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Comprehensive mapping of functional epitopes on dengue virus glycoprotein E DIII for binding to broadly neutralizing antibodies 4E11 and 4E5A by phage display

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ABSTRACT

Here we investigated the binding of Dengue virus envelope glycoprotein domain III (DIII) by two broadly neutralizing antibodies (bNAbs), 4E11 and 4E5A. There are four serotypes of Dengue virus (DENV-1 to -4), whose DIII sequences vary by up to 49%. We used combinatorial alanine scanning mutagenesis, a phage display approach, to map functional epitopes (those residues that contribute most significantly to the energetics of antibody–antigen interaction) on these four serotypes. Our results showed that 4E11, which binds strongly to DENV-1, -2, and -3, and moderately to DENV-4, recognized a common conserved core functional epitope involving DIII residues K310, L/I387, L389, and W391. There were also unique recognition features for each serotype, suggesting that 4E11 has flexible recognition requirements. Similar scanning studies for the related bNAb 4E5A, which binds more tightly to DENV-4, identified broader functional epitopes on DENV-1. These results provide useful information for immunogen and therapeutic antibody design.

Introduction

The specific recognition of an antigen by an antibody is the hallmark of humoral immunity; such interactions are essential for the function of numerous antibody or antibody-like molecules for use as therapeutic, diagnostic, or research reagents (French et al., 1989; Milstein and Rada, 1995; Neuberger, 2002). Significant effort has been expended toward understanding the physicochemical basis of high affinity antibody-antigen interactions (Da Silva et al., 2010; Fellouse et al., 2007; Fellouse et al., 2005; Fellouse et al., 2004; Persson et al., 2013), how these interactions develop through the process of affinity maturation in naturally-occurring antibodies, (Li et al., 2003; Wedemayer et al., 1997; Yin et al., 2003; Yin et al., 2001) and how the information from these studies can be exploited for functional design by combinatorial or computational protein engineering (Fellouse et al., 2004; Persson et al., 2013; Tharakaraman et al., 2013).

An added layer of complexity is that some antibody combining sites, natural or engineered, have "multispecific" properties in that they are able to bind in high affinity to multiple antigens with little or no non-specific activity against irrelevant targets (Bostrom et al., 2011, 2009; Cockburn et al., 2012; Scheid et al., 2011; West et al., 2012; Wu et al., 2011). For engineered bispecific antibodies, this expanded recognition appears to require separate "functional paratopes" (i.e., those residues on the antibody combining site that contribute most to the energy of the binding reaction) for each of the binding targets (Bostrom et al., 2009, 2011). Prime examples of multispecific natural antibodies are broadly neutralizing virus antibodies (bNAbs), which are frequently derived from immunization or by mining antibody repertoires of vaccinees or survivors and can bind to glycoproteins with diverse primary sequences from different viral species or strains (Bostrom et al., 2011, 2009; Cockburn et al., 2012; Scheid et al., 2011; Wu et al., 2011). In general, bNAbs target conserved and sometimes compact epitopes on viral glycoproteins (Ekiert et al., 2009; Wu et al., 2011), but, in some cases, larger structural paratopes (antigen contacting residues as determined from X-ray structures) engage structural epitopes that include positions that contain high antigenic diversity among viral strains or species (West et al., 2012). In several viral systems including Dengue, HIV-1, and influenza, a wealth of structural information on bNAb-glycoprotein complexes provides some insight into the requisite features of the structural epitopes.

