Profile of circulating levels of IL-1Ra, CXCL10/IP-10, CCL4/MIP-1β and CCL2/MCP-1 in dengue fever and parvovirosis

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Dengue virus (DENV) and parvovirus B19 (B19V) infections are acute exanthematic febrile illnesses that are not easily differentiated on clinical grounds and affect the paediatric population. Patients with these acute exanthematic diseases were studied. Fever was more frequent in DENV than in B19V-infected patients. Arthritis/arthralgias with DENV infection were shown to be significantly more frequent in adults than in children. The circulating levels of interleukin (IL)-1 receptor antagonist (Ra), CXCL10/inducible protein-10 (IP-10), CCL4/macrophage inflammatory protein-1 beta and CCL2/monocyte chemotactic protein-1 (MCP-1) were determined by multiplex immunoassay in serum samples obtained from B19V (37) and DENV-infected (36) patients and from healthy individuals (7). Forward stepwise logistic regression analysis revealed that circulating CXCL10/IP-10 tends to be associated with DENV infection and that IL-1Ra was significantly associated with DENV infection. Similar analysis showed that circulating CCL2/MCP-1 tends to be associated with B19V infection. In dengue fever, increased circulating IL-1Ra may exert antipyretic actions in an effort to counteract the already increased concentrations of IL-1β, while CXCL10/IP-10 was confirmed as a strong pro-inflammatory marker. Recruitment of monocytes/macrophages and upregulation of the humoral immune response by CCL2/MCP-1 by B19V may be involved in the persistence of the infection. Children with B19V or DENV infections had levels of these cytokines similar to those of adult patients.

Key words: dengue - parvovirus B19 - chemokines - IL-1Ra

Among the many viruses that cause acute febrile rash illnesses, the most common are measles, rubella, herpes, varicella viruses, cytomegalovirus, Epstein-Barr virus, enteroviruses, human parvovirus B19 (B19V) and dengue virus (DENV) (Oliveira et al. 2008). The latter two were studied here because these induce acute disease and because no vaccines are available for either. Indeed, the high incidence in paediatric populations is a feature common to all these viruses even when effective vaccines are available (Ahmed et al. 2010). Although these paediatric viruses are commonly regarded as mild childhood diseases, complications may occur and frequently require hospitalisation (Elena et al. 2011, May et al. 2011). Further, additional investigations have linked infection with these viruses with a risk of developing autoimmune disorders (Krone et al. 2008) or cancers. In fact, acute B19V-infection has been frequently observed in paediatric patients with haematological and/or oncological diseases. In patients with non-malignant diseases, anaemia or autoimmune disorders were diagnosed in association with B19V-infection (Jitschin et al. 2010). A retrospective hospital-based study was conducted in which the presence of spontaneous bleeding, hepatomegaly, signs of capillary leakage such as ascites and pleural effusion, leukopenia < 4.000 mm^3 and age less than five years were found out to be significant risk factors of dengue shock syndrome (DSS) in paediatric patients with dengue haemorrhagic fever (DHF) (Gupta et al. 2011).

DENV, of which four serotypes have been identified (DENV 1-4), is a single-stranded, positive-sense RNA virus belonging to the genus Flavivirus of the Flaviviridae family. DENV usually causes an acute self-limited illness known as classic dengue fever (DF) that lasts five-seven days (Halstead 2007). Symptoms include high fever, headache, retro-orbital headache, myalgia, arthralgia, abdominal pain, nausea and vomiting. Less than 3% of patients developed severe forms (DHF/DSS) after a normal acute phase, characterised by an abrupt increase in capillary permeability and resulting plasma leakage that can lead to circulatory shock and death. Dengue is the most frequent human vector-borne viral disease. Two-fifths of the world populations are at risk and an estimated 50-100 million cases of DF occur each year worldwide (Gubler 2002a, b). Brazil reported approximately 1.5 million cases in 2009-2010 (MS/SVS 2010a, b) and 155,613 cases during the two first months of 2011 (MS/SVS 2011).

