(54) Title: VIRUS LIKE PARTICLE COMPOSITION

Fig. 9

VLP-TNFalpha induced anti-TNFalpha antibodies

[Continued on next page]
Abstract: The present invention provides a particle comprising a polypeptide and at least one antigen, and a composition comprising thereof.


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— with international search report (Art. 21(3))
— with sequence listing part of description (Rule 5.2(a))
DESCRIPTION

VIRUS LIKE PARTICLE COMPOSITION

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. provisional Patent Application No.: 61/599,746 filed on February 16, 2012, the entire contents of which are incorporated by reference herein.

TECHNICAL FIELD

The present invention relates to a particle comprising a polypeptide and at least one antigen, and a composition comprising thereof.

BACKGROUND

Virus-like particles (VLPs) are multiprotein structures that mimic the organization and conformation of authentic native viruses but lack the viral genome, potentially yielding safer and cheaper vaccine candidates. A handful of prophylactic VLP-based vaccines is currently commercialized worldwide: GlaxoSmithKline's Engerix® (hepatitis B virus) and Cervarix® (human papillomavirus), and Merck and Co., Inc.'s Recombivax HB® (hepatitis B virus) and Gardasil® (human papillomavirus) are some
examples. Other VLP-based vaccine candidates are in clinical trials or undergoing preclinical evaluation, such as, influenza virus, parvovirus, Norwalk and various chimeric VLPs. Many others are still restricted to small-scale fundamental research, despite their success in preclinical tests. The implications of large-scale VLP production are discussed in the context of process control, monitorization and optimization. The main up- and downstream technical challenges are identified and discussed accordingly. Successful VLP-based vaccine blockbusters are briefly presented concomitantly with the latest results from clinical trials and the recent developments in chimeric VLP-based technology for either therapeutic or prophylactic vaccination (Expert Rev. Vaccines 9(10), 1149-1176, 2010).

Chikungunya virus (CHIKV) has infected millions of people in Africa, Europe and Asia since this alphavirus reemerged from Kenya in 2004. The severity of the disease and the spread of this epidemic virus present a serious public health threat in the absence of vaccines or antiviral therapies. It is reported that a VLP vaccine for epidemic Chikungunya virus protects non-human primates against infection (Nat Med. 2010 March; 16 (3) : 334-338) . US patent publication No. 2012/0003266 discloses a virus-
like particle (VLP) comprising one or more Chikungunya virus structural polypeptides which is useful for formulating a vaccine or antigenic composition for Chikungunya that induces immunity to an infection or at least one symptom thereof. WO2012/106356 discloses modified alphavirus or flavivirus virus-like particles (VLPs) and methods for enhancing production of modified VLPs for use in the prevention or treatment of alphavirus and flavivirus-mediated diseases, (these cited references are herein incorporated by reference).

SUMMARY OF THE INVENTION

In the first aspect, the present invention provides a particle which is capable of being self-assembled, comprising a polypeptide and at least one antigen, wherein said polypeptide comprises at least one first attachment site and said at least one antigen comprises at least one second attachment site, and wherein said polypeptide and said antigen are linked through said at least one first and said at least one second attachment site.

In the second aspect, the present invention provides a nucleic acid molecule comprising a nucleotide sequence
that encodes a particle provided in the first aspect of the present invention.

In the third aspect, the present invention provides a composition comprising the particle provided in the first aspect of the present invention and/or the nucleic acid molecule provided in the second aspect of the present invention.

In the fourth aspect, the present invention provides a method of producing an antibody, comprising contacting the particle provided in the first aspect of the present invention and/or the nucleic acid molecule provided in the second aspect of the present invention to a mammal.

In the fifth aspect, the present invention provides a method of immunomodulation, a method of treating an autoimmune disease, a method of inducing and/or enhancing immune response against an antigen in a mammal, and a method of treating cancer comprising administering the composition provided in the third aspect of the present invention to a mammal.

In sixth aspect, the present invention provides a method of passive immunization, comprising administering the antibody provided in the fourth aspect of the present invention to a mammal.
In seventh aspect, the present invention provides a method of presenting an antigen on macrophage, comprising contacting the particle provided in the first aspect of the present invention and/or the nucleic acid molecule provided in the second aspect of the present invention to a mammal.

In eighth aspect, the present invention provides a method for producing the particle provided in the first aspect of the present invention, comprising preparing a gene comprising a nucleotide sequence encoding said particle; culturing a cell which is transfected with said gene to express said particle; and recovering said particle.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows modification of TNF alpha sequence to be inserted into Venezuelan Equine Encephalitis virus (VEEV) structural polypeptide.

Fig. 2 shows results of Western Blot which indicates that TNF alpha conjugated VLP was expressed.

Fig. 3 shows VLP_CHI 512 vector.

Fig. 4 shows VLP_CHI 532 vector.
Fig. 5 shows VLP_CHI 520 vector.

Fig. 6 shows VLP_VEEV VLP 518 vector.

Fig. 7 shows VLP_VEEV VLP 519 vector.

Fig. 8 shows VLP_VEEV VLP 538 vector.

Fig. 9 shows detection of anti-TNF alpha antibodies induced by TNF alpha derived peptide-conjugated virus like particle.

Fig. 10 shows detection of anti-human CD20 antibodies induced by CD20 derived peptide-conjugated virus like particle.

Fig. 11 shows detection of anti-mouse CD20 antibodies induced by CD20 derived peptide-conjugated virus like particle.

DETAILED DESCRIPTION OF THE INVENTION

(1) A particle comprising a polypeptide and at least one antigen

In the first aspect, the present invention provides a particle which is capable of being self-assembled, comprising a polypeptide and at least one antigen, wherein said polypeptide comprises at least one first attachment
site and said at least one antigen comprises at least one second attachment site, and wherein said polypeptide and said antigen are linked through said at least one first and said at least one second attachment site.

As used herein, "a particle which is capable of being self-assembled" refers to a particle formed by at least one constituent which is spontaneously assembled. The constituent may be a polypeptide or non-peptide chemical compound. In one embodiment, "a particle which is capable of being self-assembled" may be a particle comprising or consisting of at least one polypeptide. The at least one polypeptide consists of one or more kinds of peptide. In one embodiment, said particle has a diameter of at least 10nm, for example, at least 20nm, preferably at least 50nm.

In one embodiment, molecular weight of said particle is from 100 kDa to 100,000 kDa, preferably from 400kDa to 30,000kDa.

A polypeptide used for the present invention is not limited as long as it is spontaneously assembled. The polypeptide may be a virus structural polypeptide. Thus, the particle provided by the present invention may be a virus like particle.

A virus structural polypeptide may be a naturally occurring viral polypeptide or modified polypeptide thereof. In one embodiment, the modified polypeptide has at least
70%, 75%, 80%, 85%, 90%, 95% or 98% amino acid sequence identity to a naturally occurring viral structural polypeptide including capsid and envelope protein. In one embodiment, the modified polypeptide is a mutant where at most 10% of the amino acids are deleted, substituted, and/or added to a naturally occurring viral structural polypeptide including capsid and envelope protein.

In one embodiment, virus structural polypeptide used for the present invention consists of or comprises capsid and/or envelope protein or fragment thereof. For example, an envelope protein comprises at least one selected from the group consisting of E3, E2, 6K and E1. Virus structural polypeptide used for the present invention may be derived from Alphavirus or Flavivirus. Thus, the particle provided by the present invention may be a virus like particle derived from Alphavirus or Flavivirus.

Examples of Alphavirus and Flavivirus include, but not limited to, Aura virus, Babanki virus, Barmah Forest virus (BFV), Bebaru virus, Cabassou virus, Chikungunya virus (CHIKV), Eastern equine encephalitis virus (EEEV), Eilat virus, Everglades virus, Fort Morgan virus, Getah virus, Highlands J virus, Kyzylagach virus, Mayaro virus, Me Tri virus, Middelburg virus, Mosso das Pedras virus, Mucambo virus, Ndumu virus, O'nyong-nyong virus, Pixuna virus, Rio Negro virus, Ross River virus (RRV), Salmon pancreas
disease virus, Semliki Forest virus, Sindbis virus, Southern elephant seal virus, Tonate virus, Trocara virus, Una virus, Venezuelan equine encephalitis virus (VEEV), Western equine encephalitis virus (WEEV), Whataroa virus, West Nile virus, dengue virus, tick-borne encephalitis virus and yellow fever virus.

As used herein, the term "antigen" refers to a molecule capable of being bound by an antibody or a T cell receptor (TCR) if presented by MHC molecules. The term "antigen", as used herein, also encompasses T-cell epitopes. A T-cell epitope is recognized by a T-cell receptor in the context of MHC class I, present on all cells of the body except erythrocytes, or class II, present on immune cells and in particular antigen-presenting cells. This recognition event leads to activation of T-cells and subsequent effector mechanisms such as proliferation of the T-cells, cytokine secretion, perforin secretion etc. An antigen is additionally capable of being recognized by the immune system and/or being capable of inducing a humoral immune response and/or cellular immune response leading to the activation of B- and/or T-lymphocytes. This may, however, require that, at least in certain cases, the antigen contains or is linked to a 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. Antigens, as used herein, include but are not limited to allergens, self antigens, haptens, cancer antigens (i.e. tumor antigens) and infectious disease antigens as well as small organic molecules such as drugs of abuse (like nicotine) and fragments and derivatives thereof. Furthermore, antigens used for the present invention can be peptides, proteins, domains, carbohydrates, alkaloids, lipids or small molecules such as, for example, steroid hormones and fragments and derivatives thereof, autoantibody and cytokine itself.

Examples of cytokines include, but are not limited to, interleukin (IL) including over 30 type such as IL-1α, IL-1β, IL-2, -3, -4, -5, -6, -7, -8, -9, -10, -11 to -37; interferon (IFN) such as IFN-α, IFN-β and IFN-γ; tumor necrosis factor (TNF) such as TNF-α and TNF-β; transforming growth factor (TGF) such as TGF-α and TGF-β; colony stimulating factor (CSF) such as granulocyte -colony-stimulating factor (G-CSF), granulocyte -macrophage- colony-stimulating factor (GM-CSF), macrophage -colony Stimulating
factor (M-CSF), erythropoietin (EPO), stem cell factor (SCF) and monocyte chemotactic and activating factor (MCAF); growth factor (GF) such as epidermal growth factor (EGF), fibroblast growth factor (FGF), insulin like growth factor (IGF), nerve growth factor (NGF), Brain-derived neurotrophic factor (BDNF), platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), keratinocyte growth factor (KGF), thrombopoietin (TPO), and bone morphogenic protein (BMP); and other polypeptide factors including LIF, kit ligand (KL), MPO (Myeloperoxidase) and CRP (C-reactive protein); COX (Cyclooxygenase) such as COX-1, COX-2 and COX-3, NOS (Nitric oxide synthase) such as NOS-1, NOS-2 and NOS-3; and so on.

Cytokines also includes chemokines which are cytokines that induce chemotaxis. There are two major classes of chemokines, CXC and CC. The CXC chemokines, such as neutrophil-activating protein-2 (NAP-2) and melanoma growth stimulatory activity protein (MGSA) are chemotactic primarily for neutrophils and T lymphocytes, whereas the CC chemokines, such as RANTES, Macrophage inflammatory protein (MIP) including MIP-1α and MIP-1β, keratinocyte-derived chemokine (KC), the monocyte chemotactic proteins (MCP-1, MCP-2, MCP-3, MCP-4, and MCP-5) and the eotaxins (-1 and -2) are chemotactic for, among
other cell types, macrophages, T lymphocytes, eosinophils, neutrophils, dendritic cells, and basophils. There also exist the chemokines lymphotactin-1, lymphotactin-2 (both C chemokines), and fractalkine (a CX3C chemokine) that do not fall into either of the major chemokine subfamilies.

