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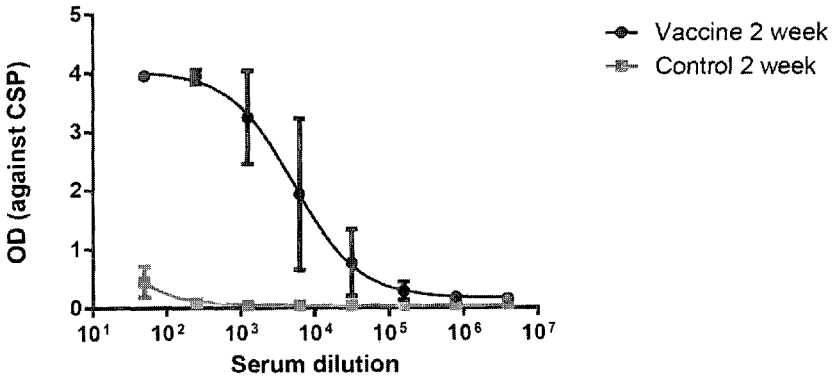
(54) Title: MALARIA VACCINE

Fig. 5



(57) Abstract: The present invention provides 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 infections.

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DESCRIPTION

Malaria vaccine

TECHNICAL FIELD

[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.

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 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 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).

[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 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, 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 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 (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 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 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 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 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).

[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 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 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 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 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

[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 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 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 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 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.

DETAILED DESCRIPTION OF THE INVENTION

[0019]

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

[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 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.

[0021] A polypeptide used for the present invention may be spontaneously assembled. The polypeptide may be a virus 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 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.

[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) (*Plasmodium vivax*)
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 enwyslkknsrslgenddgnnnngdngregkdedkrdgnnedneklrkpkhkklkqpgd gnpdpnanpnvdpnanpnvdpnanpnvdpnanpnanpnanpnanpnanpnanpnanpna npnanpnanpnanpnanpnanpnanpnanpnvdpnanpnanpnanpnanpnanpnanpn anpnanpnanpnanpnanpnanpnanpnanpnanpnanpnanpnanpnknnqgng qghnmpndpnrnvdenanannavknnnneepsdkhieqylkkiknsistewspcsvtcg ngiqvrikpgsankpkdeldyendiekkickmekcssvfnvvnssiglimvlsflflnt 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 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 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.

[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-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.

[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 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 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).

[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 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.

[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 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, 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):

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

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

mefiptqtfynrryqprpwaprptiqvirprprpqrqagqlaqlisavnkltmravpqq
kprrnrknkkqrqkkqapqndpkqkkqppqkkpaqkkkkpgrrermcmkiendc
ifevkhegkvmgyaclvgdkvmkpahvkgtidnadlaklafkrsskydlecaqipvh
mksdaskfthekpegyynwhhgavqysggrftiptgagkpgdsgrpifdnkgrvvai
vlgganegartalsvvtwnkdivtkitpegaeewslalpvlcllanttfpcsqppctpccye
kepestlrmlednvmrpgyyqllkasltcsphrqrrstkdnfnvykatrpylahcpdcg
eghschspialerirneatdgtlkiqvslqigiktddshdwtklrymdshtpadaeragll
vrtsapctitgtmghfilarcpkgetltvgftdsrkishtcthpfhheppvigrerfhsrpq
hgkelpcstyvqstaataeeievhmppdtpdrtlmtqqsgnvkitvngqtvrykcncg
gsneglttdkvinnckidqchaavtnhknwqynsplvprnaelgdrkgkihipfplan
vtcrvpkarnptvtygknqvtmllypdhptllsyrnmgqepnyheewvthkkevtltv
pteglevtwgnnepykywpqmstngtahghpheiilyyyelyptmtvvivsvasfvlls
mvgtavgmcvcarrrcitpyeltpgatvpfllsllccvrttkaatyyeaaaylwneqqplf
wlqaliplaalivlcnclkllpcccktlaflavmsigahtvsayehvtvipntvgvpyktlvn
rpgyspmvlemelqsvtleptlsldyitceyktvipspyvkccgtaeckdkslpdysckvf
tgvypfmwggaycfcdaentqlseahveksescktefasayrahtasasaklrvlyqgn
nitvaayangdhavtvkdakfvvgpmssawtpfdnkivvykgdvynmdyppfgagr
pgqfgdiqsrtpeskdvyantqlvlqrpaagtvhvpysqapsgfkywlkergaslqhta
pfgcqiatnpvravncavgnipisidipdaaftrvvdapsvtdmscevpacthssdfggv
aiikytaskkgkcavhsmtnavtireadvevegnsqlqisfstalasaefrvqvcstqvhc
aaachppkdhivnypashttlgvqdisttamswvqkitggvglivavaalilivvlcvsfs
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):

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

[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.

[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-

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 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.

[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 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 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).

[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 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, 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 NO.: 50 or SEQ ID No.:53.

[0073]

(2) Nucleotide, Vector, Host cell

In the second aspect, the present invention provides 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 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):

