METHODS AND COMPOSITIONS FOR DENGUE VIRUS VACCINES

STATEMENT OF PRIORITY

This application claims the benefit, under 35 U.S.C. § 119(e), of U.S Provisional Application Serial No. 61/839,687, filed June 26, 2013, the entire contents of which are incorporated by reference herein.

STATEMENT OF GOVERNMENT SUPPORT

This invention was made with government support under Grant No. U54 AI057157 awarded by the National Institutes of Health. The United States government has certain rights in the invention.

FIELD OF THE INVENTION

The present invention is directed to dengue virus vaccines that induce neutralizing antibodies against more than one dengue virus serotype from a single source.

BACKGROUND OF THE INVENTION

Dengue is a mosquito-borne flavivirus that is spreading at an unprecedented rate and has developed into a major health and economic burden in over 50 countries. Current DENV vaccines protecting against all four DENV serotypes must be delivered as a "tetravalent" formulation of four viruses or four recombinant proteins, each intended to confer protection against that serotype. The correct mix of serotypes in the tetravalent cocktail to achieve a balanced antibody response is not known, underscored by the recent failure of the most advanced tetravalent live attenuated chimeric virus to provide clinically meaningful protection in a large phase 2B trial in Thailand (Sabchareon A, et al., 2012). Viral interference is thought to contribute to failure as one or more virus serotypes out-compete the others. The DENV-1/3 and DENV 3/1 chimeric viruses are single viruses that present epitopes recognized by neutralizing antibodies from both DENV-1 and DENV-3 immune individuals. This indicates that single viruses should be able to elicit neutralizing antibodies targeting two serotypes at once, replacing two viruses (DENV-1 and 3) with one virus (DENV-1/3 or DENV-3/1).

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-B. For the DENV-1/3 mutant, the EDI-II hinge from DENV3 was transplanted into a DENV-1 background, WestPac'74, creating a DENV-3/1 hinge mutant. The EDI-II hinge was defined using the DENV3 specific human mAb 5J7. A) The resultant virus, rDENV-1/3, was tested against monoclonal antibody 5J7. This figure shows that DENV-1 is not neutralized by 5J7, whereas DENV-3 is. rDENV-1/3, which only contains the DENV-3 EDI/II hinge, is neutralized by 5J7 at concentrations equivalent to DENV-3 neutralizing concentrations. This demonstrates successful transplant of the 5J7 epitope into DENV-1. B) This panel shows that DENV-3 is not neutralized by mAb 1F4, DENV-1 is neutralized by 1F4, and rDENV-1/3 is also neutralized, indicating that 1F4 can still bind to and neutralize the chimeric virus.

Figures 2A-B. This figure shows primary DENV-1 and DENV-3 human immune sera tested against DENV-1, DENV-3 and the hinge chimeric virus WestPac-3001 hinge (rDENV-1/3). The Y – axis shows fold dilution of immune sera required to neutralize 50% of input virus in tissue culture. The higher values indicate more potent serum. A) DENV-1 primary immune sera potently neutralizes DENV-1 but not DENV-3. rDENV-1/3 is sensitive to neutralization by DENV-1 immune sera at concentrations similar to DENV-1, indicating that in contrast to the parental DENV-3 virus, the chimeric virus displays epitopes recognized by DENV-1 immune sera. B) DENV-3 primary immune sera does not neutralize DENV-1 but neutralizes DENV-3. rDENV-1/3 is neutralized by DENV-3 primary immune sera at concentrations similar to DENV-3, indicating that the chimeric virus rDENV-1/3 preserves the critical DENV-3 epitopes targeted by DENV-3 antibodies in DENV-3 human immune sera. * indicates not neutralized.

Figure 3. This figure shows that WestPac'74 3001-hinge induces broadly cross-neutralizing antibodies at 28, 60, 90, 120 and 180 days post infection in rhesus macaques. The Y axis shows neutralizing antibody titer as above. The X axis shows each virus serotype. Each plotted point is the neutralizing titer for a single rhesus macaque against a given serotype. The central line through each cluster of points is the geometric mean neutralizing titer for each group of macaques against each serotype. The whiskers show standard error of the mean. Each time point (28, 30, 60, 90, 120, 180 days) shows broadly cross-neutralizing antibody responses against all four serotypes.

Figures 4A-B. For the DENV-3/1 mutants, the EDI-II hinge defined by the monoclonal antibody 1F4 footprint from DENV1 (WestPac '74) was transplanted into a DENV-3 background (3001) creating a DENV-1/3 hinge mutant. This transplant was

executed for three different viruses, (1F4S, 1F4R, and 1F4E), with each variant representing a larger epitope region. The EDI-II hinge from rDENV-3 was put into a recombinant rDENV-1 virus. This figure shows enzyme linked immunosorbent assay (ELISA) data with relative binding of antibody by optical density (OD) on the Y-axis and increasing antibody concentration on the X-axis. A) Binding of mAb 1F4 to 3001-1F4S, R and E. The rising curve against the chimeric virus shows binding of the antibody, in contrast to parental 3001, which does not bind mAb 1F4. B) Binding of mAb 5J7 to parental 3001, 3001-1F4S, R and E. 5J7 binding is preserved in these viruses, whereas epitope donor icWestPac '74 does not bind 5J7.

Figures 5A-B. This figure shows primary DENV-1 and DENV-3 human immune sera tested against DENV-1, DENV-3 and the hinge chimeric virus 3001-1F4E. The Y – axis shows fold dilution of immune sera required to neutralize 50% of input virus in tissue culture. The higher values indicate more potent serum. A) DENV-1 primary immune sera potently neutralizes DENV-1 but not DENV-3. 3001 1F4E is sensitive to neutralization by DENV-1 immune sera at concentrations similar to DENV-1, indicating that in contrast to the parental DENV-3 virus, the chimeric virus displays epitopes recognized by DENV-1 immune sera. B) DENV-3 primary immune sera does not neutralize DENV-1 but neutralizes DENV-3. 3001 1F4E is neutralized by DENV-3 primary immune sera at concentrations similar to DENV-3, indicating that the chimeric virus 3001-1F4E preserves the critical DENV-3 epitopes targeted by DENV-3 antibodies in DENV-3 human immune sera.

Figure 6. Immunogenicity of 3001-1F4E in rhesus macaques. Only one time point is provided, showing broadly cross-neutralizing antibodies, consistent with what was found for WestPac-3001 hinge.

SUMMARY OF THE INVENTION

The present invention provides a chimeric dengue virus E glycoprotein comprising a dengue virus E glycoprotein backbone that comprises amino acid substitutions that introduce an epitope that is recognized by an antibody that is reactive with a dengue virus serotype that is different from the dengue virus serotype of the dengue virus E glycoprotein backbone. In one embodiment, the dengue virus E glycoprotein backbone is from dengue virus serotype 1 and in one embodiment, the dengue virus E glycoprotein backbone is from dengue virus serotype 3. In some embodiments, the antibody is reactive with dengue virus serotype 3 (e.g., monoclonal antibody 5J7) and in other embodiments, the antibody is reactive with dengue virus serotype 1 (e.g., monoclonal antibody 1F4).

The present invention further provides a chimeric dengue virus E glycoprotein, comprising the amino acid sequence:

MRCVGIGNRDFVEGLSGATWVDVVLEHGSCVTTMAKDKPTLDIELLKTEATQLATLRKLC
IEAKISNTTTDSRCPTQGEATLVEEQDTNFVCRRTFVDRGWGNGCGLFGKGSLITCAKFK
CVTKIEGKVVQYENLKYSVIVTVHTGDQHQVGNETTEHGTIATITPQAPTSEIQLTDYGA
LTLDCSPRTGLDFNEMILLTMKNKAWMVHRQWFLDLPLPWTSGASTSQETWNRQDLLVTF
KTAHAKKQEVVVLGSQEGAMHTALTGATEIQNSGGTSIFAGHLKCRLKMDKLTLKGMSYV
MCTGSFKLEKEVAETQHGTVLVQVKYEGTDAPCKIPFSSQDEKGVTQNGRLITANPIVTD
KEKPVNIEAEPPFGESYIVVGAGEKALKLSWFKKG

Also provided herein is a chimeric dengue virus E glycoprotein, comprising the amino acid sequence:

MRCVGIGNRDFVEGLSGATWVDVVLEHGGCVTTMAKNKPTLDIELFKTEVTNPAVLRKLCIE
GKITNITTDSRCPTQGEAVLPEEQDQNYVCKHTYVDRGWGNGCGLFGKGSLVTCAKFQCLEP
IEGKVVQYENLKYSVIVTVHTGDQHQVGNETTEHGTIATITPQAPTSEIQLTDYGALGLECS
PRTGLDFNEMILLTMKNKAWMVHRQWFFDLPLPWTSGATTETPTWNRKELLVTFKNAHAKKQ
EVVVLGSQEGAMHTALTGATEIQTSGTTTIFAGHLKCRLKMDKLELKGMSYAMCTNTFVLKK
EVSETQHGTILIKVEYKGEDAPCKIPFSTEDGQGKAHNGRLITANPVVTKKEEPVNIEAEPP
FGESNIVIGIGDNALKINWYKKG

Additionally provided herein is flavivirus particle or virus like particle (VLP) comprising the E glycoprotein of this invention.

An isolated nucleic acid molecule encoding the E glycoprotein of this invention is also provided herein, as well as an isolated nucleic acid molecule encoding the flavivirus particle or VLP of this invention.

The present invention also provides a composition comprising the E glycoprotein of this invention in a pharmaceutically acceptable carrier and provides a composition comprising the nucleic acid molecule of this invention in a pharmaceutically acceptable carrier.

Furthermore, the present invention provides a method of producing an immune response to a dengue virus in a subject (e.g., a subject in need thereof), comprising administering to the subject an effective amount of the E glycoprotein of this invention, the flavivirus particle of this invention, the nucleic acid molecule of this invention and/or the composition of this invention and any combination thereof.

The present invention also provides a method of treating a dengue virus infection in a subject in need thereof, comprising administering to the subject an effective amount of the E glycoprotein of this invention, the flavivirus particle of this invention, the nucleic acid molecule of any of this invention and/or the composition of this invention and any combination thereof.

Additionally provided herein is a method of preventing a dengue virus infection in a subject (e.g., a subject in need thereof), comprising administering to the subject an effective amount of the E glycoprotein of this invention, the flavivirus particle of this invention, the nucleic acid molecule of any of this invention and/or the composition of this invention and any combination thereof.

A method is also provided herein of protecting a subject (e.g., a subject in need thereof), from the effects of dengue virus infection, comprising administering to the subject an effective amount of the E glycoprotein of this invention, the flavivirus particle of this invention, the nucleic acid molecule of any of this invention and/or the composition of this invention and any combination thereof.

The present invention further provides the E glycoprotein of this invention, the flavivirus particle of this invention, the nucleic acid molecule of this invention and/or the composition of this invention for use in the manufacture of a medicament for producing an immune response to a dengue virus in a subject, for treating a dengue virus infection in a subject in need thereof, for preventing a dengue virus infection in a subject and/or for protecting a subject from the effects of dengue virus infection.

Also provided herein is the use of the E glycoprotein of this invention, the flavivirus particle of this invention, the nucleic acid molecule of this invention and/or the composition of this invention for use in producing an immune response to a dengue virus in a subject, in treating a dengue virus infection in a subject in need thereof, in preventing a dengue virus infection in a subject and/or in protecting a subject from the effects of dengue virus infection.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is based on the unexpected discovery that epitope regions that define a DENV serotype can be transferred into a protein backbone of a different DENV serotype to create a chimeric molecule that contains antibody targets for both serotypes, thereby functioning as a bivalent vaccine that can induce neutralizing antibodies against two different DENV serotypes from a single source. Thus, in one embodiment, the present invention provides a platform for construction of a chimeric dengue virus E glycoprotein backbone that comprises amino acid substitutions that introduce epitopes that are recognized by an antibody that is reactive with a dengue virus serotype that is different from the dengue virus serotype of the dengue virus E glycoprotein backbone.

In some embodiments, that dengue virus E glycoprotein backbone is from dengue virus serotype 1. In some embodiments, the dengue virus E glycoprotein backbone can be from dengue virus serotype 2, dengue virus serotype 3 or dengue virus serotype 4.

In some embodiments, the antibody that is reactive with a dengue virus serotype that is different from the dengue virus serotype of the dengue virus E glycoprotein backbone is an antibody that is reactive with dengue virus serotype 3. A nonlimiting example of such an antibody is monoclonal antibody 5J7.

In other embodiments, the antibody that is reactive with a dengue virus serotype that is different from the dengue virus serotype of the dengue virus E glycoprotein backbone is an antibody that is reactive with dengue virus serotype 1, dengue virus serotype 2 or dengue virus serotype 4.

It would be understood that any combination of a first dengue virus serotype for the dengue virus E glycoprotein backbone and a second dengue virus serotype that is the target of the antibody that recognizes the epitope introduced into the E glycoprotein backbone can be used, provided that the first dengue virus serotype and the second dengue virus serotype are different (i.e., not the same serotype).

In some embodiments, the chimeric dengue virus E glycoprotein of this invention can comprise, consist essentially of or consist of the amino acid sequence:

WestPac74-3001 hinge (rDENV-1/3)
MRCVGIGNRDFVEGLSGATWVDVVLEHGSCVTTMAKDKPTLDIELLKTEATQLATLRKLC
IEAKISNTTTDSRCPTQGEATLVEEQDTNFVCRRTFVDRGWGNGCGLFGKGSLITCAKFK
CVTKIEGKVVQYENLKYSVIVTVHTGDQHQVGNETTEHGTIATITPQAPTSEIQLTDYGA
LTLDCSPRTGLDFNEMILLTMKNKAWMVHRQWFLDLPLPWTSGASTSQETWNRQDLLVTF
KTAHAKKQEVVVLGSQEGAMHTALTGATEIQNSGGTSIFAGHLKCRLKMDKLTLKGMSYV
MCTGSFKLEKEVAETQHGTVLVQVKYEGTDAPCKIPFSSQDEKGVTQNGRLITANPIVTD
KEKPVNIEAEPPFGESYIVVGAGEKALKLSWFKKG.

