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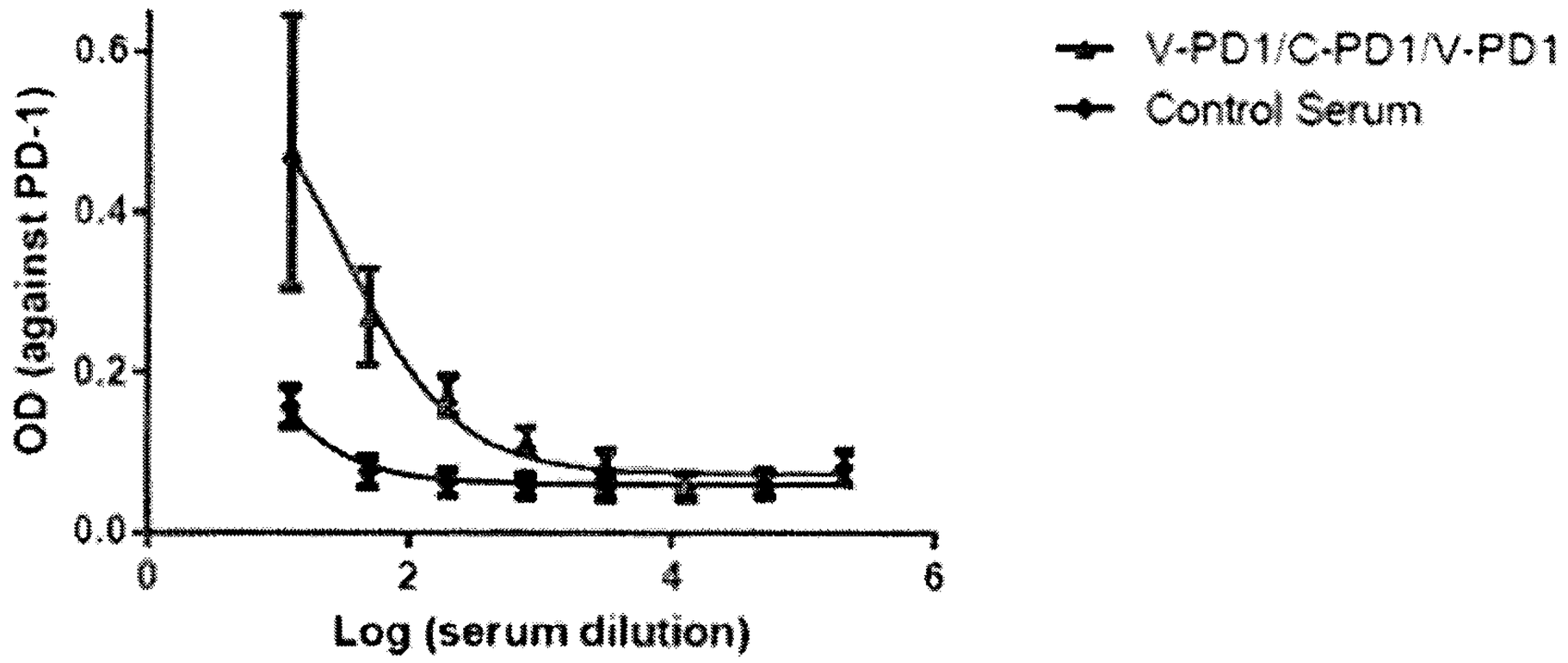
(54) **Titre : PARTICULE DE TYPE VIRUS COMPRENANT L'ANTIGENE PD-1 OU L'ANTIGENE DE LIGAND PD-1**
(54) **Title: VIRUS LIKE PARTICLE COMPRISING PD-1 ANTIGEN OR PD-1 LIGAND ANTIGEN**

Figure 1



(57) **Abrégé/Abstract:**
The present invention provides a virus like particle comprising a virus structural protein and an antigen derived from PD-1 or a ligand of PD-1, and a composition or kit comprising thereof, its use in immune response etc.

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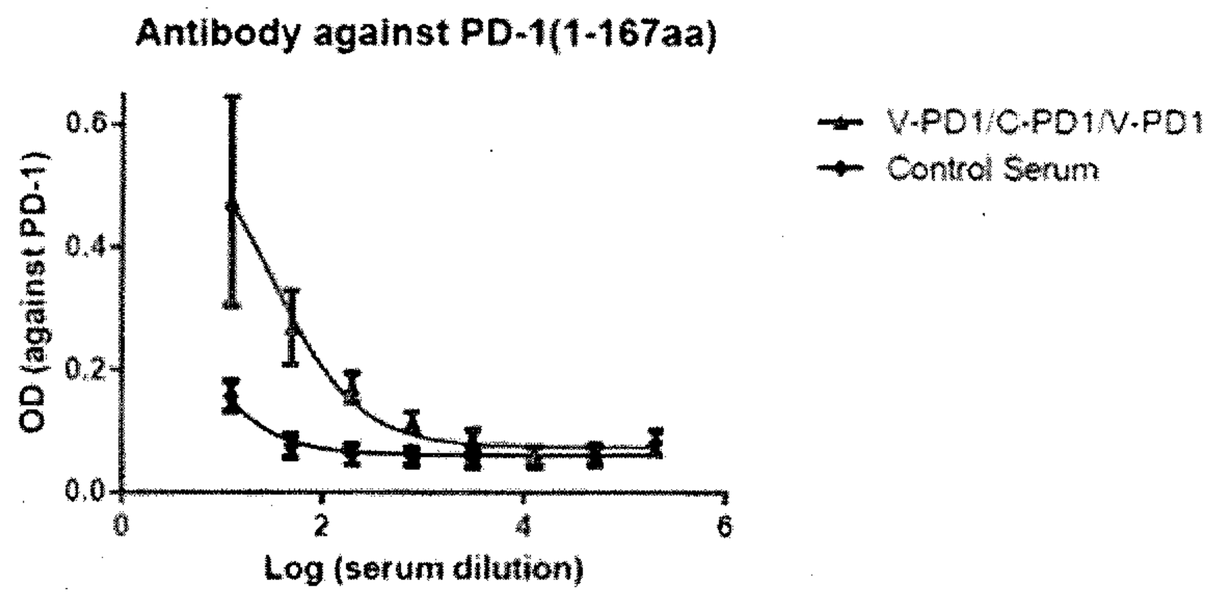
(54) Title: VIRUS LIKE PARTICLE COMPRISING PD-1 ANTIGEN OR PD-1 LIGAND ANTIGEN

Figure 1



(57) Abstract: The present invention provides a virus like particle comprising a virus structural protein and an antigen derived from PD-1 or a ligand of PD-1, and a composition or kit comprising thereof, its use in immune response etc.

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DESCRIPTION

Virus like particle comprising PD-1 antigen or PD-1 ligand antigen

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application No.: 61/845,712 filed on July 12, 2013, the entire contents of which are incorporated by reference herein.

TECHNICAL FIELD

[0002] The present invention relates to a virus like particle comprising a virus structural protein and an antigen derived from PD-1 or a ligand of PD-1, and a composition or a kit comprising thereof, its use in immune response etc.

BACKGROUND ART

[0003] Programmed Cell Death 1 or PD-1 (also referred to as PDCD1) is a 50 to 55 kDa type I transmembrane receptor of the CD28 superfamily that negatively regulates T cell antigen receptor signaling by interacting with the specific ligands and is suggested to play a role in the maintenance of self-tolerance.

[0004] PD-1 antigen relates to almost every aspect of immune responses including autoimmunity, tumor immunity,

infectious immunity, transplantation immunity, allergy and immunological privilege.

[0005] PD-1 is expressed on the surface of activated T cells, B cells, and macrophages, (Y. Agata et al., International Immunology vol.8, No. 5 p765-772, 1996) suggesting that compared to CTLA-4 that also plays an important regulatory role in the immune system, PD-1 more broadly negatively regulates immune responses.

[0006] In general, a need exists to provide safe and effective therapeutic methods for immune disorders such as, for example, autoimmune diseases, inflammatory disorders, allergies, transplant rejection, cancer, immune deficiency, and other immune system-related disorders. Modulation of the immune responses involved in these disorders can be accomplished by manipulation of the PD-1 pathway.

[0007] PD-1 has two ligands, PD-L1 (Programmed Death Ligand for PDCD1L1 or B7-H1) and PD-L2 (Programmed Death Ligand 2 or PDCD1L2 or B7-DC), which are members of the B7 family ligands.

[0008] In one approach, blocking the interaction of PD-1 as well as its ligand (PD-L1, PD-L2 or both) may provide an effective way for tumor and viral immunotherapy.

[0009] US 5,629,204 and US 5,698,520 relate to a membrane protein related to human PD-1 and DNA encoding the said protein, and indicates that PD-1 protein may be

useful for the treatment of various infections, immunological depression or acceleration, or tumors etc.

[0010] US 7,595,048 and US 2011/0081341 relate to immunopotentiation characterized by inhibiting immunosuppressive signals induced by PD-1, PD-L1 or PD-L2, compositions for cancer or infection treatment, and therapies that use them.

[0011] Several big pharmaceutical companies are pitting their PD-1 antibodies against each other in a race to be first to market. Here are 3 monoclonal antibodies such as Merck's Lambrolizumab (MK-3475) which is a humanized monoclonal IgG4 antibody that acts against PD-1, Bristol-Myers Squibb's Nivolumab (BMS-936558) which is fully anti human PD-1 antibody and Roche Genentech's MPDL3280A which doesn't block PD-1, but rather blocks PD-L1.

[0012] However, more potent and safe compound targeting PD-1 is strongly desired.

[0013] 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).

