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 down-stream 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 a 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-lymphocytss 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

Target	Use
GD2	neuroblastoma
CA-125 (imitation)	ovarian cancer
CD41 (integrin alpha-IIb)	platelet aggregation inhibitor
TNF-α	rheumatoid arthritis etc.
EpCAM	prostate and breast cancer
TNF-α	sepsis
CD20	lymphoma
VEGFR2	cancer
IL-6	rheumatoid arthritis
CD52	CLL, CTCL
CEA	colorectal cancer
TAG-72	non-small cell lung carcinoma
HLA-DR	hematological cancers
CEA	gastrointestinal cancers
L-selectin (CD62L)	severely injured patients
IL-6 receptor	rheumatoid arthritis
Rhesus factor	hemolytic disease of the newborn
beta amyloid	Alzheimer's disease
CD25 (α chain of IL-2 receptor)	prevention of organ transplant rejections
phosphatidylserine	cancer, viral infections
CD22	non-Hodgkin's lymphoma
BAFF	non-Hodgkin lymphoma etc.
CD125	asthma
CCL11 (eotaxin-1)	severe allergic disorders
CEA-related antigen	inflammatory lesions and metastases
VEGF-A	metastatic cancer
fibrin II, beta chain	thromboembolism
CD44 v6	squamous cell carcinoma
CD19	cancer
CD30 (TNFRSF8)	hematologic cancers
IL-12, IL-23	psoriasis, rheumatoid arthritis, inflammatory bowel diseases, multiple sclerosis
IL-1	rheumatoid arthritis
mucin CanAg	colorectal cancer etc.
prostatic carcinoma cells	prostate cancer
EpCAM, CD3	ovarian cancer, malignant ascites, gastric cancer
TAG-72	tumor detection
CD4	prevention of organ transplant rejections, treatment of autoimmune diseases
TNF-α	Crohn's disease
EGFR	metastatic colorectal cancer and head and neck cancer
EpCAM	ovarian cancer and other solid tumors
IGF-1 receptor	solid tumors
CD4	rheumatoid arthritis
MUC1	pancreatic cancer
TRAIL-R2	cancer
Influenza A hemagglutinin	infectious disease/influenza A
CD40	hematologic cancers
CD25 (α chain of IL-2 receptor)	prevention of organ transplant rejections
RANKL	osteoporosis, bone metastases etc.
B-lymphoma cell	lymphoma
GD3 ganglioside	malignant melanoma
C5	paroxysmal nocturnal hemoglobinuria
endotoxin	sepsis caused by Gram-negative bacteria
EpCAM	colorectal carcinoma
LFA-1 (CD11a)	psoriasis (blocks T-cell migration)
Hsp90	invasive Candida infection
SLAMF7	multiple myeloma
CD22	cancer, SLE
ITGB2 (CD18)	heart attack, stroke, traumatic shock
HER2/neu, CD3	breast cancer etc.
integrin αvβ3	melanoma, prostate cancer, ovarian cancer etc.
hepatitis B surface antigen	hepatitis B
CD15	appendicitis
folate receptor 1	ovarian cancer

Table 1 -continued-

Target	Use
respiratory syncytial virus	respiratory syncytial virus infection
IL-22	rheumatoid arthritis, psoriasis
IGF-1 receptor	adrenocortical carcinoma, non-small cell lung carcinoma etc.
IFN-γ	Crohn's disease etc.
rabies virus glycoprotein	rabies (prophylaxis)
TGF-β	idiopathic pulmonary fibrosis, focal segmental glomerulosclerosis, cancer
CD80	B-cell lymphoma
beta amyloid	Alzheimer's disease
CD147 (basigin)	graft versus host disease
CD33	acute myelogenous leukemia
carbonic anhydrase 9 (CA-IX)	clear cell renal cell carcinoma
GPNMB	melanoma, breast cancer
TNF-α	rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis
CD23 (IgE receptor)	allergic asthma
CD4	HIV infection
CD20	non-Hodgkin's lymphoma
CA-125	ovarian cancer
cardiac myosin	cardiac imaging
TNF-α	rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, psoriasis, Crohn's disease, ulcerative colitis
CD51	solid tumors (prostate cancer, melanoma)
CD25 (α chain of IL-2 receptor)	graft versus host disease
CD22	cancer
CD152	melanoma
CD30 (TNFRSF8)	Hodgkin's lymphoma
CD4	chronic asthma
CEA	colorectal cancer
IL-13	asthma
NCA-90 (granulocyte antigen)	diagnostic agent
TGF beta 2	reduction of scarring after glaucoma surgery
TRAIL-R2	cancer
hepatitis B surface antigen	hepatitis B
CD33	cancer
CD56	cancer
CD40	multiple myeloma, non-Hodgkin's lymphoma, Hodgkin's lymphoma
CD23 (IgE receptor)	chronic lymphocytic leukemia
TRAIL-R1	cancer
EGFR	colorectal, lung and stomach cancer
IL-5	asthma and white blood cell diseases
TGF beta 1	systemic scleroderma
CD74	multiple myeloma and other hematological malignancies
GD3 ganglioside	small cell lung carcinoma
respiratory syncytial virus	respiratory syncytial virus (prevention)
CD3	prevention of organ transplant rejections
C242 antigen	colorectal cancer
5T4	non-small cell lung carcinoma, renal cell carcinoma
integrin α4	multiple sclerosis, Crohn's disease
endotoxin	sepsis
EGFR	non-small cell lung carcinoma
EGFR	squamous cell carcinoma, head and neck cancer, nasopharyngeal cancer, glioma
CD20	rheumatoid arthritis, lupus erythematosus etc.
LFA-1 (CD11a)	prevention of organ transplant rejections, immunological diseases
CD20	chronic lymphocytic leukemia etc.
PDGF-R α	cancer
IgE Fc region	allergic asthma
EpCAM	cancer
CA-125	ovarian cancer
CD3	diabetes mellitus type 1
lipoteichoic acid	sepsis (Staphylococcus)
respiratory syncytial virus	respiratory syncytial virus (prevention)
EGFR	colorectal cancer

