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Review

The development of therapeutic antibodies against dengue virus

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ABSTRACT

Dengue virus, a positive-sense RNA virus, is one of the major human pathogens transmitted by mosquitoes. However, no fully effective licensed dengue vaccines or therapeutics are currently available. Several potent neutralizing antibodies against DENV have been isolated from mice and humans, and the characterization of their properties by biochemical and biophysical methods have revealed important insights for development of therapeutic antibodies. In this review, we summarize recently reported antibody–antigen complex structures, their likely neutralization mechanisms and enhancement propensities, as well as their prophylactic and therapeutic capabilities in mouse models. This article forms part of a symposium on flavivirus drug discovery in the journal *Antiviral Research*.

Contents

1. Introduction

Dengue virus (DENV), a member of the family *Flaviviridae*, is transmitted to humans by the bite of an infected *Aedes aegypti* or

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A. albopictus mosquito. There are up to 390 million cases of DENV infection detected in the world annually, leading to approximately 100 million cases of dengue fever and 21,000 deaths (Bhatt et al., 2013; Thomas and Endy, 2011). Weakly neutralizing or non-neutralizing antibodies were shown to be able to enhance infection of dengue virus to Fcγ-positive cells such as monocytes via a mechanism known as antibody-dependent enhancement (ADE, see section 6.1) (Halstead, 2003). Therefore, a safe and effective vaccine should induce strong neutralizing antibodies against all four DENV (DENV1-4) serotypes. A potential tetravalent dengue vaccine developed by Sanofi-Pasteur has completed phase III clinical trials (Capeding et al., 2014; Guy et al., 2015; Sabchareon et al., 2012; Villar et al., 2015) and is awaiting approval to be used in humans. Its efficacy against DENV1, DENV3 and DENV4 is moderate: approximately 50–55%, 65–78%, and 72–80% respectively, while that to DENV2 is poor (approximately 35–50%) (Capeding et al., 2014; Villar et al., 2015). It was shown to be able to reduce hospitalization for vaccinees above 9 years old. However, the vaccine is not recommended for children younger than 9 years old, as it can increase the severity of disease when the vaccinee is later infected with DENV.

Structural studies, using either X-ray crystallography or cryo-electron microscopy (cryo-EM) of potent antibody-virus complexes can identify the virus epitopes that will elicit a protective response and thus be useful for vaccine design. When combined with biochemical assays, the mechanism of neutralization of these antibodies can be further evaluated, helping in the selection of those that could potentially be used as therapeutic agents. In addition, structural information can aid the design of modified antibodies to improve their neutralization capabilities against all dengue serotypes (Robinson et al., 2015).

2. Role of E proteins in the virus infection cycle

The flavivirus envelope (E) glycoprotein is the major target for neutralizing antibodies (Feighny et al., 1994; Roehrig, 2003). Cryo-EM structures of the mature DENV showed that the E protein is anchored in the virus lipid bilayer membrane and displayed on the outside of the particle (Fibriansah et al., 2015b; Kostyuchenko et al., 2014; Kostyuchenko et al., 2013; Kuhn et al., 2002; Zhang et al., 2013a). Crystal structures of the E protein ectodomain showed that it consists of three domains: DI, DII and DIII (Modis et al., 2003; Rey et al., 1995). Each of these E protein domains plays important roles in attachment and fusion of virus during its entry into the cell.

