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IL-18 and IL-18 binding protein levels in patients with dengue virus infection

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ABSTRACT

Objective: To determine the levels of IL-18 and IL-18 binding protein(BP) in patients with dengue virus infection. **Methods:** Acute and convalescent sera were collected from each patient. Control group was sera from blood donors. The levels of both IL-18 and IL-18BP were measured by ELISA assays. **Results:** It was shown that IL-18 and IL-18BP levels were significantly higher in patients when compared with controls. In addition, the level of IL-18BP was lower in convalescent than in acute sera. **Conclusions:** These data suggest that both IL-18 and IL-18BP production was induced following dengue virus infection. Investigating the regulation of IL-18 by its natural regulator could lead to further understanding of the immune response or immunopathogenesis following dengue virus infection.

1. Introduction

Dengue virus (DENV) is a single-stranded RNA virus belonging to the genus *Flavivirus* and the family *Flaviviridae*. Dengue virus infection is an increasing cause of morbidity and mortality in tropical and subtropical countries. Patients infected with dengue viruses can be asymptomatic or present with two forms of illness, dengue fever (DF) and dengue hemorrhagic fever (DHF).

DF is a self-limited febrile illness. Patients may demonstrate fever, headache, myalgias and thrombocytopenia and recover from the symptoms about a week after disease onset. Some patients infected with dengue virus demonstrate plasma leakage, thrombocytopenia, and hemorrhagic manifestation. This severe syndrome is called DHF[1].

Pathologies observed in DHF can be mediated by the virulence of infecting viruses and by host immune response. It has been shown that plasma leakage is caused by vascular endothelial cell damage from the immune response to dengue virus infection[2]. Tumor necrotic factor-α (TNF-α) has been known to be involved in plasma leakage. Monocytes and endothelial cells infected with dengue viruses produced TNF-α [2, 3]. Monocytes produced TNF-α in the presence of enhancing antibody and the produced TNF-α induced plasma leakage in *in vitro* experiments[4]. Levels of plasma cytokines such as TNF-α, IL-2, IL-6, IL-8, IL-12 and IFN-γ were significantly higher in DHF than in DF[5]. It has been shown that mast cells and basophil infected with dengue virus produced IL-1 and IL-6[6]. Dengue virus-specific T lymphocytes also secreted IFN-γ, IL-2 and TNF-α [2, 7]. These suggested that various cell types are involved in increased cytokine production.

In addition, it has been demonstrated that the levels of IL-6 and IL-1 receptor antagonist were higher in DSS non-survivors than in the survivors. The level of IL-1 receptor antagonist was shown to associate with disease mortality[8]. IL-1β, IFN-γ, IL-4, IL-6, IL-13, IL-7 and granulocyte-macrophage colony stimulating factor (GM-CSF) were significantly increased in patients with severe manifestations compared with patients with mild disease. IL-1β, IL-8, TNF-α and monocyte-chemotactic protein-1 (MCP-1) were associated with marked thrombocytopenia. Bozza *et al* demonstrated that macrophage inflammatory protein-1beta (MIP-1β) was a good prognostic marker whereas IFN-γ was associated with disease severity[9].

The increase of cytokines in dengue virus infection can be the results of the increased cytokine production or down-regulation of cytokine regulator. IL-18 is a member of the IL-1 family. It was originally known as an interferon-gamma inducing cytokine. IFN-γ enhances macrophage, natural killer (NK) cell and cytotoxic T cell activity. For those

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reasons, IL-18 has been studied as a cytokine involved in host defense especially in killing of intracellular organisms and eradication of cancer cells. The predominant cell sources of IL-18 are macrophages and dendritic cells[10]. IL-18 binding protein (BP) is a natural IL-18 antagonist. Binding of IL-18BP to IL-18 prevents IL-18 from binding to IL-18R α on the target cell membrane[11]. Functional homologues of human IL-18BP encoded by viruses such as orthopoxviruses have been shown to inhibit IL-18 activity[12]. There are four isotypes (IL-18BPa, b, c and d) of human IL-18BP generated from alternative mRNA splicing.Only IL-18BPa and IL-18BPc have intact Ig-like domains and can neutralize IL-18. The role of the other two isoforms is still unknown[13].

