The plates are blocked and then incubated with serially diluted mouse sera. Bound antibodies are detected with enzymatically labeled anti-canine IgG. As a control, pre-immune sera of the same dogs are also tested (data not shown). All dogs make a clear and strong IgG response against cIL-5 as well as CMV-Ntt830.

EXAMPLE 21

Coupling of canine IL-4 to CMV-Ntt830 and induction of immune response

Canine IL-4 (cIL-4) (SEQ ID NO:62) is modified to contain a free Cys expressed analogously to as described for murine IL-5 in Zou Y, et al., Vaccine 28 (2010) 3192–3200. Coupling of the so modified cIL-4 to the VLP CMV-Ntt830 is performed as follows. In order to be coupled to cIL-4 CMV-Ntt830 VLPs are first derivatized with 30-fold excess of a heterobifunctional chemical cross-linker, succinimidyl-6-(β -maleimidopropionamido)hexanoate (SMPH). The unbound SMPH is removed by dialysis against PBS. cIL-4 was reduced for 1 h with an equimolar amount of tri(2-carboxyethyl)phosphine hydrochloride (TCEP) in PBS (pH 8.0). Reduced cIL-4 (80 μ M) was incubated for 4 h at 22°C with 40 μ M of SMPH derivatized CMV-Ntt830. The reaction was dialysed 12 h against PBS pH 8.0.

Immunization of dogs with cIL-4 coupled to VLPs of CMV-Ntt830

Groups of four female outbreed dogs are immunized with CMV-Ntt830 VLPs coupled via SMPH to cIL-4 as described above. 50 μ g of the composition are diluted in 20 mM HEPES, 50 mM NaCl, pH 7.3 to 500 μ l and injected subcutaneously on day 0 and day 14. Dogs are bled

on days 0 (pre-immune), day 14, and day 21, and sera are analyzed using cIL-4 and CMV-Ntt830 – specific ELISA.

ELISA

ELISA plates are coated either with cIL-4 at a concentration of 10 µg/ml or CMV-Ntt830 VLPs at a concentration of 2 µg/ml. The plates are blocked and then incubated with serially diluted dog sera. Bound antibodies are detected with enzymatically labeled anti-canine IgG. As a control, pre-immune sera of the same dogs are also tested (data not shown). All dogs make a clear and strong IgG response against cIL-4 as well as CMV-Ntt830.

EXAMPLE 22

Coupling of canine IL-13 to CMV-Ntt830 and induction of immune response

Canine IL-13 (cIL-13) (SEQ ID NO:63) is modified to contain a free Cys and expressed analogously to as described for murine IL-5 in Zou Y, et al., Vaccine 28 (2010) 3192–3200.

Coupling of the so modified cIL-13 to the VLP CMV-Ntt830 is performed as follows. In order to be coupled to cIL-13 CMV-Ntt830 VLPs are first derivatized with 30-fold excess of a heterobifunctional chemical cross-linker, succinimidyl-6-(β-maleimidopropionamido)hexanoate (SMPH). The unbound SMPH is removed by dialysis against PBS. cIL-13 was reduced for 1 h with an equimolar amount of tri(2-carboxyethyl)phosphine hydrochloride (TCEP) in PBS (pH 8.0). Reduced cIL-13 (80 µM) was incubated for 4 h at 22°C with 40 µM of SMPH derivatized CMV-Ntt830. The reaction was dialysed 12 h against PBS pH 8.0.

Immunization of dogs with cIL-13 coupled to VLPs of CMV-Ntt830

Groups of four female outbred dogs are immunized with CMV-Ntt830 VLPs coupled via SMPH to cIL-13 as described above. 50 µg of the composition are diluted in 20 mM HEPES, 50 mM NaCl, pH 7.3 to 500 µl and injected subcutaneously on day 0 and day 113. Dogs are bled on days 0 (pre-immune), day 14, and day 21, and sera are analyzed using cIL-13 and CMV-Ntt830 – specific ELISA.