Table 1 -continued-

Target	Use
respiratory syncytial virus	respiratory syncytial virus (prevention)
EGFR	colorectal cancer
Pseudomonas aeruginosa	Pseudomonas aeruginosa infection
IL-4	asthma
MUC1	cancer
HER2/neu	cancer
C5	reduction of side effects of cardiac surgery
adenocarcinoma antigen	adenocarcinoma
CD4	Crohn's disease, multiple sclerosis
vimentin	brain cancer
CCR5	HIV infection
rabies virus glycoprotein	rabies (prophylaxis)
VEGFR2	solid tumors
VEGF-A	macular degeneration (wet form)
anthrax toxin, protective antigen	anthrax (prophylaxis and treatment)
cytomegalovirus glycoprotein B	cytomegalovirus infection
IL-5	inflammations of the airways, skin and gastrointestinal tract
HGF	solid tumors
CD20	lymphomas, leukemias, some autoimmune disorders
IGF-1 receptor	cancer
IFN-α	systemic lupus erythematosus
CD11, CD18	haemorrhagic shock etc.
CD154 (CD40L)	rheumatic diseases
TAG-72	cancer
cytomegalovirus	cytomegalovirus infection
FAP	cancer
IFN-α	SLE, dermatomyositis, polymyositis
CD2	psoriasis, graft-versus-host disease (prevention)
beta amyloid	Alzheimer's disease
sphingosine-1-phosphate	choroidal and retinal neovascularization
myostatin	muscular dystrophy
NCA-90 (granulocyte antigen)	osteomyelitis
alpha-fetoprotein	cancer
integrin αIIbβ3	percutaneous coronary intervention
IgE	allergic reaction
NGF	pain
CD19	cancer
clumping factor A	Staphylococcus aureus infection
tenascin C	cancer
CD3	diabetes mellitus type 1
CD28	chronic lymphocytic leukemia, rheumatoid arthritis
CTLA-4	cancer
TRAIL-R2	cancer
IL-13	Hodgkin's lymphoma
IL-6 receptor	rheumatoid arthritis
CD154 (CD40L)	rheumatoid arthritis, lupus nephritis etc.
CD20	follicular lymphoma
HER2/neu	breast cancer
CTLA-4	cancer
EpCAM	cancer
hepatitis B virus	chronic hepatitis B
Escherichia coli	diarrhoea caused by E. coli
IL-12, IL-23	multiple sclerosis, psoriasis, psoriatic arthritis
integrin $\alpha4\beta7$	Crohn's disease, ulcerative colitis
CD20	non-Hodgkin's lymphoma
AOC3 (VAP-1)	inflammation
CD3	Crohn's disease, ulcerative colitis
integrin $\alpha5\beta1$	solid tumors
tumor antigen CTAA16.88	colorectal tumors
EGFR	squamous cell carcinoma of the head and neck
CD4	rheumatoid arthritis, psoriasis, T-cell lymphoma
CD5	systemic lupus erythematosus, graft-versus-host disease

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 11 Å or 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 mutatation 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 37997, 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):

