Epitope Mapping of Dengue Viral Envelope

Introduction

Considered as a neglected disease by the World Health Organization, dengue fever is caused by a mosquito-borne flavivirus endemic to sub-tropical and tropical parts of the world, including many parts of Central and South America, South and Southeast Asia, and the western Pacific (World Health Organization). An estimated 2.5 billion people worldwide are at risk of dengue infection, and of the 100 million reported cases of dengue fever per year, up to 500,000 develop life threatening dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS) (Leong et al., 2007). DHF and DSS, with symptoms that include hemorrhagic manifestations, thrombocytopenia, and haemoconcentration, have become the leading cause of death and hospitalization among children in several countries (World Health Organization). While effective and safe vaccines currently exist for other flaviviruses including yellow fever virus, Japanese encephalitis virus, and tick-borne encephalitis virus, there is no vaccine or effective antiviral treatment for the dengue virus (World Health Organization). With a significantly increased incidence of DHF/DSS over the past 50 years, an efficacious vaccine is needed now more than ever before (Kyle and Harris, 2008; Gubler, 2006).

The development of a dengue vaccine is complicated by the need to simultaneously protect against four serotypes of the virus and the potential of a suboptimal vaccine (one that does not neutralize all 4 serotypes) to exacerbate the disease. Previous studies suggest that individuals who experience a second infection by a different viral serotype suffer a significantly increased risk of severe disease, in the form of DHF and DSS (Vaugh et al., 2000). Despite these complications and our incomplete understanding of the immunology involved in a dengue infection, knowledge of other flaviviruses and the existence of naturally acquired protective immunity make the development of a dengue vaccine feasible.

Background

While much of the pathogenesis and immunology involved in a dengue infection remain unknown, previous evidences suggest that antibodies play a significant role in the clearance of the virus and the protection of the host. The passive transfer of antibodies, either monoclonal or polyclonal, against viral envelope protein protects mice against lethal challenges of several flavivirus (Pierson and Diamond, 2008). Additionally, mice lacking B cells are more susceptible to flavivirus infections (Chambers et al., 2008). Some mechanisms of antibody mediated protection include direct neutralization of receptor binding, inhibition of viral fusion, Fc-γ-receptor dependent viral clearance, complement-mediated lysis of virus or infected cells, and antibody-dependent cytotoxicity of infected cells.

Virions of the genus flavivirus generally consists of a caspid, premembrane/membrane and envelope structural proteins, viral RNA, and a lipid envelope derived from endoplasmic reticulum. Of particular immunological interest are the envelope structural proteins, which have
been shown to be the primary target for neutralizing anti-flavivirus antibodies (Roehrig, 2003). These envelope proteins are very dynamic and are believed to play a role in viral assembly, viral fusion, receptor attachment, and entry. From X-ray crystallography, the envelope proteins are thought to be composed of three domains: E-DI, E-DII, and E-DIII (reviewed in Pierson et al., 2008). E-DIII is suggested to play a critical role in receptor interactions; E-DII contains a highly conserved 13 amino acid fusion loop; and E-DI connects E-DIII and E-DII.

While epitopes recognized by neutralizing antibodies have been mapped to all three domains, many of the more potent type specific antibodies map to E-DIII (Gromowski and Barrett, 2007). Using a combination of physical and biological methods, Gromowski and Barrett (2007) mapped the epitopes recognized by seven neutralizing murine monoclonal antibodies (mAbs) to E-DIII of dengue type 2. More specifically, they showed that the seven antibodies had overlapping epitopes on E-DIII and that two different amino acid residues were critical for the binding affinity of all seven antibodies. The two residues were highly conserved among dengue type 2 strains and cluster together on the upper lateral face of E-DIII, a region thought to play a role in attachment to cell receptor. Using the same methods, Matsui et al. (2008) found similar results with the E-DIII of dengue type 3, with five different amino acid residues critical for the binding affinity of seven neutralizing antibodies. Surprisingly, even though the structural folds of E-DIII of dengue type 2 and 3 are identical, the critical residues for binding are different, giving rise to the type specificity.

