VIRUS LIKE PARTICLE COMPOSITIONS AND METHODS OF USE

CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit of the following U.S. Provisional Application Nos.:61/118,206 and 61/201,118, filed on November 26, 2008 and December 5, 2008, respectively, the entire contents of each of which are incorporated herein by reference.

STATEMENT OF RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH

Research supporting this application was carried out by the United States of America as represented by the Secretary, Department of Health and Human Services. This research was supported by the Intramural Research Program, Vaccine Research Center, NIAID of the National Institute of Health. The Government has certain rights in this invention.

BACKGROUND OF THE INVENTION

Chikungunya virus (CHIKV), a mosquito-borne alphavirus in the family Togaviridae, was first isolated in Tanzania in 1952. Infection by this virus causes human disease that is characterized by rash, high fever and, its hallmark feature, severe arthritis that can persist for years. Chikungunya virus (CHIKV) has infected millions of people in Africa, Europe, and Asia since its re-emergence in Kenya in 2004. The evolution and spread of the virus into new geographic areas, and the disease severity present a serious public health in the absence of a vaccines or anti-viral therapies. Therefore, the development of anti-viral therapies for CHIKV and vaccine development remains a high priority. Phylogenetic analysis of CHIKV showed that there are three genotypes: Asian, East/Central/South African and West African. The Asian and East/Central/South African genotypes are most similar, whereas the West African strains are more divergent. Therapeutic and/or prophylactic methods for treating or preventing Chikungunya viral disease are urgently required.

SUMMARY ~~OF THE INVENTION~~

As described below, the present disclosure~~invention~~ features compositions and methods for the prevention or treatment of one or more strains of Chikungunya virus, as well as other alphavirus-mediated diseases.

In one embodiment~~aspect~~, the present disclosure~~invention~~ provides a virus-like particle (VLP) containing one or more (e.g., one, two, three, four, five) Chikungunya virus structural polypeptides. In one

embodiment, the structural polypeptides are any one or more of capsid and envelope proteins E3, E2, 6K and E1.

In one aspect, there is provided a virus-like particle (VLP) comprising one or more Chikungunya virus structural polypeptides, wherein the VLP does not carry genetic information encoding the VLP proteins.

In another embodimentaspect, the present disclosureinvention provides an isolated polynucleotide encoding a virus- like particle of the previous aspect or any other VLP delineated herein. In one embodiment, the polynucleotide encodes a Chikungunya virus polyprotein containing C-E3-E2-6K-E1.

In a related embodimentaspect, the present disclosureinvention provides an expression vector containing a polynucleotide encoding one or more Chikungunya virus structural polypeptides.

In another embodimentaspect, the present disclosureinvention provides a prokaryotic or eukaryotic cell (e.g., mammalian, human, insect) containing the expression vector of any previous embodimentaspect or any other expression vector delineated herein. In one embodiment, the cell is *in vitro*.

In another aspect, the present disclosureinvention provides an immunogenic composition containing an effective amount of a virus-like particle of any previous aspect or any other VLP delineated herein.

In a related embodimentaspect, the present disclosureinvention provides an immunogenic composition containing an effective amount of a VLP containing a Chikungunya structural polyprotein containing C-E3- E2-6K-E1 and an adjuvant.

In another embodimentaspect, the present disclosureinvention provides an immunogenic composition containing an effective amount of an expression vector of any previous aspect embodiment or otherwise delineated herein (e.g., a DNA vaccine).

In another embodimentaspect, the present disclosureinvention provides a vaccine containing an effective amount of one or more Chikungunya virus structural polypeptides that is any one or more of capsid (C) and envelope proteins El , E2, E3 and 6K.

In another aspect, the present disclosureinvention provides a vaccine containing an effective amount of a virus-like particle of any previous aspect or containing a polyprotein containing C-E3-E2- 6K-E 1.

In another embodimentaspect, the present disclosureinvention provides a vaccine containing a polynucleotide encoding a Chikungunya structural polyprotein or fragment thereof. In one embodiment, the Chikungunya structural polyprotein is encoded by an expression vector of any previous aspectembodiment. In one embodiment, the expression vector comprises a CMV/R promoter. In another embodiment, the vaccine is a DNA vaccine.

In another embodimentaspect, the present disclosureinvention provides a method of inducing an immune response against Chikungunya in a subject (e.g. human), the method involving administering to the subject an effective amount of an immunogenic composition of any previous aspect or any other immunogenic composition delineated herein. In one embodiment, the immunogenic

composition contains one or more Chikungunya virus structural polypeptides that is any one or more of capsid (C) and envelope proteins E1, E2, E3 and 6K. In another embodiment, the immunogenic composition comprises a polyprotein containing C-E3-E2-6K-E1. In another embodiment, the method induces neutralizing antibodies in a subject.

