

# Coagulopathy in Dengue Infection and The Role of Interleukin-6

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## ABSTRACT

*Interleukin-6 (IL-6) is a multifunctional cytokine that plays a central role in host defense due to its wide range of immune and hematopoietic activities and also its potential ability to induce the acute phase response.*

*The high concentration of IL-6 has implication in the pathology of some diseases especially in DHF/DSS patients. This cytokine play a crucial role in the enhanced production of anti-platelet or anti-endothelial cell auto antibodies local or systemic, elevated levels of tPA, as well as a deficiency in coagulation, leading to plasma leakage and bleeding.*

*We describe the pathogenic, characteristic and mechanism of IL-6 during DV infection.*

**Key words:** IL-6, dengue hemorrhagic fever/dengue shock syndrome.

## INTRODUCTION

Dengue is a mosquito-borne viral disease which has a high impact worldwide, with over 2.5 billion people running the risk of dengue virus (DV) infection. Every year, an estimated 100 million cases are reported with 250,000 developing into the severe and potentially fatal form of dengue hemorrhagic fever or dengue shock syndrome (DHF/DSS).<sup>1</sup> In Indonesia, 79,462 cases of dengue fever and approximately 954 died in the year 2005.<sup>2</sup> The almost regular outbreaks of this disease and expected ensuing deaths has prompted many researchers to try to answer questions regarding its method of spread, pathogenesis and pathophysiology.

Several studies have demonstrated that plasma leakage due to increasing vascular permeability and bleeding tendency due to hemostasis deregulation, are the two major pathophysiological changes in Dengue infection.<sup>3,4</sup> There are overproductions of cytokines and

adhesion molecules which are released by endothelial cell during immune response to DV infection. Interleukin-6 (IL-6) is one of several inflammatory mediators that responsible for this condition. Refer to other studies, high level of IL-6 during critical conditions and complications of DHF/DSS.<sup>3, 5-7</sup>

Huang et al, in the study of Dengue virus-infected HUVEC, showed a significant increase in the production of IL-6 and IL-8 in patients with DHF/DSS.<sup>6</sup> In the previous study, increase IL-6 and IL-8 which observed in both DHF/DSS patient's sera and supernatants of DV-infected HUVEC, proved the involvement of these cytokines in the progress of hemorrhage and shock syndrome.<sup>4</sup> Suharti et al, also found increase IL-6 in Dengue shock syndrome (DSS) non survivors.<sup>8</sup>

IL-6 could increase vessel permeability, fluid-phase endocytosis and transcytosis of CNS-derived endothelial cells.<sup>9</sup> Furthermore, IL-6 could intensify leukocyte recruitment and therefore plays a positive role in local inflammatory reactions.<sup>10</sup> There is evidence that local secretion of IL-6 along with IL-8 by DV-infected endothelial cells might enhance the recruitment of leukocytes and activate or damage endothelial cells themselves.

In this review, we describe the role of IL-6 in DV infection whereby IL-6 are released in CD4<sup>+</sup> T lymphocytes and endothelial cells. To obtain a better understanding on the subject of IL-6, we shall discuss the characteristic of IL-6 before discussing pathological activity of IL-6 during DV infection.

## IL-6: FUNCTION RELATIONSHIPS

Interleukin-6 (IL-6) is an extremely varied immunomodulatory cytokine produced by mast cells fibroblasts, endothelial cells, monocytes and both benign and malignant lymphocytes of B- and T cell origin during infection, trauma, and immunological challenge.<sup>10-12</sup> It plays an essential role in host defense due to its wide range of immune and hematopoietic activities and its potential ability to stimulate the acute phase response.<sup>12-15</sup>

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Often, together with the proinflammatory cytokines TNF- $\alpha$  and IL-1, IL-6 is induced in many alarm conditions. The induction by IL-6 of these acute phase reactions has been regarded as part of an effort to maintain homeostasis and also involved in the modulation of other aspect of inflammation, mainly cytokine responses and tissue inflammatory infiltration.<sup>16</sup> In this regard, clinically, serum IL-6 levels appear to correlate with mortality of patients with DSS.<sup>17</sup>

High concentrations of IL-6 has implications in the pathology of several diseases such as multiple myeloma, rheumatoid arthritis.<sup>12</sup> An elevated serum level of IL-6 may also be associated with the generation of anti platelet, anti endothelial autoantibody, coagulation defects leading to bleeding and plasma leakage in DV infection.<sup>18</sup>

