

Editorial

Shift to Th2 cytokine response in dengue haemorrhagic fever

Fully differentiated CD4⁺ helper T (Th)-cells have two major subsets of Th1 and Th-2 cells (now Th17 are also appearing on the scene). Th-1 cells are responsible for cell-mediated inflammatory reactions, delayed type hypersensitivity and tissue injury in infections and autoimmune diseases and secrete interferon-gamma (IFN- γ), interleukin-2 (IL-2) and tumour necrosis factor-beta (TNF- β). Th-2 cells are associated with help for B cell antibody production and secrete IL-4, IL-5, IL-6, IL-10, and IL-13. Cytokine secretion profiles correlate well with the distinctive functions of these cells. IL-10 and IFN- γ mediate cross-regulation of the two types of cells in which TNF- α is an inducer of IL-10, and IL-10 is a downregulator of TNF- α . Infections with a dominant humoral immune response induce a higher expression of Th2-related cytokines and are associated with low levels of IFN- γ and IL-2, whereas those characterized by delayed-type hypersensitivity response show a higher expression of Th1 cytokines, IFN- γ and IL-2, and low levels of IL-4. In a number of viral infections such as human immunodeficiency virus (HIV), herpes simplex and influenza viruses, a Th1 response is linked to recovery from infection while a Th2-type response tends to lead to severe pathology and exacerbation of the disease¹.

Dengue virus (DV) infection produces a mild self-limiting acute febrile illness, dengue fever (DF), and a life threatening severe illness, dengue haemorrhagic fever (DHF). DHF has emerged as the most important arbovirus disease in man in the last two decades. The frequency of dengue epidemics has markedly increased with hyperendemic transmission and expansion to newer geographical areas. DHF has been classified into four grades on the basis of the clinical presentation and laboratory findings; the mildest is grade I and the most severe is grade IV. The pathognomonic features of DHF are capillary leakage, cerebral oedema, increased

haematocrit, thrombocytopenia and altered number and functions of leucocytes. Plasma leakage in various serous cavities of the body including the pleura, pericardium and peritoneal cavities in DHF grades III and IV may result in profound shock²⁻⁴.

Cytokines are crucially involved in resistance to, and exacerbation of infectious diseases, therefore, a comprehensive study was undertaken on patients during the wide spread dengue epidemic at Lucknow in 1996⁵. At that time information on the Th1-type of cytokines in DHF was inadequate and no information on the Th2-type response was available in the literature. The levels of Th1- (TNF- α , IFN- γ , IL-2) and Th2-type (IL-4, IL-6, and IL-10) cytokines in the serum samples of 117 patients with various grades of dengue illness were estimated and correlated with the severity and duration of illness. Serum levels of IFN- γ and IL-2 were the highest in DF while in the most severe cases of DHF (*i.e.*, grade IV) serum levels of IL-4, IL-6 and IL-10 were maximum. Levels of IL-4 and IL-10 were negligible in patients with DF and levels of IFN- γ were lowest in patients with DHF grade IV. The levels of TNF- α were higher in cases of DHF grades II, III, and IV as compared to those of normal healthy controls. The levels to increase first were of IFN- γ and TNF- α while IL-4, IL-6 and IL-10 levels tended to increase during the 4th to 8th day of the illness. The most significant findings of this study was a shift of the predominant Th1-type response observed in 66 per cent of DF patients to the Th2-type response seen in the 71 per cent of DHF grade IV patients (Fig.), thus indicating a role for Th2 in the pathogenesis of DHF⁶. Similar findings were reported in an *in vitro* study on human peripheral blood leucocyte cultures infected with dengue virus⁷. These findings were confirmed nine months later by Green *et al*⁸ and then followed a spurt of studies showing direct correlation of Th-2 cytokine

