vaccines against flavivirus may exponentially increase incidence of flavivirus infection and illness.

[0006] Accordingly, there is an urgent need for therapeutics and methods for preventing flavivirus infection, and diseases and disorders related thereto, without increasing the risk of antibody-dependent enhancement of infection.

SUMMARY ~~OF THE INVENTION~~

[0007] The ~~invention~~ present disclosure is based upon the discovery of monoclonal antibodies which bind and neutralize flavivirus, and do not contribute to the antibody-dependent enhancement of flavivirus infection. The monoclonal antibody is fully human. The antibodies recognize the West Nile virus envelope protein E (WNE), and have broad cross-reactivity across other members of the flavivirus family. Importantly, the antibodies contain mutations in the Fc region that prevent binding to the Fcγ receptor. The antibodies are referred to herein as huFV antibodies.

[0008] The present disclosure ~~invention~~ provides an isolated humanized monoclonal antibody having a heavy chain with three CDRs, wherein the CDR1 includes amino acid sequence GYSTH (SEQ ID NO:4), wherein the CDR2 includes amino acid sequence WDNPSSGDTTYAENFRG (SEQ ID NO:5), and wherein the CDR3 includes amino acid sequence GGDDYSFDH (SEQ ID NO:6) respectively; a light chain with three CDRs, wherein the CDR1 includes amino acid sequence RGDSLRSYYAS (SEQ ID NO:7), wherein the CDR2 includes amino acid sequence GENNRPS (SEQ ID NO:8), and wherein the CDR3 includes amino acid sequence NSRDSSDHLLL (SEQ ID NO:8) respectively. The antibody has a modified Fc region such that the Fc region does not bind to the Fcγ receptor, and binds to a flavivirus. Exemplary Fc regions are disclosed herein.

[0009] ~~In one aspect, t~~The present disclosure ~~invention~~ provides an isolated humanized monoclonal antibody having a V_H amino acid sequence having SEQ ID NO: 1, a V_L amino acid sequence having SEQ ID NO: 3. ~~In another aspect, t~~The ~~invention~~ present disclosure provides an isolated humanized monoclonal antibody comprising a V_H nucleotide sequence having SEQ ID NO: 2, a V_L nucleotide sequence having SEQ ID NO: 4. The antibody further comprises a modified Fc region such that the Fc region does not bind to the Fcγ receptor, and binds to a flavivirus.

[0009a] In one aspect, the present disclosure provides an isolated humanized monoclonal antibody comprising:

a. a heavy chain with three CDRs comprising the amino acid sequences GYSTH (SEQ ID NO:4), WDNPSSGDTTYAENFRG (SEQ ID NO:5), and GGDDYSFDH (SEQ ID NO:6) respectively;

b. a light chain with three CDRs comprising the amino acid sequences RGDSLRSYYAS (SEQ ID NO:7), GENNRPS (SEQ ID NO:8), and NSRDSSDHLLL (SEQ ID NO:8) respectively; and

c. a modified Fc region such that the Fc region does not bind to the Fcγ receptor,

and wherein the antibody binds a flavivirus.

[0009b] In a further aspect, the present disclosure provides an isolated humanized monoclonal antibody comprising a V_H amino acid sequence having SEQ ID NO: 1, a V_L amino acid sequence having SEQ ID NO: 3, and a modified Fc region such that the Fc region does not bind to the Fcγ receptor, and wherein the antibody binds a flavivirus.

[0009c] In another aspect, the present disclosure provides an isolated humanized monoclonal antibody comprising a V_H nucleotide sequence having SEQ ID NO: 2, a V_L nucleotide sequence having SEQ ID NO: 4, and a modified Fc region such that the Fc region does not bind to the Fcγ receptor, and wherein the antibody binds a flavivirus.

[0010] The present ~~invention~~ disclosure further provides an isolated humanized monoclonal antibody that neutralizes a flavivirus.

[0011] The present ~~invention~~ disclosure also provides antibodies with a modified Fc region such that the Fc region does not bind to the Fcγ receptor. The modified Fc region contains mutations at amino acid positions 234 and 235. In one ~~aspect~~embodiment, the mutations are L234A and L235A. In one embodiment, the modified Fc region comprises a CH2 region wherein the amino acids at positions 4 and 5 of the CH2 region are mutated. For example, the leucine amino acids at positions 4 and 5 are mutated to a different amino acid, preferably, an alanine. In other embodiments, the modified Fc region comprises an Fc region where the amino acids at positions 108 and 109 are mutated. For example, the leucines at positions 108 and 109 of the Fc region are mutated to a different amino acid, preferably, an alanine. In another embodiment, the modified Fc region comprises the amino acid sequence of SEQ ID NO: 7. The modified Fc region binds to the neonatal Fc receptor (FcRn).