Abbreviations: DENV, Dengue virus; DHF, Dengue hemorrhagic fever; DSS, Dengue shock syndrome; ADE, antibody dependent enhancement of infection; bNAb, broadly neutralizing antibody; DIII, domain III; DI, domain I; DII, domain II; mAb, monoclonal antibody; IgH, immunoglobulin heavy chain; IgL, immunoglobulin light chain; HEK, human embryonic kidney cells; PEI, polyethylenimine; PFU, plaque forming units; PEG, polyethylene glycol; PBS, phosphate buffered saline; PBS–T, phosphate buffered saline with tween 20; BSA, bovine serum albumin; HRP, horse radish peroxidase; TMB, 3,3′5,5′-Tetramethylbenzidine; WT, wildtype; Ala, alanine; RT, room temperature; ELISA, enzyme-linked immunosorbent assay

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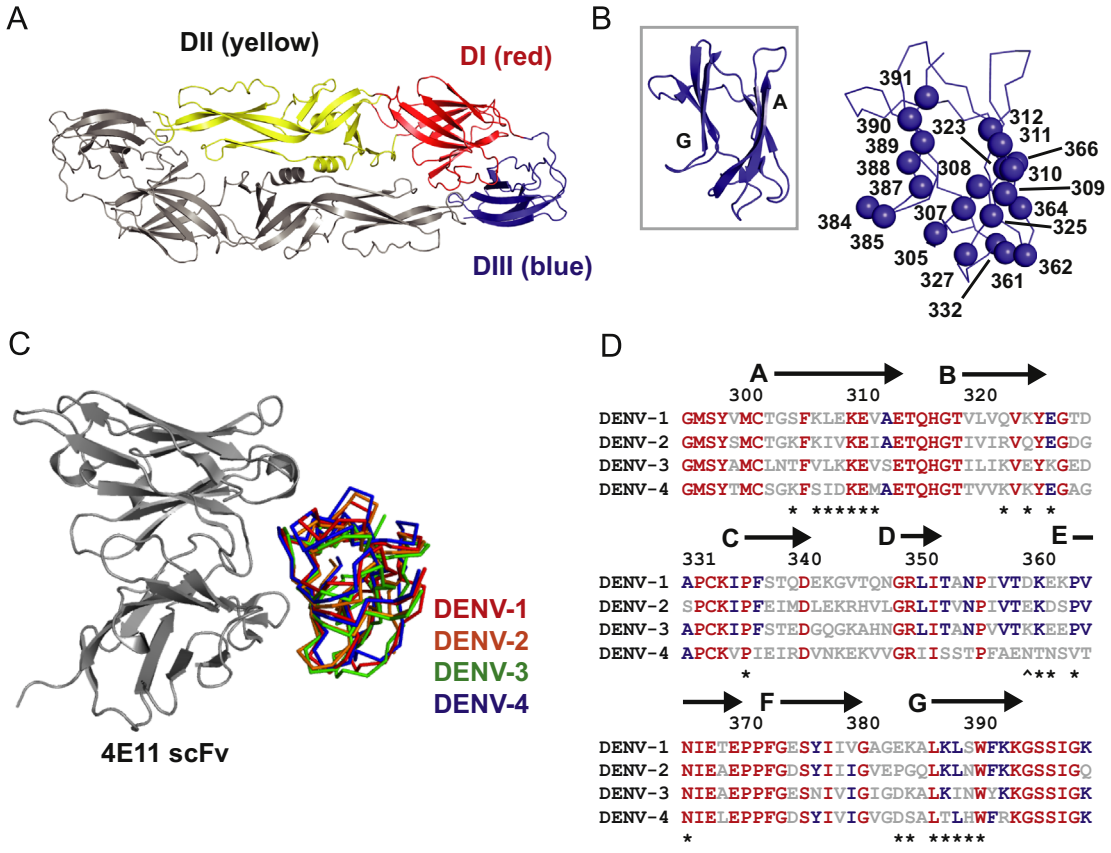

Fig. 1. Structure of DENV DIII and library design. (A) Prefusion structure of homodimeric E, with the three domains on one of the subunits colored (DENV-2 shown as an example, from PDB code 1OKE, Modis et al., 2003). (B) View of the DENV-2 DIII as an example down the 4E11 structural epitope. The A- and G-strands are labeled in the inset, and the locations of the residues included in the combinatorial alanine scanning mutagenesis analysis are shown on the larger graphic, which has the same orientation as the inset. Position 360 (not shown) was included in the analysis for DENV-2 only. (C) Overlay of the four DENV DIII domains bound to the 4E11 scFv (PDB codes 3UZQ, 3UZV, 3UZE, and 3UYP, Cockburn et al., 2012). (D) Amino acid alignment of the four DENV DIII domains. Residues that are absolutely conserved are colored red, those that are conserved in three of the serotypes are colored blue. The location of the β-strands is indicated with arrows; the residues subjected to combinatorial scanning mutagenesis are indicated with asterisks except for position 360 (^), which was analyzed in DENV-2 only.

