

Abstract

An effective vaccine for dengue is currently a priority need, worldwide. However, the development of anti-dengue vaccines is challenged by the observed high variation in immune response against infection by different dengue virus (DENV) serotypes or their genetic variants. These immune responses are non-neutralizing and at the same time rather disease enhancing. This study proposes an alternative strategy to the commonly used tetravalent-type vaccine design strategy; to focus on conserved epitopes. Therefore this project aims to identify a conserved DENV protein epitope that elicit broadly cross-reactive and neutralizing antibodies during natural dengue infections.

B-cell epitopes on the Envelope (E) and Pre-membrane (prM) proteins, are the ones exposed on the virion surface, of DENV. The epitopes were predicted using three bioinformatic tools: BepiPred, Ellipro and SVMTriP. Protein sequences of fifty strains from each serotype when analyzed, yielded thirty two (32) and seventeen (17) epitopes respectively for E and prM proteins. Each epitope was bioinformatically analyzed for their level of conservation across and within each serotype. The predicted epitopes were then evaluated for their natural immunogenicity. Using short peptides representing those epitopes, the antibody responses against those epitopes in immunesera from people having well-defined past infections with one of the four serotypes were measured by ELISA assays. Of the predicted epitopes having more than 50% conservancy across the serotypes, six (6) E peptides and two (2) prM peptides gave positive antibody responses to sera of all the four serotypes, showing broadly cross-reactive antibody responses.

Mice immunesera generated against those broadly immunogenic peptides were assessed for their ability to neutralize DENV. Immunesera against five (5) E peptides and one (1) prM peptide neutralized the virus. Some of these neutralizing peptides represent protein regions that are important in host cell attachment, such as the fusion loop and the bc loop of the DII domain of the E protein. The functional significance of these broadly immunogenic neutralizing epitopes strengthens their potential as epitope based universal vaccine targets.

In addition to the primary objective of this study, antibody responses of the epitopes which are weakly conserved across the four DENV serotypes but highly conserved within a serotype were also evaluated. Several such peptides showed antibody responses that are specific to the same serotype, indicating their usefulness as diagnostic markers for identifying the serotype of a dengue infection. This study further characterized the antigenicity of the Capsid (C) protein as assessed by different bioinformatics tools; Parker hydrophilicity prediction, Emini surface accessibility prediction and Karplus & Schulz flexibility predictions, and ELISA using immune sera and an array of overlapping DENV2 C peptides. Three C protein peptides with broader cross-reactive antibody responses were identified. They are located in the amino and carboxy terminal regions of C protein that were initially predicted as antigenic during the bioinformatic analysis. The results offer new insight into the human antibody response to an internal DENV structural protein.

Keywords: dengue, E protein, prM protein, C protein, bioinformatics, natural infections, microneutralization