certain effector molecules in the body. Effector molecules are so named because they carry out the pathogenic effects of immune complexes. By inhibiting the formation of large, insoluble immune complexes, or by inhibiting the binding of immune complexes to effector molecules, the tissue damaging effects of immune complexes may be prevented.

SUMMARY

This document is based in part on the discovery that polypeptides having amino acid sequences based on those set forth in SEQ ID NO:2 and SEQ ID NO:20 (also referred to herein as NB-406) can bind specifically and with high affinity to the C_H2-C_H3 domain of immunoglobulin molecules, thus inhibiting the formation of insoluble immune complexes containing antibodies and antigens, and preventing the binding of such complexes to effector molecules. This document provides such polypeptides, other C_H2-C_H3 binding compounds, compostions containing the polypeptides and/or compounds, and methods for using the polypeptides and compositions to inhibit immune complex formation and therapeutic use in treating viral infections.

In one aspect, this document features a method for inhibiting immune complex formation in a subject, the method comprising administering to the subject a composition comprising a purified polypeptide, the polypeptide comprising the amino acid sequence $(Xaa_1)_m$-Cys-Ala-Xaa_2-His-Leu-Gly-Glu-Leu-Val-Trp-Cys-Thr-$(Xaa_3)_n$ (SEQ ID NO:53), wherein Xaa_1 is any amino acid, Xaa_2 is Trp, Tyr or Phe, 5-Hydroxytrphophan (5-HTP), 5-hydroxytryptamine (5-HT), or another amino acid derivative, Xaa_3 is any amino acid, and m and n independently are 0, 1, 2, 3, 4, or 5, and wherein the immune complex formation is associated with a viral infection. The immune complex formation can be associated with Antibody Dependent Enchancement (ADE) of Dengue Fever or infection by Dengue Virus (DENV), or contributes to the enhacement of a DENV infection. The peptide can cause clinical or ~~histiological~~ histological improvement of a DENV infection. The peptide can cause an improvement in or delay the onset of one or more of the ~~histiological~~ histological characteristics of a DENV infection. The peptide can decrease the ADE of a DENV infection.

The DENV infection can be Dengue ~~Hemorragic~~ Hemorrhagic Fever (DHF) or Dengue Shock Syndrome (DSS). The peptide can inhibit binding of an anti-DENV heterologous IgG immune complex (IC) to an FcγR. The peptide can inhibit formation of IC that contribute to ~~imunopathogenesis~~ immunopathogenesis of the ADE of DENV infections. The peptide can inhibit binding of DENV ~~virons~~ virions to IgG IC. The

peptide can inhibit binding of a DENV protein (e.g., NS1) to an IgG immune complex. The peptide can inhibit binding of TNF-α to IgG IC. The peptide can inhibit binding of a DENV-IgG IC to FcγI, FcγIIa H131 allele, FcγIIa R131 allele, FcγRIIb, FcγRIIc, FcγRIIIa, or FcyγIIIb. The peptide can inhibit binding of DENV-IgG IC to mC1q or sC1q.

The polypeptide can comprise a terminal stabilizing group. The terminal stabilizing group can be at the amino terminus of the polypeptide and can be a tripeptide having the amino acid sequence Xaa-Pro-Pro, wherein Xaa is any amino acid (e.g., Ala). The terminal stabilizing group can be at the carboxy terminus of the polypeptide and can be a tripeptide having the amino acid sequence Pro-Pro-Xaa, wherein Xaa is any amino acid (e.g., Ala).

The method can further comprise the step of monitoring the subject for one or more clinical, ~~histiopatholigical~~ histopathological or molecular characteristics of hemorrhagic fever. The one or more clinical, ~~histiopathological~~ histopathological, or molecular characteristics of hemorrhagic fever can be selected from the group consisting of a decrease in platelets, hemoconcentration, or an increase in FcγR+ effector cells.

The polypeptide can comprise the amino acid sequence Xaa-Pro-Pro-Asp-Cys-Ala-Trp-His-Leu-Gly-Glu-Leu-Val-Trp-Cys-Thr (SEQ ID NO:19), wherein Xaa is any amino acid. The polypeptide can comprise the amino acid sequence Ala-Pro-Pro-Asp-Cys-Ala-Trp-His-Leu-Gly-Glu-Leu-Val-Trp-Cys-Thr (SEQ ID NO:20). The polypeptide can comprise the amino acid sequence Asp-Cys-Ala-Trp-His-Leu-Gly-Glu-Leu-Val-Trp-Cys-Thr (SEQ ID NO:2).