mefiptqtfynrryqprpwtprptiqvirprprpqrqagqlaqlisavnkltmravpqq
kprrnrknkkqkqkqqapqnntnqkkqppkkkpaqkkkkpgrrermcmkiendcifevk
hegkvtgyaclvgdkvmkpahvkgtidnadlaklafkrsskydlecaqipvhmksdask
fthekpegyynwhhgavqysggrftiptgagkpgdsgrpifdnkgrvvaivlgganega
rtalsvvtwnkdivtkitpegaeewslaipvmcllanttfpcsqppctpccyekepeet
lrmlednvmrpgyyqllqasltcsphrqrrstkdnfnvykatrpylahcpdcgeghsch
spvalerirneatdgtlkiqvslqigiktddshdwtklrymdnhmpadaeraglfvrts
apctitgtmghfilarcpkgetltvgftdsrkishscthpfhhdppvigrekfhsrpqh
gkelpcstyvqstaatteeievhmppdtpdrtlmsqqsgnvkitvngqtvrykcncggs
neglttdkvinnckvdqchaavtnhkkwqynsplvprnaelgdrkgkihipfplanvt
crvpkarnptvtygknqvimllypdhptllsyrnmgeepnyqeewvmhkkevvltvpte
glevtwgnnepykywpqlstngtahghpheiilyyyelyptmtvvvvsvatfillsmvg
maagmcmcarrrcitpyeltpgatvpfllsliccirtakaatyqeaaiylwneqqplfw
lqaliplaalivlcnclrllpcccktlaflavmsvgahtvsayehvtvipntvgvpykt
lvnrpgyspmvlemellsvtleptlsldyitceyktvipspyvkccgtaeckdknlpdy
sckvftgvypfmwggaycfcdaentqlseahveksescktefasayrahtasasaklrv
lyqgnnitvtayangdhavtvkdakfivgpmssawtpfdnkivvykgdvynmdyppfga
grpgqfgdiqsrtpeskdvyantqlvlqrpavgtvhvpysqapsgfkywlkergaslqh
tapfgcqiatnpvravncavgnmpisidipeaaftrvvdapsltdmscevpacthssdf
ggvaiikyaaskkgkcavhsmtnavtireaeievegnsqlqisfstalasaefrvqvcs
tqvhcaaechppkdhivnypashttlgvqdisatamswvqkitggvglvvavaaliliv
vlcvsfsrh

Another exemplary Chikungunya virus structural polypeptide sequence is provided at Genbank Accession No. ABX40011.1, which is described below (SEQ ID No.:2):

mefiptqtfynrryqprpwaprptiqvirprprpqrqagqlaqlisavnkltmravpqq
kprrnrknkkqrqkkqapqndpkqkkqppqkkpaqkkkkpgrrermcmkiendcifevk

hegkvmgyaclvgdkvmkpahvkgtidnadlaklafkrsskydlecaqipvhmksdask
fthekpegyynwhhgavqysggrftiptgagkpgdsgrpifdnkgrvvaivlgganega
rtalsvvtwnkdivtkitpegaeewslalpvlcllanttfpcsqppctpccyekepest
lrmlednvmrpgyyqllkasltcsphrqrrstkdnfnvykatrpylahcpdcgeghsch
spialerirneatdgtlkiqvslqigiktddshdwtklrymdshtpadaeragllvrts
apctitgtmghfilarcpkgetltvgftdsrkishtcthpfhheppvigrerfhsrpqh
gkelpcstyvqstaataeeievhmppdtpdrtlmtqqsgnvkitvngqtvrykcncggs
negltttdkvinnckidqchaavtnhknwqynsplvprnaelgdrkgkihipfplanvt
crvpkarnptvtygknqvtmllypdhptllsyrnmgqepnyheewvthkkevtltvpte
glevtwgnnepykywpqmstngtahghpheiilyyyelyptmtvvivsvasfvllsmvg
tavgmcvcarrrcitpyeltpgatvpfllsllccvrttkaatyyeaaaylwneqqplfw
lqaliplaalivlcnclkllpcccktlaflavmsigahtvsayehvtvipntvgvpykt
lvnrpgyspmvlemelqsvtleptlsldyitceyktvipspyvkccgtaeckdkslpdy
sckvftgvypfmwggaycfcdaentqlseahveksescktefasayrahtasasaklrv
lyqgnnitvaayangdhavtvkdakfvvgpmssawtpfdnkivvykgdvynmdyppfga
grpgqfgdiqsrtpeskdvyantqlvlqrpaagtvhvpysqapsgfkywlkergaslqh
tapfgcqiatnpvravncavgnipisidipdaaftrvvdapsvtdmscevpacthssdf
ggvaiikytaskkgkcavhsmtnavtireadvevegnsqlqisfstalasaefrvqvcs
tqvhcaaachppkdhivnypashttlgvqdisttamswvqkitggvglivavaaliliv
vlcvsfsrh.

An exemplary Venezuelan equine encephalitis virus structural polypeptide is provided at Genbank Accession No. L01443.1 (http://www.ncbi.nlm.nih.gov/nuccore/L01443.1), which is described below (SEQ ID No.:3):