Atgagcctcgccctcccggtcttgtgcctgttggcaaacactacattcccctgctctcagccgccttgcacaccctgctgctacga
aaaggaaccggaaagcaccttgcgcatgcttgaggacaacgtgatgagacccggatactaccagctactaaaagcatcgct
gacttgctctccccaccgccaaagacgcagtactaaggacaattttaatgtctataaagccacaagaccatatctagctcattg
tcctgactgcggagaagggcattcgtgccacagccctatcgcattggagcgcatcagaaatgaagcaacggacggaacgctg
aaaatccaggtctctttgcagatcgggataaagacagatgacagccacgattggaccaagctgcgctatatggatagccata
cgccagcggacgcggagcgagccggattgcttgtaaggacttcagcaccgtgcacgatcaccgggaccatgggacactttatt
ctcgcccgatgcccgaaaggagagacgctgacagtgggatttacggacagcagaaagatcagccacacatgcacacacccg
ttccatcatgaaccacctgtgataggtagggagaggttccactctcgaccacaacatggtaaagagttaccttgcagcacgta
cgtgcagagcaccgctgccactgctgaggagatagaggtgcatatgcccccagatactcctgaccgcacgctgatgacgcag
cagtctggcaacgtgaagatcacagttaatgggcagacggtgcggtacaagtgcaactgcggtggctcaaacgagggactg
acaaccacagacaaagtgatcaataactgcaaaattgatcagtgccatgctgcagtcactaatcacaagaattggcaatac
aactcccctttagtcccgcgcaacgctgaactcggggaccgtaaaggaaagatccacatcccattccattggcaaacgtgac
ttgcagagtgccaaaagcaagaaaccctacagtaacttacggaaaaaaccaagtcaccatgctgctgtatcctgaccatccg
acactcttgtcttaccgtaacatgggacaggaaccaaattaccacgaggagtgggtgacacacaagaaggaggttaccttg
accgtgcctactgagggtctggaggtcacttggggcaacaacgaaccatacaagtactggccgcagatgtctacgaacggta
ctgctcatggtcacccacatgagataatcttgtactattatgagctgtaccccactatgactgtagtcattgtgtcggtggcctcg
ttcgtgcttctgtcgatggtgggcacagcagtgggaatgtgtgtgtgcgcacggcgcagatgcattacaccatatgaattaac
accaggagccactgttcccttcctgctcagcctgctatgctgcgtcagaacgaccaaggcggccacatattacgaggctgcggc
atatctatggaacgaacagcagccccctgttctggttgcaggctcttatcccgctggccgccttgatcgtcctgtgcaactgtctg
aaactcttgccatgctgctgtaagaccctggctttttagccgtaatgagcatcggtgcccacactgtgagcgcgtacgaacac
gtaacagtgatcccgaacacggtgggagtaccgtataagactcttgtcaacagaccgggttacagccccatggtgttggaga
tggagctacaatcagtcaccttggaaccaacactgtcacttgactacatcacgtgcgagtacaaaactgtcatccccctccccgt
acgtgaagtgctgtggtacagcagagtgcaaggacaagagcctaccagactacagctgcaaggtctttactggagtctaccc
atttatgtggggcggcgcctactgcttttgcgacgccgaaaatacgcaattgagcgaggcacatgtagagaaatctgaatctt
gcaaaacagagtttgcatcggcctacagagcccacaccgcatcggcgtcggcgaagctccgcgtcctttaccaaggaaacaa
cattaccgtagctgcctacgctaacggtgaccatgccgtcacagtaaaggacgccaagtttgtcgtgggcccaatgtcctccgc
ctggacacctttgacaacaaaatcgtggtgtacaaaggcgacgtctacaacatggactacccacctttggcgcaggaagac
caggacaatttggtgacattcaaagtcgtacaccggaaagtaaagacgtttatgccaacactcagttggtactacagaggcc
agcagcaggcacggtacatgtaccatactctcaggcaccatctggcttcaagtattggctgaaggaacgaggagcatcgcta
cagcacacggcaccgttcggttgccagattgcgacaaacccggtaagagctgtaaattgcgctgtggggaacataccaatttc
catcgacataccggatgcggcctttactagggttgtcgatgcaccctctgtaacggacatgtcatgcgaagtaccagcctgcac
tcactcctccgactttgggggcgtcgccatcatcaaatacacagctagcaagaaaggtaaatgtgcagtacattcgatgacca
acgccgttaccattcgagaagccgacgtagaagtagaggggaactcccagctgcaaatatccttctcaacagccctggcaag
cgccgagtttcgcgtgcaagtgtgctccacacaagtacactgcgcagccgcatgccaccctccaaaggaccacatagtcaatt
acccagcatcacacaccacccttggggtccaggatatatccacaacggcaatgtcttgggtgcagaagattacgggaggagt
aggattaattgttgctgttgctgccttaattttaattgtggtgctatgcgtgtcgtttagcaggcac

[0077] Regarding Chikungunya virus, another exemplary nucleotide sequence that encodes envelope is described below (SEQ ID No.:5):

