

DESCRIPTION

Malaria vaccine

TECHNICAL FIELD

5 [0001] The present invention relates to a particle comprising a polypeptide and at least one malaria antigen, and a composition or vaccine comprising thereof, its use in medicine, particularly in the prevention or treatment of malaria.

10

BACKGROUND

[0002] Malaria is one of the world's most prevalent serious infectious diseases, with approximately 250 million cases and 1 million deaths per year (WHO, 2009). Mortality is primarily in children under the age of five and in
15 pregnant women. Every 45 seconds, an African child dies of malaria. The disease is transmitted from person to person by infected mosquitoes, so past eradication efforts involved massive insecticide campaigns. These were successful in the Southeast U.S. for example, but failed in
20 most poorly developed tropical countries. Current efforts involve distribution of bednets, particularly bednets impregnated with insecticide, to prevent mosquito bites at night. However, resistance to insecticides and to anti-malarial drugs for both prevention and treatment is rapidly

rising. Thus, the need for a malaria vaccine is imperative for protection of millions of people from disease (<http://www.globalvaccines.org/content/malaria+vaccine+program/19614>).

5 [0003] Malaria caused by *Plasmodium falciparum* remains a major public health threat, especially among children and pregnant women in Africa. An effective malaria vaccine would be a valuable tool to reduce the disease burden and could contribute to elimination of malaria in some regions
10 of the world. Current malaria vaccine candidates are directed against human and mosquito stages of the parasite life cycle, but thus far, relatively few proteins have been studied for potential vaccine development.

[0004] The most advanced vaccine candidate, RTS,S,
15 conferred partial protection against malaria in phase II clinical trials and is currently being evaluated in a phase III trial in Africa. (The Journal of Clinical Investigation 120(12) 4168-4178, 2010).

[0005] The CSP is the predominant surface antigen on
20 sporozoites. CSP is composed of an N-terminal region that binds heparin sulfate proteoglycans (RI), a central region containing a four-amino-acid (NPNA) repeat, and a GPI-anchored C-terminal region containing a thrombospondin-like

domain (RII). The region of the CSP included in the RTS,S vaccine includes the last 16 NPNA repeats and the entire flanking C-terminus. HBsAg particles serve as the matrix carrier for RTS,S, 25% of which is fused to the CSP segment
5 (The Journal of Clinical Investigation 120(12) 4168-4178, 2010).

[0006] In a series of phase II clinical trials for RTS,S, 30%-50% of malaria-naive adults immunized with RTS,S were protected against challenge by mosquitoes infected with the
10 homologous *P. falciparum* clone. In phase II field trials in the Gambia and Kenya, RTS,S conferred short-lived protection against malaria infection in approximately 35% of adults, although results from the Kenya trial did not reach statistical significance. Approximately 30%-50% of
15 children and infants immunized with RTS,S in phase II trials conducted in Mozambique, Tanzania, and Kenya were protected from clinical malaria, however, protection was generally short-lived (The Journal of Clinical Investigation 120(12) 4168-4178, 2010). Results from a
20 pivotal, large-scale Phase III trial, published November 9, 2012, online in the *New England Journal of Medicine (NEJM)*, show that the RTS,S malaria vaccine candidate can help protect African infants against malaria. When compared to immunization with a control vaccine, infants (aged 6-12

weeks at first vaccination) vaccinated with RTS,S had one-third fewer episodes of both clinical and severe malaria and had similar reactions to the injection.

[0007] There are currently no licensed vaccines against malaria. Highly effective malaria vaccine is strongly desired.

[0008] Virus-like particles (VLPs) are multiprotein structures that mimic the organization and conformation of authentic native viruses but lack the viral genome, potentially yielding safer and cheaper vaccine candidates. A handful of prophylactic VLP-based vaccines is currently commercialized worldwide: GlaxoSmithKline's Engerix® (hepatitis B virus) and Cervarix® (human papillomavirus), and Merck and Co., Inc.'s Recombivax HB® (hepatitis B virus) and Gardasil® (human papillomavirus) are some examples. Other VLP-based vaccine candidates are in clinical trials or undergoing preclinical evaluation, such as, influenza virus, parvovirus, Norwalk and various chimeric VLPs. Many others are still restricted to small-scale fundamental research, despite their success in preclinical tests. The implications of large-scale VLP production are discussed in the context of process control, monitorization and optimization. The main up- and downstream technical challenges are identified and discussed

accordingly. Successful VLP-based vaccine blockbusters are briefly presented concomitantly with the latest results from clinical trials and the recent developments in chimeric VLP-based technology for either therapeutic or prophylactic vaccination (Expert Rev. Vaccines 9(10), 1149-1176, 2010).

[0009] 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

[0010] In the first aspect, the present invention provides
5 a particle which is capable of being self-assembled,
comprising a polypeptide and at least one malaria antigen,
wherein said polypeptide comprises at least one first
attachment site and said at least one malaria antigen
comprises at least one second attachment site, and wherein
10 said polypeptide and said malaria antigen are linked
through said at least one first and said at least one
second attachment site.

[0011] In the second aspect, the present invention
provides a nucleic acid molecule which is designed for
15 expression of a particle provided in the first aspect of
the present invention.

[0012] In the third aspect, the present invention provides
a composition or vaccine comprising the particle provided
in the first aspect of the present invention and/or the
20 nucleic acid molecule provided in the second aspect of the
present invention.

[0013] 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.

[0014] In the fifth aspect, the present invention provides a method of immunomodulation, a method of treating malaria, a method of inducing and/or enhancing immune response against a malaria antigen in a mammal, comprising administering the composition provided in the third aspect of the present invention to a mammal.

[0015] In sixth aspect, the present invention provides a method of passive immunization against a malaria-causing pathogen, comprising administering the antibody provided in the fourth aspect of the present invention to a mammal.

[0016] 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.

[0017] 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

vector which is designed for expression of said particle;
culturing a cell which is transfected with said vector to
express said particle; and recovering said particle.

BRIEF DESCRIPTION OF THE INVENTION

5 [0018]

Fig 1 shows pVLP74_15 (VLP_CHI 532 NPNAx6) vector.

Fig 2 shows pVLP78_15 (VLP_CHI 532 NPNAx25) vector.

Fig 3 shows pVLP74_25 (VLP_VEEV 519 NPNAx6) vector.

Fig 4 shows that the serum from individual monkeys
10 immunized with Malaria VLPs after 2 weeks induced high
titer of antibodies against CSP.

Fig 5 shows mean value and SD of the data shown in Fig 4.

Fig 6 shows effects of combined immunization of CHIKV VLP
and VEEV VLP on induction of antibodies against CSP. In
15 the figure, Adj indicates adjuvant.

Fig 7 shows effects of administered VLP fused with no
malaria antigen on induction of antibodies against CSP. In
the figure, 4w, 6w, 10w and 14w indicate 4 weeks after
immunization, 6 weeks after immunization, 10 weeks after
20 immunization and 14 weeks after immunization, respectively.

Fig 8 shows effects of administered VLP fused with malaria
antigen on induction of antibodies against CSP. In the
figure, 4w, 6w, 10w and 14w indicate 4 weeks after
immunization, 6 weeks after immunization, 10 weeks after

immunization and 14 weeks after immunization, respectively.
Fig 9 shows effects of administered VLP fused with malaria
antigen together with adjuvant on induction of antibodies
against CSP. In the figure, 4w, 6w, 10w and 14w indicate 4
5 weeks after immunization, 6 weeks after immunization, 10
weeks after immunization and 14 weeks after immunization,
respectively.

Fig 10 shows schedule of the experiment.

Fig 11 shows detection of 18S malaria DNA by means of PCR.

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DETAILED DESCRIPTION OF THE INVENTION

[0019]

(1) A particle comprising a polypeptide and at least one
15 malaria 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 malaria antigen,
wherein said polypeptide comprises at least one first
20 attachment site and said at least one antigen comprises at
least one second attachment site, and wherein said
polypeptide and said malaria antigen are linked through
said at least one first and said at least one second
attachment site.

25 [0020] 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
5 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.

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

[0021] A polypeptide used for the present invention may be spontaneously assembled. The polypeptide may be a virus
15 structural polypeptide. Thus, the particle provided by the present invention may be a virus like particle.

[0022] A virus structural polypeptide may be a naturally occurring viral polypeptide or modified polypeptide thereof. In one embodiment, the modified polypeptide has at least
20 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,
25 and/or added to a naturally occurring viral structural

polypeptide including capsid and envelope protein.

[0023] 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, virus structural polypeptide used for the present invention consists of or comprises capsid and E2 and E1.