The DENV entry process starts with the attachment of E protein to host cell receptors (reviewed in (Perera-Lecoin et al., 2014). The virus attachment step is likely to be a process that involves multiple molecules, which work in combination or in sequence. Thus far, a few receptors that can promote DENV infection have been identified. A few ancillary receptors that concentrate DENV particles on the cell surface but do not stimulate endocytosis have been identified. For example, DENV particles make initial contact with heparan-sulfate on host cell surfaces (Chen et al., 1997; Germi et al., 2002; Hilgard and Stockert, 2000; Lee et al., 2006). The negative charges of the sulfated groups interact with positively charged residues on DIII of the E protein (Watterson et al., 2012). These sulfated polysaccharides bind and concentrate DENV particles on the cell surface thereby increasing the chances of the virus to bind to other receptors (Putnak et al., 1997). Other ancillary receptors that use a different mode of binding (sugar moieties on E proteins) have been identified and they are the C-type lectin molecules (Robinson et al., 2006), such as DC-SIGN in dendritic cells (Lozach et al., 2005; Navarro-Sanchez et al., 2003; Tassaneetrithep et al., 2003), L-SIGN in liver or lymph node cells (Dejnirattisai et al., 2011; Tassaneetrithep et al., 2003), and the mannose receptor in macrophages (Miller et al., 2008). DC-SIGN receptor binds simultaneously to glycosylation sites at residue N67 of two neighboring E proteins (Mondotte et al., 2007; Pokidysheva et al., 2006) on the mature virus particles and it was shown that the distance between these two sites determines binding capability. It was shown to be able to bind to the immature DENV, however, the E protein arrangement on the immature virus is very different from the mature virus, it is thus unknown how DC-SIGN could interact with the immature virus (Richter et al., 2014).

The lipid envelope of DENV contains phosphatidylserine (PS). Receptors that bind PS can significantly enhance virus infection. (Jemielity et al., 2013; Meertens et al., 2012). There are two types of PS receptor: receptors that are able to bind PS directly and those that require adapter molecules to engage with PS. Direct PS receptors include the T-cell immunoglobulin and mucin receptor (TIM) proteins and the CD300a and CD300f family proteins. TIM proteins also facilitate the entry of other enveloped viruses (Jemielity et al., 2013; Kondratowicz et al., 2011; Meertens et al., 2012; Moller-Tank et al., 2013; Morizono and Chen, 2014). Beside PS, phosphatidylethanolamine, which is also present in DENV lipid envelope, can bind the TIM (Richard et al., 2015) and CD300a (Carnec et al., 2015) receptors and assist in the virus entry process. The tyrosine protein kinase receptor 3 (TYRO3)-AXL-MER (TAM) family is an example of the indirect PS receptors that require GAS6 and protein S (PROS) to engage with PS receptors (Lemke and Rothlin, 2008; Meertens et al., 2012).

Following the virus attachment to receptors, the virus is endocytosed into the cell. DENV uses the clathrin-mediated endocytosis as the main route for internalization (Chu and Ng, 2004; Krishnan et al., 2007; van der Schaar et al., 2008). Fusion of virus with the endosomal membrane is facilitated by the exposure of E proteins to the low pH environment of the endosome. The high flexibility of DI-DII hinge allows E protein to flip up thereby exposing its fusion loop (Klein et al., 2013; Modis et al., 2004). Once the dimer comes apart to allow DII to flip up, the rearrangement from a dimer to a trimer configuration has begun (Modis et al., 2004), leading to the fusion between the virus and the host endosomal membranes. Antibodies that can interfere and disrupt any of these steps may thus prevent the entry of the virus into the cell.

3. The quaternary structures of DENV

Cryo-electron microscopy structures of mature DENV1-4 show that the E proteins are tightly packed on the virus surface forming a smooth surface morphology (Fig. 1A) (Fibriansah et al., 2015b; Kostyuchenko et al., 2014; Kostyuchenko et al., 2013; Kuhn et al., 2002; Zhang et al., 2013a). The viruses used in these structural studies were grown in mosquito cells at 28 °C, purified and kept at 4 °C before freezing on cryo-EM grids. Interestingly, when particles of the DENV2 New Guinea-C strain, and some others strains, were incubated at 37 °C their smooth surface morphology changed to a "bumpy" surface (Fibriansah et al., 2013; Zhang et al., 2013b). It is important to study these different virus morphologies, as they may elicit distinctly different types of antibodies, which may not be cross-protective against the different morphologies within a serotype.

3.1. The structure of DENV at 28 °C

At a temperature of 28 °C, the E protein dimers lie flat on the DENV surface (Fig. 1A). In total, there are 180 copies each of the E and M proteins arranged in icosahedral symmetry, with each asymmetric unit consisting of three pairs of E and M proteins. The three individual E proteins from one asymmetric unit are named molecules (mols) A, B and C (Fig. 1A). Three E protein dimers are