mfpfqpmypmqpmpyrnpfaaprrpwfprtdpflamqvqeltrsmanltfkqrrdappe
gpsaakpkkeasqkqkgggqgkkknqgkkkaktgppnpkaqngnkkktnkkpgkrqrm
vmklesdktfpimlegkingyacvvggklfrpmhvegkidndvlaalktkkaskydley
advpqnmradtfkythekpqgyyswhhgavqyengrftvpkgvgakgdsgrpildnqgr
vvaivlggvnegsrtalsvvmwnekgvtvkytpenceqwslvttmcllanvtfpcaqpp
icydrkpaetlamlsvnvdnpgydelleaavkcpgrkrrsteelfneykltrpymarci
rcavgschspiaieavksdghdgyvrlqtssqygldssgnlkgrtmrydmhgtikeipl
hqvslytsrpchivdghgyfllarcpagdsitmefkkdsvrhscsvpyevkfnpvgrel
ythppehgveqacqvyahdaqnrgayvemhlpgsevdsslvslsgssvtvtppdgtsal
vececggtkisetinktkqfsqctkkeqcrayrlqndkwvynsdklpkaagatlkgklh
vpflladgkctvplapepmitfgfrsvslklhpknptylitrqladephythelisepa
vrnftvtekgwefvwgnhppkrfwaqetapgnphglphevithyyhrypmstilglsic
aaiatvsvaastwlfcrsrvacltpyrltpnaripfclavlccartaraettwesldhl
wnnnqqmfwiqlliplaalivvtrllrcvccvvpflvmagaagagayehattmpsqagi
syntivnragyaplpisitptkikliptvnleyvtchyktgmdspaikccgsqectpty
rpdeqckvftgvypfmwggaycfcdtentqvskayvmksddcladhaeaykahtasvqa
flnitvgehsivttvyvngetpvnfngvkitagplstawtpfdrkivqyageiynydfp
eygagqpgafgdiqsrtvsssdlyantnlvlqrpkagaihvpytqapsgfeqwkkdkap
slkftapfgceiytnpiraencavgsiplafdipdalftrvsetptlsaaectlnecvy
ssdfggiatvkysasksgkcavhvpsgtatlkeaavelteqgsatihfstanihpefrl
qictsyvtckgdchppkdhivthpqyhaqtftaavsktawtwltsllggsaviiiiglv
lativamyvltnqkhn.

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 a cysteine.

In one embodiment, a conjugation of more than two substances (e.g. antigen and Chikungunya 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-α, 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-α;

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-α; 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-α, 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 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-α, 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-α, 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-α ,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-SGG-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

i) a fusion protein of a polypeptide derived from Chikungunya virus (CHIKV) and a polypeptide derived from TNF-α, 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

Capsid-E3-E2-6K-E1 of Venezuelan equine encephalitis virus TC-83.

Regarding Chikungunya virus, an exemplary nucleotide sequence that encodes E3-E2-6K-E1 is described below (SEQ ID No.:9):

Atgagcctcgccctcccggtcttgtgcctgtggcaaacactacattcccctgctctcagccgccttgcacaccctgctgctacgaaaaggaaccgg
aaagcaccttgcgcatgcttgaggacaacgtgatgagacccggatactaccagctactaaaagcatcgctgacttgctctccccaccgccaaagac
gcagtactaaggacaatttaatgtctataaagccacaagaccatatctagctcattgtcctgactgcggagaagggcattcgtgccacagccctatc
gcattggagcgcatcagaaatgaagcaacggacggaacgctgaaaatccaggtctcttgcagatcgggataaagacagatgacagccacgattg
gaccaagctgcgctatatggatagccatacgccagcggacgcggagcgagccggattgcttgtaaggacttcagcaccgtgcacgatcaccgggg
accatgggacacttttatctcgcccgatgcccgaaaggagagacgctgacagtgggatttacggacagcagaaagatcagccacacatgcacaca
cccgttccatcatgaaccacctgtgataggtagggagaggttccactctcgaccacaacatggtaaagagttaccttgcagcacgtacgtgcagag
caccgctgccactgctgaggagatagaggtgcatatgccccagatactcctgaccgcacgctgatgacgcagcagtctggcaacgtgaagatca
cagttaatgggcagacggtgcggtacaagtgcaactgcggtggctcaaacgagggactgacaaccacagacaaagtgatcaataactgcaaaatt
gatcagtgccatgctgcagtcactaatcacaagaattggcaatacaactcccctttagtcccgcgcaacgctgaactcggggaccgtaaaggaaag

atccacatcccattcccattggcaaacgtgacttgcagagtgccaaaagcaagaaacccctacagtaacttacggaaaaaaccaagtcaccatgctg
ctgtatcctgaccatccgacactcttgtcttaccgtaacatgggacaggaaccaaattaccacgaggagtgggtgacacacaagaaggaggttacct
tgaccgtgcctactgagggtctggaggtcacttggggcaacaacgaaccatacaagtactggccgcagatgtctacgaacggtactgctcatggtc
acccacatgagataatcttgtactattatgagctgtaccccactatgactgtagtcattgtgtcggtggcctcgttcgtgcttctgtcgatggtgggcaca
gcagtgggaatgtgtgtgtgcgcacggcgcagatgcattacaccatatgaattaacaccaggagccactgttccctcctgctcagcctgctatgctg
cgtcagaacgaccaaggcggccacatattacgaggctgcggcatatctatggaacgaacagcagccccgttctggttgcaggctcttatcccgct
ggccgccttgatcgtcctgtgcaactgtctgaaactcttgccatgctgctgtaagaccctggcttttttagccgtaatgagcatcggtgcccacactgtg
agcgcgtacgaacacgtaacagtgatcccgaacacggtgggagtaccgtataagactcttgtcaacagaccgggttacagccccatggtgttgga
gatggagctacaatcagtcaccttggaaccaacactgtcacttgactacatcacgtgcgagtacaaaactgtcatcccctccccgtacgtgaagtgct
gtggtacagcagagtgcaaggacaagagcctaccagactacagctgcaaggtctttactggagtctacccatttatgtggggcggcgcctacttgctt
ttgcgacgccgaaaatacgcaattgagcgaggcacatgtagagaaatctgaatcttgcaaaacagagtttgcatcggcctacagagcccacaccg
catcggcgtcggcgaagctccgcgtccttaccaaggaaacaacattaccgtagctgcctacgctaacggtgaccatgccgtcacagtaaaggac
gccaagtttgtcgtgggcccaatgtcctccgcctggacaccttttgacaacaaaatcgtggtgtacaaaggcgacgtctacaacatggactacccac
ctttggcgcaggaagaccaggacaatttggtgacattcaaagtcgtacaccggaaagtaaagacgttatgccaacactcagttggtactacagag
gccagcagcaggcacggtacatgtaccatactctcaggcaccatctggcttcaagtattggctgaaggaacgaggagcatcgctacagcacacgg
caccgttcggttgccagattgcgacaaacccggtaagagctgtaaattgcgctgtggggaacataccaatttccatcgacataccggatgcggcctt
tactagggttgtcgatgcacccctctgtaacggacatgtcatgcgaagtaccagcctgcactcactcctccgacttgggggcgtcgccatcatcaaat
acacagctagcaagaaaggtaaatgtgcagtacattcgatgaccaacgccgttaccattcgagaagccgacgtagaagtagaggggaactccca
gctgcaaatatccttctcaacagccctggcaagcgccgagtttcgcgtgcaagtgtgctccacacaagtacactgcgcagccgcatgccacccctcc
aaaggaccacatagtcaattacccagcatcacacaccacccttggggtccaggatatatccacaacggcaatgtcttgggtgcagaagattacggg
aggagtaggattaattgttgctgttgctgccttaatttaattgtggtgctatgcgtgtcgtttagcaggcac