An antigen may be inserted into E2. In one embodiment, a particle provided by the first aspect of the present invention can be formed by assembling 240 capsids, 240 E1 proteins and 240 E2 proteins where a malaria antigen is inserted into each of E2 proteins.

[0024] 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.

[0025] Malaria is a disease which human or other animal (e.g. mouse) suffers from. Example of malaria include, but are not limited to, a disease caused by Plasmodium (P.) species including P. falciparum, P. malariae, P. ovale, P. vivax, P. knowlesi, P. berghei, P. chabaudi and P. yoelii

[0026] As used herein, the term "malaria antigen" refers to any antigen or fragment thereof. The term antigen or fragment thereof, means any peptide-based sequence that can be recognized by the immune system and/or that stimulates a cell-mediated immune response and/or stimulates the generation of antibodies.

[0027] According to Scand. J. Immunol. 56, 327-343, 2002, considering the whole parasite life cycle, there are essentially six targets for a malaria vaccine: (1) sporozoites; (2) liver stages; (3) merozoites; (4) infected RBC; (5) parasite toxins; (6) sexual stages.

[0028] Table summarizes the main candidate antigens of each stage identified.

Table 1. Main vaccine candidates from the different phases of *Plasmodium* life cycle

Targets	Candidate antigens
Sporozoite	Circumsporozoite protein (CSP)
	Thrombospondin-related adhesive protein (TRAP)
	Sporozoite and liver-stage antigen (SALSA)
	Sporozoite threonine- and asparagine-rich protein (STARP)
Liver stage	CSP
	Liver-stage antigen (LSA)-1 and -3
	SALSA
	STARP
Merozoite	Merozoite surface protein (MSP)-1, -2, -3, -4 and -5
	Erythrocyte-binding antigen (EBA)-175
	Apical membrane antigen (AMA)-1
	Rhoptry-associated protein (RAP)-1 and -2
	Acidic-basic repeat antigen (ABRA)
	Duffy-binding protein (DBP) (<i>Plasmodium vivax</i>)
Blood stage	Ring erythrocyte surface antigen (RESA)
	Serine-rich protein (SERP)
	Erythrocyte membrane protein (EMP)-1, -2 and -3
	Glutamate-rich protein (GLURP)
Toxins	Glycosilphosphatidylinositol (GPI)

Table 1. Main vaccine candidates from the different phases of *Plasmodium* life cycle

Targets	Candidate antigens
Sexual stages	Ps25, Ps28, Ps48/45 and Ps230

(Scand. J. Immunol. 56, 327-343, 2002)

[0029] According to the present invention, one or more antigens listed above can be used as long as it is formed to a particle. For example, a circumsporozoite protein and a fragment thereof can be used as an antigen. Examples of circumsporozoite protein include, but are not limited to, *Plasmodium falciparum* circumsporozoite protein consisting of amino acid sequence described below (SEQ ID No.:56):

Mmrklailsvssflfvealfqeyqcygsssntrvlnelnydnagtnlynelemnyygkq
 enwyslkknsrslgenddgnnngdngregkdedkrdggnedneklrkpkhkkklkqpgd
 gnpdpnanpnvdpnanpnvdpnanpnvdpnanpnanpnanpnanpnanpnanpn
 npnanpnanpnanpnanpnanpnanpnanpnvdpnanpnanpnanpnanpnanpn
 anpnanpnanpnanpnanpnanpnanpnanpnanpnanpnanpnanpnknnggng
 qghnmpndphrnvdenanannavknnnnneepsdkhieqylkkiknsistewspcsvtcg
 ngiqvrikpgsankpkdelldyendiekkickmekcssvfnvnssiglimvlsflflnt
 r.

[0030] In one embodiment, malaria antigen is a *Plasmodium falciparum* circumsporozoite protein B cell epitope. Example of *Plasmodium falciparum* circumsporozoite protein B cell epitope may be a repeat sequence of NPNA, including

(NPNA)₄₋₃₀ (i.e. 4xNPNA, 5xNPNA, 6xNPNA, 7xNPNA, 8xNPNA, 9xNPNA, 10xNPNA, 11xNPNA, 12xNPNA, 13xNPNA, 14xNPNA, 15xNPNA, 16xNPNA, 17xNPNA, 18xNPNA, 19xNPNA, 20xNPNA, 21xNPNA, 22xNPNA, 23xNPNA, 24xNPNA, 25xNPNA, 26xNPNA, 27xNPNA, 28xNPNA, 29xNPNA or 30xNPNA).

[0031] In one embodiment, malaria antigen is a Plasmodium yoelii circumsporozoite protein B cell epitope including (QGPGAP)₃₋₁₂.

[0032] In one embodiment, malaria antigen is a Plasmodium vivax circumsporozoite protein B cell epitope including (ANGAGNQPG)₁₋₁₂.

[0033] In one embodiment, malaria antigen is a Plasmodium malariae circumsporozoite protein B cell epitope including (NAAG)₄₋₃₀.

[0034] In one embodiment, malaria antigen is a Plasmodium falciparum circumsporozoite protein T cell epitope. Example of Plasmodium falciparum circumsporozoite protein T cell epitope may be EYLNKIQNSLSTEWSPCSVT (SEQ ID No.:44). (EYLNKIQNSLSTEWSPCSVT)₁₋₆ may be also used as a malaria antigen.

[0035] In one embodiment, malaria antigen is a Plasmodium yoelii circumsporozoite protein T cell epitope which is YNRNIVNRLLGDALNGPEEK (SEQ ID No.45). (YNRNIVNRLLGDALNGPEEK)₁₋₆ may be also used as a malaria

antigen.

[0036] The present invention addresses one or more of the above needs by providing antigens, vectors encoding the antigens, and antibodies (and antibody-like molecules including aptamers and peptides) that specifically bind to the antigen, together with the uses thereof (either alone or in combination) in the prevention or treatment of malaria infections. 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.

[0037] 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.

[0038] 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.

[0039] 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.

[0040] 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.

[0041] 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
5 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.

[0042] In one embodiment, a nucleic acid molecule, in
10 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
15 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
20 through a linker peptide including SG, GS, SGG, GGS and SGSG, the polypeptide is conjugated with the antigen.

[0043] 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-
25 linker which is a chemical compound.

[0044] 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.

5 [0045] 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
10 antigen or a naturally occurring protein containing the antigen or modified protein therefrom.

[0046] 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
15 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
20 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
25 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).

[0047]

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 malaria 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 malaria 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.

[0048] In one embodiment, a spatial distance between the N-terminal residue and C-terminal residue of the malaria 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 malaria antigen or a naturally occurring protein containing the malaria antigen or modified protein therefrom.

[0049] In one embodiment, the malaria 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.

[0050] 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.

[0051] 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.

[0052] 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
5 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
10 polypeptide is a mutant where at most 10% of the amino acids are deleted, substituted, and/or added based on a naturally occurring viral capsid and/or envelope protein. For example, K64A or K64N mutation may be introduced into a capsid of Venezuelan equine encephalitis virus structural
15 polypeptide used in the present invention.

[0053] Chikungunya or Venezuelan equine encephalitis virus structural polypeptide may consist of or comprise a capsid, E2 and E1.

[0054] Examples of Chikungunya virus structural
20 polypeptide include, but are not limited to, Capsid- E2-E1 of Chikungunya virus Strain 37997, and Capsid- E2-E1 of Chikungunya virus LR2006 OPY-1.

[0055] Examples of Venezuelan equine encephalitis virus structural polypeptide include, but are not limited to,
25 Capsid- E2-E1 of Venezuelan equine encephalitis virus

Strain TC-83.

