Human antibodies stop dengue virus by jamming its mechanics

Shee-Mei Lok¹

Program in Emerging Infectious Diseases, Duke-National University of Singapore Graduate Medical School, Singapore 169857; and Center for Bioimaging Sciences, Department of Biological Sciences, National University of Singapore, Singapore 117557

Dengue virus (DENV) is a mosquito-borne flavivirus that infects up to 400 million people each year, leading to 100 million cases of dengue fever and 21,000 deaths (1). Thus far, there are no approved therapeutics or vaccine available. Current treatment of the disease is mainly supportive, e.g., bed rest and electrolyte replacement for relief of symptoms. DENV consists of four serotypes; DENV1 to DENV4. Infection with one serotype confers lifelong protection against the homologous serotype. However, the antibodies generated against this virus serotype may cause the development of a more severe disease, dengue hemorrhagic fever, if the host is infected with another serotype (2). This, in part, is due to the binding of weakly neutralizing serotype cross-reactive antibodies to the new DENV serotype. The antibody is unable to neutralize the virus; instead, it

helps to concentrate the virus on the host macrophage cell surface by the binding of antibody to the macrophage Fc receptors, thereby causing enhanced infection. This complicates the development of a vaccine as it would need to stimulate strong neutralizing antibody response against all serotypes. Therefore, for the last few decades, there is a quest in identifying neutralizing epitopes for all serotypes. With the recent availability of technologies to generate dengue virus infectious clones and human monoclonal antibodies (HMAb), the identification of immuno-dominant neutralizing epitopes recognized by humans is made possible. In PNAS, Messer et al. (3) identify a major epitope commonly recognized by most neutralizing human antibodies.

The mature DENV particle consists of multiple copies of three proteins: the enve-

lope (E), the membrane (M), and the capsid proteins (4). The positive sense genomic RNA complexed with capsid proteins forms the inside of the virus particle. It is surrounded by a bilayer lipid membrane. Anchored on the outside of the membrane are the M and E proteins. The M protein is a small protein that is hidden under the E protein. The major protein that stimulates antibody response is the E protein (Fig. 1A). Each E protein monomer has three domains: DI, DII, and DIII (5, 6). DIII is thought to be involved in receptor binding. DII contains the fusion loop, which interacts with the endosomal membrane to facilitate fusion of the virus with the endosomal membrane during virus entry into the cell (7). The hinge that connects DI to DII is very flexible and is used to flex DII in the low pH environment of the endosome, leading to the exposure of its fusion loop (5). Cryo-electron microscopy (cryoEM) reconstruction of the DENV particle showed that there are 180 copies of E proteins on the surface of the virion (Fig. 1A) forming an icosahedral symmetry shell (4, 8). The E proteins exist as dimers; three of these dimers lie parallel to each other forming a raft. There are 30 rafts on the surface of the virus, and they are arranged in a herringbone pattern.

In the past, only mouse monoclonal antibodies were available; therefore, epitope mapping work was done with these antibodies (9). DIII was found to contain the most highly neutralizing epitopes. One site on DIII (the lateral ridge) induces serotype-specific antibody response, whereas another site (the A-strand) induces serotype cross-reactive response. The antibodies stimulated by these sites neutralize virus differently. The DIII lateral ridge antibodies stop virus fusion process (10), whereas those directed to A-strand cause the E proteins to undergo structural rearrangement (11).

Recent technological developments that enable generation of human monoclonal antibodies showed that human sera contain

Fig. 1. (A) Organization of the E proteins on the surface of DENV. DI, DII, and DIII are colored in red, yellow, and blue, respectively. Fusion loop on DII is colored in green. Three E protein dimers lie parallel to each other forming a raft. On the virus surface, the 30 E protein rafts are arranged in a herringbone pattern (4). The E proteins are organized in icosahedral symmetry; the black triangle represents one asymmetric unit. The 5-, 3-fold icosahedral symmetry vertices are indicated. (B) Location of the residues transplanted by Messer et al. (3) (spheres) and the footprints of HMAbs 14c10 (14) and CR4354 (15) (both shown by a cyan circle) on E protein raft. The predicted footprint of a chimpanzee antibody 5H12 on DENV is also shown (gray circle) (16). The fivefold and threefold vertices are indicated.