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

As used herein, the term "antibody" refers to molecules which are capable of binding an epitope or antigenic determinant. The term is meant to include whole antibodies and antigen-binding fragments thereof, including single-chain antibodies. Such antibodies include human antigen binding antibody fragments and include, but are not limited to, Fab, Fab' and F(ab')2, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a VL or VH domain. The antibodies can be from any animal origin including
birds and mammals. Preferably, the antibodies are mammalian e.g. human, murine, rabbit, goat, guinea pig, camel, horse and the like, or other suitable animals e.g. chicken. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulins and that do not express endogenous immunoglobulins, as described, for example, in U.S. Patent No. 5,939,598, the disclosure of which is incorporated herein by reference in its entirety.

Antigen may be a substance (e.g. protein) which is not derived from virus (e.g. Chikungunya virus, Venezuelan equine encephalitis virus).

In one embodiment, antigen which is used for the present invention is at least one target or a polypeptide therefrom as listed in Table 1.

[Table 1]
Table 1

<table>
<thead>
<tr>
<th>Target</th>
<th>Use</th>
</tr>
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<tbody>
<tr>
<td>GD2</td>
<td>neuroblastoma</td>
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<tr>
<td>CA-125 (mutation)</td>
<td>ovarian cancer</td>
</tr>
<tr>
<td>CD41 (integrin alpha-2b)</td>
<td>platelet aggregation inhibitor</td>
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<tr>
<td>TNF-α</td>
<td>rheumatoid arthritis etc.</td>
</tr>
<tr>
<td>EpCAM</td>
<td>prostate and breast cancer</td>
</tr>
<tr>
<td>TNF-α</td>
<td>sepsis</td>
</tr>
<tr>
<td>CD20</td>
<td>lymphoma</td>
</tr>
<tr>
<td>VEGFR2</td>
<td>cancer</td>
</tr>
<tr>
<td>IL-6</td>
<td>rheumatoid arthritis</td>
</tr>
<tr>
<td>CD25</td>
<td>CLL, CTCL</td>
</tr>
<tr>
<td>CEA</td>
<td>colorectal cancer</td>
</tr>
<tr>
<td>TAG-72</td>
<td>non-small cell lung carcinoma</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>hematological cancers</td>
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<tr>
<td>CEA</td>
<td>gastrointestinal cancers</td>
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<td>L-selectin (CD62L)</td>
<td>severely injured patients</td>
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<td>IL-6 receptor</td>
<td>rheumatoid arthritis</td>
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<tr>
<td>Rhesus factor</td>
<td>hemolytic disease of the newborn</td>
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<tr>
<td>beta amyloid</td>
<td>Alzheimer’s disease</td>
</tr>
<tr>
<td>CD25 (α chain of IL-2 receptor)</td>
<td>prevention of organ transplant rejections</td>
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<tr>
<td>phosphatidylserine</td>
<td>cancer, viral infections</td>
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<tr>
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<td>non-Hodgkin’s lymphoma</td>
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<tr>
<td>BAFF</td>
<td>non-Hodgkin lymphoma etc.</td>
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<tr>
<td>CD125</td>
<td>asthma</td>
</tr>
<tr>
<td>OCL11 (eotaxin-1)</td>
<td>severe allergic disorders</td>
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<tr>
<td>CEA-related antigen</td>
<td>inflammatory lesions and metastases</td>
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<td>VEGF-A</td>
<td>metastatic cancer</td>
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<tr>
<td>fibrin II beta chain</td>
<td>thromboembolism</td>
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<tr>
<td>CD44 v6</td>
<td>squamous cell carcinoma</td>
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<tr>
<td>CD19</td>
<td>cancer</td>
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<td>CD30 (TNFRSF8)</td>
<td>hematologic cancers</td>
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<td>IL-12, IL-23</td>
<td>psoriasis, rheumatoid arthritis, inflammatory bowel diseases, multiple sclerosis</td>
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<tr>
<td>mucin CanAg</td>
<td>colorectal cancer etc.</td>
</tr>
<tr>
<td>prostatic carcinoma cells</td>
<td>prostate cancer</td>
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<tr>
<td>EpCAM, CD3</td>
<td>ovarian cancer, malignant ascites, gastric cancer</td>
</tr>
<tr>
<td>TAG-72</td>
<td>tumor detection</td>
</tr>
<tr>
<td>CD4</td>
<td>prevention of organ transplant rejections, treatment of autoimmune diseases</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Crohn’s disease</td>
</tr>
<tr>
<td>EGFR</td>
<td>metastatic colorectal cancer and head and neck cancer</td>
</tr>
<tr>
<td>EpCAM</td>
<td>ovarian cancer and other solid tumors</td>
</tr>
<tr>
<td>IGF-1 receptor</td>
<td>solid tumors</td>
</tr>
<tr>
<td>CD4</td>
<td>rheumatoid arthritis</td>
</tr>
<tr>
<td>MUC1</td>
<td>pancreatic cancer</td>
</tr>
<tr>
<td>TRAIL-R2</td>
<td>cancer</td>
</tr>
<tr>
<td>Influenza A haemagglutinin</td>
<td>infectious disease/influenza A</td>
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<tr>
<td>CD40</td>
<td>hematologic cancers</td>
</tr>
<tr>
<td>CD25 (α chain of IL-2 receptor)</td>
<td>prevention of organ transplant rejections</td>
</tr>
<tr>
<td>RANKL</td>
<td>osteoporosis, bone metastases etc.</td>
</tr>
<tr>
<td>B-lymphoma cell</td>
<td>lymphoma</td>
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<tr>
<td>GD3 ganglioside</td>
<td>malignant melanoma</td>
</tr>
<tr>
<td>C5</td>
<td>paroxysmal nocturnal hemoglobinuria</td>
</tr>
<tr>
<td>endotoxin</td>
<td>sepsis caused by Gram-negative bacteria</td>
</tr>
<tr>
<td>EpCAM</td>
<td>colorectal carcinoma</td>
</tr>
<tr>
<td>LFA-1 (CD11a)</td>
<td>psoriasis (blocks T-cell migration)</td>
</tr>
<tr>
<td>Haplo</td>
<td>invasive Candida infection</td>
</tr>
<tr>
<td>SLAMF7</td>
<td>multiple myeloma</td>
</tr>
<tr>
<td>CD22</td>
<td>cancer, SLE</td>
</tr>
<tr>
<td>TIGB2 (CD18)</td>
<td>heart attack, stroke, traumatic shock</td>
</tr>
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<td>HER2/neu, CD3</td>
<td>breast cancer etc.</td>
</tr>
<tr>
<td>integrin α v β 3</td>
<td>melanoma, prostate cancer, ovarian cancer etc.</td>
</tr>
<tr>
<td>hepatitis B surface antigen</td>
<td>hepatitis B</td>
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<td>CD15</td>
<td>appendicitis</td>
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<tr>
<td>folate receptor 1</td>
<td>ovarian cancer</td>
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<td>Table 1 -continued-</td>
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<tr>
<td>---------------------</td>
<td>-------------------</td>
</tr>
<tr>
<td><strong>Target</strong></td>
<td><strong>Use</strong></td>
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<tr>
<td>respiratory syncytial virus</td>
<td>respiratory syncytial virus infection</td>
</tr>
<tr>
<td>IL-22</td>
<td>rheumatoid arthritis, psoriasis</td>
</tr>
<tr>
<td>TGF-β receptor</td>
<td>adenocortical carcinoma, non-small cell lung carcinoma etc.</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Crohn’s disease etc.</td>
</tr>
<tr>
<td>rabies virus glycoprotein</td>
<td>rabies (prophyaxis)</td>
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<tr>
<td>TGF-β</td>
<td>idiopathic pulmonary fibrosis, focal segmental glomerulosclerosis, cancer</td>
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<tr>
<td>CD80</td>
<td>B-cell lymphoma</td>
</tr>
<tr>
<td>beta amyloid</td>
<td>Alzheimer’s disease</td>
</tr>
<tr>
<td>CD147 (basigin)</td>
<td>graft versus host disease</td>
</tr>
<tr>
<td>CD33</td>
<td>acute myelogenous leukemia</td>
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<tr>
<td>carbonic anhydrase 9 (CA-IX)</td>
<td>clear cell renal cell carcinoma</td>
</tr>
<tr>
<td>GPA4B</td>
<td>melanoma, breast cancer</td>
</tr>
<tr>
<td>TNF-α</td>
<td>rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis</td>
</tr>
<tr>
<td>CD23 (IgE receptor)</td>
<td>allergic asthma</td>
</tr>
<tr>
<td>CD4</td>
<td>HIV infection</td>
</tr>
<tr>
<td>CD20</td>
<td>non-Hodgkin’s lymphoma</td>
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<tr>
<td>CA-125</td>
<td>ovarian cancer</td>
</tr>
<tr>
<td>cardiac myosin</td>
<td>cardiac imaging</td>
</tr>
<tr>
<td>TNF-α</td>
<td>rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, psoriasis, Crohn’s disease, ulcerative colitis</td>
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<tr>
<td>CD51</td>
<td>solid tumors (prostate cancer, melanoma)</td>
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<td>CD25 (α chain of IL-2 receptor)</td>
<td>graft versus host disease</td>
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<tr>
<td>CD52</td>
<td>cancer</td>
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<tr>
<td>CD152</td>
<td>melanoma</td>
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<td>CD30 (TNFRSF8)</td>
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<tr>
<td>CD4</td>
<td>chronic asthma</td>
</tr>
<tr>
<td>GEA</td>
<td>colorectal cancer</td>
</tr>
<tr>
<td>IL-13</td>
<td>asthma</td>
</tr>
<tr>
<td>NCA-90 (granulocyte antigen)</td>
<td>diagnostic agent</td>
</tr>
<tr>
<td>TGF beta 2</td>
<td>reduction of scarring after glaucoma surgery</td>
</tr>
<tr>
<td>TRAIL-R2</td>
<td>cancer</td>
</tr>
<tr>
<td>hepatitis B surface antigen</td>
<td>hepatitis B</td>
</tr>
<tr>
<td>CD33</td>
<td>cancer</td>
</tr>
<tr>
<td>CD56</td>
<td>cancer</td>
</tr>
<tr>
<td>CD40</td>
<td>multiple myeloma, non-Hodgkin’s lymphoma, Hodgkin’s lymphoma</td>
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<td>CD23 (IgE receptor)</td>
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</tr>
<tr>
<td>TRAIL-R1</td>
<td>cancer</td>
</tr>
<tr>
<td>EGFR</td>
<td>colorectal, lung and stomach cancer</td>
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<tr>
<td>IL-5</td>
<td>asthma and white blood cell diseases</td>
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<tr>
<td>TGF beta 1</td>
<td>systemic scleroderma</td>
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<td>CD74</td>
<td>multiple myeloma and other hematological malignancies</td>
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<td>D10e (pentosido)</td>
<td>small cell lung carcinoma</td>
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<td>respiratory syncytial virus</td>
<td>respiratory syncytial virus (prevention)</td>
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<td>CD3</td>
<td>prevention of organ transplant rejections</td>
</tr>
<tr>
<td>C242 antigen</td>
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<td>STS</td>
<td>non-small cell lung carcinoma, renal cell carcinoma</td>
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<td>integrin α6</td>
<td>multiple sclerosis, Crohn’s disease</td>
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<tr>
<td>etodoxin</td>
<td>sepsis</td>
</tr>
<tr>
<td>EGFR</td>
<td>non-small cell lung carcinoma</td>
</tr>
<tr>
<td>EGFR</td>
<td>squamous cell carcinoma, head and neck cancer, nasopharyngeal cancer, glioma</td>
</tr>
<tr>
<td>CD20</td>
<td>rheumatoid arthritis, lupus erythematosus etc.</td>
</tr>
<tr>
<td>LFA-1 (CD11a)</td>
<td>prevention of organ transplant rejections, immunological diseases</td>
</tr>
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<td>CD20</td>
<td>chronic lymphocytic leukemia etc.</td>
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<td>PDGF-R α</td>
<td>cancer</td>
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<td>IgE Fc region</td>
<td>allergic asthma</td>
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<td>diabetes mellitus type 1</td>
</tr>
<tr>
<td>lipoteichoic acid</td>
<td>sepsis (Staphylococcus)</td>
</tr>
<tr>
<td>respiratory syncytial virus</td>
<td>respiratory syncytial virus (prevention)</td>
</tr>
<tr>
<td>EGFR</td>
<td>colorectal cancer</td>
</tr>
</tbody>
</table>
In one embodiment, antigen which is used for the
present invention is at least one protein or a polypeptide therefrom selected from the group consisting of CTLA-4, PD-1, TIM-3, BTLA, VISTA, LAG-3, CD28, OX40, GITR, CD137, CD27 and HVEM. CTLA-4, PD-1, TIM-3, BTLA, VISTA and LAG-3 are inhibitory receptors for T-cell stimulation, and CD28, OX40, GITR, CD137, CD27 and HVEM are activating receptors for T-cell stimulation (see Mellman et al., Nature 480, 480-489 (2011)).

