CYTOKINE-RELATED GENE EXPRESSION IN PERIPHERAL BLOOD LEUKOCYTES AND DENGUE INFECTION SEVERITY

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Abstract. Dengue virus (DENV) can produce a wide spectrum of clinical manifestations ranging from mild severity to a severe form with plasma leakage and shock. The mechanism underlying the increased disease severity is not fully understood. However, it is thought to be mediated by various host factors, particularly cytokine-related gene expression. This study aimed to elucidate the cellular gene responses to dengue infection at the transcriptional level and to correlate expression levels with disease activity. Patients with confirmed dengue infection and controls were enrolled during a calendar year at King Chulalongkorn Memorial Hospital. RNA was extracted from peripheral blood leukocytes and was analyzed on the day of defervescence. The mRNA expression levels of interleukin (IL)-8, IL-1ß and matrix metalloproteinase-9 (MMP-9) were assayed in 30 children with DF, 19 children with DHF and 10 unaffected controls by real time reverse transcription quantitative polymerase chain reaction. A level of $p < 0.05$ was considered to be statistically significant. All expression data were analyzed using the Q-gene software. IL-8 and IL-1ß mRNA levels were not significantly different between children with DF and DHF, but those with DHF had significantly higher levels of MMP-9 mRNA. MMP-9 might have an important role in dengue pathogenesis. To gain further insight into the pathogenesis of dengue disease severity, serial transcription profiling of additional selected genes in peripheral blood mononuclear cells (PBMCs) might serve as a predictor of dengue infection as well as disease activity.

Keywords: cytokine-related gene expression, IL-8, IL-1ß, MMP-9, dengue infection severity

INTRODUCTION

Dengue virus (DENV) is the causative agent of the mosquito-borne viral diseases and has become a serious public health problem worldwide. This virus has four major serotypes (DENV-1, DENV-2, DENV-3 and DENV-4) and can produce a wide spectrum of clinical manifestations ranging from mild acute febrile illness, classical dengue fever (DF), dengue hemorrhagic fever (DHF) to a severe form with plasma leakage and shock (dengue shock syndrome, DSS) (Hemunkorn *et al*, 2007). Current hypotheses have been proposed to explain the pathogenesis of DHF/DSS including immune enhancement, virus virulence, autoimmune responses against dengue non-structural 1 (NS1) protein and host genetic predisposition (Prommalikit *et al*, 2004; Prommalikit and Thisyakorn, 2015). The mechanism underlying the increased disease severity is however not fully understood. It is thought to be mediated by various host factors, particularly cytokine-related gene expression. Previous studies have suggested an involvement of immune response mediators in the severity of dengue infection (Basu and Chaturvedi, 2008). Elevated levels of serum interleukin (IL)-8, IL-1ß and matrix metalloproteinase-9 (MMP-9) are associated with the feature of severe dengue infection (Talavera *et al*, 2004; Luplertlop *et al*, 2006; Bozza *et al*, 2008).

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This study aimed to elucidate the cellular gene responses to dengue infection at the transcriptional level and to correlate expression levels with disease activity.

MATERIALS AND METHODS

Patients with serologically and virologically confirmed dengue infection by an enzyme linked immunosorbent assay (ELISA) and real time polymerase chain reaction (RT-PCR) were enrolled at the Department of Pediatrics, King Chulalongkorn Memorial Hospital during a calendar year. Clinical diagnosis of dengue infection and its severity were based on the 1997 World Health Organization criteria (WHO, 1997). Informed consent was obtained from the parents of the subjects and controls recruited into the study. After informed consent, whole blood was drawn. RNA was extracted from 3 ml of peripheral blood leukocytes using the QIAamp RNA Blood Mini Kit® (Qiagen, Hilden, Germany) and was analyzed on the day of defervescence.

