vaccines, there is still a need for further distinct VLP systems. In particular, vaccines induce variable antibody responses in immunized subjects and individuals often spanning 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 associated with certain MHC class II molecules and failure to induce good T helper (Th) cell responses is believed to be responsible for poor antibody responses seen in these individuals (Goncalves L, et al., Virology (2004) 326:20–28). Furthermore, elderly 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. Moreover, one further associated very important problem yet to be solved during the vaccine construction process is antigen spatial conformation. For vaccine construction, it is important to achieve optimal peptide presentation on the particle surface without affecting the overall viral structure. This becomes evident since only in exceptional cases, VLPs are able to accommodate more than 50 or 70 amino acids-long protein domains or even still longer and retain the typical VLP morphology (I. Balke et al., Adv. Drug Deliv. Rev. (2018), https://doi.org/10.1016/j.addr.2018.08.007; I. Kalnciema, et al. Mol. Biotechnol. 52 (2012) 129–139).

SUMMARY

We have now surprisingly found that antigenic polypeptides of various and very different length and nature can be inserted at a specific position of CMV polypeptides already modified by the incorporation of a T helper cell epitope, and that said resulting fusion proteins are still capable of forming and assembling to modified virus-like particles (VLPs) which are, in addition, highly immunogenic. Preferably and further surprisingly, mosaic modified virus-like particles have been generated comprising said described fusion proteins with the in-fused antigenic polypeptides and further comprising CMV proteins with no such in-fused antigenic polypeptides. These mosaic modified CMV VLPs were found to be highly beneficial for and allow even the incorporation of antigenic polypeptides of very high length such as the incorporation of antigenic polypeptides of up to more than 200 amino acids in length.

In a first example, the present disclosure provides a modified virus-like particle (VLP) of cucumber mosaic virus (CMV) comprising at least one fusion protein, wherein said at

least one fusion protein comprises, or preferably consists of,

a) a chimeric CMV polypeptide, wherein said chimeric CMV polypeptide comprises, or preferably consists of

(i) a CMV polypeptide, wherein said CMV polypeptide comprises, or preferably consists of, a coat protein of CMV, wherein preferably said coat protein of CMV comprises, or preferably consists of, SEQ ID NO:62; 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% with SEQ ID NO:62; and

(ii) an antigenic polypeptide, wherein said antigenic polypeptide is inserted into said CMV polypeptide,

wherein said insertion of said antigenic polypeptide is between amino acid residues of said CMV polypeptide corresponding to amino acid residues of position 84 and position 85 of SEQ ID NO:62; and

(iii) a T helper cell epitope, wherein said T helper cell epitope replaces a N-terminal region of said CMV polypeptide, and wherein preferably said N-terminal region of said CMV polypeptide corresponds to amino acids 2-12 of SEQ ID NO:62.

In one aspect, the present disclosure refers to a modified virus-like particle (VLP) of cucumber mosaic virus (CMV) comprising

(a) at least one fusion protein, wherein said at least one fusion protein comprises

a) a chimeric CMV polypeptide comprising

(i) a CMV polypeptide, wherein said CMV polypeptide comprises a coat protein of CMV or an amino acid sequence having a sequence identity of at least 75% with SEQ ID NO:62; and

(ii) an antigenic polypeptide, wherein said antigenic polypeptide is inserted into said CMV polypeptide, and said antigenic polypeptide has a length of at least 40 amino acids;

wherein said insertion of said antigenic polypeptide is between amino acid residues of said CMV polypeptide corresponding to amino acid residues of

position 84 and position 85 of SEQ ID NO:62; and

(iii) a T helper cell epitope, wherein said T helper cell epitope replaces a N-terminal region of said CMV polypeptide, wherein said Th cell epitope is selected from the sequences SEQ ID NO:64, SEQ ID NO:65, and SEQ ID NOs:67-71, and wherein said N-terminal region of said CMV polypeptide corresponds to amino acids 2-12 of SEQ ID NO:62; and

(b) at least one CMV protein, wherein said at least one CMV protein comprises a coat protein of CMV or an amino acid sequence having a sequence identity of at least 75% with SEQ ID NO:62, and wherein said CMV protein is optionally modified by a T helper cell epitope, and wherein preferably said coat protein of CMV comprises SEQ ID NO:62.