In some embodiments, the chimeric dengue virus E glycoprotein of this invention can comprise, consist essentially of or consist of the amino acid sequence:

3001-1F4E (rDENV-3/1)
MRCVGIGNRDFVEGLSGATWVDVVLEHGGCVTTMAKNKPTLDIELFKTEVTNPAVLRKLCIE
GKITNITTDSRCPTQGEAVLPEEQDQNYVCKHTYVDRGWGNGCGLFGKGSLVTCAKFQCLEP
IEGKVVQYENLKYSVIVTVHTGDQHQVGNETTEHGTIATITPQAPTSEIQLTDYGALGLECS
PRTGLDFNEMILLTMKNKAWMVHRQWFFDLPLPWTSGATTETPTWNRKELLVTFKNAHAKKQ
EVVVLGSQEGAMHTALTGATEIQTSGTTTIFAGHLKCRLKMDKLELKGMSYAMCTNTFVLKK
EVSETQHGTILIKVEYKGEDAPCKIPFSTEDGQGKAHNGRLITANPVVTKKEEPVNIEAEPP
FGESNIVIGIGDNALKINWYKKG

The present invention also provides a flavivirus particle or virus like particle (VLP) comprising the chimeric E glycoprotein of this invention.

Production of the chimeras of this invention can be carried out by introducing some (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, etc.) or all of the amino acid substitutions identified in **Table 1** into a dengue virus E glycoprotein backbone or flavivirus E glycoprotein backbone. Not every amino acid identified in **Table 1** is required to be substituted to produce a chimeric protein of this invention. For example, in some embodiments further substitutions and/or omission of substitutions of about 1, 2, 3, 4 or 5 amino acids at either end of the contiguous amino acid sequences identified in **Table 1** as the respective epitope regions can be included in production of a chimera of this invention. The number of substitutions necessary to produce the desired conformational epitope can be readily determined by one of ordinary skill in the art according to the teachings herein and according to protocols well known in the art. The amino acid position numbering in **Table 1** is based on the amino acid sequence of WestPac74 (DENV-1), or the amino acid sequence of UNC 3001 (DENV-3), as provided herein. However it would be readily understood by one of ordinary skill in the art that the equivalent amino acid positions in other dengue virus E glycoprotein amino acid sequences or other flavivirus E glycoprotein amino acid sequences can be readily identified and employed in the production of the chimeric proteins of this invention.

Table 2 shows one example of modifications that can be made to the nucleotide sequence encoding the DENV-1 E glycoprotein to introduce the epitope that is recognized by the monoclonal antibody 5J7, which is reactive with DENV-3. The amino acid sequence that results from translation of a nucleotide sequence comprising these substitutions is:

MRCVGIGNRDFVEGLSGATWVDVVLEHGSCVTTMAKDKPTLDIELLKTE**A**T**Q**L**A**TLRKLC
IEAKISNTTTDSRCPTQGEATLVEEQDTNFVCRRTFVDRGWGNGCGLFGKGSLITCAKFK
CVTK**I**EGK**V**VQYENLKYSVIVTVHTGDQHQVGNETTEHGT**I**ATITPQAPTSEIQLTDYGA
LTLDCSPRTGLDFNEM**I**LLTM**KN**K**A**W**M**VHRQWFLDLPLPWTSGASTSQETWNRQDLLVTF
KTAHAKKQEVVVLGSQEGAMHTALTGATEIQ**N**SG**G**T**S**IFAGHLKCRLKMDKLTLKGMSYV
MCTGSFKLEKEVAETQHGTVLVQVKYEGTDAPCKIPFSSQDEKGVTQNGRLITANPIVTD
KEKPVNIEAEPPFGESYIVVGAGEKALKLSWFKKG.

It would be understood that the modifications provided in **Table 2** provide one example of how the amino acid sequence above can be obtained and that, due to the degeneracy of the amino acid codons, numerous other modifications can be made to the nucleotide sequence encoding the DENV-3 E glycoprotein to obtain this amino acid sequence.

Table 3 shows that WestPac'74 3001-hinge is infectious in rhesus macaques infected subcutaneously with 500,000 infectious units of virus. The reported values for each day are log transformed monkey serum virus titers quantified by immunofocus assay.

Table 4. Attenuation of 3001-1F4E in rhesus macaques. This table shows that 3001-1F4E is infectious in rhesus macaques infected subcutaneously with 500,000 infectious units of virus. *However*, this virus was below quantitative level of detection (50 infectious virus/mL serum). A more sensitive assay, the delayed focus assay, is capable of detecting virus <50 infectious units/mL, but is not capable of quantifying the low level of virus present. Consequently days for which virus was detected by our most sensitive assay are scored as positive with "+". Total number of days infected are shown in the left column. The low level of viremia and low mean number of days infected (2.25 days) are consistent with virus attenuation in macaques.

Table 5. To further characterize the chimeric virus DENV 1/3, it was probed with a DENV-1 specific monoclonal antibody, 1F4. 1F4 is serotype specific and its target epitope is in the EDI-II hinge. If the transplanted DENV-3 EDI-II hinge disrupts the 1F4 epitope, 1F4 should no longer neutralize the chimeric WestPac74/3001 virus.

In some embodiments, the present invention provides a chimeric flavivirus E glycoprotein in which amino acid substitutions are made to introduce a dengue virus epitope into a flavivirus E glycoprotein from a flavivirus that is not a dengue virus. Thus, in some embodiments, the present invention provides a flavivirus E glycoprotein comprising a chimeric E glycoprotein comprising a flavivirus E glycoprotein backbone that is not a dengue virus E glycoprotein backbone, wherein the flavivirus E glycoprotein backbone comprises amino acid substitutes that introduce an epitope that is recognized by an antibody that is reactive with a dengue virus.

Nonlimiting examples of flaviviruses that can be used include yellow fever virus (YFV) (e.g., GenBank® Database Accession No. JX503529) Japanese encephalitis virus (JEV) (e.g., GenBank® Database Accession No. U14163), West Nile virus (WNV) (e.g., GenBank® Database Accession No. DQ211652) and any other flavivirus now known or later identified.

It is known in the art that many attempts to produce dengue virus vaccines result in the production of non-neutralizing antibodies, which may increase the likelihood of pathology upon subsequence exposure to natural infection or vaccine. Another approach to provide an engineered epitope is to deliver all or a portion of the dengue virus E protein incorporated into another flavivirus particle or VLP. In representative embodiments, the heterologous flavivirus is West Nile virus or Yellow Fever virus. Portions of the E protein can be grafted into the E protein of the heterologous flavivirus backbone, *e.g.*, to reduce the

generation of non-neutralizing dengue virus antibodies to non-neutralizing epitopes present in the dengue virus E protein and/or other dengue virus structural proteins.

Thus, a chimeric flavivirus or chimeric flavivirus VLP can present the quaternary dengue virus epitope in proper conformation while reducing the generation of non-neutralizing antibodies to other portions of the dengue virus E protein and/or other structural proteins that are not presented in the chimeric flavivirus or flavivirus VLP.

In some embodiments of the invention the individual and conformational epitopes of the flavivirus E glycoprotein or dengue virus E glycoprotein can be presented on a synthetic backbone or support structure so that the epitopes within the synthetic backbone or support structure mimic the conformation and arrangement of the epitopes within the structure of the E glycoprotein, virus particle or VLP.

In still further embodiments of the invention, the present invention provides peptide mimitopes (see, Meloen et al. (2000) *J. Mol. Recognit.* 13, 352–359) that mimic the individual and conformational epitopes of the E glycoproteins of the invention. Mimitopes may be identified using any technique known in the art, such as by surface stimulation, random peptide libraries or phage display libraries, using an antibody or antibodies to the individual and conformational epitopes of the E glycoproteins of the invention.

The invention further provides a nucleic acid (*e.g.*, isolated nucleic acid) encoding a dengue virus epitope or a polypeptide of the invention.

The invention further provides a nucleic acid (*e.g.*, an isolated nucleic acid) encoding a chimeric flavivirus VLP or a chimeric flavivirus particle (e.g., a viral coat of the flavivirus particle) of the invention.

Also provided are vectors encoding the nucleic acids of the invention.

Also provided are cells comprising the vectors, nucleic acids, dengue virus epitopes, polypeptides, chimeric flavivirus VLPs or chimeric flavivirus particles of the invention.

The invention also provides immunogenic compositions comprising the cells, vectors, nucleic acids, dengue virus epitopes, polypeptides, chimeric flavivirus VLPs or chimeric flavivirus particles of the invention. In embodiments, the immunogenic composition is monovalent. In embodiments, the immunogenic composition is multivalent (*e.g.*, tetravalent) for dengue virus serotypes DEN1, DEN2, DEN 3 and/or DEN4.

The invention encompasses methods of producing an immune response to a dengue virus in a subject, the method comprising administering to the subject an effective amount of a dengue virus epitope, a polypeptide, a chimeric flavivirus VLP or chimeric flavivirus particle, nucleic acid, vector, cell or immunogenic composition of the invention.

Further, the present invention can advantageously be practiced to induce an immune response against one, two, three or all four of DEN1, DEN2, DEN3 and DEN4. It is well-known in the art that effective and safe multivalent dengue vaccines have been a challenge to design because of the problem of interference among serotypes. For example, the immune response may be predominantly directed against only some of the target serotypes. Multiple vaccinations are then required to try to achieve a response against all serotypes; however, in the case of dengue virus, this approach can be dangerous because repeated administrations to a subject with pre-existing antibodies can lead to dengue hemorrhagic fever.

A still further aspect of the invention is a method of treating a dengue virus infection, comprising administering to the subject an effective amount of a dengue virus epitope, a polypeptide, a chimeric flavivirus VLP or chimeric flavivirus particle, nucleic acid, vector, cell, or immunogenic composition of the invention.

A still further aspect of the invention is a method of preventing a dengue virus infection, comprising administering to the subject an effective amount of a dengue virus epitope, a polypeptide, a chimeric flavivirus VLP or chimeric flavivirus particle, nucleic acid, vector, cell, or immunogenic composition of the invention.

A still further aspect of the invention is a method of protecting a subject from the effects of dengue virus infection, comprising administering to the subject an effective amount of a dengue virus epitope, a polypeptide, a chimeric flavivirus VLP or chimeric flavivirus particle, nucleic acid, vector, cell, or immunogenic composition of the invention.

There are four serotypes of dengue virus (DENV-1, DENV-2, DENV-3 and DENV-4). Within each serotype there are a number of different strains or genotypes. The dengue virus antigens and epitopes of the invention can be derived from any dengue virus, including all serotypes, strains and genotypes, now known or later identified.

In embodiments of the invention, the dengue virus is UNC1017 strain (DEN1), West Pacific 74 strain (DEN1), S16803 strain (DEN2), UNC2005 strain (DEN2), UNC3001 strain (DEN3), UNC3043 (DEN3 strain 059.AP-2 from Philippines, 1984), UNC3009 strain (DEN3, D2863, Sri Lanka 1989), UNC3066 (DEN3, strain 1342 from Puerto Rico 1977), CH53489 strain (DEN3), UNC4019 strain (DEN4), or TVP-360 (DEN4).

In embodiments of the invention, an "immunogenically active fragment" of a dengue virus polypeptide (*e.g.*, the E protein) comprises, consists essentially of or consists of at least about 6, 8, 10, 12, 15, 20, 30, 50, 75, 100, 125, 150, 200, 250, 300, 350, 400, 450 or more amino acids, optionally contiguous amino acids, and/or less than about 495, 475, 450, 425, 400, 350, 300, 250, 200, 150, 100, 75 or 50 amino acids, optionally contiguous amino acids,

including any combination of the foregoing as long as the lower limit is less than the upper limit, and the "immunogenically active fragment" induces an immune response (*e.g.*, IgG and/or IgA that react with the native antigen), optionally a protective immune response, against dengue virus in a host and induces the production of antibodies that specifically bind to the quaternary dengue virus epitope newly identified by the inventors.

The term "epitope" as used herein means a specific amino acid sequence that, when present in the proper conformation, provides a reactive site for an antibody (*e.g.*, B cell epitope) or T cell receptor (*e.g.*, T cell epitope).

Portions of a given polypeptide that include a B-cell epitope can be identified using any number of epitope mapping techniques that are known in the art. (*See, e.g.*, Epitope Mapping Protocols in Methods in Molecular Biology, Vol. 66, Glenn E. Morris, Ed., 1996, Humana Press, Totowa, N.J.). For example, linear epitopes can be determined by, *e.g.*, concurrently synthesizing large numbers of peptides on solid supports, the peptides corresponding to portions of the protein molecule, and reacting the peptides with antibodies while the peptides are still attached to the supports. Such techniques are known in the art and described in, *e.g.*, U.S. Pat. No. 4,708,871; Geysen et al. (1984) *Proc. Natl. Acad. Sci.* USA 81:3998-4002; Geysen et al. (1986) *Molec. Immunol.* 23:709-715.

Similarly, conformational epitopes can be readily identified by determining spatial conformation of amino acids such as by, *e.g.*, x-ray crystallography and 2-dimensional nuclear magnetic resonance. Antigenic regions of proteins can also be identified using standard antigenicity and hydropathy plots, such as those calculated using, *e.g.*, the Omiga version 1.0 software program available from the Oxford Molecular Group. This computer program employs the Hopp/Woods method (Hopp et al., *Proc. Natl. Acad. Sci* USA (1981) 78:3824-3828) for determining antigenicity profiles and the Kyte-Doolittle technique (Kyte et al., *J. Mol. Biol.* (1982) 157:105-132) for hydropathy plots.

Generally, T-cell epitopes that are involved in stimulating the cellular arm of a subject's immune system are short peptides of about 8-25 amino acids. A common way to identify T-cell epitopes is to use overlapping synthetic peptides and analyze pools of these peptides, or the individual ones, that are recognized by T cells from animals that are immune to the antigen of interest, using, for example, an enzyme-linked immunospot assay (ELISPOT). These overlapping peptides can also be used in other assays such as the stimulation of cytokine release or secretion, or evaluated by constructing major histocompatibility (MHC) tetramers containing the peptide. Such immunogenically active

fragments can also be identified based on their ability to stimulate lymphocyte proliferation in response to stimulation by various fragments from the antigen of interest.