The antigen used for the present invention can be modified polypeptide derived from a naturally occurring protein. The modified polypeptide may be a fragment of the naturally occurring protein. In one embodiment, the modified polypeptide has at least 70%, 75%, 80%, 85%, 90%, 95% or 98% amino acid sequence identity to a polypeptide derived from a naturally occurring protein. In one embodiment, the modified polypeptide derived is a mutant where at most 10% of the amino acids are deleted, substituted, and/or added based on a polypeptide derived from naturally occurring protein.

In the particle as provided by the present invention, a polypeptide and an antigen may be linked through at least one first attachment site which is present in the polypeptide and at least one second attachment site which is present in the antigen.

As used herein, each of "a first attachment site"
and "a second attachment site" refers to a site where more than one substance is linked each other.

In one embodiment, the polypeptide and the antigen are directly fused. Alternatively, one or two linkers may intervene between N-terminal residue of the antigen and the polypeptide and/or between C-terminal residue of the antigen and the polypeptide.

The antigen or the polypeptide can be truncated and replaced by short linkers. In some embodiments, the antigen or the polypeptide include one or more peptide linkers. Typically, a linker consists of from 2 to 25 amino acids. Usually, it is from 2 to 15 amino acids in length, although in certain circumstances, it can be only one, such as a single glycine residue.

In one embodiment, a nucleic acid molecule, in which polynucleotide encoding the polypeptide is genetically fused with polynucleotide encoding the antigen, is expressed in a host cell so that the first attachment site and the second attachment site are linked through a peptide bond. In this case, the polypeptide and the antigen are linked through a peptide bond. Relating to this embodiment, the first attachment site and/or the second attachment site may be genetically modified from the original polypeptide or antigen. For example, the first attachment site is modified from the polypeptide so that through a linker
peptide including SG, GS, SGG, GGS and SGSG, the polypeptide is conjugated with the antigen.

When the polypeptide are chemically conjugated with the antigen, the first attachment site and the second attachment site may be linked through a chemical cross-linker which is a chemical compound.

Examples of the cross-linker include, but are not limited to, SMPH, Sulfo-MBS, Sulfo-EMCS, Sulfo-GMBS, Sulfo-SIAB, Sulfo-SMPB, Sulfo-SMCC, SVSB, SIA and other cross-linkers available from the Pierce Chemical Company.

In one embodiment, the particle provided by the present invention comprises a polypeptide linked to an antigen, wherein spatial distance between the N-terminal residue and C-terminal residue of the antigen is 30Å or less when the distance is determined in a crystal of the antigen or a naturally occurring protein containing the antigen or modified protein therefrom.

The antigen used for the present invention can be designed by a person skilled in the art. For example, the antigen used for the present invention may be a naturally occurring protein or a fragment thereof. Alternatively, the antigen used for the present invention may be a protein modified from a naturally occurring protein or a fragment thereof. A person skilled in the art can design the antigen so that spatial distance between the N-terminal
residue and C-terminal residue of the antigen is 30Å or less when the distance is determined in a crystal of the antigen or a naturally occurring protein containing the antigen or modified protein therefrom. For example, the antigen used for the particle provided by the present invention can be designed using a free software including PyMOL (e.g. PyMOL v0.99: http://www.pymol.org). In one embodiment, the spatial distance between the N-terminal residue and C-terminal residue of the antigen is 30Å (angstrom) or less, 20Å or less, or 10Å or less (e.g. from 5 Å to 15 Å, from 5 Å to 12 Å, from 5 Å to 11 Å, from 5 Å to 10 Å, from 5 Å to 8 Å, from 8 Å to 15 Å, from 8 Å to 13 Å, from 8 Å to 12 Å, from 8 Å to 11 Å, from 9 Å to 12 Å, from 9 Å to 11 Å, from 9 Å to 10 Å or from 10 Å to 11 Å).

Chikungunya virus like particle or a Venezuelan equine encephalitis virus like particle

In one embodiment, the present invention provides a Chikungunya virus like particle or a Venezuelan equine encephalitis virus like particle comprising a Chikungunya or Venezuelan equine encephalitis virus structural polypeptide and at least one antigen, wherein said Chikungunya virus structural polypeptide or said Venezuelan equine encephalitis virus structural polypeptide comprises at least one first attachment site and said at least one
antigen comprises at least one second attachment site, and wherein said Chikungunya or Venezuelan equine encephalitis virus structural polypeptide and said at least one antigen are linked through said at least one first and said at least one second attachment site.

In one embodiment, a spatial distance between the N-terminal residue and C-terminal residue of the antigen may be 30 Å or less; 25 Å or less; 20 Å or less; 15 Å or less; 14 Å or less; 13 Å or less; 12 Å or less; 11 Å or less; 10 Å or less; 9 Å or less; or 8 Å or less (e.g. from 5 Å to 15 Å, from 5 Å to 12 Å, from 5 Å to 11 Å, from 5 Å to 10 Å, from 5 Å to 8 Å, from 8 Å to 15 Å, from 8 Å to 13 Å, from 8 Å to 12 Å, from 8 Å to 11 Å, from 9 Å to 12 Å, from 9 Å to 11 Å, from 9 Å to 10 Å, from 10 Å to 11 Å) when the distance is determined in a crystal of the antigen or a naturally occurring protein containing the antigen or modified protein therefrom.

In one embodiment, the antigen is linked to the Chikungunya or Venezuelan equine encephalitis virus structural polypeptide by way of chemical cross-linking or as a fusion protein produced by way of genetic engineering.

A Chikungunya or Venezuelan equine encephalitis virus structural polypeptide used in the present invention may comprise a Chikungunya or Venezuelan equine encephalitis virus envelope protein and/or a capsid.
Examples of Chikungunya virus include, but are not limited to, strains of 37997 and LR2006 OPY-1.

Examples of Venezuelan equine encephalitis virus include, but are not limited to, TC-83.

Chikungunya or Venezuelan equine encephalitis virus structural polypeptide used in the present invention may naturally occurring virus structural polypeptide or modified polypeptide thereof. The modified polypeptide may be a fragment of the naturally occurring virus structural polypeptide. In one embodiment, the modified polypeptide has at least 70%, 75%, 80%, 85%, 90%, 95% or 98% amino acid sequence identity to a naturally occurring viral capsid and/or envelope protein. In one embodiment, the modified polypeptide is a mutant where at most 10% of the amino acids are deleted, substituted, and/or added based on a naturally occurring viral capsid and/or envelope protein. For example, K64A or K64N mutation may be introduced into a capsid of Venezuelan equine encephalitis virus structural polypeptide used in the present invention.

Chikungunya or Venezuelan equine encephalitis virus envelope protein may comprise at least one selected from the group consisting of E3, E2, 6K and E1.

Examples of Chikungunya virus structural polypeptide include, but are not limited to, E3-E2-6K-E1 of Chikungunya virus Strain_37997, Capsid-E3-E2-6K-E1 of Chikungunya virus
Strain 31991, E3-E2-6K-E1 of Chikungunya virus Strain LR2006 OPY-1 and Capsid-E3-E2-6K-E1 of Chikungunya virus LR2006 OPY-1.

Examples of Venezuelan equine encephalitis virus structural polypeptide include, but are not limited to, E3-E2-6K-E1 of Venezuelan equine encephalitis virus Strain TC-83 and Capsid-E3-E2-6K-E1 of Venezuelan equine encephalitis virus Strain TC-83.

An exemplary Chikungunya virus structural polypeptide sequence is provided at Genbank Accession No. ABX40006.1, which is described below (SEQ ID No.: 1):

```plaintext
meftptqtfnrryprwptprptiqvi rprprqpraqgqlaqil isavnkltmravpqq kprnknkkqkqkqapqqnctcckqppaapkppkppqrrermcmkiendcf evk
hegkvtgycalvdkvmpahvkgtidnaldklaf krskskydecaqipvhmksdask fthekpewyynwhagvqysgrf tiptaagkqpdsgripf dnlkewaivelngagenaqk rtalswntnkdivtkpegaeewslaipmcianttfpcscqppctpcykepeet
lrmlednvmrppgyyqllqasltcspshqrrstkdfnqyvkyatrpypahcdggeqs spvalerineatdgtlkiqsylqigictddhstdklymdnhmpadaeraglivrt
aptctigtmghf ilarcnpkgetltygftdsrkishstchpflhddppvigrekfharpgqhkelpcstvyqstaatteeeieehmpdtptdrtmaqqsngvktivngtvkgcnevccgs
negtttddkvinnckvdqaavtnhkkwqympnvprnaeqlrdkrkhipfplanf
crvpkarnptvtykgnqvimilypdhptilysrnmegepnqeeewmhkekevtiplt
glevtvempteykywpqqli stngtahghpeiiilyytlyptntw vswtatfillsmvgtmaagmcmcarrc ltpyeltpgtavfpllliliceirtkayrgeaaiylwneqqpfl
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Another exemplary Chikungunya virus structural polypeptide sequence is provided at Genbank Accession No. ABX40011.1, which is described below (SEQ ID No.: 2):