[0056] An exemplary Chikungunya virus structural polypeptide sequence is provided at Genbank Accession No. ABX40006.1, which is described below (SEQ ID No.:1):

mefiptqtftynrryqprpwtprptiqvirprprpqrqagqlaqlisavnkltmravpqq
kprnrknkkqkqkqgapqnnntngkkqppkkkpaqkkkkpgrrrermcmkiendcifevk
hegkvtgyaclvgdkvmkpahvkgtidnadlaklafkrsskydlecaqipvhmksdask
fthekpegyynwhhgavqysggrftiptgagkpgdsgrpifdnkgrvvaivlgganega
rtalsvvtwnkdivtkitpegaeewslaipvmcllanttfpcsqppctpcceyekepet
lrmllednvmrpgyyqllqasltcsphrqrrstkdnfnvykatrpylahcpdcgegshsch
spvalerirneatdgtlkiqvsllqigiktddshdwtklrymdnhmpadaeraglfvrt
apctitgtmghfilarcpkgetltvgftdsrkishscthpfhhdppvigrekfhsrpqh
gkelpcstyvgstaatteeievhmppdtpdrtlmsqqsgnvnkitvngqtvrykcncggs
neglttttdkvinncvkdqchaavtnhkkwqynsplvprnaelgdrkgkihipfplanvt
crvpkarnptvtvygknqvimllypdhptllsyrmngeepnyqeewvmhkkevltvpte
glevtwgnnepkykypqlstngtahghpheiillyyelyptmtvsvsvatfillsmvg
maagmcmcarrrcitpyeltpgatvpfllslliccirtakaatygeaaiylwneqqplfw
lqalipaalivlcnclrlpcccktlaflavmsvgahtvsayehvtvipntvgvpykt
lvnrpgyspmvlemellsvtleptlsldytceyktvipspykccgtaeckdknlpd
sckvftgvypfmwggaycfcdadentqlseahveksescktefasayrahtasasaklr
lyqgnnitvtayangdhavtvkdakfivgpmssawtpfdnkivvykgdvynmdyppfga
grpqgfgdiqsrtpeskdvyantqlvlqrpavgtvhvpysqapsqgfywfkergaslqh
tapfgcqiatsnpvranvavgnmpisidipeaafttrvvdapsltdmscevpacthssdf
ggvaiikyaaskkgkcavhsmtnavtireaeievegnsqqlqisfstalasaefrvqvc
tqvhcaaechppkdhivnypashttlgvqdisatamswvqkitggvgglvavaaliliv
vlcvsfshr

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[0057] Another exemplary Chikungunya virus structural polypeptide sequence is provided at Genbank Accession No. ABX40011.1, which is described below (SEQ ID No.:2):

mefiptqtfnrryqprpwaprptiqvirprprpqrqagqlaqlisavnkltmravpqq
kprnrknkkqrqkkqapqndpkqkkqppqkkpaqkkkkpgrrrermcmkiende
ifevkhegkvmgyaclvgdkvmpahvkgtidnadlaklafkrsskydlecaqipvh
mksdaskfthekepegyynwhhgavqysggrftiptgagkpgdsgrpifdnkgrvvai
vlgganegartalsvvtwnkdivtkitpegaeewslalpvlcllanttfpcsqppctpcye
kepestlrmlendnvmrpgyyqllkasltcsphrqrrstkdnfnvykatrpylahcpdeg
eghschspialerirneatdgtlkiqvsliqigiktdsdhwtklrymdshtpadaeragll
vrtsapctitgtmghfilarcpkgetltvgftdsrkishtcthpfhheppvigrerfhsrpq
hgkelpcstyvqstaataeeievhmppdtpdrtlmtqqsgnkitvngqtvrykcncg
gsnegltttdkvinneckidqchaavtnhknwqynsplvprnaelgdrkgkihipfplan
vtrvpkarnptvtygknqvtmllypdhptllsyrnmgqepnyheewvthkkevltlv
pteglevtwgnnepykywpqmstngtahghpheiiyyelyptmtvvivsvasfvlls
mvgtavgmvcarrcitpyeltpgatvpfllslccvrttkaatyeeaaaylwneqqplf
wlqalipaalivlcnclllpcccktlaflavmsigahtvsayehvtvipntvgvpyktlvn
rpgyspmvlemelqsvtleptlsldyitceyktvipspyvkcgcgtaeckdkslpdysckvf
tgvyppfmwggaycfcdadentqlseahveksescktefasayrahtasasaklrlyqgn
nitvaayangdhavtvkdakfvvgpmssawtpfdnkivvykgdvynmdyppfgagr
pgqfgdiqsrtpeskdvyantqlvlqrpaagtvhvpysqapsgfkywlkergaslqhta
pfgcqiatsnpvrvancavgnipisidipdaaftrvvdapsvtdmscevpackhssdfggv
aiikytaskkgkcavhsmtnavtireadvevegnsqqlqisfstalasaefrvqvcestqvhc
aaachppkdhivnypashttlgvqdisttamswwqkitggvgglivavaalilivlvsvfs
rh

[0058] 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):

mfpfqpmypmqmpyrnpfaaprrpwfprtdpflamqvqeltrsmantlftkqrrdappe
gpsaakpkkeasqkqkggggkknqgkktaktgppnpkaqngnkkktnkkpgkrqrm
vmklesdktfpimlegkingyacvvggklfrpmhvegkidndvlaalktkkaskydney
advpqnmradtfkylthekpqgyyswhhgavqyengrftvpkgvgakgdsgrpildnqgr
vvaivlggvnegsrtalsvwmwnekgvtvkytpenceqwslvttmcllanvtfpcaqpp
icydrkpaetlamlsvnvdnpgydelleaavkcpgrkrrsteelfneykltrpymarci
rcavgschspiaieavksdghdgyvrlqtssqyglldssgnlkgrtmrydmhgtikeipl
hqvslytsrphchivdghgyfillarcpagdsitmeffkksvrhscsvpyevkfnpvgrel
ythppeghgveqacqvayahdaqnrqayvemhlpqsevdsslvslsgssvtvtpdpdgtal
vececggtkisetinktkqfsqctkqecqrayrlqndkwvynsdklpkaagatlkgklh
vpflladgkctvplapepmittfgfrsvslklhpkntylitrqladephythelisepa
vrnftvtekgwefvwgnhpkrfwaqetapgnphglphevithyhyhrypmstilglsic
aaiatvsvaastwlfcrsrvacltpyrlltpnaripfclavlc cartaraettwesldhl
wnnnqgmfwiglliplaalivvtrllrcvccvvpflvmagaagagayehattmpsqagi
syntivnragyaplpisitptkikliptvnleyvtchyktgmdspaikkccgsqectpty
rpdeqckvftgvypfmwggaycfc dtentqvskayvmksddcladhaeaykahtasvqa
flnitvgehsivttvyvnggetpvnfngvkitagplstawtpfdrkivqyageiynydfp
eygagqpgafgdiqsrtvsssdlyantnlvlqrpkagaihvpvtqapsgfeqwkdkkap
slkftapfgceiytnpiracnecavgsiplafdipldalftrvsetptlsaaectlnecvy
ssdfggiatvkysasksgkcavhvpstatlkeaavelteggssatihfstanihpfeirl
qictsyvtckgdchppkdhivthpqyhaqtftaavsktawtwltsllggsaviiiiglv
lativamyvltngkhn.

5

[0059] 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.

10

[0060] 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-

15

MBS, Sulfo-EMCS, Sulfo-GMBS, Sulfo-SIAB, Sulfo-SMPB, Sulfo-SMCC, SVSB, SIA and other cross-linkers available from the Pierce Chemical Company.

[0061] According to the present invention, a Chikungunya
5 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
10 fusion protein can be provided.

[0062] 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
15 C- terminal of the Chikungunya or Venezuelan equine
encephalitis virus structural polypeptide, or the antigen
may be inserted into Chikungunya or Venezuelan equine
encephalitis virus structural protein.

[0063] In one embodiment, at least one antigen is
20 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-
25 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).

[0064] 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).

[0065] 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.

[0066] 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 malaria antigen 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.

[0067] In one embodiment, the modified polypeptide derived from malaria antigen 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 malaria antigen 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 malaria antigen.

[0068] 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.

[0069] In one embodiment, the present invention provides a virus like particle comprising a fusion protein of a polypeptide derived from Chikungunya virus (CHIKV) or Venezuelan equine encephalitis virus (VEEV) and a polypeptide derived from malaria antigen, wherein the virus like particle is prepared by transfecting an expression vector comprising a nucleic acid molecule corresponding to the amino acid sequence represented by SEQ ID NO. 28, 31, 34, 37, 39, 41 or 43 into a mammalian cell (e.g. 293F cell). Regarding this embodiment, modified fusion protein can be also used for a virus like particle provided by the present

invention, which can be prepared by transfecting an expression vector comprising a nucleic acid molecule corresponding to the amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95% or 98% amino acid sequence identity to SEQ ID NO. 28, 31, 34, 37, 39, 41 or 43 into a mammalian cell (e.g. 293F cell).