Atgagtcttgccatcccagttatgtgcctgttggcaaacaccacgttcccctgctcccagccccctttgcacgccctgctgctacga
aaaggaaccggaggaaaccctacgcatgcttgaggacaacgtcatgagacctgggtactatcagctgctacaagcatcctta
acatgttctccccaccgccagcgacgcagcaccaaggacaacttcaatgtctataaagccacaagaccatacttagctcactgt
cccgactgtggagaagggcactcgtgccatagtcccgtagcactagaacgcatcagaaatgaagcgacagacgggacgctg
aaaatccaggtctccttgcaaatcggaataaagacggatgacagccacgattggaccaagctgcgttatatggacaaccaca
tgccagcagacgcagagagggcggggctatttgtaagaacatcagcaccgtgtacgattactggaacaatgggacacttcat
cctggcccgatgtccaaaagggaaactctgacggtgggattcactgacagtaggaagattagtcactcatgtacgcacccat
ttcaccacgaccctcctgtgataggtcgggaaaaattccattcccgaccgcagcacggtaaagagctaccttgcagcacgtacg
tgcagagcaccgccgcaactaccgaggagatagaggtacacatgcccccagacacccctgatcgcacattaatgtcacaaca
gtccggcaacgtaaagatcacagtcaatggccagacggtgcggtacaagtgtaattgcggtggctcaaatgaaggactaaca
actacagacaaagtgattaataactgcaaggttgatcaatgtcatgccgcggtcaccaatcacaaaaagtggcagtataact
cccctctggtcccgcgtaatgctgaacttggggaccgaaaaggaaaaattcacatcccgtttccgctggcaaatgtaacatgca
gggtgcctaaagcaaggaaccccaccgtgacgtacgggaaaaaccaagtcatcatgctactgtatcctgaccacccaacactc
ctgtcctaccggaatatgggagaagaaccaaactatcaagaagagtgggtgatgcataagaaggaagtcgtgctaaccgtg
ccgactgaagggctcgaggtcacgtggggcaacaacgagccgtataagtattggccgcagttatctacaaacggtacagccc
atggccacccgcatgagataattctgtattattatgagctgtaccccactatgactgtagtagttgtgtcagtggccacgttcat
actcctgtcgatggtgggtatggcagcggggatgtgcatgtgtgcacgacgcagatgcatcacaccgtatgaactgacaccag
gagctaccgtcccttcctgcttagcctaatatgctgcatcagaacagctaaagcggccacataccaagaggctgcgatatacc
tgtggaacgagcagcaaccttgttttggctacaagcccttattccgctggcagccctgattgttctatgcaactgtctgagactc
ttaccatgctgctgtaaaacgttggctttttttagccgtaatgagcgtcggtgcccacactgtgagcgcgtacgaacacgtaaca
gtgatcccgaacacggtgggagtaccgtataagactctagtcaatagacctggctacagccccatggtattggagatggaact
actgtcagtcactttggagccaacactatcgcttgattacatcacgtgcgagtacaaaaccgtcatcccgtctccgtacgtgaag
tgctgcggtacagcagagtgcaaggacaaaaacctacctgactacagctgtaaggtcttcaccggcgtctacccatttatgtgg
ggcggcgcctactgcttctgcgacgctgaaaacacgcagttgagcgaagcacacgtggagaagtccgaatcatgcaaaacag
aatttgcatcagcatacagggctcataccgcatctgcatcagctaagctccgcgtcctttaccaaggaaataacatcactgtaa
ctgcctatgcaaacggcgaccatgccgtcacagttaaggacgccaaattcattgtggggccaatgtcttcagcctggacaccttt
cgacaacaaaattgtggtgtacaaaggtgacgtctataacatggactacccgcccttggcgcaggaagaccaggacaattt
ggcgatatccaaagtcgcacacctgagagtaaagacgtctatgctaatacacaactggtactgcagagaccggctgtgggta
cggtacacgtgccatactctcaggcaccatctggctttaagtattggctaaaagaacgcggggcgtcgctgcagcacacagca
ccatttggctgccaaatagcaacaaacccggtaagagcggtgaactgcgccgtagggaacatgcccatctccatcgacatacc
ggaagcggccttcactagggtcgtcgacgcgccctctttaacggacatgtcgtgcgaggtaccagcctgcacccattcctcagac
tttgggggcgtcgccattattaaatatgcagccagcaagaaaggcaagtgtgcggtgcattcgatgactaacgccgtcactatt
cgggaagctgagatagaagttgaagggaattctcagctgcaaatctctttctcgacggccttagccagcgccgaattccgcgta
caagtctgttctacacaagtacactgtgcagccgagtgccacccccgaaggaccacatagtcaactacccggcgtcacatacc
accctcggggtccaggacatctccgctacggcgatgtcatgggtgcagaagatcacgggaggtgtgggactggttgttgctgtt
gccgcactgattctaatcgtggtgctatgcgtgtcgttcagcaggcac

[0078] Regarding Chikungunya virus, an exemplary nucleotide sequence that encodes a Capsid-envelope is described below (SEQ ID No.:6):

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

ttgcgcatgcttgaggacaacgtgatgagacccggatactaccagctactaaaagcatc
gctgacttgctctccccaccgccaaagacgcagtactaaggacaattttaatgtctata
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.

[0079] Regarding Chikungunya virus, another exemplary nucleotide sequence that encodes a Capsid-envelope is described below (SEQ ID No.:7):

atggagttcatcccaacccaaacttttacaataggaggtaccagcctcgaccctggac
tccgcgccctactatccaagtcatcaggcccagaccgcgccctcagaggcaagctgggc
aacttgcccagctgatctcagcagttaataaactgacaatgcgcgcggtaccacaacag
aagccacgcaggaatcggaagaataagaagcaaaagcaaaaacaacaggcgccacaaaa
caacacaaatcaaaagaagcagccacctaaaaagaaaccggctcaaaagaaaaagaagc
cgggccgcagagagaggatgtgcatgaaaatcgaaaatgattgtattttcgaagtcaag
cacgaaggtaaggtaacaggttacgcgtgcctggtgggggacaaagtaatgaaaccagc
acacgtaaaggggaccatcgataacgcggacctggccaaactggcctttaagcggtcat
ctaagtatgaccttgaatgcgcgcagatacccgtgcacatgaagtccgacgcttcgaag
ttcacccatgagaaaccggaggggtactacaactggcaccacggagcagtacagtactc
aggaggccggttcaccatccctacaggtgctggcaaaccaggggacagcggcagaccga
tcttcgacaacaagggacgcgtggtggccatagtcttaggaggagctaatgaaggagcc
cgtacagccctctcggtggtgacctggaataaagacattgtcactaaaatcacccccga
gggggccgaagagtggagtcttgccatcccagttatgtgcctgttggcaaacaccacgt
tcccctgctcccagccccttgcacgccctgctgctacgaaaaggaaccggaggaaaacc
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
accatgctgctgtaaaacgttggctttttagccgtaatgagcgtcggtgcccacactg
tgagcgcgtacgaacacgtaacagtgatcccgaacacggtgggagtaccgtataagact
ctagtcaatagacctggctacagccccatggtattggagatggaactactgtcagtcac
tttggagccaacactatcgcttgattacatcacgtgcgagtacaaaaccgtcatcccgt
ctccgtacgtgaagtgctgcggtacagcagagtgcaaggacaaaaacctacctgactac
agctgtaaggtcttcaccggcgtctacccatttatgtggggcggcgcctactgcttctg
cgacgctgaaaacacgcagttgagcgaagcacacgtggagaagtccgaatcatgcaaaa
cagaatttgcatcagcatacagggctcataccgcatctgcatcagctaagctccgcgtc
ctttaccaaggaaataacatcactgtaactgcctatgcaaacggcgaccatgccgtcac
agttaaggacgccaaattcattgtggggccaatgtcttcagcctggacacctttcgaca
acaaaattgtggtgtacaaaggtgacgtctataacatggactacccgcccttggcgca
ggaagaccaggacaatttggcgatatccaaagtcgcacacctgagagtaaagacgtcta