Author contributions: S.-M.L. wrote the paper.

The author declares no conflict of interest.

See companion article on page 1939.

¹E-mail: sheemei.lok@duke-nus.edu.sg.

mostly weakly neutralizing, cross-reactive antibodies (12, 13). Only a small fraction of the antibodies is serotype specific and highly neutralizing. DIII antibodies, although highly neutralizing, are only present in the human sera at low levels. Preabsorption of DIII antibodies do not significantly reduce neutralizing activity of the sera, suggesting that neutralizing epitopes recognized by humans are on other sites of the E proteins (13). Neutralizing human monoclonal antibodies also predominantly recognize quaternary structure dependent epitopes (13) as they only bind to virus particles and not soluble recombinant E proteins (rE).

The conventional way of mutating rE protein will not be able to identify quaternary structure epitopes. To overcome this, Messer et al. use the DENV infectious clone to mutate the E proteins on virus particle surface for epitope mapping. Using a potent DENV3-specific antibody 5J7 that binds to both virus and rE protein, the first epitope mapping was done by testing for binding of this antibody to a library of rE protein mutants. They also selected and sequenced neutralization escape mutants. With these methods, they identified eight residues that are located in the hinge region. Messer et al. then replaced 25 residues within 12 Å of the center of this epitope in the DENV3 infectious clone with DENV4 residues (DENV3/h4 mutant). These mutations clustered at the DI–DII hinge (Fig. 1B). The chimeric virus grows well in both mosquito and green monkey kidney cells, indicating that the hinge is fully functional. The switch in the DI–DII hinge in DENV3/h4 led to a switch in their neutralization profile using DENV3 and DENV4 human and primate sera compared with WT DENV3 and DENV4. The authors also injected the DENV3/h4 mutant virus into primates that have been previously exposed to DENV4. Viremia was not detected, indicating that the primates were protected. Together, these experiments indicated that the DI–DII hinge contained the major neutralizing epitopes recognized by antibodies from humans and primates.

The result from Messer et al. is consistent with the cryoEM studies of potent human monoclonal antibodies complexed with dengue and West Nile viruses (Fig. 1B). Part of

the epitope recognized by these antibodies is localized on the DI–DII hinge. HMAb 14c10 is a DENV1-specific highly neutralizing antibody (14). It recognizes an epitope across two E proteins, which is only available on the

The study by Messer et al. makes a breakthrough in the identification of the approximate position of the common neutralizing epitope on DENV recognized by human antibodies.

virus particle and not in the rE protein. The footprint of the Fab fragment is on DI, the DI–DII hinge of one E protein, and DIII of a neighboring E protein. HMAb CR4354 is a highly neutralizing West Nile virus-specific antibody (15); although it binds to non-overlapping residues as 14c10, the footprint lies in a similar region. Another potent antibody from chimpanzee (5H12) complexed with dengue rE protein was solved by X-ray crystallography (16). The structure showed that the epitope is localized at DI and does not extend to the hinge region. Because the complex was formed by incubating

antibody with the rE protein, it is possible that it only showed part of the footprint, whereas the actual footprint on the virus particle may be bigger.

The study by Messer et al. makes a breakthrough in the identification of the approximate position of the common neutralizing epitope on DENV recognized by human antibodies. This will contribute significantly to vaccine design. It also shed light on the possible common neutralization mechanism of DENV by human antibodies. The E protein of DENV had been shown to undergo major structural changes when exposed to physiological temperature in humans (17, 18). Also, when virus is endocytosed into the cell, to facilitate fusion of the virus to the endosomal membrane, the E proteins have to expose the fusion loop and undergo reorganization from dimeric to trimeric and then to the postfusion structures (19). The DI–DII hinge had been shown to be highly flexible and may play an important role in facilitating the structural change in these steps of the virus infection (5). The preference of human neutralizing antibodies to bind to this site suggests that the antibodies may lock the DENV particles, thus preventing important structural changes essential for entry and/or release of the viral RNA into cells.