The expression levels of IL-8, IL-1ß and MMP-9 were assayed in 30 children with DF, 19 children with DHF and 10 controls with other febrile illness (OFI) by real time reverse transcription quantitative polymerase chain reaction. Data on patients' characteristics and laboratory results were compared between two groups by using the Mann-Whitney *U* test. A $p < 0.05$ was considered to be statistically significant. All expression data were analyzed using the Q-gene software (http://www.biotechniques.com/softlib/qgene.html).

RESULTS

The mRNA expression levels of IL-8, IL-1ß and MMP-9 were examined in 30 DF patients, 19 DHF patients and 10 controls.

There was no significant difference among the characteristics of children with dengue infection at the time of admission, except for white blood cell count and hematocrit which were significantly higher among children with DHF (Table 1).

Concerning gene expression pattern in dengue infection, mRNA levels analyzed on the day of defervescence (arbitrary units relative to 18SrRNA expression; presented as mean±SEM) showed that IL-8 and IL-1ß mRNA levels were not significantly different between children with DF and DHF, but those with DHF had significantly higher levels of MMP-9 mRNA (Figs 1-3).

DISCUSSION

Dengue virus infection is a systemic and dynamic disease with a wide spectrum of clinical manifestations. The exact pathogenesis of DHF/DSS

Table 1. Characteristics of children with dengue infection at the time of admission.

Variables	DF (*n*=30)	DHF (*n*=19)	*p*-value
Age; median years (range)	11 (3-14)	11 (4-11)	>0.05
Sex (M:F)	14:16	11:8	
Illness day prior to study entry	4.5 days	4.9 days	>0.05
Temperature at study entry (range)	38.9°C (36.4-41.0)	38.5°C (37.0-41.0)	>0.05
The median white blood cell count at study entry (cells/mm^3)	2,445	3,530	<0.05
The median hematocrit at study entry (range)	39.5% (30.9-45.0)	42.5% (35.0-49.8)	<0.01
The median platelet count at study entry (range) (cells/mm^3)	88,500 (38,000-294,000)	76,000 (14,000-199,000)	>0.05

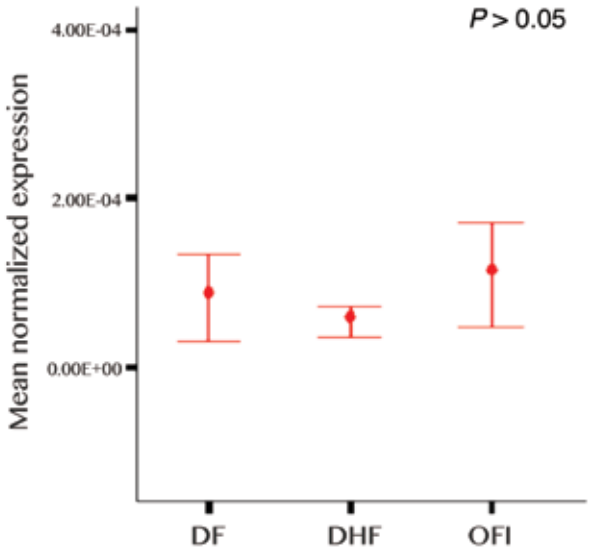

Fig 1–The mRNA expression levels of IL-8 in dengue patients and controls. DF, dengue fever, DHF, dengue hemorrhagic fever, OFI, other febrile illness.

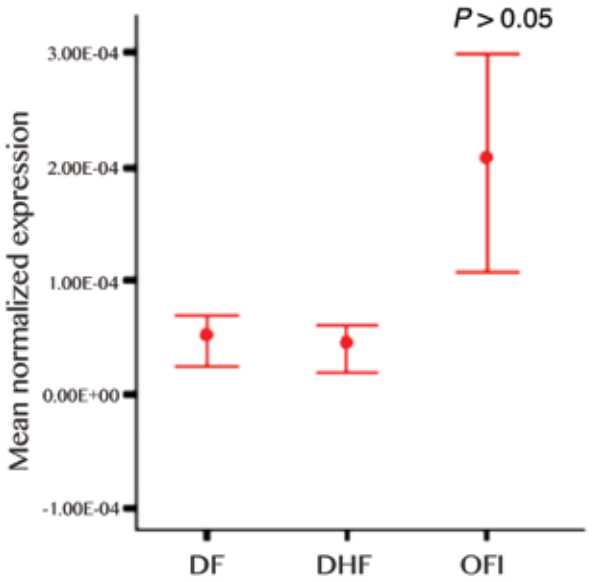

Fig 2–The mRNA expression levels of IL-1ß in dengue patients and controls.