tgctaatacacaactggtactgcagagaccggctgtgggtacggtacacgtgccatact
ctcaggcaccatctggctttaagtattggctaaaagaacgcggggcgtcgctgcagcac
acagcaccatttggctgccaaatagcaacaaacccggtaagagcggtgaactgcgccgt
agggaacatgcccatctccatcgacataccggaagcggccttcactagggtcgtcgacg
cgccctctttaacggacatgtcgtgcgaggtaccagcctgcacccattcctcagacttt
gggggcgtcgccattattaaatatgcagccagcaagaaaggcaagtgtgcggtgcattc
gatgactaacgccgtcactattcgggaagctgagatagaagttgaagggaattctcagc
tgcaaatctctttctcgacggccttagccagcgccgaattccgcgtacaagtctgttct
acacaagtacactgtgcagccgagtgccacccccgaaggaccacatagtcaactaccc
ggcgtcacataccaccctcggggtccaggacatctccgctacggcgatgtcatgggtgc
agaagatcacgggaggtgtgggactggttgttgctgttgccgcactgattctaatcgtg
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 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 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 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 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, 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 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

(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 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 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 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

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.

[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.

[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 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, 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 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, 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 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.

[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. 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 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 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 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 Chikingunya or Venezuelan equine 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 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.

[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]

EXAMPLES

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

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)

Sggnpnanpnanpnanpnanpnanpnaggs (SEQ ID No.:46)

(Tccggaggaaacccgaatgccaatcccaacgcgaaccccaatgctaacccaaatgcca acccaaacgccaaccccaacgctggtggatcc) (SEQ ID No.:47)

VLP78 (25 repeat of NPNA amino acid sequence)

Sggnpnanpnanpnanpnanpnanpnvdpnanpnanpnanpnanpnanpnanpnanpna npnanpnanpnanpnanpnanpnanpnanpnanpnanpnanpnaggs (SEQ ID No.:48)

(tccggaggaaacccgaatgccaatcccaacgcgaaccccaacgctaaccccaacgcca atccgaatgcaaacccgaacgttgacccaaacgccaacccgaatgccaatcccaacgcg aaccccaatgctaacccaaatgccaacccaaacgccaaccccaacgctaatccaaacgc caaccctaacgccaatcccaacgcgaatcctaacgctaatcccaacgcaaatcccaatg ctaatccgaacgcgaaccctaatgcaaaccccaacgccaacccgaacgctaacccgaac gctaatcccaacgccggtggatcc) (SEQ ID No.:49)

[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.

[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 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 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 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.

[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 H_2SO_4 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 Week 2	After 2nd immunization 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, 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 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

[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/μl. 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 Venezuelan equine encephalitis virus (VEEV) like particle comprising 6x NPNA was prepared according to Example 2. To prepare a vaccine composition, 80μg 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.

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

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.

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

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.

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

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 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 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 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.

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, 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 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.

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 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 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 inducing and/or enhancing immune response against a malaria antigen in a mammal, comprising administering an effective amount of the composition of any 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 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 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 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.

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 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.

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

(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 (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 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 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 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.