The present invention can be practiced for prophylactic, therapeutic and/or diagnostic purposes. In addition, the invention can be practiced to produce antibodies for any purpose, such as diagnostic or research purposes, or for passive immunization by transfer to another subject.

The present invention further provides a kit comprising one or more compositions of this invention. It would be well understood by one of ordinary skill in the art that the kit of this invention can comprise one or more containers and/or receptacles to hold the reagents (e.g., antibodies, antigens, nucleic acids) of the kit, along with appropriate buffers and/or diluents and/or other solutions and directions for using the kit, as would be well known in the art. Such kits can further comprise adjuvants and/or other immunostimulatory or immunomodulating agents, as are well known in the art.

The compositions and kits of the present invention can also include other medicinal agents, pharmaceutical agents, carriers, diluents, immunostimulatory cytokines, etc. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in this art.

Administration to a subject can be by any route known in the art. As non-limiting examples, the route of administration can be by inhalation (*e.g.*, oral and/or nasal inhalation), oral, buccal (*e.g.*, sublingual), rectal, vaginal, topical (including administration to the airways), intraocular, transdermal, by parenteral (*e.g.*, intramuscular [*e.g.*, administration to skeletal muscle], intravenous, intra-arterial, intraperitoneal and the like), subcutaneous (including administration into the footpad), intradermal, intrapleural, intracerebral, and/or intrathecal routes.

The epitopes, polypeptides, VLPs and viral vectors of the invention can be delivered *per se* or by delivering a nucleic acid (*e.g.*, DNA) that encodes the same.

Immunomodulatory compounds, such as immunomodulatory chemokines and cytokines (preferably, CTL inductive cytokines) can be administered concurrently to a subject.

Cytokines may be administered by any method known in the art. Exogenous cytokines may be administered to the subject, or alternatively, a nucleic acid encoding a cytokine may be delivered to the subject using a suitable vector, and the cytokine produced *in vivo*. In particular embodiments, a viral adjuvant expresses the cytokine.

In embodiments of the invention, multiple dosages (*e.g.*, two, three or more) of a composition of the invention can be administered without detectable pathogenicity (*e.g.*, Dengue Shock Syndrome/Dengue Hemorrhagic Fever).

In embodiments of the invention, the multivalent vaccines of the invention do not result in immune interference, *e.g.*, a balanced immune response is induced against all antigens presented. In embodiments of the invention, the balanced response results in protective immunity against DENV-1, DENV-2, DENV-3 and DENV-4.

In embodiments of the invention, the multivalent vaccine can be administered to a subject that has anti-dengue maternal antibodies present.

It should be appreciated that the invention can be embodied in different forms and should not be construed as limited to the embodiments set forth herein. Rather, these embodiments are provided so that this disclosure will be thorough and complete, and will fully convey the scope of the invention to those skilled in the art.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. The terminology used in the description of the invention herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention.

As used herein, "a," "an" or "the" can mean one or more than one. For example, "a" cell can mean a single cell or a multiplicity of cells.

Also as used herein, "and/or" refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative ("or").

The term "about," as used herein when referring to a measurable value such as an amount of dose (*e.g.*, an amount of a fatty acid) and the like, is meant to encompass variations of ± 20%, ± 10%, ± 5%, ± 1%, ± 0.5%, or even ± 0.1% of the specified amount.

As used herein, the transitional phrase "consisting essentially of" means that the scope of a claim is to be interpreted to encompass the specified materials or steps recited in the claim, "and those that do not materially affect the basic and novel characteristic(s)" of the claimed invention. *See*, *In re Herz*, 537 F.2d 549, 551-52, 190 U.S.P.Q. 461, 463 (CCPA 1976) (emphasis in the original); *see also* MPEP § 2111.03. Thus, the term "consisting essentially of" when used in a claim of this invention is not intended to be interpreted to be equivalent to "comprising."

As used herein, the term "nucleic acid" encompasses both RNA and DNA, including cDNA, genomic DNA, synthetic (*e.g.*, chemically synthesized) DNA and chimeras of RNA and DNA. The nucleic acid may be double-stranded or single-stranded. The nucleic acid may be synthesized using nucleotide analogs or derivatives (e.g., inosine or phosphorothioate nucleotides). Such nucleotides can be used, for example, to prepare nucleic acids that have altered base-pairing abilities or increased resistance to nucleases.

As used herein, the term "polypeptide" encompasses both peptides and proteins (including fusion proteins), unless indicated otherwise.

A "fusion protein" is a polypeptide produced when two heterologous nucleotide sequences or fragments thereof coding for two (or more) different polypeptides not found fused together in nature are fused together in the correct translational reading frame.

A "recombinant" nucleic acid, polynucleotide or nucleotide sequence is one produced by genetic engineering techniques.

A "recombinant" polypeptide is produced from a recombinant nucleic acid, polypeptide or nucleotide sequence.

As used herein, an "isolated" polynucleotide (*e.g.*, an "isolated nucleic acid" or an "isolated nucleotide sequence") means a polynucleotide at least partially separated from at least some of the other components of the naturally occurring organism or virus, for example, the cell or viral structural components or other polypeptides or nucleic acids commonly found associated with the polynucleotide. Optionally, but not necessarily, the "isolated" polynucleotide is present at a greater concentration (*i.e.*, is enriched) as compared with the starting material (*e.g.*, at least about a two-fold, three-fold, four-fold, ten-fold, twenty-fold, fifty-fold, one-hundred-fold, five-hundred-fold, one thousand-fold, ten thousand-fold or greater concentration). In representative embodiments, the isolated polynucleotide is at least about 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or more pure.

An "isolated" polypeptide means a polypeptide that is at least partially separated from at least some of the other components of the naturally occurring organism or virus, for example, the cell or viral structural components or other polypeptides or nucleic acids commonly found associated with the polypeptide. Optionally, but not necessarily, the "isolated" polypeptide is present at a greater concentration (*i.e.*, is enriched) as compared with the starting material (*e.g.*, at least about a two-fold, three-fold, four-fold, ten-fold, twenty-fold, fifty-fold, one-hundred-fold, five-hundred-fold, one thousand-fold, ten thousand-fold or greater concentration). In representative embodiments, the isolated polypeptide is at least about 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or more pure.

Furthermore, an "isolated" cell is a cell that has been partially or completely separated from other components with which it is normally associated in nature. For example, an isolated cell can be a cell in culture medium and/or a cell in a pharmaceutically acceptable carrier.

The terms "immunogen" and "antigen" are used interchangeably herein and mean any compound (including polypeptides) to which a cellular and/or humoral immune response can be directed. In particular embodiments, an immunogen or antigen can induce a protective immune response against the effects of dengue virus infection.

"Effective amount" as used herein refers to an amount of a vector, nucleic acid, epitope, polypeptide, cell, particle, VLP, composition or formulation of the invention that is sufficient to produce a desired effect, which can be a therapeutic and/or beneficial effect. The effective amount will vary with the age, general condition of the subject, the severity of the condition being treated, the particular agent administered, the duration of the treatment, the nature of any concurrent treatment, the pharmaceutically acceptable carrier used, and like factors within the knowledge and expertise of those skilled in the art. As appropriate, an "effective amount" in any individual case can be determined by one of ordinary skill in the art by reference to the pertinent texts and literature and/or by using routine experimentation.

The term "immunogenic amount" or "effective immunizing dose," as used herein, unless otherwise indicated, means an amount or dose sufficient to induce an immune response (which can optionally be a protective response) in the treated subject that is greater than the inherent immunity of non-immunized subjects. An immunogenic amount or effective immunizing dose in any particular context can be routinely determined using methods known in the art.

The terms "vaccine," "vaccination" and "immunization" are well-understood in the art, and are used interchangeably herein. For example, the terms vaccine, vaccination or immunization can be understood to be a process or composition that increases a subject's immune reaction to an immunogen (*e.g.*, by providing an active immune response), and therefore its ability to resist, overcome and/or recover from infection (*i.e.*, a protective immune response).

By the terms "treat," "treating" or "treatment of" (and grammatical variations thereof) it is meant that the severity of the subject's condition is reduced, at least partially improved or ameliorated and/or that some alleviation, mitigation or decrease in at least one clinical symptom is achieved and/or there is a delay in the progression of the disease or disorder. In representative embodiments, the terms "treat," "treating" or "treatment of" (and grammatical

variations thereof) refer to a reduction in the severity of viremia and/or a delay in the progression of viremia, with or without other signs of clinical disease.

A "treatment effective" amount as used herein is an amount that is sufficient to treat (as defined herein) the subject. Those skilled in the art will appreciate that the therapeutic effects need not be complete or curative, as long as some benefit is provided to the subject.

The term "prevent," "preventing" or "prevention of" (and grammatical variations thereof) refer to prevention and/or delay of the onset and/or progression of a disease, disorder and/or a clinical symptom(s) in a subject and/or a reduction in the severity of the onset and/or progression of the disease, disorder and/or clinical symptom(s) relative to what would occur in the absence of the methods of the invention. In representative embodiments, the terms "prevent," "preventing" or "prevention of" (and grammatical variations thereof) refer to prevention and/or delay of the onset and/or progression of viremia in the subject, with or without other signs of clinical disease. The prevention can be complete, *e.g.*, the total absence of the disease, disorder and/or clinical symptom(s). The prevention can also be partial, such that the occurrence of the disease, disorder and/or clinical symptom(s) in the subject and/or the severity of onset and/or the progression is less than what would occur in the absence of the present invention.

A "prevention effective" amount as used herein is an amount that is sufficient to prevent (as defined herein) the disease, disorder and/or clinical symptom in the subject. Those skilled in the art will appreciate that the level of prevention need not be complete, as long as some benefit is provided to the subject.

The efficacy of treating and/or preventing dengue virus infection by the methods of the present invention can be determined by detecting a clinical improvement as indicated by a change in the subject's symptoms and/or clinical parameters (*e.g.*, viremia), as would be well known to one of skill in the art.

Unless indicated otherwise, the terms "protect," "protecting," "protection" and "protective" (and grammatical variations thereof) encompass both methods of preventing and treating dengue virus infection in a subject, whether against one or multiple strains, genotypes or serotypes of dengue virus.

The terms "protective" immune response or "protective" immunity as used herein indicates that the immune response confers some benefit to the subject in that it prevents or reduces the incidence and/or severity and/or duration of disease or any other manifestation of infection. For example, in representative embodiments, a protective immune response or protective immunity results in reduced viremia, whether or not accompanied by clinical

disease. Alternatively, a protective immune response or protective immunity may be useful in the therapeutic treatment of existing disease.

An "active immune response" or "active immunity" is characterized by "participation of host tissues and cells after an encounter with the immunogen. It involves differentiation and proliferation of immunocompetent cells in lymphoreticular tissues, which lead to synthesis of antibody or the development of cell-mediated reactivity, or both." Herbert B. Herscowitz, *Immunophysiology: Cell Function and Cellular Interactions in Antibody Formation, in* IMMUNOLOGY: BASIC PROCESSES 117 (Joseph A. Bellanti ed., 1985). Alternatively stated, an active immune response is mounted by the host after exposure to immunogens by infection or by vaccination. Active immunity can be contrasted with passive immunity, which is acquired through the "transfer of preformed substances (antibody, transfer factor, thymic graft, interleukin-2) from an actively immunized host to a non-immune host." *Id.*

A "subject" of the invention includes any animal susceptible to dengue virus infection. Such a subject is generally a mammalian subject (*e.g.*, a laboratory animal such as a rat, mouse, guinea pig, rabbit, primates, *etc.*), a farm or commercial animal (*e.g.*, a cow, horse, goat, donkey, sheep, *etc.*), or a domestic animal (*e.g.*, cat, dog, ferret, *etc.*). In particular embodiments, the subject is a primate subject, a non-human primate subject (*e.g.*, a chimpanzee, baboon, monkey, gorilla, *etc.*) or a human. Subjects of the invention can be a subject known or believed to be at risk of infection by dengue virus. Alternatively, a subject according to the invention can also include a subject not previously known or suspected to be infected by dengue virus or in need of treatment for dengue virus infection.

Subjects may be treated for any purpose, such as for eliciting a protective immune response or for eliciting the production of antibodies in that subject, which antibodies can be collected and used for other purposes such as research or diagnostic purposes or for administering to other subjects to produce passive immunity therein, *etc.*

Subjects include males and/or females of any age, including neonates, juvenile, mature and geriatric subjects. With respect to human subjects, in representative embodiments, the subject can be an infant (*e.g.*, less than about 12 months, 10 months, 9 months, 8 months, 7 months, 6 months, or younger), a toddler (*e.g.*, at least about 12, 18 or 24 months and/or less than about 36, 30 or 24 months), or a child (*e.g.*, at least about 1, 2, 3, 4 or 5 years of age and/or less than about 14, 12, 10, 8, 7, 6, 5, or 4 years of age). In embodiments of the invention, the subject is a human subject that is from about 0 to 3, 4, 5, 6, 9, 12, 15, 18, 24, 30, 36, 48 or 60 months of age, from about 3 to 6, 9, 12, 15, 18, 24, 30, 36, 48 or 60 months

of age, from about 6 to 9, 12, 15, 18, 24, 30, 36, 48 or 60 months of age, from about 9 to 12, 15, 18, 24, 30, 36, 48 or 60 months of age, from about 12 to 18, 24, 36, 48 or 60 months of age, from about 18 to 24, 30, 36, 48 or 60 months of age, or from about 24 to 30, 36, 48 or 60 months of age.

In embodiments of the invention, the subject has maternal antibodies to dengue virus.

A "subject in need" of the methods of the invention can be a subject known to be, or suspected of being, infected with, or at risk of being infected with, dengue virus.