[0012] ~~In one aspect,~~ According to the present disclosure, the antibody does not contribute to an antibody-dependent enhancement of a flavivirus infection.

[0013] ~~In one aspect,~~ According to the present disclosure, the antibody is linked to a therapeutic agent. The therapeutic agent is a toxin, a radiolabel, a siRNA, a small molecule, or a cytokine. For example, the cytokine is TGF-beta.

[0014] The present ~~invention~~ disclosure further provides a cell producing a huFV antibody. The cell may be a mammalian cell (*i.e.*, a mouse, rabbit, goat, or sheep), or a plant cell (*i.e.* tobacco plant).

[0014a] In one aspect, the present disclosure provides a cell producing an antibody disclosed herein.

[0014b] In another aspect, the present disclosure provides a use of an antibody disclosed herein in the manufacture of a medicament for preventing antibody-dependent enhancement of a flavivirus infection in a subject.

[0014c] In a further aspect, the present disclosure provides a use of an antibody disclosed herein and a vaccine in the manufacture of a medicament for increasing vaccine efficiency in a subject.

[0014d] In another aspect, the present disclosure provides a use of an antibody disclosed herein in the manufacture of a medicament for treating or alleviating a symptom of a flavivirus infection in a subject.

[0014e] In a further aspect, the present disclosure provides a use of an antibody disclosed herein in the manufacture of a medicament for delaying the onset of one or more symptoms of a flavivirus infection in a subject.

[0015] The present ~~invention~~ disclosure provides a method for preventing antibody-dependent enhancement of a flavivirus infection by administering a huFv antibody to a subject. In one ~~aspect~~embodiment, the antibody is administered after a first infection by a flavivirus.

[0016] Additionally, the present disclosure ~~invention~~ provides a method of increasing vaccine efficiency by administering to a subject a huFV antibody and a vaccine. In one ~~aspect~~embodiment, the huFV antibody and the vaccine are administered sequentially or concurrently. In one ~~aspect~~embodiment, the vaccine is a viral vaccine.

[0017] The present disclosure ~~invention~~ further provides a method for treating or alleviating a symptom of a flavivirus infection by administering to a subject in need thereof a composition containing a huFV antibody. ~~In another aspect, t~~The present ~~invention~~ disclosure features a method for delaying the onset or progression of one or more symptoms of a flavivirus infection. Symptoms of flavivirus infection include, but are not limited to, weight loss, paralysis, fever, headaches, nausea, vomiting, skin rash, and body aches. In one ~~aspect~~embodiment, an anti-viral agent is also administered to the subject. In one ~~aspect~~embodiment, the huFV antibody and the

anti-viral agent are administered sequentially or concurrently. The anti-viral agent is an antibody, an antibody linked to a therapeutic agent, or a small molecule.

[0018] The flavivirus is West Nile virus, Dengue virus (serotypes 1-4), St. Louis encephalitis virus, yellow fever virus, Japanese encephalitis virus, and Murray Valley encephalitis virus.

[0019] In one aspect, ~~T~~the ~~invention~~ present disclosure ~~further~~ provides a nucleic acid sequence containing a nucleic acid sequence of SEQ ID NO: 2, 4 and 8.

[0020] The present disclosure ~~invention~~ further provides a nucleic acid sequence encoding a polypeptide of SEQ ID NO: 1 and 3. The ~~invention~~ present disclosure further provides a polypeptide containing the amino acid sequence of SEQ ID NO: 1, 3 and 7.

[0020a] In one aspect, the present disclosure provides a nucleic acid sequence encoding a polypeptide comprising SEQ ID NO: 1, 3 and 7 or 13.

[0020b] In another aspect, the present disclosure provides a polypeptide comprising the amino acid sequence comprising SEQ ID NO: 1, 3 and 7 or 13.

[0021] The present disclosure ~~invention~~ further provides a vector containing the nucleic acid sequence containing SEQ ID NO: 2, 4 and 8, or encoding a polypeptide of SEQ ID NO: 1, 3 and 7.

[0021a] In one aspect, the present disclosure provides a vector comprising a nucleic acid disclosed herein.

[0022] Additionally, the present disclosure ~~invention~~ provides a cell containing a vector containing the nucleic acid sequence containing SEQ ID NO: 2, 4 and 8, or encoding a polypeptide of SEQ ID NO: 1, 3 and 7.

[0022a] In one aspect, the present disclosure provides a cell comprising a vector disclosed herein.

[0023] Other features and advantages of the present disclosure ~~invention~~ will be apparent from and are encompassed by the following detailed description and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0024] **Figure 1** shows a schematic of an expression vector containing wild-type mAb-11.

