

to become a life-threatening disease. It has been reported that an anti-DENV antibody with mutations in the Fc region that prevent binding to FcγR failed to enhance DENV infection (WO2010/043977). However, there is still a need for antibody therapeutics without increasing the risk of antibody-dependent enhancement of infection.

SUMMARY

The invention provides anti-DENV antibodies, polypeptides containing variant Fc regions, and methods of using the same.

In one aspect, there is provided an isolated antibody that binds to dengue virus (DENV) E protein, wherein the antibody comprises: (i) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 14, (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 17, (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 24 and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 27; (ii) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 15, (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 17, (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 24 and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 27; (iii) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 15, (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 18, (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 24 and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 27; (iv) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 15, (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 19, (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 43, (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 44 and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 45; (v) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 15, (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 17, (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 25 and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 28; (vi) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 15, (c)

HVR-H3 comprising the amino acid sequence of SEQ ID NO: 17, (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 22, (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 25 and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 29; (vii) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 12, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 15, (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 20, (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 23, (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 26 and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 30; or (viii) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 12, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 15, (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 20, (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 24 and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 27.

In another aspect, there is provided an isolated antibody, comprising (a) a VH sequence having the amino acid sequence of any one of SEQ ID NOs: 2-6; (b) a VL sequence having the amino acid sequence of any one of SEQ ID NOs: 8-10; or (c) a VH sequence of any one of SEQ ID NOs: 2-6 and a VL sequence of any one of SEQ ID NOs: 7-10.

In another aspect, there is provided an isolated antibody that binds to dengue virus (DENV) E protein, wherein the antibody comprises: (a) VH comprising the amino acid sequence of SEQ ID NO: 2, VL comprising the amino acid sequence of SEQ ID NO: 7, CH comprising the amino acid sequence of SEQ ID NO: 46 and CL comprising the amino acid sequence of SEQ ID NO: 60, (b) VH comprising the amino acid sequence of SEQ ID NO: 3, VL comprising the amino acid sequence of SEQ ID NO: 7, CH comprising the amino acid sequence of SEQ ID NO: 46 and CL comprising the amino acid sequence of SEQ ID NO: 60, (c) VH comprising the amino acid sequence of SEQ ID NO: 3, VL comprising the amino acid sequence of SEQ ID NO: 7, CH comprising the amino acid sequence of SEQ ID NO: 54 and CL comprising the amino acid sequence of SEQ ID NO: 60, (d) VH comprising the amino acid sequence of SEQ ID NO: 3, VL comprising the amino acid sequence of SEQ ID NO: 7, CH comprising the amino acid sequence of SEQ ID NO: 59 and CL comprising the amino acid sequence of SEQ ID NO: 60, (e) VH comprising the amino acid sequence of SEQ ID NO: 4, VL comprising the amino acid sequence of SEQ ID NO: 7, CH comprising the amino acid sequence of SEQ ID NO: 46 and CL comprising the amino acid sequence of SEQ ID NO: 60, (f) VH comprising the amino acid sequence of SEQ ID NO: 5, VL comprising the amino acid