[0014] 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 proteins 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 INVENTION

[0015] In a first aspect, the present invention provides a particle comprising a virus structural protein and an antigen selected from the group consisting of an antigen derived from PD-1 and an antigen derived from a ligand of PD-1.

[0016] In a second aspect, the present invention provides a nucleic acid molecule comprising a nucleotide sequence for expressing a particle which comprises a virus structural protein and an antigen selected from the group consisting of an antigen derived from PD-1 and an antigen derived from a ligand of PD-1.

[0017] In a third aspect, the present invention provides a pharmaceutical composition and a kit comprising a pharmaceutical composition, wherein the pharmaceutical composition comprises a particle comprising a virus

structural protein and an antigen selected from the group consisting of an antigen derived from PD-1 and an antigen derived from a ligand of PD-1; or a nucleic acid molecule comprising a nucleotide sequence for expressing a particle which comprises a virus structural protein and an antigen selected from the group consisting of an antigen derived from PD-1 and an antigen derived from a ligand of PD-1; and a pharmaceutically acceptable carrier.

[0018] In a forth aspect, the present invention provides a use of a particle comprising a virus structural protein and an antigen selected from the group consisting of an antigen derived from PD-1 and an antigen derived from a ligand of PD-1; or a nucleic acid molecule comprising a nucleotide sequence for expressing a particle which comprises a virus structural protein and an antigen selected from the group consisting of an antigen derived from PD-1 and an antigen derived from a ligand of PD-1 for the manufacture of a pharmaceutical composition or a kit for modulating an immuno response, treating cancer or treating an infectious disease in a subject.

BRIEF DESCRIPTION OF DRAWINGS

[0019] Figure 1 shows results of ELISA where an antibody which binds to N-terminal PD-1 (1-167aa) was detected.

Figure 2 shows results of ELISA where an antibody which binds to PD-1-Fc was detected.

DESCRIPTION OF EMBODIMENT

[0020]

(1) Particle comprising a virus structural protein and an antigen selected from the group consisting of an antigen derived from PD-1 and an antigen derived from a ligand of PD-1

In a first aspect, the present invention provides a particle comprising a virus structural protein and an antigen selected from the group consisting of an antigen derived from PD-1 and an antigen derived from a ligand of PD-1.

A derivative of the above-described particle which can be prepared by modulating the above-described particle is also provided by the present invention. Examples of the modification include, but are not limited to, addition, deletion or replacement of one or more amino acid residues.

[0021] The particle provided by the present invention may be a particle which consists of or comprises i) at least one virus structural protein and ii) at least one antigen derived from PD-1 or at least one antigen derived from a ligand of PD-1. The at least one virus structural protein may consist of one or more kinds of protein or peptide and spontaneously assembled to form the particle provided by

the present invention. In one embodiment, the particle provided by the present invention has a diameter of at least 10nm, for example, at least 20nm, preferably at least 50nm. In one embodiment, molecular weight of the particle is from 100 kDa to 100,000 kDa, preferably from 400kDa to 30,000kDa.

[0022] Preferably, a virus structural protein used for the present invention may be a virus structural protein derived from Alphavirus or Flavivirus. Thus, the particle provided by the present invention may be a virus like particle including 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.

[0023] Virus structural protein may be a capsid protein, an envelope protein, a fragment thereof or a complex thereof. Thus, virus structural protein used for the present invention may consist of or comprise a capsid protein and/or an envelope protein and/or a fragment thereof. In one embodiment, the virus like particle provided by the present invention consists of or comprises capsid, E2 and E1. An antigen may be inserted into E2. For example, the virus like particle provided by the present invention may be formed by assembling 240 capsids, 240 E1 proteins and 240 E2 proteins where a PD-1 antigen is inserted into each of E2 proteins.

[0024] As used herein, the term "PD-1 antigen" refers to an antigen derived from PD-1. Preferably, PD-1 is a human PD-1. An antigen derived from PD-1 may be a fragment of PD-1 or a derivative of a fragment of PD-1.

[0025] As used herein, the term "PD-1 ligand antigen" refers to an antigen derived from a ligand of PD-1. Examples of a ligand of PD-1 include, but are not limited to, PD-L1 and PD-L2. Preferably, a ligand of PD-1 is human PD-L1 or human PD-L2. An antigen derived from PD-L1 may be a fragment of PD-L1 or PD-L2; or a derivative of a fragment of PD-L1 or PD-L2.

[0026] A fragment of PD-1, PD-L1 or PD-L2 for use in an antigen contained in the particle provided by the present invention may be selected based on the amino acid sequence of PD-1, PD-L1 or PD-L2 and/or tertiary structure thereof.

[0027] For example, a fragment for use in the antigen may consist of or comprise a fragment located in the surface of PD-1, PD-L1 or PD-L2. Preferably, an antibody against an antigen contained in the particle provided by the present invention blocks an interaction between PD-1 and PD-L1 or PD-1 and PD-L2. A fragment of PD-1, PD-L1 or PD-L2 for use in the antigen may be 10-300 amino acid residues (e.g. 10-120, 10-30 or 15-30 amino acid residues) in length.

[0028] In one embodiment, a fragment for use in the antigen may be selected 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, an 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, a spatial distance between 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).

[0029] Examples of a fragment of PD-1 for use in the antigen include, but are not limited to, lnwyrmspsnqtdklaaf (SEQ ID No.:4), mlnwyrmspsnqtdklaafs (SEQ ID No.:5), vlnwyrmspsnqtdklaafp (SEQ ID No.:6), gaislhpkakiees (SEQ ID No.:7), cgaislhpkakieec (SEQ ID No.:8), VLNWYRMSPSNQTDKLAAF (SEQ ID No.:9), GAISLAPKAQIKES (SEQ ID No.:10), RNDSGTYLCGAISLAPKAQIKESLRAELRVT (SEQ ID No.:11) and RNDSGIYLCGAISLHPKAKIEESPGAELVVT (SEQ ID No.:12). Examples of a fragment of PD-L1 for use in the antigen include, but are not limited to, ciisyggadyc (SEQ ID No.:13), CMISYGGADYC (SEQ ID No.:14), LQDAGVYRCMISYGGADYKRITVKVN (SEQ ID No.:15), LQDAGVYRAMISYGGADYKRITVKVN (SEQ ID No.:16), DLAALIVYWEMEDKNIIQFVH (SEQ ID No.:17), DLAALIVYWEMEDKNIIQFVHGG (SEQ ID No.:18), FTVTVPKDLYVVEYGSNMTIECKFPVE (SEQ ID No.:19), Lqdagvycciisyggadykritlkvn (SEQ ID No.: 20), lqdagvyaaiisyggadykritlkvn (SEQ ID No.:21),

dllalvvywekedeqviqfva (SEQ ID No.: 22), dllalvvywekedeqviqfvagg (SEQ ID No.: 23) and ftitapkdlyvveygsnvtmecrfpve (SEQ ID No.: 24).

[0030] A derivative of a fragment of PD-1, PD-L1 or PD-L2 may be prepared by addition, deletion or replacement of one or several amino acid residues in the fragment of PD-1, PD-L1 or PD-L2. In one embodiment, a derivative of a fragment of PD-1, PD-L1 or PD-L2 has at least 70%, 75%, 80%, 85%, 90%, 95% or 98% amino acid sequence identity to the corresponding fragment of a naturally occurring PD-1, PD-L1 or PD-L2. In one embodiment, a derivative of a fragment of PD-1, PD-L1 or PD-L2 is a mutant where at most 10% of the amino acids are deleted, substituted, and/or added based on the corresponding fragment of naturally occurring PD-1, PD-L1 or PD-L2.

[0031] In the particle as provided by the present invention, a virus structural protein and an antigen may be linked through at least one first attachment site which is present in the virus structural protein and at least one second attachment site which is present in the antigen.

[0032] As used herein, each of "a first attachment site" and "a second attachment site" refers to a site where more than one substance is linked each other.

In one embodiment, a virus structural protein and an antigen are directly fused. In one embodiment, one or two

linkers may intervene between N-terminal residue of an antigen and a virus structural protein and/or between C-terminal residue of an antigen and a virus structural protein.

[0033] An antigen or a virus structural protein can be truncated and replaced by short linkers. In some embodiments, an antigen or a virus structural protein 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.

[0034] In one embodiment, a nucleic acid molecule, in which polynucleotide encoding the virus structural protein 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 virus structural protein 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 protein or antigen. For example, the first attachment site is modified from the virus structural protein so that through a linker peptide including SG, GS, SGG, GGS and SGSG, the protein is conjugated with the antigen.

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

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

[0035] Preferably, an antigen may be linked to the Chikungunya virus structural protein or Venezuelan equine encephalitis virus structural protein as a fusion protein produced by way of genetic engineering.

[0036] A Chikungunya virus structural protein or Venezuelan equine encephalitis virus structural protein used in the present invention may be a Chikungunya or Venezuelan equine encephalitis virus envelope protein or a capsid or a complex of one or more envelope proteins and/or a capsid protein.

[0037] 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.

[0038] Chikungunya virus structural protein or Venezuelan equine encephalitis virus structural protein used in the present invention may naturally occurring virus

structural protein or modified protein thereof. The modified protein may be a fragment of the naturally occurring virus structural protein. In one embodiment, the modified protein 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 protein 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 protein used in the present invention.

[0039] Chikungunya or Venezuelan equine encephalitis virus structural protein may consist of or comprise a capsid, E2 and El.

[0040] Examples of Chikungunya virus structural protein include, but are not limited to, Capsid-E2-E1 of Chikungunya virus Strain 37997, and Capsid-E2-E1 of Chikungunya virus LR2006 OPY-1.

[0041] Examples of Venezuelan equine encephalitis virus structural protein include, but are not limited to, Capsid-E2-E1 of Venezuelan equine encephalitis virus Strain TC-83.