In another embodimentaspect, the present disclosureinvention provides a method for treating or preventing a Chikungunya infection in a subject, the method involving administering to the subject an effective amount of a vaccine of any previous aspect or an immunogenic composition of any previous aspect. In one embodiment, wherein the vaccine or immunogenic composition is administered in one or more doses.

In another aspect, there is provided the use of an effective amount of an immunogenic composition as defined above in the manufacture of a medicament for inducing an immune response against Chikungunya in a subject.

In another aspect, there is provided the use of an effective amount of a vaccine as defined above in the manufacture of a medicament for treating or preventing a Chikungunya infection in a subject.

In another aspect, there is provided the use of a virus-like particle as defined above in the manufacture of a medicament for treating or preventing a Chikungunya infection.

In another embodimentaspect, the present disclosureinvention provides a method for producing a virus-like particle, the method involves expressing in a cell one or more Chikungunya structural protein capable of self-assembly to form a virus-like particle. In one embodiment, the method further involves isolating the virus-like particle.

In an aspect, there is provided an in vitro method for producing a virus-like particle (VLP), the method comprising expression in a cell a polynucleotide encoding one or more Chikungunya virus structural polypeptides selected from the group consisting of capsid (C) and envelop proteins E3, E2, 6K and E1 under conditions that allow formation of a virus-like particle lacking genetic information encoding the VLP proteins; and isolating the VLP.

In another aspect, there is provided a virus-like particle produced as defined above.

In another embodimentaspect, the present disclosureinvention provides a virus-like particle (VLP) containing one or more alphavirus structural polypeptides (e.g., capsid or envelope polypeptide). In one embodiment, the alphavirus is any one or more of Chikungunya virus, Sindbis virus, Eastern equine encephalitis (EEE) virus, Western equine encephalitis (WEE) virus, and Venezuelan equine encephalitis (VEE) virus.

In another embodimentaspect, the present disclosureinvention provides an isolated polynucleotide encoding a virus- like particle of the previous aspect or otherwise delineated herein.

In another embodimentaspect, the present disclosureinvention provides an expression vector containing a polynucleotide encoding one or more alphavirus structural polypeptides wherein the alphavirus is selected from the group consisting of Chikungunya virus, Sindbis virus, Eastern equine encephalitis (EEE) virus, Western equine encephalitis (WEE) virus, and Venezuelan equine encephalitis (VEE) virus.

In another embodimentaspect, the present disclosureinvention provides an immunogenic composition containing an effective amount of a virus-like particle of any previous aspect or otherwise delineated herein.

In another embodimentaspect, the present disclosureinvention provides a vaccine containing an effective amount of one or more alphavirus structural polypeptides or a polynucleotide encoding one or more alphavirus structural proteins, wherein the alphavirus is selected from the group consisting of Chikungunya virus, Sindbis virus, Eastern equine encephalitis (EEE) virus, Western equine encephalitis (WEE) virus, and Venezuelan equine encephalitis (VEE) virus.

In another embodimentaspect, the present disclosureinvention provides a method of inducing an immune response against an alphavirus in a subject, the method involving administering to the subject an effective amount of an immunogenic composition of a previous aspect. In one embodiment, the immunogenic composition contains one or more alphavirus structural polypeptides (e.g. envelope or capsid).

In another embodimentaspect, the present disclosureinvention provides a method for treating or preventing an alphavirus infection in a subject, the method involving administering to the subject an effective amount of a vaccine or an immunogenic composition of any previous aspect.

In another embodimentaspect, the present disclosureinvention provides a kit containing a VLP of any previous aspect, and instructions for use.

In another embodimentaspect, the present disclosureinvention provides a kit containing an immunogenic composition of any previous aspect, and instructions for use in a subject. In one embodiment, the immunogenic composition is provided in a first container and a second immunogenic composition is provided in a second container, and instructions for use in a prime boost immunization. In another embodiment, the immunogenic composition in the second container contains a VLP, viral polypeptide, or viral polynucleotide.

In another embodimentaspect, the present disclosureinvention provides a method for identifying inhibitors of Chikungunya virus entry into a eukaryotic cell, the method involving contacting a cell that expresses a Chikungunya virus receptor with a Chikungunya polypeptide selected from the group consisting of C, E3, E2, 6K, and El and a candidate compound, and assaying for viral entry, wherein a candidate compound that reduces viral entry in the cell relative to a control cell is identified as an inhibitor of Chikungunya virus entry. In one embodiment, the candidate inhibitor is an antibody, or fragment thereof or small molecule.

In another embodimentaspect, the present disclosureinvention provides a method for identifying inhibitors of Chikungunya viral entry involving contacting a cell that expresses a Chikungunya virus receptor with a candidate inhibitor and a pseudotyped virus containing a reporter gene; and measuring expression of the reporter gene in the cell, wherein a compound that reduces expression of the reporter gene relative to a control cell is identified as inhibiting viral entry. In one embodiment, the pseudotyped virus (e.g., lentivirus) contains one or more Chikungunya virus envelope proteins (e.g., E3, E2, 6K and El). In one embodiment, the candidate inhibitor is an antibody, or fragment thereof or small molecule.