sequence of SEQ ID NO: 7, CH comprising the amino acid sequence of SEQ ID NO: 46 and CL comprising the amino acid sequence of SEQ ID NO: 60, (g) VH comprising the amino acid sequence of SEQ ID NO: 3, VL comprising the amino acid sequence of SEQ ID NO: 8, CH comprising the amino acid sequence of SEQ ID NO: 46 and CL comprising the amino acid sequence of SEQ ID NO: 60, (h) VH comprising the amino acid sequence of SEQ ID NO: 3, VL comprising the amino acid sequence of SEQ ID NO: 9, CH comprising the amino acid sequence of SEQ ID NO: 46 and CL comprising the amino acid sequence of SEQ ID NO: 60, (i) VH comprising the amino acid sequence of SEQ ID NO: 6, VL comprising the amino acid sequence of SEQ ID NO: 10, CH comprising the amino acid sequence of SEQ ID NO: 46 and CL comprising the amino acid sequence of SEQ ID NO: 60, (j) VH comprising the amino acid sequence of SEQ ID NO: 6, VL comprising the amino acid sequence of SEQ ID NO: 10, CH comprising the amino acid sequence of SEQ ID NO: 59 and CL comprising the amino acid sequence of SEQ ID NO: 60, (k) VH comprising the amino acid sequence of SEQ ID NO: 6, VL comprising the amino acid sequence of SEQ ID NO: 7, CH comprising the amino acid sequence of SEQ ID NO: 46 and CL comprising the amino acid sequence of SEQ ID NO: 60, (l) VH comprising the amino acid sequence of SEQ ID NO: 6, VL comprising the amino acid sequence of SEQ ID NO: 7, CH comprising the amino acid sequence of SEQ ID NO: 54 and CL comprising the amino acid sequence of SEQ ID NO: 60, or (m) VH comprising the amino acid sequence of SEQ ID NO: 6, VL comprising the amino acid sequence of SEQ ID NO: 7, CH comprising the amino acid sequence of SEQ ID NO: 59 and CL comprising the amino acid sequence of SEQ ID NO: 60.

In some examples, an isolated anti-DENV antibody as disclosed herein binds to DENV E protein. In some examples, an anti-DENV antibody as disclosed herein comprises:

- (a) (i) HVR-H3 comprising the amino acid sequence GGX₁ALFYDSYTPX₂DX₃GSWWFDP, wherein X₁ is R or E, X₂ is R or F, X₃ is G, D or L (SEQ ID NO: 42), (ii) HVR-L3 comprising the amino acid sequence QQFX₁X₂LPIT, wherein X₁ is D, S or E, X₂ is D or A (SEQ ID NO: 45), and (iii) HVR-H2 comprising the amino acid sequence VINPRGGSX₁X₂SAQKFQG, wherein X₁ is T or R, X₂ is A or R (SEQ ID NO: 41);
- (b) (i) HVR-H1 comprising the amino acid sequence SX₁YX₂H, wherein X₁ is N or Y, X₂ is I or M (SEQ ID NO: 40), (ii) HVR-H2 comprising the amino acid sequence VINPRGGSX₁X₂SAQKFQG, wherein X₁ is T or R, X₂ is A or R (SEQ ID NO: 41), and (iii)

HVR-H3 comprising the amino acid sequence GGX₁ALFYDSYTPX₂DX₃GSWWFDP, wherein X₁ is R or E, X₂ is R or F, X₃ is G, D or L (SEQ ID NO: 42);

(c) (i) HVR-H1 comprising the amino acid sequence SX₁YX₂H, wherein X₁ is N or Y, X₂ is I or M (SEQ ID NO: 40), (ii) HVR-H2 comprising the amino acid sequence

- 5 VINPRGGSX₁X₂SAQKFQG, wherein X₁ is T or R, X₂ is A or R (SEQ ID NO: 41), (iii) HVR-H3 comprising the amino acid sequence GGX₁ALFYDSYTPX₂DX₃GSWWFDP, wherein X₁ is R or E, X₂ is R or F, X₃ is G, D or L (SEQ ID NO: 42), (iv) HVR-L1 comprising the amino acid sequence QASQX₁IRX₂YLN, wherein X₁ is D or E, X₂ is K or Q (SEQ ID NO: 43); (v) HVR-L2 comprising the amino acid sequence DASX₁LKX₂, wherein X₁ is N or E, X₂ is T or F (SEQ ID NO: 44); and (vi) HVR-L3 comprising the amino acid sequence QQFX₁X₂LPIT, wherein X₁ is D, S or E, X₂ is D or A (SEQ ID NO: 45); or

(d) (i) HVR-L1 comprising the amino acid sequence QASQX₁IRX₂YLN, wherein X₁ is D or E, X₂ is K or Q (SEQ ID NO: 43); (ii) HVR-L2 comprising the amino acid sequence DASX₁LKX₂, wherein X₁ is N or E, X₂ is T or F (SEQ ID NO: 44); and (iii) HVR-L3

- 15 comprising the amino acid sequence QQFX₁X₂LPIT, wherein X₁ is D, S or E, X₂ is D or A

(SEQ ID NO: 45). In some examples, the antibody as disclosed herein is not an antibody comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 13, (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 16, (iv) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (v) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 24, and (vi) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 27.