Pharmaceutical formulations (*e.g.*, immunogenic formulation) comprising the dengue virus epitopes, polypeptides, chimeric flavivirus VLPs or chimeric flavivirus particles, nucleic acids, vectors, cells or compositions of the invention and a pharmaceutically acceptable carrier are also provided, and can be formulated for administration in a pharmaceutical carrier in accordance with known techniques. *See, e.g.,* Remington, *The Science And Practice of Pharmacy* (latest edition). In the manufacture of a pharmaceutical composition according to embodiments of the present invention, the composition of the invention is typically admixed with, *inter alia*, a pharmaceutically acceptable carrier. By "pharmaceutically acceptable carrier" is meant a carrier that is compatible with other ingredients in the pharmaceutical composition and that is not harmful or deleterious to the subject. The carrier may be a solid or a liquid, or both, and is preferably formulated with the composition of the invention as a unit-dose formulation, for example, a tablet, which may contain from about 0.01 or 0.5% to about 95% or 99% by weight of the composition. The pharmaceutical compositions are prepared by any of the well-known techniques of pharmacy including, but not limited to, admixing the components, optionally including one or more accessory ingredients. In certain embodiments, the pharmaceutically acceptable carrier is sterile and would be deemed suitable for administration into human subjects according to regulatory guidelines for pharmaceutical compositions comprising the carrier.

Furthermore, a "pharmaceutically acceptable" component such as a salt, carrier, excipient or diluent of a composition according to the present invention is a component that (i) is compatible with the other ingredients of the composition in that it can be combined with the compositions of the present invention without rendering the composition unsuitable for its intended purpose, and (ii) is suitable for use with subjects as provided herein without undue adverse side effects (such as toxicity, irritation, and allergic response). Side effects are "undue" when their risk outweighs the benefit provided by the composition. Non-limiting examples of pharmaceutically acceptable components include any of the standard

pharmaceutical carriers such as phosphate buffered saline solutions, water, emulsions such as oil/water emulsion, microemulsions and various types of wetting agents.

In some embodiments, the compositions of the invention can further comprise one or more than one adjuvant. The adjuvants of the present invention can be in the form of an amino acid sequence, and/or in the form or a nucleic acid encoding an adjuvant. When in the form of a nucleic acid, the adjuvant can be a component of a nucleic acid encoding the polypeptide(s) or fragment(s) or epitope(s) and/or a separate component of the composition comprising the nucleic acid encoding the polypeptide(s) or fragment(s) or epitope(s) of the invention. According to the present invention, the adjuvant can also be an amino acid sequence that is a peptide, a protein fragment or a whole protein that functions as an adjuvant, and/or the adjuvant can be a nucleic acid encoding a peptide, protein fragment or whole protein that functions as an adjuvant. As used herein, "adjuvant" describes a substance, which can be any immunomodulating substance capable of being combined with a composition of the invention to enhance, improve or otherwise modulate an immune response in a subject.

In further embodiments, the adjuvant can be, but is not limited to, an immunostimulatory cytokine (including, but not limited to, GM/CSF, interleukin-2, interleukin-12, interferon-gamma, interleukin-4, tumor necrosis factor-alpha, interleukin-1, hematopoietic factor flt3L, CD40L, B7.1 co-stimulatory molecules and B7.2 co-stimulatory molecules), SYNTEX adjuvant formulation 1 (SAF-1) composed of 5 percent (wt/vol) squalene (DASF, Parsippany, N.J.), 2.5 percent Pluronic, L121 polymer (Aldrich Chemical, Milwaukee), and 0.2 percent polysorbate (Tween 80, Sigma) in phosphate-buffered saline. Suitable adjuvants also include an aluminum salt such as aluminum hydroxide gel (alum), aluminum phosphate, or algammulin, but may also be a salt of calcium, iron or zinc, or may be an insoluble suspension of acylated tyrosine, or acylated sugars, cationically or anionically derivatized polysaccharides, or polyphosphazenes.

Other adjuvants are well known in the art and include without limitation MF 59, LT-K63, LT-R72 (Pal et al., *Vaccine* 24(6):766-75 (2005)), QS-21, Freund's adjuvant (complete and incomplete), aluminum hydroxide, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-isoglutamine (CGP 11637, referred to as nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn -glycero-3-hydroxyphosphoryloxy)-ethylamine (CGP 19835A, referred to as MTP-PE) and RIBI, which contains three components extracted from bacteria, monophosphoryl lipid A,

trealose dimycolate and cell wall skeleton (MPL+TDM+CWS) in 2% squalene/Tween 80 emulsion.

Additional adjuvants can include, for example, a combination of monophosphoryl lipid A, preferably 3-de-O-acylated monophosphoryl. lipid A (3D-MPL) together with an aluminum salt. An enhanced adjuvant system involves the combination of a monophosphoryl lipid A and a saponin derivative, particularly the combination of QS21 and 3D-MPL as disclosed in PCT publication number WO 94/00153, or a less reactogenic composition where the QS21 is quenched with cholesterol as disclosed in PCT publication number WO 96/33739. A particularly potent adjuvant formulation involving QS21 3D-MPL & tocopherol in an oil in water emulsion is described in PCT publication number WO 95/17210. In addition, the nucleic acid compositions of the invention can include an adjuvant by comprising a nucleotide sequence encoding the antigen and a nucleotide sequence that provides an adjuvant function, such as CpG sequences. Such CpG sequences, or motifs, are well known in the art.

An adjuvant for use with the present invention, such as, for example, an immunostimulatory cytokine, can be administered before, concurrent with, and/or within a few hours, several hours, and/or 1, 2, 3, 4, 5, 6, 7, 8, 9, and/or 10 days before and/or after the administration of a composition of the invention to a subject.

Furthermore, any combination of adjuvants, such as immunostimulatory cytokines, can be co-administered to the subject before, after and/or concurrent with the administration of an immunogenic composition of the invention. For example, combinations of immunostimulatory cytokines, can consist of two or more immunostimulatory cytokines, such as GM/CSF, interleukin-2, interleukin-12, interferon-gamma, interleukin-4, tumor necrosis factor-alpha, interleukin-1, hematopoietic factor flt3L, CD40L, B7.1 co-stimulatory molecules and B7.2 co-stimulatory molecules. The effectiveness of an adjuvant or combination of adjuvants can be determined by measuring the immune response produced in response to administration of a composition of this invention to a subject with and without the adjuvant or combination of adjuvants, using standard procedures, as described herein and as known in the art.

In embodiments of the invention, the adjuvant comprises an alphavirus adjuvant as described, for example in U.S. 7,862,829.

Boosting dosages can further be administered over a time course of days, weeks, months or years. In chronic infection, initial high doses followed by boosting doses may be advantageous.

The pharmaceutical formulations of the invention can optionally comprise other medicinal agents, pharmaceutical agents, stabilizing agents, buffers, carriers, diluents, salts, tonicity adjusting agents, wetting agents, and the like, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, sorbitan monolaurate, triethanolamine oleate, *etc.*

For injection, the carrier will typically be a liquid. For other methods of administration, the carrier may be either solid or liquid. For inhalation administration, the carrier will be respirable, and is typically in a solid or liquid particulate form.

The compositions of the invention can be formulated for administration in a pharmaceutical carrier in accordance with known techniques. See, e.g., Remington, The Science And Practice of Pharmacy (9^{th} Ed. 1995). In the manufacture of a pharmaceutical composition according to the invention, the VLPs are typically admixed with, inter alia, an acceptable carrier. The carrier can be a solid or a liquid, or both, and is optionally formulated with the compound as a unit-dose formulation, for example, a tablet. A variety of pharmaceutically acceptable aqueous carriers can be used, e.g., water, buffered water, 0.9% saline, 0.3% glycine, hyaluronic acid, pyrogen-free water, pyrogen-free phosphate-buffered saline solution, bacteriostatic water, or Cremophor EL[R] (BASF, Parsippany, N.J.), and the like. These compositions can be sterilized by conventional techniques. The formulations of the invention can be prepared by any of the well-known techniques of pharmacy.

The pharmaceutical formulations can be packaged for use as is, or lyophilized, the lyophilized preparation generally being combined with a sterile aqueous solution prior to administration. The compositions can further be packaged in unit/dose or multi-dose containers, for example, in sealed ampoules and vials.

The pharmaceutical formulations can be formulated for administration by any method known in the art according to conventional techniques of pharmacy. For example, the compositions can be formulated to be administered intranasally, by inhalation (*e.g.*, oral inhalation), orally, buccally (*e.g.*, sublingually), rectally, vaginally, topically, intrathecally, intraocularly, transdermally, by parenteral administration (*e.g.*, intramuscular [*e.g.*, skeletal muscle], intravenous, subcutaneous, intradermal, intrapleural, intracerebral and intra-arterial, intrathecal), or topically (*e.g.*, to both skin and mucosal surfaces, including airway surfaces).

For intranasal or inhalation administration, the pharmaceutical formulation can be formulated as an aerosol (this term including both liquid and dry powder aerosols). For example, the pharmaceutical formulation can be provided in a finely divided form along with a surfactant and propellant. Typical percentages of the composition are 0.01-20% by weight,

preferably 1-10%. The surfactant is generally nontoxic and soluble in the propellant. Representative of such agents are the esters or partial esters of fatty acids containing from 6 to 22 carbon atoms, such as caproic, octanoic, lauric, palmitic, stearic, linoleic, linolenic, olesteric and oleic acids with an aliphatic polyhydric alcohol or its cyclic anhydride. Mixed esters, such as mixed or natural glycerides may be employed. The surfactant may constitute 0.1-20% by weight of the composition, preferably 0.25-5%. The balance of the composition is ordinarily propellant. A carrier can also be included, if desired, as with lecithin for intranasal delivery. Aerosols of liquid particles can be produced by any suitable means, such as with a pressure-driven aerosol nebulizer or an ultrasonic nebulizer, as is known to those of skill in the art. *See, e.g.*, U.S. Patent No. 4,501,729. Aerosols of solid particles can likewise be produced with any solid particulate medicament aerosol generator, by techniques known in the pharmaceutical art. Intranasal administration can also be by droplet administration to a nasal surface.

Injectable formulations can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. Alternatively, one can administer the pharmaceutical formulations in a local rather than systemic manner, for example, in a depot or sustained-release formulation.

Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules and tablets of the kind previously described. For example, an injectable, stable, sterile formulation of the invention in a unit dosage form in a sealed container can be provided. The formulation can be provided in the form of a lyophilizate, which can be reconstituted with a suitable pharmaceutically acceptable carrier to form a liquid composition suitable for injection into a subject. The unit dosage form can be from about 1 μg to about 10 grams of the formulation. When the formulation is substantially water-insoluble, a sufficient amount of emulsifying agent, which is pharmaceutically acceptable, can be included in sufficient quantity to emulsify the formulation in an aqueous carrier. One such useful emulsifying agent is phosphatidyl choline.

Pharmaceutical formulations suitable for oral administration can be presented in discrete units, such as capsules, cachets, lozenges, or tables, as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water or water-in-oil emulsion. Oral delivery can be performed by complexing a compound(s) of the present invention to a carrier capable of withstanding degradation by digestive enzymes in the gut of an animal. Examples of such carriers include plastic capsules or tablets, as known in the art.

Such formulations are prepared by any suitable method of pharmacy, which includes the step of bringing into association the protein(s) and a suitable carrier (which may contain one or more accessory ingredients as noted above). In general, the pharmaceutical formulations are prepared by uniformly and intimately admixing the compound(s) with a liquid or finely divided solid carrier, or both, and then, if necessary, shaping the resulting mixture. For example, a tablet can be prepared by compressing or molding a powder or granules, optionally with one or more accessory ingredients. Compressed tablets are prepared by compressing, in a suitable machine, the formulation in a free-flowing form, such as a powder or granules optionally mixed with a binder, lubricant, inert diluent, and/or surface active/dispersing agent(s). Molded tablets are made by molding, in a suitable machine, the powdered protein moistened with an inert liquid binder.

Pharmaceutical formulations suitable for buccal (sub-lingual) administration include lozenges comprising the compound(s) in a flavored base, usually sucrose and acacia or tragacanth; and pastilles in an inert base such as gelatin and glycerin or sucrose and acacia.

Pharmaceutical formulations suitable for parenteral administration can comprise sterile aqueous and non-aqueous injection solutions, which preparations are preferably isotonic with the blood of the intended recipient. These preparations can contain anti-oxidants, buffers, bacteriostats and solutes, which render the composition isotonic with the blood of the intended recipient. Aqueous and non-aqueous sterile suspensions, solutions and emulsions can include suspending agents and thickening agents. Examples of nonaqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like.

Pharmaceutical formulations suitable for rectal administration are optionally presented as unit dose suppositories. These can be prepared by admixing the active agent with one or more conventional solid carriers, such as for example, cocoa butter and then shaping the resulting mixture.

Pharmaceutical formulations suitable for topical application to the skin preferably take the form of an ointment, cream, lotion, paste, gel, spray, aerosol, or oil. Carriers that can

be used include, but are not limited to, petroleum jelly, lanoline, polyethylene glycols, alcohols, transdermal enhancers, and combinations of two or more thereof. In some embodiments, for example, topical delivery can be performed by mixing a pharmaceutical formulation of the present invention with a lipophilic reagent (e.g., DMSO) that is capable of passing into the skin.

Pharmaceutical formulations suitable for transdermal administration can be in the form of discrete patches adapted to remain in intimate contact with the epidermis of the subject for a prolonged period of time. Formulations suitable for transdermal administration can also be delivered by iontophoresis (*see*, for example, *Pharmaceutical Research* 3:318 (1986)) and typically take the form of a buffered aqueous solution of the compound(s). Suitable formulations can comprise citrate or bis\tris buffer (pH 6) or ethanol/water and can contain from 0.1 to 0.2M active ingredient.

In embodiments of the invention, the dosage of a virus particle of this invention can be in a range of about 10^4 to about 10^7 plaque forming units (PFUs). In embodiments of this invention, the dosage of a VLP of this invention can be in a range of about 500 micrograms to about 5 milligrams. In embodiments of this invention, the dosage of a protein of this invention can be in a range of about 10^0 to about 10^4 micrograms +/- adjuvant.

Further, the composition can be formulated as a liposomal formulation. The lipid layer employed can be of any conventional composition and can either contain cholesterol or can be cholesterol-free. The liposomes that are produced can be reduced in size, for example, through the use of standard sonication and homogenization techniques.

The liposomal formulations can be lyophilized to produce a lyophilizate which can be reconstituted with a pharmaceutically acceptable carrier, such as water, to regenerate a liposomal suspension.