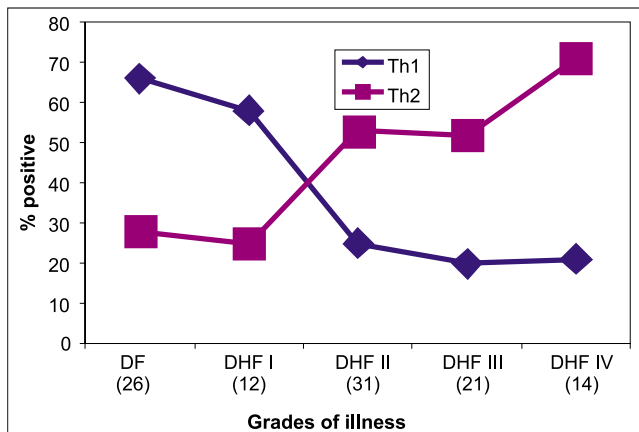


Fig. Analysis of total cases of different grades of illness on the basis of their cytokine profile. The figures in the parenthesis represent total number of the cases in each group (Source: Adapted from Ref. 6 with permission).

response with severe dengue disease⁹⁻¹⁶. Another study showed that a Th-2 immune response occurs in patients with dengue around the time of defervescence¹⁷ which again confirmed our *in vivo* and *in vitro* findings^{6,7}. Interestingly, this data packed study⁶ was rejected by one American and two British Journals delaying the publication by one year resulting in others publishing later claimed to be the first⁸. But within a decade's time this study has become a landmark in the pathogenesis of DHF with around acceptance of results, and has changed the direction of research, opening up of new area for investigation.

A number of studies have been done to understand the mechanism of the shift to Th-2 response in DHF. The presence of high levels of IL-13 and IL-18¹⁸ and transforming growth factor-beta (TGF- β)¹⁹ and early upregulation of IL-13 transcripts in samples from DHF patients²⁰ during severe illness and late phases of the disease suggests that these cytokines may contribute to the shift from a Th1- to Th2-type response and thus to the pathogenesis of DHF. The shift from Th1 to Th2 is regulated by the relative levels of IFN- γ and IL-10 and between IL-12²¹ and TGF- β , which show an inverse relationship in patients with DF²². Macrophages are profoundly affected by the cytokine profile in their immediate environment. Pre-treatment of human monocytes or macrophages with Th2 cytokines (IL-4 or IL-13) enhances their susceptibility to productive DV infection²³. The mechanisms resulting in increased infection in response to IL-4 and IL-13 are not known. Exposure of macrophages to IL-4 or IL-13 elicits an 'alternate type of activation', as opposed to the classical activation induced by IFN- γ ²⁴.

The precise mechanism of DHF is not yet fully known. The important hypotheses put forward regarding the role of host factors are antibody-dependent enhancement (ADE) of DV replication², shift of Th1 to Th2-type cytokine response^{6,22} and other T cell responses resulting into Cytokine Tsunami²³. The viral factors include genotypic mutation, for example, the Southeast Asian type of DV produces DHF in children while the American genotype does not. Non neutralizing anti-DV antibodies lasting from an earlier infection by a heterologous serotype of DV mediate ADE. They form complexes with the infecting DV leading to greater uptake by macrophages and consequent greater number of DV-infected cells². DV infection of THP-1 cells via ADE disrupts the transcription of the iNOS gene transcription factor, IRF-1, and blocks the activation of STAT-1, suppressing nitric oxide radicals. It also suppresses the transcription and translation of IL-12, IFN- γ and TNF- α , while the expression and synthesis of the anti-inflammatory cytokines IL-6 and IL-10 are enhanced. ADE infection modifies innate and adaptive intracellular antiviral mechanisms, besides facilitating the entry process of DV resulting in unrestricted DV replication in THP-1 cells²⁵. Thus, ADE may also be responsible for the shift. Further, DV have been divided into two groups on the basis of their sensitivity to the inhibitory effect of nitric oxide radicals on the virus replication²⁶. The nitric oxide-resistant DV are virulent, have higher replication rate and have stronger influence on host genetic response as compared to the nitric oxide-susceptible DV. Nitric oxide-resistant DV induce greater expression of immune response-related genes, for example, genes involving cytokines/chemokines, activation of T cells, B cells, platelets, and inflammatory cells. Nitric oxide-resistant DV significantly up-regulate IL-6, IL-7, IL-8, RANTES, and MCP-3 that correlate with increased DHF^{26,27}. Is nitric oxide the master switch that triggers the shift from Th1 to Th2 in DHF? To date, why Th2 shift is initially produced and how it acts to enhance the severity of the dengue disease remains unclear. More studies are required to study this phenomenon.

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