problem of inducing relevant protective immune responses in vaccinated individuals while maintaining an acceptable safety profile, in particular for those subjects at highest risk of severe disease, including infants, the elderly, and immunocompromised.

~~All publications mentioned herein are incorporated by reference for the purpose of describing and disclosing methodologies and materials that might be used in connection with the present invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.~~

Having described preferred embodiments of the invention with reference to the accompanying drawings, it is to be understood that the invention is not limited to those precise embodiments, and that various changes and modifications may be effected therein by one skilled in the art without departing from the scope or spirit of the invention as defined in the appended claims.

The following examples illustrate, but do not limit the invention.

Example 1

Expression and Purification of Dengue 80E Proteins in the Drosophila S2 system

The expression plasmid pMttbns (derived from pMttPA) contains the following elements: *Drosophila melanogaster* metallothionein promoter, the human tissue plasminogen activator secretion leader (tPAL) and the SV40 early polyadenylation signal. A 14 base pair *BamH*I (restriction enzyme from *Bacillus amyloliqufacience*) fragment was excised from the pMttbns vector to yield pMttΔXho that contains a unique *Xho*I (restriction enzyme from *Xanthomonas holicicola*) site in addition to an existing unique *Bgl*II (restriction enzyme from *Bacillus globigii*) site. This expression vector promotes the secretion of expressed proteins into the culture medium. Dengue sequences were introduced into the pMttΔXho vector using these unique *Bgl*II and *Xho*I sites. Dengue sequences used for studies described herein are represented by SEQ ID NO's 1-5, as follows: (1) SEQ ID NO:1; DEN1 prM-80E; (2) SEQ ID NO:2; DEN2 prM-80E; (3) SEQ ID NO:3; DEN3 prM-80E; (4) SEQ ID NO:4; DEN4 prM-80E; and (5) SEQ ID NO:5; DEN4 prM-80EZip. For the expression of carboxy-truncated dengue envelope proteins, the relevant gene fragment was amplified from viral RNA or cDNA clones. The synthetic prM80E (pre-membrane protein-80% glycoprotein E) gene fragment included two stop codons immediately following the last dengue codon.

S2 cells were co-transformed with both the expression plasmid and the pCoHygro selection plasmid that encodes hygromycin resistance utilizing the (i) calcium phosphate co-precipitation method or (ii) Cellfectin (Invitrogen Kits, Carlsbad, CA) according to the manufacturer's recommendations. Cells were co-transformed with 20 μg total DNA with a 20:1 ratio of expression plasmid to selection plasmid. Transformants were selected with hygromycin

WHAT IS CLAIMED IS:

~~1. An immunogenic composition comprising an effective amount of purified dengue virus envelope ("E") proteins of serotype DEN-1, DEN-2, DEN3, and DEN-4, a pharmaceutically acceptable excipient, and an effective amount of adjuvant; wherein the E proteins each constitute about 80% of the length of wild type E starting from amino acid residue 1 at its N-terminus; wherein the DEN-4 E protein is dimeric; and wherein the composition induces the production of neutralizing antibodies in human subjects.~~

~~2. The composition of claim 1 wherein the E protein is recombinantly produced and expressed in insect host cells.~~

~~3. The composition of claim 1 wherein the E protein is recombinantly produced and expressed in *Drosophila melanogaster* Schneider 2 (S2) host cells.~~

~~4. The composition of any preceding claim, wherein the adjuvant is an aluminum salt adjuvant.~~

~~5. The composition of any preceding claim, wherein the adjuvant is an ISCOM-type adjuvant.~~

~~6. The composition of claim 5; wherein the adjuvant is ISCOMATRIX.~~

~~7. The composition of claim 5, wherein the amount of DEN4 protein is about 1.5 to about 3 times the individual amounts of DEN1, DEN2, and DEN3 proteins.~~

~~8. The composition of claim 7, wherin the amount of DEN4 protein is about twice the individual amounts of DEN1, DEN2, and DEN3 proteins.~~

~~9~~1. An immunogenic composition comprising an effective amount of purified dengue virus envelope ("E") protein monomers of serotype DEN-1, DEN-2, DEN-3, and DEN-4, a pharmaceutically acceptable excipient, and an effective amount of adjuvant; wherein the E proteins each constitute approximately 80% of the length of wild type E starting from amino acid

residue 1 at its N-terminus, such that said E protein is secretable into growth medium when expressed recombinantly in a host cell; wherein the amount of DEN4 E protein is about 1.5 to about 3 times the individual amounts of DEN1, DEN2, and DEN3 E proteins, and wherein the composition induces the production of neutralizing antibodies in human subjects.