The immunogenic formulations of the invention can optionally be sterile, and can further be provided in a closed pathogen-impermeable container.

EXAMPLES

Synthetic biology offers unparalleled genetic control over the genome structure, expression and organization of viral genomes. The dengue virus (DENV) complex consists of four closely related viruses designated DENV serotypes 1-4, which are antigenically similar yet induce complex patterns of cross reactive neutralizing and enhancing antibody responses in human populations. To study the antigenic relationships among the DENV serotypes, we describe the construction and characterization of a panel of stable DENV1-4

molecular clones and recombinant viruses based on a low passage clinical isolates. Recombinant viruses replicated like wildtype viruses and encoded appropriate marker mutations. To evaluate the role of natural variation in DENV3, four synthetically designed isogenic constructs were made by replacing the parent envelope (E) glycoprotein gene with E genes based on the four genetically and geographically distinct DENV-3 genotypes. Recombinant viruses were viable, evaluated for growth on insect and mammalian hosts, and monoclonal and polyclonal neutralization tests demonstrate that natural microvariation among DEN3 neutralization influences cross neutralization susceptibility patterns. To evaluate the use of recombinant DNA technology to map defined epitopes, we used escape mutations and epitope mapping to map the coordinates of several epitopes. Then, we exchanged these epitopes between strains. Recombinant viruses were viable and gain and loss of function assays with monoclonal and polyclonal sera revealed antigenic patterns that reveal important considerations in vaccine design.

The anti-dengue virus (DENV) human monoclonal antibody (mAb) 5J7 potently neutralizes DENV serotype 3 (DENV-3) by binding to an epitope on the DENV-3 envelope (E) glycoprotein. This epitope spans the E region known as the E domain I-II (EDI-II) hinge. Using a DENV infection clone platform, the DENV-3 5J7 epitope was transplanted into a DENV serotype 1 (DENV-1) E glycoprotein. This transplant makes the recombinant DENV-1/3 virus sensitive to neutralization by mAb 5J7. Significantly, the transplant does not disrupt the native DENV-1 antigenic structure, and the recombinant virus is sensitive to both DENV-1 and DENV-3 human polyclonal sera. This sensitivity indicates that the DENV-1/3 chimeric E glycoprotein may function as a bivalent vaccine capable of inducing neutralizing antibodies against two virus serotypes - DENV-1 and DENV-3.

The foregoing is illustrative of the present invention, and is not to be construed as limiting thereof. The invention is defined by the following claims, with equivalents of the claims to be included therein.

All publications, patent applications, patents and other references cited herein are incorporated by reference in their entireties for the teachings relevant to the sentence and/or paragraph in which the reference is presented.

TABLE 1. Amino acid substitutions to produce DENV-1/3 and DENV-3/1

E AA #	50	52	53	55	125	129	161	197	202	203	205	207	210	272	275	277
WestPac'74	V	N	P	V	L	I	T	V	E	K	W	L	K	T	T	T
DENV-1/3 hinge	A	Q	L	T	I	V	I	I	K	N	A	M	R	N	G	S

E AA #	46	50	52	53	138	141	155	156	157	160	163	169	171	173	174	176	177	180	272	275	277
3001	Q	A	Q	L	T	I	T	-	-	V	E	S	T	A	I	P	E	T	N	G	S
Denv-3/1	L	V	N	P	S	V	V	T	E	T	T	P	S	I	Q	T	D	A	T	T	T

TABLE 2. Nucleotide substitutions in WestPac'74 (DENV-1) CDS to produce DENV 1-3 hinge

nt. Position	1083	1087	1088	1090	1092	1093	1096	1097	1098	1102
WestPac'74	T	A	A	C	C	T	C	G	T	G
DENV-1/3 hinge	C	C	C	A	T	G	G	A	C	A

nt. Position	1103	1105	1108	1111	1307	1309	1311	1318	1319	1321
WestPac'74	C	C	A	G	C	G	A	G	A	A
DENV-1/3 hinge	A	G	G	A	A	A	G	A	G	G

nt. Position	1324	1416	1510	1513	1519	1523	1525	2528	2529	1531
WestPac'74	C	C	C	T	G	G	G	G	T	G
DENV-1/3 hinge	G	T	T	C	A	A	C	A	C	A

nt. Position	1538	1543	1547	1553	1555	1558	1561	1563	1724	1729
WestPac'74	G	A	T	C	C	C	C	A	T	T
DENV-1/3 hinge	A	C	G	A	G	A	T	G	C	A

nt. Position	1735	1749	1750	1753	1757	1758	1759	1764	1765	1774
WestPac'74	G	C	G	T	A	C	G	C	A	A
DENV-1/3 hinge	C	A	C	A	G	G	C	G	C	G

TABLE 3.

RM I.D.	Challenge Virus	Viremia (Log FFU/mL) Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Days viremia
BM05		-	2.0	-	2.1	1.7	2.4	-	1.4	-	-	5
BP34	rDENV1/3	-	-	1.7	-	2.1	2.4	1.9	-	-	-	4
BP73		-	1.7	-	2.0	1.9	2.6	1.4	-	-	-	5
BS69		-	2.3	1.9	2.0	2.4	-	2.1	-	-	-	5

TABLE 4.

RM I.D. OL3	Challenge Virus	Viremia (Log FFU/mL) Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Days viremia
		-	-	-	+	+	+	+	-	-	-	4
3J6	3001-F4E	-	+	-	+	+	-	-	-	-	-	3
8K2		-	-	-	-	+	-	-	-	-	-	1
7L2		-	-	-	+	-	-	-	-	-	-	1

TABLE 5.

Mabs	Donor	Virus Binding	Protein binding rE	EDI II	DV1	DV2	Neut50 (μg/ml) DV3	DV4
1B19	HD184	Complex	+	-	1.2	1.8	2.9	5.7
1B22	HD184	Complex	-	-	>10	>10	>10	>10
1B23	19	Complex	+	+	7.7	9.77	3.1	18.6
1C6	Harris Acute	Complex	-	-	>10	>10	1.55	>10
10000	HD184	Complex	+	+	1.1	1	3.4	4
1F4	**HD184**	**DENV-1**	**-**	**-**	**0.11**	**>10**	**>10**	**>10**
1F16.2	Harris Acute	Complex	+	+	3.93	5	12	20.9
1G10	Harris Acute	Complex	+	-	>10	>10	0.093	>10
1H10	HD184	Complex	-	-	>10	>10	0.37	4.3
1H16	Harris Acute	Complex	-	-	>10	>10	>10	>10
1I12	HD184	Complex	-	-	>10	>10	0.36	>10
1L6	HD184	Complex	+	-	2.34	6.7	1.1	6.25
1L13	Vaccine	Complex	-	-	>10	>10	0.24	>10
1M19	19	Complex	+	-	4.6	6.7	0.28	5.9
1N5	Harris Acute	Complex	+	-	0.27	.04	0.98	0.85
1N8	HD184	Complex	+	-	4.5	4.1	7.65	5.95
2M11	HD184	Complex	+	-	1.72	2.62	3.61	4.36
3B4	HD184	Complex	+	-	1.77	2.23	1.26	1.61
5C8	HD184	Complex	+	-	1.07	1.65	0.95	3.31
5J7	**105**	**Complex**	**-**	**-**	**>10**	**>10**	**0.09**	**>10**
5K17	HD184	Complex	+	-	2.28	3.16	6.21	4.71

SEQUENCES

UNC 3001 (DENV-3) AMINO ACID SEQUENCE

MRCVGIGNRDFVEGLSGATWVDVVLEHGGCVTTMAKNKPTLDIELQKTEATQLATLRKLC
IEGKITNITTDSRCPTQGEAVLPEEQDQNYVCKHTYVDRGWGNGCGLFGKGSLVTCAKFQ
CLEPIEGKVVQYENLKYTVIITVHTGDQHQVGNETQGVTAIIT--PQASTTEAILPEYGT
LGLECSPRTGLDFNEMILLTMKNKAWMVHRQWFFDLPLPWTSGATTETPTWNRKELLVTF
KNAHAKKQEVVVLGSQEGAMHTALTGATEIQNSGGTSIFAGHLKCRLKMDKLELKGMSYA
MCTNTFVLKKEVSETQHGTILIKVEYKGEDAPCKIPFSTEDGQGKAHNGRLITANPVVTK
KEEPVNIEAEPPFGESNIVIGIGDNALKINWYKKG

WestPac74 (DENV-1) AMINO ACID SEQUENCE

MRCVGIGNRDFVEGLSGATWVDVVLEHGSCVTTMAKDKPTLDIELLKTEVTNPAVLRKLC
IEAKISNTTTDSRCPTQGEATLVEEQDTNFVCRRTFVDRGWGNGCGLFGKGSLITCAKFK
CVTKLEGKIVQYENLKYSVIVTVHTGDQHQVGNETTEHGTTATITPQAPTSEIQLTDYGA
LTLDCSPRTGLDFNEMVLLTMEKKSWLVHKQWFLDLPLPWTSGASTSQETWNRQDLLVTF
KTAHAKKQEVVVLGSQEGAMHTALTGATEIQTSGTTTIFAGHLKCRLKMDKLTLKGMSYV
MCTGSFKLEKEVAETQHGTVLVQVKYEGTDAPCKIPFSSQDEKGVTQNGRLITANPIVTD
KEKPVNIEAEPPFGESYIVVGAGEKALKLSWFKKG

WestPac74 hinge (DENV 1/3) AMINO ACID SEQUENCE

MRCVGIGNRDFVEGLSGATWVDVVLEHGSCVTTMAKDKPTLDIELLKTEATQLATLRKLC
IEAKISNTTTDSRCPTQGEATLVEEQDTNFVCRRTFVDRGWGNGCGLFGKGSLITCAKFK
CVTKIEGKVVQYENLKYSVIVTVHTGDQHQVGNETTEHGTIATITPQAPTSEIQLTDYGA
LTLDCSPRTGLDFNEMILLTMKNKAWMVHRQWFLDLPLPWTSGASTSQETWNRQDLLVTF
KTAHAKKQEVVVLGSQEGAMHTALTGATEIQNSGGTSIFAGHLKCRLKMDKLTLKGMSYV
MCTGSFKLEKEVAETQHGTVLVQVKYEGTDAPCKIPFSSQDEKGVTQNGRLITANPIVTD
KEKPVNIEAEPPFGESYIVVGAGEKALKLSWFKKG

3001-1F4E (DENV 3/1) AMINO ACID SEQUENCE

MRCVGIGNRDFVEGLSGATWVDVVLEHGGCVTTMAKNKPTLDIELFKTEVINPAVLRKLCIEGK
ITNITTDSRCPTQGEAVLPEEQDQNYVCKHTYVDRGWGNGCGLFGKGSLVTCAKFQCLEPIEGK
VVQYENLKYSVIVTVHTGDQHQVGNETTEHGTIATITPQAPTSEIQLTDYGALGLECSPRTGLD
FNEMILLTMKNKAWMVHRQWFFDLPLPWTSGATTETPTWNRKELLVTFKNAHAKKQEVVVLGSQ
EGAMHTALTGATEIQTSGTTTIFAGHLKCRLKMDKLELKGMSYAMCTNTFVLKKEVSETQHGTI
LIKVEYKGEDAPCKIPFSTEDGQGKAHNGRLITANPVVTKKEEPVNIEAEPPFGESNIVIGIGD
NALKINWYKKG