Despite the major health and economic impacts of this disease, there is currently no vaccine or specific treatment (Duyen et al. 2011). Severe disease may occasionally be seen during primary DENV infection, particularly in infants (Kliks et al. 1988, Chau et al. 2010); however, complications are usually described in association with sequential or secondary infections (Sangkaw-

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ibha et al. 1984, Halstead 2007). Antibody-dependent enhancement is thought to underlie this phenomenon (Halstead et al. 1970, Halstead & O'Rourke 1977, Dejnirattisai et al. 2010). Moreover, the rapid activation of cross-reactive DENV-specific memory T cells generated during primary DENV infection appears to trigger strong production of proinflammatory cytokines, such as interferon (IFN)-γ, tumour necrosis factor (TNF)-α and interleukin (IL)-6, which can directly damage vascular endothelial cells, further resulting in plasma leakage (Mangada et al. 2002, Webster et al. 2009). DENV-specific T cells may have a dual role, both helping to clear the virus and causing bystander tissue damage. Numerous studies have explored the cytokine response to DENV. Proinflammatory cytokines, such as TNF-α, IFN-γ, IL-6, IL-18 and macrophage migration inhibitory factor (MIF), are also known to be involved during the acute phase of the illness (Pinto et al. 1999, Braga et al. 2001, Bozza et al. 2008, Levy et al. 2010) and many chemokines involved in leukocyte recruitment to sites of infection, such as CXCL8/IL-8, CXCL10/inducible protein-10 (IP-10) and CCL2/monocyte chemotactic protein-1 (MCP-1), are produced during inflammation (Raghupathy et al. 1998, Hsieh et al. 2006, Lee et al. 2006). One study showed elevated IFN-α plasma levels shortly after symptom onset in DF children (Kurane et al. 1993). The severity of DENV infection seems to be due more to disproportionate inflammatory cytokine production than direct viral effects (Chaturvedi et al. 2007, Rothman 2009). Recently, a study was conducted in African DF patients infected during the first Gabonese DENV-2 outbreak in 2007. The concentrations of 50 cytokines, chemokines and growth factors were measured in plasma by Luminex technology (Bio-Rad). These data showed that relative to the controls, the patients had significantly elevated levels of growth factors (G-CSF, GM-CSF and VEGF-A), pro-inflammatory and antiviral cytokines (IL-6, Il-17 and IFN-α2), anti-inflammatory cytokines [IL-1 receptor antagonist (Ra), IL-2rα and IL-13], chemokines (IL-16, CCL2/MCP-1, CXCL10/IP-10, SDF-1α, MIF and CCL5/RANTES) and cytokines associated with adaptive responses (IL-7, IL-12p40 and IFN-γ) (Becquart et al. 2010).

B19V, a member of the family Parvoviridae and the genus Erythrovirus, is a non-enveloped, small single-stranded DNA virus. B19V is the aetiological agent of erythema infectiosum, a childhood rash disease and involved in a wide spectrum of other diseases (Young & Brown 2004). Due to the destruction of erythroid precursor cells in bone marrow, the main targets for B19V replication affected patients develop anaemia, resulting in haematopoietic disorders (e.g., transient red cell aplasia, thrombocytopenia and pancytopenia in children and adults or hydrops fetalis in pregnant women) (Chorba et al. 1986, Yaegashi et al. 1998). Chronic immune activation and cytokine dysregulation occur in patients during and following symptomatic acute B19V-infection. It was shown that circulating IL-6, IFN-γ and TNF-α are detectable in patients with symptomatic B19V-infection and that raised levels of IFN-γ and TNF-α correlate with prolonged and chronic fatigue (Kerr et al. 2001). Cytokine dysregulation has been linked with B19V-associated haemophagocytosis and pancytopenia (Watanabe et al. 1994), arthritis (Wagner et al. 1995) and myocarditis (Nigro et al. 2000). Lymphocytes from convalescent adults produce high levels of IL-2 and IFN-γ in response to both B19V viral capsid VP1 and VP2 proteins (Corcoran et al. 2000). The levels of IL-6, TNF-α and GM-CSF at acute infection were lower in patients suffering from arthritis. There was also a trend towards lower levels of IFN-γ, CCL2/MCP-1 and TGF-β1 in acute B19V-associated arthritis and decreased level of serum TGF-β1 in patients with rash. Moreover, a mixed Th1/Th2 cytokine profile was observed, as demonstrated by elevated levels of IFN-γ/TNF-α and IL-4, respectively, in the sera of acute symptomatic B19V-infected patients (Kerr et al. 2004).