```plaintext
meftptqtfnrryprwprwptprptiqvi rprprqpraqgqlaqil isavnkltmravpqq kprnknkkqkqkqapqqnctcckqppaapkppkppqrrermcmkiendcf evk
```
hegkvragyaclvgdkvmkpahvkgtidnadlaklaf krsskydlecaqipvhmksdask
f thekpegyynwhhagvypsggrf tiptgakpgdsgrpif dngkrwaivlvgganega
rtalswnkwndikvitkipeagewslalpvlclllanttfcpsqcpppcycekepest
lmlednvarpgpyqlklasltcsphrqrstrkdnfnyvaktyphalpcqcgghsch
spialerineadgtklqygsigiki1ddshdwtklymdshtpadaeraiglvrts
apctgtmgfhf ilarcpkgetitvf/ttsrksishtcchpf hheppvigrfr fsrphpg
kelpcstvyqstaateaeieehmmpdptdrtmtgqsngvktngttvrykncggs
negllttdkvinncidqchaatvnhknwnqytplvnraelgrkgkihipf planvtt
crvpkarnptvtqvgkqnyttml ylpdhpt llssrnmgeqnpnyhe ewtvkheeyt1lypte
glevtwgngpekywpqmstngtahgpsheiiyyelypeymtltiwsvasfvismsmg
 tavmgccarrctpiycytheipgtatvpf wllslccvrttkaattyearaayalwneqplf
 wqlaliplaalivlcnclklppcccttlf lavmsiaghtvasehvtipntvqpykt
 lvnprpgpqlabez gridSizeeiyypvytosemk0g0dnggr
 waivlgvny.nrslatswalswnwnmekyqvyktytenceqswlvtmmcllanvtpfpcaqp
 icydrkpaetlamlqsnvdpqydelleavkpcgkrkrsteelf neylktrypmraci
rcavgschpsiaeeavksdgdyvgvrylgsqygg1ldsngktrmythmhtkeiiepl
 hqvsltyrsrpchivdhgyfs llarcpqsditsmeff kksdrhscvpyvevfk npvpgr
 ythhephqvegacqcyahdagnrgrayvemhpsevdsllsvsgssvtttcpdtsal
 vececgqtkisetntkqf sgctkkekcrayrlqndkwkysnlkapqatlkkgki1
 vplflldaktgpvlpapepmfortff stormsllkhhpkntylitrqladephthelisepa
 vnrftvtekgwefvwnhnpkp wpagetapqngphlphevthyyhrypmstilgisic
 aaivatsaaswlf crrsnaltpttynrapirpf clavlcarratarrettwsldh
 wnnnnqgmfiwqililipallaiu vqtrllrllccwcf lvmqaagaaqyehatmtpsgaqi
 syntvnrnayapilspitkikittltptynlevtytchtkgmdsapaickcqsctepy
 rpdeqckvtftqvypffwmggacyf cdentqsvskaymksddcladhayaektasvq
 flntvyheisvthvyyvynetpvnf ngykitaqplstawtf dpkrkvyageiynidfp
 eygaggqpgqdfgidsrlsytvstsvdiyantl1vrlqkapalhpytqapsgf eqwkkkap
 slkftapfgeiytnpairaenqvsipal dipdalf ttrsetpltasactyelnev
 ssdfggiaqtikyasksngkcavhpststlakleaeltggsatifh stanihepf
 rl gictsyvcktqgcdhpdkhivhplqhayqtf taaasvktawtwltltslggssviiigly
 lativamyv1ngkhn .

An exemplary Venezuelan equine encephalitis virus structural polypeptide is treated at Genbank Accession No.
L01443.1 (http://www.ncbi.nlm.nih.gOv/nuccore/L01443.1),
which is described below (SEQ ID No.:):

mfpqfpmypmpqmpyrynpfe aaprrpwfprtdpf lamqvqeltrsmanl t kqrrdappe
gpsaakpkkeasqkgkggkkkkkqngkkkktkkngppnpkaqnggknkntknpkgkrqm
vmlklesdktfpeimqlgkyacwggkfl rpmhvegkinncvlaal1kkaskydley
advpqnmradbxfkythekpgyyswhwghagvngengrf tvpkyvgavagkgdsgrpildnogr
waivlgvny.nrslatswalswnwnmekyqvyktytenceqswlvtmmcllanvtpfpcaqp
 icydrkpaetlamlqsnvdpqydelleavkpcgkrkrsteelf neylktrypmraci
rcavgschpsiaeeavksdgdyvgvrylgsqygg1ldsngktrmythmhtkeiiepl
 hqvsltyrsrpchivdhgyfs llarcpqsditsmeff kksdrhscvpyvevfk npvpgr
 ythhephqvegacqcyahdagnrgrayvemhpsevdsllsvsgssvtttcpdtsal
 vececgqtkisetntkqf sgctkkekcrayrlqndkwkysnlkapqatlkkgki1
 vplflldaktgpvlpapepmfortff stormsllkhhpkntylitrqladephthelisepa
 vnrftvtekgwefvwnhnpkp wpagetapqngphlphevthyyhrypmstilgisic
 aaivatsaaswlf crrsnaltpttynrapirpf clavlcarratarrettwsldh
 wnnnnqgmfiwqililipallaiu vqtrllrllccwcf lvmqaagaaqyehatmtpsgaqi
 syntvnrnayapilspitkikittltptynlevtytchtkgmdsapaickcqsctepy
 rpdeqckvtftqvypffwmggacyf cdentqsvskaymksddcladhayaektasvq
 flntvyheisvthvyyvynetpvnf ngykitaqplstawtf dpkrkvyageiynidfp
 eygaggqpgqdfgidsrlsytvstsvdiyantl1vrlqkapalhpytqapsgf eqwkkkap
 slkftapfgeiytnpairaenqvsipal dipdalf ttrsetpltasactyelnev
 ssdfggiaqtikyasksngkcavhpststlakleaeltggsatifh stanihepf
 rl gictsyvcktqgcdhpdkhivhplqhayqtf taaasvktawtwltltslggssviiigly
 lativamyv1ngkhn .

In one embodiment, a first attachment site comprises an amino group, preferably an amino group of a lysine
residue. In one embodiment, the second attachment site comprises sulfhydryl group, preferably, a sulfhydryl group of cysteine.

In one embodiment, a conjugation of more than two substances (e.g. antigen and Chikunguniya or Venezuelan equine encephalitis virus structural polypeptide) through a first attachment site or a second attachment site is achieved using chemical cross linker. Examples of the cross-linker include, but are not limited to, SMPH, Sulfo-MBS, Sulfo-EMCS, Sulfo-GMBS, Sulfo-SIAB, Sulfo-SMPB, Sulfo-SMCC, SVSB, SIA and other cross-linkers available from the Pierce Chemical Company.

According to the present invention, a Chikungunya or Venezuelan equine encephalitis virus like particle comprising a Chikungunya or Venezuelan equine encephalitis virus structural polypeptide and an antigen, wherein said Chikungunya or Venezuelan equine encephalitis virus structural polypeptide and said antigen are expressed as a fusion protein can be provided.

In one embodiment, the antigen can be fused with any site of the Chikungunya or Venezuelan equine encephalitis virus structural polypeptide. For example, the antigen may be directly or indirectly linked to N- or C-terminal of the Chikungunya or Venezuelan equine encephalitis virus structural polypeptide (e.g. capsid, E3, E2, 6K or E1), or
the antigen may be inserted into Chikungunya or Venezuelan equine encephalitis virus structural protein (e.g. capsid, E3, E2, 6K, or E1).

In one embodiment, at least one antigen is inserted into E2 of Chikungunya or Venezuelan equine encephalitis virus structural protein. For example, regarding Chikungunya virus structural protein, at least one antigen is inserted between residues 519 and 520 of SEQ ID Nos.1 or 2 (i.e. between G at 519-position and Q at 520-position of SEQ ID Nos.1 or 2); between residues 530 and 531 of SEQ ID Nos.1 or 2 (i.e. between G at 530-position and S at 531-position of SEQ ID Nos.1 or 2); between residues 531 and 532 of SEQ ID Nos.1 or 2 (i.e. between S at 531-position and N at 532-position of SEQ ID Nos.1 or 2); between residues 529 and 530 of SEQ ID Nos.1 or 2 (i.e. between G at 529-position and G at 530-position of SEQ ID Nos.1 or 2); or between residues 510 and 511 of SEQ ID Nos.1 or 2 (i.e. between S at 510-position and G at 511-position of SEQ ID Nos.1 or 2); or between residues 511 and 512 of SEQ ID Nos.1 or 2 (i.e. between G at 511-position and N at 512-position of SEQ ID Nos.1 or 2); or between residues 509 and 510 of SEQ ID Nos.1 or 2 (i.e. between Q at 509-position and S at 510-position of SEQ ID Nos.1 or 2).

For example, regarding Venezuelan equine encephalitis virus structural protein, at least one antigen
is inserted between residues 517 and 518 of SEQ ID No. 3 (i.e. between G at 517-position and S at 518-position of SEQ ID No. 3); between residues 518 and 519 of SEQ ID No. 3 (i.e. between S at 518-position and S at 519-position of SEQ ID No. 3); between residues 519 and 520 of SEQ ID No. 3 (i.e. between S at 519-position and V at 520-position of SEQ ID No. 3); between residues 515 and 516 of SEQ ID No. 3 (i.e. between L at 515-position and S at 516-position of SEQ ID No. 3); between residues 516 and 517 of SEQ ID No. 3 (i.e. between S at 516-position and G at 517-position of SEQ ID No. 3); between residues 536 and 537 of SEQ ID No. 3 (i.e. between C at 536-position and G at 537-position of SEQ ID No. 3); between residues 537 and 538 of SEQ ID No. 3 (i.e. between G at 537-position and G at 538-position of SEQ ID No. 3); between residues 538 and 539 of SEQ ID No. 3 (i.e. between G at 538-position and T at 539-position of SEQ ID No. 3).

The fusion protein may be expressed using a conventional technique in the art. A variety of expression systems can be used for the expression of the fusion protein. For example, the fusion protein can be expressed in 293 cells, Sf9 cells or E.coli.

In one embodiment, antigen is a substance (e.g. protein) which is not derived from Chikungunya or Venezuelan equine encephalitis virus. Antigen may be at
least one selected from the group consisting of self antigens and cancer antigens. For example, antigen is a polypeptide derived from TNF-a, CD20 or CTLA4. Thus, examples of combinations of the polypeptide and the antigen used for the present invention include, but are not limited to, i) a polypeptide derived from Chikungunya virus (CHIKV) and a polypeptide derived from TNF-a; ii) a polypeptide derived from Chikungunya virus (CHIKV) and a polypeptide derived from CD20; iii) a polypeptide derived from Venezuelan equine encephalitis virus (VEEV) and a polypeptide derived from TNF-a; or iv) a polypeptide derived from Venezuelan equine encephalitis virus (VEEV) and a polypeptide derived from CD20. v) a polypeptide derived from Venezuelan equine encephalitis virus (VEEV) and a polypeptide derived from CTLA4.

A polypeptide derived from Chikungunya virus (CHIKV) or Venezuelan equine encephalitis virus (VEEV) may be a naturally occurring viral polypeptide or modified polypeptide thereof. In addition, a polypeptide derived from TNF-a, CD20 or CTLA4 may be a naturally occurring polypeptide or modified polypeptide of the naturally occurring polypeptide or a fragment of the naturally occurring polypeptide.
occurring polypeptide or the modified peptide. The modified polypeptide may be a fragment of the naturally occurring virus structural polypeptide.

In one embodiment, the modified polypeptide derived from TNF-a, CD20 or CTLA4 has at least 70%, 75%, 80%, 85%, 90%, 95% or 98% amino acid sequence identity to a naturally occurring polypeptide. In one embodiment, the modified peptide derived from TNF-a, CD20 or CTLA4 is a mutant where at most 10% of the amino acids are deleted, substituted, and/or added based on a naturally occurring polypeptide derived from TNF-a, CD20 or CTLA4.

When a polypeptide derived from a virus is conjugated with a polypeptide derived from an antigen, a linker peptide including SG, GS, SGG, GGS, SGSG and TRGGS may be used. Examples of conjugation of the polypeptide derived from a virus (referred to as "PFV" below) with the polypeptide derived from the antigen (referred to as "PFA" below) include, but not limited to: PFV-SG-PFA-GS-PFV; PFV-SG-PFA-GGS-PFV; PFV-SSG-PFA-GS-PFV; PFV-SSG-PFA-GGS-PFV; PFV-SGSG-PFA-GS-PFV; and PFA-SGG-PFA-TRGGS-PFV.

In one embodiment, the present invention provides a virus like particle comprising

1) a fusion protein of a polypeptide derived from Chikungunya virus (CHIKV) and a polypeptide derived from TNF-a, which consists of an amino acid sequence
represented by SEQ ID No. 4;
ii) a fusion protein of a polypeptide derived from Chikungunya virus (CHIKV) and a polypeptide derived from CD20, which consists of an amino acid sequence represented by SEQ ID No. 5;
iii) a fusion protein of a polypeptide derived from Venezuelan equine encephalitis virus (VEEV) and a polypeptide derived from TNF-α, which consists of an amino acid sequence represented by SEQ ID No. 6; or
iv) a fusion protein of a polypeptide derived from Venezuelan equine encephalitis virus (VEEV) and a polypeptide derived from CD20, which consists of an amino acid sequence represented by SEQ ID No. 7;
v) a fusion protein of a polypeptide derived from Venezuelan equine encephalitis virus (VEEV) and a polypeptide derived from CTLA4, which consists of an amino acid sequence represented by SEQ ID No. 8.

In one embodiment, the present invention provides a virus-like particle comprising a fusion protein which is modified from the fusion protein having an amino acid sequence represented by any one of SEQ ID Nos. 4-8. The modified fusion protein may have at least 70%, 75%, 80%, 85%, 90%, 95% or 98% amino acid sequence identity to the fusion protein having an amino acid sequence represented by any one of SEQ ID Nos. 4-8. Also, the modified fusion
protein may be a mutant where at most 10% of the amino acids are deleted, substituted, and/or added based on the fusion protein having an amino acid sequence represented by any one of SEQ ID Nos.4-8.

(2) Nucleotide, Vector, Host cell

In the second aspect, the present invention provides a nucleic acid molecule comprising a nucleotide sequence that encodes a particle as provided in the first aspect of the present invention.

In one embodiment, the present invention provides a nucleic acid molecule comprising a nucleotide sequence that encodes the Chikungunya or Venezuelan equine encephalitis virus like particle as described above.

Examples of the nucleotide sequence that encodes the Chikungunya or Venezuelan equine encephalitis virus like particle include, but are not limited to, a nucleotide sequence encoding E3-E2-6K-E1 of Chikungunya virus Strain 37997, a nucleotide sequence encoding Capsid-E3-E2-6K-E1 of Chikungunya virus Strain 37997, a nucleotide sequence encoding E3-E2-6K-E1 of Chikungunya virus Strain LR2006 OPY-1, a nucleotide sequence encoding Capsid-E3-E2-6K-E1 of Chikungunya virus LR2006 OPY-1, a nucleotide sequence encoding E3-E2-6K-E1 of Venezuelan equine encephalitis virus Strain TC-83 and a nucleotide sequence encoding
Regarding Chikungunya virus, another exemplary nucleotide sequence that encodes E3-E2-6K-E1 is described below (SEQ ID No.: 10):

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ATgagcgcctccctgcgtttgtgtgcctgllgacacacacagtgaactaacatcataacagaccaagacagggcgcgtcttgcctgllgacacaggttgcgtctgctcggtcgggacttgcgtgtggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcgtgttggtttgcg
Regarding Chikungunya virus, an exemplary nucleotide sequence that encodes a Capsid-E3-E2-6K-El is described below (SEQ ID No.: 11):

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atggagttcatccgcacgcacaaacttcttataacaggaaggtaccaacccgggctcaggttcatcgcagcttggtgcgtgtctgtcgcgtgtc
acatggagttcatccgcacgcacaaacttcttataacaggaaggtaccaacccgggctcaggttcatcgcagcttggtgcgtgtctgtcgcgtgtc
acatggagttcatccgcacgcacaaacttcttataacaggaaggtaccaacccgggctcaggttcatcgcagcttggtgcgtgtctgtcgcgtgtc
acatggagttcatccgcacgcacaaacttcttataacaggaaggtaccaacccgggctcaggttcatcgcagcttggtgcgtgtctgtcgcgtgtc
acatggagttcatccgcacgcacaaacttcttataacaggaaggtaccaacccgggctcaggttcatcgcagcttggtgcgtgtctgtcgcgtgtc
acatggagttcatccgcacgcacaaacttcttataacaggaaggtaccaacccgggctcaggttcatcgcagcttggtgcgtgtctgtcgcgtgtc
acatggagttcatccgcacgcacaaacttcttataacaggaaggtaccaacccgggctcaggttcatcgcagcttggtgcgtgtctgtcgcgtgtc
acatggagttcatccgcacgcacaaacttcttataacaggaaggtaccaacccgggctcaggttcatcgcagcttggtgcgtgtctgtcgcgtgtc
acatggagttcatccgcacgcacaaacttcttataacaggaaggtaccaacccgggctcaggttcatcgcagcttggtgcgtgtctgtcgcgtgtc
acatggagttcatccgcacgcacaaacttcttataacaggaaggtaccaacccgggctcaggttcatcgcagcttggtgcgtgtctgtcgcgtgtc
acatggagttcatccgcacgcacaaacttcttataacaggaaggtaccaacccgggctcaggttcatcgcagcttggtgcgtgtctgtcgcgtgtc
acatggagttcatccgcacgcacaaacttcttataacaggaaggtaccaacccgggctcaggttcatcgcagcttggtgcgtgtctgtcgcgtgtc
acatggagttcatccgcacgcacaaacttcttataacaggaaggtaccaacccgggctcaggttcatcgcagcttggtgcgtgtctgtcgcgtgtc
acatggagttcatccgcacgcacaaacttcttataacaggaaggtaccaacccgggctcaggttcatcgcagcttggtgcgtgtctgtcgcgtgtc
acatggagttcatccgcacgcacaaacttcttataacaggaaggtaccaacccgggctcaggttcatcgcagcttggtgcgtgtctgtcgcgtgtc
acatggagttcatccgcacgcacaaacttcttataacaggaaggtaccaacccgggctcaggttcatcgcagcttggtgcgtgtctgtcgcgtgtc
acatggagttcatccgcacgcacaaacttcttataacaggaaggtaccaacccgggctcaggttcatcgcagcttggtgcgtgtctgtcgcgtgtc
acatggagttcatccgcacgcacaaacttcttataacaggaaggtaccaacccgggctcaggttcatcgcagcttggtgcgtgtctgtcgcgtgtc
acatggagttcatccgcacgcacaaacttcttataacaggaaggtaccaacccgggctcaggttcatcgcagcttggtgcgtgtctgtcgcgtgtc
acatggagttcatccgcacgcacaaacttcttataacaggaaggtaccaacccgggctcaggttcatcgcagcttggtgcgtgtctgtcgcgtgtc
acatggagttcatccgcacgcacaaacttcttataacaggaaggtaccaacccgggctcaggttcatcgcagcttggtgcgtgtctgtcgcgtgtc
acatggagttcatccgcacgcacaaacttcttataacaggaaggtaccaacccgggctcaggttcatcgcagcttggtgcgtgtctgtcgcgtgtc
acatggagttcatccgcacgcacaaacttcttataacaggaaggtaccaacccgggctcaggttcatcgcagcttggtgcgtgtctgtcgcgtgtc
acatggagttcatccgcacgcacaaacttcttataacaggaaggtaccaacccgggctcaggttcatcgcagcttggtgcgtgtctgtcgcgtgtc
acatggagttcatccgcacgcacaaacttcttataacaggaaggtaccaacccgggctcaggttcatcgcagcttggtgcgtgtctgtcgcgtgtc
```
ttgcgcatgcttgaggacaacgtgatgagacccggatactaccagctactaaaagcatc
gctgacttgctctccccaccgccaaagacgcagtactaaggacaattttaatgtctata
... virus, another exemplary
nucleotide sequence that encodes a Capsid-E3-E2-6K-El is
described below (SEQ ID No.: 12)