icDengueIII UNC 3001

```
agttgttagt ctacgtggac cgacaagaac agtttcgact cggaagcttg cttaacgtag      60
tgctaacagt tttttattag agagcagatc tctgatgaac aaccaacgga agaagacggg     120
aaaaccgtct atcaatatgc tgaaacgcgt gagaaaccgt gtgtcaactg gaccacagtt     180
ggcgaagaga ttctcaaaag gactgctgaa cggccaggga ccaatgaaat tggttatggc     240
gttcatagct ttcctcagat ttctagccat tccaccaaca gcaggagtct tggctagatg     300
gggaaccttc aagaagtcgg gagccattaa ggtcctgaaa ggcttcaaga aggagatctc     360
aaacatgctg agcataatca acaaacggaa aaagacatcg ctctgtctca tgatgatatt     420
gccagcagca cttgctttcc acttgacttc acgagatgga gagccgcgca tgattgtggg     480
gaagaatgaa agaggaaaat ccctactttt taagacagcc tctggaatta acatgtgcac     540
actcatagcc atggacttgg gagagatgtg tgatgacacg gtcacttaca aatgccccca     600
cattaccgaa gtggaacctg aagacattga ctgctggtgc aacgttacat caacatgggt     660
gacttatgga acgtgcaatc aagctggaga gcatagacgc gacaaaagat cagtggcgtt     720
agctcctcat gtcggcatgg gactggacac acgcacccaa acctggatgt cggctgaagg     780
agcttggaga caagtcgaga aggtagagac atgggcccta aggcacccag ggttcaccat     840
actagcccta tttcttgccc attacatagg cacttccttg acccagaagg tggttatttt     900
tatactacta atgctggtca ccccatccat gacaatgaga tgtgtgggaa taggaaacag     960
agattttgtg gaaggtctat caggagctac gtgggttgac gtggtgctcg agcacggggg    1020
gtgtgtgact accatggcta agaacaagcc cacgctggat atagagcttc agaagaccga    1080
ggccacccaa ctggcgaccc taaggaagct atgcattgag gggaaaatta ccaacataac    1140
aactgactca agatgtccta cccaagggga agcggttttg cctgaggagc aggaccagaa    1200
ctacgtgtgt aagcatacat acgtagacag aggctggggg aacggttgtg gcttgtttgg    1260
caagggaagc ttggtaacgt gtgcgaaatt tcaatgcctg gaaccaatag agggaaaagt    1320
ggtgcaatat gagaacctca aatacaccgt catcattaca gtgcacacag gagaccaaca    1380
ccaggtagga aatgaaacgc agggagtcac ggctgagata acacctcagg catcaaccac    1440
tgaagccatc ttgcctgaat atggaaccct tgggctagaa tgctcaccac ggacaggttt    1500
ggatttcaat gaaatgatct tactaacaat gaagaacaaa gcatggatgg tacatagaca    1560
atggtttttt gacctacctc taccatggac atcaggagct acaacagaaa cgccaacctg    1620
gaacaggaag gagcttcttg tgacattcaa aaacgcacat gcgaaaaaac aagaagtagt    1680
cgtccttgga tcgcaagagg gagcaatgca taccgcactg acaggagcca cagaaatcca    1740
aaactcagga ggcacaagca tttttgcggg gcacttaaaa tgtagactta agatggacaa    1800
attggaactc aaggggatga gctatgcaat gtgcacgaat accttttgtgt tgaagaaaga    1860
agtctcagaa acgcagcatg ggacaatact cattaaggtc gagtacaagg gggaagatgc    1920
gccttgcaag attcctttct ccacagagga tggacaaggg aaagctcaca atggcagact    1980
gatcacagcc aacccagtgg tgactaagaa ggaggagcct gtcaatattg aggctgaacc    2040
tcctttgggg gaaagtaata tagtaattgg aattggagac aacgccttga aaatcaactg    2100
gtacaagaag ggaagctcta ttgggaagat gttcgaggcc actgccagag gtgcaaggcg    2160
catggccatc ttgggagaca cagcttggga ctttggatca gtgggtggtg ttctgaactc    2220
attaggcaaa atggtgcacc aaatattcgg aagtgcttac acagccctat tcagtggagt    2280
ctcttgggtg atgaaaattg gaataggtgt tctcttgact tggatagggt tgaattcaaa    2340
aaacacatcc atgtcatttt catgcattgc gataggaatc attacactct atctgggagc    2400
tgtggtacaa gctgacatgg ggtgtgtcat aaactggaaa ggcaaagaac tcaaatgtgg    2460
aagtggaatt ttcgtcacca acgaggtcca tcctggacca gagcaataca aattccaagc    2520
agactcccca aaaagattgg cgacagccat tgcaggcgct tgggagaatg gagtgtgcgg    2580
aattaggtca acaaccagaa tggagaatct cctgtggaag caaatagcca atgaactgaa    2640
ctacatatta tgggaaaaca atatcaaatt aacggtagtt gtgggcgata caattggggt    2700
cttagagcaa ggaaaaagaa cactaacacc acaacccatg gagctaaaat actcatggaa    2760
aacatgggga aaggcaaaaa tagtgacagc tgaaacacaa aattcctcct tcataataga    2820
cgggccaaac acaccggagt gtccaagtgc ctcaagagca tggaatgtgt gggaggtgga    2880
agattacggg ttcggagtct tcacaaccaa catatggctg aaactccgag atgtgtacac    2940
ccaactatgt gaccataggc taatgtcggc agccgtcaag gatgagaggg ccgtacacgc    3000
cgacatgggc tattggatag aaagccaaaa gaatggaagt tggaagctag aaaaagcatc    3060
cctcatagag gtgaaaacct gcacatggcc aaaatcacac actctttgga gcaatggtgt    3120
gctagagagt gacatgatca tcccaaagag cctcgctggc cctatttcgc aacacaacta    3180
caggcctggg taccacaccc aaacagcagg accctggcac ttaggaaaat tggagctgga    3240
cttcaactat tgtgaaggaa caacagttgt catcacagaa aactgtggga caagaggccc    3300
atcattgaga acaacaacag tgtcagggaa gttgatacac gaatggtgtt gccgctcgtg    3360
cacacttcct ccctgcgat acatgggaga agacggctgc tggtatggca tggaaatcag    3420
acccatcagt gagaaagaag agaacatggt aaagtcttta gtctcagcgg gaagtggaaa    3480
ggtggacaac ttcacaatgg gtgtcttgtg tttggcaatc ctctttgaag aggtgatgag    3540
```

aggaaaattt gggaagaaac acatgattgc aggggttctc ttcacgtttg tgctccttct 3600
ctcagggcaa ataacatgga gagacatggc gcacacacta ataatgattg ggtccaacgc 3660
ctctgacagg atgggaatgg gcgtcacctа cctagcttta attgcaacat ttaaaatcca 3720
gccattcttg gctttgggat ttttcctaag aaaactgaca tctagagaaa atttattgtt 3780
aggagttggg ctggctatgg caacaacgtt acaactgcca gaggacattg aacaaatggc 3840
aaatggaatc gctctggggc tcatggctct taaactgata acacaatttg aaacatacca 3900
attatggacg gcattagtct ccttaacgtg ttcaaataca attcttacgt tgactgttgc 3960
ctggagaaca gccaccctga ttttggccgg agtttcgctt ttaccagtgt gccagtcttc 4020
gagcatgagg aaaacagact ggcttccaat gacagtggca gctatgggag ttccaccct 4080
accacttttt atttttagct tgaaagacac actcaaaagg agaagctggc cactgaatga 4140
aggggtgatg gctgttgggc ttgtgagcat tctggccagt tctctcctta gaaatgatgt 4200
gcccatggct ggaccattag tggccggggg cttgctgata gcgtgctacg tcataactgg 4260
cacgtcagca gacctcactg tggaaaaagc agcagatgta acatgggagg aagaggctga 4320
gcaaaacagga gtgtcccaca acttaatgat cacagttgat gatgatggaa caatgagaat 4380
aaaagatgat gagactgaga acatcctaac agtgctttta aaaacagcat tactaatagt 4440
atcaggcatc tttccatact ccataccgc aacattgttg gtctggcata cttggcagaa 4500
gcaaacccaa aggtccggcg ttctgtggga cgtacccagc ccccagaga cacagaaagc 4560
agaactggaa gaaggggttt ataggatcaa acagcaagga attcttggga aaacccaagt 4620
aggggttgga gtacagaaag aaggagtctt ccacaccatg tggcacgtca caagaggggc 4680
agtgttgaca cataatggga aaagactgga accaaactgg gctagcgtga aaaaagatct 4740
gatttcatac ggaggaggat ggagattgag cgcgcaatgg caaaaggggg aggaggtgca 4800
ggttattgcc gtggagcctg ggaagaaccc aaagaacttt caaaccatgc caggcacttt 4860
tcagactaca acaggggaaa taggagcaat tgcactggat ttcaagcctg gaacttcagg 4920
atctcctatc ataaacagag agggaaaggt agtgggactg tatggcaatg gagtggttac 4980
aaagaatggt ggctacgtca gcggaatagc gcaaacaaat gcagaaccag atggaccgac 5040
accagagttg gaagaagaga tgttcaaaaa gcgaaatcta accataatgg atcttcatcc 5100
tgggtcagga aagacacgga aatacctttcc agctattgtt agaggaggcaa tcaagagacg 5160
tttaagaact ctaattttgg caccgacaag ggtggttgca gctgagatgg aagaagcatt 5220
gaaagggctc ccaataaggt accaaacaac agcaacaaaa tctgaacaca caggaagaga 5280
gattgttgat ctaatgtgcc acgcaacgtt cacaatgcgc ttgctgtcac cagttagggt 5340
tccaaattat aacttgataa taatggatga ggcccattтc acagacccag ccagcatagc 5400
ggctagaggg tacatatcaa ctcgtgttgg aatgggagag gcagccgcaa ttttcatgac 5460
agcaacgccc cctggaacag ctgatgcctt tcctcagagc aacgctccaa ttcaagatga 5520
agaaagggac ataccagaac gctcatggaa ttcaggcaat gaatggatta ccgacttcgc 5580
tgggaaaacg gtgtggtttg tccccagcat taaagccgga aatgacatag caaactgctt 5640
gcggaaaaac ggaaaaaagg tcattcaact tagtaggaag acttttgaca cagaatatca 5700
gaagactaaa ctgaatgatt gggacttcgt ggtgacaact gacatttcag aaatgggggc 5760
caatttcaaa gcagatagag tgatcgaccc aagaagatgt ctcaaaccag tgatcctgac 5820
agatggacca gagcgggtga tcctggctgg accaatgcca gtcaccgcgg cgagtgctgc 5880
gcaaaggaga ggaagagttg gcaggaaccc acaaaaagaa aatgaccagt acatattcac 5940
gggccagcct ctcaacaatg atgaagacca tgctcactgg acagaagcaa aaatgctgct 6000
ggacaacatt aacacaccag aagggattat accagctctc tttgaaccag aaagggagaa 6060
gtcagccgcc atagacggtg agtatcgcct gaagggtgag tccaggaaga ctttcgtgga 6120
actcatgagg aggggtgacc ttccagtttg gttagcccat aaagtagcat cagaagggat 6180
caaatataca gatagaaaat ggtgctttga tggacaacgc aataatcaaa ttttagagga 6240
gaacatggat gtggaaatct ggacaaagga aggagaaaag aaaaaattga gacctaggtg 6300
gcttgatgcc cgcacttatt cagatccctt agcactcaag gaatttaagg actttgcggc 6360
tggcagaaag tcaatcgccc ttgatcttgt gacagaaata ggaagagtgc cttcacacct 6420
agcccacaga acgagaaacg ctctggacaa tctggtgatg ctgcatacgt cagaacatgg 6480
cggtagggcc tacaggcatg cggtggagga actaccagaa acaatggaaa cacttttact 6540
cttgggactc atgatcttgt tgacaggtgg agcaatgctt ttcttgatat caggaaaagg 6600
gattggaaag acttcaatag gactcatttg tgtaattgcc tccagcggca tgttgtggat 6660
ggccgaaatc ccactccagt ggatcgcgtc ggctatagtc ctggagtttt ttatgatggt 6720
gttgcttata ccagaaccag aaaagcagag aacccccaa gacaaccaac tcgcatatgt 6780
cgtgataggc atacttacat tggctgcaat aatagcagcc aatgaaatgg gactgttgga 6840
aactacaaag agagatttag gaatgtctaa ggagccaggt gttgtttctc caaccagcta 6900
tttggatgtg gacttgcacc cagcatcagc ctggacattg tacgccgtgg ccactacagt 6960
aataacacca atgttaagac ataccataga gaattctaca gcaaatgtgt ccctggcagc 7020
tatagccaac caggcagtgg tcctgatggg tttggacaaa ggatggccaa tatcaaaaat 7080
ggacttaggc gtaccactac tggcattggg ttgctattca caagtgaacc cactgactct 7140
aacagcggca gtacttttgc taatcacaca ttatgctatt ataggtccag gattgcaggc 7200

```
aaaagccact cgtgaagctc agaaaaggac agctgctgga ataatgaaga atccaacggt  7260
ggatgggata atgacaatag acctagatcc tgtaatatat gattcaaaat ttgaaaagca  7320
actgggacag gttatgctcc tggttttgtg tgcagttcaa cttttgttaa tgagaacatc  7380
atgggccttg tgtgaagctt taactctagc tacaggacca ataacaacac tctgggaagg  7440
atcacctgga aagttttgga acaccacgat agctgtttcc atggcgaaca tttttagagg  7500
gagctatttа gcaggagctg ggcttgcttt ttctattatg aaatcagttg gaacaggaaa  7560
aagaggaaca ggttcacaag gcgaaacttt aggagaaaaa tggaaaaaga aattaaatca  7620
attatcccgg aaagagtttg acctttacaa gaaatctgga atcactgaag tggatagaac  7680
agaagccaaa gaagggttga aaagaggaga aataacacat catgccgtgt ccagaggtag  7740
cgcaaaactt caatggtttg tggagagaaa catggtcatt cccgaaggaa gagtcataga  7800
cttgggctgt ggaagaggag gctggtcata ctactgtgca ggactgaaaa aagtcacaga  7860
agtgcgagga tacacaaaag gcggtccagg acacgaagaa ccagtaccta tgtctacata  7920
tggatggaac atagttaagt taatgagtgg aaaggatgtg ttttatcttc cacctgaaaa  7980
gtgtgatacc ctgttgtgtg acatcggaga atcttcacca agcccaacag tggaagaaag  8040
cagaactata agagttttga agatggttga accatggcta aaaaacaacc agttttgcat  8100
taaagtattg aacccttaca tgccaactgt gattgagcac ctagaaagac tacaaaggaa  8160
acatggagga atgcttgtga gaaatccact ttcacgaaac tccacgcacg aaatgtactg  8220
gatatctaat ggcacaggta acattgtctc ttcagtcaac atggtatcta gattgctact  8280
gaacaggttc acgatgacac acaggagacc taccatagag aaagatgtgg atttaggagc  8340
aggaactcga catgttaatg cggaaccaga aacacccaac atggatgtca ttggggaaag  8400
aataaaaagg atcaaggagg agcacaattc aacatggcac tatgatgacg aaaacccctа  8460
caaaacgtgg gcttaccacg gatcctatga agtcaaagcc acaggctcag cctcctccat  8520
gataaatgga gtcgtgaaac tcctcactaa accatgggat gtggtgccca tggtgacaca  8580
gatggcaatg acagatacaa ctccatttgg ccagcagaga gtctttaaag agaaagtgga  8640
caccaggaca cccaggccca tgccagggac aagaaaggtt atggggatca cagcggagtg  8700
gctctggaga accctgggaa ggaacaaaag acccaggtta tgcacaaggg aagagtttac  8760
aaaaaaggtc agaactaacg cagccatggg cgccgttttc acagaggaga accaatggga  8820
cagtgcgaaa gctgctgttg aggatgaaga attttggaaa cttgtggaca gagaacgtga  8880
actccacaaa ttgggcaagt gtggaagctg tgtttacaac atgatgggca agagagagaa  8940
gaaacttgga gagtttggca aagcaaaagg cagtagagct atatggtaca tgtggttggg  9000
agccaggtac cttgagttcg aagcccttgg attcctaaat gaagaccact ggttctcgcg  9060
tgacaactct tacagtggag tagaaggaga aggactgcac aagctaggct acatattaag  9120
ggacatttcc aagatacccg gaggagctat gtatgctgat gacacagctg gttgggacac  9180
aagaataaca gaagatgacc tgcacaatga ggaaaagatc acacagcaaa tggaccctga  9240
acacaggcag ttagcgaacg ctatatttaa gctcacatac caaaacaaag tggtcaaagt  9300
tcaacgaccg actccaacgg gcacggtaat ggacatcata tctaggaaag accaaagagg  9360
cagtggacag gtgggaactt atggtctgaa tacattcacc aacatggaag tccagttagt  9420
cagacaaatg gaaggagaag gtgtgctgtc aaaggcagac ctcgagaacc ctcatctgcc  9480
agagaagaaa attacacaat ggttggaaac caaaggagtg gagaggttaa aaagaatggc  9540
cattagcggg gatgattgtg tagtgaaacc aatcgatgac aggttcgcta atgccctgct  9600
tgctctgaac gatatgggaa aggttcggaa agacatacct caatggcagc catcaaaggg  9660
atggcatgat tggcaacagg ttcctttctg ctcccaccac tttcatgaat tgatcatgaa  9720
agatggaaga aagttagtgg ttccctgtag accccaggac gaactaatag gaagagcaag  9780
aatctctcaa ggagcgggat ggagccttag agagaccgca tgtctgggga aagcctacgc  9840
tcaaatgtgg agtctcatgt actttcacag aagagatctc agactagcat ccaacgccat  9900
atgttcagca gtaccagtcc actgggtccc cacaagtaga acgacatggt ctattcatgc  9960
tcaccatcag tggatgacta cagaagacat gcttactgtc tggaacaggg tgtggatcga 10020
ggacaatcca tggatggaag acaaaactcc agttacaacc tgggaaaatg ttccatatct 10080
agggaagaga gaagaccaat ggtgcggatc acttattggt ctcacctcca gagcaacctg 10140
ggcccagaac atacccacag caattcaaca ggtgagaagt cttataggga atgaagagtt 10200
tctggattac atgccttcaa tgaagagatt caggaaggag gaggagtcgg aaggagccat 10260
ttggtaaacg taggaagtga aaaagaggca aactgtcagg ccaccttaag ccacagtacg 10320
gaagaagctg tgctgcctgt gagccccgtc caaggacgtt aaaagaagaa gtcaggcccc 10380
aaagccacgg tttgagcaaa ccgtgctgcc tgtagctccg tcgtggggac gtaaaacctg 10440
ggaggctgca aactgtggaa gctgtacgca cggtgtagca gactagcggt tagaggagac 10500
ccctcccatg acacaacgca gcagcggggc ccgagcactg agggaagctg tacctccttg 10560
caaaggacta gaggttagag gagacccccc gcaaacaaaa acagcatatt gacgctggga 10620
gagaccagag atcctgctgt ctcctcagca tcattccagg cacagaacgc cagaaaatgg 10680
aatggtgctg ttgaatcaac aggttcttaa aagagacg                          10718
```