B19V and DENV have common features, as both are acute paediatric exanthematous diseases. Our first goal was to characterise a profile of chemokines and IL-1Ra in each of the viruses. We then investigated the association of the plasma levels of these soluble proteins in age subgroups (< 15 years or ≥ 15 years) with clinical manifestations such as fever and arthritis/arthralgia.

PATIENTS, MATERIALS AND METHODS

Patient enrolment, assessment and serum collection - From 2001-2008, we prospectively included 37 patients with acute B19V infection and 36 patients with acute DENV infection. All patients were assisted at University Hospital Antônio Pedro (HUAP) at Fluminense Federal University, Niterói, state of Rio de Janeiro, Brazil. All patients presented acute exanthematic manifestations and were bled at the time or shortly after the onset of their symptoms, irrespective of their presumed clinical diagnosis. The disease history and physical examination of each patient were recorded. Patients with DF had DENV infection confirmed by anti-DENV enzyme-linked immunosorbent assay (ELISA)-IgM (Panbio, Inc, INDX® IVD™ Microwell ELISA Dengue Fever IgM Test, Columbia, USA) and all other viral infections (e.g., measles, rubella or B19V) were excluded by serology. All patients had mild clinical manifestations; no haemorrhagic manifestations were observed and no hospitalisations were necessary. In B19V cases, the serum anti-B19V IgM test was positive (Biotrin Parvovirus Enzyme Immunoassay, Dublin, Ireland), while markers of other acute exanthematic infections (e.g., measles virus, rubella or DENVs) were negative. Seven healthy male controls (mean age of 31.6 years) were also enrolled in the study. The study was approved by HUAP Ethical Committee (CMM/HUAP 134/05). A written informed consent was signed by all patients or their guardians prior to blood collection.

Blood samples and chemokine/cytokine detection by multiplex microbead immunoassay - Blood samples were collected in pyrogen-free blood collection tubes and kept on ice. Serum was collected after centrifugation, divided in aliquots and stored at -20ºC until the assay. A multiplex biometric immunoassay containing fluorescent microspheres conjugated with monoclonal antibodies specific to the target proteins was used for cytokine measurement according to the manufacturer's

instructions (Beadlyte® Human Multi-Cytokine Flex Kit; Upstate, Lake Placid, NY, USA). The cytokines measured were as follows: IFN-IP-10 (CXCL10/IP-10), MCP-1 (CCL2/MCP-1), macrophage inflammatory protein-1 beta (CCL4/MIP-1β) and IL-1Ra. Briefly, 10 µL plasma samples were diluted 1:10 and incubated with an antibody-coupled bead pool. The complexes were sequentially washed, incubated with a biotinylated detection antibody pool and streptavidin-phycoerythrin to detect the cytokine titres in sera. A range of 2.3-5.000 pg/mL of the recombinant cytokines provided by the vendor (Upstate) was used to establish the standard curves, maximising the sensitivity and the dynamic range of the assay. Cytokine levels were determined using a multiplex array reader from Luminex® 200™ System (Luminex Corp). The analyte concentration was calculated from the respective standard curve by five-parameter logistic analysis using software provided by the manufacturer (xPONENT® v3.1 software).

Statistical analyses - The relationships between sex, days of onset symptoms and clinical manifestations were examined using Fisher's exact test. The nonparametric Kruskal-Wallis test was used to evaluate differences between cytokine ratios from acute B19V and DENV infected-patients. Generalised linear models (GLMs) were used to evaluate factors independently associated with quantitative variables. Analysis of factors independently associated with the two different viruses studied and other clinical manifestations was performed with GLMs with logistic or Gaussian regression. The results from the logistic regressions are given as odds ratio. The confidence interval was established at 95%. Alternatively, for a GLM, Gaussian family t values were recorded. A probability value of $p < 0.05$ was considered to be significant. The distributions of cytokine levels in the control and disease groups were normally distributed; comparisons of cytokine levels associated with clinical manifestations were carried out using the non-parametric Kruskal-Wallis test. Models were also built/constructed using logistic regression analysis to determine the clinical variables most strongly associated with the development of symptomatic infection. The statistical programs R (R: A language and environment for statistical computing) [R Foundation for Statistical Computing, Vienna, Austria, ISBN 3-900051-07-0 (R-project.org)] and Prism 4 (Graph-Pad Software, San Diego, CA, USA) were used.