[0042] An exemplary Chikungunya virus structural protein sequence is provided at Genbank Accession No.

ABX40006.1, which is described below (SEQ ID No.:1):

mefiptqtfynrryqprpwtprptiqvirprprpqrqaggflaqlisavnkltmravpqq
kprrnrknkkqkqkqqapqnntnqkkqppkkkpaqkkkkpgrrermcmkiendcifevk
hegkvtgyaclvgdkvmkpahvkgtidnadlaklafkrsskydlecaqipvhmksdask
fthekpegyynwhhgavqysggrftiptgagkpgdsgrpifdnkgrvvaivlgganega
rtalsvvtwnkdivtkitpegaeewslalpvmclianttfpcsqppctpccyekepeet
lrmlednvmrpgyyqllqasltcsphrqrrstkdnfnvykatrpylahcpdcgeghsch
spvaleirneatdgtlkiqvslqigiktddshdwtklrymdnhmpadaeraglfvrts
apctitgtmghfilarcpkgetltvgftdsrkishscthpfhhdppvigrekfhsrpqh
gkelpcstyvqstaatteelevhmppdtpdrtlmsqqsgnvkitvngqtvrykcncggs
negltttdkvinnckvdqchaavtnhkkwqynsplvprnaelgdrkgkihipfplanvt
crvpkarnptvtygknqvimllypdhptllsyrnmgeepnyqeewvmhkkevvltvpte
glevtwgnnepykywpqlstngtahghpheiillyyyelyptmtvvvvsvatfillsmvg
maagmcmcarrrcitpyeltpgatvpfllsliccirtakaatyqeaalylwneqqplfw
lqaliplaalivlcnclrllpccckktlaflavmsvgahtvsayehvtvipntvgvpykt
lvnrpgyspmvlemellsvtleptlsldyitceyktvipspyvkccgtaeckdknlpdy
sckvftgvypfmwggaycfcdaentqlseahvekseschtefasayrahtasasaklrv
lyqgnnitvtayangdhavtvkdakfivgpmssawtpfdnklvvykgdvynmdyppfga
grpgqfgdiqsrtpeskdvyantqlvlqrpavgtvhvpysqapsgfkywlkergaslqh
tapfgcqiatnpvtavncavgmmpisidlpeaaftrvvdapsltdmscevpacthssdf
ggvafikyaaskkgkcavhsmtnavtireaelevegnsqlqisfstalasaefrvqvcs
tqvhcaaechppkdhivnypashttlgvqdisatamswvqkitggvglvvavaalliiv
vlcvsfsrh

[0043] Another exemplary Chikungunya virus structural protein sequence is provided at Genbank Accession No. ABX40011.1, which is described below (SEQ ID No.:2):

mefiptqtfynrryqprpwaprptiqvirprprpqrqaggqlaqlisavnkltmravpqq
kprrnrknkkqrqkkqapqndpkqkkqppqkkpaqkkkkpgrrermcmkiendcifevk

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

[0044] An exemplary Venezuelan equine encephalitis

virus structural protein 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
gpsaakpkkeasqkqkgggqgkkkknqgkkkaktgppnpkaqngnkkktnkkpgkrqrm
vmklesdktfpimlegkingyacvvggklfrpmhvegkidndvlaalktkkaskydley
advpqnmradtfkythekpqgyyswhhgavqyengrfttvpkgvgakgdsgrpildnggr
vvaivlggvnegsrtalsvvmwnekgvtvkytpenceqwslvttmcllanvtfpcaqpp
icydrkpaetlamlsvnvdnpgydelleaavkcpgrkrrsteelfneykltrpymarci
rcavgschspiaieavksdghdgyvrlqtssqyqldssgnlkgrtmrydmhgtikeipl
hqvslytsrpchivdghgyfllarcpagdsitmefkkdsvrhscsvpyevkfnpvgrel
ythppehgveqacqvyahdaqnrgayvemhlpgsevdsslvslsgssvtvtppdgtsal
vececggtkisetinktkqtsqctkkeqcrayrlqndkwvynsdklpkaagatlkgklh
vpflladgkctvplapepmitfgfrsvslklhpknptylitrqladephythelisepa
vrnftvtekgwefvwgnhppkrfwaqetapgnphglphevithyyhrypmstilglsic
aalatvsvaastwlfcrsrvacltpyrltpnaripfclavlccartaraettwesldhl
wnnhqqmfwiqlliplaalivvtrllrcvccvvpflvmagaagagayehattmpsqagi
syntivnragyaplpisitptkikliptvnleyvtchyktgmdspaikccgsqectpty
rpdeqckvftgvypfmwggaycfcdtentqvskayvmksddcladhaeaykahtasvqa
flnitvgehsivttvyvngetpvnfngvkitagplstawtpfdrkivqyageiymydfp
eygagqpgafgdiqsrtvsssdlyantnlvlqrpkagaihvpytqapsgfeqwkkdkap
slkftapfgceiytnpiraencavgsiplafdipdalfttrvsetptlsaaectlnecvy
ssdfggiatvkysasksgkcavhvpsgtatlkeaavelteqgsatihfstanihpefrl
qictsyvtckgdchppkdhivthpqyhaqtftaavsktawtwltsllggsavliiiglv
lativamyvltnqkhn.

[0045] 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.

[0046] According to the present invention, a Chikungunya virus like particle or Venezuelan equine encephalitis virus like particle comprising a Chikungunya or Venezuelan equine encephalitis virus structural protein and an antigen selected from the group consisting of an antigen derived from PD-1 and an antigen derived from a ligand of

PD-1 (e.g. PD-L1, PD-L2), wherein the Chikungunya virus structural protein or Venezuelan equine encephalitis virus structural protein and the antigen are expressed as a fusion protein can be provided.

An antigen selected from the group consisting of an antigen derived from PD-1 and an antigen derived from a ligand of PD-1 (e.g. PD-L1, PD-L2) can be fused with any site of the Chikungunya virus structural protein or Venezuelan equine encephalitis virus structural protein. For example, an antigen selected from the group consisting of an antigen derived from PD-1 and an antigen derived from a ligand of PD-1 (e.g. PD-L1, PD-L2) may be directly or indirectly linked to N- or C- terminal of a Chikungunya virus structural protein or Venezuelan equine encephalitis virus structural protein, or an antigen selected from the group consisting of an antigen derived from PD-1 and an antigen derived from a ligand of PD-1 (e.g. PD-L1, PD-L2) may be inserted into Chikungunya virus structural protein or Venezuelan equine encephalitis virus structural protein.

[0047] In one embodiment, at least one antigen selected from the group consisting of an antigen derived from PD-1 and an antigen derived from a ligand of PD-1 (e.g. PD-L1, PD-L2) is inserted into E2 of Chikungunya virus structural protein or Venezuelan equine encephalitis virus structural protein. For example, regarding Chikungunya virus

structural protein, at least one antigen selected from the group consisting of an antigen derived from PD-1 and an antigen derived from a ligand of PD-1 (e.g. PD-L1, PD-L2) 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). VLP_CHI 532 vector (SEQ ID No.: 25) may be used for preparing Chikungunya virus like particle where the antigen is inserted between residues 531 and 532 of SEQ ID Nos.1 or 2.

[0048] For example, regarding Venezuelan equine encephalitis virus structural protein, at least one antigen

selected from the group consisting of an antigen derived from PD-1 and an antigen derived from a ligand of PD-1 (e.g. PD-L1, PD-L2) 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). VLP_VEEV VLP 518 vector (SEQ ID No. :26) may be used for preparing Venezuelan equine encephalitis virus like particle where the antigen is inserted between residues 517 and 518 of SEQ ID No.3.

[0049] 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.

[0050] A protein derived from Chikungunya virus (CHIKV) or Venezuelan equine encephalitis virus (VEEV) may be a naturally occurring viral protein or modified protein thereof.

[0051] When a protein derived from a virus is conjugated with a protein derived from an antigen, a linker peptide including SG, GS, SGG, GGS SGSG and TRGGS may be used. Examples of conjugation of the protein derived from a virus (referred to as "PFV" below) with the protein 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.

[0052] In one embodiment, the present invention provides a virus like particle comprising a fusion protein of a protein derived from Chikungunya virus (CHIKV) or Venezuelan equine encephalitis virus (VEEV) and a protein derived from PD-1 or PD-L1, wherein the virus like particle is prepared by transfecting an expression vector comprising a nucleic acid molecule comprising a nulceotide sequence represented by SEQ ID Nos.:27-32 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 comprising a nulceotide sequence having at least 70%, 75%, 80%, 85%, 90%, 95% or 98% amino acid sequence identity to SEQ ID Nos.: 27-32 into a mammalian cell (e.g. 293F cell).

[0053] 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 an antigen selected from the group consisting of an antigen derived from PD-1 and an antigen derived from a ligand of PD-1 (e.g. PD-L1, PD-L2) 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 an antigen selected from the group consisting of an antigen derived from PD-1 and an antigen derived from a ligand of PD-1 (e.g. PD-L1, PD-L2) is inserted into each of E2s of Chikungunya virus (CHIKV) or Venezuelan equine encephalitis virus (VEEV).

[0054] In this embodiment, the E2 into which the antigen is inserted may consist of an amino acid sequence represented by SEQ ID Nos.:33-36; the E1 may consist of an amino acid sequence represented by SEQ ID No.:37; and the capsid may consist of an amino acid sequence represented by SEQ ID No.: 38; or

the E2 into which the antigen is inserted may consist of an amino acid sequence represented by SEQ ID Nos.:39-42; the E1 may consist of an amino acid sequence represented by SEQ ID No.:43; and the capsid may consist of an amino acid sequence represented by SEQ ID No.:44.