Regarding Chikungunya virus, another exemplary nucleotide sequence that encodes E3-E2-6K-E1 is described below (SEQ ID No.:10):

Atgagtcttgccatcccagttatgtgcctgttggcaaacaccacgttcccctgctcccagccccctttgcacgcccctgctgctacgaaaaggaaccgg
aggaaaccctacgcatgcttgaggacaacgtcatgagacctgggtactatcagctgctacaagcatccttaacatgttctccccaccgccagcgac
gcagcaccaaggacaacttcaatgtctataaagccacaagaccatacttagctcactgtcccgacigtggagaagggcactcgtgccatagtcccg
tagcactagaacgcatcagaaatgaagcgacagacgggacgctgaaaatccaggtctccttgcaaatcggaataaagacggatgacagccacga
ttggaccaagctgcgttatatggacaaccacatgccagcagacgcagagagggcggggctatttgtaagaacatcagcaccgtgtacgattactgg

aacaatgggacacttcatcctggcccgatgtccaaaaggggaaactctgacggtgggattcactgacagtaggaagattagtcactcatgtacgca
cccatttcaccacgaccctcctgtgataggtcgggaaaaattccattcccgaccgcagcacggtaaagagctaccttgcagcacgtacgtgcagag
caccgccgcaactaccgaggagatagaggtacacatgccccccagacaccccctgatcgcacattaatgtcacaacagtccggcaacgtaaagatc
acagtcaatggccagacggtgcggtacaagtgtaattgcggtggctcaaatgaaggactaacaactacagacaaagtgattaataactgcaaggtt
gatcaatgtcatgccgcggtcaccaatcacaaaaagtggcagtataactcccctctggtcccgcgtaatgctgaacttggggaccgaaaaggaaaa
attcacatcccgtttccgctggcaaatgtaacatgcagggtgcctaaagcaaggaaccccaccgtgacgtacgggaaaaaccaagtcatcatgcta
ctgtatcctgaccacccaacactcctgtcctaccggaatatgggagaagaaccaaactatcaagaagagtgggtgatgcataagaaggaagtcgtg
ctaacegtgccgactgaagggctcgaggtcacgtggggcaacaacgagccgtataagtattggccgcagttatctacaaacggtacagcccatgg
ccacccgcatgagataattctgtattattatgagctgtaccccactatgactgtagtagttgtgtcagtggccacgttcatactcctgtcgatggtgggta
tggcagcggggatgtgcatgtgtgcacgacgcagatgcatcacaccgtatgaactgacaccaggagctaccgtcccttcctgcttagcctaatatg
ctgcatcagaacagctaaagcggccacataccaagaggctgcgatatacctgtggaacgagcagcaaccttgtttttggctacaagccctattccg
ctggcagccctgattgttctatgcaactgtctgagactcttaccatgctgctgtaaaacgttggctttttiagccgtaatgagcgtcggtgcccacactgt
gagcgcgtacgaacacgtaacagtgatcccgaacacggtgggagtaccgtataagactctagtcaatagacctggctacagccccatggtattgg
agatggaactactgtcagtcactttggagccaacactatcgcttgattacatcacgtgcgagtacaaaaccgtcatcccgtctccgtacgtgaagtgct
gcggtacagcagagtgcaaggacaaaaacctacctgactacagctgtaaggtcttcaccggcgtctacccatttatgtggggcggcgcctactgctt
ctgcgacgctgaaaacacgcagttgagcgaagcacacgtggagaagtccgaatcatgcaaaacagaatttgcatcagcatacagggctcataccg
catctgcatcagctaagctccgcgtcctttaccaaggaaataacatcactgtaactgcctatgcaaacggcgaccatgccgtcacagttaaggacgc
caaattcattgtggggccaatgtcttcagcctggacacctttcgacaacaaaattgtggtgtacaaaggtgacgtctataacatggactacccgcccti
tggcgcaggaagaccaggacaatttggcgatatccaaagtcgcacacctgagagtaaagacgtctatgctaatacacaactggtactgcagagac
cggctgtgggtacggtacacgtgccatactctcaggcaccatctggctttaagtattggctaaaagaacgcggggcgtcgctgcagcacacagcac
catttggctgccaaatagcaacaaacccggtaagagcggtgaactgcgccgtagggaacatgcccatctccatcgacataccggaagcggccttc
actagggtcgtcgacgcgcccctctttaacggacatgtcgtgcgaggtaccagcctgcacccattcctcagacttgggggcgtcgccattattaaata
tgcagccagcaagaaaggcaagtgtgcggtgcattcgatgactaacgccgtcactattcgggaagctgagatagaagttgaagggaatictcagct
gcaaatctctttctcgacggccttagccagcgccgaattccgcgtacaagtctgttctacacaagtacactgtgcagccgagtgccaccccccgaag
gaccacatagtcaactacccggcgtcacataccacccicggggtccaggacatctccgctacggcgatgtcatgggtgcagaagatcacgggag
gtgtgggactggttgttgctgttgccgcactgattctaatcgtggtgctatgcgtgtcgttcagcaggcac