[0025] **Figure 2** shows a schematic of an expression vector containing engineered mAb-11-LALA.

[0026] **Figure 3** is a series of three graphs showing the mAb11(LALA) protection against lethal Dengue 2 virus infection. (A) Kaplan Meier survival curve compares the survival of mice administered with mAb11 with wild-type (wt) or mutant (mut) Fc regions. (B) Percent of original weight was monitored in mice administered with mAb11 with wild-type (wt) or mutant (mut) Fc regions. (C) Clinical scores were determined based on appearance, mobility and general attitude of the mice administered with mAb11 with wild-type (wt) or mutant (mut) Fc regions.

[0027] **Figure 4** is a series of three graphs comparing the survival rate of mice administered mammalian mAb11 with LALA mutation (mutAb) derived from mammalian cells or plant cells, and then challenged with lethal Dengue 2 virus infection. (A) Mammalian-derived mutant mAb11 antibody compared to plant-derived mutant mAb11

8. The antibody of claim 6, wherein the Fc region comprises the amino acid sequence of SEQ ID NO: 7 or SEQ ID NO: 13.

9. The antibody of any of the preceding claims, wherein the modified Fc region binds to the neonatal Fc receptor.

10. The antibody of any of the preceding claims, wherein the flavivirus is West Nile virus, Dengue virus (serotypes 1-4), St. Louis encephalitis virus, yellow fever virus, Japanese encephalitis virus, and Murray Valley encephalitis virus.~~\~~

11. The antibody of any of the preceding claims, wherein said humanized monoclonal antibody is produced in a plant.

12. The antibody according to any one of the previous claims linked to a therapeutic agent.

13. The antibody of claim 12, wherein said therapeutic agent is a toxin, a radiolabel, a siRNA, a small molecule, or a cytokine.

14. The antibody of claim 13, wherein said cytokine is TGF-beta.

15. A cell producing the antibody of the previous claims.

16. Use of an antibody of any one of claims 1-14 in the manufacture of a medicament for ~~A method of~~ preventing antibody-dependent enhancement of a flavivirus infection ~~comprising administering the antibody of any one of claims 1-14 to~~ in a subject.

17. The ~~method~~ use of claim 16, wherein said antibody is to be administered to said subject after a first infection by a flavivirus.

18. Use of an antibody of any one of claims 1 to 14 and a vaccine in the manufacture of a medicament for ~~A method of~~ increasing vaccine efficiency ~~comprising administering to~~ in a subject ~~an antibody according to any of claims 1-13 and a vaccine~~.

19. The ~~method~~ use of claim 18, wherein said antibody and said vaccine are to be administered to said subject sequentially or concurrently.

20. The ~~method~~ use of claim 18, wherein said vaccine is a viral vaccine.

21. Use of an antibody according to any one of claims 1-14 in the manufacture of a medicament for ~~A method of~~ treating or alleviating a symptom of a flavivirus infection~~, comprising administering to~~ in a subject ~~in need thereof a composition comprising an antibody according to any one of claims 1-14~~.

22. Use of an antibody according to any one of claims 1-14 in the manufacture of a medicament for ~~A method of~~ delaying the onset of one or more symptoms of a flavivirus infection~~, comprising administering to~~ in a subject ~~in need thereof a composition comprising an antibody according to any one of claims 1-14~~.

23. The ~~method~~ use of claim 21 or 22, wherein ~~further comprising administering~~ an anti-viral agent is to be further administered to said subject.

24. The ~~method~~ use of claim 23, wherein the anti-viral agent is an antibody, an antibody linked to a therapeutic agent, or a small molecule.

25. The ~~method~~ use of claim 23, wherein said antibody and the anti-viral agent are to be administered sequentially or concurrently.

26. The ~~method~~ use of any one of claims 21-25, wherein said one or more symptom comprises weight loss, paralysis, fever, headache, nausea, vomiting, skin rash, and body aches.

27. The ~~method~~ use of any one of claims 1-26, wherein said flavivirus is West Nile virus, Dengue virus (serotypes 1-4), St. Louis encephalitis virus, yellow fever virus, Japanese encephalitis virus, or Murray Valley encephalitis virus.

28. A nucleic acid sequence comprising a nucleic acid sequence of SEQ ID NO: 2, 4 and 8.

29. A nucleic acid sequence encoding a polypeptide comprising SEQ ID NO: 1, 3 and 7 or 13.

30. A polypeptide comprising the amino acid sequence comprising SEQ ID NO: 1, 3 and 7 or 13.

31. A vector comprising the nucleic acid claims 28 and 29.

32. A cell comprising the vector of claim 31.

33. The cell of claim 15 or 32, wherein said cell is a plant cell.