[0055] 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.:38 or SEQ ID No.:44; 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.:37 or SEQ ID No.:43; 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 Nos.:33-36 or SEQ ID Nos.:39-42. 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.:38 or SEQ ID No.:44; E1 consisting of an amino acid sequence represented by SEQ ID No.:37 or SEQ ID No.:43; and/or E2 consisting of consisting of an amino acid sequence represented by SEQ ID Nos.:33-36 or SEQ ID Nos.:39-42.

Virus like particle may be prepared by introducing an expression vector comprising a DNA molecule having a

nucleotide sequence encoding the virus like particle into a cell (e.g. 293 cell) and recovering the virus like particle from the conditioned medium using ultracentrifugal method.

[0056]

(2) Nucleotide, Vector, Host cell

In a second aspect, the present invention provides a nucleic acid molecule comprising a nucleotide sequence for expressing the disclosed particle which comprises a virus structural protein and an antigen selected from the group consisting of an antigen derived from PD-1 and an antigen derived from a ligand of PD-1.

[0057] The nucleic acid molecule provided by the present invention may be an isolated nucleic acid molecule for expressing a Chikungunya virus like particle or Venezuelan equine encephalitis virus like particle which comprises a virus structural protein and an antigen selected from the group consisting of an antigen derived from PD-1 and an antigen derived from a ligand of PD-1.

[0058] One skilled in the art may prepare the nucleic acid molecule provided by the present invention described above based on an exemplary nucleotide sequences that encode capsid and/or envelope represented by SEQ ID Nos.:63-64.

[0059] For example, one skilled in the art may introduce a nucleotide sequence encoding an antigen derived from

PD-1 or PD-L1 into nucleotide sequence encoding E2 of Chikungunya or Venezuelan equine virus structural protein for preparing a nucleic acid molecule which is introduced into a vector to express Chikungunya virus like particle or Venezuelan equine virus like particle which comprises a virus structural protein and an antigen selected from the group consisting of an antigen derived from PD-1 and an antigen derived from a ligand of PD-1. Examples of the nucleotide sequence where antigen-derived sequence is introduced into E2 as described above include, but are not limited to, nucleotide sequence represented by SEQ ID Nos.:27 or 29 (for expressing Chikungunya virus like particle comprising PD-1 antigen); nucleotide sequence represented by SEQ ID No.:31 (for expressing Chikungunya virus like particle comprising PD-L1 antigen); nucleotide sequence represented by SEQ ID Nos.:28 or 30 (for expressing Venezuelan equine encephalitis virus like particle comprising PD-1 antigen); and nucleotide sequence represented by SEQ ID No.:32 (for expressing Venezuelan equine encephalitis virus like particle comprising PD-L1 antigen).

[0060] 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.

[0061] 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.

[0062] 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 present invention.

[0063] 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.

[0064] Examples of vectors which can be used for expressing a virus like particle comprising a fusion protein of a protein derived from Chikungunya virus (CHIKV) and an antigen derived from PD-1 include a vector shown in VLP31.11 vector (SEQ ID No.:45) and VLP274.11 vector (SEQ ID No.:46).

[0065] Examples of vectors which can be used for expressing a virus like particle comprising a fusion protein of a protein derived from Chikungunya virus (CHIKV) and an antigen derived from PD-L1 include a vector shown in

VLP299.15 vector (SEQ ID No.:47).

[0066] Examples of vectors which can be used for expressing a virus like particle comprising a fusion protein of a protein derived from Venezuelan equine encephalitis virus (VEEV) and an antigen derived from PD-1 include a vector shown in VLP31.21 vector (SEQ ID No.:48) and VLP274.21 vector (SEQ ID No.:49).

[0067] Examples of vectors which can be used for expressing a virus like particle comprising a fusion protein of a protein derived from Venezuelan equine encephalitis virus (VEEV) and an antigen derived from PD-L1 include a vector shown in VLP299.25 vector (SEQ ID No.:50).

[0068] A nucleic acid molecule having 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:45-50 and a nucleic acid molecule which 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.:45-50 are also provided by the present invention.

[0069] In addition, a recombinant cell prepared by introducing the above-described vector into a host cell is provided by the present invention. For example, CHO cells or 293 cells are used as host cells.

[0070]

(3) Pharmaceutical composition, kit

In a third aspect, the present invention provides a pharmaceutical composition and a kit comprising a pharmaceutical composition, wherein the pharmaceutical composition comprises a particle comprising a virus structural protein and an antigen selected from the group consisting of an antigen derived from PD-1 and an antigen derived from a ligand of PD-1; or a nucleic acid molecule comprising a nucleotide sequence for expressing a particle which comprises a virus structural protein and an antigen selected from the group consisting of an antigen derived from PD-1 and an antigen derived from a ligand of PD-1; and a pharmaceutically acceptable carrier.

[0071] In one embodiment, the present invention provides a pharmaceutical composition or a kit comprising a pharmaceutical composition, wherein the pharmaceutical composition comprises 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; and a pharmaceutically acceptable carrier. 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% of the pharmaceutical composition.

[0072] Dosage amount of the particle provided by the present invention (e.g. CHIKV VLP or VEEV VLP) may be 1-500μg/day.

[0073] One or more PD-1 antigens or PD-1 ligand antigens may be used for one pharmaceutical composition provided by the present invention.

[0074] The pharmaceutical composition may further comprise adjuvant. Examples of adjuvant include, but are not limited to, Ribi solution (Sigma Adjuvant system, Sigma-Aldrich). The pharmaceutical composition provided by 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 pharmaceutical composition is an aqueous solution containing 0.001-1 w/w% of a particle (e.g. CHIKV VLP or VEEV VLP) comprising a virus structural protein and an antigen selected from the group consisting of an antigen derived from PD-1 or an antigen derived from a ligand of PD-1, 1-10w/w% of buffering agent, 0.01-1w/w% of adjuvant and 0.00001-0.001w/w% of preserving agent.

[0075] A skilled person can prepare the pharmaceutical composition using conventional technique. For example, a particle (e.g. CHIKV VLP or VEEV VLP) comprising a virus structural protein and an antigen selected from the group

consisting of an antigen derived from PD-1 or an antigen derived from a ligand of PD-1 is mixed with buffer solution having physiological pH (e.g. pH 5-9, pH7) to prepare the pharmaceutical composition provided by the present invention.

In one embodiment, the pharmaceutical composition is a vaccine or an immunostimulant comprising a particle comprising a virus structural protein and an antigen selected from the group consisting of an antigen derived from PD-1 and an antigen derived from a ligand of PD-1. For example, the vaccine composition provided by the present invention can be used for immunotherapy (e.g. treating cancer).

[0076] In one embodiment, the pharmaceutical composition is a DNA vaccine comprising a nucleic acid molecule comprising a nucleotide sequence for expressing a particle which comprises a virus structural protein and an antigen selected from the group consisting of an antigen derived from PD-1 and an antigen derived from a ligand of PD-1. In one embodiment, the DNA vaccine provided by the present invention comprises CpG containing oligonucleotide.

[0077] The pharmaceutical composition provided in the third aspect of the present invention can be administered one or more times. When the pharmaceutical composition 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.

[0078] A skilled person can determine timing of immunization using the composition or vaccine provided by the present invention. For example, 2nd immunization is performed 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 weeks after 1st immunization.

In one embodiment, the present invention provides a kit comprising

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

(b) another pharmaceutical 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.

[0079] In one embodiment, the present invention provides a kit comprising

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

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

(c) one or more pharmaceutical 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).

[0080] The respective pharmaceutical compositions contained in the above-described kit may be administered simultaneously, separately or sequentially.

[0081] 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 pharmaceutical composition provided in the third aspect of the present invention.

[0082] 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 PD-1 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 Nos.:33-36; the E1 may consist of an amino acid sequence represented by SEQ ID No.:37; and the capsid may consist of an amino acid sequence represented by SEQ ID No.:38; or

the E2 into which the antigen is inserted may consist of an

amino acid sequence represented by SEQ ID Nos.:39-42; the E1 may consist of an amino acid sequence represented by SEQ ID No.:43; and the capsid may consist of an amino acid sequence represented by SEQ ID No.:44.

[0083]

(4) Use of the disclosed particle etc.

In a forth aspect, the present invention provides a use of a particle comprising a virus structural protein and an antigen selected from the group consisting of an antigen derived from PD-1 and an antigen derived from a ligand of PD-1; or a nucleic acid molecule comprising a nucleotide sequence for expressing a particle which comprises a virus structural protein and an antigen selected from the group consisting of an antigen derived from PD-1 and an antigen derived from a ligand of PD-1 for the manufacture of a pharmaceutical composition or a kit for treating or preventing cancer or infectious disease; producing an antibody against PD-1 or a ligand of PD-1 in a mammal (e.g. human); modulating an immune response; immunostimulation; inhibiting an interaction between PD-1 and a ligand of PD-1; or inhibiting a PD-1 activity. The particle, the pharmaceutical composition or the kit disclosed herein for use in a method of treating or preventing cancer or infectious disease; producing an antibody against PD-1 or a ligand of PD-1 in a mammal (e.g.

human); modulating an immune response; immunostimulation; inhibiting an interaction between PD-1 and a ligand of PD-1; or inhibiting a PD-1 activity is also provided.

[0084] The pharmaceutical composition may be administered to a mammal (e.g. human) intramuscularly (i.m.), intracutaneously (i.c.), subcutaneously (s.c.), intradermally (i.d.) or intraperitoneally (i.p.).

In one embodiment, the pharmaceutical composition is a vaccine, which can be applied to immunotherapy (e.g. treating cancer).

