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Correlation of IFN-Inducible Protein 10 Levels in Sera with Disease Severity and Clinical Outcome of the Dengue Patients

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Abstract: Dengue Virus (DV) infection may cause life-threatening Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS). Although many cytokines involved in the pathogenesis of DHF/DSS have been reported, the expression status of IFN-inducible protein-10 (IP-10) in dengue patients is still unknown. This is the first report to unravel that IP-10 level was significantly elevated in the sera of Dengue Fever (DF) patients. However, the level declined while the disease proceeds to severe DHF/DSS, which is different from the expression profiles of other DV related cytokines in the patients. IP-10 may be used to monitor the severity of dengue disease.

Key words: IP-10, dengue virus

INTRODUCTION

Dengue virus, a positive single strand RNA virus with four serotypes (DV1.to DV4), belongs to the family of Flaviviridae. DV is transmitted to human through the mosquito. DVs are prevalent in tropical and subtropical areas of the world and there are 50-100 million people per year infected by DV. Most of DV infections are asymptomatic. Some causes only mild DF, which shows the symptoms of high fever, headache, rash, bone pain and myalgia. In 5-30% of the patients, the disease proceeds to life-threatening DHF/DSS, and shows the symptoms of thrombocytopenia, plasma leakage, bleeding and shock.

Several hypotheses have been reported to interpret the pathogenesis of dengue virus induced DHF/DSS. Briefly, it includes high virus load, antibody-dependent enhancement (ADE), hypersensitivity, immune enhancement and autoimmunity[1]. Virus load has been considered as the major inducer of many virus infection related pathogenesis. During dengue virus infection, the virus load correlates with disease severity[1]. Moreover, the abnormal immune responses can be induced in response to severe viral infection. The ADE hypothesis states that anti-DV-antibodies can enhance the viral infection and cause DHF/DSS. Taken together, all the reports indicate a close relationship between virus load and severity of the disease.

IP-10 has been studied in various viral infections. It plays a key role in the trafficking and recruitment of effector T cells[2-5]. Furthermore, IP-10 is required for the resistance of the host to the primary DV infection in DV infected mice. IP-10 inhibited DV infection through competition with DV for heparan sulfate on the host cell membrane[6,7]. Above findings both *in vitro* and *in vivo* can not interpret the role of IP-10 in DV infected patients. Based on previous reports, we speculate that IP-10 expression level in DV patients' sera may correlate with the viral load and affect the outcome of patients. In this study, IP-10 levels in DF, DHF and DSS patients as well as in normal controls were measured and their correlation with clinical outcome and disease severity were analyzed.

MATERIALS AND METHODS

Dengue patients and serum samples: Twenty patients were admitted to the Department of Dengue Hemorrhagic Fever at Children's Hospital No. 1-Ho Chi Minh City (Ho Chi Minh City, Vietnam) in year 2002 with a clinical diagnosis of DHF/DSS, sixteen patients were admitted to the National Cheng Kung

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University Hospital, Tainan Taiwan in 2002 with a clinical diagnosis of DF based on the criteria of the World Health Organization (WHO). All of the 36 patients showed high fever, headache, muscle and joint pain, but only DHF/DSS patients were hospitalized for treatment. Additional serum samples of healthy adults (n = 8) without antibodies against dengue virus obtained during routine health examinations were used as the controls. All sera were collected and stored at -80°C.

Cytokine measurement: Sixteen serum samples were collected from DF patients, fifteen serum samples were collected from DHF patients and five serum samples were collected from DSS patients. Eight sera from healthy volunteers were used as the negative controls.

The CXCL10/IP-10 levels in the double-blind serum samples were measured using the commercial ELISA kit (R&D Systems, Minneapolis, MN). Briefly, a monoclonal antibody to CXCL10/IP-10 (concentration: 2.0 μg/mL) was used as a capture antibody in combination with biotinylated goat anti-human IP-10 polyclonal detection antibody (concentration: 50 ng/mL). A standard curve was drawn using a two-fold serial dilution of recombinant human IP-10 between 2 ng/mL and 62.5 pg/mL. IFN-γ, TNF-α, IL-10 and IL-6 levels in sera were also measured using the ELISA kits (R&D Systems) according to the manufacturer's instruction.

Statistical analysis: Data are presented as the mean ± SE. Differences between the test and control groups were analyzed using the Student's t-test. Significance was set at $p<0.05$.