In some examples, an isolated anti-DENV antibody as disclosed herein comprises:

(a) (i) HVR-H3 from the VH sequence of any one of SEQ ID NOs: 2-6, (ii) HVR-L3 from the VL sequence of any one of SEQ ID NOs: 8-10, and (iii) HVR-H2 from the VH sequence of any one of SEQ ID NOs: 2-6;

(b) (i) HVR-H1 from the VH sequence of any one of SEQ ID NOs: 2-6, (ii) HVR-H2 from the VH sequence of any one of SEQ ID NOs: 2-6, and (iii) HVR-H3 from the VH sequence of any one of SEQ ID NOs: 2-6;

(c) (i) HVR-H1 from the VH sequence of any one of SEQ ID NOs: 2-6, (ii) HVR-H2 from the VH sequence of any one of SEQ ID NOs: 2-6, (iii) HVR-H3 from the VH sequence of any one of SEQ ID NOs: 2-6, (iv) HVR-L1 from the VL sequence of any one of SEQ ID NOs: 8-10; (v) HVR-L2 from the VL sequence of any one of SEQ ID NOs: 8-10; and (vi) HVR-L3 from the VL sequence of any one of SEQ ID NOs: 8-10;

(d) (i) HVR-L1 from the VL sequence of any one of SEQ ID NOs: 8-10; (ii) HVR-L2 from the VL sequence of any one of SEQ ID NOs: 8-10; and (iii) HVR-L3 from the VL sequence of any one of SEQ ID NOs: 8-10; or

(e) (i) HVR-H1 from the VH sequence of any one of SEQ ID NOs: 2-6, (ii) HVR-H2 from the VH sequence of any one of SEQ ID NOs: 2-6, (iii) HVR-H3 from the VH sequence of any one of SEQ ID NOs: 2-6, (iv) HVR-L1 from the VL sequence of any one of SEQ ID NOs: 7; (v) HVR-L2 from the VL sequence of any one of SEQ ID NOs: 7; and (vi) HVR-L3 from the VL sequence of any one of SEQ ID NOs: 7.

In some examples, the antibody as disclosed herein is not an antibody comprising (i) HVR-H1 from the VH sequence of SEQ ID NO: 1, (ii) HVR-H2 from the VH sequence of SEQ ID NO: 1, (iii) HVR-H3 from the VH sequence of SEQ ID NO: 1, (iv) HVR-L1 from the VL sequence of SEQ ID NO: 7, (v) HVR-L2 from the VL sequence of SEQ ID NO: 7, and (vi) HVR-L3 from the VL sequence of SEQ ID NO: 7.

In some examples, an isolated anti-DENV antibody as disclosed herein further comprises a heavy chain variable domain framework FR1 comprising the amino acid sequence of SEQ ID NO: 31, FR2 comprising the amino acid sequence of SEQ ID NO: 32, FR3

comprising the amino acid sequence of SEQ ID NO: 33 or 34, FR4 comprising the amino acid sequence of SEQ ID NO: 35. In some examples, an isolated anti-DENV antibody as disclosed hereinfurther comprises a light chain variable domain framework FR1 comprising the amino acid sequence of SEQ ID NO: 36, FR2 comprising the amino acid sequence of SEQ ID NO: 37, FR3 comprising the amino acid sequence of SEQ ID NO: 38, FR4 comprising the amino acid sequence of SEQ ID NO: 39.

In some examples, an isolated anti-DENV antibody as disclosed herein comprises (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 2-6; (b) a VL sequence having at least 95% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 8-10; (c) a VH sequence of any one of SEQ ID NOs: 2-6 and a VL sequence of any one of SEQ ID NOs: 8-10; or (d) a VH sequence of any one of SEQ ID NOs: 2-6 and a VL sequence of any one of SEQ ID NOs: 7.