In a further example, the present disclosure provides a modified VLP of CMV comprising at least one fusion protein, wherein said at least one fusion protein comprises, or preferably consists of,

a) a chimeric CMV polypeptide, wherein said chimeric CMV polypeptide comprises, or preferably consists of

(i) a CMV polypeptide, wherein said CMV polypeptide comprises, or preferably consists of, a coat protein of CMV, wherein preferably said coat protein of CMV comprises, or preferably consists of, SEQ ID NO:62; 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% with said coat protein, preferably with said SEQ ID NO:62; and

(ii) an antigenic polypeptide, wherein said antigenic polypeptide is inserted into said CMV polypeptide,

wherein said insertion of said antigenic polypeptide is between amino acid residues of said CMV polypeptide corresponding to amino acid residues of position 84 and position 85 of SEQ ID NO:62; and

(iii) a first amino acid linker, preferably a first amino acid linker and a second amino acid linker, wherein said first amino acid linker is positioned at the N- or at the C-terminus of said antigenic polypeptide, and wherein preferably said first amino acid linker is selected from the group consisting of:

(a.) a polyglycine linker $(Gly)_n$ of a length of n=2-10;

(b.) a glycine-serine linker (GS-linker) comprising at least one glycine and at least one serine, wherein preferably said GS linker has an amino acid sequence of $(GS)_r(G_sS)_t(GS)_u$ with r=0 or 1, s=1-5, t=1-5 and u=0 or 1; and

(c.) an amino acid linker comprising at least one Gly, at least one Ser, and at least one amino acid selected from Thr, Ala, Lys, Asp and Glu.

In another example, the present disclosure provides a modified virus-like particle (VLP) of cucumber mosaic virus (CMV) comprising

(a) at least one fusion protein, wherein said at least one fusion protein comprises, or preferably consists of,

a) a chimeric CMV polypeptide, wherein said chimeric CMV polypeptide comprises, or preferably consists of

(i) a CMV polypeptide, wherein said CMV polypeptide comprises, or preferably consists of, a coat protein of CMV, wherein preferably said coat protein of CMV comprises, or preferably consists of, SEQ ID NO:62; 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% with SEQ ID NO:62; and

(ii) an antigenic polypeptide, wherein said antigenic polypeptide is inserted into said CMV polypeptide,

wherein said insertion of said antigenic polypeptide is between amino acid residues of said CMV polypeptide corresponding to amino acid residues of position 84 and position 85 of SEQ ID NO:62; and

(b) at least one CMV protein, wherein said CMV protein comprises, or preferably consists of, a coat protein of CMV, wherein preferably said coat protein of CMV comprises, or preferably consists of, SEQ ID NO:62; 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% with SEQ ID NO:62, and wherein said CMV protein is optionally modified by a T helper cell epitope, wherein said T helper cell epitope replaces a N-terminal region of said CMV protein, and wherein preferably said N-terminal region of said CMV protein corresponds to amino acids 2-12 of SEQ ID NO:62.

Further examples of the present disclosure will be become apparent as this description continues.

BRIEF DESCRIPTION OF FIGURES

FIG. 1: Description of pET-CMV-Ntt830-Ab36 plasmid map with single-cut restriction enzyme sites. All other maps (pET-CMV-Ntt830-Ab15; pET-CMV-Ntt830-Ab16; pET-CMV-Ntt830-Ab17) have essentially the same sequences and gene organisations; they differ only in nucleotide sequences of coding for amyloid (beta) peptides.