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 pertains. Although methods and materials similar or equivalent to those described herein can be used to practice the invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and from the claims.

et al. (2007) *Virol.* 369:143-152). To evaluate susceptibility to DENV infection, the humanized mice were challenged with DEN-2 serotype. Viremia lasting up to 3 weeks was detected in infected mice with viral titers reaching up to $10^{6.3}$ RNA copies/ml, and fever characteristic of dengue also was noted. In addition, an antibody capture ELISA to evaluate the presence of human anti-dengue antibodies showed anti-dengue IgM by 2 weeks post-infection, followed by IgG at 6 weeks. Sera from some of the infected mice also were found to be capable of dengue virus neutralization.

Macrophages are important in dengue pathogenesis, but the initiation of DENV infections appears to begin before the involvement of macrophages. In particular, dendritic cells (DC) have been shown to by 10-fold more permissive for DENV infection than monocytes or macrophages. Mast cells also are present in dermal and subdermal tissues, which would be expected to come into contact with Dengue ~~Virons~~ Virions shortly after a mosquito bite. In addition to DC and blood monocytes and macrophages, mast cells are susceptible to antibody-enhanced dengue virus infection, producing a number of inflammatory mediators (e.g., IL-1, IL-6, and CCL5). The participating FcRs in antibody-enhanced mast cell dengue virus infection appear to be FcγRIIa/b. As shown in Table 3 of Example 2 herein, SEQ ID NO:20 was about 90% effective in blocking the binding of immune complexes to FcγRIIa/b. Thus, polypeptides such as this may have a significant impact on ADE of DENV infection in DC, mast cells, macrophages, and monocytes.

Circulating immune complexes (CIC) also may play a role in the immunopathogenesis of DHF. CIC have been detected in the serum of DHF patients based on the inhibitory activity of CIC on agglutination of IgG-coated latex particles by Clq (Agnello et al. (1970) *Immunol.* 19:909-919; and Agnello et al. (1971) *J. Exp. Med.* 134:228-241). This latex technique provides a simple, semi-quantitative method which can demonstrate the presence of IC in the serum of patients during the early course of DHF (Ruangjirachuporn et al. (1979) *Clin. Exp. Immunol.* 36:46-53). In addition, large the Raji cell immunofluorescent method (Theophilopoulos (1976) *J. Clin. Invest.* 57:169-82) has shown that large size CIC develop in the later stage of DHF. Removal of these complexes by absorption with Raji cells reduced or eliminated the reactivity in the Raji cell immunofluorescence assay, as well as in the latex technique. Thus, these studies confirmed the specificity of the reaction between the dengue antigen-antibody complexes in DHF serum and the surface of the Raji cells. In addition, virus-containing IC have been shown to persist at defervescence for patients with DHF.

As discussed in Example 3 herein, inactivated DENV2 virons (DENV-2 strain 16681) can avidly bind heterologous, non-DENV-associated, peroxidase-anti-peroxidase immune complexes. A likely reason for the avid binding, based on structural analysis (Zhang et al. (2004) *Structure* 12:1607-1618) is that DENV envelope protein domain III may be responsible for homologous and heterologous IgG immune complex binding. The second weakly IC binding protein is non-structural protein 1 (NS1), which is released in high amounts to the serum of DENV infected individuals and also is found within the DENV ~~viron~~ virion itself and on the surface of DENV infected cells. The results of DENV NS1 binding to non-DENV related immune complexes (PAP-IC), and the inhibition of NS1 binding to IC by NB406 (SEQ ID NO:20; APPDCAWHLGELVWCT) are shown in Example 6 below. In addition, Table 3 in Example 2 shows that SEQ ID NO:20 inhibited binding of either FcγRIIa or FcγRIIb to immune complexed IgG Fc by more than 90%, suggesting that SEQ ID NO:20 may abrogate ADE in DC caused by sub-neutralizing DENV antibody titers.

Methods for using C_H2-C_H3 binding polypeptides to inhibit Fc-mediated immune complex formation

C_H2-C_H3 binding polypeptides can be used in *in vitro* assays of Fc-mediated immune complex (IC) formation. Such methods are useful to, for example, evaluate the ability of a C_H2-C_H3 cleft-binding polypeptide to block Fc-mediated IC formation. *In vitro* methods can involve, for example, contacting an immunoglobulin molecule (e.g., an antigen bound immunoglobulin molecule) with an effector molecule (e.g., mC1q, sC1q, FcRs and FcRn, or another antibody) in the presence and absence of a polypeptide as provided herein, and determining the level of IC formation in each sample. Levels of IC formation can be evaluated by, for example, polyacrylamide gel electrophoresis with Coomassie blue or silver staining, or by co-immunoprecipitation. Such methods are known to those of ordinary skill in the art, and can be used to test the ability of a candidate polypeptide or compound to inhibit IC formation associated with an infectious viral disease, for example.