ELISA

ELISA plates were coated either with cIL-13 at a concentration of 10 µg/ml or CMV-Ntt830 VLPs at a concentration of 2 µg/ml. The plates are blocked and then incubated with serially diluted canine sera. Bound antibodies are detected with enzymatically labeled anti-

canine IgG. As a control, pre-immune sera of the same dogs are also tested (data not shown). All dogs make a clear and strong IgG response against cIL-13 as well as CMV-Ntt830.

EXAMPLE 23

5 **Coupling of human IL-31 to CMV-Ntt830 and induction of immune response**

Human IL-31 (hIL-31) (SEQ ID NO:43) is modified to contain a free Cys and expressed analogously to as described for murine IL-5 in Zou Y, et al., Vaccine 28 (2010) 3192–3200. Coupling of the so modified hIL-31 to the VLP CMV-Ntt830 is performed as follows. In order to be coupled to hIL-31 CMV-Ntt830 VLPs are first derivatized with 30-fold excess of a
10 heterobifunctional chemical cross-linker, succinimidyl-6-(β -maleimidopropionamido)hexanoate (SMPH). The unbound SMPH is removed by dialysis against PBS. hIL-31 was reduced for 1 h with an equimolar amount of tri(2-carboxyethyl)phosphine hydrochloride (TCEP) in PBS (pH 8.0). Reduced hIL-31 (80 μ M) is incubated for 4 h at 22°C with 40 μ M of SMPH derivatized CMV-Ntt830. The reaction was dialysed 12 h against PBS pH 8.0.

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Immunization of mice with hIL-31 coupled to VLPs of CMV-Ntt830

Groups of four female BALB/c mice are immunized with CMV-Ntt830 VLPs coupled via SMPH to IL-31 as described above. 50 μ g of the composition are diluted in 20 mM HEPES, 50 mM NaCl, pH 7.3 to 500 μ l and injected subcutaneously on day 0 and day 113. Dogs are bled
20 on days 0 (pre-immune), day 14, and day 21, and sera are analyzed using hIL-31 and CMV-Ntt830 – specific ELISA.

ELISA

ELISA plates were coated either with hIL-31 at a concentration of 10 μ g/ml or CMV-Ntt830 VLPs at a concentration of 2 μ g/ml. The plates are blocked and then incubated with
25 serially diluted mouse sera. Bound antibodies are detected with enzymatically labeled anti-mouse IgG. As a control, pre-immune sera of the same mice are also tested (data not shown). All mice make a clear and strong IgG response against hIL-31 as well as CMV-Ntt830.

CLAIMS

1. A modified virus-like particle (VLP) of cucumber mosaic virus (CMV), wherein said modified VLP of CMV comprises, essentially consists of, or alternatively consists of, at least one modified CMV polypeptide, wherein said modified CMV polypeptide comprises,
5 or preferably consists of,
 - (a) a CMV polypeptide, and
 - (b) a T helper cell epitope; andwherein said CMV polypeptide comprises, or preferably consists of,
 - (i) an amino acid sequence of a coat protein of CMV; or
 - 10 (ii) a mutated amino acid sequence, wherein the amino acid sequence to be mutated is an amino acid sequence of a coat protein of CMV, and wherein said mutated amino acid sequence and said coat protein of CMV show a sequence identity of at least 90 %, preferably of at least 95%, further preferably of at least 98% and again more preferably of at least 99%.
- 15 2. The modified VLP of CMV of claim 1, wherein said CMV polypeptide comprises, preferably consists of, an amino acid sequence of a coat protein of CMV.
3. The modified VLP of CMV of claim 1, wherein said CMV polypeptide comprises,
20 preferably consists of a mutated amino acid sequence, wherein the amino acid sequence to be mutated is an amino acid sequence of a coat protein of CMV, and wherein said mutated amino acid sequence and said coat protein of CMV show a sequence identity of at least 90 %, preferably of at least 95%, further preferably of at least 98% and again more preferably of at least 99%.
- 25 4. The modified VLP of CMV of claim 3, wherein said mutated amino acid sequence and said amino acid sequence to be mutated differ in least one and in at most 11, 10, 9, 8, 7, 6, 5, 4, 3, or 2 amino acid residues, and wherein preferably these differences are selected from (i) insertion, (ii) deletion, (iii) amino acid exchange, and (iv) any combination of (i) to (iii).
- 30 5. The modified VLP of CMV of any one of the preceding claims, wherein said CMV polypeptide comprises, or preferably consists of,