FIG. 2A: SDS-PAGE gel analysis of the purification of the VLP derived from the expression of CMV-Ntt830-Ab36. M - protein size marker PageRuler (Thermo Fisher Scientific, # 26620); S – soluble proteins in *E. coli* C2566 cells after 18h cultivation at 20°C in cell extract before sucrose gradient (20-60%); P - insoluble proteins; 1 - 6 - sucrose gradient fractions (from 60% at the bottom of tube to 0% at the top. The asterisc

CLAIMS

1. A modified virus-like particle (VLP) of cucumber mosaic virus (CMV) comprising

(a) at least one fusion protein, wherein said at least one fusion protein comprises

a) a chimeric CMV polypeptide comprising

(i) a CMV polypeptide, wherein said CMV polypeptide comprises a coat protein of CMV or an amino acid sequence having a sequence identity of at least 75% with SEQ ID NO:62; and

(ii) an antigenic polypeptide, wherein said antigenic polypeptide is inserted into said CMV polypeptide, and said antigenic polypeptide has a length of at least 40 amino acids;

wherein said insertion of said antigenic polypeptide is between amino acid residues of said CMV polypeptide corresponding to amino acid residues of position 84 and position 85 of SEQ ID NO:62; and

(iii) a T helper cell epitope, wherein said T helper cell epitope replaces a N-terminal region of said CMV polypeptide, wherein said Th cell epitope is selected from the sequences SEQ ID NO:64, SEQ ID NO:65, and SEQ ID NOs:67-71, and wherein said N-terminal region of said CMV polypeptide corresponds to amino acids 2-12 of SEQ ID NO:62; and

(b) at least one CMV protein, wherein said at least one CMV protein comprises a coat protein of CMV or an amino acid sequence having a sequence identity of at least 75% with SEQ ID NO:62, and wherein said CMV protein is optionally modified by a T helper cell epitope, and wherein preferably said coat protein of CMV comprises SEQ ID NO:62.

2. The modified VLP of CMV of claim 1, wherein said chimeric CMV polypeptide further comprises a first amino acid linker, wherein said first amino acid linker is positioned at the N- or at the C-terminus of said antigenic polypeptide, and wherein said first amino acid linker is selected from the group consisting of:

(a.) a polyglycine linker $(Gly)_n$ of a length of n=2-10;

(b.) a glycine-serine linker (GS-linker) comprising at least one glycine and at least one serine, wherein said GS linker has an amino acid sequence of $(GS)_r(G_sS)_t(GS)_u$ with r=0 or 1, s=1-5, t=1-5 and u=0 or 1; and

(c.) an amino acid linker comprising at least one Gly, at least one Ser, and at least one amino acid selected from Thr, Ala, Lys, Asp and Glu, wherein said linker comprises an amino acid sequence of $(DED)_x(G_sS)_t(G)_y(DED)_z(GS)_u$ with s=3 or 4, t=1, 2 or 3, u=0 or 1, x=0, y=1-5 and z= 1, or said linker comprises an amino acid sequence of $(TS)(DED)_x(G_sS)_t(G)_y(DED)_z(GS)_u$ with s=1-5, t=1-5, u=0 or 1, x=0 or 1, y=0-5 and z=0 or 1;

and wherein said first amino acid linker has a length of at most 30 amino acids.

3. The modified VLP of CMV of claim 2, wherein said chimeric CMV polypeptide further comprises a second amino acid linker, wherein said first amino acid linker is positioned at the N-terminus of said antigenic polypeptide, and said second amino acid linker is positioned at the C-terminus of said antigenic polypeptide, and wherein said second amino acid linker is selected from the group consisting of:

(a.) a polyglycine linker $(Gly)_n$ of a length of n=2-10;

(b.) a glycine-serine linker (GS-linker) comprising at least one glycine and at least one serine, wherein said GS linker has an amino acid sequence of $(GS)_r(G_sS)_t(GS)_u$ with r=0 or 1, s=1-5, t=1-5 and u=0 or 1; and

(c.) an amino acid linker comprising at least one Gly, at least one Ser, and at least one amino acid selected from Thr, Ala, Lys, Asp and Glu, wherein said linker comprises an amino acid sequence of $(DED)_x(G_sS)_t(G)_y(DED)_z(GS)_u$ with s=3 or 4, t=1, 2 or 3, u=0 or 1, x=0, y=1-5 and z= 1, or said linker comprises an amino acid sequence of $(TS)(DED)_x(G_sS)_t(G)_y(DED)_z(GS)_u$ with s=1-5, t=1-5, u=0 or 1, x=0 or 1, y=0-5 and z=0 or 1;

and wherein said second amino acid linker has a length of at most 30 amino acids.