RESULTS

The clinical characteristics and the laboratory analysis of 8 normal controls, 16 DF patients, 15 DHF patients and 5 DSS patients are shown in Table 1. All patients showed high and continuous fever that lasted an average of 3.1 to 4.8 days. In DHF and DSS groups, all patients had petechiae on the skin (100%), which was not observed in DF group. All patients did not have the symptom of gastrointestinal (GI) bleeding (hematemesis or melena). Hepatomegaly was clearly diagnosed in each DHF and DSS patient (100%). Only 1 patient in DF group (6.3%) showed mild hepatomegaly. The severity of hepatomegaly increased while the symptoms of dengue patients proceed from DF to DHF and DSS. The average increase of liver size was from 1 cm to 5 cm below the right costal margin in these groups.

In the laboratory analysis of the patient sera, the thrombocytopenia was diagnosed in 69% DF (11/16), 80% DHF (12/15) and 100% DSS (5/5) patients. The difference among these three groups was not significant. We also detected the levels of aspartate aminotransaminase (AST) and alanine aminotransaminase (ALT), the indicators of liver function. Our data showed that both AST (75% DF, 80% DHF and 100% DSS patients) and ALT (81% DF, 70% DHF and 100% DSS patients) levels were elevated in the sera of all three DV infected groups, however, the difference detected among these groups was not significant.

The cytokine profiles of INF-γ, TNF-α , IL-6, IL-10 and IP-10 in the sera of the above patients were determined. Table 1 shows that IP-10 expression levels in the serum of DF, DHF and

Table 1: Clinical features and laboratory analysis of the patients with DF, DHF or DSS and normal controls

Variable Clinical characteristics	DF patients (n = 16)	DHF patients (n = 15)	DSS patients (n = 5)	Normal control (n = 8)	P
Age, year (range)	50.0±18.4 (10-63)	10.9±3.0 (5-15)	8.4±2.3 (6-11)	26.1±1.7 (24-29)	ND
Duration of fever (range)	3.1±2.8 (0-7)	4.3±1.0 (2-5)	4.8±0.8 (4-6)	ND	ND
Bleeding manifestations					
Petechiae % (no. of patients)	0 (0/16)	100 (15/15)	100 (5/5)	ND	ND
Gastrointestinal bleeding	0	0	0	ND	ND
Liver size, cm (%)	0.1±0.3 (100)	1.8±0.7 (100)	2.8±1.5 (6.3)	ND	0.00^{b}; 0.00^{c}; 0.06^{d}
Laboratory analysis					
IP-10 level, pg/mL	5188.1±2630.2	3075.0±1485.7	2584.1±805.4	874.1±265.0	0.00^{a}; 0.01^{b}; 0.04^{c}; 0.49^{d}
Platelet count ($\times10^4/mm^3$)	7.6±5.3	7.0±4.1	6.0±2.9	ND	0.58^{b}; 0.54^{c}; 0.78^{d}
AST level (U/L)	151.6±110.8	164.6±183.6	149.3±45.7	ND	0.82^{b}; 0.97^{c}; 0.89^{d}
ALT level (U/L)	116.9±136.6	76.9±108.1	56.0±19.3	ND	0.44^{b}; 0.46^{c}; 0.75^{d}
WBC ($\times10^3$)	3.9±1.2	4.3±1.8	5.3±1.1	ND	0.42^{b}; 0.03^{c}; 0.25^{d}

*DF: dengue fever; DHF: dengue hemorrhagic fever; DSS: dengue shock syndrome; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ND, not determined, a: P value of comparison with health group vs. dengue patients group; calculated using the Student's t-test. b: P value of comparison between DF group and. DHF group. c: P value of comparison between DF group and. DSS group. d: P value of comparison between DHF group and. DSS group. P value is calculated by Student's t-test.

Table 2: Cytokine profiles in the patients with DF, DHF or DSS

Cytokine	DF patients (n = 5) (pg/mL)	DHF patients (n = 7) (pg/mL)	DSS patients (n = 4) (pg/mL)	P
IFN-γ	295.7±305.3	44.9±43.7	27.4±26.4	0.054[a]; 0.128[b]; 0.490[c]
TNF-α	0.0±0.0	1.7±2.2	2.5±0.3	0.122[a]; 0.000[b]; 0.501[c]
IL-10	25.0±22.6	85.4±90.2	49.7±36.6	0.179[a]; 0.252[b]; 0.476[c]
IL-6	17.0±31.9	4.0±2.0	9.0±1.6	0.297[a]; 0.634[b]; 0.002[c]