[0070] In one embodiment, the present invention provides a virus like particle comprising or consisting of:

one or more capsid of Chikungunya virus (CHIKV) or Venezuelan equine encephalitis virus (VEEV);

one or more E1 of Chikungunya virus (CHIKV) or Venezuelan equine encephalitis virus (VEEV); and

one or more E2 of Chikungunya virus (CHIKV) or Venezuelan equine encephalitis virus (VEEV), wherein malaria antigen is inserted into E2 of Chikungunya virus (CHIKV) or Venezuelan equine encephalitis virus (VEEV). For example, present invention provides a virus like particle comprising or consisting of:

240 capsids of Chikungunya virus (CHIKV) or Venezuelan equine encephalitis virus (VEEV);

240 E1s of Chikungunya virus (CHIKV) or Venezuelan equine encephalitis virus (VEEV); and

240 E2s of Chikungunya virus (CHIKV) or Venezuelan equine encephalitis virus (VEEV), wherein malaria antigen is inserted into each of E2s of Chikungunya virus (CHIKV) or

Venezuelan equine encephalitis virus (VEEV).

[0071] In this embodiment, the E2 into which the antigen is inserted may consist of an amino acid sequence represented by SEQ ID No.50; the E1 may consist of an amino acid sequence represented by SEQ ID No.51; and the capsid may consist of an amino acid sequence represented by SEQ ID NO.: 52; or

the E2 into which the antigen is inserted may consist of an amino acid sequence represented by SEQ ID NO.53; the E1 may consist of an amino acid sequence represented by SEQ ID NO.54; and the capsid may consist of an amino acid sequence represented by SEQ ID NO.: 55.

[0072] Further, regarding this embodiment, modified capsid of Chikungunya virus (CHIKV) or Venezuelan equine encephalitis virus (VEEV), modified E1 of Chikungunya virus (CHIKV) or Venezuelan equine encephalitis virus (VEEV) and modified E2 of Chikungunya virus (CHIKV) or Venezuelan equine encephalitis virus (VEEV) may be used for the virus like particle. For example, the modified capsid of Chikungunya virus (CHIKV) or Venezuelan equine encephalitis virus (VEEV) may have at least 70%, 75%, 80%, 85%, 90%, 95% or 98% amino acid sequence identity to the amino acid sequence represented by SEQ ID NO.: 52 or SEQ ID No.:55; the modified E1 of Chikungunya virus (CHIKV) or Venezuelan equine encephalitis virus (VEEV) may have at least 70%, 75%,

80%, 85%, 90%, 95% or 98% amino acid sequence identity to the amino acid sequence represented by SEQ ID NO.: 51 or SEQ ID No.:54; and/or the modified E2 of Chikungunya virus (CHIKV) or Venezuelan equine encephalitis virus (VEEV) may
5 have at least 70%, 75%, 80%, 85%, 90%, 95% or 98% amino acid sequence identity to the amino acid sequence represented by SEQ ID NO.: 50 or SEQ ID No.:53.

Also, the modified capsid, E1 or E2 may be a mutant where at most 10% of the amino acids are deleted, substituted,
10 and/or added based on the capsid consisting of an amino acid sequence represented by SEQ ID NO.: 52 or SEQ ID No.:55; E1 consisting of an amino acid sequence represented by SEQ ID NO.: 51 or SEQ ID No.:54; and/or E2 consisting of consisting of an amino acid sequence represented by SEQ ID
15 NO.: 50 or SEQ ID No.:53.

[0073]

(2) Nucleotide, Vector, Host cell

In the second aspect, the present invention provides
20 a nucleic acid molecule which is designed for expression of a particle as provided in the first aspect of the present invention.

[0074] In one embodiment, the present invention provides a nucleic acid molecule comprising a nucleotide sequence
25 that encodes the Chikungunya or Venezuelan equine

encephalitis virus like particle as described above.

[0075] 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 envelope of Chikungunya virus Strain 37997, a nucleotide sequence encoding Capsid-envelope of Chikungunya virus Strain 37997, a nucleotide sequence encoding envelope of Chikungunya virus Strain LR2006 OPY-1, a nucleotide sequence encoding Capsid-envelope of Chikungunya virus LR2006 OPY-1, a nucleotide sequence encoding envelope of Venezuelan equine encephalitis virus Strain TC-83 and a nucleotide sequence encoding Capsid-envelope of Venezuelan equine encephalitis virus TC-83.

[0076] Regarding Chikungunya virus, an exemplary nucleotide sequence that encodes envelope is described below (SEQ ID No.:4):

Atgagcctcgccctcccggtcttctgtcctgttggcaaacactacattccctgctctcagccgcttgcacacctgctgctacga
 aaaggaaccggaaagcacccttgcgcattgctgaggacaacgtgatgagaccggatactaccagctactaaaagcctcgt
 gacttgcctccccaccgcaaaagacgcagctactaaggacaattttaattgtctataaagccacaagaccatatctagctcattg
 tctgactcgggagaaggcgcttctgcccacagccctatcgcattggagcgcacatcagaaatgaagcaacggacggaaacgtg
 aaaatccaggtctctttgcagatcgggataaagacagatgacagccacgattggaccaagctgcgctatatggatagccata
 cggcagcggacgcggagcagccggattgcttgaaggacttcagcaccgtgcacgacacccggaccatgggacactttatt
 ctgcgccgatgccgaaaggagagacgctgacagtggtgatttacggacagcagaaagatcagccacacatgcacacaccg
 ttccatcatgaaccacctgtgataggtaggagaggttccactctgcaccacaacatggtaagagttaccttgcagcagcga
 cgtgcagagcaccgctgccactgctgaggagatagaggtgcataatgccccagatactcctgaccgacgctgatgacgag
 cagtctggcaacgtgaagatcacagttaatgggcagacggtgctgtacaagtgaactgcggtggctcaaacgagggactg
 acaaccacagacaagtgatcaataactgcaaaattgatcagtgccatgctgcagtcactaatcacaagaattggcaatac
 aactcccccttagtcccgccgaacgctgaactcggggaccgtaaaaggaaagatccacatcccattcccattggcaaacgtgac
 ttgcagagtgcacaaagcaagaaacctacagtaacttaacggaaaaaaccaagtcaccatgctgctgtatcctgaccatccg
 aactcttcttaccgtaacatgggacaggaaccaaattaccacagaggagtggtgacacacaagaaggaggttaccttg
 accgtgctactgagggctctggaggtcacttggggcaacaacgaaccatacaagtaactggcgcagatgcttacgaacggt
 ctgctcatggtcaccacatgagataatcttctactattatgagctgtacccactatgactgtagtcttctgctggtggcctcg
 ttctgcttctctgctgagtggtgggcacagcagtggtgaatgtgtgtgtgcgcacggcgagatgcattacaccatatgaattac
 accaggagccactgttcccttctgctcagcctgctatgctgcgtcagaacgaccaaggcgccacatattacgaggtcgccgc
 atatctatggaacgaacagcagccctgttctggttgcaggtcttatacccgctggcgcccttgatcgtctgtgcaactgtctg
 aaactcttgccatgctgctgaagacctggcttttttagccgtaatgagcagctgggtgccacactgtgagcgcgtaacgaac
 gtaacagtgatcccgaaacacgggtgggagtagcgtataagactcttgcacacagaccgggttacagccccatggtgttgga
 tggagctacaatcagtcaccttggaaaccaactgtcacttgactacatcagctgcgagtaacaaactgtcatccccccccgt
 acgtgaagtgctgtgtgtacagcagagtgcaaggacaagagcctaccagactacagctgcaagggtctttactggagtctacc
 atttatgtggggcgccgctactgcttttgcgacgcgaaaatacgaattgagcgaggccacatgtagagaaatctgaattct
 gcaaaacagagtttgcagcggctacagagccacacccgcatcgccgctggcggaagctcccgctctttaccaaggaaacaa
 cattaccgtagctgctacgctaacgggtgacctgcccgtacagtaaaaggacgccaagtttgcgtgggcccattgtctccgc
 ctggacaccttttgacaacaaaatcgtgtgtacaaaggcgacgtctacaacatggactaccaccttttggcgagggaagac
 caggacaatttgggtgacattcaaagtcgtacaccggaaagttaaagacgtttatgccaacactcagttggtactacagaggcc
 agcagcaggcagcggtacatgtaccatactctcaggcaccatctggttcaagtaattggctgaagggaacgaggagcagctga
 cagcacacggcaccgttgcgttccagattgcgacaaacccggtaagagctgtaaattgcgctgtggggaacataccaatttc
 cagcacataccggatgcggccttactaggttgcgtatgcacctctgtacggacatgtcatgcgaagtaccagcctgcac
 tcaactctccgactttggggggcgtgcacatcatcaaatcacagctagcaagaaaggtaaatgtgcagtaacattcgatgacca
 acgcccgttaccattcgagaagccgacgtagaagtagagggggaactcccgagctgcaaatatccttctcaacagccctggcaag
 cgccgagtttcgctgcaagtgtgtctccacacaagtacactgcgcagccgcatgccacctccaaaggaccacatagtcaatt
 acccagcatcacacaccaccttgggggtcaggatataccacaacggcaatgtcttgggtgcagaagattacgggaggaggt
 aggattaattgttctgttgccttaattttaattgtgtgtatgcgtgtcgttttagcaggcac