Methods provided herein also can be used to inhibit IC formation in a subject, and to treat an infectious viral disease in a subject by inhibiting Fc-mediated IC formation. Such methods can include, for example, administering any of the polypeptides described herein, or a composition containing any of the polypeptides described herein, to a subject having or being at risk for having or developing an infectious viral disease (e.g., Dengue

Fever). For example, a method can include administering to an individual a composition containing a polypeptide that includes the amino acid sequence Cys-Ala-Trp-His-Leu-Gly-Glu-Leu-Val-Trp-Cys-Thr (SEQ ID NO:10). Alternatively, a method can include administering to a subject a polypeptide that contains the amino acid sequence Asp-Cys-Ala-Trp-His-Leu-Gly-Glu-Leu-Val-Trp-Cys-Thr (SEQ ID NO:2), Xaa-Pro-Pro-Asp-Cys-Ala-Trp-His-Leu-Gly-Glu-Leu-Val-Trp-Cys-Thr (SEQ ID NO:19; where Xaa is any amino acid), or Ala-Pro-Pro-Asp-Cys-Ala-Trp-His-Leu-Gly-Glu-Leu-Val-Trp-Cys-Thr (SEQ ID NO:20).

In some embodiments, when the viral disease is Dengue Fever, a polypeptide can be administered to inhibit IC formation that is associated with ADE of Dengue Fever or infection by DENV, or contributes to the enhancement of a DENV infection. The polypeptide can, for example, result in clinical or ~~histiological~~ histological improvement of a DENV infection, result in an improvement or delay of onset of one or more of the ~~histiological~~ histological characteristics of a DENV infection, and/or decrease the ADE of a DENV infection (e.g., a DENV infection such as DHF or DSS). In some cases, the polypeptide can inhibit binding of an anti-DENV heterologous IgG IC to an FcγR, inhibit formation of IC that contribute to ~~imunopathogenesis~~ immunopathogenesis of the ADE of DENV infections, inhibit binding of DENV ~~virons~~ virions to IgG IC, inhibit binding of a DENV protein (e.g., NS1) to an IgG immune complex, inhibit binding of TNF-α to IgG IC, inhibit binding of a DENV-IgG IC to FcγI, a FcγIIa H131 allele, a FcγIIa R131 allele, FcγRIIb, FcγRIIc, FcγRIIIa, or FcγγIIIb, and/or inhibit binding of DENV-IgG IC to mC1q or sC1q.

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

EXAMPLES

In vitro assays for measuring ligand binding to the C_H2-C_H3 cleft. *In vitro* assays involving enzyme-linked immunosorbent assay (ELISA) have been used to demonstrate (competitive) inhibition of immune complexed IgG Fc binding to immune mediating factors such as FcRs: (FcγRI, FcγIIa, FcγRIIb/c, FcγRIIIa/b), FcRn, mC1q, and sC1q by the polypeptides and compounds described herein. See, e.g., Examples __ herein, as well as U.S. Patent Publication Nos. 20070225231 and 20070276125, and PCT Publication No. WO2007/030475. Standardized reagents and ELISA kits are useful to reduce costs and increase the reproducibility of the experiments.

Table 2

Peptide	SEQ ID	OD_{405}		
		FcγIIa (R131 allele)	FcγIIb	FcγIIIa
DCAWHLGELVWCT	2	0.561	0.532	0.741
APPCARHLGELVWCT	16	0.956	0.768	0.709
DCAFHLGELVWCT	3	0.660	0.510	0.810
APPDCAWHLGELVWCT	**20**	**0.509**	**0.496**	**0.670**
APPCAFHLGELVWCT	18	0.605	0.380	0.880
APPCAWHLGELVWCT	14	0.658	0.562	0.530
Positive Control	-	**1.599**	**1.394**	**1.588**

Peptide APPDCAWHLGELVWCT (SEQ ID NO:20) appeared to result in the greatest overall inhibition of FcR binding to PAP, followed by peptide DCAWHLGELVWCT (SEQ ID NO:2).