[0085] Examples of the cancer which may be treated include, but are not limited to, melanoma, renal cancer, prostate cancer, breast cancer, colon cancer and non-small cell lung cancer. Other examples of the cancer include, but are not limited to, include bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, non-Hodgkin's lymphoma, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the

parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, chronic or acute leukemias including acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, solid tumors of childhood, lymphocytic lymphoma, cancer of bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally induced cancers including those induced by asbestos, and combinations thereof.

[0086] Examples of infectious disease which may be treated include, but are not limited to, HIV, Influenza, Herpes, Güardia, Malaria, Leishmania, the pathogenic infection by the virus Hepatitis (A, B and C), herpes virus (e.g., VZV, HSV-I, HAV-6, HSV-II, and CMV, Epstein Barr virus), adenovirus, influenza virus, flaviviruses, echovirus, rhinovirus, coxsackie virus, cornovirus, respiratory syncytial virus, mumps virus, rotavirus, measles virus, rubella virus, parvovirus, vaccinia virus, HTLV virus, dengue virus, papillomavirus, molluscum virus, poliovirus, rabies virus, JC virus and arboviral encephalitis virus, pathogenic infection by the bacteria chlamydia, rickettsial

bacteria, mycobacteria, staphylococci, streptococci, pneumonococci, meningococci and conococci, klebsiella, proteus, serratia, pseudomonas, legionella, diphtheria, salmonella, bacilli, cholera, tetanus, botulism, anthrax, plague, leptospirosis, and Lymes disease bacteria, pathogenic infection by the fungi Candida (albicans, krusei, glabrata, tropicalis, etc.), Cryptococcus neoformans, Aspergillus (fumigatus, niger, etc.), Genus Mucorales (mucor, absidia, rhizophus), Sporothrix schenkii, Blastomyces dermatitidis, Paracoccidioides brasiliensis, Coccidioides immitis and Histoplasma capsulatum, and pathogenic infection by the parasites Entamoeba histolytica, Balantidium coli, Naegleriafowleri, Acanthamoeba sp., Giardia lambia, Cryptosporidium sp., Pneumocystis carinii, Plasmodium vivax, Babesia microti, Trypanosoma brucei, Trypanosoma cruzi, Leishmania donovani, Toxoplasma gondi, and Nippostrongylus brasiliensis.

[0087] When a pharmaceutical composition comprising a virus structural protein and an antigen selected from the group consisting of an antigen derived from PD-1 or a ligand of PD-1 is administered to a mammal (e.g. human), an antibody against PD-1 or a ligand of PD-1 is produced in blood of the mammal. The produced antibody may modulate an immune response; show immunostimulating effects; inhibit an interaction between PD-1 and a ligand of PD-1

(e.g. PD-L1, PD-L2); or inhibit a PD-1 activity.

[0088] The produced antibody may be humanized using a conventional technique. Using the particle provided in a first aspect of the present invention, monoclonal antibody or polyclonal antibody can be prepared. In one embodiment, the present invention provides a method for producing an antibody against PD-1 or a ligand of PD-1 comprising administering the particle provided in a first aspect of the present invention to a non-human mammal and humanizing non-human mammal produced antibody.

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.

[0089] The term "PD-1 activity" refers to one or more immunoregulatory activities associated with PD-1. For example, PD-1 is a negative regulator of the TcR/CD28-mediated immune response. Thus, examples of modulation of immune response include, but are not limited to, enhancing the TcR/CD28-mediated immune response.

[0090] In one embodiment, the present invention provides a method for producing Chikungunya or Venezuelan equine encephalitis virus like particle provided in a first aspect of the present invention, comprising preparing a vector designed for expression of the particle; culturing a cell which is transfected with the vector to express the particle; and recovering the 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.

[0091] The following exemplary embodiments (1)-(35) are further provided by the present invention:

(1) A particle comprising a virus structural protein and at least one antigen selected from the group consisting of an antigen derived from PD-1 and an antigen derived from a ligand of PD-1;

(2) A particle, which is a derivative of the article according to (1);

(3) The particle according to (1) or (2), wherein the antigen is an antigen derived from PD-1, PD-L1 or PD-L2;

(4) The particle according to any one of (1) -(3), wherein administration of the particle to animals induces an antibody against the antigen and the antibody blocks PD-1 and PD-L1 interaction or PD-1 and PD-L2 interaction;

(5) The particle according to any one of (1)-(4), wherein said particle is virus like particle;

(6) The particle according to any one of (1)-(5), wherein said particle is a virus like particle derived from alphavirus or flavivirus;

(7) The particle according to any one of (1)-(6), wherein said particle is a virus like particle derived from Chikungunya virus or Venezuelan equine encephalitis virus;

(8) The particle according to any one of (1)-(7), wherein the virus structural protein comprises at least one first attachment site and the at least one antigen comprises at

least one second attachment site, and wherein the virus structural protein and the antigen are linked through the at least one first and the at least one second attachment site, and wherein the particle is virus like particle;

(9) The particle according to any one of (1)-(8), wherein the virus structural protein comprises the capsid and/or the envelope proteins E1 and E2;

(10) The particle according to (9), wherein at least one antigen derived from PD-1 or at least one antigen derived from PD-L1 is inserted into E2 of the envelope protein;

(11) The particle according to any one of (1)-(10), wherein the virus structural protein is a protein derived from Chikungunya virus (CHIKV) or Venezuelan equine encephalitis virus (VEEV);

(12) The particle according to any one of (1)-(11), wherein the particle is Chikungunya virus like particle consisting of one or more envelope protein E2 into which the antigen derived from PD-1 is inserted, one or more envelope protein E1 and one or more capsid, and

wherein the envelope protein E2 into which the antigen derived from PD-1 is inserted consists of an amino acid sequence represented by SEQ ID Nos.:33-35; the envelope protein E1 consists of an amino acid sequence represented by SEQ ID No.:37; and the capsid consists of an amino acid sequence represented by SEQ ID No.:38;

(13) The particle according to any one of (1)-(11), wherein the particle is Venezuelan equine virus like particle consisting of one or more envelope protein E2 into which the antigen derived from PD-1 is inserted, one or more envelope protein E1 and one or more capsid, and

wherein the envelope protein E2 into which the antigen derived from PD-1 is inserted consists of an amino acid sequence represented by SEQ ID Nos.:39-41; the envelope protein E1 consists of an amino acid sequence represented by SEQ ID No.:43; and the capsid consists of an amino acid sequence represented by SEQ ID No.:44;

(14) The particle according to any one of (1)-(11), wherein the particle is Chikungunya virus like particle consisting of one or more envelope protein E2 into which the antigen derived from PD-L1 is inserted, one or more envelope protein E1 and one or more capsid, and

wherein the envelope protein E2 into which the antigen derived from PD-L1 is inserted consists of an amino acid sequence represented by SEQ ID No.:36; the envelope protein E1 consists of an amino acid sequence represented by SEQ ID No.:37; and the capsid consists of an amino acid sequence represented by SEQ ID No.:38;

(15) The particle according to any one of (1)-(11), wherein the particle is Venezuelan equine virus like particle consisting of one or more envelope protein E2 into which

the antigen derived from PD-L1 is inserted, one or more envelope protein E1 and one or more capsid, and wherein the envelope protein E2 into which the antigen derived from PD-L1 is inserted consists of an amino acid sequence represented by SEQ ID No.:42; the envelope protein E1 consists of an amino acid sequence represented by SEQ ID No.:43; and the capsid consists of an amino acid sequence represented by SEQ ID No.44;

(16) A particle consisting of an amino acid sequence which has a sequence identity of 90% or more (or 95% or more) with an amino acid sequence of the particle according to any one of (1)-(15).

(17) An isolated nucleic acid molecule comprising a nucleotide sequence for expressing the particle according to any one of (1)-(16);

(18) An isolated nucleic acid molecule consisting of a nucleotide sequence which has a sequence identity of 90% or more with a nucleotide sequence represented by any one of SEQ ID Nos.:27-32;

(19) The nucleic acid molecule according to (18), wherein the nucleic acid molecule consists of a nucleotide sequence represented by any one of SEQ ID Nos.:27-32;

(20) A vector comprising the nucleic acid molecule according to any one of (17)-(19), wherein the vector optionally comprises an expression control sequence

operably linked to the nucleic acid molecule (e.g. a vector consisting of a nucleotide sequence represented by SEQ ID Nos. 45, 46, 47, 48, 49 or 50);

(21) A pharmaceutical composition comprising:

(a) the particle according to any one of (1)-(16), the nucleic acid molecule according to any one of (17)-(19) and/or the vector according to (20); and

(b) a pharmaceutically acceptable carrier;

(22) The pharmaceutical composition (e.g. vaccine) according to (16), wherein the pharmaceutical composition comprises the particle according to any one of (1)-(16) and a pharmaceutically acceptable carrier,

(23) Use of the particle according to any one of (1)-(16), the nucleic acid molecule according to any one of (17)-(19) and/or the vector according to (20) for the manufacture of a pharmaceutical composition or a kit for treating or preventing cancer or infectious disease; producing an antibody against PD-1 or a ligand of PD-1 in a mammal; modulating an immune response; immunostimulation; inhibiting an interaction between PD-1 and a ligand of PD-1; or inhibiting a PD-1 activity;

(24) The use according to (23), wherein the pharmaceutical composition is administered to inhibit binding of PD-L1 and/or PD-L2 to PD-1;

(25) The use according to (23) or (24), wherein the cancer

is melanoma, renal cancer, prostate cancer, breast cancer, colon cancer or non-small cell lung cancer;

(26) The use according to (23) or (24), wherein the cancer is selected from the group consisting of bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, non-Hodgkin's lymphoma, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, chronic or acute leukemias including acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, solid tumors of childhood, lymphocytic lymphoma, cancer of bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally induced cancers including those

induced by asbestos, and combinations thereof;