In some examples, an isolated anti-DENV antibody as disclosed herein is a monoclonal antibody. In some examples, an isolated anti-DENV antibody as disclosed herein is a human, humanized, or chimeric antibody. In some examples, an isolated anti-DENV antibody as disclosed herein is an antibody fragment that binds to DENV or DENV E protein. In some examples, an isolated anti-DENV antibody as disclosed herein is a full length IgG antibody.

In some examples, an Fc region of an anti-DENV antibody as disclosed herein comprises Ala at position 234 and Ala at position 235 according to EU numbering. In some examples, an Fc region of an anti-DENV antibody as disclosed herein may be selected from variant Fc regions described herein.

The disclosure also provides isolated nucleic acids encoding an anti-DENV antibody as disclosed herein. The disclosure also provides host cells comprising a nucleic acid as disclosed herein. The disclosure also provides a method of producing an antibody comprising culturing a host cell as disclosed herein so that the antibody is produced.

The disclosure also provides a pharmaceutical formulation comprising an anti-DENV antibody as disclosed herein and a pharmaceutically acceptable carrier.

Anti-DENV antibodies as disclosed herein may be for use as a medicament. In some examples, anti-DENV antibodies as disclosed herein may be for use in treating DENV infection.

Anti-DENV antibodies as disclosed herein may be used in the manufacture of a medicament. In some examples, the medicament is for treatment of a DENV infection.

The disclosure also provides a method of treating an individual having a DENV infection. In some examples, the method comprises administering to the individual an effective amount of an anti-DENV antibody as disclosed herein. In some examples, the method further comprises administering to the individual an additional therapeutic agent, e.g., as described below.

In some examples, a variant Fc region as disclosed herein comprises at least one amino acid alteration in a parent Fc region. In further examples, the variant Fc region has a substantially decreased FcγR-binding activity when compared to the parent Fc region. In further examples, the variant Fc region does not have a substantially decreased C1q-binding activity when compared to the parent Fc region.

In some examples, a variant Fc region as disclosed herein comprises Ala at position 234, Ala at position 235, according to EU numbering. In further examples, the variant Fc region comprises amino acid alterations of any one of the following (a)-(c): (a) positions 267, 268, and 324, (b) positions 236, 267, 268, 324, and 332, and (c) positions 326 and 333; according to EU numbering.

In some examples, a variant Fc region as disclosed herein comprises amino acids selected from the group consisting of: (a) Glu at position 267, (b) Phe at position 268, (c) Thr at position 324, (d) Ala at position 236, (e) Glu at position 332, (f) Ala, Asp, Glu, Met, or Trp at position 326, and (g) Ser at position 333; according to EU numbering.

In some examples, a variant Fc region as disclosed herein further comprises amino acids selected from the group consisting of: (a) Ala at position 434, (b) Ala at position 434, Thr at position 436, Arg at position 438, and Glu at position 440, (c) Leu at position 428, Ala at position 434, Thr at position 436, Arg at position 438, and Glu at position 440, and (d) Leu at position 428, Ala at position 434, Arg at position 438, and Glu at position 440; according to EU numbering.

In some examples, a variant Fc region as disclosed herein comprises any of the amino acid alterations, singly or in combination, as described in Table 4. In some examples, a parent Fc region described herein is derived from human IgG1. The disclosure provides a polypeptide comprising the amino acid sequence of any one of SEQ ID NOs: 51-59.

In some examples, a polypeptide comprising a variant Fc region as disclosed herein is an antibody. In further examples, the antibody is an anti-virus antibody. In

further examples, the antibody comprises a variable region derived from an anti-DENV antibody described herein.

In further examples, the antibody comprises:

- (a) (i) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 16, (ii) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 27, and (iii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 13;
- (b) (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 13, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 16;
- (c) (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 13, (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 16, (iv) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21; (v) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 24; and (vi) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 27; or
- (d) (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21; (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 24; and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 27.