Experiments with SEQ ID NO:20 were repeated. Costar microtiter plates were coated with 1:10 dilutions of highly purified FcγIIa (His 131 allele aka H161), FcγIIb and FcγIIIb and incubated for 24 hours. The plates were washed and then blocked with 10 mg/ml BSA blocking solution for 24 hours. PAP immune complexes were formed as described in Example 2. PAP (100 µl) were pre-incubated with 100 µl of peptide for one hour. PAP/peptide mixtures were added to the FcγR coated plates and incubated for one hour. After washing, plates were incubated with ABTS substrate for 15 minutes and read at 405 nm. Results are shown in Table 3.

Table 3

Peptide	SEQ ID	Fcγ1	FCγIIa*	FCγIIb/c	FCγIIIb (NA2)
APPDCAWHLGELVWCT	20	0.264	0.209	0.266	0.266
Positive control	-	2.333	3.509	2.218	3.060
% inhibition of IC binding	-	89	94	88	91

*Note: (H131 allele)

Thus, SEQ ID NO:20 inhibited binding of all three major classes of Fc receptor (FcγI, FcγIIa/FcγIIb, and FcγIII) to soluble PAP immune complexes.

Example 3 - Inactivated DENV2 ~~virons~~ virions bind non-DENV immune complexes

About 1 ng of DENV2 (strain 16681; Microbix Corp.) was coated onto Costar microtiter plates using ELISA coating buffer (Alpha Diagnostic International) for 24 hours at 4°C. After 24 hours, the plates were washed 5 times with ELISA wash solution

(Quidel Corp.), and blocked with highly purified BSA for 1 hour. After 5 washes, PAP IC were incubated with the DENV-coated microtiter plates for one hour. After an hour, the plates were again washed 5 times with Quidel ELISA wash solution, and then incubated with ABTS (Immunochem Corp.) for twenty minutes. The higher the OD_{405nm}, the higher the binding of DENV non-related immune complex (PAP-IC) to DENV2 (16681) ~~virons~~ virions. Results are shown in Table 4.

Table 4

Sample	**1**	**2**	**3**	**4**
PAP-Immune Complexes (n=4)	3.106	3.086	3.046	3.081
Avg. = 3.080				
(Neg avg. = 0.050)				

Thus, DENV2 (16681) ~~virons~~ virions bound avidly to non-DENV-related immune complexes, making DENV ADE in non DENV, IC+ individuals possible in DENV endemic areas.

Example 4 - Inhibition of DENV-IgG-IC binding to FcγIIa Coated Plates

Highly purified recombinant human FcγIIa (R&D Systems) was immunoadsorbed onto plastic microwells (Costar Microtiter plates) using ELISA coating buffer (Alpha Diagnostic International) for 24 hours at 4°C. After 24 hours, the plates were washed 5 times with ELISA wash solution (Quidel Corp.), blocked with highly purified BSA for 1 hour, and washed 5 times. Human anti-DENV2 IC were formed by combining inactivated DENV-2 ~~virons~~ virions (strain 16681, Microbix Corp.) with neutralizing polyclonal, pooled, affinity-purified IgG from DENV convalescent individuals (Calbiotech Dengue Fever IgG ELISA; Cat. No. DE050G4). The results of 1:250 and 1:500 serial dilutions (ADE was most evident at serum dilutions of 1:250 to 1:500) of the DENV-IgG-IC were tested against inhibition of DENV-IgG-IC binding to huFcγRIIa coated microtiter wells. Results using huFcγRIIa coated wells with either a 1:250 dilution of DENV-IgG-IC with no inhibitor, a 1:250 dilution of DENV-IgG-IC with SEQ ID NO:20, a 1:500 dilution of DENV-IgG-IC with no inhibitor, and a 1:500 dilution of DENV-IgG-IC with SEQ ID NO:20 are shown in Table 5.

Thus, SEQ ID NO:20 showed a marked ability to inhibit the binding of IC comprised PAP-IC to recombinant DENV NS1 coated plates.

Example 6 - Inhibition of PAP-IC Binding to TNFα Coated Plates by SEQ ID NO:20

Highly purified recombinant TNF-α Sigma-Aldrich) was immunoadsorbed onto plastic microwells (Costar Microtiter plates) using ELISA coating buffer (Alpha Diagnostic International) for 24 hours at 4°C. After 24 hours, the plates were washed 5 times with ELISA wash solution (Quidel Corp), blocked with highly purified BSA for 1 hour, and washed 5 times. PAP IC were incubated with either no peptide or with peptide SEQ ID NO:20 for one hour. After an hour, the plates were washed 5 times with Quidel ELISA wash solution and then incubated with ABTS (Immunochem, Corp) for twenty minutes. The higher the OD_{405nm}, the higher the binding of TNF-α non-related immune complex (PAP-IC) to TNF-α was observed. Results are shown in Table 7.