Fig. 1

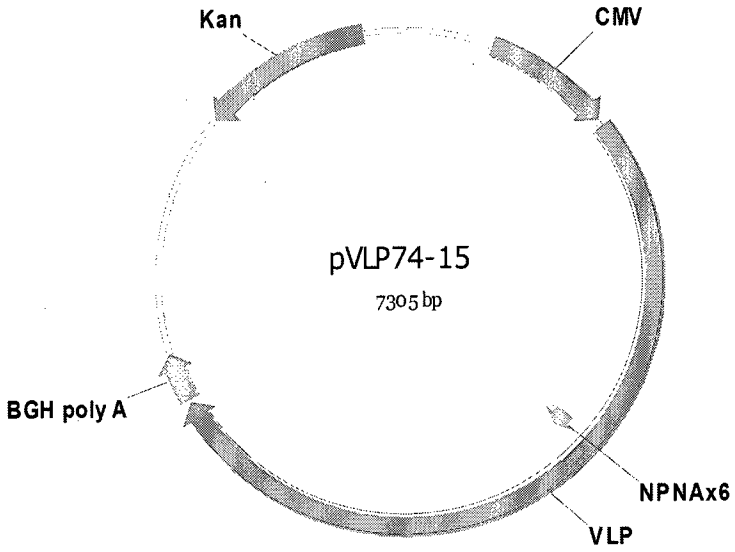

Kan
CMV
pVLP74-15
7305 bp
BGH poly A
NPNAx6
VLP

Fig. 2

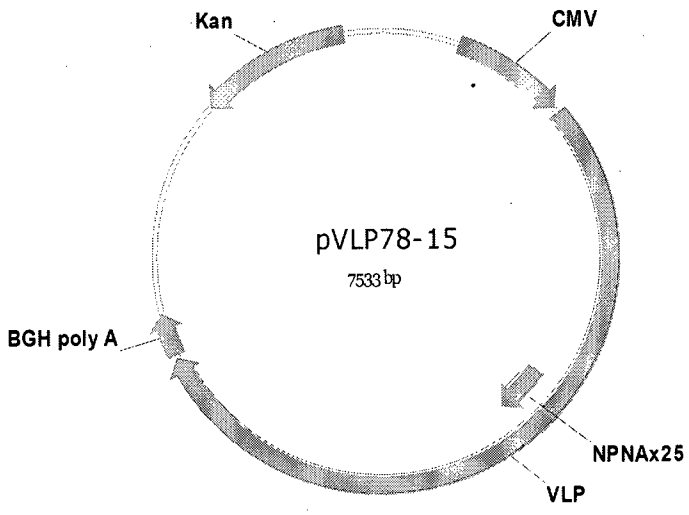

Kan
CMV
pVLP78-15
7533 bp
BGH poly A
NPNAx25
VLP

Fig. 3

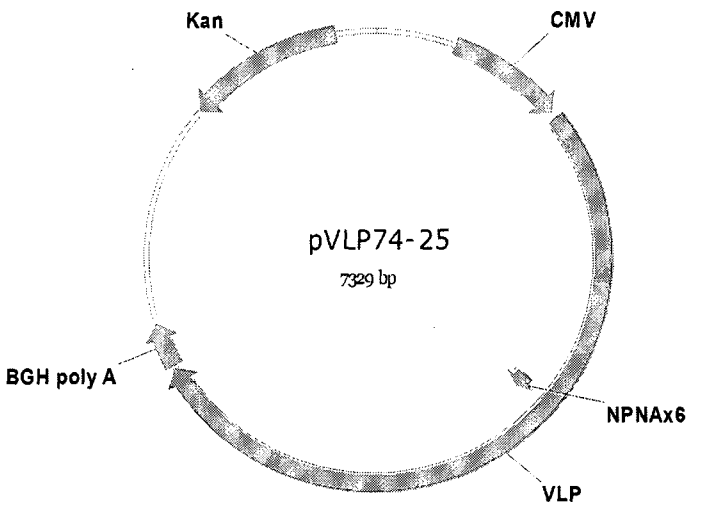

Kan
CMV
pVLP74-25
7329 bp
BGH poly A
NPNAx6
VLP

Fig.4

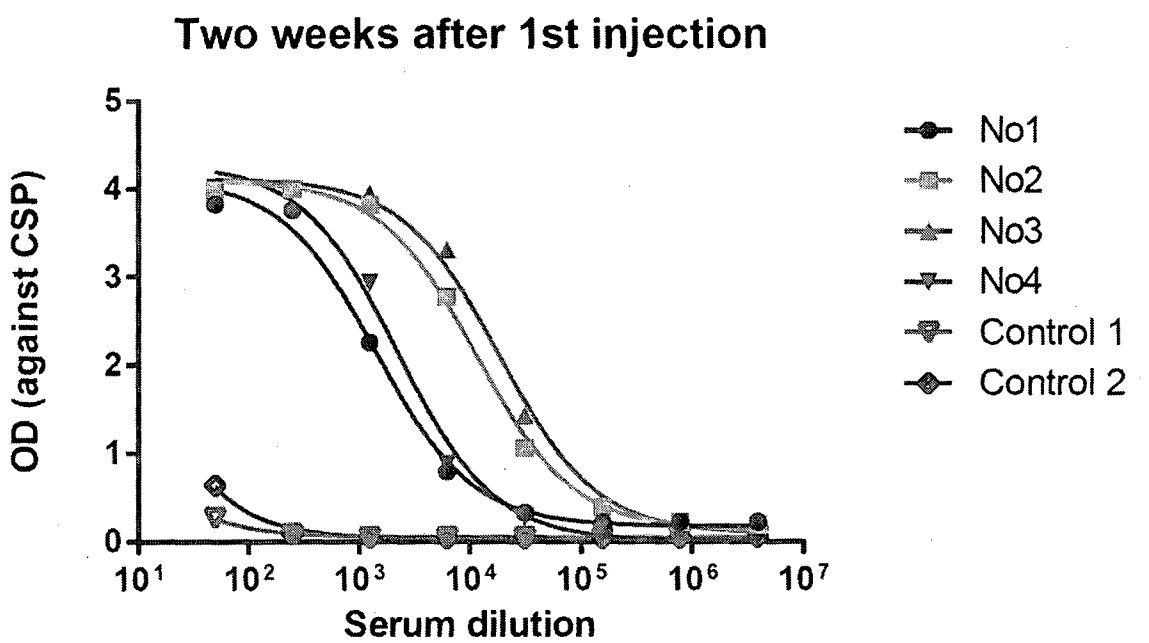

Two weeks after 1st injection
OD (against CSP)
5
4
3
2
1
0
10¹
10²
10³
10⁴
10⁵
10⁶
10⁷
Serum dilution
No1
No2
No3
No4
Control 1
Control 2

