

# DETECTION AND EVALUATION OF PROTECTIVE HUMORAL IMMUNE RESPONSES DURING DENGUE INFECTION

Lee S. K.<sup>1</sup>, Fong M. Y.<sup>2</sup>, Sekaran S. D.<sup>1\*</sup>

<sup>1</sup> Department of Medical Microbiology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia.

<sup>2</sup> Department of Parasitology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia.

(shamala@ummc.edu.my)

## INTRODUCTION

Dengue (DENV) infection is a mosquito-borne viral disease that causes major public health problems worldwide where more than 2.5 billion people are at risk of dengue virus infection each year. Annually, there are approximately 50-100 million cases of dengue fever occurring throughout the whole worldwide. Among these number of cases, 250,000 to 500,000 cases are related to Dengue Hemorrhagic Fever/Dengue Shock Syndrome (DHF/DSS) (Rigau-Perez *et al.*, 1998; Vaughn *et al.*, 2000). Thus far, there is no cure or vaccine that is efficient to treat DENV infection.

Infection of DENV can be asymptomatic or cause diseases ranging from flu-like syndrome to severe disease which can be life-threatening. Besides the DF/DHF cases reported, there are also silent DENV infections being reported especially in DENV endemic countries. Clinic-based studies done by Vaughn *et al.* (2000) and Buchy *et al.* (2005) revealed that primary DENV-2 or DENV-4 infections in children were usually silent. During an outbreak in Santiago de Cuba in 1997, over 95% of primary infections of circulating Southeast Asian genotype DENV-2 in adults were silent (Guzman *et al.*, 2000). In a study on 103 school children in Costa Rica from July 2002 to July 2003, Inturrino-Monge *et al.* (2006) found a substantial number of asymptomatic infections in these children. This will greatly increase the risk of occurrence of DHF/DSS in these children in the future, as the previous DENV infection had gone undetected.

Viral virulence and host genetic background are factors affecting the pathogenicity of dengue infection. Therefore study of host genetic factors is necessary to understand the genetic involvement in providing protection in dengue infections.

## OBJECTIVES

This study is aimed at determining the level of neutralising antibodies in dengue patients compared to dengue asymptomatic individuals. At the same time, we are also looking into the genetic involvements in dengue infections by comparing the gene expression of the patients and also of their household members who are asymptomatic towards dengue infections to determine the genetic factor that constitute protection in the asymptomatic individuals during DENV infections.

## **METHODOLOGY**

Blood samples were collected from three study groups: (1) the DF/DHF group, (2) dengue asymptomatic group, consisting of patients' household members who might have been exposed to DENV but did not develop any clinical symptoms of DENV infection, and (3) uninfected group, which consisted of healthy blood donors.

Peripheral Blood Mononuclear Cells (PBMC) were extracted and cryopreserved for future use in microarray analysis. Serum was collected and stored at -80 °C until use.

Dengue viral RNA was extracted from serum and subjected to Real Time RT-PCR to identify DENV and its serotype (Yong *et al.*, 2007). In-house IgM-captured ELISA was carried out to confirm the occurrence of dengue infection (Lam *et al.*, 1987). Based on the detected IgG antibody titers in acute serum sample and also convalescent serum sample using haemagglutination inhibition (HI) test, primary and secondary DENV infections were distinguished (Clarke and Casals, 1958).

Plaque reduction neutralisation test (PRNT) and focus reduction neutralisation test (FRNT) were performed to determine the level of neutralising antibodies present (Vorndam and Kuno, 1997; Russell *et al.*, 1967; Lambeth *et al.*, 2005).

The stored PBMC samples of 10 chosen samples were shipped to Miltenyi Biotech GmbH in dry ice for gene expression analysis. Genes that were >2.0-fold up- or down-regulated represented putative candidate genes.

## **RESULTS AND DISCUSSION**

In the neutralising antibody tests, increase in serotype specific neutralising antibodies titers were detected in 30 out of 56 samples of the patients' household members. This indicates that these individuals had been infected with DENV, but did not develop any apparent clinical symptoms. The neutralising antibodies titers in asymptomatic individuals were not as high as the patients'.

Ten of the asymptomatic individuals' samples which have shown elevation in the neutralising antibodies titers toward DENV were chosen for further genetic study in microarray analysis. In the analysis, we found that the genes up-regulated were involved in both innate and humoral immune responses. In the gene expression of the asymptomatic individuals, we identified differentially expressed genes in macrophage initiated inflammatory response, T cell signaling pathway, and B cell signaling pathway.

Genes involved in macrophage initiated inflammatory responses that had been expressed were the C-C motif chemokine ligands (CCL26 and CCL5), C-C chemokine receptors (CCR5 and CCR2), genes related to macrophage activation (INHA) and macrophage derived growth factor (MDGF/PPBP). The cytokines or chemokines secreted function as chemo-attractant for other immune cell. Macrophages also act as antigen-presenting cells

in conjunction with major histocompatibility complex (MHC) Class II molecules to recruit T-cell and B-cell responses during DENV infection (Chaturvedi *et al.*, 2006).

We found expression of CCL5, CCR2, CCR5, CD8B, CD96, CX3CR1, IL-16, IL-17A, KLRK1, STAT4, TBLYM, and TNFRSF25 genes in T cells signaling pathways. CCL5, CCR5, STAT4, and IL-17A are involved in T helper cell differentiation and T-cell polarisation. CD8B is required in the development of cytotoxic T-cells, and IL-16 is a chemo-attractant that modulates T-cell activation. T-cell specific T-box transcription factor (TBLYM) plays a role in T-cell signaling while TNFRSF25 plays a role in the removal of self-reactive T cells in the thymus. CD8<sup>+</sup> T lymphocytes have virus-specific cytotoxic activity during DENV infection (Gubler, 1998).

The up-regulated genes involved in B-cell signaling pathway are B-cell activation regulatory genes (CD72, CD79B, and BLK), CCL5, CCR2, ASB2, EBI2, INHA, KLRK1, TNFRSF25, and class II major histocompatibility complex, DQ alpha1 (HLA-DQA1). CD72 negatively regulates signaling through the B-cell receptor and may play a role in regulation of B-cell activation. CD79B is part of the B-cell antigen receptor which might play a role in binding to antigen presented on antigen-presenting cells during DENV infection. EBI2 promotes B-cell localisation in the outer follicle and the interfollicular regions. HLA-DQA1 is MHC class II molecules that is expressed on the antigen presenting cells, such as B lymphocytes, dendritic cells, and macrophages. When B cells are activated upon DENV infection, humoral immunity is mediated by producing different classes of antibodies (Noisakran and Perng, 2007). The nonspecific circulating natural antibodies can provide early host protection by binding to pathogen (Gobet *et al.*, 1988).

There are also three other up-regulated genes that may exhibit some functions in inhibition of vascular leakage. The ABIN-2 gene has been shown to interact with the TIE-2 receptor found on endothelial cells (Hughes *et al.*, 2003). The TIE-2 receptor promotes endothelial survival because of its potent anti-inflammatory effects on endothelial cells and suppresses the expression of leukocyte adhesion molecules and procoagulant tissue factor induced by VEGF and TNF-alpha, and therefore inhibits vascular leakage. ABIN-2 also inhibits cell death by apoptosis of endothelial cells elicited by growth factor deprivation (Tadros *et al.*, 2003).

From the microarray analysis, it is observed that innate immune responses play a major role in the immune response during DENV infection in the asymptomatic individuals, followed by cell-mediated immune response and humoral immune response.