[0077] Regarding Chikungunya virus, another exemplary nucleotide sequence that encodes envelope is described

5 below (SEQ ID No.:5):

atggaggttcattcccgacgcaaaactttctataacagaaggtaccaaccgccaccctgggc
cccacgcccctacaattcaagtaattagacctagaccacgtccacagaggcaggctgggc
aactcgcccgctgatctccgcagtcaacaaattgaccatgcgcgcggtacctcaacag
aagcctcgagaaatcggaaaaaacaagaagcaaaaggcagaagaagcaggcgccgcaaaa
cgacccaaagcaaaagaagcaaccaccacaaaagaagccggctcaaaagaagaagaaac
caggccgtagggagagaatgtgcatgaaaattgaaaatgattgcatcttcgaagtcaag
catgaaggc aaagtgatgggctacgcatgcctgggtgggggataaagtaatgaaaccagc
acatgtgaagggaactatcgacaatgccgatctggctaaactggcctttaagcggctgt
ctaaatacgatcttgaatgtgcacagataccgggtgcacatgaagtctgatgcctcgaag
tttaccacagagaaaccgaggggtactataactggcatcacggagcagtgacgtattc
aggaggccggttcaactatcccgacgggtgcaggcaagccgggagacagcggcagaccga
tcttcgacaacaaaggacgggtggtggccatcgtcctaggagggggccaacgaaggtgcc
cgcacggccctctccgtggtgacgtggaacaaagacatcgtcacaaaaattaccctga
gggagccgaagagtggagcctcgccctccgggtcttgtgcctgtttggcaaacactacat
tccctgtctctcagccgccttgcacaccctgctgctacgaaaaggaaccggaaagcacc

nucleotide sequence that encodes a capsid-envelope is

described below (SEQ ID No.: 7):

tgctaatacacaaactgggtactgcagagaccggctgtgggtacgggtacacgtgccatact
ctcagggcaccatctggctttaagtattggctaaaagaacgcggggcgctcgctgcagcac
acagcaccatttggctgccaaatagcaacaaacccggtaagagcgggtgaactgcgcgct
agggaacatgcccattctccatcgacataccggaagcggccttcactagggctcgtcgacg
cgccctctttaacggacatgtcgtgcgaggtaccagcctgcacccattcctcagacttt
gggggcgtcgccattattaaatatgcagccagcaagaaaggcaagtgtgcggtgcattc
gatgactaacgcccgtcactattcggaagctgagatagaagttgaagggaattctcagc
tgcaaatctctttctcgacggccttagccagcgccgaattccgcgtacaagtctgttct
acacaagtacactgtgcagccgagtgccacccccgaaggaccacatagtcaactaccc
ggcgtcacataccaccctcggggtccaggacatctccgctacggcgatgtcatgggtgc
agaagatcacgggaggtgtgggactggttgttgcgtgttgccgcactgattctaatacgtg
gtgctatgcgtgtcgttcagcaggcactaa.

[0080] 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.

[0081] 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.

[0082] In this embodiment, the vector comprising an
expression control sequence operably linked to the nucleic
acid molecule as described above can be used as an
expression vector for preparing the particle provided by
the first aspect of the present invention.

[0083] 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.

[0084] Examples of vectors which can be used for expressing a virus like particle comprising 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.:8) containing CHIKV VLP polynucleotide (SEQ ID No. 13; corresponding amino acid sequence represented by SEQ ID No.:14); and VLP_CHI 532 vector (SEQ ID No.: 9) containing CHIKV VLP polynucleotide (SEQ ID No. 15; corresponding amino acid sequence represented by SEQ ID No.:16).

[0085] 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.

[0086] Examples of vectors which can be used for expressing a virus like particle comprising 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.:10) containing VEEV VLP polynucleotide (SEQ ID No. 17; corresponding amino acid sequence represented by SEQ ID No.:18); VLP_VEEV VLP 519 vector (SEQ ID No.11) containing VEEV VLP polynucleotide (SEQ ID No. 19; corresponding amino acid sequence represented by SEQ ID No.:20); and VLP_VEEV VLP 538 vector (SEQ ID No.: 12) containing VEEV VLP

polynucleotide (SEQ ID No. 21; corresponding amino acid sequence represented by SEQ ID No.:22).

[0087] In one embodiment, the present invention provides a nucleic acid molecule which is designed for expression of a virus like particle comprising a fusion protein of a polypeptide derived from Chikungunya virus (CHIKV) or Venezuela equine encephalitis virus (VEEV) and a polypeptide derived from malaria antigen, which consists of a nucleotide sequence represented by SEQ ID Nos.26-27, 29-30, 32-33, 35-36, 38, 40 or 42.

[0088] 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.26-27, 29-30, 32-33 or 35-36, 38, 40 or 42. 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.26-27, 29-30, 32-33, 35-36, 38, 40 or 42. 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.26-27, 29-30, 32-33, 35-36, 38, 40 or 42.

[0089]

(3) Composition or vaccine

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

[0090] In one embodiment, the present invention provides a composition or vaccine comprising the Alphavirus or
10 Flavivirus virus like particle (e.g. Chikungunya virus like particle or Venezuelan equine encephalitis virus like particle) as described above or the nucleic acid molecule as described above. The content of the Alphavirus or Flavivirus virus like particle and the content of the
15 nucleic acid molecule may be 0.00001-1 w/w%.

[0091] Dosage amount of the particle provided in the first aspect of the present invention (e.g. CHIKV VLP or VEEV VLP) may be 1-500µg/day.

[0092] One or more malaria antigens may be used for one
20 composition or one vaccine provided by the third aspect of the present invention.

[0093] The composition or vaccine may further comprise a pharmaceutical acceptable carrier and/or adjuvant. Examples of adjuvant include, but are not limited to,
25 aluminium salts, sodium hydroxide, Freund's complete adjuva

nt, Freund's incomplete adjuvant and Ribi solution (Sigma Adjuvant system, Sigma-Aldrich). The composition or vaccine provided in the third aspect of the present invention may contain buffering agent such as dibasic sodium phosphate hydrate, sodium dihydrogen phosphate and sodium chloride; and preserving agent such as thimerosal. In one embodiment, the composition or vaccine is an aqueous solution containing 0.001-1 w/w% of the particle provided in the first aspect of the present invention (e.g. CHIKV VLP or VEEV VLP), 1-10w/w% of buffering agent, 0.01-1w/w% of adjuvant and 0.00001-0.001w/w% of preserving agent.

[0094] A skilled person can prepare the pharmaceutical composition and vaccine using conventional technique. For example, the particle provided in the first aspect of the present invention is mixed with buffer solution having physiological pH (e.g. pH 5-9, pH7) to prepare the pharmaceutical composition and vaccine provided in the third aspect of the present invention.

[0095] 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.

[0096] In a combination of plural active ingredients, their respective contents may be suitably increased or decreased in consideration of their therapeutic effects and safety.

[0097] 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.

[0098] 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.

[0099] The composition or vaccine provided in the third aspect of the present invention can be administered one or more times. When the composition or vaccine provided in

the third aspect of the present invention is administered more than one time, different particle provided in the first aspect of the present invention (e.g. CHIKV VLP or VEEV VLP) may be used for each of the administration. In one embodiment, combination of immunization using CHIKV VLP provided in the first aspect of the invention and immunization using VEEV VLP provided in the first aspect of the invention is employed. For example, CHIKV VLP provided in the first aspect of the present invention may be used for the 1st immunization and VEEV VLP provided in the first aspect of the present invention may be used for the 2nd immunization, or VEEV VLP provided in the first aspect of the present invention may be used for the 1st immunization and CHIKV VLP provided in the first aspect of the present invention may be used for the 2nd immunization.

[0100] A skilled person can determine timing of immunization using the composition or vaccine provided in the third aspect of the present invention. For example, 2nd immunization is performed 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 weeks after 1st immunization.

[0101] In one embodiment, the present invention provides a kit comprising

- (a) a vaccine composition comprising the particle provided in the first aspect of the present invention; and
- (b) another vaccine composition comprising the particle

provided in the first aspect of the present invention,
wherein the particle contained in (a) is a virus like
particle which is different from the particle contained in
(b). In this embodiment, the particle contained in (a) may
5 be Chikungunya virus like particle and the particle
contained in (b) may be Venezuelan equine encephalitis
virus like particle.