(27) The use according to (23) or (24), wherein the infectious disease is selected from the group consisting of HIV, Influenza, Herpes, Güardia, Malaria, Leishmania, the pathogenic infection by the virus Hepatitis (A, B and C), herpes virus (e.g., VZV, HSV-I, HAV-6, HSV-II, and CMV, Epstein Barr virus), adenovirus, influenza virus, flaviviruses, echovirus, rhinovirus, coxsackie virus, cornovirus, respiratory syncytial virus, mumps virus, rotavirus, measles virus, rubella virus, parvovirus, vaccinia virus, HTLV virus, dengue virus, papillomavirus, molluscum virus, poliovirus, rabies virus, JC virus and arboviral encephalitis virus, pathogenic infection by the bacteria chlamydia, rickettsial bacteria, mycobacteria, staphylococci, streptococci, pneumonococci, meningococci and conococci, klebsiella, proteus, serratia, pseudomonas, legionella, diphtheria, salmonella, bacilli, cholera, tetanus, botulism, anthrax, plague, leptospirosis, and Lymes disease bacteria, pathogenic infection by the fungi Candida (albicans, krusei, glabrata, tropicalis, etc.), Cryptococcus neoformans, Aspergillus (fumigatus, niger, etc.), Genus Mucorales (mucor, absidia, rhizophus), Sporothrix schenkii, Blastomyces dermatitidis,
Paracoccidioides brasiliensis, Coccidioides immitis and Histoplasma capsulatum, and pathogenic infection by the

parasites Entamoeba histolytica, Balantidium coli, Naegleriafowleri, Acanthamoeba sp., Giardia lambia, Cryptosporidium sp., Pneumocystis carinii, Plasmodium vivax, Babesia microti, Trypanosoma brucei, Trypanosoma cruzi, Leishmania donovani, Toxoplasma gondi, and Nippostrongylus brasiliensis;

(28) A kit comprising

(a) a pharmaceutical composition comprising the particle according to any one of (1)-(16); and

(b) another pharmaceutical composition comprising the particle according to any one of (1)-(16),

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

(29) The kit according to (28), 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.;

(30) The kit according to (28) or (29), further comprising

(c) one or more pharmaceutical compositions, each of which comprises the particle according to any one of (1)-(16), 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);

(31) The kit according to any one of (28)-(30), wherein the respective pharmaceutical compositions are administered simultaneously, separately or sequentially;

(32) The particle according to any one of (1)-(16), the nucleic acid molecule according to any one of (17)-(20), the vector according to (20), the pharmaceutical composition according to (21) or (22) or the kit according to any one of (28)-(31) for use in a method of treating or preventing cancer or infectious disease; producing an antibody against PD-1 or a ligand of PD-1 in a mammal; modulating an immune response; immunostimulation; inhibiting an interaction between PD-1 and a ligand of PD-1; or inhibiting a PD-1 activity;

(33) The particle according to any one of (1)-(16), the nucleic acid molecule according to any one of (17)-(20), the vector according to (20), the pharmaceutical composition according to (21) or (22) or the kit according to any one of (28)-(31) for use in a method of treating or preventing cancer, wherein the cancer is selected from the cancers described in (25) or (26);

(34) The particle according to any one of (1)-(16), the nucleic acid molecule according to any one of (17)-(20), the vector according to (20), the pharmaceutical composition

according to (21) or (22) or the kit according to any one of (28)-(31) for use in a method of treating or preventing infectious disease, wherein the infectious disease is selected from the infectious disease described in (27);

(35) A method for producing Chikungunya virus like particle or Venezuelan equine encephalitis virus like particle, comprising culturing a cell which is transfected with the vector according to (20) to express the particle; and purifying the particle using ultracentrifugation.

[0092] The present invention will be described in detail with reference to the following examples, which, however, are not intended to limit the scope of the present invention.

[0093]

EXAMPLES

EXAMPLE 1: Preparation of Chikungunya virus (CHIKV) like particle comprising a virus structural protein and a fragment of PD-1 antigen

The following polynucleotides of PD-1 were used to be inserted into VLP_CHI 532 vector (SEQ ID No.:25). N terminal linker is SGG in amino acid sequence (TCCGGAGGA in nuclear sequence) and C terminal linker is GGS in amino acid sequence (GGAGGATCC in nuclear sequence).

[0094]

1. VLP31 (PD-1 No.1 sequence): A sequence of a PD-1

fragment attaching linker, which was used for an antigen:

Nuclear sequence

Tccggaggactaaactggtaccgcatgagccccagcaaccagacggacaagct ggccgccttcggaggatcc (SEQ ID No.:51)

Amino acid sequence

sgglnwyrmspsnqtdklaafggs (SEQ ID No.:52)

2. VLP32 (PD-1 No.2 sequence): Another sequence of a PD-1 fragment attaching linker, which was used for an antigen:

Nuclear Sequence

Tccggaggaatgctaaactggtaccgcatgagccccagcaaccagacggacaa gctggccgccttctcaggaggatcc (SEQ ID No.:53)

Amino acid sequence

sggmlnwyrmspsnqtdklaafsggs (SEQ ID No.:54)

3. VLP33 (PD-1 No.3 sequence): Another sequence of a PD-1 fragment attaching linker, which was used for an antigen:

Nuclear Sequence

Tccggaggagtgctaaactggtaccgcatgagccccagcaaccagacggacaa gctggccgccttccccggaggatcc (SEQ ID No.:55)

Amino Acid sequence

sggvlnwyrmspsnqtdklaafpggs (SEQ ID No.:56)

[0095] The respective polynucleotides was inserted between the codons encoding Ser at 531-position and Asn at 532-position of SEQ ID No.2 to construct a plasmid

(hereinafter referred to as VLP31_11, VLP32_11 or VLP33_11) for expressing Chikungunya virus like particle where the modified PD-1-derived peptide is inserted into E2 of Chikungunya virus structural protein.

[0096] 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.

[0097] The expression of VLP comprising VLP31, 32 or 33 conjugated with Chikungunya virus structural protein was confirmed by Western Blot using an antibody specific for CHIKV (ATCC: VR-1241AF).

[0098]

<u>EXAMPLE 2: Preparation of Venezuelan equine encephalitis virus (VEEV) like particle comprising a virus structural protein and a fragment of PD-1 antigen</u>

The same polynucleotides of PD-1 used in EXAMPLE 1 were used to be inserted into VLP_VEEV VLP 518 vector (SEQ ID No.:26). N terminal linker and C terminal linker are same in EXAMPLE 1.

[0099] The respective polynucleotides was inserted between the codons encoding Ser at 518-position and Ser at 519-position of SEQ ID No.3 to construct a plasmid (hereinafter referred to as VLP31_21, VLP32_21 or VLP33_21) for expressing Venezuelan equine encephalitis virus like particle where the modified PD-1 -derived peptide is inserted into E2 of Venezuelan equine encephalitis structural protein.

[0100] 293F cells were transfected with the plasmid like EXAMPLE 1. The expression of VLP comprising VLP 31, 32 or 33 conjugated with Venezuelan equine encephalitis virus structural protein was confirmed by Western Blot using an antibody specific for VEEV.

[0101]

<u>EXAMPLE 3: Preparation of Chikungunya virus (CHIKV) like particle and Venezuelan equine encephalitis virus (VEEV) like particle comprising a virus structural protein and a fragment of PD-1 antigen or PD-L1 antigen</u>

The following polynucleotides of mouse PD-1 and mouse PD-L1 were used to be inserted into VLP CHI 532 vector (SEQ ID No.:25) or to be inserted into VLP_VEEV VLP 518 vector (SEQ ID No.:26). N terminal linker is SGG in amino acid sequence (TCCGGAGGA in nuclear sequence) and C terminal linker is GGS in amino acid sequence (GGAGGATCC in nuclear sequence).

1. VLP299 (mousePD-L1 sequence): A sequence of a fragment of mouse PD-L1 Domain3S attaching linker, which was used for an antigen:

Nuclear Sequence

Tccggaggatgcatcatcagctacggcggagccgactacggaggatcc (SEQ ID No.:57)

Amino Acid sequence

SGG-ciisyggadyC-GGS (SEQ ID No.:58)

2. VLP274 (mousePD-1 sequence): A sequence of a fragment of mouse PD-1 domain2short attaching linker, which was used for an antigen:

Nuclear Sequence

Tccggaggaggcgccatcagcctgcaccccaaggccaagatcgaggaatctgg aggatcc (SEQ ID No.:59)

Amino Acid sequence

SGG-gaislhpkakiees-GS (SEQ ID No.:60)

3. VLP275 (mousePD-1 sequence): A sequence of a fragment of mouse PD-1 domain2short_v2 attaching linker, which was used for an antigen:

Nuclear Sequence

Tccggaggatgtggcgccatcagcctgcaccccaaggccaagatcgaggaagg aggatcc (SEQ ID No.:61)

Amino Acid sequence

SGG-cgaislhpkakieeC-GGS (SEQ ID No.:62)

[0102] The respective polynucleotides was inserted

between the codons encoding Ser at 531-position and Asn at 532-position of SEQ ID No.2 to construct a plasmid (hereinafter referred to as VLP299_15, VLP299_25 for VLP299-inserted vector; VLP274_11, VLP274_15 for VLP274-inserted vector; VLP275_11, VLP275_15 for VLP275-inserted vector) for expressing Chikungunya virus like particle where the modified PD-1-derived peptide or the modified PD-L1-derived peptide is inserted into E2 of Chikungunya virus structural protein.

