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Increased expression of cytokines, soluble cytokine receptors, soluble apoptosis ligand and apoptosis in dengue

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ABSTRACT

Several studies have been performed to determine biomarkers that define the risk factors to developing severe forms of dengue. In this study, the levels of TNF-α, IL-6, IL-1, IL-17, soluble interleukin-1 receptor like 1 protein (sST2), soluble TNF-related apoptosis-inducing ligand (sTRAIL), IL-12 and soluble receptors for TNF (sTNF-RI and sTNF-RII) were determined by ELISA in dengue patients and monocyte/macrophage cultures. Dengue was classified as dengue without warning symptoms (DNWS), with warning symptoms (DWWS) and severe dengue (SD). High values of IL-6, sTNFRI, sTNFRII and sST2 were observed in DWWS and/or SD and IL-12 and sTRAIL in DNWS. TNF-α and IL-17 were increased not associated to the disease severity. High production of TNF-α, IL-1β, IL-12, IL-17, sST2 and sTRAIL and apoptosis expression were observed in dengue monocyte/macrophage cultures. This study shows that beneficial or deleterious biomarkers can be present in dengue regardless the disease severity and that monocytes may be in part the source of studied molecules.

Introduction

Dengue virus (DENV) is a single-stranded RNA virus that causes disease in humans. DENV infection results in different clinical manifestations ranking from benign disease (Halstead et al., 1970; WHO, 2009). Due to the lack of efficient biomarkers that define the degree of severity, there is the need to find relevant biological markers of the disease. Several investigations have been focused in DENV serotypes, type of infection and risk factors to developing severe forms of the disease (Halstead et al., 1970; Monath, 1994; Guzman et al., 1990). However, there are few studies relate to serum levels of soluble biomarkers with possible beneficial or deleterious activities, during the course of dengue and theirs association with the severity of disease, type of infection and DENV infection.

Several cytokines such as tumor necrosis factor alpha (TNF-α), gamma interferon, interleukin (IL)-6, IL-1, IL-17, soluble interleukin-1 receptor like 1 protein (sST2) and soluble TNF-related apoptosis-inducing ligand (sTRAIL) have been associated to deleterious effect during dengue (Halstead et al., 1970; Gagnon et al., 2002; Espina et al., 2003; Levy et al., 2010; Rachman and Rinaldi 2006; Huang et al., 2003; Wu et al., 2013; Jain et al., 2013; Amatucci et al., 2007; Tajima et al., 2007; Wajant et al., 2001), and IL-12 and soluble receptors for TNF (TNF-RI and TNF-RII) with beneficial effects (Pacsa et al., 2000; Herbein and O'Brien, 2000; Vandenabeele et al., 1995). Therefore, the aim of this study was to determine the serum levels of TNF-α, IL-6, IL-1, IL-17, sST2, sTRAIL, IL-12, TNF-RI and TNF-RII in patients infected by DENV and their association with the severity of disease, type of infection (primary, secondary) and DENV type infection. In addition, to determine the production of those biomarkers and the apoptosis expression by monocyte isolated from dengue patients and healthy controls.

Results

Hematological and biochemical parameters of dengue patients and healthy control are shown in Table 1. Decreased numbers of platelets were observed in acute DNWS, DWWS and SD patients compared to healthy controls. The lowest counts were observed in SD patients. Prothrombin time (PT) and partial thromboplastin time (PTT) were found to be increased in acute SD and PTT in acute DWWS and SD. Counts of leukocytes were decreased in acute DNWS, DWWS and SD. Liver enzymes were observed increased in

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Table 1
Age, gender and laboratory parameters of healthy controls and patients with dengue according to severity and evolution of disease.

	Control	Dengue								
		DNWS			DWWS			SD		
n	10	12			10			8		
Age (years)	18 (2–42)	20 (5–33)			12 (1–39)			10 (4–26)		
Gender Female/male	6/4	6/6			6/4			4/4		
Laboratory parameters		**Acute**	**Convalescence**	**Recovery**	**Acute**	**Convalescence**	**Recovery**	**Acute**	**Convalescence**	**Recovery**
Leukocyte counts $\times 10^3/\mu l$	6.9 ± 1.3	3.7 ± 1.3[a]	6.0 ± 1.9[b,c,d]	6.9 ± 1.3[b,c,d]	3.7 ± 0.8[a]	6.2 ± 1.4[b,c,d]	6.0 ± 3.1[b,d]	3.0 ± 0.6[a]	5.93 ± 1.8[d]	6.72 ± 1.1[b,c,d]
Platelet counts $\times 10^3/\mu l$	274.5 ± 50.1	150.9 ± 34.2[a]	306.0 ± 160.6[b,c,d]	321.1 ± 97.5[b,c,d]	64.5 ± 32.2[a]	212.0 ± 86.7[c,d]	262.4 ± 38.9[c,d]	44.0 ± 15.9[a]	164.2 ± 71.3	262.2 ± 36.3[c,d]
Hemoglobin (g/dl)	13.1 ± 1.9	13.4 ± 1.5	12.8 ± 1.5	12.6 ± 1.3	12.4 ± 1.2	11.7 ± 0.8	12.5 ± 1.6	11.1 ± 1.7[b]	11.7 ± 1.3	11.8 ± 1.1
Hematocrit (%)	41.3 ± 6.0	42.8 ± 4.7	40.2 ± 4.0	39.1 ± 3.0	40.4 ± 3.1	39.1 ± 1.2	38.7 ± 5.5	38.1 ± 5.6	38.0 ± 4.9	37.3 ± 2.9
PT (s)	12.4 ± 0.9				12.8 ± 2.0	12.3 ± 0.4[d]		14.7 ± 1.2[a,c]	12.4 ± 0.8[d]	
PTT (sec)	30.4 ± 3.8				39.7 ± 6.2[a]	27.9 ± 2.3[c,d]		36.6 ± 5.6[a]	29.9 ± 0.7[c,d]	
Glycemia (mg/dl)	78.9 ± 6.6	86.5 ± 7.9	89.2 ± 6.2	86.3 ± 7.8	92.7 ± 15.8	93.0 ± 6.7	90.8 ± 7.6	107.4 ± 41.5[a]	88.6 ± 15.2	89.0 ± 12.7
Creatinine (mg/dl)	0.9 ± 0.2	0.65 ± 0.3	0.7 ± 0.2	0.7 ± 0.09	0.7 ± 0.2	0.6 ± 0.2	0.6 ± 0.2	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1
AST (IU/L)	21.2 ± 4.2	49.7 ± 22.9	23.2 ± 6.2[b,c,d]	19.1 ± 3.7[c,d]	156.3 ± 105[a,b]	82.2 ± 41.1[a,d]	21.0 ± 3.5[c,d]	233.9 ± 160.7[a,b]	107.3 ± 67.6[a,d]	18.7 ± 4.32[c,d]
ALT (IU/L)	23.0 ± 5.3	59.6 ± 26.1[a]	28.8 ± 8.7[c,d]	21.1 ± 4.0[c,d]	146.1 ± 113[a,b]	94.2 ± 59.6[a]	24.0 ± 4.1[c,d]	152.5 ± 115.6[a,b]	96.7 ± 55.7[a]	21.7 ± 5.9[c,d]