- (i) (a) an amino acid sequence of a coat protein of CMV, wherein said amino acid sequence comprises, or preferably consists of, SEQ ID NO:1 or (b) an amino acid sequence having a sequence identity of at least 75%, preferably of at least 80%, more preferably of at least 85%, again further preferably of at least 90 %, again more preferably of at least 95%, still further preferably of at least 98% and still again further more preferably of at least 99% of SEQ ID NO:1; or
- (ii) a mutated amino acid sequence, wherein said amino acid sequence to be mutated is said amino acid sequence as defined in (i) of this claim, and wherein said mutated amino acid sequence and said amino acid sequence to be mutated show a sequence identity of at least 95%, preferably of at least 98%, and more preferably of at least 99%.
6. The modified VLP of CMV of any one of the preceding claims, wherein said CMV polypeptide comprises, or preferably consists of, (a) an amino acid sequence of a coat protein of CMV, wherein said amino acid sequence comprises, or preferably consists of, SEQ ID NO:1 or (b) an amino acid sequence having a sequence identity of at least 75%, preferably of at least 80%, more preferably of at least 85%, again further preferably of at least 90 %, again more preferably of at least 95%, still further preferably of at least 98% and still again further more preferably of at least 99% of SEQ ID NO:1.
7. The modified VLP of CMV of any one of the preceding claims, wherein said CMV polypeptide comprises, or preferably consists of,
- (i) (a) an amino acid sequence of a coat protein of CMV, wherein said amino acid sequence comprises SEQ ID NO:21, or (b) an amino acid sequence of a coat protein of CMV comprising an amino acid sequence region, wherein said amino acid sequence region has a sequence identity of at least 75%, preferably of at least 80%, more preferably of at least 85%, again further preferably of at least 90 %, again more preferably of at least 95%, still further preferably of at least 98% and still again further more preferably of at least 99% with SEQ ID NO:21; or
- (ii) a mutated amino acid sequence, wherein said amino acid sequence to be mutated is said amino acid sequence as defined in (i) of this claim, and wherein said mutated amino acid sequence and said amino acid sequence to be mutated

show a sequence identity of at least 95%, preferably of at least 98%, and more preferably of at least 99%.

8. The modified VLP of CMV of any one of the preceding claims, wherein said CMV polypeptide comprises, or preferably consists of, (a) an amino acid sequence of a coat protein of CMV, wherein said amino acid sequence comprises SEQ ID NO:21, or (b) an amino acid sequence of a coat protein of CMV comprising an amino acid sequence region, wherein said amino acid sequence region has a sequence identity of at least 75%, preferably of at least 80%, more preferably of at least 85%, again further preferably of at least 90 %, again more preferably of at least 95%, still further preferably of at least 98% and still again further more preferably of at least 99% with SEQ ID NO:21.
9. The modified VLP of CMV of any one of the preceding claims, wherein said CMV polypeptide comprises, or preferably consists of,
- (i) (a) an amino acid sequence of a coat protein of CMV, wherein said amino acid sequence comprises, or preferably consists of, SEQ ID NO:1 or (b) an amino acid sequence having a sequence identity of at least 75%, preferably of at least 80%, more preferably of at least 85%, again further preferably of at least 90 %, again more preferably of at least 95%, still further preferably of at least 98% and still again further more preferably of at least 99% of SEQ ID NO:1; and wherein said amino sequence as defined in (a) or (b) in this claim comprises SEQ ID NO:21; or wherein said amino sequence as defined in (a) or (b) in this claim comprises an amino acid sequence region, wherein said amino acid sequence region has a sequence identity of at least 75%, preferably of at least 80%, more preferably of at least 85%, again further preferably of at least 90 %, again more preferably of at least 95%, still further preferably of at least 98% and still again further more preferably of at least 99% with SEQ ID NO:21; or
- (ii) a mutated amino acid sequence, wherein said amino acid sequence to be mutated is said amino acid sequence as defined in (i) of this claim, and wherein said mutated amino acid sequence and said amino acid sequence to be mutated show a sequence identity of at least 98% preferably of at least 99%.