[0103] 293F cells were transfected with the plasmid like EXAMPLE 1. The expression of VLP comprising VLP299, 274 or 275 conjugated with Chikungunya virus structural protein was confirmed by Western Blot using an antibody specific for CHIKV or VEEV.

[0104]

EXAMPLE 4: Immunogenicity of PD-1

The following polynucleotides of human PD-1, which is also found in the mouse PD-1 gene, was used to be inserted into VLP_CHI 532 vector (SEQ ID No.:25) or to be inserted into VLP_VEEV VLP 518 vector (SEQ ID No.:26). N terminal linker is SGG in amino acid sequence (TCCGGAGGA in nuclear sequence) and C terminal linker is GGS in amino acid sequence (GGAGGATCC in nuclear sequence).

Nuclear Sequence

Tccggaggactaaactggtaccgcatgagccccagcaaccagacggacaagct
ggccgccttcggaggatcc (SEQ ID No.:51)
Amino Acid sequence
SGGLNWYRMSPSNQTDKLAAFGGS (SEQ ID No.:52)

[0105] The polynucleotides was inserted between the codons encoding Ser at 531-position and Asn at 532-position of SEQ ID No.:2 to construct a plasmid (hereinafter referred to pCHIKV-hPD-1) for expressing Chikungunya virus like particle where the modified PD-1-derived peptide is inserted into E2 of Chikungunya virus structural protein. Also, the polynucleotides was inserted between the codons encoding Ser at 518-position and Ser at 519-position of SEQ ID No.:3 to construct a plasmid (hereinafter referred to as pVEEV-hPD-1) for expressing Venezuelan equine encephalitis virus like particle where the modified PD-1 -derived peptide is inserted into E2 of Venezuelan equine encephalitis structural protein.

[0106] 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. The purified virus like particles were further concentrated using spin column (Molecular Weight-cutoff: 100kDa) to prepare the virus like particles for the immunization (CHIKV-hPD-1 and VEEV-hPD-1).

[0107] The mice (4 week old male) were immunized with the VEEV-hPD-1 2 times at 0 and 8 week (20ug VLP per mouse) by intramuscle injection with Adjuvant Ribi, and immunized with CHIKV-hPD-1 once at 4 week (20ug VLP per mouse) by intramuscle injection with Adjuvant Ribi(Sigma Adjuvant system, Sigma-Aldrich). The blood was taken at 10 weeks after the first immunization.

[0108] 96 well ELISA plate were coated with 50ng of Recombinant N-terminal fragment of PD-1 (1-167aa) or PD-1-Fc conjugate 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 mice were added in the blocking buffer and incubated for 1 h at room temperature. After washing three times, peroxidase labeled goat 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).

[0109] The immunogenicities are shown in Figures 1 and 2. As seen from Figures 1 and 2, induction of antibodies against PD-1 was found in the serum of mice immunized with CHIKV-PD-1 and VEEV-PD-1.

[0110]

Example 5: Preparation of a pharmaceutical composition comprising Chikungunya virus (CHIKV) like particle or Venezuelan equine encephalitis virus (VEEV) like particle comprising a virus structural protein and a fragment of PD-1 antigen

Chikungunya virus (CHIKV) like particle comprising a virus structural protein and a fragment of PD-1 antigen and Venezuelan equine encephalitis virus (VEEV) like particle a virus structural protein and a fragment of PD-1 antigen were prepared according to Example 4.

To prepare a pharmaceutical composition which is a vaccine composition, 80 μg 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 protein and an antigen selected from the group consisting of an antigen derived from PD-1 and an antigen derived from a ligand of PD-1.

2. The particle according to Claim 1, wherein the virus structural protein comprises the capsid and/or the envelope proteins E1 and E2.

3. The particle according to Claim 2, wherein at least one antigen derived from PD-1 or at least one antigen derived from PD-L1 is inserted into E2 of the envelope protein.

4. The particle according to any one of Claims 1-3, wherein the particle is Chikungunya virus like particle consisting of one or more envelope protein E2 into which the antigen derived from PD-1 is inserted, one or more envelope protein E1 and one or more capsid, and
wherein the envelope protein E2 into which the antigen derived from PD-1 is inserted consists of an amino acid sequence represented by SEQ ID Nos.:33-35; the envelope protein E1 consists of an amino acid sequence represented by SEQ ID No.:37; and the capsid consists of an amino acid sequence represented by SEQ ID No.:38.

5. The particle according to any one of Claims 1-3, wherein the particle is Venezuelan equine virus like particle consisting of one or more envelope protein E2 into which the antigen derived from PD-1 is inserted, one or more envelope protein E1 and one or more capsid, and
wherein the envelope protein E2 into which the antigen derived from PD-1 is inserted consists of an amino acid sequence represented by SEQ ID Nos.:39-41; the envelope protein E1 consists of an amino acid sequence represented by SEQ ID No.:43; and the capsid consists of an amino acid sequence represented by SEQ ID No.:44.

6. The particle according to any one of Claims 1-3, wherein the particle is Chikungunya virus like particle consisting of one or more envelope protein E2 into which the antigen derived from PD-L1 is inserted, one or more envelope protein E1 and one or more capsid, and
wherein the envelope protein E2 into which the antigen derived from PD-L1 is inserted consists of an amino acid sequence represented by SEQ ID No.:36; the envelope protein E1 consists of an amino acid sequence represented by SEQ ID No.:37; and the capsid consists of an amino acid sequence represented by SEQ ID No.:38.

7. The particle according to any one of Claims 1-3, wherein the particle is Venezuelan equine virus like particle consisting of one or more envelope protein E2 into which the antigen derived from PD-L1 is inserted, one or more envelope protein E1 and one or more capsid, and wherein the envelope protein E2 into which the antigen derived from PD-L1 is inserted consists of an amino acid sequence represented by SEQ ID No.:42; the envelope protein E1 consists of an amino acid sequence represented by SEQ ID No.:43; and the capsid consists of an amino acid sequence represented by SEQ ID No.:44.

8. A particle consisting of an amino acid sequence which has a sequence identity of 90% or more with an amino acid sequence of the particle according to any one of Claims 4-7.

9. An isolated nucleic acid molecule comprising a nucleotide sequence for expressing the particle according to any one of Claims 1-8.

10. An isolated nucleic acid molecule consisting of a nucleotide sequence which has a sequence identity of 90% or more with a nucleotide sequence represented by SEQ ID Nos.:27-32.

11. A vector comprising the nucleic acid molecule according to any one of Claims 9-10, wherein the vector optionally comprises an expression control sequence operably linked to the nucleic acid molecule.

12. A pharmaceutical composition comprising:
(a) the particle according to any one of Claims 1-8 the nucleic acid molecule according to any one of Claims 9-10 and/or the vector according to Claim 11; and
(b) a pharmaceutically acceptable carrier.

13. The pharmaceutical composition according to claim 12, wherein the pharmaceutical composition is a vaccine.

14. Use of the particle according to any one of Claims 1-8 the nucleic acid molecule according to any one of Claims 9-10 and/or the vector according to Claim 11 for the manufacture of a pharmaceutical composition or a kit for treating or preventing cancer or infectious disease; producing an antibody against PD-1 or a ligand of PD-1 in a mammal; modulating an immune response; immunostimulation; inhibiting an interaction between PD-1 and a ligand of PD-1; or inhibiting a PD-1 activity.

15. A kit comprising

(a) a pharmaceutical composition comprising the particle according to any one of Claims 1-8; and

(b) another pharmaceutical composition comprising the particle according to any one of Claims 1-8,

