DHF and DSS with secondary dengue infection has been hypothesized to be due to non-neutralizing antibodies induced by infection with one virus type enhancing infectivity of a second dengue virus type (antibody-dependent enhancement – ADE). This concept has important implications for vaccine development, as an effective dengue vaccine must simultaneously induce balanced specific neutralizing antibodies and specific memory cells against all four dengue serotypes (Halstead and Deen, 2002). This has proven to be a major problem in dengue vaccine development.

To date, the majority of the vaccines tested clinically are live, attenuated vaccines, which present safety concerns common to all live viral vaccines given to healthy subjects. Under-attenuation of the virus may result in virus-related adverse events, whereas over-attenuation may abrogate vaccine efficacy. Also, reversion to wild type or mutation to increased virulence (or decreased efficacy) may occur. Moreover, even if properly attenuated, live viral vaccines are contraindicated for specific patient populations, such as immune deficient or immune suppressed patients, as well as particular segments of the normal population, such as pregnant women, infants, or elderly individuals.

Further issues with live attenuated virus approaches for dengue include the challenges associated with combination of four independently replicating viruses in a tetravalent vaccine. Issues with interference have plagued all tetravalent formulations tested to date and have resulted in unbalanced tetravalent immunity and the requirement for multiple doses administered at an extended interval (e.g. 0, 6, 12 months). This is less than ideal and could present safety issues for individuals who have been partially immunized and become exposed to wild type virus as these individuals may be at higher risk of exacerbated disease (e.g. dengue hemorrhagic fever).

Ivy et al. (U.S. Patent 6,432,411) disclose a tetravalent subunit vaccine comprising DEN1-4 80% E (equivalent to amino acids 1-395 of the DEN-2 envelope polypeptide) proteins. Ivy et al, *supra*, also report compositions comprising DEN 1-4 80% E and ISCOMATRIX® adjuvant. There remains a need; however, for stable, tetravalent vaccines that can induce a balanced immune response against all four dengue serotypes.

SUMMARY

The present disclosure provides vaccines and immunogenic compositions for use in human patient populations for the prevention and/or treatment of disease associated with dengue virus infections. The vaccines are formed by the combination of recombinant subunit protein(s) derived from dengue virus envelope protein(s) and an adjuvant. The dengue virus vaccines of the present disclosure are designed to induce balanced, protective, tetravalent immune responses against DEN1, DEN2, DEN3, and DEN4, while providing an acceptable safety profile.

The unique vaccine formulation depends upon novel, properly folded recombinant envelope subunit proteins ("dengue 80E" or "DEN-80E" or "DEN1-80E" or "DEN2-80E" or "DEN3-80E" or "DEN4-80E" or "DEN4-80EZip") combined with adjuvants to produce the vaccine formulations. The unique combination in varying ratios of monomeric and/or dimeric forms of the recombinant envelope proteins of the formulation are designed specifically to address the need for balanced tetravalent responses. The vaccines are designed to induce relevant, balanced, tetravalent protective immune responses, such as virus neutralizing antibody in healthy human volunteers and to maintain an acceptable safety profile for administration to healthy and immunocompromised individuals. An additional advantage of the vaccine compositions described herein is that they do not contain significant quantities of the pre-membrane (prM) protein, potentially minimizing risk of ADE which has recently been linked to anti-prM antibodies (Dejnirattisai *et al.*, *Science* 328:745-748 (2010); Rodenhuis-Zybert *et al.*, PLos Pathogens 6:1-9 (2010)). The 80E proteins are expressed co-translationally with prM, but the polyprotein is cleaved as it transits the secretory pathway at the prM-E junction by host cell signalase releasing the 80E component into the culture medium for purification (Clements *et al.*, 2010 *Vaccine* 28:2705).

In one aspect, there is provided 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 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 DEN4 E protein is about 1.5 to about 3 times the individual amounts of DEN1, DEN2, and DEN3 proteins; and

wherein the composition induces the production of neutralizing antibodies in human subjects.

The disclosure also includes use of therapeutically effective amounts of the vaccines in an acceptable carrier as an immunoprophylactic against disease caused by dengue virus infection and use of the therapeutically effective amount of the vaccines in an acceptable carrier as a pharmaceutical composition.

In another aspect, there is provided use of a composition as defined herein for the manufacture of a medicament for the treatment or prevention of dengue infection or disease.

As used throughout the specification and in the appended claims, the singular forms "a," "an," and "the" include the plural reference unless the context clearly dictates otherwise.

As used throughout the specification and appended claims, the following definitions and abbreviations apply:

The term "treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Individuals in need of treatment include those already with the dengue infection, whether or not manifesting any clinical symptoms, as well as those at risk of being infected with dengue, i.e. those subjects/patients in which dengue infection and/or the clinical manifestations thereof are to be prevented. Treatment of a patient with the dengue vaccines of the invention includes one or more of the following: inducing/increasing an immune response against dengue in the patient, preventing, ameliorating, abrogating, or reducing the likelihood of the clinical manifestations of dengue in patients who have been infected with dengue, preventing or reducing the likelihood of developing dengue fever, DHF, or DSS and/or other disease or complication associated with dengue infection, reducing the severity or duration of the clinical symptoms of dengue infection and/or other disease or complication associated with the dengue, and preventing or reducing the likelihood of dengue infection.

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 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 DEN4 E protein is about 1.5 to about 3 times the individual amounts of DEN1, DEN2, and DEN3 proteins; and

wherein the composition induces the production of neutralizing antibodies in human subjects.

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

3. The composition of claim 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 one of the preceding claims, wherein the adjuvant is an aluminum salt adjuvant.

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

8. Use of a composition of any one of claims 1-3 for the manufacture of a medicament for the treatment or prevention of dengue infection or disease.