1 Bhatt S, et al. (2013) The global distribution and burden of dengue. *Nature* 496(7446):504–507.

2 Halstead SB, O'Rourke EJ (1977) Dengue viruses and mononuclear phagocytes. I. Infection enhancement by non-neutralizing antibody. *J Exp Med* 146(1):201–217.

3 Messer WB, et al. (2014) Dengue virus envelope protein domain II hinge determines long-lived serotype specific dengue immunity. *Proc Natl Acad Sci USA* 111:1939–1944.

4 Kuhn RJ, et al. (2002) Structure of dengue virus: Implications for flavivirus organization, maturation, and fusion. *Cell* 108(5):717–725.

5 Zhang Y, et al. (2004) Conformational changes of the flavivirus E glycoprotein. *Structure* 12(9):1607–1618.

6 Modis Y, Ogata S, Clements D, Harrison SC (2003) A ligand-binding pocket in the dengue virus envelope glycoprotein. *Proc Natl Acad Sci USA* 100(12):6986–6991.

7 Rey FA, Heinz FX, Mandl C, Kunz C, Harrison SC (1995) The envelope glycoprotein from tick-borne encephalitis virus at 2 Å resolution. *Nature* 375(6529):291–298.

8 Kostyuchenko VA, Zhang Q, Tan JL, Ng TS, Lok SM (2013) Immature and mature dengue serotype 1 virus structures provide insight into the maturation process. *J Virol* 87(13):7700–7707.

9 Sukupolvi-Petty S, et al. (2007) Type- and subcomplex-specific neutralizing antibodies against domain III of dengue virus type 2 envelope protein recognize adjacent epitopes. *J Virol* 81(23):12816–12826.

10 Kaufmann B, et al. (2006) West Nile virus in complex with the Fab fragment of a neutralizing monoclonal antibody. *Proc Natl Acad Sci USA* 103(33):12400–12404.

11 Lok SM, et al. (2008) Binding of a neutralizing antibody to dengue virus alters the arrangement of surface glycoproteins. *Nat Struct Mol Biol* 15(3):312–317.

12 Beltramello M, et al. (2010) The human immune response to Dengue virus is dominated by highly cross-reactive antibodies endowed with neutralizing and enhancing activity. *Cell Host Microbe* 8(3):271–283.

13 de Alwis R, et al. (2012) Identification of human neutralizing antibodies that bind to complex epitopes on dengue virions. *Proc Natl Acad Sci USA* 109(19):7439–7444.

14 Teoh EP, et al. (2012) The structural basis for serotype-specific neutralization of dengue virus by a human antibody. *Sci Transl Med* 4(139):139ra83.

15 Kaufmann B, et al. (2010) Neutralization of West Nile virus by cross-linking of its surface proteins with Fab fragments of the human monoclonal antibody CR4354. *Proc Natl Acad Sci USA* 107(44):18950–18955.

16 Cockburn JJ, et al. (2012) Structural insights into the neutralization mechanism of a higher primate antibody against dengue virus. *EMBO J* 31(3):767–779.

17 Zhang X, et al. (2013) Dengue structure differs at the temperatures of its human and mosquito hosts. *Proc Natl Acad Sci USA* 110(17):6795–6799.

18 Fibriansah G, et al. (2013) Structural changes in dengue virus when exposed to a temperature of 37°C. *J Virol* 87(13):7585–7592.

19 Modis Y, Ogata S, Clements D, Harrison SC (2004) Structure of the dengue virus envelope protein after membrane fusion. *Nature* 427(6972):313–319.