icDengueI WestPac'74

```
agttgttagt ctacgtggac cgacaagaac agtttcgaat cggaagcttg cttaacgtag      60
ttctaacagt tttttattag agagcagatc tctgatgaac aaccaacgga aaaagacggg     120
tcgaccgtct ttcaatatgc tgaaacgcgc gagaaaccgc gtgtcaactg tttcacagtt     180
ggcgaagaga ttctcaaaag gattgctttc aggccaagga cccatgaaat tggtgatggc     240
ttttatagca ttcctaagat ttctagccat acctccaaca gcaggaattt tggctagatg     300
gggctcattc aagaagaatg gagcgatcaa agtgttacgg ggtttcaaga aagaaatctc     360
aaacatgttg aacataatga acaggaggaa aagatctgtg accatgctcc tcatgctgct     420
gcccacagcc ctggcgttcc atctgaccac ccgaggggga gagccgcaca tgatagttag     480
caagcaggaa agaggaaaat cacttttgtt taagacctct gcaggtgtca acatgtgcac     540
ccttattgca atggatttgg gagagttatg tgaggacaca atgacctaca aatgcccccg     600
gatcactgag acggaaccag atgacgttga ctgttggtgc aatgccacgg agacatgggt     660
gacctatgga acatgttctc aaactggtga acaccgacga gacaaacgtt ccgtcgcact     720
ggcaccacac gtagggcttg gtctagaaac aagaaccgaa acgtggatgt cctctgaagg     780
cgcttggaaa caaatacaaa aagtggagac ctgggctctg agacacccag gattcacggt     840
gatagccctt tttctagcac atgccatagg aacatccatc acccagaaag ggatcatttt     900
tattttgctg atgctggtaa ctccatccat ggccatgcgg tgcgtgggaa taggcaacag     960
agacttcgtg gaaggactgt caggagctac gtgggtggat gtggtactgg agcatggaag    1020
ttgcgtcact accatggcaa aagacaaacc aacactggac attgaactct tgaagacgga    1080
ggtcacaaac cctgccgtcc tgcgcaaact gtgcattgaa gctaaaatat caaacaccac    1140
cacccgttcg agatgtccaa cacaaggaga agccacgctg gtggaagaac aggacacgaa    1200
ctttgtgtgt cgacgaacgt tcgtggacag aggctggggc aatggttgtg ggctattcgg    1260
aaaaggtagc ttaataacgt gtgctaagtt taagtgtgtg acaaaactgg aaggaaagat    1320
agtccaatat gaaaacttaa aatattcagt gatagtcacc gtacacactg gagaccagca    1380
ccaagttgga aatgagacca cagaacatgg aacaattgca accataacac ctcaagctcc    1440
cacgtcggaa atacagctga cagactacgg agctctaaca ttggattgtt cacctagaac    1500
agggctagac tttaatgaga tggtgttgtt gacaatgaaa aaaaaatcat ggctcgtcca    1560
caaacaatgg tttctagact taccactgcc ttggacctcg ggggcttcaa catcccaaga    1620
gacttggaat agacaagact tgctggtcac atttaagaca gctcatgcaa aaaagcagga    1680
agtagtcgta ctaggatcac aagaaggagc aatgcacact gcgttgactg gagcgacaga    1740
aatccaaacg tctggaacga caacaatttt tgcaggacac ctgaaatgca gactaaaaat    1800
ggataaactg actttaaaag ggatgtcata tgtaatgtgc acagggtcat tcaagttaga    1860
gaaggaagtg gctgagaccc agcatggaac tgttctagtg caggttaaat acgaaggaac    1920
agatgcacca tgcaagatcc ccttctcgtc ccaagatgag aagggagtaa cccagaatgg    1980
gagattgata acagccaacc ccatagtcac tgacaaagaa aaaccagtca acattgaagc    2040
ggagccacct tttggtgaga gctacattgt ggtaggagca ggtgaaaaag ctttgaaact    2100
aagctggttc aagaagggaa gcagtatagg gaaaatgttt gaagcaactg cccgtggagc    2160
acgaaggatg gccatcctgg gagacactgc atgggacttc ggttctatag gaggggtgtt    2220
cacgtctgtg ggaaaactga tacaccagat ttttgggact gcgtatggag ttttgttcag    2280
cggtgtttct tggaccatga agataggaat agggattctg ctgacatggc taggattaaa    2340
ctcaaggagc acgtccottt caatgacgtg tatcgcagtt ggcatggtca cgctgtacct    2400
aggagtcatg gttcaggcgg actcgggatg tgtaatcaac tggaaaggca gagaactcaa    2460
atgtggaagc ggcatttttg tcaccaatga agtccacacc tggacagagc aatataaatt    2520
ccaggccgac tcccctaaga gactatcagc ggccattggg aaggcatggg aggagggtgt    2580
gtgtggaatt cgatcagcca ctcgtctcga gaacatcatg tggaagcaaa tatcaaatga    2640
attaaaccac atcttacttg aaaatgacat gaaatttaca gtggtcgtag gagacgttag    2700
tggaatcttg gcccaaggaa agaaaatgat taggccacaa cccatggaac acaaatactc    2760
gtggaaaagc tggggaaaag ccaaaatcat aggagcagat gtacagaata ccaccttcat    2820
catcgacggc ccaaacaccc cagaatgccc tgataaccaa agagcatgga acatttggga    2880
agttgaagac tatggatttg gaattttcac gacaaacata tggttgaaat tgcgtgactc    2940
ctacactcaa gtgtgtgacc accggctaat gtcagctgcc atcaaggata gcaaagcagt    3000
ccatgctgac atggggtatt ggatagaaag tgaaaagaac gagacttgga agttggcaag    3060
agcctccttc atagaagtta agacatgcat ctggccaaaa tcccacactc tatggagcaa    3120
tggagtcctg gaaagtgaga tgataatccc aaagatatat ggaggaccaa tatctcagca    3180
caactacaga ccaggatatt tcacacaaac agcagggccg tggcacttgg gcaagttaga    3240
actagatttt gatttatgtg aaggtaccac tgttgttgtg gatgaacatt gtggaaatcg    3300
aggaccatct cttagaacca caacagtcac aggaaagaca atccatgaat ggtgctgtag    3360
atcttgcacg ttaccccccc tacgtttcaa aggagaagac gggtgctggt acggcatgga    3420
aatcagacca gtcaaggaga aggaagagaa cctagttaag tcaatggtct ctgcagggtc    3480
aggagaagtg gacagttttt cactaggact gctatgcata tcaataatga tcgaagaggt    3540
```

aatgagatcc agatggagca gaaaaatgct gatgactgga acattggctg tgttcctcct 3600
tcttacaatg ggacaattga catggaatga tctgatcagg ctatgtatca tggttggagc 3660
caacgcttca gacaagatgg ggatgggaac aacgtaccta gctttgatgg ccactttcag 3720
aatgagacca atgttcgcag tcgggctact gtttcgcaga ttaacatcta gagaagttct 3780
tcttcttaca gttggattga gtctggtggc atctgtagaa ctaccaaatt ccttagagga 3840
gctaggggat ggacttgcaa tgggcatcat gatgttgaaa ttactgactg attttcagtc 3900
acatcagcta tgggctacct tgctgtcttt aacatttgtc aaaacaactt tttcattgca 3960
ctatgcatgg aagacaatgg ctatgatact gtcaattgta tctctcttcc ctttatgcct 4020
gtccacgact tctcaaaaaa caacatggct tccggtgttg ctgggatctc ttggatgcaa 4080
accactaacc atgtttctta taacagaaaa caaaatctgg ggaaggaaaa gctggcctct 4140
caatgaagga attatggctg ttggaatagt tagcattctt ctaagttcac ttctcaagaa 4200
tgatgtgcca ctagctggcc cactaatagc tggaggcatg ctaatagcat gttatgtcat 4260
atctggaagc tcggccgatt tatcactgga gaaagcggct gaggtctcct gggaagaaga 4320
agcagaacac tctggtgcct cacacaacat actagtggag gtccaagatg atggaaccat 4380
gaagataaag gatgaagaga gagatgacac actcaccatt ctcctcaaag caactctgct 4440
agcaatctca ggggtatacc caatgtcaat accggcgacc ctctttgtgt ggtatttttg 4500
gcagaaaaag aaacagagat caggagtgct atgggacaca cccagccctc cagaagtgga 4560
aagagcagtc cttgatgatg gcatttatag aattctccaa agaggattgt tgggcaggtc 4620
tcaagtagga gtaggagttt ttcaagaagg cgtgttccac acaatgtggc acgtcaccag 4680
gggagctgtc ctcatgtacc aagggaagag actggaacca agttgggcca gtgtcaaaaa 4740
agacttgatc tcatatggag gaggttggag gtttcaagga tcctggaacg cgggagaaga 4800
agtgcaggtg attgctgttg aaccggggaa gaaccccaaa aatgtacaga cagcgccggg 4860
taccttcaag accctgaag gcgaagttgg agccataget ctagacttta aaccccggcac 4920
atctggatct cctatcgtga acagagaggg aaaaatagta ggtctttatg gaaatggagt 4980
ggtgacaaca agtggtacct acgtcagtgc catagctcaa gctaaagcat cacaagaagg 5040
gcctctacca gagattgagg acgaggtgtt taggaaaaga aacttaacaa taatggacct 5100
acatccagga tcgggaaaaa caagaagata ccttccagcc atagtccgtg aggccataaa 5160
aagaaagctg cgcacgctag tcttagctcc cacaagagtt gtcgcttctg aaatggcaga 5220
ggcgctcaag ggaatgccaa taaggtatca gacaacagca gtgaagagtg aacacacggg 5280
aaaggagata gttgaccttа tgtgtcacgc cactttcact atgcgtctcc tgtctcctgt 5340
gagagttccc aattataata tgattatcat ggatgaagca catttcaccg atccagccag 5400
catagcagcc agagggtata tctcaacccg agtgggtatg ggtgaagcag ctgcgatttt 5460
catgacagcc actccccccg gatcggtgga ggcctttcca cagagcaatg cagttatcca 5520
agatgaggaa agagacattc ctgaaagatc atggaactca ggctatgact ggatcactga 5580
tttcccaggt aaaaacagtct ggtttgttcc aagcatcaaa tcaggaaatg acattgccaa 5640
ctgtttaaga aagaatggga aacgggtggt ccaattgagc agaaaaactt ttgacactga 5700
gtaccagaaa acaaaaaata acgactggga ctatgttgtc acaacagaca tatccgaaat 5760
gggagcaaac ttccgagccg acagggtaat agaccccgagg cggtgcctga aacggtaat 5820
actaaaagat ggcccagagc gtgtcattct agccggaccg atgccagtga ctgtggctag 5880
cgccgcccag aggagaggaa gaattggaag gaaccaaaat aaggaaggcg atcagtatat 5940
ttacatggga cagcctctaa acaatgatga ggaccacgcc cattggacag aagcaaaaat 6000
gctccttgac aacataaaca caccagaagg gattatccca gccctctttg agccggagag 6060
agaaaagagt gcagcaatag acggggaata cagactacgg ggtgaagcga ggaaaacgtt 6120
cgtggagctc atgagaagag gagatttacc tgtctggcta tcctacaaag ttgcctcaga 6180
aggcttccag tactccgaca gaaggtggtg ctttgatggg gaaaggaaca accaggtgtt 6240
ggaggagaac atggacgtgg agatctggac aaaagaagga gaaagaaaga aactacgacc 6300
ccgctggctg gatgccagaa catactctga cccactggct ctgcgcgaat tcaaagagtt 6360
cgcagcagga agaagaagcg tctcaggtga cctaatatta gaaatаggga aacttccaca 6420
acatttaacg caaagggccc agaacgcctt ggacaatctg gttatgttgc acaactctga 6480
acaaggagga aaagcctata gacacgccat ggaagaacta ccagacacca tagagacgtt 6540
aatgctccta gctttgatag ctgtgctgac tggtggagtg acgttgttct tcctatcagg 6600
aaggggtcta ggaaaaacat ccattggcct actctgcgtg attgcctcaa gtgcactgtt 6660
atggatggcc agtgtggaac cccattggat agcggcctct atcatactgg agttctttct 6720
gatggtgttg cttattccag agccggacag acagcgcact ccacaagaca accagctagc 6780
atacgtggtg ataggtctgt tattcatgat attgacagtg gcagccaatg agatgggatt 6840
actggaaacc acaaagaagg acctggggat tggtcatgca gctgctgaaa accaccatca 6900
tgctgcaatg ctggacgtag acctacatcc agcttcagcc tggactctct atgcagtggc 6960
cacaacaatt atcactccca tgatgagaca cacaattgaa aacacaacgg cgaatatttc 7020
cctgacagct attgcaaacc aggcagctat attgatggga cttgacaagg gatggccaat 7080
atcaaagatg gacataggag ttccacttct cgccttgggg tgctattctc aggtgaaccc 7140
gctgacgctg acagcggcgg tatttatgct agtggctcat tatgccataa ttggacccgg 7200