Regarding Chikungunya virus, an exemplary nucleotide sequence that encodes a Capsid-E3-E2-6K-E1 is described below (SEQ ID No.:11):

atggagttcatcccgacgcaaactttctataacagaaggtaccaaccccgaccctgggc
cccacgccctacaattcaagtaattagacctagaccacgtccacagaggcaggctgggc
aactcgcccagctgatctccgcagtcaacaaattgaccatgcgcgcggtacctcaacag
aagcctcgcagaaatcggaaaaacaagaagcaaaggcagaagaagcaggcgccgcaaaa
cgacccaaagcaaaagaagcaaccaccacaaaagaagccggctcaaaagaagaagaaac
caggccgtagggagagaatgtgcatgaaaattgaaaatgattgcatcttcgaagtcaag
catgaaggcaaagtgatgggctacgcatgcctggtgggggataaagtaatgaaaccagc
acatgtgaagggaactatcgacaatgccgatctggctaaactggcctttaagcggtcgt
ctaaatacgatcttgaatgtgcacagataccggtgcacatgaagtctgatgcctcgaag
tttacccacgagaaacccgaggggtactataactggcatcacggagcagtgcagtattc
aggaggccggttcactatcccgacgggtgcaggcaagccgggagacagcggcagaccga
tcttcgacaacaaaggacgggtggtggccatcgtcctaggaggggccaacgaaggtgcc
cgcacggccctctccgtggtgacgtggaacaaagacatcgtcacaaaaattacccctga
gggagccgaagagtggagcctcgccctcccggtcttgtgcctgttggcaaacactacat
tcccctgctctcagccgccttgcacaccctgctgctacgaaaaggaaccggaaagcacc