In fact, IL-6 promotes inflammatory actions through the expansion and activation of T cells, differentiation of B cells and the induction of acute-phase reactants (C-reactive protein) by hepatocytes. Xing, et al found IL-6 down-regulates proinflammatory cytokine expression while simultaneously inducing the expression of IL-1 receptor antagonist and TNF- $\alpha$  receptor.<sup>16</sup> In contrast, IL-6 also performs a protective role during disease and counteracts the manifestation of certain inflammatory responses.<sup>14-16</sup> IL-6 also functions as growth factors together with stem cell factor (SCF), IL-11, IL-3, GM-CSF, IL-4, IL-9 and Erythropoietin induce transformation quiescent stem cell to activated stem cell in erythroid lineage.

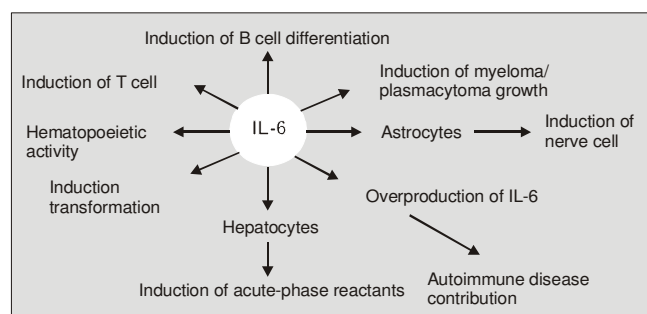


Figure 1. The Biological Activity of IL-6

## PLASMA LEAKAGE IN DENGUE INFECTION

The primary pathophysiologic abnormality seen in DHF/DSS is an acute increase in vascular permeability that leads to plasma leakage into extra vascular compartment. The pathogenesis mechanism that responsible for the increase vascular permeability observed in DHF and DSS is not known, but it has been found that cytokines and chemical mediators such as tumor necrosis factor (TNF), interleukin-1 (IL-1), IL-2, IL-6, platelet-activating factor (PAF), complement activation products C3a and C5a, and histamine may have a role.

Kurane and Ennis have proposed a model of immunopathogenesis based on these observations. Briefly, it is hypothesized that dengue virus infections of monocytes/macrophages is enhanced by Antibodies Dependent Enhancement (ADE). This enhancement is facilitated by dengue virus-specific CD41 T lymphocytes which produce IFN- $\gamma$  and in turn regulates the expression of FC-g receptors. The increase number of dengue virus infected by monocytes/macrophages results in increase T-cell activation, with results in the release of increased levels of cytokines and chemical mediators. Kurane and Ennis hypothesized that the rapid increase of the levels and the synergistic effects of mediators (such as TNF, IL-2, IL-6, IFN- $\gamma$ , PAF, C3a, C5a, and histamine) cause increase vascular permeability, plasma leakage and shock.<sup>19</sup>

The overproduced IL-6 might play a crucial role in capillary leakage. M. Juffrie et, al. found that for patients with elevated IL-6 levels, the incidence of ascites was increased.<sup>20</sup> For patients with the highest secretory phospholipase A2 (sPLA2) levels, the incidence of both pleural effusion and ascites were increased. Both of them are signal of plasma leakage. IL-6, most likely exert pro-inflammatory effects by induction of mediators such as sPLA2.

Phospholipase A2 plays a role in phospholipids digestion. Patients with the highest sPLA2 levels have higher incidence of ascites and pleural effusion and lower plasma protein concentrations compare to patients with lower sPLA2 levels. Although our findings only represent correlation, but it is still consistent with the hypothesis sPLA2 could be involved in the pathogenesis of plasma leakage.<sup>20</sup>

Recently, it has been shown that sPLA2 is involved in the production of pro-inflammatory cytokines and nitric oxide (NO). Production of NO caused up-regulation of p53 and Bax and down-regulation of Bcl-2 and Bcl-xL. the pro- and anti-apoptotic factors that lead to cytochrome *c* release and caspase-3 activation. Activation of caspases is involved in the apoptotic pathway.<sup>21</sup>

Chiou-Feng Lin et al's study demonstrated that Abs against dengue virus nonstructural protein 1 (NS1) generated in mice cross-reacted with human endothelial cells and mouse vessel endothelium. After binding, mouse anti-NS1 Abs induced endothelial cell apoptosis in a caspase-dependent manner. Inducible NO synthase expression could be observed; it showed a time- and dose-dependent correlation with NO production that anti-NS1 induced endothelial cell death via a caspase-dependent pathway.<sup>21</sup>