DNWS: dengue without warning symptoms; DWWS: dengue with warning symptoms; SD: severe dengue; PT: Prothrombin time; PTT: Partial Thromboplastin time; AST: Aspartate transaminase; ALT: Alanine transaminase.

[a] $p < 0.01$ vs. control.
[b] $p < 0.01$ vs. DNWS in acute period.
[c] $p < 0.01$ vs. DWWS in acute period.
[d] $p < 0.01$ vs. SD in acute period.

Table 2
Dengue patients according to severity of disease, type of infection and dengue type infection.

Dengue	DNWS		DWWS		SD		Total	
	n	%	*n*	%	*n*	%	*n*	%
Infection:								
Primary	5	42	3	30	0	0	8	27
Secondary	7	58	7	70	8	100	22	73
Viral type[a]								
DENV-1	3	37.5	2	25	0	0	5	21
DENV-2	4	50	2	25	2	25	8	33
DENV-3	1	12.5	2	25	2	25	5	21
DENV-4	0	0	2	25	4	50	6	25

DNWS: dengue without warning symptoms; DWWS: dengue with warning symptoms; SD: severe dengue.

[a] Viral isolation was obtained in 24 out of 30 dengue patients.

Table 3
Total serum cytokine and soluble receptor and ligand values in dengue and healthy controls.

Cytokine[a]	Healthy control ($n=10$)	Dengue ($n=30$)
TNF-α	20.38 ± 3.24	47.36 ± 21.17[b]
IL-1β	5.18 ± 0.45	5.80 ± 0.87
IL-6	2.21 ± 1.03	6.17 ± 2.07[b]
IL-12	42.72 ± 9.85	86.25 ± 40.65[b]
IL-17	3.27 ± 2.19	45.93 ± 25.11[b]
sTNF-RI	1583 ± 458.30	3352 ± 1720[b]
sTNF-RII	2547 ± 697.20	10,858 ± 5978[b]
sST2	22.08 ± 5.16	10,950 ± 9248[b]
sTRAIL	51.71 ± 8.02	95.81 ± 26.93[b]

[a] Cytokine values are expressed as pg/mL and media ± standard deviation. TNF-α: Tumor necrosis factor-alpha; IL-1β: Interleukin-1 beta; IL-6: Interleukin-6; IL-12: Interleukin-12; IL-17: Interleukin-17; sTNF-RI: soluble tumor necrosis factor receptor type I; sTNF-RII: soluble tumor necrosis factor receptor type II; sST2: Interleukin-1 receptor like 1 protein; sTRAIL: soluble tumor necrosis factor -related apoptosis inducing ligand

[b] $p < 0.05$ vs. healthy control.

dengue patients. Incremented AST values in acute DWWS and acute and convalescence SD were observed. ALT was observed increased in DNWS, DWWS (acute and convalescence) and SD (acute and convalescence). Table 2 shows dengue patient distribution according to severity of disease, type of infection and dengue type infection. Values of total studied serum molecules were found to be increased, except IL-1β (Table 3). In general, biomarkers were found to be increased in the different grades of dengue severity (Fig. 1). High values of IL-6, sTNFRI, sTNFRII and sST2 were observed in DWWS and/or SD and IL-12 and sTRAIL in DNWS. TNF-α and IL-17 values were found to be increased compared to healthy controls, but, no differences between the different grades of the disease severity were observed. Values of studied compounds were found to be increased in the acute period of disease, reaching similar values to those observed in healthy controls in the recovery period (Fig. 2). Dengue patients having secondary infection showed increased values of sTNFRI, sTNFRII and sST2, however, TNF-α, IL-6, IL-12, IL-17 and sTRAIL values remained similar in primary and secondary infections (Fig. 3). All DENV types were capable of inducing biomarker production in infected patients. However, DENV-1 was associated to higher values of IL-12 and sTRAIL; DENV-2 to sTNFRI, sTNFRII and sST2; DENV-3 to IL-12 and DENV-4 to IL-6 and sST2 (Fig. 4).

Monocytes from dengue patients and healthy controls were cultured for 5 days and biomarker contents were determined. Increased contents of TNF-α, IL-1β, IL-12, IL-17, sST2 and sTRAIL