Fig. 5

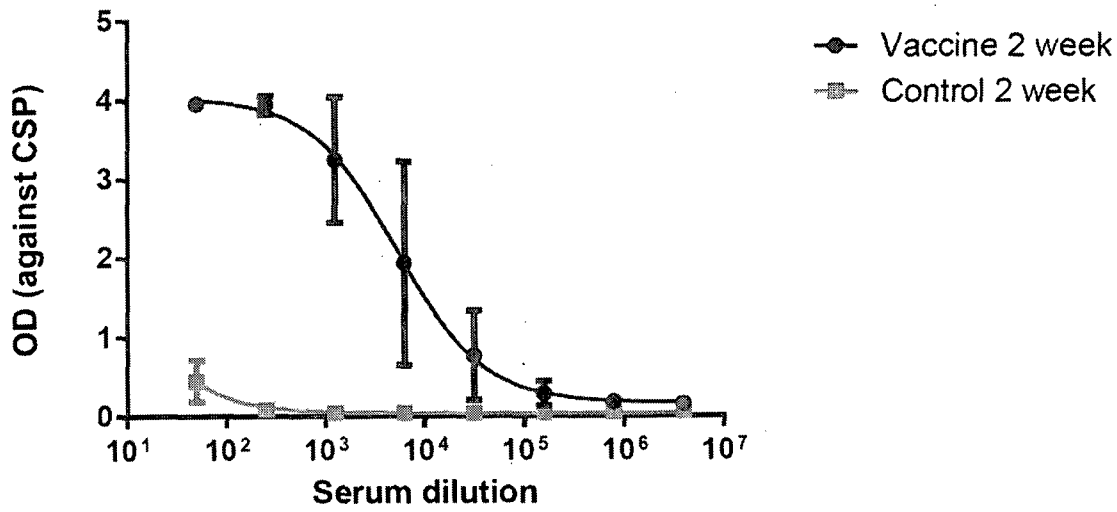

OD (against CSP)
Serum dilution
Vaccine 2 week
Control 2 week

Fig.6

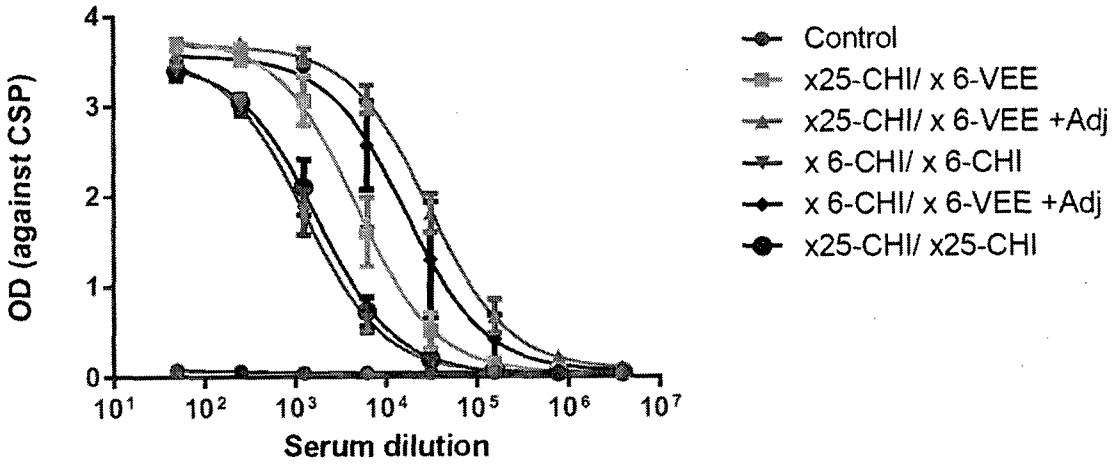

OD (against CSP)
Serum dilution
Control
x25-CHI/ x 6-VEE
x25-CHI/ x 6-VEE +Adj
x 6-CHI/ x 6-CHI
x 6-CHI/ x 6-VEE +Adj
x25-CHI/ x25-CHI

Fig. 7

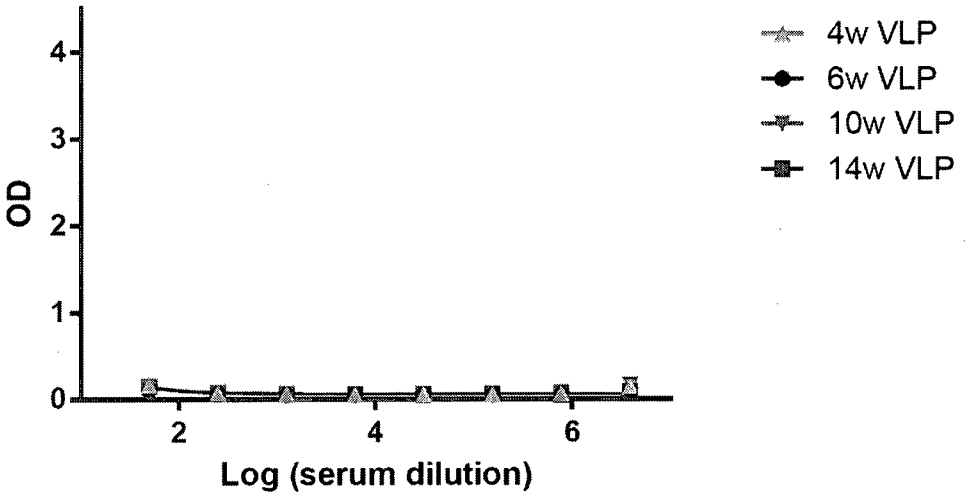

4w VLP
6w VLP
10w VLP
14w VLP
OD
0
1
2
3
4
2
4
6
Log (serum dilution)

Fig. 8

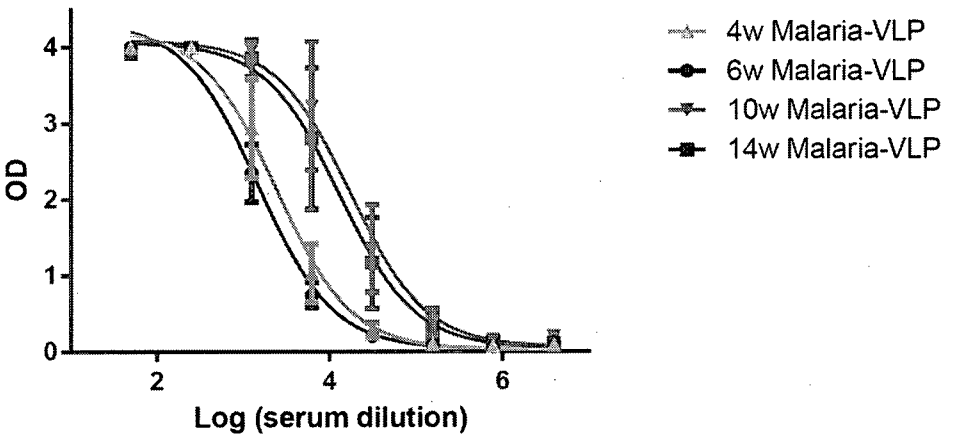

4w Malaria-VLP
6w Malaria-VLP
10w Malaria-VLP
14w Malaria-VLP
OD
0
1
2
3
4
2
4
6
Log (serum dilution)