*DF: dengue fever; DHF: dengue hemorrhagic fever; DSS: dengue shock syndrome, a: P value of comparison with DF group vs. DHF group; calculated by Student's t-test. b: P value of comparison between DF group and. DSS group. c: P value of comparison between DHF group and. DSS group. P value is calculated by Student's t-test

DSS groups were all significantly higher than that in normal group ($p<0.01$). Moreover, the expression level of IP-10 in DF group was also significantly higher than those in DHF group ($p<0.05$) and in DSS group ($p<0.05$), respectively. However, the difference of IP-10 levels between DHF and DSS groups was not significant ($p = 0.49$). In addition, Table 2 shows the levels of IFN-γ, TNF-α, IL-10 and IL-6 in DF (n = 5), DHF (n = 7) and DSS (n = 4) patients. The level of TNF-α was significantly higher in DSS patients than in DF patients ($p<0.01$). The level of IL-6 was significantly higher in DHF than in DSS groups ($p<0.01$). The levels of IFN-γ and IL-10 in DF, DHF and DSS groups were no significant difference (Table 2).

DISCUSSION

High fever, petechiae on the skin and hepatomegaly are the common features of DHF and DSS patients[8]. Similar symptoms were also observed in this study. Platelet abnormalities correlate with the severity of the hemorrhage. Patients with GI bleeding normally showed significantly lower platelet counts compared to those without GI bleeding[9,10]. However, the platelet counts in our DF, DHF and DSS patients were no significant difference, which may be related to our patients without GI bleeding symptom (Table 1). In addition, thrombocytopenia was detected in DF, DHF and DSS groups. Both AST and ALT levels were increased in DF, DHF and DSS patients, which are consistent with other's report that DV infection could elevate AST and ALT expression levels[8]. In summary, the clinical characteristics and the laboratory analysis results of the DF, DHF and DSS patients are properly fit WHO case definition.

Previous studies showed that cytokine levels (IFN-γ, TNF-α , IL-6, IL-10, etc.) in the blood of the DF patients were different from those in DHF and DSS patients[11-13]. Abnormal high levels of cytokines in the serum may be important for pathogenesis of DHF and DSS, because the levels of these cytokines in the dengue patients correlated with the severity of the clinical symptoms. However, none of above cytokines seems to be DV infection specific. The reason that the average age of DF group (50 years old) was significantly higher than the ages of DHF (10.9 years old) and DSS (8.4 years old) groups ($p<0.05$) is that lack of children DF patients in Taiwan. In this study, our observation of the expression levels of IFN-γ, TNF-α , IL-10 and IL-6 in DF, DHF and DSS patients, were consistent with the results of other reports[8,14].

This is the first study to provide the expression profile of IP-10 levels in the sera of DF, DHF and DSS patients. The levels of IP-10 were significantly elevated in DF, DHF and DSS patients compared to normal controls. Moreover, the level of IP-10 in DF patient was significantly higher than in DHF and DSS patients. This phenomenon indicates an inverse correlation between the IP-10 expression level and the disease severity. Because IP-10 expression level was also elevated in the sera of enterovirus 71 (EV71) infected patients (data not shown), suggesting that IP-10 overexpression is not DV infection specific. Differently, IP-10 expression level in EV71 patients was positively correlated with the disease severity (data not shown). Based on the previous studies, IP-10 seems to play dual-roles during DV infection. Initially, IP-10 may contribute to the resistance of the host cells against primary dengue virus infection by effectively recruiting effector T cells to the site of DV infection[7]. Subsequently, it can inhibit the binding of dengue viruses by competing with heparin sulfate (a receptor of DV) on the target cells[6]. All together, overexpression of IP-10 during DV infection may counterbalance viral load. The end point result will determine the severity of the disease. The relationship between the level of IP-10 and the viral load in DV infected patients needs further investigation.

On the other hand, the Single Nucleotide Polymorphism (SNP) of IP-10 in Caucasian population has been identified. There are over 5% frequency of G → C and T → C SNPs in exon 4 of IP-10 gene[15]. Therefore, it is interesting to clarify whether SNP

determines the IP-10 expression levels during virus infection in different human populations.

In conclusion, we demonstrate that DV infection may induce IP-10 expression, however its level counterbalances virus load. The disease severity in DV patients is dependent on how the IP-10 level and virus load are compromised. Therefore, IP-10 may have the potential to be used as a diagnostic marker to predict the outcome of DV infection. However, more analysis of the clinical samples is needed to establish the relationship between the level of IP-10 and the severity of DV patients.

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