4. The modified VLP of CMV of claim 2 or claim 3, wherein said first and/or said second amino acid linker is independently selected from (i) a glycine-serine linker (GS-linker) comprising at least one glycine and at least one serine, wherein said GS linker has an amino acid sequence of $(GS)_r(G_sS)_t(GS)_u$ with r=0 or 1, s=1-5, t=1-5 and u=0 or 1, and (ii) a glycine-serine-glutamic acid-aspartic acid linker (GSED-linker) comprising at least one glycine, at least one serine, at least one glutamic acid and at least one aspartic acid, wherein said GSED linker comprises an amino acid sequence of $(DED)_x(G_sS)_t(G)_y(DED)_z(GS)_u$ with s=3, t=1, 2 or 3, u=0 or 1, x=0, y=1-5 and z= 1,

or said GSED linker comprises an amino acid sequence of $(TS)(DED)_x(G_sS)_t(G)_y(DED)_z(GS)_u$ with s=1-5, t=1-5, u=0 or 1, x=0 or 1, y=0-5 and z=0 or 1.

5. The modified VLP of CMV of any one of the preceding claims, wherein said CMV polypeptide is a coat protein of CMV or an amino acid sequence having a sequence identity of at least 90%, preferably 95% with SEQ ID NO:62.

6. The modified VLP of CMV of any one of the preceding claims, wherein said CMV polypeptide comprises, or preferably consists of,

(i) an amino acid sequence of a coat protein of CMV, wherein said amino acid sequence comprises, or preferably consists of, SEQ ID NO:62; or

(ii) an amino acid sequence having a sequence identity of at least 90 % of SEQ ID NO:62; and

wherein said amino sequence as defined in (i) or (ii) comprises SEQ ID NO:63 or an amino acid sequence region, wherein said amino acid sequence region has a sequence identity of at least 90% with SEQ ID NO:63.

7. The modified VLP of CMV of any one of the preceding claims, wherein said Th cell epitope is derived from tetanus toxin or is a PADRE sequence.

8. The modified VLP of CMV of any one of the preceding claims, wherein said Th cell epitope comprises the amino acid sequence of SEQ ID NO:64 or SEQ ID NO:65.

9. The modified VLP of CMV of any one of the preceding claims, wherein said chimeric CMV polypeptide comprises the amino acid sequence of SEQ ID NO:5 or SEQ ID NO:66, wherein said antigenic polypeptide is inserted into said chimeric CMV polypeptide of SEQ ID NO:5 between amino acid residues of position 88 and position 89 of SEQ ID NO:5 or wherein said antigenic polypeptide is inserted into said chimeric CMV polypeptide of SEQ ID NO:66 between amino acid residues of position 86 and position 87 of SEQ ID NO:66.

10. The modified VLP of CMV of any one of the preceding claims, wherein said antigenic polypeptide is a polypeptide derived from the group consisting of: (a) allergens; (b)

viruses; (c) bacteria; (d) parasites; (e) tumors; (f) self-molecules; (g) hormones; (h) cytokines; and (i) chemokines, 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 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.

11. The modified VLP of CMV of any one of the preceding claims, wherein said antigenic polypeptide is an allergen, a self antigen, a tumor antigen, or a polypeptide of a pathogen, wherein 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.

12. The modified VLP of CMV of any one of the preceding claims, wherein said antigenic polypeptide is a peanut allergen.

13. The modified VLP of CMV of claim 12, wherein said peanut allergen comprises, or preferably consists of, a protein with the amino sequence selected from SEQ ID NO:27, SEQ ID NO:72 or SEQ ID NO:73 or a protein with an amino acid sequence of at least 90% amino acid sequence identity with SEQ ID NO:27, SEQ ID NO:72 or SEQ ID NO:73.

14. The modified VLP of CMV of claim 1, wherein said chimeric CMV polypeptide is selected from the group consisting of SEQ ID NO:29, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:128, SEQ ID NO:132, SEQ ID NO:135 or SEQ ID NO:139.

15. The modified VLP of CMV of claim 1, wherein said chimeric CMV polypeptide consists of SEQ ID NO:29, and wherein said CMV protein consists of SEQ ID NO:5.