

THE ROLE OF CROSS-REACTING ANTIBODY RESPONSES AND CYTOKINE GENE POLYMORPHISMS IN DENGUE VIRUS INFECTION

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ABSTRACT

In Malaysia, where dengue has been endemic, major outbreaks involving heterogenotypic and homogenotypic viruses occur in cyclical pattern. All four dengue virus types have caused major outbreaks in the last three decades. Two major outbreaks involving dengue virus type-2 (DENV-2) had occurred in Klang Valley, Malaysia; the first outbreak was in 1989-1991 and the second outbreak was in 1998-2000. The recurrence of DENV-2 homogenotypic outbreak suggests the possibility of viral evolution that may contribute to the immunity escape, and lack of cross-protective immunity in host population. Non-neutralizing cross-reactive antibody response may enhance the viral infection via antibody-dependent enhancement (ADE) during secondary infection thus predispose individuals to severe form of dengue. The present study, therefore, aimed to investigate the role of the cross-reacting antibody responses in immune protection and immune enhancement of dengue virus infection. In addition, as an increasing number of studies have implicated the genetic association of cytokine genes to infectious diseases, the correlation between cytokine gene polymorphisms and individuals' predisposition to severe dengue disease were studied.

DENV-2 isolates from the two outbreaks were recovered from archival collections of the University Malaya Medical Centre Diagnostic Virology repository. Viruses from only the first passage in C6/36 mosquito cells were used in the study. Viral genomic RNAs were extracted and the envelope (E) gene was amplified, purified and sequenced. The sequences were aligned and phylogenetic trees were constructed using the neighbor-joining (NJ) method. Mice were inoculated with the DENV-2 virus three times at 2-week intervals by intramuscular inoculation. All mice were bled twice per week up to 40 days after the start of immunisation. Neutralisation capacity of sera of mice inoculated with dengue virus against the respective outbreak virus was examined by focus reduction neutralisation test (FRNT). The ADE assay was also performed to determine the capacity of sera from the two outbreaks to enhance DENV infection. For cytokine gene polymorphism genotyping, two hundred and sixty five patients tested dengue IgM positive from year 2006-2007 were enrolled in this study. Dengue patients were graded as DF and DHF/DSS following the WHO severity grading criteria. The genotyping of both IL-12B and IL-10 promoter polymorphisms were performed using polymerase chain reaction (PCR)-coupled restriction fragment length polymorphism (RFLP) method and genome sequencing.

Phylogenetic analysis from the study revealed the presence of two DENV-2 genotypes; the well established Cosmopolitan genotype and Asian I genotype. Majority (>80%) of the DENV-2 isolates sampled from both outbreaks grouped into the Cosmopolitan genotype, with only few of the isolates belonging to the Asian I genotype. Amino acid substitution

from valine to isoleucine at position 129 of the E protein differentiated viruses of the two outbreaks. These findings highlight the potential influence of specific intragenotypic variations in eliciting varied host immune responses against different DENV subgenotypes. Sera of patients from the first outbreak and sera of mice inoculated with virus from the same outbreak had poorer neutralisation activity against virus of the second outbreak. Conversely, patient sera from the second outbreak showed higher neutralisation titer against virus of the early outbreak. At subneutralising concentrations, sera of mice immunised with second outbreak virus did not significantly enhance infection with viruses from the earlier outbreak. On the other hand, our present study showed a high frequency of heterozygous genotypes in both IL-12B promoter and 3'UTR in the studied cohort of dengue patients. The non-GCC haplotypes in IL-10 promoter region were found dominant in these cohort of patients. The prevalence of the specific gene polymorphisms in the Malaysian population is important for further examination of the potential influence of genetic factors in determining the severity of dengue. In summary, there are potentially a number of factors that could influence the outcome of dengue. In our study, we showed that immunity against DENV-2 may not be completely protective against subsequent DENV-2 infection and specific gene polymorphism could influence the outcome of dengue.