Regarding Chikungunya virus, another exemplary
nucleotide sequence that encodes a Capsid-E3-E2-6K-El is
described below (SEQ ID No.: 12):
atggagttcatcccaacccaaactttttacaataggaggtaccagcctcgaccctggac
tccgcgccctactatccaagtcatcaggcccagaccgcgccctcagaggcaagctgggc
aacttgcccagctgatctcagcagttaataaactgacaatgcgcgcggtaccacaacag
aagccacgcaggaatcggaagaataagaagcgcacccaccccaaaaaaacacagggcc
cttcgcaacaacagaccgttggtgcccatagttgagggagctaatgaaggagcc
cttcgctcttggagcaacgctcttggggtattagcaatgcgcgcacaaaattgtggtgtacaaaggtgacgtctataacatggactacccgccctttggcgca
ggaagaccaggacaatttggcgatatccaaagtcgcacacctgagagtaaagacgtcta
ctgcagctatgctgtaacagccccatggtattggagatggaactactgtcagtcac
tttggagccaacctatcgcttgattacatcacgtgcgagtacaaaaccgtcatcccgt
tccgtacgtgaagtgctgcggtacagcagagtgcaaggacaaaaacctacctgactac
agctgtaaggtcttcaccggcgtctacccattatatggggctggcgcctactgcttctgcagcctgaaaacacgcagttgagcgaagcacacgtggagaagtccgaatcatgcaaaa
cagaatttgcatcagcatacagggct
In one embodiment, the present invention provides a vector comprising the nucleic acid molecule as described above, wherein the vector optionally comprises an expression control sequence operably linked to the nucleic acid molecule.

Examples of an expression control sequence include, but are not limited to, promoter such as CMV promoter, phage lambda PL promoter, the E. coli lac, phoA and tac promoters, the SV40 early and late promoters, and promoters of retroviral LTRs.

The expression vectors can be prepared by a person skilled in the art based on WO/2012/006180, the entire contents of which are incorporated by reference herein.

Examples of vectors which can be used for expressing a fusion protein of a polypeptide derived from Chikungunya virus (CHIKV) and a polypeptide of antigen include a vector shown in VLP_CHI 512 vector (SEQ ID No.:23) containing CHIKV VLP polynucleotide (SEQ ID No. 28; corresponding amino acid sequence represented by SEQ ID No.:29); and
The expression vectors can be prepared by a person skilled in the art based on US2012/0003266, the entire contents of which are incorporated by reference herein. Examples of vectors which can be used for expressing a fusion protein of a polypeptide derived from Venezuelan equine encephalitis virus (VEEV) and a polypeptide of antigen include a vector shown in VLP_VEEV VLP 518 vector (SEQ ID No.:25) containing VEEV VLP polynucleotide (SEQ ID No. 32; corresponding amino acid sequence represented by SEQ ID No.: 33); VLP_VEEV VLP 519 vector (SEQ ID No. 26) containing VEEV VLP polynucleotide (SEQ ID No. 34; corresponding amino acid sequence represented by SEQ ID No. -35); and VLP_VEEV VLP 538 vector (SEQ ID No.: 27) containing VEEV VLP polynucleotide (SEQ ID No. 36; corresponding amino acid sequence represented by SEQ ID No. :37).