In further examples, the antibody comprises:

- (a) (i) HVR-H3 from the VH sequence of SEQ ID NO: 6, (ii) HVR-L3 from the VL sequence of SEQ ID NO: 10, and (iii) HVR-H2 from the VH sequence of SEQ ID NO: 6;
- (b) (i) HVR-H1 from the VH sequence of SEQ ID NO: 6, (ii) HVR-H2 from the VH sequence of SEQ ID NO: 6, and (iii) HVR-H3 from the VH sequence of SEQ ID NO: 6;
- (c) (i) HVR-H1 from the VH sequence of SEQ ID NO: 6, (ii) HVR-H2 from the VH sequence of SEQ ID NO: 6, (iii) HVR-H3 from the VH sequence of SEQ ID NO: 6, (iv) HVR-L1 from the VL sequence of SEQ ID NO: 10; (v) HVR-L2 from the VL sequence of SEQ ID NO: 10; and (vi) HVR-L3 from the VL sequence of SEQ ID NO: 10;
- (d) (i) HVR-L1 from the VL sequence of SEQ ID NO: 10; (ii) HVR-L2 from the VL sequence of SEQ ID NO: 10; and (iii) HVR-L3 from the VL sequence of SEQ ID NO: 10; or
- (e) (i) HVR-H1 from the VH sequence of SEQ ID NO: 6, (ii) HVR-H2 from the VH sequence of SEQ ID NO: 6, (iii) HVR-H3 from the VH sequence of SEQ ID NO: 6, (iv) HVR-L1 from the VL sequence of SEQ ID NO: 7; (v) HVR-L2 from the VL sequence of SEQ ID NO: 7; and (vi) HVR-L3 from the VL sequence of SEQ ID NO: 7;

The disclosure also provides isolated nucleic acids encoding a polypeptide comprising a variant Fc region as disclosed herein. The disclosure also provides host cells comprising a nucleic acid as disclosed herein. The disclosure also provides a method of producing a polypeptide comprising a variant Fc region comprising culturing a host as disclosed herein so that the polypeptide is produced.

The disclosure also provides a pharmaceutical formulation comprising a polypeptide comprising a variant Fc region as disclosed herein and a pharmaceutically acceptable carrier.

Polypeptides comprising variant Fc regions as disclosed herein may be for use as a medicament. In some examples, polypeptides comprising variant Fc regions as disclosed herein may be for use in treating a viral infection.

Polypeptides comprising variant Fc regions as disclosed herein may be used in the manufacture of a medicament. In some examples, the medicament is for treatment of a viral infection.

The disclosure also provides a method of treating an individual having a viral infection. In some examples, the method comprises administering to the individual an effective amount of a polypeptide comprising a variant Fc region as disclosed herein.

The disclosure also provides an anti-DENV antibody described herein, further comprising a polypeptide comprising a variant Fc region as disclosed herein.

In one example the present disclosure refers to a process for preparing an antibody comprising: (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 12, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 15, (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 20, (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 24 and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 27, the process comprising the steps of:

(a) combining a VH variant sequence selected from the group consisting of: 3CH1047 (SEQ ID NO: 6), and 3CH1049 (SEQ ID NO: 95) with a human IgG1 CH sequence selected from the group consisting of: SG182 (SEQ ID NO: 46), SG1095 (SEQ ID NO: 54) and SG1106 (SEQ ID NO: 59);

(b) combining a VL variant sequence selected from the group consisting of 3CL (SEQ ID NO: 7) and 3CL633 (SEQ ID NO: 98), with a human CL sequence SK1 (SEQ ID NO: 60);

(c) cloning each one of the combinations in an expression vector;

(d) expressing the resulting expression vectors in co-transfected cells (host cells); and

(e) purifying the resulting antibody from step (d).