10. The modified VLP of CMV of any one of the preceding claims, wherein said CMV

polypeptide comprises, or preferably consists of,

(a) an amino acid sequence of a coat protein of CMV, wherein said amino acid sequence comprises, or preferably consists of, SEQ ID NO:1 or

(b) an amino acid sequence having a sequence identity of at least 90 % of SEQ ID NO:1; and

wherein said amino sequence as defined in (a) or (b) in this claim comprises SEQ ID NO:21; or

wherein said amino sequence as defined in (a) or (b) in this claim comprises an amino acid sequence region, wherein said amino acid sequence region has a sequence identity of at least 90% with SEQ ID NO:21.

11. The modified VLP of CMV of any one of the preceding claims, wherein said T helper cell epitope replaces a N-terminal region of said CMV polypeptide.

12. The modified VLP of CMV of any one of the preceding claims, wherein the number of amino acids of said N-terminal region replaced is equal to or lower than the number of amino acids of which said T helper cell epitope consists.

13. The modified VLP of CMV of any one of the preceding claims, wherein said replaced N-terminal region of said CMV polypeptide consists of 5 to 15 consecutive amino acids, preferably of 9 to 14 consecutive amino acids, more preferably of 11 to 13 consecutive amino acids.

14. The modified VLP of CMV of any one of the preceding claims, wherein said N-terminal region of said CMV polypeptide corresponds to amino acids 2-12 of SEQ ID NO:1.

15. The modified VLP of CMV of any one of the preceding claims, wherein said T helper cell epitope is a universal T helper cell epitope.

16. The modified VLP of CMV of any one of the preceding claims, wherein said T helper cell epitope consists of at most 20 amino acids.

17. The modified VLP of CMV of any one of the preceding claims, wherein said Th cell epitope is a PADRE sequence.

18. The modified VLP of CMV of any one of the preceding claims, wherein said Th cell epitope comprises, preferably consists of, the amino acid sequence of SEQ ID NO:5.

5 19. The modified VLP of CMV of any one of the preceding claims, wherein said T helper cell epitope is derived from a human vaccine.

20. The modified VLP of CMV of any one of the preceding claims, wherein said Th cell epitope is derived from tetanus toxin.

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21. The modified VLP of CMV of any one of the preceding claims, wherein said Th cell epitope has, preferably consists of, the amino acid sequence of SEQ ID NO:4.

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22. The modified VLP of CMV of any one of the preceding claims, wherein said CMV polypeptide comprises, or preferably consists of, an amino acid sequence of a coat protein of CMV, wherein said amino acid sequence comprises, or preferably consists of, SEQ ID NO:1 or an amino acid sequence having a sequence identity of at least 95 % of SEQ ID NO:1; and wherein said amino sequence comprises SEQ ID NO:21, and wherein said T helper cell epitope replaces the N-terminal region of said CMV polypeptide, and wherein
20 said replaced N-terminal region of said CMV polypeptide consists of 11 to 13 consecutive amino acids, preferably of 11 consecutive amino acids, and wherein further preferably said N-terminal region of said CMV polypeptide corresponds to amino acids 2-12 of SEQ ID NO:1.

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23. The modified VLP of CMV of claim 1, wherein said modified CMV polypeptide comprises, preferably consists of, an amino acid sequence of SEQ ID NO:6.

24. The modified VLP of CMV of claim 1, wherein said modified CMV polypeptide comprises, preferably consists of, an amino acid sequence of SEQ ID NO:7.

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25. A composition comprising a modified virus-like particle of any one of the claims 1 to 24.

26. The composition of claim 25, wherein said composition comprises:

- (a) a modified virus-like particle of any one of claims 1 to 24, and wherein said modified virus-like particle comprises at least one first attachment site; and
- (b) at least one antigen, wherein said antigen comprises at least one second attachment site;

5 wherein (a) and (b) are linked through said at least one first and said at least one second attachment site.

