

(I) Dengue hemorrhagic fever and hemostasis

There are an estimated 100 million cases of dengue virus infection per year that can be caused by any one of the four serotypes of dengue virus (DEN-1 to 4) (1). An infection may result in a self-limiting febrile infection known as dengue fever (DF) (1-4). However, some infections can lead to dengue hemorrhagic fever (DHF), which is characterized by increased vascular permeability and abnormal hemostasis (5). WHO categorized DHF into 4 grades (1). Plasma leakage and the consequent decreased intravascular volume in DHF grades 3 and 4, can be so profound that shock (undetectable blood pressure) can occur. These grades of DHF, also known as dengue shock syndrome (DSS), can be fatal unless plasma leakage is corrected early, and has a case fatality rate as high as 44% (1, 2). Severity of DHF has been correlated with high viremia titer, secondary infection, and DEN-2 virus serotype (6). **DHF immunopathogenesis: the involvement of cytokines and dengue envelope E glycoprotein.** Viral virulence (10, 11), host genetic factors represented by human leukocyte antigen (HLA) class I alleles (12, 13) as well HLA class II alleles (14), and host immune response (15, 16, 17), have all been implicated in the pathogenesis of DHF. Cytokine production has been shown by many clinical studies to be important in immunopathogenesis of DHF (7). Plasma leakage, the main manifestation of DHF grade 4, has been correlated to malfunction of vascular endothelial cells, believed to be caused by exposure to elevated levels of certain cytokines (2). The cytokines elevated in DHF, but not DF patient sera are TNF- α (17), IL-2 (18)

, IL-4 (17), IL-6 (19), IL-8 (19, 20), IL-10 (21b), and IFN- γ . Some of these cytokines mediate direct and indirect effects on vascular endothelium, giving rise to plasma leakage (2, 7). TNF- α has been shown to induce plasma leakage and shock in animal models (5). IFN- γ enhances TNF- α production by activated monocytes, and interacts with TNF- α to activate endothelial cells *in-vitro* (22). Dengue virus can infect endothelial cells *in-vitro* and induce production of IL-6 and IL-8 (19, 20, 21). Other than effects of cytokines, other studies propose that the dengue virus, specifically the envelope glycoprotein E, play an important role in the establishment of hemorrhagic manifestations of DHF (23, 7). **Impaired hemostasis contribute to DHF manifestations** Hemostatic changes observed in DHF involve mainly three factors: vascular alterations, thrombocytopenia and multiple defects in the coagulation-fibrinolysis system (23). Hemostasis is maintained by a balance between activation of coagulation and fibrinolysis (24). In the coagulation system, thrombin that is formed from prothrombin converts fibrinogen to fibrin, which is later broken down in the fibrinolytic system. Normal endothelium produces TM and tPA, which are inhibitors of blood coagulation and modulators of fibrinolysis (25). Systemic infections cause an imbalance between coagulation and fibrinolysis systems, by producing TF, PAI-1 and vWF. Excessive expression of these factors can disrupt hemostasis, and lead to intravascular thrombosis, bleeding, or both (26). The coagulation and fibrinolytic systems as measured by elevated tPA, were found to be activated in the acute stage of dengue infection (25). PAI-1 level, which is elevated in DHF patients remain high in lethal DHF (27). In these cases, PAI-1 is proposed to prevent the

switch from the 'procoagulant' to 'profibrinolytic' states, giving rise to hemorrhagic manifestations prevalent in DHF patients. Elevation of protein levels of TM, TF and PAI-1 (28) suggests that dengue infection may activate fibrinolysis primarily, which prompted secondary activation of the procoagulant homeostatic mechanisms (28). A very recent study found that IL-6 can regulate dengue virus-induced tPA production of endothelial cells (29).

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(II) Mechanism of dengue immunopathogenesis; specific T-cell restricted epitopes correlate with disease severity.

Viral virulence, host genetic factors represented by the human leukocyte antigen (HLA) genes, and host immune response, have all been implicated in the immunopathogenesis of DHF (1, 2). HLA-A polymorphisms are significantly associated with susceptibility to DHF (3). Various T-cell epitopes have been traced

to multiple dengue proteins. Most of these epitopes are localized on dengue non-structural proteins, which show greater sequence homology between the dengue serotypes. In another study, T cell responses to an HLA-B*07 restricted (221-232) epitope on the dengue NS3 protein, which is an important target of CD8(+)T cells, correlated with disease severity (4). These studies support the hypothesis that activation of dengue-virus-specific CD8+ T cells, in response to dengue-specific peptide-presentation via MHC Class I molecule, play important roles in the pathogenesis of DHF.

Identification of dengue-specific T-cell epitope using “reverse-immunology”;
towards development of peptide-based vaccine. Major histocompatibility complex (MHC) class I ligands have a typical length of 8-12 amino acids. A ligand can be defined as specific “T-cell epitope” where a specific peptide presentation by MHC molecule results in T-cell activation, which then triggers T-cell-mediated immune response. Thus far, several dengue-specific T-cell epitopes have been identified but the approach undertaken has not exhausted the possibility of identifying the crucial T-cell epitopes that could be utilized for the construction of a much-needed, highly effective peptide-based vaccine. The use of “reverse-immunology” has now become a successful strategy for the identification of T cell epitopes (5). Such predictive strategy would identify peptide sequences within a protein that will effectively elicit T-cell responses, which in practice represent only a very small proportion of the total potential peptide sequences that can possibly be derived from a given protein.

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