In one embodiment, the present invention provides i) a nucleic acid molecule encoding a fusion protein of a polypeptide derived from Chikungunya virus (CHIKV) and a polypeptide derived from TNF-α, which consists of a nucleotide sequence represented by SEQ ID No.13; ii) a nucleic acid molecule encoding a fusion protein of a
polypeptide derived from Chikungunya virus (CHIKV) and a polypeptide derived from CD20, which consists of a nucleotide sequence represented by SEQ ID No.14;

iii) a nucleic acid molecule encoding a fusion protein of a polypeptide derived from Venezuelan equine encephalitis virus (VEEV) and a polypeptide derived from TNF-a, which consists of a nucleotide sequence represented by SEQ ID No.15;

iv) a nucleic acid molecule encoding a fusion protein of a polypeptide derived from Venezuelan equine encephalitis virus (VEEV) and a polypeptide derived from CD20, which consists of a nucleotide sequence represented by SEQ ID No.16; or

v) a nucleic acid molecule encoding a fusion protein of a polypeptide derived from Venezuelan equine encephalitis virus (VEEV) and a polypeptide derived from CTLA4, which consists of a nucleotide sequence represented by SEQ ID No.17.

In one embodiment, the present invention provides a nucleic acid molecule which is modified from the nucleic acid molecule having a nucleotide sequence represented by any one of SEQ ID Nos.13-17. The modified nucleic acid molecule may have at least 70%, 75%, 80%, 85%, 90%, 95% or 98% nucleotide sequence identity to the nucleic acid molecule having a nucleotide sequence represented by any
one of SEQ ID Nos. 13-17. Also, the modified nucleic acid molecule may be a mutant where at most 10% of the amino acids are deleted, substituted, and/or added based on the nucleic acid molecule having a nucleotide sequence represented by any one of SEQ ID Nos. 13-17.

(3) Composition

In the third aspect, the present invention provides a composition comprising the particle provided in the first aspect of the present invention and/or the nucleic acid molecule provided in the second aspect of the present invention.

In one embodiment, the present invention provides a composition comprising the Chikungunya or Venezuelan equine encephalitis virus like particle as described above or the nucleic acid molecule as described above.

The composition may further comprise a pharmaceutical acceptable carrier and/or adjuvant. Examples of adjuvant include, but are not limited to Ribi solution (Sigma Adjuvant system, Sigma-Aldrich).

The pharmaceutical composition of the present invention may contain a single active ingredient or a combination of two or more active ingredients, as far as they are not contrary to the objects of the present invention. For example, cytokines including chemokines,
anti-body of cytokines such as anti TNF antibody (e.g. infliximab, adalimumab), anti-VEGF antibody (e.g. bevacizumab and ranibizumab), cytokine receptor antagonist such as anti HER2 antibody (e.g. Trastuzumab), anti EGF receptor antibody (e.g. Cetuximab), anti VEGF aptamer (e.g. Pegaptanib) and immunomodulator such as cyclosporine, tacrolimus, ubenimex may be used for the combination therapy.

In a combination of plural active ingredients, their respective contents may be suitably increased or decreased in consideration of their therapeutic effects and safety.

The term "combination" used herein means two or more active ingredient are administered to a patient simultaneously in the form of a single entity or dosage, or are both administered to a patient as separate entities either simultaneously or sequentially with no specific time limits, wherein such administration provides therapeutically effective levels of the two components in the body, preferably at the same time.

In one embodiment, the composition is a vaccine composition including a DNA vaccine. In one embodiment, the DNA vaccine provided by the present invention comprises CpG containing oligonucleotide.

(4) Method of producing an antibody, Method of
immunomodulation, Method of treating an autoimmune disease, Method of inducing and/or enhancing immune response against an antigen in a mammal, Method of treating cancer, Method of passive immunization, Method of presenting an antigen on macrophage, and Method for producing a particle

In the fourth aspect, the present invention provides a method of producing an antibody, comprising contacting the particle provided in the first aspect of the present invention and/or the nucleic acid molecule provided in the second aspect of the present invention to a mammal.

The antibody produced in the fourth aspect of the present invention may be humanized using a conventional technique. Thus, in one embodiment, the method provided in the fourth aspect of the invention further comprises a step of humanizing non-human mammal produced antibody.

The particle provided in the first aspect of the present invention and/or the nucleic acid molecule provided in the second aspect of the present invention may be administered directly into the patient, into the affected organ or systemically, or applied ex vivo to cells derived from the patient or a human cell line which are subsequently administered to the patient, or used in vitro to select a subpopulation from immune cells such as B-cell
and T-cell derived from the patient, which are then re-administered to the patient.

According to the present invention, the virus like particle can be applied for the immune therapy.

In the fifth aspect, the present invention provides a method of immunomodulation, a method of treating an autoimmune disease, a method of inducing and/or enhancing immune response against an antigen in a mammal, and a method of treating cancer comprising administering the composition provided in the third aspect of the present invention to a mammal.

In sixth aspect, the present invention provides a method of passive immunization, comprising administering the antibody provided in the fourth aspect of the present invention to a mammal.

In seventh aspect, the present invention provides a method of presenting an antigen on macrophage, comprising contacting the particle provided in the first aspect of the present invention and/or the nucleic acid molecule provided in the second aspect of the present invention to a mammal.

In eighth aspect, the present invention provides a method for producing the particle provided in the first
aspect of the present invention, comprising preparing a gene comprising a nucleotide sequence encoding said particle; culturing a cell which is transfected with said gene to express said particle; and recovering said particle.

In one embodiment, the present invention provides a method of producing an antibody, comprising contacting the Chikungunya or Venezuelan equine encephalitis virus like particle as described above and/or the nucleic acid molecule as described above to a mammal. The produced antibody may be an antibody which can specifically bind to the antigen comprised in the Chikungunya or Venezuelan equine encephalitis virus like particle or the antigen encoded by the nucleic acid molecule. The method of producing an antibody provided by the present invention may be a useful method for producing a monoclonal or polyclonal antibody against an antigen (e.g. TNFα, CD20 and CLTA4).

In one embodiment, the antibody obtained by the method of producing an antibody according to the present invention is used for passive immunization. The method of passive immunization may comprise administering the obtained antibody to a mammal.

According to the present invention, the composition of the present invention is useful for immunomodulation. Especially said immunomodulation is for the treatment of
autoimmune disease, neural disease, inflammatory disease such as inflammatory lung disease, including the acute respiratory distress syndrome, chronic obstructive pulmonary disease and asthma, angiogenesis associated diseases including neoplasm.

In one preferred embodiment, the immunomodulation provided by the present invention is inducing and/or enhancing immune response against an antigen in a mammal. Thus, in one embodiment, the present invention provides a method of inducing and/or enhancing immune response against an antigen in a mammal, comprising administering an effective amount of the composition as described above to the mammal. Examples of mammal include, but are not limited to, a human.

Since many antibodies are useful for the treatment of disease, the method and the composition which are provided by the present invention can be useful for the treatment of diseases. For example, an antibody which specifically binds the target as listed in Table 1 or an antibody which binds an epitope on the target as listed in Table 1 is useful for the treatment of the disease as listed in Table 1.
In one embodiment, at least one antigen which is used for the present invention is at least one target as listed in Table 1. When at least one antigen which is used for the present invention is at least one target as listed in Table 1, the particle, the isolated nucleic acid, the vector, the composition and the method provided by the present invention can be useful for the treatment of the disease or the condition as listed in Table 1 (see "Use" of Table 1).

For example, when at least one antigen used for the present invention is one or more cancer antigen, the particle, the isolated nucleic acid, the vector, the composition and the method provided by the present invention can be useful for the treatment of cancer.

Examples of cancer antigen include, but are not limited to, VEGF, epidermal growth factor receptor, CD33, CD20 and ErbB2. When the composition of the present invention comprising two or more cancer antigens is administered to a mammal, antibodies directed to the two or more cancer antigens can attack the cancer.

For example, when at least one antigen used for the present invention is amyloid β, the isolated nucleic acid, the vector, the composition and the method provided by the
present invention can be useful for the treatment of Alzheimer's disease.

For example, when at least one antigen used for the present invention is TNF alpha, the isolated nucleic acid, the vector, the composition and the method provided by the present invention can be useful for the treatment of inflammation; auto immune disease including rheumatoid arthritis; psoriasis, Crohn's disease; ulcerative colitis etc.

For example, when at least one antigen used for the present invention is CD20, the isolated nucleic acid, the vector, the composition and the method provided by the present invention can be useful for the treatment of auto immune disease including rheumatoid arthritis and SLE; cancer including Non-Hodgkin lymphoma etc.

For example, when at least one antigen used for the present invention is CTLA4, the isolated nucleic acid, the vector, the composition and the method provided by the present invention can be useful for the treatment of cancer including melanoma; and useful for activating T cells etc.

Given the symptom of patients infected with Chikungunya or Venezuelan equine encephalitis together with unusual big molecule of Chikingunya or Venezuelan equine
encephalitis, this VLP can act effectively and efficiently to target macrophage and its composition such as cytokines and immunomodulative compounds.

In one aspect, the present invention provides a method of presenting an antigen on macrophage, comprising administering the Chikungunya or Venezuelan equine encephalitis virus like particle as described above and/or the nucleic acid molecule as described above to a mammal. The Chikungunya or Venezuelan equine encephalitis virus like particle provided by the present invention is good to target macrophage. In one embodiment, the Chikungunya or Venezuelan equine encephalitis virus like particle provided by the present invention is a kind of delivery system of the at least one antigen, which is comprised in the Chikungunya or Venezuelan equine encephalitis virus like particle, to macrophage.

In one embodiment, the present invention provides a method for producing Chikungunya or Venezuelan equine encephalitis virus like particle provided in the first aspect of the present invention, comprising preparing a gene comprising a nucleotide sequence encoding said particle; culturing a cell which is transfected with said gene to express said particle; and recovering said particle. In this embodiment, transfection can be conducted using a
conventional method. Cells using for the transfection may be 293 cells. Recovering VLP may include collecting a conditioned medium after cells are transfected with a plasmid comprising a gene, and may further include purify VLP from the conditioned medium using ultracentrifugation.

In one embodiment, further step may be included in the method for producing Chikungunya or Venezuelan equine encephalitis virus like particle provided in the eighth aspect of the present invention, where a polynucleotide encoding an antigen is designed so that spatial distance between the N-terminal residue and C-terminal residue of the antigen is 30Å or less (e.g. from 5Å to 15Å, from 5Å to 12Å, from 5Å to 11Å, from 5Å to 10Å, from 5Å to 8Å, from 8Å to 15Å, from 8Å to 13Å, from 8Å to 12Å, from 8Å to 11Å, from 9Å to 12Å, from 9Å to 11Å, from 9Å to 10Å, or from 10Å to 11Å) when the distance is determined in a crystal of the antigen or a naturally occurring protein containing the antigen or modified protein therefrom.