ttgcgcatgcttgaggacaacgtgatgagacccggatactaccagctactaaaagcatc
gctgacttgctctccccaccgccaaagacgcagtactaaggacaatttttaatgtctata
aagccacaagaccatatctagctcattgtcctgactgcggagaagggcattcgtgccac
agccctatcgcattggagcgcatcagaaatgaagcaacggacggaacgctgaaaatcca
ggtctctttgcagatcgggataaagacagatgacagccacgattggaccaagctgcgct
atatggatagccatacgccagcggacgcggagcgagccggattgcttgtaaggacttca
gcaccgtgcacgatcaccgggaccatgggacactttattctcgcccgatgcccgaaagg
agagacgctgacagtgggatttacggacagcagaaagatcagccacacatgcacacacc
cgttccatcatgaaccacctgtgataggtagggagaggttccactctcgaccacaacat
ggtaaagagttaccttgcagcacgtacgtgcagagcaccgctgccactgctgaggagat
agaggtgcatatgcccccagatactcctgaccgcacgctgatgacgcagcagtctggca
acgtgaagatcacagttaatgggcagacggtgcggtacaagtgcaactgcggtggctca
aacgagggactgacaaccacagacaaagtgatcaataactgcaaaattgatcagtgcca
tgctgcagtcactaatcacaagaattggcaatacaactccccttttagtcccgcgcaacg
ctgaactcggggaccgtaaaggaaagatccacatcccattcccattggcaaacgtgact
tgcagagtgccaaaagcaagaaaccctacagtaacttacggaaaaaaccaagtcaccat
gctgctgtatcctgaccatccgacactcttgtcttaccgtaacatgggacaggaaccaa
attaccacgaggagtgggtgacacacaagaaggaggttaccttgaccgtgcctactgag
ggtctggaggtcacttggggcaacaacgaaccatacaagtactggccgcagatgtctac
gaacggtactgctcatggtcacccacatgagataatcttgtactattatgagctgtacc
ccactatgactgtagtcattgtgtcggtggcctcgttcgtgcttctgtcgatggtgggc
acagcagtgggaatgtgtgtgtgcgcacggcgcagatgcattacaccatatgaattaac
accaggagccactgttcccttcctgctcagcctgctatgctgcgtcagaacgaccaagg
cggccacatattacgaggctgcggcatatctatggaacgaacagcagcccctgttctgg
ttgcaggctcttatcccgctggccgccttgatcgtcctgtgcaactgtctgaaactctt
gccatgctgctgtaagaccctggctttttagccgtaatgagcatcggtgcccacactg
tgagcgcgtacgaacacgtaacagtgatcccgaacacggtgggagtaccgtataagact
cttgtcaacagaccgggttacagccccatggtgttggagatggagctacaatcagtcac
cttggaaccaacactgtcacttgactacatcacgtgcgagtacaaaactgtcatcccct
ccccgtacgtgaagtgctgtggtacagcagagtgcaaggacaagagcctaccagactac
agctgcaaggtctttactggagtctacccatttatgtggggcggcgcctactgcttttg
cgacgccgaaaatacgcaattgagcgaggcacatgtagagaaatctgaatcttgcaaaa
cagagtttgcatcggcctacagagcccacaccgcatcggcgtcggcgaagctccgcgtc
ctttaccaaggaaacaacattaccgtagctgcctacgctaacggtgaccatgccgtcac
agtaaaggacgccaagtttgtcgtgggcccaatgtcctccgcctggacacctttgaca
acaaaatcgtggtgtacaaaggcgacgtctacaacatggactacccacctttggcgca
ggaagaccaggacaatttggtgacattcaaagtcgtacaccggaaagtaaagacgttta
tgccaacactcagttggtactacagaggccagcagcaggcacggtacatgtaccatact
ctcaggcaccatctggcttcaagtattggctgaaggaacgaggagcatcgctacagcac
acggcaccgttcggttgccagattgcgacaaacccggtaagagctgtaaattgcgctgt
ggggaacataccaatttccatcgacataccggatgcggcctttactagggttgtcgatg
caccctctgtaacggacatgtcatgcgaagtaccagcctgcactcactcctccgacttt
gggggcgtcgccatcatcaaatacacagctagcaagaaaggtaaatgtgcagtacattc
gatgaccaacgccgttaccattcgagaagccgacgtagaagtagaggggaactcccagc
tgcaaatatccttctcaacagccctggcaagcgccgagtttcgcgtgcaagtgtgctcc
acacaagtacactgcgcagccgcatgccaccctccaaaggaccacatagtcaattaccc
agcatcacacaccacccttggggtccaggatatatccacaacggcaatgtcttgggtgc
agaagattacgggaggagtaggattaattgttgctgttgctgccttaattttaattgtg
gtgctatgcgtgtcgtttagcaggcactaa.

Regarding Chikungunya virus, another exemplary nucleotide sequence that encodes a Capsid-E3-E2-6K-E1 is described below (SEQ ID No.:12):