LALA + ACT3, LALA + ACT5, LALA + KAES, LALA + KAES + ACT3, and LALA + KAES + ACT5. These Fc variants were tested for binding to FcγRs including: (a) mouse FcγR1, (b) mouse FcγR2b, (c) mouse FcγR3, and (d) mouse FcγR4. Each Fc variant was evaluated both in the form of an antibody alone with the Fc variant (described as Ab alone) and in the form of an immune complex formed with the antibody and a trimeric CD154 antigen (described as CD154 IC).

Figure 8 illustrates Biacore analysis for Fc variants binding to human FcRn, as described in Example 5. Fc variants tested are: WT (described as hIgG1 in the figure), LALA, LALA + ACT3, LALA + ACT5, LALA + KAES, LALA + KAES + ACT3, and LALA + KAES + ACT5. Each Fc variant was evaluated both in the form of an antibody alone with the Fc variant (described as Ab alone) and in the form of an immune complex formed with the antibody and a trimeric CD154 antigen (described as CD154 IC).

Figure 9 illustrates viremia at day 3 after DENV-2 virus infection in AG129 mice, as described in Example 6. Anti-DENV antibodies 3C and 3Cam in combination with Fc variants of WT (described as hIgG1), LALA, and LALA + KAES, or PBS as a negative control were administered at day 2 after virus infection.

Figure 10 (A)-(D) illustrate BIACORE[®] sensorgrams of anti-DENV antibodies 3C and 3Cam2 towards DENV-1 E protein (A), DENV-2 E protein (B), DENV-3 E protein (C), and DENV-4 E protein (D), as described in Example 2.

DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

The techniques and procedures described or referenced herein are generally well understood and commonly employed using conventional methodology by those skilled in the art, such as, for example, the widely utilized methodologies described in Sambrook et al., *Molecular Cloning: A Laboratory Manual* 3d edition (2001) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; *Current Protocols in Molecular Biology* (F.M. Ausubel, et al., eds., (2003)); the series *Methods in Enzymology* (Academic Press, Inc.): *PCR 2: A Practical*

The affinity of anti-DENV E protein antibodies binding to E protein of DENV-1, DENV-2, DENV-3, and DENV-4 were determined at 25°C using Biacore T200 instrument (GE Healthcare). Anti-histidine antibody (GE Healthcare) was immobilized onto all flow cells of a CM4 sensor chip using an amine coupling kit (GE Healthcare). All antibodies and
 5 analytes were prepared in PBS, pH 7.4, containing 20 mM sodium phosphate, 150 mM NaCl, 0.05% Tween 20 and 0.005% NaN₃. E protein of DENV-1, DENV-2, DENV-3, and DENV-4 with C-terminal His-tag was captured onto flow cell 2 or 3, with flow cell 1 as reference flow cell. Capture level for E protein was aimed at 200 resonance unit (RU). Anti-DENV E protein antibodies were injected at 250 nM over the entire sensor surface for 180 seconds, followed by
 10 300 seconds of dissociation. The sensor surface was regenerated after each cycle using 10 mM Gly-HCl pH1.5. Binding affinity was determined by processing and fitting the data to 1:1 binding model using Biacore T200 Evaluation software, version 2.0 (GE Healthcare).

Tables 3a and 3b show the affinity (K_d) of anti-DENV E protein antibodies binding to E protein of DENV-1 (described as DV1 in the Table), DENV-2 (described as DV2 in the Table),
 15 DENV-3 (described as DV3 in the Table), and DENV-4 (described as DV4 in the Table). Each 3C variant showed increased binding affinity against all four DENV serotypes, compared to the parent 3C. As examples, the sensorgrams of the parent antibody 3C (DG_3CH-SG182/3CL-SK1) and one of the variant antibody 3Cam (DG_3CH1047-SG182/3CL658-SK1) are shown in Figure 1. Also, the sensorgrams of the parent antibody 3C (DG_3CH-SG182/3CL-SK1) and
 20 one of the variant antibody 3Cam2 (DG_3CH1047-SG182/3CL-SK1) are shown in Figure 10.