In contrast to the two previous studies, cross-reactive antibodies are generally shown to map to E-DII (Lai et al., 2008). Lai et al. (2008) showed that over 90% of anti-Envelope human polyclonal antibodies in most cases after primary infection were cross-reactive and non-neutralizing, and single point mutations at two different sites on the fusion loop of E-DII abrogated cross-reactivity. The critical role of the E-DII fusion loop in the cross-reactivity of murine monoclonal antibodies has also been demonstrated in the West Nile virus, which belongs to the same genus as dengue (Oliphant et al., 2005).

While previous studies greatly advance our understanding of the immunology of a dengue infection, they are limited in that the monoclonal antibodies used were derived from mouse and not humans. Compared to mouse antibodies, antibodies developed in people infected with dengue or immunized with dengue antigens may recognize different epitopes. In hopes of addressing these limitations, the de Silva lab has identified many unique human monoclonal antibodies that neutralize dengue infection (unpublished data). Two of these antibodies, DVC 3.7 and DVC 23.13, are of particular interest. DVC 3.7 is strongly neutralizing against infection by and only by dengue type 2, while DVC 23.13 is broadly neutralizing against all four serotypes (albeit at lower neutralizing capabilities). As expected, DVC 3.7 binds E-DIII specifically, while DVC 23.13 binds E-DII. Determining exactly where on E-DIII and E-DII DVC 3.7 and DVC 23.13 bind, respectively, will advance our understanding of dengue immunology and aid in future vaccine development.

Hypothesis

We propose that the dengue type 3 specific DVC 3.7 antibody is likely to bind the upper lateral face of E-DIII. Likewise, the broadly neutralizing DVC 23.13 antibody is likely to bind in the highly conserved 13 amino acids fusion loop of E-DII.
Specific Aim

To determine the binding sites of the two given antibodies through the creation, sequencing, and analysis of escape mutants.

Research Design and Methods

The strategies and methods used to create, sequence, and study antibody escape mutants have been well-described elsewhere (Rockx et al., 2008; Meulen et al., 2006). In brief, escape variants may be generated by incubating serial dilutions of virus with a constant concentration of the monoclonal antibody (mAb) for a determined amount of time. Next, the virus-antibody mixture is incubated with human cells. The supernatant of cells incubated will the highest virus dilution showing cytopathic effect is harvested. These virus samples are then diluted serially further and incubated with wells containing human cells, in the presence of mAb. Wells are then overlaid with agarose containing the same mAb. Escape mutants can then be picked from the agarose and amplified. The escape mutant’s and wild type’s DNA can be sequenced by isolating the viral RNA and converting it cDNA. When compared to wild type DNA, any differences in the escape mutant’s DNA may account for its ability to evade neutralization by the mAbs. Generally, escape mutants of the two antibodies will exhibit point mutations in the gene encoding the envelope protein. These mutations alter the binding affinity of the antibodies to the envelope protein. Through sequencing the escape mutants and comparing them to the wild type, we can determine the residues that are critical for antibody binding (and hence the binding sites).

The altered binding affinity of the antibody to the escape variant’s envelope protein may be determined through ELISA based techniques. First, recombinant envelope protein of the escape variants can be made from the determined sequence and then purified. ELISA can then be used to determine the relative binding affinity of the antibody to wild type’s and escape variant’s envelope proteins. Alternatively, ELISA can be performed on whole viruses instead of just the recombinant proteins. By comparing these binding affinities, we can determine the effects of the point mutations on antibody binding to the mutant’s envelope proteins. If multiple mutations to a single envelope protein are determined through the sequencing process, the effects of each mutation on antibody binding may be determined through site directed mutagenesis of the recombinant protein (or entire virus). Particularly, through using more ELISA, one can look at whether or not a recombinant protein (or entire virus) with one of the mutated sites converted back to wild-type will recover antibody binding. If antibody binding is recovered, then that mutation is important for envelope protein-antibody interactions. If antibody binding is not recovered, then that mutation is not important for envelope protein-antibody interactions.


Lai, C., et al. (2008). Antibodies to envelope glycoprotein of dengue virus during the natural course of infection are predominantly cross-reactive and recognize epitopes containing highly conserved residues at the fusion loop of domain II. *Journal of Virology* 82, 13: 6631-6643.