is not well understood. However, the risk factors are ever mentioned for severe dengue including age, the genetic background of the host, dengue virus virulence (viral serotype and genotype), autoimmune responses and antibody-dependent enhancement (Sangkawibha *et al*, 1984; Rico-Hesse *et al*, 1997; Gubler, 1998; Guzman *et al*, 2002). Based on previous evidence, the disease symptoms in dengue may be a consequence of the immune response against the virus (Basu and Chaturvedi, 2008). Elevated serum levels of many different cytokines including IL-8, IL-1ß and

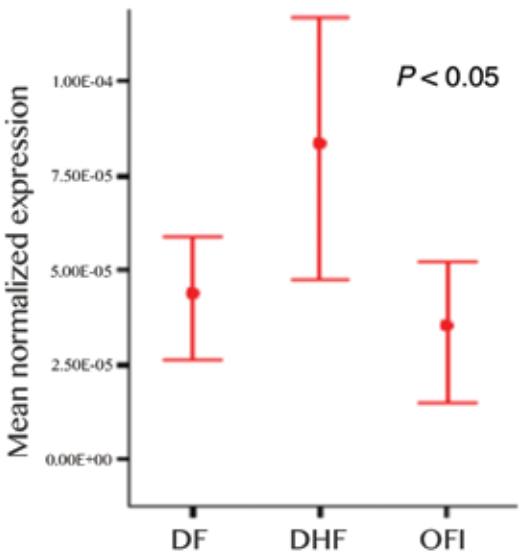

Fig 3- The mRNA expression levels of MMP-9 in dengue patients and controls.

also MMP-9 are associated with severe dengue pathology (Talavera *et al*, 2004; Bozza *et al*, 2008; Luplertlop and Missé, 2008). However, it is not fully understood how these cytokines and MMP-9 cause abnormal pathology in severe dengue virus infection (Bäck and Lundkvist, 2013).

Elevated serum levels of IL-8 are associated with secondary DENV infections, deregulated coagulation and fibrinolysis, and autoimmune responses in dengue virus infection which are correlated with disease severity (Bäck and Lundkvist, 2013). IL-1ß is a potent cytokine that is regulated and can be induced by DENV in macrophages and monocytes (Dinarello, 1997). Elevated serum levels of IL-1ß increase vascular permeability that can lead to leakage of plasma as described in a mouse model of severe dengue pathology (Martin *et al*, 1988; Bozza *et al*, 2008). But in the present study, the mRNA expression levels of IL-8 and IL-1ß were not significantly different between children with DF and those with DHF.

MMP-9 is a matrixin, a class of enzymes that belong to the zinc-metalloproteinases family involved in the degradation of the extracellular matrix. This enzyme is concerned in embryonic development, reproduction, angiogenesis, bone development and wound healing. *In vitro* studies and mouse model experiments have found that MMP-9 could induce vascular leakage in dengue virus infection (Luplertlop *et al*, 2006). MMP-9 may play a role in the mechanism of plasma leakage in DHF. Our study has demonstrated that children with DHF have significantly higher levels of the MMP-9 mRNA expression than children with DF. This mediator might have an important role in dengue pathogenesis. However, Voraphani *et al* (2010) reported that there was no significant difference between serum MMP-9 levels in patients with DHF and those with DF at any stage of the disease.

To gain further insight into the pathogenesis of dengue disease severity, serial transcription profiling of additional selected genes in PBMCs might serve as a predictor of dengue infection as well as disease activity.

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