Table 3a

K_d values of 3C and 3C variants against four DENV serotypes

of KAES or KWES mutants were not greatly reduced compared to that of WT IgG for most of the FcγRs tested. The results for human FcRn are shown in Figure 8. The binding of a wild type Fc region (described as hIgG1) to human FcRn did not appear to be affected, even after introducing LALA or LALA +KAES mutations into the Fc region. The binding was slightly enhanced by further introducing ACT3 or ACT5 mutations but still remained comparatively low (Figure 8).

EXAMPLE 6: *In vivo* efficacy of anti-DENV antibodies against DENV infection

6-8weeks old AG129 mice were infected intraperitoneally with 10^6 plaque-forming units (pfu) DENV-2 strain D2Y98P. 48 hours later, the mice were treated with 25μg antibody in PBS. The antibody was injected intravenously via the retro-orbital route. Further 24 hours later, that is 72 hours after the initial infection, blood was collected. In previous studies (Zust et al, J Virol (2014) 88, 7276-7285; Tan et al, PLOS Negl Trop Dis (2010) 4, e672), it has been established that peak viremia after infection with D2Y98P is reached between day 3-4 after infection. Viral RNA was extracted from the plasma from each mouse and a quantitative PCR was performed and compared against a DENV-2 standard with known infectivity in a plaque assay. Both 3C and 3Cam antibodies greatly reduced viremia, compared to PBS control in this mice model, and the efficacy of both antibodies were comparable. The antibody having the LALA + KAES mutation in Fc region showed stronger efficacy compared to the antibody with only the LALA mutation. This was the case for both the 3C and the 3Cam antibodies (Figure 9). This result indicates that recovery of C1q binding activity by adding KAES mutation to LALA mutation contributes to antiviral efficacy of the antibodies.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the invention. The disclosures of all patent and scientific literature cited herein are expressly incorporated in their entirety by

5 reference.

WHAT IS CLAIMED IS:

1. An isolated antibody that binds to dengue virus (DENV) E protein, wherein the antibody comprises:

(i) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 14, (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 17, (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 24 and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 27;

(ii) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 15, (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 17, (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 24 and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 27;

(iii) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 15, (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 18, (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 24 and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 27;

(iv) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 15, (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 19, (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 43, (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 44 and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 45;

(v) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 15, (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 17, (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 25 and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 28;

(vi) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 15, (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 17, (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 22, (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 25 and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 29;

(vii) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 12, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 15, (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 20, (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 23, (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 26 and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 30; or

(viii) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 12, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 15, (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 20, (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 24 and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 27.

2. An isolated antibody, comprising (a) a VH sequence having the amino acid sequence of any one of SEQ ID NOs: 2-6; (b) a VL sequence having the amino acid sequence of any one of SEQ ID NOs: 8-10; or (c) a VH sequence of any one of SEQ ID NOs: 2-6 and a VL sequence of any one of SEQ ID NOs: 7-10.

3. The antibody of claim 1 or 2, comprising a variant Fc region comprising at least one amino acid alteration in a parent Fc region, wherein the variant Fc region has a substantially decreased FcγR-binding activity and does not have a substantially decreased C1q-binding activity when compared to the parent Fc region.

4. The antibody of claim 3, wherein the variant Fc region comprises Ala at position 234, Ala at position 235 and further amino acid alterations of any one of the following (a)-(c):

(a) positions 267, 268, and 324;

(b) positions 236, 267, 268, 324, and 332; and

(c) positions 326 and 333;

according to EU numbering.

5. The antibody of claim 4, wherein the variant Fc region comprises amino acids selected from the group consisting of:

- (a) Glu at position 267;
- (b) Phe at position 268;
- (c) Thr at position 324;
- (d) Ala at position 236;
- (e) Glu at position 332;
- (f) Ala, Asp, Glu, Met, or Trp at position 326; and
- (g) Ser at position 333;

according to EU numbering.