[0102] In one embodiment, the present invention provides
a kit comprising

10 (a) a vaccine composition comprising the particle provided
in the first aspect of the present invention; and

(b) another vaccine composition comprising the particle
provided in the first aspect of the present invention,

(c) one or more vaccine composition, each of which
15 comprises the particle provided in the first aspect of the
present invention,

wherein (a) is used for priming immunization and (b) and
(c) are used for boosting immunization; and the particle
contained in (a) is a virus like particle which is
20 different from the particle contained in (b); and the
particle contained in (c) is different from the particle
contained in (a) and (b), or the same as the particle
contained in (a) or (b).

[0103] The respective vaccine compositions contained in
25 the above-described kit may be administered simultaneously,

separately or sequentially.

[0104] The Alphavirus or Flavivirus virus like particle (e.g. Chikungunya virus or Venezuelan equine encephalitis virus) provided in the first aspect of the present invention or the nucleic acid molecule provided by the second aspect of the invention can be used for the composition and vaccine provided in the third aspect of the present invention.

[0105] For example, Chikungunya or Venezuelan equine encephalitis virus like particle comprising or consisting of:

one or more (e.g. 240) capsid of Chikungunya virus (CHIKV) or Venezuelan equine encephalitis virus (VEEV);

one or more (e.g. 240) E1 of Chikungunya virus (CHIKV) or Venezuelan equine encephalitis virus (VEEV); and

one or more (e.g. 240) E2 of Chikungunya virus (CHIKV) or Venezuelan equine encephalitis virus (VEEV), wherein malaria antigen is inserted into E2 of Chikungunya virus (CHIKV) or Venezuelan equine encephalitis virus (VEEV) may

be used for preparing the composition or vaccine provided in the third aspect of the present invention. The E2 into which the antigen is inserted may consist of an amino acid sequence represented by SEQ ID No.50; the E1 may consist of an amino acid sequence represented by SEQ ID No.51; and the capsid may consist of an amino acid sequence represented by

SEQ ID NO.: 52; or

the E2 into which the antigen is inserted may consist of an amino acid sequence represented by SEQ ID NO.53; the E1 may consist of an amino acid sequence represented by SEQ ID NO.54; and the capsid may consist of an amino acid sequence represented by SEQ ID NO.: 55.

[0106] The composition or vaccine provided in the third aspect of the present invention can be administered to a mammal (e.g. human) intramuscularly (i.m.), intracutaneously (i.c.), subcutaneously (s.c.), intradermally (i.d.) or intraperitoneally (i.p.).

[0107] The composition or vaccine provided in the third aspect of the present invention may be used for treating or preventing malaria.

[0108] Thus, use of the Alphavirus or Flavivirus (e.g. Chikungunya virus or Venezuelan equine encephalitis virus) virus like particle provided in the first aspect of the present invention or the nucleic acid molecule provided by the second aspect of the invention for manufacturing a pharmaceutical composition or vaccine for treating or preventing malaria is also provided by the present invention.

[0109]

(4) Method of producing an antibody, Method of immunomodulation, Method of treating malaria, Method of

inducing and/or enhancing immune response against a malaria antigen in a mammal, Method of passive immunization, Method of presenting an antigen on macrophage, and Method for producing a particle

- 5 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.
- 10 [0110] 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.
- 15 [0111] 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
- 20 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.

[0112] According to the present invention, the virus like particle can be applied for the immune therapy.

[0113] In the fifth aspect, the present invention provides a method of immunomodulation, a method of treating malaria,
5 a method of inducing and/or enhancing immune response against a malaria antigen in a mammal comprising administering the composition provided in the third aspect of the present invention to a mammal.

[0114] In sixth aspect, the present invention provides a
10 method of passive immunization against a malaria-causing pathogen, comprising administering the antibody provided in the fourth aspect of the present invention to a mammal.

[0115] In seventh aspect, the present invention provides a method of presenting a malaria antigen on macrophage,
15 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.

[0116] In eighth aspect, the present invention provides a
20 method for producing the particle provided in the first aspect of the present invention, comprising preparing a vector designed for expression of said particle; culturing

a cell which is transfected with said vector to express said particle; and recovering said particle.

[0117] Examples of mammal include, but are not limited to, a human.

5 [0118] In one embodiment, the present invention provides a method of producing an antibody against malaria antigen, 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.

10 The produced antibody may be an antibody which can specifically bind to a malaria antigen comprised in the Chikungunya or Venezuelan equine encephalitis virus like particle or a malaria antigen encoded by the nucleic acid molecule. The method of producing an antibody provided by
15 the present invention may be a useful for producing a monoclonal or polyclonal antibody against a malaria antigen.

[0119] In one embodiment, an antibody against malaria antigen obtained by the method of producing an antibody according to the present invention is used for passive
20 immunization. The method of passive immunization may comprise administering the obtained antibody to a mammal.

[0120] In one preferred embodiment, the immunomodulation provided by the present invention is inducing and/or

enhancing immune response against malaria antigen in a mammal. Thus, in one embodiment, the present invention provides a method of inducing and/or enhancing immune response against malaria antigen in a mammal, comprising
5 administering an effective amount of the composition as described above to the mammal.

[0121] Given the symptom of patients infected with Chikungunya or Venezuelan equine encephalitis together with unusual big molecule of Chikungunya or Venezuelan equine
10 encephalitis, this VLP can act effectively and efficiently to target macrophage and its composition such as cytokines and immunomodulative compounds.

[0122] In one aspect, the present invention provides a method of presenting an antigen on macrophage, comprising
15 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
20 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.

[0123] 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 vector designed for expression of said particle; culturing a cell which is transfected with said vector 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 vector, and may further include purify VLP from the conditioned medium using ultracentrifugation.

[0124] 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.

[0125]

20 EXAMPLES

EXAMPLE 1: Preparation of Chikungunya virus (CHIKV) like particle comprising a virus structural polypeptide and a fragment of malaria antigen

The following polynucleotides of malaria CSP1 proteins are used. N terminal linker is SGG and C terminal linker is GGS.

VLP74 (6 repeat of NPNA amino acid sequence)

5 Sggnpnanpnanpnanpnanpnanpnaggs (SEQ ID No.:46)

(Tccggaggaaacccgaatgccaatcccaacgcgaaccccaatgctaaccctaatgccaaacgaacgccaaccccaacgctggtggatcc) (SEQ ID No.:47)

VLP78 (25 repeat of NPNA amino acid sequence)

10 Sggnpnanpnanpnanpnanpnanpnvdpnanpnanpnanpnanpnanpnanpnanpnanpnanpnanpnanpnaggs (SEQ ID No.:48)

(tccggaggaaacccgaatgccaatcccaacgcgaaccccaacgctaaccctaacgccaatccgaatgcaaaccgaacggttgacccaaacgccaacccgaatgccaatcccaacgcgaaccccaatgctaaccctaatgccaaacgccaaccccaacgctaataccaaacgccaaccctaacgccaatcccaacgcgaatcctaacgctaatacccaacgcaaatacccaatgctaataccgaacgcgaaccctaatacgaaccccaacgccaacccgaacgctaaccgaacgctaatacccaacgcccgtggatcc) (SEQ ID No.:49)

15

[0126] The respective polynucleotides was inserted between the codons encoding Ser at 531-position and Asn at 532-position of SEQ ID Nos.15 or 16 (SEQ ID Nos.1 or 2) to construct a plasmid (hereinafter referred to as CHIKV-VLP74 or 78) for expressing Chikungunya virus like particle where the modified VLP74 or 78-derived peptide is inserted into E2 of Chikungunya virus structural polypeptide.

20

[0127] 293F cells (Lifetechnology) were transfected with the plasmid using PEI (GE Healthcare) or GeneX (ATCC). 4 days after the transfection, the conditioned medium was collected and centrifuged at 3000rpm for 15 minutes to separate it from cells. The supernatant was filtrated using 0.45µm filter to obtain virus like particles. The virus like particles were concentrated using TFF column and purified using QXL column (GE Healthcare) to obtain purified virus like particles. When animals were immunized with virus like particles, the purified virus like particles were further concentrated using spin column (Molecular Weight-cutoff: 100kDa) to prepare the virus like particles for the immunization.

[0128] The expression of VLP comprising VLP74 or 78 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 VLP74 or 78.