Fig. 9

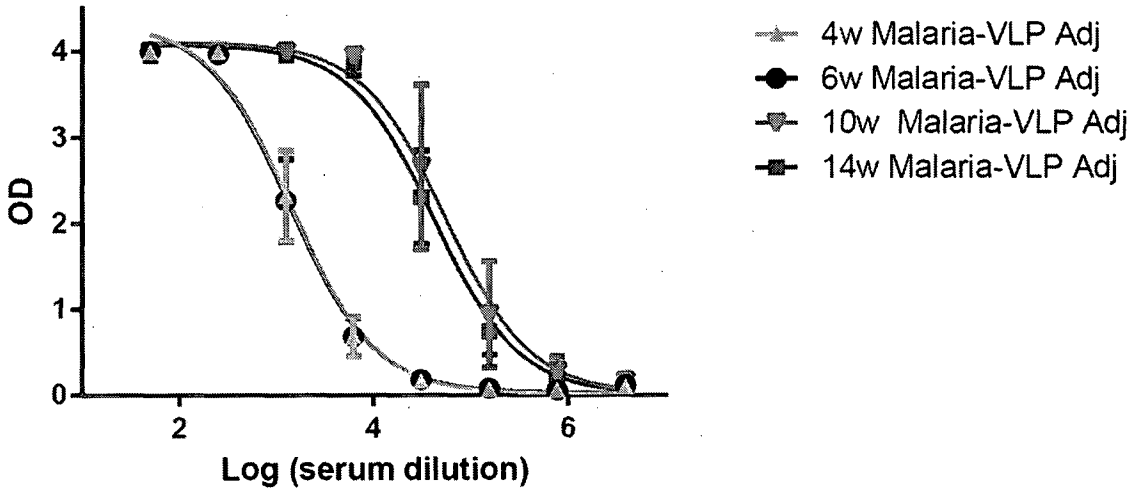


Figure 10

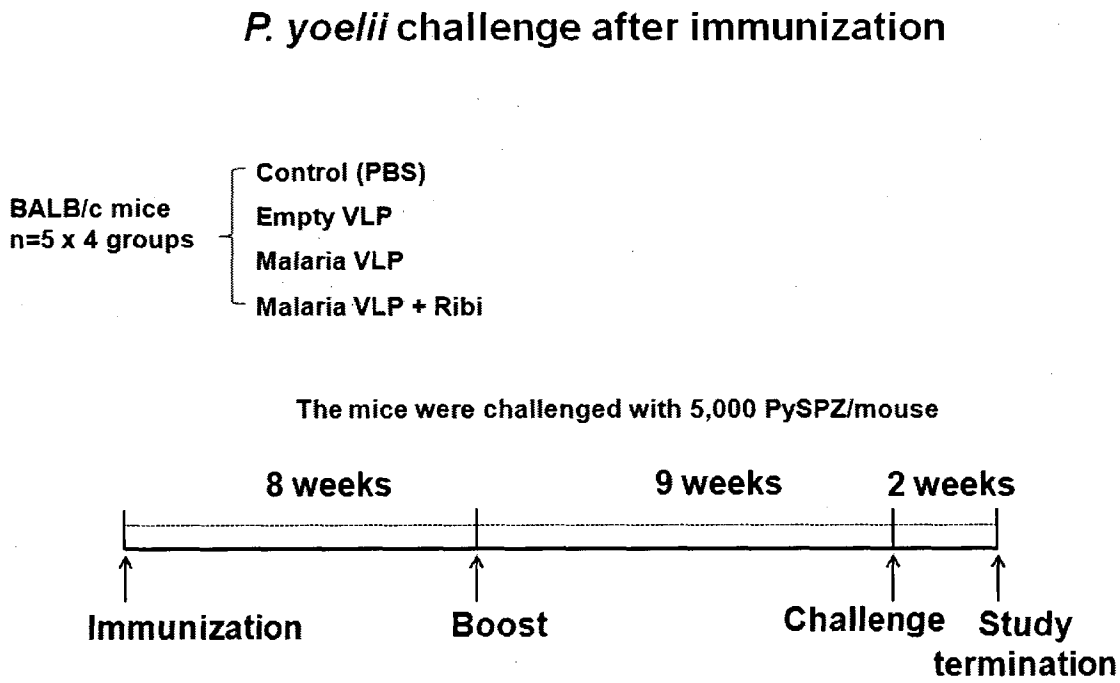
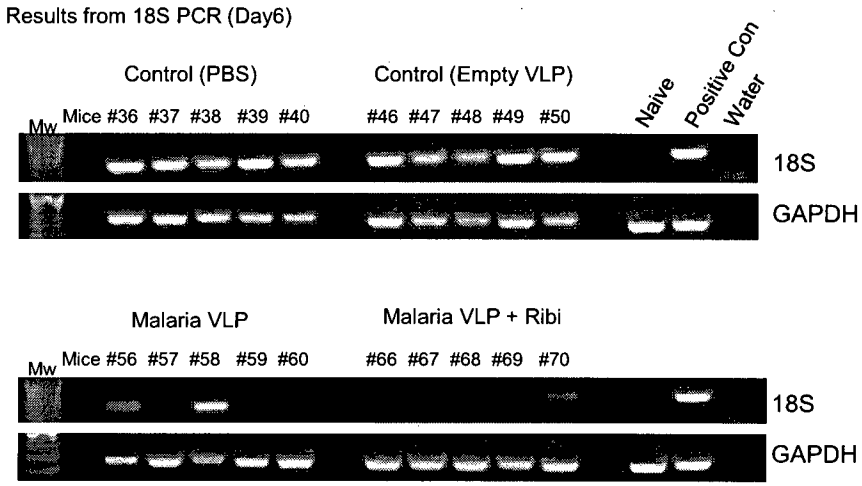


