Understanding the Role of Host Genetic Factors and Specific Dengue Virus Epitopes During Dengue Infections

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Abstract

This thesis outlines studies to identify host genetic factors that influence severe disease in dengue and important dengue virus T cell epitopes based on circulating dengue strains. Chapter two of this study evaluates genetic association between various host genetic polymorphisms and dengue disease severity in Sri Lankan patients. Specific polymorphisms studied are located on the gene for transporters associated with antigen presentation (TAP), the promoter of tumor necrosis factor α (TNF-α), and the promoter of Interleukin 10 (IL-10). Previously they are reported to be associated with dengue fever elsewhere but not in Sri Lanka. The polymorphisms were typed by amplification refractory mutation system PCR in 107 dengue haemorrhagic fever patients and 62 healthy controls. Results implied that neither TAP nor IL-10 individual polymorphisms could significantly affect dengue disease outcome with regard to severity. However IL-10 genotype combination, IL-10 (-592/-819/-1082) CCA/ATA was significantly associated with development of severe dengue, suggesting a risk factor. Genotype combination IL-10 (-592/-819/-1082) ATA/ATG suggested a possibility for protection from severe dengue and the TNF-α (-308) GG genotype was significantly associated with severe dengue, suggesting another significant risk factor. Results are specific for the Sri Lankan population.

A full genome sequencing of DENV-1 viral isolates collected from the years 1983 to 2014 was done and phylogeographic methods were used to characterise a particularly virulent DENV-1 strain circulating in Sri Lanka. Our results confirm that the virus isolated from 1983 is DENV-1 and belongs to genotype III. We can confirm that by 1997 a new DENV-1 strain had entered Sri Lanka, belonging to genotype IV, and continued to circulate until 2006. We also confirm that a DENV-1, genotype I caused the bulk of serious disease in Colombo and out lying areas from 2012 to 2014 and the same strain caused the 2009 epidemic. Our analysis suggests that genotype I spread directly or indirectly from Thailand, to China and then to Sri Lanka around 2007 and subsequently spread to Pakistan and Singapore. Analysis of the DENV-1 sequences isolated, found 82 sites of non-synonymous amino acid mutations, post 2007. As a result of this change in the currently circulating DENV-1 genotype, we found sequence changes in 13 validated MHC class I T cell epitopes and our results suggest the new genotype I is capable of producing a change in the host immune response from that of previous DENV-1 genotypes. This host response that the new virus is capable of producing could explain the clinical outcome of the dengue infection. We also demonstrate the possibility of using a T cell ELISPOT assay to determine the past serotype(s) of dengue infection in dengue patients.

The phenotype of $CD8^+$ T cells during DENV infections is not fully understood and we confirm through flow-cytometric analysis that DENV specific $CD8^+$ T cells produce IFNγ, TNFα and perforin and show that these cells express the surface marker PD-1 in response to DENV infections. This study shows that host genetic background, host immune response and viral factors can play a role in influencing dengue pathogenesis.