atggagttcatcccaacccaaacttttacaataggaggtaccagcctcgaccctggac
tccgcgccctactatccaagtcatcaggcccagaccgcgccctcagaggcaagctgggc
aacttgcccagctgatctcagcagttaataaactgacaatgcgcgcggtaccacaacag
aagccacgcaggaatcggaagaataagaagcaaaagcaaaaacaacaggcgccacaaaa
caacacaaatcaaaagaagcagccacctaaaaagaaaccggctcaaaagaaaaagaagc
cgggccgcagagagaggatgtgcatgaaaatcgaaaatgattgtattttcgaagtcaag
cacgaaggtaaggtaacaggttacgcgtgcctggtgggggacaaagtaatgaaaccagc
acacgtaaaggggaccatcgataacgcggacctggccaaactggcctttaagcggtcat
ctaagtatgaccttgaatgcgcgcagatacccgtgcacatgaagtccgacgcttcgaag
ttcacccatgagaaaccggaggggtactacaactggcaccacggagcagtacagtactc
aggaggccggttcaccatccctacaggtgctggcaaaccaggggacagcggcagaccga
tcttcgacaacaagggacgcgtggtggccatagtcttaggaggagctaatgaaggagcc
cgtacagccctctcggtggtgacctggaataaagacattgtcactaaaatcacccccga
gggggccgaagagtggagtcttgccatcccagttatgtgcctgttggcaaacaccacgt
tcccctgctcccagccccttgcacgccctgctgctacgaaaaggaaccggaggaaacc
ctacgcatgcttgaggacaacgtcatgagacctgggtactatcagctgctacaagcatc
cttaacatgttctccccaccgccagcgacgcagcaccaaggacaacttcaatgtctata
aagccacaagaccatacttagctcactgtcccgactgtggagaagggcactcgtgccat
agtcccgtagcactagaacgcatcagaaatgaagcgacagacgggacgctgaaaatcca
ggtctccttgcaaatcggaataaagacggatgacagccacgattggaccaagctgcgtt
atatggacaaccacatgccagcagacgcagagagggcggggctatttgtaagaacatca
gcaccgtgtacgattactggaacaatgggacacttcatcctggcccgatgtccaaaagg
ggaaactctgacggtgggattcactgacagtaggaagattagtcactcatgtacgcacc
catttcaccacgaccctcctgtgataggtcgggaaaaattccattcccgaccgcagcac
ggtaaagagctaccttgcagcacgtacgtgcagagcaccgccgcaactaccgaggagat
agaggtacacatgcccccagacacccctgatcgcacattaatgtcacaacagtccggca
acgtaaagatcacagtcaatggccagacggtgcggtacaagtgtaattgcggtggctca
aatgaaggactaacaactacagacaaagtgattaataactgcaaggttgatcaatgtca
tgccgcggtcaccaatcacaaaaagtggcagtataactcccctctggtcccgcgtaatg
ctgaacttggggaccgaaaaggaaaaattcacatcccgtttccgctggcaaatgtaaca
tgcagggtgcctaaagcaaggaaccccaccgtgacgtacgggaaaaaccaagtcatcat
gctactgtatcctgaccacccaacactcctgtcctaccggaatatgggagaagaaccaa
actatcaagaagagtgggtgatgcataagaaggaagtcgtgctaaccgtgccgactgaa
gggctcgaggtcacgtggggcaacaacgagccgtataagtattggccgcagttatctac
aaacggtacagcccatggccacccgcatgagataattctgtattattatgagctgtacc
ccactatgactgtagtagttgtgtcagtggccacgttcatactcctgtcgatggtgggt
atggcagcggggatgtgcatgtgtgcacgacgcagatgcatcacaccgtatgaactgac
accaggagctaccgtccctttcctgcttagcctaatatgctgcatcagaacagctaaag
cggccacataccaagaggctgcgatatacctgtggaacgagcagcaacctttgttttgg
ctacaagcccttattccgctggcagccctgattgttctatgcaactgtctgagactctt
accatgctgctgtaaaacgttggctttttttagccgtaatgagcgtcggtgcccacactg
tgagcgcgtacgaacacgtaacagtgatcccgaacacggtgggagtaccgtataagact
ctagtcaatagacctggctacagccccatggtattggagatggaactactgtcagtcac
tttggagccaacactatcgcttgattacatcacgtgcgagtacaaaaccgtcatcccgt
ctccgtacgtgaagtgctgcggtacagcagagtgcaaggacaaaaacctacctgactac
agctgtaaggtcttcaccggcgtctacccatttatgtggggcggcgcctactgcttctg
cgacgctgaaaacacgcagttgagcgaagcacacgtggagaagtccgaatcatgcaaaa
cagaatttgcatcagcatacagggctcataccgcatctgcatcagctaagctccgcgtc
ctttaccaaggaaataacatcactgtaactgcctatgcaaacggcgaccatgccgtcac
agttaaggacgccaaattcattgtggggccaatgtcttcagcctggacacctttcgaca
acaaaattgtggtgtacaaaggtgacgtctataacatggactacccgcccttggcgca
ggaagaccaggacaatttggcgatatccaaagtcgcacacctgagagtaaagacgtcta

tgctaatacacaactggtactgcagagaccggctgtgggtacggtacacgtgccatact
ctcaggcaccatctggctttaagtattggctaaaagaacgcggggcgtcgctgcagcac
acagcaccatttggctgccaaatagcaacaaacccggtaagagcggtgaactgcgccgt
agggaacatgcccatctccatcgacataccggaagcggccttcactagggtcgtcgacg
cgccctctttaacggacatgtcgtgcgaggtaccagcctgcacccattcctcagacttt
gggggcgtcgccattattaaatatgcagccagcaagaaaggcaagtgtgcggtgcattc
gatgactaacgccgtcactattcgggaagctgagatagaagttgaagggaattctcagc
tgcaaatctctttctcgacggccttagccagcgccgaattccgcgtacaagtctgttct
acacaagtacactgtgcagccgagtgccacccccgaaggaccacatagtcaactaccc
ggcgtcacataccaccctcggggtccaggacatctccgctacggcgatgtcatgggtgc
agaagatcacgggaggtgtgggactggttgttgctgttgccgcactgattctaatcgtg
gtgctatgcgtgtcgttcagcaggcactaa.

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

VLP_CHI 532 vector (SEQ ID No.: 24) containing CHIKV VLP polynucleotide (SEQ ID No. 30; corresponding amino acid sequence represented by SEQ ID No.:31).

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-α, 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 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) 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-

TNFa4. 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-TNFa 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-TNFa 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 TNFa 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).

<u>(4) Preparation of Venezuelan equine encephalitis virus like particle comprising a virus structural polypeptide and a fragment of human CD20</u>

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: CKVELMYPPPYYLGIG(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 CLTA4.

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 39.6Å 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 iyncepanpseknspstqycysiq (SEQ ID No.:21); CD20 Mouse ydcepsnsseknspstqycnsi (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: SGGiyncepanpseknspstqycysiqGS (SEQ ID No.:42)

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

CD20 Mouse version3: SGGydcepsnsseknspstqycnsiGS (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 100μg 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, 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.

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 E1.

9. The particle according to Claim 8, wherein said at least one antigen is inserted in to 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-α, 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-α;

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-α; 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.