Figure 11


Results from 18S PCR (Day6)
Control (PBS)
Control (Empty VLP)
Naive
Positive Con
Water
Mw
Mice #36 #37 #38 #39 #40
#46 #47 #48 #49 #50
18S
GAPDH
Malaria VLP
Malaria VLP + Ribi
Mw
Mice #56 #57 #58 #59 #60
#66 #67 #68 #69 #70
18S
GAPDH

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2014/065166

A. CLASSIFICATION OF SUBJECT MATTER

Int.Cl. C12N15/09(2006.01)i, A61K39/015(2006.01)i, C07K14/18(2006.01)i, C07K14/445(2006.01)i, C07K19/00(2006.01)i, C12N7/00(2006.01)i

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Int.Cl. C12N15/09, A61K39/015, C07K14/18, C07K14/445, C07K19/00, C12N7/00

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

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

CAplus/MEDLINE/WPIDS/BIOSIS(STN), JSTPlus/JST7580(JDreamIII)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	RODRIGUEZ D et al., Vaccine efficacy against malaria by the combination of porcine parvovirus-like particles and vaccinia virus vectors expressing CS of Plasmodium, PLoS One, 2012.04.17, Vol.7, No.4, e34445, doi:10.1371/journal.pone.0034445	1, 6, 12, 13, 19, 21, 24-27, 38-40 1-28, 36-44
X Y	OLIVEIRA GA et al., Safety and enhanced immunogenicity of a hepatitis B core particle Plasmodium falciparum malaria vaccine formulated in adjuvant Montanide ISA 720 in a phase I trial, Infect. Immun., 2005, Vol.73, No.6, pp.3587-3597	1, 6, 12, 13, 19, 21, 24-27 1-28, 36-44

☑ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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Date of the actual completion of the international search	Date of mailing of the international search report
02.09.2014	16.09.2014

Name and mailing address of the ISA/JP	Authorized officer	
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2014/065166

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	WO 2011/035004 A1 (FRAUNHOFER USA INC.) 2011.03.24, & US 2012/0219579 A1 & EP 2477650 A1	1, 6, 12, 13, 21, 24-27 1-28, 36-44
X Y	WO 2008/025067 A1 (HEPGENICS PTY LTD) 2008.03.06, & US 2010/0120092 A1	1, 6, 12, 13, 21, 24-27 1-28, 36-44
Y	WO 2012/006180 A1 (THE UNITED STATES OF AMERICA, AS REPRESENTED BY THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES) 2012.01.12, (no family)	1-28, 36-44
Y	WO 2012/106356 A2 (GOVERNMENT OF THE USA, as represented by THE SECRETARY, DEPARTMENT OF HEALTH & HUMAN SERVICES) 2012.08.09, (no family)	1-28, 36-44
P,X P,Y	JONES RM et al., A plant-produced Pfs25 VLP malaria vaccine candidate induces persistent transmission blocking antibodies against Plasmodium falciparum in immunized mice, PLoS One, 2013.11.18, Vol.8, No.11, e79538, doi: 10.1371/journal.pone.0079538	1, 6, 12, 13, 21, 24-27 1-28, 36-44
P,Y	WO 2013/122262 A1 (VLP THERAPEUTICS, LLC) 2013.08.22, & US 2013/0251744 A1	1-28, 36-44
A	CROMPTON PD et al., Advances and challenges in malaria vaccine development, J. Clin. Invest., 2010, Vol.120, No.12, pp.4168-4178	1-28, 36-44
A	GHASPARIAN A et al., Engineered synthetic virus-like particles and their use in vaccine delivery, Chembiochem, 2011, Vol.12, No.1, pp.100-109	1-28, 36-44
A	ALLSOPP CE et al., Comparison of numerous delivery systems for the induction of cytotoxic T lymphocytes by immunization, Eur. J. Immunol., 1996, Vol.26, No.8, pp.1951-1959	1-28, 36-44

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2014/065166

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GILBERT SC et al., A protein particle vaccine containing multiple malaria epitopes, Nat. Biotechnol., 1997, Vol.15, No.12, pp.1280-1284	1-28, 36-44
A	RODRIGUES M et al., Influenza and vaccinia viruses expressing malaria CD8+ T and B cell epitopes. Comparison of their immunogenicity and capacity to induce protective immunity, J. Immunol., 1994, Vol.153, No.10, pp.4636-4648	1-28, 36-44
A	PFEIFFER B et al., A virosome-mimotope approach to synthetic vaccine design and optimization: synthesis, conformation, and immune recognition of a potential malaria-vaccine candidate, Angew. Chem. Int. Ed., 2003, Vol.42, No.21, pp.2368-2371	1-28, 36-44
A	DOBANO C et al., Alphavirus replicon particles are highly immunogenic in the murine malariamodel by homologous or heterologous immunization, Open Vaccine Journal, Vol.1, 2008, pp.27-37	1-28, 36-44
A	LECHNER F et al., Virus-like particles as a modular system for novel vaccines, Intervirology, 2002, Vol.45, No.4-6, pp.212-217	1-28, 36-44
A	AKAHATA W et al., A virus-like particle vaccine for epidemic Chikungunya virus protects nonhuman primates against infection, Nat. Med., 2010, Vol.16, No.3, pp.334-338	1-28, 36-44

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2014/065166

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

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

1. ☑ Claims Nos.: 29-35, 37(partially)
because they relate to subject matter not required to be searched by this Authority, namely:

The subject matter of claims 29-35, 37(partially) relates to a method for treatment of the human or animal body by surgery or therapy, which does not require an international search by the International Searching Authority in accordance with PCT Article 17(2)(a)(i) and [Rule 39.1(iv)].

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

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

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

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

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