Immune system evolves to recognize foreign antigens for killing pathogens such as viruses or bacteria. It also evolves not to recognize self proteins to protect self proteins. It is called immune tolerance system. Therefore it is difficult to induce antibodies against self-antigen.
by traditional immunization methods. To overcome the immune tolerance, we developed a novel vaccine method using a self-assembly subunit containing an self-antigen. The self-assembly subunit spontaneously assembles and forms a stable organized unit that presents highly repetitive antigens on the surface. Highly repetitive antigens immunogen strengthen signal pathways in B cells and result in stimulation of antibody responses than single antigen immunogen such as traditional immunization methods. Applying this mechanism to vaccine development not only increases antibody responses against target immunogens but also overcome self-antigen tolerance.

The present invention will be described in detail with reference to the following example, which, however, is not intended to limit the scope of the present invention.

EXAMPLES

(1) Preparation of Chikungunya virus like particle comprising a virus structural polypeptide and a fragment of human TNF alpha

It was expected that a monomer of TNF alpha polypeptide fused with Chikungunya virus structural polypeptide is difficult to be stably expressed because TNF
alpha is found as a trimer under natural conditions. However, use of a fusion protein, in which TNF alpha monomer peptide (a fragment of TNF alpha monomer peptide) is fused with the Chikungunya virus structural polypeptide through linkers at N- and C-terminal of TNF alpha-derived peptide for attaching TNF alpha-derived peptide to the Chikungunya virus structural polypeptide, resulted in stable expression of a Chikungunya virus like particle comprising a virus structural polypeptide and TNF alpha monomer-derived peptide.

In detail, polynucleotide encoding the original human TNF alpha was modified to prepare a polynucleotide encoding modified TNF alpha-derived peptide where RTPSD which is N-terminal sequence of the original TNF alpha-derived peptide is replaced with SGG and TRGGS is attached to C-terminal of the TNF alpha (see SEQ ID Nos.18-20 as shown in Fig.1). The resulting polynucleotide was inserted between the codons encoding G at 519-position and Q at 520-position of SEQ ID No. 2 to construct a plasmid (hereinafter referred to as CHIKV-TNFa4) for expressing Chikungunya virus like particle where the modified TNF alpha-derived peptide is inserted into E2 of Chikungunya virus structural polypeptide (C-E3-E2-6K-E1). Subsequently, 293F cells (1.5x10^6 cells/ml) was transfected with 250μg of CHIKV-
After culturing for 4 days, the supernatant was collected. The obtained supernatant was overlaid onto Opti Prep (Sigma D1556) followed by being ultracentrifuged (20000rpm, 120min) using SW28 rotor to concentrate VLP (i.e. virus like particle). The concentrated VLP was mixed with Opti Prep to form density gradient followed by ultracentrifuged (75000rpm, 4hours) using NVT100 rotor. After the ultracentrifugation, purified VLP was collected. The expression of VLP comprising TNF alpha conjugated with Chikungunya virus structural polypeptide was confirmed by Western Blot using an antibody specific for CHIVK (ATCC: VR-1241AF) and an antibody specific for TNF alpha (Cell Signal: #6945).

The spatial distance between the N-terminal residue and C-terminal residue of the TNF alpha-derived peptide is 8.27Å when the distance is determined in a crystal of TNF alpha.

(2) Preparation of Venezuelan equine encephalitis virus like particle comprising a virus structural polypeptide and human TNF alpha-derived peptide (referred to as "VEEV-TNFα VLPs")

According to the above-described (1), polynucleotide encoding modified TNF alpha-derived peptide fused with
polynucleotide encoding Venezuelan equine encephalitis virus structural polypeptide was prepared (see SEQ ID No. 15) to construct an expression vector followed by transfection in 293F cells.

VEEV-TNFα VLPs were purified by density gradient centrifuge. As seen in Lane 4, TNF alpha-derived peptide and VEEV expression was confirmed by Western blot using TNFα monoclonal antibody (top panel) and VEEV polyclonal antibodies (bottom panel), respectively (see Fig. 2).

The spatial distance between the N-terminal residue and C-terminal residue of the TNF alpha-derived peptide is 8.27Å when the distance is determined in a crystal of TNF alpha.

(3) Detection of anti-human TNF alpha antibody in immunized mouse

Mice were divided into three groups (n=5 for each group). The Chikungunya virus like particle comprising a virus structural polypeptide and human TNF alpha-derived polypeptide prepared according to the above-described (1) (referred to as "CHIKV-TNF alpha" below), Chikungunya virus like particle without comprising human TNF alpha-derived polypeptide (referred to as "CHIKV-VLP" below), Venezuelan equine encephalitis virus like particle comprising a virus
structural polypeptide and human TNF alpha-derived polypeptide prepared according to the above-described (2) (referred to as "VEEV-TNF alpha" below), Venezuelan equine encephalitis virus like particle without comprising human TNF alpha-derived polypeptide (referred to as "VEEV-VLP" below) or vehicle (i.e. PBS) were intramuscularly administered to each group of mice. The mice were administered at the beginning of the experiment (referred to as "0 week" below) and three weeks after the first administration (referred to as "3 week" below) as described below: Group 1: VEEV-TNF alpha (0 week), CHIKV-TNF alpha (3 week); Group 2: VEEV-VLP (0 week), CHIKV-VLP (3 week); and Group 3: PBS (0 week), PBS (3 week).

6 weeks after the beginning of the experiment, blood sample was obtained from each mouse and serum was prepared. Produced anti-human TNF alpha antibody was detected using ELISA where TNF alpha protein was coated on ELISA plate. The results show that the virus like particle comprising a virus structural polypeptide and human TNF alpha-derived polypeptide induced anti-human TNF alpha antibodies in mouse (see Fig. 9).

(4) Preparation of Venezuelan equine encephalitis virus like particle comprising a virus structural polypeptide and a fragment of human CD20
According to the above-described (1) and (2), VEEV-CD20 VLPs were purified by density gradient centrifuge, and a fragment of CD20 and VEEV expression was confirmed by Western blot. IYNCEPANPSEKNSPSTQYCYSIQ (SEQ ID No.: 21), which is a fragment of CD20, was used as an antigen fused with Venezuelan equine encephalitis virus structural polypeptide.

The spatial distance between the N-terminal residue and C-terminal residue of the CD20 fragment is 10.07Å when the distance is determined in a crystal of CD20.

(5) Preparation of Venezuelan equine encephalitis virus like particle comprising a virus structural polypeptide and a fragment of human CTLA4

A fragment of CTLA4: CKVELMYPYLYLGIG (SEQ ID No.: 22) was selected based on the full-length CTLA4 amino acid sequence so that spatial distance between the N-terminal residue and the C-terminal residue of the fragment, which is fused into Venezuelan equine encephalitis virus structural polypeptide E2, is about 5.6Å when the distance is determined in a crystal of CTLA4.

A polynucleotide encoding the fragment of CTLA4 was introduced into VLP_VEEV VLP 518 vector to construct a plasmid for the expression of the fragment of CTLA4 fused
with Venezuelan equine encephalitis virus structural polypeptide consisting of the amino acid sequence by SEQ ID No.:8.

(6) Preparation of Venezuelan equine encephalitis virus like particle comprising a virus structural polypeptide and full length human CTLA4

A polynucleotide encoding full length human CTLA4 was introduced into VLP_VEEV VLP 518 vector to construct a plasmid for the expression of CTLA4 fused with Venezuelan equine encephalitis virus structural polypeptide.

The spatial distance between the N-terminal residue and the C-terminal residue of full length human CTLA4 is about 39.6 Å when the distance is determined in a crystal of CLTA4.

Expression of CTLA4 fused with Venezuelan equine encephalitis virus structural polypeptide was not be able to be detected by ELISA after transfecting 293 cells with the prepared plasmid.

(7) Preparation of Chikungunya virus like particle comprising a virus structural polypeptide and a fragment of human or mouse CD20 and detection of anti-human or mouse CD20 antibody
Chikungunya virus like particles comprising a virus structural polypeptide and a fragment of human or mouse CD20 were prepared using VLP_CHI 532 vector and a fragment of human or mouse TNF alpha antibody. The fragment of human and mouse TNF alpha antibody are described below:

CD20 Human iyncepanpsekspstqycysi (SEQ ID No.:21); CD20 Mouse ydcepsnssekspstqycysi (SEQ ID No.:41).

Likers (e.g. SGG, SG, GS or GGS) were used to insert the fragment of CD20 as described below between G at 519-position and Q at 520-position of SEQ ID No.2:

CD20 Human: SGGiyncepanpsekspstqycysiGS (SEQ ID No.:42)

CD20 Mouse version2: SGYdcepsnssekspstqycysiGGS (SEQ ID No.:43)

CD20 Mouse version3: SGGYdcepsnssekspstqycysiGS (SEQ ID No.:44).

Plasmids: VLP_CHI VLP 532 CD20H, VLP_CHI VLP 532 CD20-2 mouse and VLP_CHI VLP 532 CD20-3 mouse were used for the expression of Chikungunya virus like particles comprising a virus structural polypeptide and a fragment of human or mouse CD20 (SEQ ID No.: 45 (the amino acid sequence of the expressed polypeptide is represented by SEQ ID No. : 46), SEQ ID No.: 47 (the amino acid sequence of the expressed
polypeptide is represented by SEQ ID No.: 48) and SEQ ID No.: 49 (the amino acid sequence of the expressed polypeptide is represented by SEQ ID No.: 50). 

Virus like particles were purified according to the method as described in (1). Mice were immunized once with 100pg of the purified VLPs; 100µg of the fragment of human or mouse CD20; or PBS (control). Ten days after the immunization, blood sample were obtained from the mice and serum was prepared. Anti-human CD20 antibody induced by the immunization was detected by ELISA coated with the fragment of human CD20, and anti-mouse CD20 antibody induced by the immunization was detected by ELISA coated with the fragment of mouse CD20. The results showed that anti-human CD20 antibodies and anti-mouse CD20 antibodies were adequately induced by administration of the Chikungunya virus like particle comprising the fragment of human or mouse CD20 fused with virus structural polypeptide. Also, the results showed that antibody specific for a self antigen can be adequately induced by administering Chikungunya virus like particle comprising a fragment of the self antigen fused with virus structural polypeptide (see Fig. 10 and Fig. 11).
CLAIMS

1. A particle which is capable of being self-assembled, comprising a polypeptide and at least one antigen, wherein said polypeptide comprises at least one first attachment site and said at least one antigen comprises at least one second attachment site, and wherein said polypeptide and said antigen are linked through said at least one first and said at least one second attachment site, and wherein spatial distance between the N-terminal residue and C-terminal residue of the antigen is 30 Å or less when the distance is determined in a crystal of the antigen or a naturally occurring protein containing the antigen or modified protein therefrom.

2. The particle according to Claim 1, wherein said particle is virus like particle.

3. A particle comprising a virus structural polypeptide and at least one antigen, wherein said virus structural polypeptide comprises at least one first attachment site and said at least one antigen comprises at least one second attachment site, and wherein said virus structural polypeptide and said antigen are linked through said at least one first and said at least one second attachment site, and wherein said particle is virus like particle.
4. The particle according to Claim 2 or Claim 3, wherein said virus like particle is derived from alphavirus or Flavivirus.