RESULTS

Clinical characterisation of acute exanthematic diseases caused by DENV or B19V in Brazilian patients - Detailed demographic, clinical and laboratory data from acutely DENV and B19-infected patients are summarised in Table I. DENV-infected patients were more likely to develop fever, independent of age, as compared to acutely B19V-infected patients. The development of arthritis or arthralgias in DENV infections was shown to be significantly more frequent in adults (≥ 15 years) than in children (< 15 years).

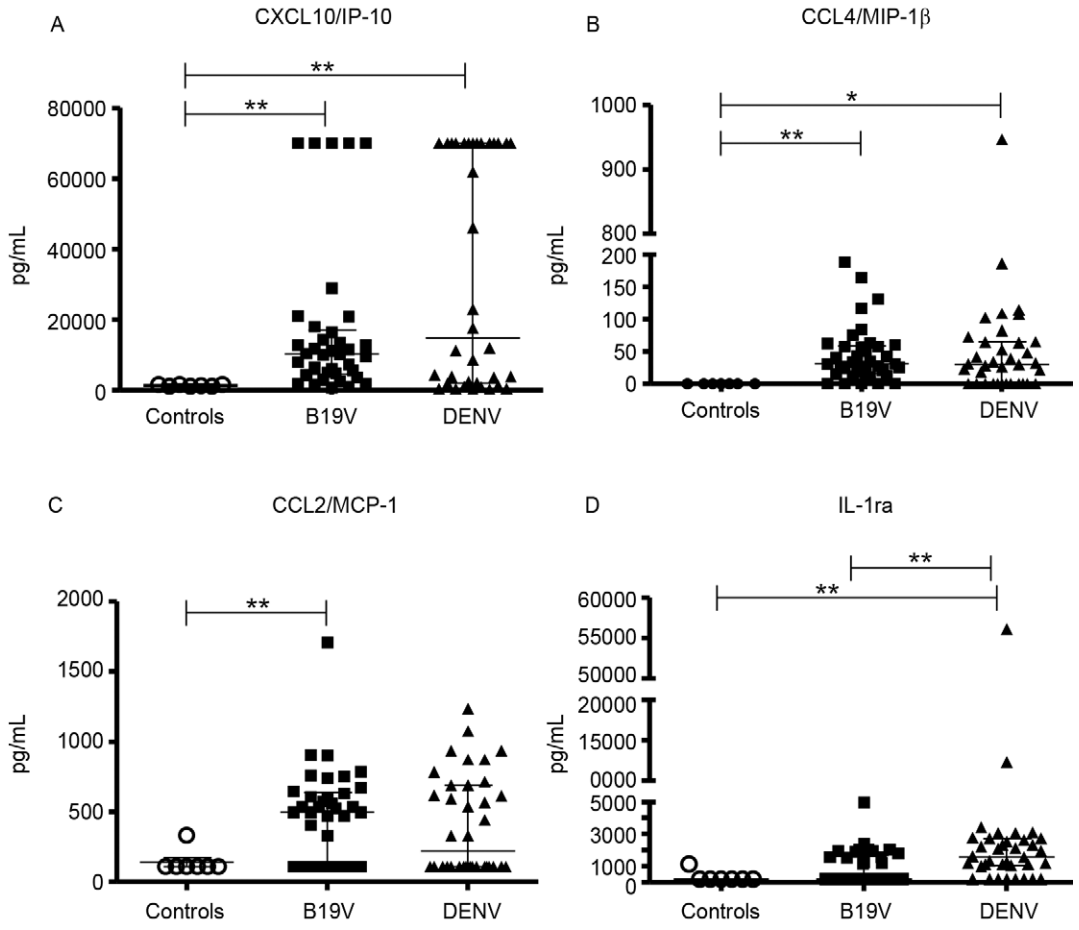
Circulating cytokines in DENV and B19V patients - Acutely DENV and B19V-infected patients showed elevated serum levels of CXCL10/IP-10 and CCL4/MIP-1β (Figure, Table II) as compared to healthy individuals that usually have low or undetectable levels of these cytokines. CCL2/MCP-1 was significantly increased only in B19V patients as compared to controls, while IL-1Ra levels were higher in DENV-infected patients as compared to controls and B19V-infected patients. There was also a tendency towards increased levels of CCL2/MCP-1 in DENV-infected patients and IL-1Ra in B19V-infected patients, although the differences are not significant as compared to controls. In general, children with