However, less effort has been devoted toward understanding the functional features (i.e., epitope residues that contribute most significantly to the energy of the bNAb–glycoprotein interaction) by mutagenesis studies (Bedouelle et al., 2006; Lisova et al., 2007, 2014).

Here, we obtained comprehensive insight into functional epitope requirements for recognition of Dengue virus (DENV) envelope glycoprotein E domain III (DIII) by two related broadly neutralizing antibodies, 4E11 and 4E5A, as a model system (Fig. 1) (Bedouelle et al., 2006; Cockburn et al., 2012; Lisova et al., 2007, 2014; Tharakaraman et al., 2013). There are four co-circulating serotypes of Dengue virus (DENV-1, -2, -3, and -4), a mosquito-spread flavivirus that causes significant disease in endemic subtropical regions (Dengue Shock Syndrome, DSS, and Dengue Hemorrhagic Fever, DHF) (Gubler et al., 2007; Halstead and O'rourke, 1977; Sangkawibha et al., 1984). A particular challenge for DENV vaccine and immunotherapeutic development is antibody-dependent enhancement of infection (ADE), a phenomenon whereby serotype-specific antibodies resulting from a primary infection worsen the severity of secondary infections caused by other serotypes. In the current model for ADE, primary infection results in a febrile illness and subsequent lifelong immunity against the infecting serotype. However, antibodies from the primary infection are weakly cross-reactive and non-neutralizing against the other serotypes and lead to increased uptake of DENV by Fcγ-receptor positive cells and thus increased viremia and pathology upon secondary infection (Halstead and O'rourke, 1977; Sangkawibha et al., 1984). There is significant interest in identification and characterization of potent and broadly neutralizing antibodies, which would inhibit the infectious cycle of all serotypes and prevent ADE.

A number of murine-derived DENV neutralizing antibodies, both serotype-specific as well as bNAbs, target DIII. However, potent bNAbs against other regions of the glycoprotein, such as the glycoprotein prefusion dimer interface, arise during the course of natural human infection (Austin et al., 2012; Beltramello et al., 2010; Brien et al., 2010; Dejnirattisai et al., 2015; Edeling et al., 2014; Gentry et al., 1982; Gromowski et al., 2010; Henchal et al., 1985; Kaufman et al., 1987; Li et al., 2012; Lok et al., 2008; Megret et al., 1992; Roehrig et al., 1998; Rouvinski et al., 2015; Shrestha et al., 2010; Sukupolvi-Petty et al., 2007; Wahala and de Silva, 2011; Williams et al., 2012). Glycoprotein E mediates entry into host cells and is located on the surface of the virus in an array of 90 homodimers parallel to the virus membrane (Fig. 1A) (Kuhn et al., 2002). The E ectodomain is composed of three domains: domain I (DI) functions as a molecular hinge that links domains II and III to one another, domain II (DII) is the dimerization domain and contains the hydrophobic fusion loop necessary for virus fusion with host cell endosomal membranes, and domain III (DIII) has been implicated in receptor binding (Crill and Roehrig, 2001; Kuhn et al., 2002; Rey et al., 1995). DIII connects to a stem region and the transmembrane (TM) domain. After adsorption to host cells, DENV is taken up via receptor-mediated endocytosis and infects through pH-dependent virus membrane fusion in the late endosome (Harrison, 2008; van der Schaar et al., 2008; Zaitseva et al., 2010). Monoclonal antibody (mAb) 4E11 is a murine antibody that was isolated from a