27. The composition of claim 26, wherein said first attachment site and said second attachment site are linked via at least one covalent peptide-bond.

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28. The composition of claim 26, wherein said first attachment site and said second attachment site are linked via at least one covalent non-peptide-bond.

29. The composition of any one of the claims 26 to 28, wherein said first attachment site is an amino group, preferably an amino group of a lysine residue.

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30. The composition of any one of the claims 26 to 29, wherein said second attachment site is a sulfhydryl group, preferably a sulfhydryl group of a cysteine residue.

20 31. The composition of any one of the claims 26 to 30, wherein said first attachment site is not a sulfhydryl group.

32. The composition of any one of the claims 26 to 31, 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 modified 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 modified virus-like particle interact through said association to form an ordered and repetitive antigen array.

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33. The composition of any one of the claims 26 to 32, wherein said second attachment sites is a sulfhydryl group attached by means of chemical coupling.

34. The composition of any one of the claims 26 to 33 further comprising a linker, wherein

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said linker associates said antigen with said second attachment site, and wherein preferably said linker comprises or alternatively consists of said second attachment site.

- 5 35. The composition of any one of the claims 25 to 34, further comprising at least one immunostimulatory substance, wherein preferably said immunostimulatory substance is bound to, mixed with or packaged into said virus-like, and wherein preferably said immunostimulatory substance is packaged into said modified virus-like particle.
- 10 36. The composition of claim 35, wherein said immunostimulatory substance consists of DNA or RNA of non-eukaryotic origin.
- 15 37. The composition of claim 35 or claim 36, wherein said immunostimulatory substance is an unmethylated CpG-containing oligonucleotide, and wherein preferably said unmethylated CpG-containing oligonucleotide comprises a palindromic sequence, and wherein further preferably the CpG motif of said unmethylated CpG-containing oligonucleotide is part of a palindromic sequence, and wherein again further preferably said palindromic sequence is GACGATCGTC (SEQ ID NO: 31).
- 20 38. The composition of claim 37, wherein said palindromic sequence is flanked at its 5'-terminus by at least 3 and at most 15 guanosine entities, and wherein said palindromic sequence is flanked at its 3'-terminus by at least 3 and at most 15 guanosine entities.
- 25 39. The composition of any one of the claims 26 to 38, wherein said antigen is a tumor antigen, a self antigen, a polypeptide of a pathogen, an allergen or a hapten.
40. The composition of any one of the claims 26 to 39, wherein said antigen is IL- 17, and wherein preferably said antigen comprises, or preferably consists of, SEQ ID NO:42.
- 30 41. The composition of any one of the claims 26 to 39, wherein said antigen is IL-5, and wherein preferably said antigen comprises, or preferably consists of, SEQ ID NO:41.
42. The composition of any one of the claims 26 to 39, wherein said antigen is TNF α .
43. The composition of any one of the claims 26 to 39, wherein said antigen is IL- 1 α .

44. The composition of any one of the claims 26 to 39, wherein said antigen is IL-13, and wherein preferably said antigen comprises, or preferably consists of SEQ ID NO:46.

5 45. The composition of any one of the claims 26 to 39, wherein said antigen is a peptide derived from A β -1-42.

46. The composition of any one of the claims 26 to 39, wherein said antigen is IgE or a peptide or domain comprised in IgE.

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47. The composition of any one of the claims 26 to 39, wherein said antigen is α -synuclein or a peptide derived from α -synuclein, and wherein preferably said peptide consists of 6 to 14 amino acids, and wherein further preferably said antigen is a peptide derived from α -synuclein selected from any one of SEQ D NO:32, SEQ ID NO:33 and SEQ ID NO:34.

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48. The composition of any one of the claims 26 to 39, wherein said antigen is IL-4, and wherein preferably said antigen comprises, or preferably consists of SEQ ID NO:45.

49. The composition of any one of the claims 26 to 39, wherein said antigen is GnRH.

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50. The composition of any one of the claims 26 to 39, wherein said antigen is eotaxin.

51. The composition of any one of the claims 26 to 39, wherein said antigen is the extracellular domain of Influenza A virus M2 protein, or an antigenic fragment thereof.