TABLE I

Demographic information about the study population with acute dengue virus (DENV) or parvovirus B19 (B19V) infection[a]

	DENV infection		B19V infection	
	Children	Adults	Children	Adults
Patients (n)	18	18	17	20
Mean age (range in years)	6.5 (0-12)	32.5 (15-77)	6.3 (1-14)	26.8 (16-55)
Female:male ratio	1.3:1	2:1	0.5:1	9:1[b]
Mean days of disease (range in days)	5.4 (2-10)	6.4 (1-15)	8.9 (1-25)	6.5 (1-14)
Fever [n (%)]	13 (72)	16 (89)	8 (47)	6 (30)
Rash [n (% total)]	100	100	100	100
Arthritis or arthralgias [n (% total)]	0	13 (65)[c]	2 (11)	7 (39)
Warning signs [n (% total)]	7 (41)	4 (20)	5 (28)	6 (33)
Serum anti-B19V VP2 IgM	0	0	100	100
Serum anti-DENV IgM	100	100	0	0

a: children < 15 years, adults ≥ 15 years; *b*: $p < 0.001$; *c*: $p < 0.0001$. Significant difference between children and adults by Fisher's exact test analysis between children and adults within the same virus disease.



Chemokines and interleukin-1 receptor antagonist (IL-1Ra) levels in serum from patients with parvovirus B19 (B19V) or dengue scatter dot plots graph. The lines extend from the 25th to the 75th percentile and the line at the middle is the median. Non-parametric Kruskal-Wallis test was used to evaluate differences between circulating proteins concentrations from B19V, dengue virus (DENV) patients and controls. IP-10: inducible protein-10; MCP-1: monocyte chemotactic protein-1; MIP-1β: macrophage inflammatory protein-1 beta; *: $p < 0.05$; **: $p < 0.01$ and < 0.001.

B19V or DENV infections had levels of these cytokines similar to those of adult patients. However, compared to CXCL10/IP-10 plasma levels in DENV infection, the median values of this chemokine in adult patients [9.897 (1.986-70.000) pg/mL] were lower than those in children [53.972 (1.163-70.000) pg/mL], although this difference is not significant. The high cytokine levels in patients with acute B19V or DENV infection demonstrate the importance of this host response. It remains to be determined which cytokines, if any, are associated with clinical manifestations.

Distinctive associations between circulating cytokines and clinical manifestations in exanthematic diseases caused by DENV and B19V - Multivariate logistic regression models were constructed to determine the individual, clinical and circulating cytokines/chemokines variables most strongly associated with the development of symptomatic infection, while also correcting for the effect of possible confounding variables. Fever was positively associated with IL-1Ra levels but negatively associated with CCL4/MIP-1β production irrespective of the associated virus (Table III). No association was found between CXCL10/IP-10 and CCL2/MCP-1 levels and fever. Another logistic regression model confirmed that arthritis/arthralgias were associated with adult patients, as observed previously (de Oliveira et al. 2009). Significant associations of arthritis/arthralgias with cytokines/chemokines (Table IV) or with premonitory signs (data not shown) could not be found in either infection.

A forward stepwise logistic regression revealed a third model in which age, sex and disease duration were parameters independently associated with the infecting virus type. In this model, there was a weak direct association between CCL2/MCP-1 levels and B19V infection. Furthermore, we found a negative association between fever, the levels of CXCL10/IP-10 (weakly) and IL-1-Ra with B19V infection, indicating that these variables could influence the manifestations of DENV infection more than those induced by B19V infection (Table V).