Table 7

Sample	**SEQ ID**	**OD_{405}**
No peptide inhibitor (+ control)	-	3.180
APPDCAWHLGELVWCT	20	0.086

Thus, SEQ ID NO:20 showed a marked ability to inhibit the binding of IC comprised PAP-IC to recombinant TNFα coated plates.

Example 7 - Demonstration of ADE in DENV(2) infected *in vivo* mouse model

The RAG2−/−γc−/− (RAG-hu) mouse model for dengue virus described above provides a unique research tool for addressing many important and timely questions about Dengue Virus infection and immunity (Kuruvilla et. al. (2007) *Virology* 369:143-152. RAG-hu mice are passively immunized with heterologous DENV IgG (i.e., neutralizing IgG for DENV1; the sera could be from any DENV heterogous neutralizing DENV infection, such as DENV1 IgG and DENV2 infective ~~virons~~ virions or DENV2 IgG and DENV1 infective ~~virons~~ virions). The mice are passively immunized with anti-DENV1 IgG, for example, and then inoculated with a lethal dose of, e.g., DENV 2 virus. A peptide having the sequence APPDCAWHLGELVWCT (SEQ ID NO:20) or XPPDCAWHLGELVWCT (SEQ ID NO:19) is administered i.p. at a daily dose of, e.g., 1 mg/kg, 10 mg/kg, 100 mg/kg and 1,000 mg/kg from the time of DENV infection. Gross clinical signs, temperature, etc., are monitored daily. Serum ELISA or RT-PCR for DENV NS1 or

WHAT IS CLAIMED IS:

1. A composition comprising a purified polypeptide for use in treating or preventing Dengue Fever or infection by Dengue Virus (DENV) in a subject having or being at risk for having or developing Dengue Fever or infection by DENV, said polypeptide comprising the amino acid sequence Xaa-Pro-Pro-Asp-Cys-Ala-Trp-His-Leu-Gly-Glu-Leu-Val-Trp-Cys-Thr (SEQ ID NO:19), wherein Xaa is any amino acid.

2. The composition of claim 1, for use in causing clinical or ~~histiological~~ histological improvement of a DENV infection in the subject.

3. The composition of claim 1, for use in causing an improvement in or a delay in the onset of one or more of the ~~histiological~~ histological characteristics of a DENV infection in the subject.

4. The composition of claim 1, for use in decreasing the antibody dependent enhancement (ADE) of a DENV infection.

5. The composition of any one of claims 1 to 4, wherein said infection by DENV is Dengue ~~Hemorragic~~ Hemorrhagic Fever (DHF) or Dengue Shock Syndrome (DSS).

6. The composition of claim 1, for use in inhibiting binding of an anti-DENV heterologous IgG immune complex (IC) to an FcγR.

7. The composition of claim 1, for use in inhibiting formation of IC that contribute to ~~imunopathogenesis~~ immunopathogenesis of the ADE of DENV infections.

8. The composition of claim 1, for use in inhibiting binding of DENV ~~virons~~ virions to IgG IC.

9. The composition of claim 1, for use in inhibiting binding of a DENV protein to an IgG immune complex.

10. The composition of claim 9, wherein said DENV protein is NS1.

11. The composition of claim 1, for use in inhibiting binding of TNF-α to IgG IC.

12. The composition of claim 1, for use in inhibiting binding of a DENV-IgG IC to FcγI, FcγIIa H131 allele, ~~FcγIIa R131 allele~~ FcγIIa R131 allele, ~~FcγRIIb~~ FcγRIIb, ~~FcγRIIc~~ FcγRIIc, ~~FcγRIIIa~~ FcγRIIIa, or ~~FcγIIIb~~ FcγIIIb.

13. The composition of claim 1, for use in inhibiting binding of DENV-IgG IC to mC1q or sC1q.

14. The composition of claim 1, wherein said polypeptide comprises the amino acid sequence Ala-Pro-Pro-Asp-Cys-Ala-Trp-His-Leu-Gly-Glu-Leu-Val-Trp-Cys-Thr (SEQ ID NO:20).