5. The particle according to Claim 4, wherein said alphavirus or Flavivirus is selected from the group consisting of Aura virus, Babanki virus, Barmah Forest virus (BFV), Bebaru virus, Cabassou virus, Chikungunya virus (CHIKV), Eastern equine encephalitis virus (EEEV), Eilat virus, Everglades virus, Fort Morgan virus, Getah virus, Highlands J virus, Kyzylagach virus, Mayaro virus, Me Tri virus, Middelburg virus, Mosso das Pedras virus, Mucambo virus, Ndumu virus, O’nyong-nyong virus, Pixuna virus, Rio Negro virus, Ross River virus (RRV), Salmon pancreas disease virus, Semliki Forest virus, Sindbis virus, Southern elephant seal virus, Tontate virus, Trocara virus, Una virus, Venezuelan equine encephalitis virus (VEEV), Western equine encephalitis virus (WEEV), Whataroa virus, West Nile virus, dengue virus, tick-borne encephalitis virus and yellow fever virus.

6. The particle according to Claim 5, wherein said alphavirus is Chikungunya virus (CHIKV) or Venezuelan equine encephalitis virus (VEEV).

7. The particle according to any one of Claims 2–6, wherein said polypeptide is virus structural polypeptide comprising an envelope protein.
8. The particle according to any one of Claims 2-7, wherein said polypeptide is virus structural polypeptide comprising the capsid and/or the envelope proteins E3, E2, 6K and El.

9. The particle according to Claim 8, wherein said at least one antigen is inserted into E2 of the envelope protein.

10. The particle according to any one of Claims 1-9, wherein said antigen is at least one selected from the group consisting of self antigens and cancer antigens.

11. The particle according to any one of Claims 1-10, wherein said antigen is a polypeptide derived from TNF-a, CD20 or CTLA4.

12. The particle according to any one of Claims 1-11, wherein said polypeptide is a polypeptide derived from Chikungunya virus (CHIKV) or Venezuelan equine encephalitis virus (VEEV).

13. The particle according to any one of Claims 1-12, wherein said polypeptide and said at least one antigen is
   i) a polypeptide derived from Chikungunya virus (CHIKV) and a polypeptide of TNF-a;
   ii) a polypeptide derived from Chikungunya virus (CHIKV) and a polypeptide of CD20;
   iii) a polypeptide derived from Venezuelan equine encephalitis virus (VEEV) and a polypeptide of TNF-a; or
iv) a polypeptide derived from Venezuelan equine encephalitis virus (VEEV) and a polypeptide of CD20
v) a polypeptide derived from Venezuelan equine encephalitis virus (VEEV) and a polypeptide of CTLA4.

14. The particle according to any one of Claims 1-13, wherein said at least one antigen and said polypeptide are expressed as a fusion protein.

15. The particle according to Claim 14, wherein said polypeptide and said at least one antigen are directly fused.

16. The particle according to Claim 14, wherein said at least one antigen are fused with said polypeptide, wherein one or two linkers intervenes between N-terminal residue of said antigen and said polypeptide and/or between C-terminal residue of said antigen and said polypeptide.

17. The particle according to Claims 15 or 16, wherein said at least one antigen is inserted between residues 519 and 520 of SEQ ID Nos. 1 or 2, between residues 530 and 531 of SEQ ID Nos. 1 or 2, between residues 531 and 532 of SEQ ID Nos. 1 or 2 or between residues 532 and 533 of SEQ ID Nos. 1 or 2.

18. The particle according to any one of Claims 14-17, wherein said fusion protein is a protein consisting of an amino acid sequence represented by SEQ ID Nos. 4, 5, 6, 7 or 8.
19. The particle according to any one of Claims 14-17, wherein said fusion protein is derived from a protein consisting of an amino acid sequence which has a sequence identity of 90% or more with an amino acid sequence represented by SEQ ID Nos. 4, 5, 6, 7 or 8.

20. The particle according to any one of Claims 1-13, wherein said at least one antigen is linked to said polypeptide by way of chemical cross-linking.

21. An isolated nucleic acid molecule comprising a nucleotide sequence that encodes the particle according to any one of Claims 1-20.

22. An isolated nucleic acid molecule consisting of a nucleotide sequence represented by SEQ ID Nos. 13, 14, 15, 16 or 17.

23. An isolated nucleic acid molecule consisting of a nucleotide sequence which has a sequence identity of 90% or more with a nucleotide sequence encoding represented by SEQ ID Nos. 13, 14, 15, 16 or 17.

24. A vector comprising the nucleic acid molecule according to Claim 23, wherein the vector optionally comprises an expression control sequence operably linked to the nucleic acid molecule.

25. A composition comprising the particle according to any one of Claims 1-20 and/or the nucleic acid molecule according to any one of Claims 21-24.
26. A pharmaceutical composition comprising:
(a) the particle according to any one of Claims 1-20 and/or
the nucleic acid molecule according to any one of Claims 21-24; and
(b) a pharmaceutically acceptable carrier.
27. A vaccine composition comprising the particle
according to any one of Claims 1-20.
28. A DNA vaccine composition comprising the nucleic acid
molecule according to any one of Claims 21-24.
29. A method of producing an antibody, comprising
contacting the particle according to any one of Claims 1-20
and/or the nucleic acid molecule according to any one of
Claims 21-24 to a mammal.
30. The method according to Claim 29, wherein said
antibody, is a monoclonal antibody.
31. A method of immunomodulation, comprising administering
an immunologically effective amount of the composition of
any one of claims 25-28 to a mammal.
32. A method of treating an autoimmune disease, comprising
administering an immunologically effective amount of the
composition of any one of claims 25-28 to a mammal.
33. A method of inducing and/or enhancing immune response
against an antigen in a mammal, comprising administering an
effective amount of the composition of any one of claims
25-28 to the mammal.
34. A method of treating cancer, comprising administering an effective amount of the composition of any one of claims 25-28 to a mammal.

35. A method of passive immunization, comprising administering the antibody obtained by the method according to Claim 29 or Claim 30 to a mammal.

36. The method according to Claim 35, for the treatment of cancer.

37. The method according to any one of Claims 34-36, wherein said at least one antigen is a cancer antigen.

38. The method according to any one of Claims 34-36, wherein said at least one antigen is one or more cancer antigen.

39. A method of presenting an antigen on macrophage, comprising contacting the particle according to any one of Claims 1-20 and/or the nucleic acid molecule according to any one of Claims 21-24 to a mammal.

40. A method for producing the particle according to Claims 14-19, comprising preparing a gene comprising a nucleotide sequence encoding said particle; culturing a cell which is transfected with said gene to express said particle; and recovering said particle.
Fig. 1

Original TNFalpha sequence

RTPSDkpvahvvanpqeqglqwlrranallangvelrdnq1vvpseglyliysqvlfgqgcp
sthvlthtsriavsytqkvnllsaiakspcqcrtpegaeakpwyepyeliygvgfqlkekgdr ISA rnp
dylfdaesgqvyfgiial

Modified TNFalpha sequence inserted into CHIKV

SGGkpvahvvanpqeqglqwlrranallangvelrdnq1vvpseglyliysqvlfgqgcpst
hvllhtthtsriavsytqkvnllsaiakspcqcrtpegaeakpwyepyeliygvgfqlkekgdr ISA rnp dy
ldfdaesgqvyfgiialTRGGS

Nucleotide sequence encoding the modified TNF-alpha

Tccggaggtaagcctgtagccccatgtttagcaaaacctcaagctgaggccagctcagttgctgaacgc
ccggcccaatgccctctggcagggctggagctgagagataaaaccctagctggtgggtgctgcccatcagaggctg
tatactctactccagagtctcttcaagggcagctgcgggccctccaccccaatgtgctgctctccacccacacac
tagccgcatacgcgtctctctaccagaaccaaggtcaacctctctcttgccatcaagccccctgccacaggggaga
ccccagaggggctgaggccaggggctgggtatggaccccatctatcttgaggggtcctccagctggagaaggg
gtggacccagtacgctgtcagatcatatcggccgccgcaactactctgttgcgagttgcttgactctttttgg
atcattgcctgtacgcgtttgaggtacc
Fig. 2

Anti-TNFalpha

Marker 1 2 3 4 5 6 7 8 9 Fraction

75kDa 50 37

E2-TNFalpha

Anti-Venezuelan equine encephalitis

Marker 1 2 3 4 5 6 7 8 9 Fraction

75kDa 50 37 25

E2-TNFalpha

E1

Capsid
Fig. 3

CMV promoter

AmpR promoter

Ampcillin

Origin

VLP_CHI 512 vector
8404 bp

poly A

CHI 512
Fig. 5

VLP_CHI 520 vector
8416 bp

CMV promoter

AmpR promoter

Ampicillin

Origin

Insert

CHI 520

poly A
Fig. 7

VLP VEEV VLP 519 vector
8431 bp

CMV promoter
AmpR promoter
Ampicillin
Origin
polyA
insert
VEEV VLP
Fig. 9

VLP-TNFalpha induced anti-TNFalpha antibodies

- VLP-TNFα
- VLP(Control)
- PBS(Control)

O.D. (450nm)

Log (serum dilution)
VLP-CD20 Induced anti-CD20 (Human) antibodies

anti-Human CD20 antibodies

- CD20 (Human) peptide
- VLP-CD20 (Human)
- CD20 (Mouse) peptide
- VLP-CD20 (Mouse)
- Control

log (serum dilution)

against Human CD20 peptide O.D. (450nm)
Fig. 11

VLP-CD20 Induced anti-CD20 (Mouse) antibodies

anti-Mouse CD20 antibodies

- CD20 (Human) peptide
- VLP-CD20 (Human)
- CD20 (Mouse) peptide
- VLP-CD20 (Mouse)
- Control

against Mouse CD20 peptide: O.D. (A500nm)

log (serum dilution)
INTERNATIONAL SEARCH REPORT

PCT/JP2013/054422

A. CLASSIFICATION OF SUBJECT MATTER

Int.Cl. See extra sheet

According to International Patent Classification (IPC) or to both national classification and IPC

ß FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Int.Cl. C07K1/9/00, A61K3/9/00, A61P35/00, A61P37/00, C07K1/4/18, C07K1/4/525, C07K1/4/705, C12N1/5/09, C12P21/08

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Published examined utility model applications of Japan 1922-1996
Published unexamined utility model applications of Japan 1971-2013
Registered utility model specifications of Japan 1996-2013
Published registered utility model applications of Japan 1994-2013

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAplus /MEDLINE/ EMBASE/ BIOS 1S (ETN)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
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<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<td>X /A</td>
<td>WO 1997/012048 A1 (MEDICAL RESEARCH COUNCIL), 1997.04.03, Claims, Example 6 and Fig. 18 &amp; JP 11-513249 A &amp; EP 854929 A1</td>
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☑ Special categories of cited documents:
- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier application or patent but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search

21.05.2013

Date of mailing of the international search report

28.05.2013

Name and mailing address of the ISA/JP

Japan Patent Office

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Form PCT/ISA/210 (second sheet) (July 2009)
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<td>WO 2012/106356 A2 (GOVERNMENT OF THE UNITED STATES OF AMERICA, as represented by THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES) 2012.08.09, A whole document (No patent family)</td>
<td>1-28,40</td>
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**INTERNATIONAL SEARCH REPORT**

**Box No. II**  
**Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ✓ Claims Nos.: 29-39  
   because they relate to subject matter not required to be searched by this Authority, namely:
   
   The subject matter of claim 29-39 relates to a method for treatment of the human or animal body by surgery or therapy, which does not require an international search by the International Searching Authority in accordance with PCT Article 17 (2) (a) (i) and [Rule 39.1 (iv) ].

2. □ Claims Nos.:  
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. □ Claims Nos.:  
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III**  
**Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. □ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. □ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. □ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:  

4. □ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  

**Remark on Protest**

- □ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- □ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- □ No protest accompanied the payment of additional search fees.
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