[0129]

EXAMPLE 2: Preparation of Venezuelan equine encephalitis virus (VEEV) like particle comprising a virus structural polypeptide and a fragment of malaria antigen

The same polynucleotides of malaria CSP1 proteins (VLP74 and VLP78) used in EXAMPLE 1 are used. N terminal linker is SGG and C terminal linker is GGS.

[0130] The respective polynucleotides was inserted
5 between the codons encoding Ser at 518-position and Ser at
519-position of SEQ ID Nos.19 or 20 (SEQ ID No.3) to
construct a plasmid (hereinafter referred to as VEEV-VLP74
or 78) for expressing Venezuelan equine encephalitis virus
like particle where the modified VLP74 or 78 -derived
10 peptide is inserted into E2 of Venezuelan equine
encephalitis virus structural polypeptide.

[0131] 293F cells (Lifetechnology) were transfected with
the plasmid using PEI (GE Healthcare) or GeneX (ATCC). 4
days after the transfection, the conditioned medium was
15 collected and centrifuged at 3000rpm for 15 minutes to
separate it from cells. The supernatant was filtrated
using 0.45µm filter to obtain virus like particles. The
virus like particles were concentrated using TFF column and
purified using QXL column (GE Healthcare) to obtain
20 purified virus like particles. When animals were immunized
with virus like particles, the purified virus like
particles were further concentrated using spin column
(Molecular Weight-cutoff: 100kDa) to prepare the virus like
particles for the immunization.

[0132] The expression of VLP comprising VLP 74 or 78 conjugated with Venezuelan equine encephalitis virus structural polypeptide was confirmed by Western Blot using an antibody specific for VEEV and an antibody specific for VLP74 or 78.

[0133]

EXAMPLE 3: Immunogenicity in Non-human Primate (Monkey)

The monkeys were immunized with x25-CHI (80ug) at 0 week and x6-VEE (80ug) at 3 week by intramuscular injection with or without adjuvant (Sigma Adjuvant System, Sigma, S6322). X25-CHI means 25 times malaria CSP repeat amino acid NPNA on CHIKV VLP particle (VLP78_15). X6-VEE means 6 times malaria CSP repeat amino acid NPNA on VEEV VLP particle (VLP74_25). The blood is taken at 2 and 5 weeks after the first immunization.

[0134] 96 well ELISA plate were coated with 50ng of Recombinant Circumsporozoite Protein (rCSP) (Reagent Proteins, ATG-422) in 100ul PBS buffer pre well. The Plates after 2 hours incubation were washed three times TBS buffer containing 0.05% Tween-20 and blocked with TBS buffer containing 0.05% Tween-20 and 5% dry milk. The heat inactivated diluted serum from monkeys were added in the blocking buffer and incubated for 1 h at room temperature.

After washing three times, peroxidase labeled goat anti-human IgG or anti-mouse IgG was added at 1:4000 dilution and incubated for 1h at room temperature. After washing three times, Peroxidase substrate was added for development and incubated for 10 mins and 2N H₂SO₄ was added to stop the development. The data were analyzed using Gen5 (BioTek) and GraphPad Prism6 (GraphPad software Inc).

[0135] The Immunogenicities are shown in Figures 4 to 6.

[0136] Induction of antibodies against CSP was found in the serum of all monkeys immunized with Malaria VLPs (see Figure 4). The mean OD values indicating titer of antibodies against CSP is shown in Figure 5. Figure 5 shows that the serum from immunized monkeys induced high titer of antibodies against CSP.

[0137] As seen in Figure 6, higher titer of antibodies against CSP was achieved when CHIKV VLP particle comprising NPNA and VEEV VLP particle comprising NPNA were used for the priming immunization and boosting immunization, respectively, compared to when only CHIKV VLP particle comprising NPNA was used for both of the priming immunization and the boosting immunization. In addition, Figure 6 shows that use of adjuvant further enhanced the titer of antibodies against CSP. Further, Figure 6 shows

that administration of 25-repeats of NPNA induces higher titer of antibodies against CSP compared to administration of 6-repeats of CSP.

[0138] The anti-Pf CSP antibody titer in the serum from the monkeys immunized with x25-CHI (80ug) at 0 week and x6-VEE (80ug) at 3 week without using adjuvant was measured by ELISA at Malaria Serology Lab Malaria Vaccine Branch, WRAIR. In the ELISA performed at Malaria Serology Lab Malaria Vaccine Branch, WRAIR, the plates were coated with CSPrp ((NPNA)6 Peptide) [0.2µg/µL] (Supplier: Eurogentec EP070034) and Goat α-Human IgG (KPL/074-1002 LOT# 120714) was used as 2nd antibody. The final titer was determined by the dilution factor that yields an OD of 1.0 (414nm).

[0139] As a result, the anti-Pf CSP antibody titer in the serum from the monkeys was enhanced after 2nd immunization compared to 1st immunization (see Table 2). Compared to the anti-Pf CSP antibody titer in the serum from the monkeys immunized with RTS,S (GlaxoSmithKline), the anti-Pf CSP antibody titer in the serum from the monkeys immunized with x25-CHI (80ug) and x6-VEE (80ug) in the absence of adjuvant was considered to be higher even though RTS,S (GlaxoSmithKline) contains adjuvant.

[Table 2]

Animal No.	After 1st immunization	After 2nd immunization
	Week 2	Week 5
1	8990	29420
2	48210	44100
3	80400	51230
4	16260	19640
Geometric mean	27359	33801

[0140]

Example 4: Immunogenicity in mouse

The mice immunized with 10ug of VLP78_15 at week 0,
 5 10ug of VLP74_25 at week3 and 10ug of VLP78_15 at week 6
 with or without adjuvant (Sigma Adjuvant System, Sigma,
 S6322) by intramuscular injection.

[0141] The anti-Pf CSP antibody titer in the serum from
 the immunized mice were measured by ELISA at Malaria
 10 Serology Lab Malaria Vaccine Branch, WRAIR, where plates
 were coated with CSPrp ((NPNA)6 Peptide) [0.2µg/µL]
 (Supplier: Eurogentec EP070034) and Goat α-Mouse IgG
 (KPL/074-1806 LOT# 100737) is used as 2nd antibody to
 detect the antibodies in the serum.

[0142] The final titer was determined by the dilution factor that yields an OD of 1.0 (414nm).

[0143] The Immunogenicity are shown in Tables 3 and 4.

[0144] Tables 3 and 4 show that higher titer of antibodies against CSP was achieved after immunizing virus like particle three times. In addition, Tables 3 and 4 show that use of adjuvant enhanced the titer of antibodies against CSP.

[Table 3]

Mouse Week 3 (after 1st immunization)

Mouse No.	VLP	VLP+Adjuvant
1	6070	QNS
2	5850	13680
3	9610	7610
4	5440	23370
5	16320	27390
Geometric Mean	7875	16066

QNS=Quantity not sufficient to test

10

[Table 4]

Mouse Week 9 (after 1st immunization)

Mouse No.	VLP	VLP+Adjuvant
1	35510	729000
2	15040	197800
3	41650	106700
4	37250	436000
5	48200	497600
Geometric Mean	33134	319666

[0145]

Example 5: Immunogenicity of P. yoelii CSP inserted VLP in mice

QGPGAP seen in the rodent malaria CSP (P.yoelii CSP) was used as an antigen. 6x QGPGAP was inserted into CHIKV VLP. The mice were immunized with the CHIKV VLP 2 times at 0 and 8 week (20ug VLP per mouse) by intramuscle injection with or without Adjuvant Ribi.

[0146] Immunogenicity was confirmed at 4, 6, 10 and 14 weeks after the first immunization. The anti- P. yoelii CSP antibody were measured by ELISA. The ELISA was performed in the same way as ELISA described in Example 3 except that the plates were coated with P. Yoelii CSP repeat sequence peptide at 0.1 ng/ μ l. The secondary antibody was anti-mouse IgG HRP (Cell signal, #7076S). The results are shown in Figures 7-9.

[0147] Figures 7-9 show that higher titer of antibodies against CSP was achieved by intramuscler administration of CHIKV VLP comprising 6X QGPGAP. In addition, Figures 7-9 show that use of adjuvant enhanced the titer of antibodies against CSP.

[0148]

Example 6: Protection of mice against malaria by intramuscle injection of CHIKV VLP comprising P. yoelii CSP epitope: 6x QGPGAP

6x QGPGAP was inserted into CHIKV VLP. The mice (n=5) were immunized with the CHIKV VLP 2 times at 0 and 8 week (20ug VLP per mouse) by intramuscle injection with or without Adjuvant Ribi (see Figure 10). Rodent malaria: P. yoelii-challenge (i.v.) was conducted at 17 weeks (see Figure 10).