The level of IFN-γ, a T helper 1 cytokine, has been shown to correlate with severity of dengue virus infection[9]. IL-18 is an IFN-γ inducing cytokine and its level was increased in patients with dengue virus infection. Moreover, the level of IL-18 correlates with disease severity[14]. IL-18 neutralization by IL-18BP regulates IL-18 activity resulting in controlling immune response induced by IFN-γ. Regulation of IL-18 by its natural inhibitor could be another promising tool for the development of disease therapy. In this study, the levels of serum IL-18 and IL-18BP were determined in patients with DF and DHF.

2. Materials and methods

The study protocol was approved by the Ethical Committee, Faculty of Medicine, Chulalongkorn University.

2.1. Specimens

Sera from patients with suspected dengue virus infection were obtained from the Center of Excellence in Clinical Virology, Faculty of Medicine, Chulalongkorn University.

Paired sera positive by IgM ELISA and RT-PCR for dengue virus were included in this study. The samples were divided into DF and DHF groups which were composed of 32 and 48 pairs of sera, respectively.

2.2. Determination of IL-18 and IL-18BP levels by captured ELISA

The levels of IL-18 and IL-18BP were determined by captured ELISA purchased from MBL (Nagoya, Japan) and R&D Systems (MN, USA), respectively. The assays were performed according to the manufacturer's instruction. Briefly, for IL-18 determination, sera and standard solution were added into anti-IL-18-coated wells. IL-18 was then captured by anti-IL-18 conjugated with peroxidase and the presence of IL-18 was demonstrated by adding TMB substrate. The absorbance at 450/620 nm was read by a dual wavelength plate reader. A standard curve was constructed and the concentration of IL-18 in samples was read from the standard curve.

For determining the level of IL-18BP, the microtiter plate was coated with antibody to IL-18BPa, the isoform with widely known IL-18 neutralizing activity. The plate was blocked with 1%BSA in phosphate buffer saline. Sera and standard solution were then added. IL-18BPa was captured by adding biotinylated anti-IL-18BPa. The presence of IL-18BPa was demonstrated by adding streptavidin-horse radish peroxidase followed by TMB substrate. Absorbance at 450/570 nm was read and the standard curve was constructed according to the manufacturer's suggestion. The IL-18BPa level was read from the standard curve.

2.3. Statistical analysis

Paired *t*-test was used to demonstrate the difference between IL-18 or IL-18BP levels in acute and convalescent sera. The difference between the levels in control and patient groups and between DF and DHF group was determined by unpaired *t*-test. *P*-values <0.05 were considered statistically different.

3. Results

The levels of IL-18 and IL-18BP in 20 sera from healthy blood donors were determined by the same reagents and methods used in this study and previously reported[15]. IL-18 and IL-18BP levels in control, DF and DHF groups were shown in Table 1 and Figure 1. Levels of IL-18 and IL-18BP in sera from patients with dengue virus infection, both DF and DHF groups, were statistically higher than the levels in sera from the control group ($P<0.001$). This suggested an increase in IL-18 and IL-18BP levels following dengue virus infection.

Table 1
Summary of IL-18 and IL-18BP levels (mean±SD).

Infection		IL-18 (pg/mL)	IL-18BP (ng/mL)	IL-18/IL-18BP
DF	Acute	695.56 ± 349.76	2.75 ± 1.32	0.31 ± 0.20
	Convalescent	626.94 ± 357.01	2.15 ± 0.94	0.34 ± 0.26
DHF	Acute	625.00 ± 301.75	2.93 ± 1.47	0.28 ± 0.22
	Convalescent	585.16 ± 315.43	2.32 ± 1.20	0.32 ± 0.23
	Control	62.95 ± 36.63	0.50 ± 0.12	–

In both DF and DHF groups, the mean of IL-18 level in convalescent sera was lower than in acute sera. However, the lower levels observed were not statistically different. In contrast, the levels of IL-18BP in convalescent sera were significantly lower than the levels in acute sera ($P<0.001$ for both DF and DHF groups).

The ratios between IL-18/IL-18BP levels were determined. There was no statistically difference between the ratio of IL-18 and IL-18BP levels in acute and convalescent sera. The levels of IL-18, IL-18BP and IL-18/IL-18BP ratio were compared between DF and DHF groups. There was no statistically difference observed.