Furthermore, anti-DV NS1 induces the generation of MCP-1 in endothelial cells. A previous study showed

MCP-1 in DSS patient plasma and pleural fluid, but not in DV-infected endothelial cells. Chiou-Feng Lin et al study showed MCP-1 production by endothelial cells after anti-DV NS1 stimulation; MCP-1 in DHF patient sera was also confirmed. MCP-1-mediated elevation of ICAM-1 expression and facilitation of leukocyte transmigration have been suggested. Results in this study showed that MCP-1 up-regulated ICAM-1 expression in anti-DV NS1-treated HMEC-1 cells. Endothelial cell activation followed by elevated expression of ICAM-1 may contribute to the adherence of immune cells to endothelial cells in the inflammatory responses associated with dengue disease pathogenesis. In addition to the increased expression of cytokines and chemokines, a possible chemotactic effect on PBMC adherence may contribute to the indirect damage in endothelial cells.<sup>22</sup>

### COAGULOPATHY IN DENGUE VIRUS INFECTION

Hemostasis is retained if the dysregulation of coagulation and fibrinolysis persists. The high concentration of IL-6 might play a crucial role in the enhanced production of anti-platelet auto antibodies, elevated levels of tPA, and the deficiency of coagulation factor XII in the intrinsic pathway.<sup>18</sup> The hyperfibrinolysis in the acute stage of DHF/DSS is caused by increased production of tPA. Statistical analysis shows a significant association between high level of IL-6 and tPA in DHF, but not in DF. Dengue virus infection induces endothelial production of tPA as well as IL-6. The de novo synthesis of tPA is blocked by anti-IL-6 antibodies, indicating that tPA production by endothelial cells is IL-6 dependent.

IL-6 can down regulate the synthesis of coagulation factor XII, the first factor to initiate the intrinsic pathway of coagulation. APTT prolongation in DHF patients caused by a lack of intrinsic pathway is probably due to impaired synthesis of coagulation factor

Furthermore, antibodies against dengue virus E protein can bind to human plasminogen. It can either inhibit plasmin activity or enhance plasminogen activation. Therefore, both coagulation and fibrinolysis are hyper activated in the acute stage of dengue virus infection, and are counteracted by increased numbers of platelets and levels of PAI-1 in the convalescent stage. An unbalance between coagulation and fibrinolysis may cause hemorrhage in DHF/DSS.

Dengue virus antigen has been detected in hepatocytes and viral particles were recovered from the liver biopsy specimens of DHF patients. Mouse liver is also a major organ for dengue virus replication. Dengue virus can infect the liver and cause hepatitis. Elevated serum

transaminase levels were found in dengue patients, and the degree of AST level elevation correlates with that of hemorrhage. In dengue- viral hepatitis, the level of AST is higher than ALT with a ratio of around 1–1.5, while other types of virally induced hepatitis have more ALT than AST.

Statistical analysis on the levels of aspartate aminotransferase (AST)/alanine aminotransferase (ALT) and APTT shows a strong association between AST/ALT elevation and APTT prolongation in DHF patients. Dysfunction of the damaged liver might be responsible for the decreased synthesis of specific factors in the intrinsic pathway. Increased factor consumption as indicated by high levels of tPA is also associated with APTT prolongation, but in a less significant manner. Therefore, both decreased synthesis and increased consumption of coagulation factors are involved in the prolongation of APTT. APTT prolongation in DHF patients caused by a deficiency in the intrinsic pathway is probably due to impaired synthesis of coagulation factor XII in the liver.

### THROMBOCYTOPENIA IN DV INFECTION

Thrombocytopenia is common during DV infection. The appearance of IgM antiplatelet antibodies causing the destruction of platelets is a predictor for the development of thrombocytopenia.<sup>18</sup> An increased level of Platelet associated IgM (PAIgM) during acute phase of secondary infection, was associated with the development of DHF. The formation of platelet-associated immunoglobulins may play a critical role in the mechanisms of thrombocytopenia and the accompanying increased vascular permeability.<sup>23</sup>

The wealth of information on dengue fever-with new insights on the role of the immune system-is constantly updated and often changing the landscape, and the future will hopefully see also new directions in the treatment of this infection.

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