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52. The composition of any one of the claims 26 to 39, wherein said antigen is IL-31, wherein preferably said antigen comprises, or preferably consists of, SEQ ID NO:43 or SEQ ID NO:44.

30 53. The composition of any one of the claims 26 to 39, wherein said antigen is derived from Plasmodium falciparum or Plasmodium Vivax, or wherein said antigen is the derived from RSV.

54. The composition of any one of the claims 26 to 39, wherein said antigen is the cat allergen Fel d1 or a recombinant Fel d1 (rFel d1), wherein preferably said antigen is a recombinant Fel d1 (rFel d1).
- 5 55. The composition of any one of the claims 26 to 39, wherein said antigen comprises, or preferably consists of, SEQ ID NO:17 or SEQ ID NO:18, and wherein preferably said antigen comprises, or preferably consists of, SEQ ID NO:18.
- 10 56. The composition of any one of the claims 26 to 39, wherein said antigen is the dog allergen Can f1 or Can f2.
57. The composition of one of the claims 26 to 39, wherein said antigen is calcitonin gene-related peptide (CGRP).
- 15 58. The composition of one of the claims 26 to 39, wherein said antigen is Amylin.
59. A vaccine comprising, or alternatively consisting of, the modified virus-like particle of any one of claims 1 to 24 or the composition of any one of claims 25 to 58.
- 20 60. A pharmaceutical composition comprising:
(a) the modified virus-like particle of any one of claims 1 to 24, the composition of any one of claims 25 to 58, or the vaccine of claim 59; and
(b) a pharmaceutically acceptable carrier, diluent and/or excipient.
- 25 61. A method of immunization comprising administering the modified virus-like particle of any one of claims 1 to 24, the composition of any one of claims 25 to 58, the vaccine of claim 59, or the pharmaceutical composition of claim 60 to an animal, or a human.
- 30 62. The method of claim 61, wherein said modified virus-like particle, virus-like particle, said composition, said vaccine, or said pharmaceutical composition, is administered to said animal or said human subcutaneously, intravenously, intradermally, intranasally, orally, intranodally or transdermally.
63. The modified virus-like particle of any one of the claims 1 to 24, the composition of any

one of claims 25 to 58, the vaccine of claim 59, or the pharmaceutical composition of claim 60 for use as a medicament.

- 5 64. Use of the modified virus-like particle of any one of the claims 1 to 24, the composition of any one of claims 25 to 58, the vaccine of claim 59, or the pharmaceutical composition of claim 60 in the manufacture of a medicament for the treatment of a disease, disorder or condition in an animal or a human.
- 10 65. The modified virus-like particle of any one of the claims 1 to 24, the composition of any one of the claims 25 to 58, the vaccine of claim 59, or the pharmaceutical composition of claim 60 for use in a method for the treatment of a disease, disorder or condition in an animal or human.
- 15 66. The composition of any one of the claims 40 to 44 and 48 for use in a method of treating inflammatory disease in an animal or human.
67. The composition of claim 66, wherein said inflammatory disease is selected from RA, MS, Psoriasis, asthma, Crohns, Colitis, COPD, diabetes, neurodermatitis (allergic dermatitis).
- 20 68. The composition of claim 45 for use in a method of treating Alzheimer's disease in an animal or human.
69. The composition of claim 46 for use in a method of treating allergies in an animal or human.
- 25 70. The composition of claim 47 for use in a method of treating Parkinson's disease in an animal or human.
71. The composition of claims 49 to lower testosterone levels in an animal or human.
- 30 72. The composition of claim 51 or claim 52 for use in a method of treating or preventing influenza A virus infection in an animal or human.
73. The composition of claim 53 for use in a method of preventing or treating malaria.

74. The composition of claim 54 for use in a method of preventing or treating RSV infection.

5 75. The composition of claims 55 for use in a method of preventing or treating cat allergy in a human.

76. The composition of claims 56 for use in a method of preventing or treating dog allergy in a human.

10 77. The composition of claim 57 for use in a method of treating migraine in an animal or human.

78. The composition of claim 58 for use in a method of treating type II diabetes animal or human.

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