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

16. The kit according to Claim 15, 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.

Figure 1

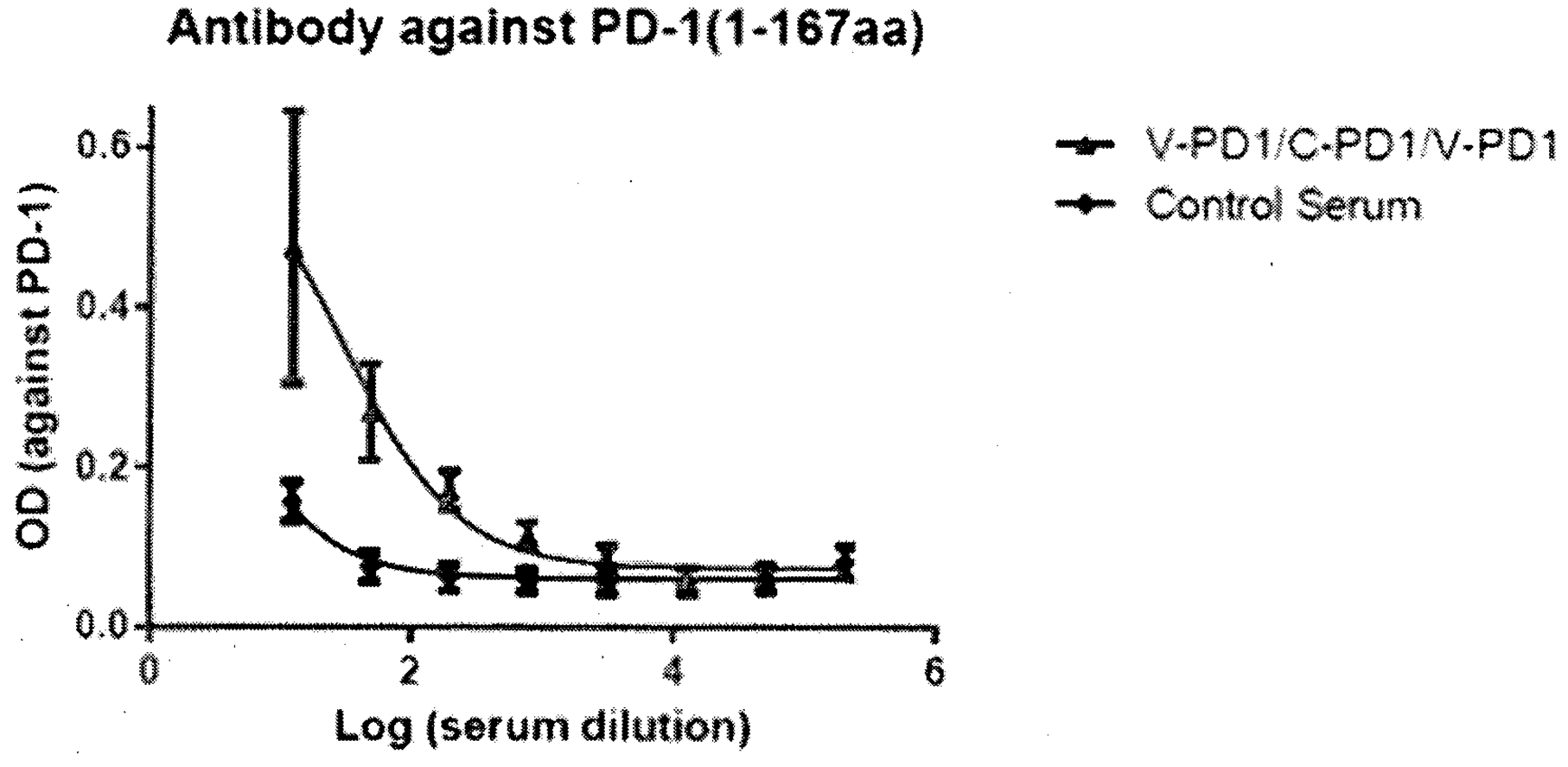


Figure 2

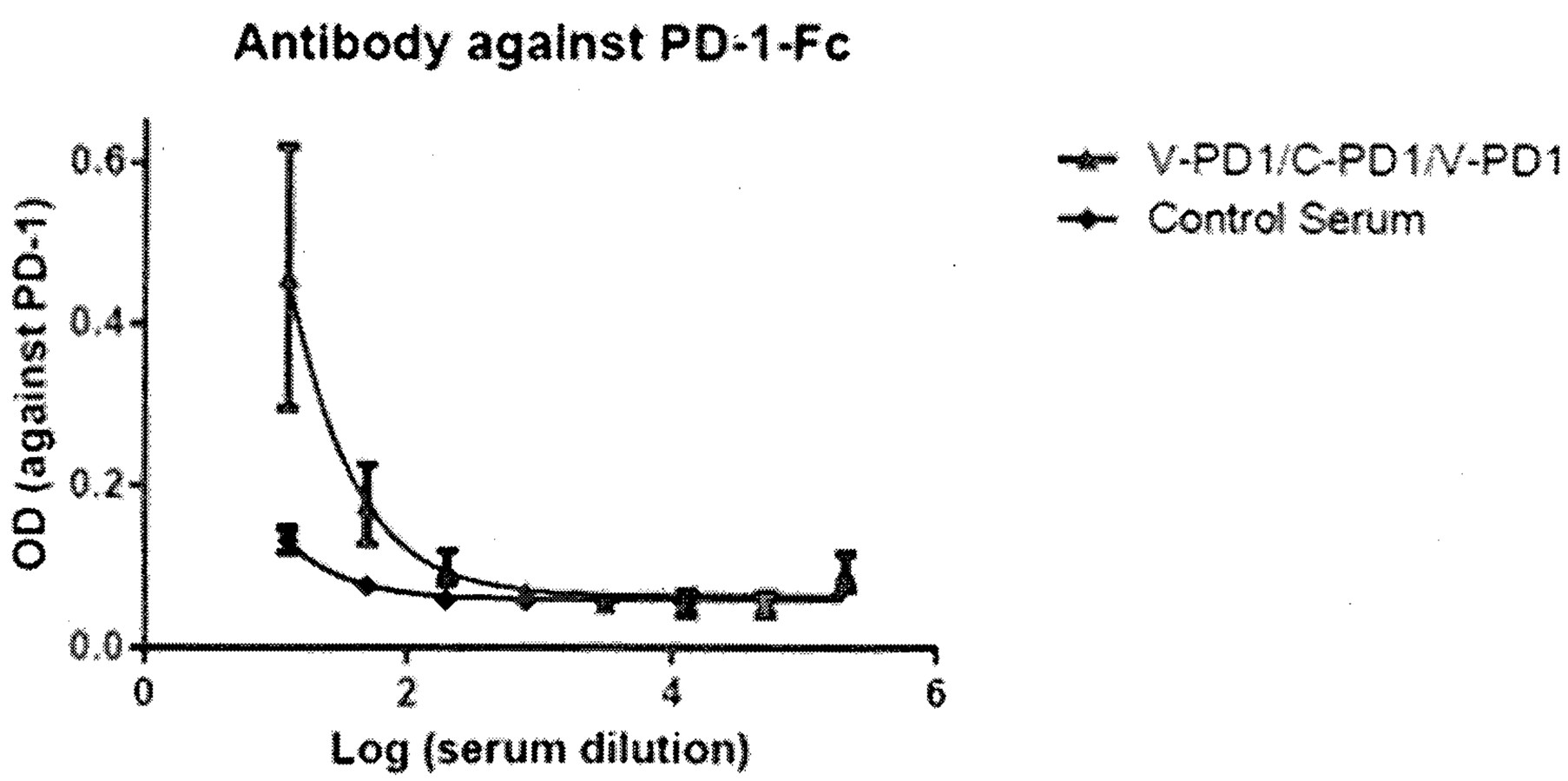
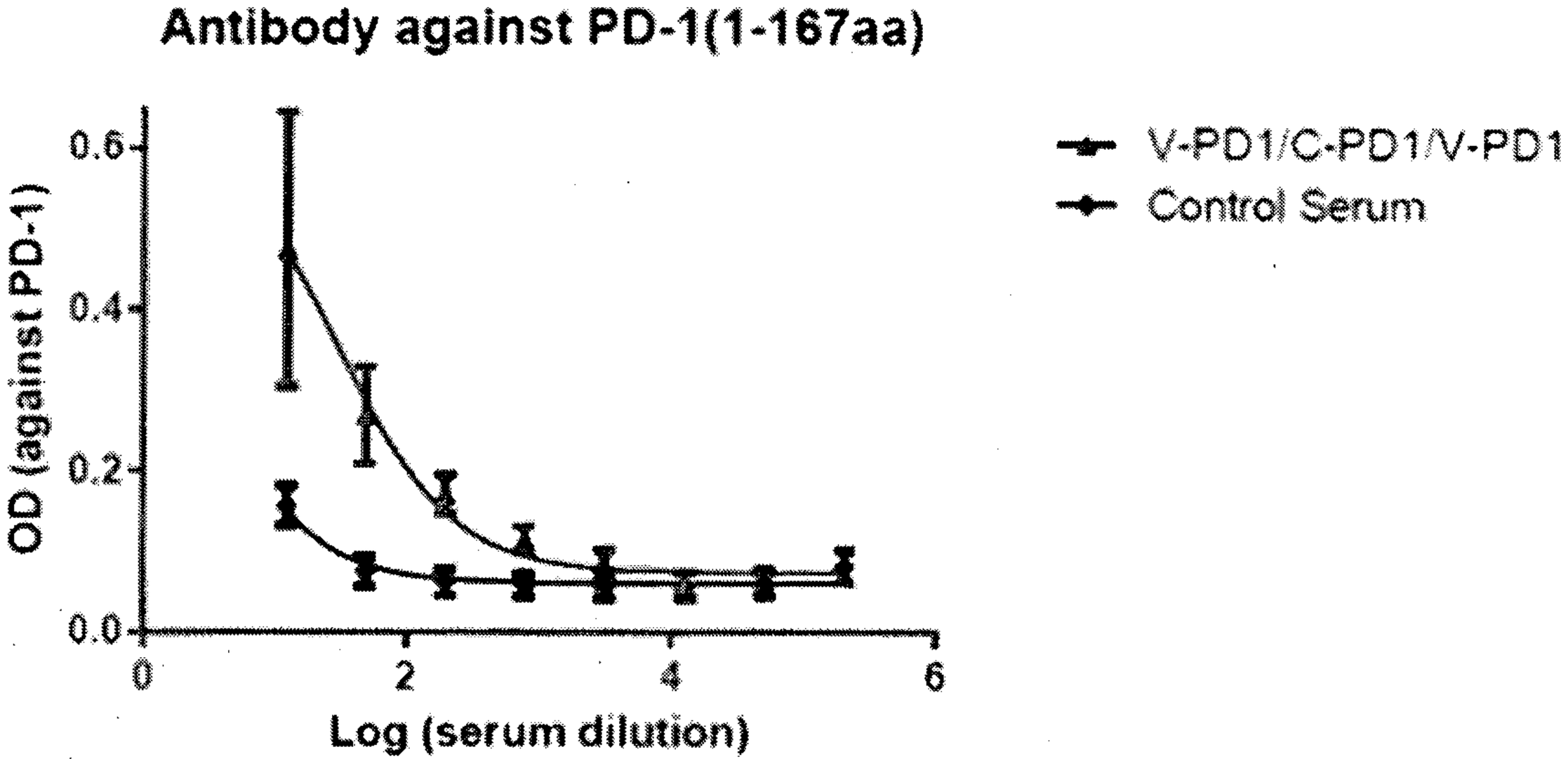


Figure 1


Antibody against PD-1(1-167aa)
OD (against PD-1)
0.6
0.4
0.2
0.0
0
2
4
6
Log (serum dilution)
V-PD1/C-PD1/V-PD1
Control Serum