6. The antibody of any one of claims 3 to 5, wherein the variant Fc region further comprises amino acids selected from the group consisting of:

- (a) Ala at position 434;
- (b) Ala at position 434, Thr at position 436, Arg at position 438, and Glu at position 440;
- (c) Leu at position 428, Ala at position 434, Thr at position 436, Arg at position 438, and Glu at position 440; and
- (d) Leu at position 428, Ala at position 434, Arg at position 438, and Glu at position 440;

according to EU numbering.

7. An isolated antibody that binds to dengue virus (DENV) E protein, wherein the antibody comprises:

- (a) VH comprising the amino acid sequence of SEQ ID NO: 2, VL comprising the amino acid sequence of SEQ ID NO: 7, CH comprising the amino acid sequence of SEQ ID NO: 46 and CL comprising the amino acid sequence of SEQ ID NO: 60,
- (b) VH comprising the amino acid sequence of SEQ ID NO: 3, VL comprising the amino acid sequence of SEQ ID NO: 7, CH comprising the amino acid sequence of SEQ ID NO: 46 and CL comprising the amino acid sequence of SEQ ID NO: 60,
- (c) VH comprising the amino acid sequence of SEQ ID NO: 3, VL comprising the amino acid sequence of SEQ ID NO: 7, CH comprising the amino acid sequence of SEQ ID NO: 54 and CL comprising the amino acid sequence of SEQ ID NO: 60,

(d) VH comprising the amino acid sequence of SEQ ID NO: 3, VL comprising the amino acid sequence of SEQ ID NO: 7, CH comprising the amino acid sequence of SEQ ID NO: 59 and CL comprising the amino acid sequence of SEQ ID NO: 60,

5 (e) VH comprising the amino acid sequence of SEQ ID NO: 4, VL comprising the amino acid sequence of SEQ ID NO: 7, CH comprising the amino acid sequence of SEQ ID NO: 46 and CL comprising the amino acid sequence of SEQ ID NO: 60,

10 (f) VH comprising the amino acid sequence of SEQ ID NO: 5, VL comprising the amino acid sequence of SEQ ID NO: 7, CH comprising the amino acid sequence of SEQ ID NO: 46 and CL comprising the amino acid sequence of SEQ ID NO: 60,

15 (g) VH comprising the amino acid sequence of SEQ ID NO: 3, VL comprising the amino acid sequence of SEQ ID NO: 8, CH comprising the amino acid sequence of SEQ ID NO: 46 and CL comprising the amino acid sequence of SEQ ID NO: 60,

20 (h) VH comprising the amino acid sequence of SEQ ID NO: 3, VL comprising the amino acid sequence of SEQ ID NO: 9, CH comprising the amino acid sequence of SEQ ID NO: 46 and CL comprising the amino acid sequence of SEQ ID NO: 60,

25 (i) VH comprising the amino acid sequence of SEQ ID NO: 6, VL comprising the amino acid sequence of SEQ ID NO: 10, CH comprising the amino acid sequence of SEQ ID NO: 46 and CL comprising the amino acid sequence of SEQ ID NO: 60,

(j) VH comprising the amino acid sequence of SEQ ID NO: 6, VL comprising the amino acid sequence of SEQ ID NO: 10, CH comprising the amino acid sequence of SEQ ID NO: 59 and CL comprising the amino acid sequence of SEQ ID NO: 60,

30 (k) VH comprising the amino acid sequence of SEQ ID NO: 6, VL comprising the amino acid sequence of SEQ ID NO: 7, CH comprising the amino acid sequence of SEQ ID NO: 46 and CL comprising the amino acid sequence of SEQ ID NO: 60,

(l) VH comprising the amino acid sequence of SEQ ID NO: 6, VL comprising the amino acid sequence of SEQ ID NO: 7, CH comprising the amino acid

sequence of SEQ ID NO: 54 and CL comprising the amino acid sequence of SEQ ID NO: 60, or

(m) VH comprising the amino acid sequence of SEQ ID NO: 6, VL comprising the amino acid sequence of SEQ ID NO: 7, CH comprising the amino acid sequence of SEQ ID NO: 59 and CL comprising the amino acid sequence of SEQ ID NO: 60.