~~10~~2. The composition of claim ~~9~~1, wherein the amount of DEN 4 E protein is about twice the individual amounts of the DEN1, DEN2, DEN3 E proteins.

~~11~~3. The composition of claim ~~10~~2, wherein the amount of DEN E proteins are selected from the group consisting of: (a) about 3 μg DEN1-80E, about 3 μg DEN2-80E, about 3 μg DEN3-80E, and about 6 μg DEN4-80E; (b) about 10 μg DEN1-80E, about 10 μg DEN2-80E, about 10 μg DEN3-80E, and about 20 μg DEN4-80E; (c) about 50 μg DEN1-80E, about 50 μg DEN2-80E, about 50 μg DEN3-80E, and about 100 μg DEN4-80E; (d) about 3 μg DEN1-80E, about 3 μg DEN2-80E, about 3 μg DEN3-80E, and about 6 μg DEN4-80EZip; (e) about 10 μg DEN1-80E, about 10 μg DEN2-80E, about 10 μg DEN3-80E, and about 20 μg DEN4-80EZip; and (f) about 50 μg DEN1-80E, about 50 μg DEN2-80E, about 50 μg DEN3-80E, and about 100 μg DEN4-80EZip.

4. The composition of claim 1 wherein the E protein is recombinantly produced and expressed in insect host cells.

5. The composition of claim 1 wherein the E protein is recombinantly produced and expressed in *Drosophila melanogaster* Schneider 2 (S2) host cells.

6. The composition of any preceding claim, wherein the adjuvant is an aluminum salt adjuvant.

7. The composition of any preceding claim, wherein the adjuvant is an ISCOM-type adjuvant.

~~12~~8. An immunogenic composition of any of the preceding claims for use in raising a protective immune response in a human patient.

~~13~~9. An immunogenic composition of any of claims 1-~~11~~7 for use in providing immune protection in humans against dengue virus induced disease.

~~14. An immunogenic composition comprising a purified dengue virus envelope ("E") protein and a pharmaceutically acceptable excipient, wherein the E protein constitutes approximately 80% of the length of wild type E starting from amino acid residue 1 at its N-terminus; and an effective amount of adjuvant, for use in raising a protective immune response in a human patient, wherein the immunogenic composition induces the production of neutralizing antibodies in human subjects.~~

~~15. The method of claim 14, wherein the immunogenic composition comprises dengue virus E proteins of serotypes DEN1, DEN2, DEN3, and DEN4, wherein the DEN4 E protein is dimeric.~~

~~16. The method of claim 14 or claim 15, wherein the amount of DEN4 protein is about twice the individual amount of DEN1, DEN2, and DEN3 proteins in the composition.~~

~~17~~10. The method of any of claims ~~13~~8-~~16~~9 wherein the composition is administered via an intramuscular, subcutaneous or intradermal route of administration.

~~18. An immunogenic composition comprising an effective amount of purified dengue virus envelope ("E") proteins of serotype DEN-1, DEN-2, DEN3, and DEN-4, a pharmaceutically acceptable excipient, and an effective amount of adjuvant; wherein the E proteins each constitute about 80% of the length of wild type E starting from amino acid residue 1 at its N-terminus,; and wherein the DEN-4 E protein is dimeric; for the prevention or treatment of dengue disease.~~

~~19. The composition of claim 16 wherein the composition is to be administered to an immunodeficient pateint.~~

~~20~~11. The use of a composition of any of claims 1-~~11~~9 for the manufacture of a medicament for the treatment or prevention of dengue infection or disease.