[0149] Malaria infection was confirmed by PCR. Genomic DNA was purified from the mice blood day 6 after challenge. 18S malaria DNA was amplified by PCR. Figure 11 shows results of the PCR, indicating that all of 5 control mice (PBS injection) were infected with malaria; all of 5 mice immunized with Control VLP were infected with malaria; among 5 mice immunized with CHIKV VLP comprising 6x QGPGAP, 2 mice were infected with malaria and 3 mice were not infected with malaria; and among 5 mice immunized with CHIKV VLP comprising 6x QGPGAP with adjuvant: Ribi, 1 mouse was infected with malaria and 4 mice were not infected with malaria.

[0150]

Example 7: Preparation of vaccine composition comprising Chikungunya virus (CHIKV) like particle comprising NPNA

repeat or Venezuelan equine encephalitis virus (VEEV) like particle comprising NPNA repeat

Chikungunya virus (CHIKV) like particle comprising 6x or 25x NPNA was prepared according to Example 1, and

5 Venezuelan equine encephalitis virus (VEEV) like particle comprising 6x NPNA was prepared according to Example 2.

To prepare a vaccine composition, 80 μ g of each of the prepared particles was mixed with 1ml of Sucrose Phosphate Solution, pH 7.2, Endotoxin Free (Teknova, SP buffer).

CLAIMS

1. A particle comprising a virus structural polypeptide and at least one malaria antigen, wherein said virus structural polypeptide comprises at least one first attachment site and said at least one malaria antigen comprises at least one second attachment site, and wherein said virus structural polypeptide and said malaria 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.
2. The particle according to Claim 1, wherein said virus like particle is derived from alphavirus or Flavivirus.
3. The particle according to Claim 2, 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.

5 4. The particle according to Claim 3, wherein said alphavirus is Chikungunya virus (CHIKV) or Venezuelan equine encephalitis virus (VEEV).

10 5. The particle according to any one of Claims 1-4, wherein said virus structural polypeptide comprises an envelope protein.

6. The particle according to any one of Claims 1-5, wherein said virus structural polypeptide comprises the capsid and/or the envelope proteins E1 and E2.

15 7. The particle according to Claim 6, wherein said at least one malaria antigen is inserted into E2 of the envelope protein.

20 8. The particle according to any one of Claims 1-7, wherein said virus structural polypeptide is a polypeptide derived from Chikungunya virus (CHIKV) or Venezuelan equine encephalitis virus (VEEV).

9. The particle according to any one of Claims 1-8, wherein the particle comprises an envelope protein E2 into which the antigen is inserted, an envelope protein E1 and a capsid.

25 10. The particle according to any one of Claims 1-8,

wherein the particle is Chikungunya virus like particle consisting of one or more envelope protein E2 into which the antigen is inserted, one or more envelope protein E1 and one or more capsid, and

5 wherein the envelope protein E2 into which the antigen is inserted consists of an amino acid sequence represented by SEQ ID No.50; the envelope protein E1 consists of an amino acid sequence represented by SEQ ID No.51; and the capsid consists of an amino acid sequence represented by SEQ ID
10 NO.: 52.

11. The particle according to any one of Claims 1-8, wherein the particle is Venezuelan equine virus like particle consisting of one or more envelope protein E2 into which the antigen is inserted, one or more envelope protein
15 E1 and one or more capsid, and

wherein the envelope protein E2 into which the antigen is inserted consists of an amino acid sequence represented by SEQ ID NO.53; the envelope protein E1 consists of an amino acid sequence represented by SEQ ID NO.54; and the capsid
20 consists of an amino acid sequence represented by SEQ ID NO.: 55.

12. The particle according to any one of Claims 1-11, wherein said at least one malaria antigen and said virus structural polypeptide are expressed as a fusion protein.

25 13. The particle according to Claim 12, wherein said virus

structural polypeptide and said at least one malaria antigen are directly fused.

14. The particle according to Claim 13, wherein said at least one malaria antigen are fused with said virus structural 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 virus structural polypeptide.

15. The particle according to any one of Claims 9-14, wherein said at least one malaria antigen is inserted between residues 531 and 532 of SEQ ID Nos.1 or 2, or between residues 518 and 519 of SEQ ID No.3.

16. The particle according to any one of Claims 9-15, wherein said particle comprising said fusion protein is expressed by transfecting a nucleic acid molecule corresponding to the amino acid sequence represented by SEQ ID Nos. 28, 31, 34, 37, 39, 41 or 43 into a mammalian cell.

17. The particle according to any one of Claims 9-15, wherein said particle comprising said fusion protein is expressed by transfecting a nucleic acid molecule corresponding to an amino acid sequence which has a sequence identity of 90% or more with an amino acid sequence represented by SEQ ID Nos. 28, 31, 34, 37, 39, 41 or 43 into a mammalian cell.

18. The particle according to any one of Claims 1-8,

wherein said at least one malaria antigen is linked to said virus structural polypeptide by way of chemical cross-linking.

19. The particle according to any one of Claims 1-18,
5 wherein said at least one malaria antigen is a fragment derived from the circumsporozoite protein.

20. The particle according to any one of Claims 1-19,
wherein said at least one malaria antigen is an antigen comprising (NPNA)_n wherein n is from 4 to 30 and/or an
10 antigen comprising (EYLNKIQSLSTEWSPCSVT)_y wherein y is from 1 to 6.

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

15 22. An isolated nucleic acid molecule consisting of a nucleotide sequence represented by SEQ ID Nos. 26-27, 29-30, 32-33, 35-36, 38, 40 or 42.

23. An isolated nucleic acid molecule consisting of a nucleotide sequence which has a sequence identity of 90% or
20 more with a nucleotide sequence encoding represented by SEQ ID Nos. 26-27, 29-30, 32-33, 35-36, 38, 40 or 42.

24. A vector comprising the nucleic acid molecule according to any one of Claims 21-23, wherein the vector optionally comprises an expression control sequence
25 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:

5 (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
10 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
15 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
20 an immunologically effective amount of the composition of any one of Claims 25-28 to a mammal.

32. A method of inducing and/or enhancing immune response against a malaria antigen in a mammal, comprising administering an effective amount of the composition of any
25 one of Claims 25-28 to the mammal.

33. A method of treating malaria, comprising administering an effective amount of the composition of any one of claims 25-28 to a mammal.

34. A method of passive immunization against a malaria-causing pathogen, comprising administering the antibody
5 obtained by the method according to Claim 29 or Claim 30 to a mammal.

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

36. A method for producing the particle according to any one of Claims 9-20, comprising preparing a vector designed for expression of said particle; culturing a cell which is
15 transfected with said vector to express said particle; and recovering said particle.

37. The method according to any one of Claims 29-36, wherein said at least one malaria antigen comprises at least a fragment of the circumsporozoite polypeptide.

20 38. A vaccine for use in the method of any one of Claims 31-33 comprising, as separate components, a priming composition comprising at least one first malaria antigen or at least one polynucleotide encoding at least one first malaria antigen; and a boosting composition comprising at
25 least one polypeptide comprising at least one second

malaria antigen or at least one polynucleotide encoding at least one second malaria antigen.

39. The vaccine of claim 38, wherein the polynucleotide encodes substantially all of the circumsporozoite protein.

5 40. The vaccine composition according to Claim 27 for use in the prevention or treatment of malaria, comprising a plurality of malaria-derived antigens.

41. A kit comprising

10 (a) a vaccine composition comprising the particle according to any one of Claims 1-20; and

(b) another vaccine composition comprising the particle according to any one of Claims 1-20,

wherein the particle contained in (a) is a virus like particle which is different from the particle contained in

15 (b).

42. The kit according to Claim 41, wherein the particle contained in (a) is Chikungunya virus like particle and the particle contained in (b) is Venezuelan equine encephalitis virus like particle, or the particle contained in (a) is
20 Venezuelan equine encephalitis virus like particle and the particle contained in (b) is Chikungunya virus like particle.

43. The kit according to Claims 41 or 42, further comprising (c) one or more vaccine compositions, each of
25 which comprises the particle according to any one of Claims

1-20, wherein (a) is used for priming immunization and (b) and (c) are used for boosting immunization, and the particle contained in (c) is different from the particle contained in (a) and (b), or the same as the particle
5 contained in (a) or (b).

44. The kit according to any one of Claims 41-43, wherein the respective vaccine compositions are administered simultaneously, separately or sequentially.