In another embodimentaspect, the present disclosureinvention provides a virus-like particle (VLP) containing one or more Chikungunya virus structural polypeptides for use in treating or preventing a Chikungunya infection.

In another embodimentaspect, the present disclosureinvention provides a method for treating or preventing a Chikungunya infection, the method involving administering a virus-like particle (VLP) containing one or more Chikungunya virus structural polypeptides prior to, subsequent to, concurrent with, or in any other sequence with the administration of one or more of another immunogenic composition, antiviral, or antibiotic agent.

In another embodimentaspect, the present disclosureinvention provides methods for treating or preventing a Chikungunya infection by administering neutralizing antibodies (e.g., mammalian, human) generated against a VLP of the invention to a subject (e.g., human).

In various embodiments of the above aspects or any other aspect of the present disclosureinvention delineated herein, the VLP contains one or more (1, 2, 3, 4) envelop proteins E3, E2, 6K and E1. In other embodiments, the VLP contains a polyprotein containing C-E3-E2-6K-E1 or a fragment thereof. In other embodiments of the above aspects or any other aspect of the invention delineated herein, a polynucleotide encodes one or more structural polypeptides that is any one or more of a alphavirus or Chikungunya virus capsid (C) and envelope proteins E3, E2, 6K and E1. In other embodiments, the polynucleotide encodes envelop proteins E3, E2, 6K and E1. In other embodiments, the polynucleotide encodes a Chikungunya virus polyprotein containing C-E3-E2-6K-E1. In still other embodiments, the expression vector is capable of expression in a prokaryotic or eukaryotic cell (e.g., mammal, human). In other embodiments, the structural polyprotein is derived from Chikungunya strain 37997 or LR2006. In other embodiments, the vector comprises the CMV/R promoter. In other embodiments, the expression vector is C-E37997 or C-EOPY-1. In other embodiments, the VLP induces an immune response (e.g., a protective immune response) in a subject. In other embodiments, the immune response treats or prevents a Chikungunya infection in a subject. In other embodiments of the above aspects, the VLP induces antibodies against homologous or heterologous strains of Chikungunya. In embodiments of the above aspects, the adjuvant is an immunostimulating agent (e.g., Ribi, aluminum salts, muramyl peptides, bacterial cell wall components, saponin adjuvants).

In other embodiments of the above aspects, the vaccine or immunogenic composition is administered in one or more priming immunizations and one or more boosting immunizations. In still another embodiment, the priming immunizations are administered at one, two, three, four, five, six, seven or eight week intervals. In still another embodiment, the boosting immunizations are administered two weeks, one month, two months or three months after the priming immunization. In other embodiments of the above aspects or any other aspect of the invention delineated herein, the immunization protects the subject against

viremia or the inflammatory consequences of infection. In other embodiments, the method protects a subject from lethality. In other embodiments, the method induces neutralizing antibodies in the subject.

The present disclosure~~invention~~ provides immunogenic compositions featuring virus-like particles comprising Chikungunya polypeptides for the prevention or treatment of Chikungunya viral disease. Compositions and articles defined by the present disclosure~~invention~~ were isolated or otherwise manufactured in connection with the examples provided below. Other features and advantages of the present disclosure~~invention~~ will be apparent from the detailed description, and from the claims.

Definitions

By "alphavirus structural protein" is meant a polypeptide or fragment thereof having at least about 40% amino acid sequence identity to a naturally occurring viral capsid or envelope protein and having immunogenic activity in a mammal. In one embodiment, the alphavirus structural protein has at least about 85%, 90%, 95% or greater amino acid sequence identity with a Chikunguna virus structural protein or immunogenic fragment thereof. In one embodiment, the protein Exemplary alphaviruses include, but are not limited to, Western, Eastern, and Venezuelan equine encephalitis viruses, o'nyong-nyong virus, Ross River virus and Sindbis virus.

By "Chikungunya virus structural protein" is meant a polypeptide or fragment thereof having at least about 85% amino acid sequence identity to a naturally occurring Chikungunya virus capsid or envelope protein. In other embodiments, the amino acid sequence identity is at least about 90%, 95%, or more.

By "agent" is meant any small molecule chemical compound, antibody, nucleic acid molecule, or polypeptide, or fragments thereof.

As used herein, the term "adjuvant" is meant to refer to a compound that, when used in combination with a specific immunogen in a formulation, will augment, alter or modify the resultant immune response. In certain embodiments, the adjuvant is used in combination with a VLP. In other embodiments, the adjuvant is used in combination with a DNA vaccine. Modification of the immune response includes intensification or broadening the specificity of either or both antibody and cellular immune responses. Modification of the immune response can also mean decreasing or suppressing certain antigen-specific immune responses. In one embodiment, the adjuvant is Ribi adjuvant.