actgcaagca aaagctacta gagaagctca aaaaaggaca gcagccggaa taatgaaaaa 7260
cccaactgtc gacgggatcg ttgcaataga tttggaccct gtggtttacg atgcaaaatt 7320
tgaaaaacag ctaggccaaa taatgttgtt gatactttgc acatcacaga tcctcctgat 7380
gcggaccaca tgggccttgt gtgaatccat cacactagcc actggacctc tgactacgct 7440
ttgggaggga tctccaggaa aattctggaa caccacgata gcggtgtcca tggcaaacat 7500
ttttagggga agttatctag caggagcagg tctggccttt tcattaatga aatctctagg 7560
aggaggtagg agaggcacgg gagcccaagg ggaaacactg ggagaaaaat ggaaaagaca 7620
gctaaaccaa ttgagcaagt cagaattcaa cacttacaaa aggagtggga ttatagaggt 7680
ggatagatct gaagccaaag aggggttaaa aagaggagaa acgactaaac acgcagtgtc 7740
gagaggaacg gccaaactga ggtggtttgt ggagaggaac cttgtgaaac cagaagggaa 7800
agtcatagac ctcggttgtg gaagaggtgg ctggtcatat tattgcgctg ggctgaagaa 7860
agtcacagaa gtgaaaggat acacgaaagg aggacctgga catgaggaac caatcccaat 7920
ggcaacctat ggatggaacc tagtaaagct atactccggg aaagatgtat tctttacacc 7980
acctgagaaa tgtgacaccc tcttgtgtga tattggtgag tcctctccga accaaactat 8040
agaagaagga agaacgttac gtgttctaaa gatggtggaa ccatggctca gaggaaacca 8100
attttgcata aaaattctaa atccctatat gccgagtgtg gtagaaactt tggagcaaat 8160
gcaaagaaaa catggaggaa tgctagtgcg aaatccactc tcaagaaact ccactcatga 8220
aatgtactgg gtttcatgtg gaacaggaaa cattgtgtca gcagtaaaca tgacatctag 8280
aatgctgcta aatcgattca caatggctca caggaagcca acatatgaaa gagacgtgga 8340
cttaggcgct ggaacaagac atgtggcagt agaaccagag gtggccaacc tagatatcat 8400
tggccagagg atagagaata taaaaaatga acacaaatca acatggcatt atgatgagga 8460
caatccatac aaaacatggg cctatcatgg atcatatgag gtcaagccat caggatcagc 8520
ctcatccatg gtcaatggtg tggtgagact gctaaccaaa ccatgggatg tcattcccat 8580
ggtcacacaa atagccatga ctgacaccac acccttttgga caacagaggg tgtttaaaga 8640
gaaagttgac acgcgtacac caaaagcgaa acgaggcaca gcacaaatta tggaggtgac 8700
agccaggtgg ttatggggtt ttctctctag aaacaaaaaa cccagaatct gcacaagaga 8760
ggagttcaca agaaaagtca ggtcaaacgc agctattgga gcagtgttcg tcgatgaaaa 8820
tcaatggaac tcagcaaaag aggcagtgga agatgaacgg ttctgggacc ttgtgcacag 8880
agagagggag cttcataaac aaggaaaatg tgccacgtgt gtctacaaca tgatgggaaa 8940
gagagagaaa aaattaggag agttcggaaa ggcaaaagga agtcgcgcaa tatggtacat 9000
gtggttgggg gcgcgctttt tagagtttga agcccttggt ttcatgaatg aagatcactg 9060
gttcagcaga gagaattcac tcagtggagt ggaaggagaa ggactccaca aacttggata 9120
catactcaga gacatatcaa agattccagg gggaaatatg tatgcagatg acacagccgg 9180
atgggacaca agaataacag aggatgatct tcagaatgag gccaaaatca ctgacatcat 9240
ggaacctgaa catgccctat tggccacgtc aatctttaag ctaacctacc aaaacaaggt 9300
agtaagggtg cagagaccag cgaaaaatgg aaccgtgatg gatgtcatat ccagacgtga 9360
ccagagagga agtggacagg ttggaaccta tggcttaaac accttcacca acatggaggc 9420
ccaactaata agacaaatgg agtctgaggg aatcttttca cccagcgaat tggaaacccc 9480
aaatctagcc gaaagagtcc tcgactggtt gaaaaaacat ggcaccgaga ggctgaaaag 9540
aatggcaatc agtggagatg actgtgtggt gaaaccaatt gatgacagat ttgcaacagc 9600
cttaacagct ttgaatgaca tgggaaaggt aagaaaagac ataccgcaat gggaaccttc 9660
aaaaggatgg aatgattggc aacaagtgcc tttctgttca caccatttcc accagctgat 9720
tatgaaggat gggagggaga tagtggtgcc atgccgcaac caagatgaac ttgtaggtag 9780
ggccagagta tcacaaggcg ccggatggag cttgagagaa actgcatgcc taggcaagtc 9840
atatgcacaa atgtggcagc tgatgtactt ccacaggaga gacttgagat tagcggctaa 9900
tgctatctgt tcagccgttc cagttgattg ggtcccaacc agccgtacca cctggtcgat 9960
ccatgcccac catcaatgga tgacaacaga agacatgttg tcagtgtgga atagggtttg 10020
gatagaggaa aacccatgga tggaggacaa gactcatgtg tccagttggg aagacgttcc 10080
atacctagga aaaagggaag atcaatggtg tggatcccta ataggcttaa cagcacgagc 10140
cacctgggcc accaacatac aagtggccat aaaccaagtg agaaggctca ttgggaatga 10200
gaattatcta gacttcatga catcaatgaa gagattcaaa aacgagagtg atcccgaagg 10260
ggcactctgg taagccaact cattcacaaa ataaaggaaa ataaaaaatc aaacaaggca 10320
agaagtcagg ccggattaag ccatagcacg gtaagagcta tgctgcctgt gagccccgtc 10380
caaggacgta aaatgaagtc aggccgaaag ccacggttcg agcaagccgt gctgcctgta 10440
gctccatcgt ggggatgtaa aaacccggga ggctgcaaac catggaagct gtacgcatgg 10500
ggtagcagac tagtggttag aggagacccc tcccaagaca caacgcagca gcgggcccca 10560
acaccagggg aagctgtacc ctggtggtaa ggactagagg ttagaggaga cccccccgcac 10620
aacaacaaac agcatattga cgctgggaga gaccagagat cctgctgtct ctacagcatc 10680
attccaggca cagaacgcca gaaaatggaa tggtgctgtt gaatcaacag gttctaaacg 10740
aagagc 10746

What is claimed is:

1. A chimeric dengue virus E glycoprotein comprising a dengue virus E glycoprotein backbone that comprises amino acid substitutions that introduce an epitope that is recognized by an antibody that is reactive with a dengue virus serotype that is different from the dengue virus serotype of the dengue virus E glycoprotein backbone,

wherein the chimeric dengue virus E glycoprotein comprises one of the following amino acid sequences:

MRCVGIGNRDFVEGLSGATWVDVVLEHGSCVTTMAKDKPTLDIELLKTE**A**T**QL**A**T**LRKLC
IEAKISNTTTDSRCPTQGEATLVEEQDTNFVCRRTFVDRGWGNGCGLFGKGSLITCAKFK
CVTK**I**EGK**V**VQYENLKYSVIVTVHTGDQHQVGNETTEHGT**I**ATITPQAPTSEIQLTDYGA
LTLDCSPRTGLDFNEM**I**LLTM**KN**K**A**W**M**VHRQWFLDLPLPWTSGASTSQETWNRQDLLVTF
KTAHAKKQEVVVLGSQEGAMHTALTGATEIQ**N**SG**G**T**S**IFAGHLKCRLKMDKLTLKGMSYV
MCTGSFKLEKEVAETQHGTVLVQVKYEGTDAPCKIPFSSQDEKGVTQNGRLITANPIVTD
KEKPVNIEAEPPFGESYIVVGAGEKALKLSWFKKG; or

MRCVGIGNRDFVEGLSGATWVDVVLEHGGCVTTMAKNKPTLDIEL**F**KTE**V**T**NP**A**V**LRKLCIEGK
ITNITTDSRCPTQGEAVLPEEQDQNYVCKHTYVDRGWGNGCGLFGKGSLVTCAKFQCLEPIEGK
VVQYENLKY**S**VI**V**TVHTGDQHQVGNET**TEH**G**TI**A**T**ITPQA**P**TSE**IQ**L**TD**YG**A**LGLECSPRTGLD
FNEMILLTMKNKAWMVHRQWFFDLPLPWTSGATTETPTWNRKELLVTFKNAHAKKQEVVVLGSQ
EGAMHTALTGATEIQ**T**SG**T**T**T**IFAGHLKCRLKMDKLELKGMSYAMCTNTFVLKKEVSETQHGTI
LIKVEYKGEDAPCKIPFSTEDGQGKAHNGRLITANPVVTKKEEPVNIEAEPPFGESNIVIGIGD
NALKINWYKKG.

2. The chimeric dengue virus E glycoprotein of claim 1, wherein the amino acid sequence is:

MRCVGIGNRDFVEGLSGATWVDVVLEHGSCVTTMAKDKPTLDIELLKTE**A**T**QL**A**T**LRKLC
IEAKISNTTTDSRCPTQGEATLVEEQDTNFVCRRTFVDRGWGNGCGLFGKGSLITCAKFK
CVTK**I**EGK**V**VQYENLKYSVIVTVHTGDQHQVGNETTEHGT**I**ATITPQAPTSEIQLTDYGA
LTLDCSPRTGLDFNEM**I**LLTM**KN**K**A**W**M**VHRQWFLDLPLPWTSGASTSQETWNRQDLLVTF
KTAHAKKQEVVVLGSQEGAMHTALTGATEIQ**N**SG**G**T**S**IFAGHLKCRLKMDKLTLKGMSYV
MCTGSFKLEKEVAETQHGTVLVQVKYEGTDAPCKIPFSSQDEKGVTQNGRLITANPIVTD
KEKPVNIEAEPPFGESYIVVGAGEKALKLSWFKKG

3. The chimeric dengue virus E glycoprotein of claim 1, wherein the amino acid sequence is:

MRCVGIGNRDFVEGLSGATWVDVVLEHGGCVTTMAKNKPTLDIEL**F**KTE**V**T**NP**A**V**LRKLCIEGK
ITNITTDSRCPTQGEAVLPEEQDQNYVCKHTYVDRGWGNGCGLFGKGSLVTCAKFQCLEPIEGK
VVQYENLKY**S**VI**V**TVHTGDQHQVGNET**TEH**G**TI**A**T**ITPQA**P**TSE**IQ**L**TD**YG**A**LGLECSPRTGLD
FNEMILLTMKNKAWMVHRQWFFDLPLPWTSGATTETPTWNRKELLVTFKNAHAKKQEVVVLGSQ
EGAMHTALTGATEIQ**T**SG**T**T**T**IFAGHLKCRLKMDKLELKGMSYAMCTNTFVLKKEVSETQHGTI
LIKVEYKGEDAPCKIPFSTEDGQGKAHNGRLITANPVVTKKEEPVNIEAEPPFGESNIVIGIGD
NALKINWYKKG

4. A flavivirus particle or virus like particle (VLP) comprising the E glycoprotein of any of claims 1-3.

5. An isolated nucleic acid molecule encoding the E glycoprotein of any of claims 1-3.

6. An isolated nucleic acid molecule encoding the flavivirus particle or VLP of claim 4.

7. A composition comprising the E glycoprotein of any of claims 1-3 in a pharmaceutically acceptable carrier.

8. A composition comprising the nucleic acid molecule of claim 5 or 6 in a pharmaceutically acceptable carrier.

9. Use of an effective amount of the E glycoprotein of any of claims 1-3, the flavivirus particle of claim 4, the nucleic acid molecule of any of claims 5-6 and/or the composition of any of claims 7-8 and any combination thereof in the manufacture of a medicament for use in producing an immune response to a dengue virus in a subject.

10. Use of an effective amount of the E glycoprotein of any of claims 1-3, the flavivirus particle of claim 4, the nucleic acid molecule of any of claims 5-6 and/or the composition of any of claims 7-8 and any combination thereof in the manufacture of a medicament for the treatment of a dengue virus infection in a subject.

11. Use of an effective amount of the E glycoprotein of any of claims 1-3, the flavivirus particle of claim 4, the nucleic acid molecule of any of claims 5-6 and/or the composition of any of claims 7-8 and any combination thereof in the manufacture of a medicament for preventing a dengue virus infection in a subject.

12. Use of an effective amount of the E glycoprotein of any of claims 1-3, the flavivirus particle of claim 4, the nucleic acid molecule of any of claims 5-6 and/or the composition of any of claims 7-8 and any combination thereof in the manufacture of a medicament for protecting a subject from the effects of dengue virus infection.