4. Discussion

IL-18 promotes IFN-γ production and the level of IFN-γ has been shown to associate with disease severity[9].According to our data, both IL-18 and IL-18BP levels were increased in patients with dengue virus infection when compared with controls. This demonstrated that dengue virus infection induces IL-18 and IL-18BP production. IL-18BP is a natural regulator of IL-18 and is induced by IFN-γ. This suggests that IFN-γ provides the negative feedback for IL-18 suppression by inducing IL-18BP production. Although the levels of IL-18BP were increased following infection, the levels of IL-18BP were lower in convalescent sera than in acute sera. The lower level of IL-18BP in convalescent sera was probably due to the loss following IL-18 neutralization. The proposed mechanism of IL-18 and IL-18BP involvement in dengue virus infection might be: Dengue virus infection induces IL-18 secretion resulting in

enhanced IFN-γ production. The increased IFN-γ induces IL-18BP production. IL-18BP negatively regulates Th1 response by neutralizing IL-18 activity. Although IL-18BP production was enhanced when compared with the controls, its amount was still not sufficient to significantly reduce the level of IL-18 in the course of infection. In addition, while IL-18BP was reduced, IL-18 level could probably be induced continuously.

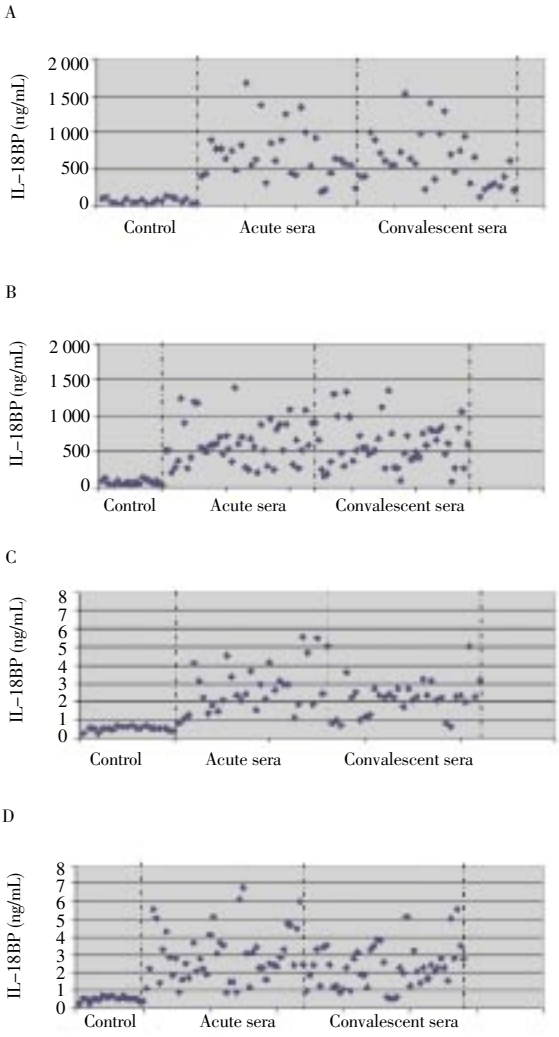

Figure 1. Comparison of IL-18 and IL-18BP levels in control, acute and convalescent sera.
The levels of IL-18 (A and B) and IL-18BP (C and D) in sera from control group and in acute and convalescent sera from patients with dengue virus infection, DF (A and C) and DHF (B and D) groups, were determined by captured ELISA as described in Materials and methods.

The inhibition of IL-18 activity by IL-18BP could be one of the mechanisms that regulate immune response. Imbalance between IL-18 and IL-18BP production could be the underlying cause of immunopathological diseases. In this study, the levels of IL-18 and IL-18BP and the ratio of IL-18/IL-18BP were not statistically different among patients with DF and DHF. It is possible that DHF samples obtained in this study were from grade I and grade II DHF patients.Further investigating in samples from patients with more severe forms of disease could better suggest whether the levels of IL-18 and IL-18BP or their ratio are useful for disease prognosis. However, the results of this study suggested the involvement of IL-18BP, a natural IL-18 regulator, in immune response following dengue virus infection. Understanding the immune response to dengue virus infection will provide further information for safe and effective vaccine development.

Conflict of interest statement

We declear that we have no conflict of interest.

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