TABLE II

Cytokines/chemokines levels from patients with acute dengue virus (DENV), parvovirus B19 (B19V) and healthy controls[a]

	DENV infection (pg/mL)		B19V infection (pg/mL)		Controls (pg/mL)
	Children	Adults	Children	Adults	
CXCL10/IP-10	53.972 (1.163-70.000)	9.897 (1.986-70.000)	8.718 (3.693-16.777)	1.1067 (5.455-18.595)	1.142 (1.035-1.455)
CCL4/MIP-1β	24.63 (0.01-55.70)	37.67 (13.72-104.0)	26.85 (13.08-54.90)	34.80 (20.82-69.30)	0.01 (0.01-0.01)
CCL2/MCP-1	276.4 (109.7-632.1)	220.4 (109.7-755.4)	496.3 (109.7-660.9)	499.3 (220.4-626.5)	109.7 (109.7-109.7)
IL-1Ra	1361 (219.5-2.572)	1885 (1191-2.849)	219.5 (219.5-1.755)	219.5 (219.5-1.736)	219.5 (219.5-219.5)

a: children < 15 years, adults ≥ 15 years; IL-1Ra: interleukin-1 receptor antagonist; IP-10: inducible protein-10; MCP-1: monocyte chemotactic protein-1; MIP-1β: macrophage inflammatory protein-1 beta.

TABLE III

Forward stepwise logistic regression analysis to determine the immunological variables most strongly associated with fever in acute dengue virus and parvovirus B19 infection[a]

Fever (n) (yes = 44; no = 29)	Regression coefficient	SE	OR (95% CI)	p
Intercept	0.095	0.475		0.842
CXCL10/IP-10	2.0e-05	1.1e-05	1.000 (1.000-1.000)	0.073
CCL4/MIP-1β	-0.017	0.008	0.983 (0.967-0.999)	0.04[b]
CCL2/MCP-1	-7.5e-05	9.1e-04	1.000 (0.998-1.002)	0.935
IL-1Ra	3.0e-04	1.4e-04	1.000 (1.000-1.001)	0.036[b]
Age (< 15 years)	0.326	0.535	1.386 (0.486-3.950)	0.542

a: models were chosen according to their best Akaike information criterion; *b*: < 0.05; CI: confidence interval; IL-1Ra: interleukin-1 receptor antagonist; IP-10: inducible protein-10; MCP-1: monocyte chemotactic protein-1; MIP-1β: macrophage inflammatory protein-1 beta; OR: odds ratio; SE: standard error.

TABLE IV

Forward stepwise logistic regression analysis to determine the clinical and laboratorial/immunological variables associated with arthritis or arthralgias in acute parvovirus B19 and dengue virus infection[a]

Arthritis/arthralgia (n) (yes = 22; no = 51)	Regression coefficient	SE	OR (95% CI)	p
Intercept	0.175	0.523		0.738
CXCL10/IP-10	-4.8e-06	1.2e-05	1.000 (0.999-1.000)	0.679
CCL4/MIP-1β	-0.005	0.0107	0.995 (0.975-1.016)	0.655
CCL2/MCP-1	3.9e-04	0.001	1.000 (0.998-1.002)	0.714
IL-1Ra	5.1e-05	2.1e-04	1.000 (0.999-1.000)	0.807
Age (< 15 years)	-2.884	0.824	0.056 (0.011-0.281)	0.001[b]

a: models were chosen according to their best Akaike information criterion; *b*: < 0.001; CI: confidence interval; IL-1Ra: interleukin-1 receptor antagonist; IP-10: inducible protein-10; MCP-1: monocyte chemotactic protein-1; MIP-1β: macrophage inflammatory protein-1 beta; OR: odds ratio; SE: standard error.

TABLE V

Forward stepwise logistic regression analysis to determine the clinical and immunological variables associated with parvovirus B19 (B19V) or dengue virus infection[a]

B19V infection (yes = 37; no = 36)	Regression coefficient	SE	OR (95% CI)	p
Intercept	8.783	2.932		0.003[b]
< 15 years	-0.917	0.831	0.400 (0.078-2.038)	0.270
Male sex	1.739	0.891	5.693 (0.994-0.326)	0.051
Days of onset symptoms	0.170	0.099	1.185 (0.975-1.440)	0.088
Fever	-3.090	0.926	0.046 (0.007-0.279)	0.001[b]
CXCL10/IP-10	-5.1e-05	1.8e-05	0.999 (0.999-1.000)	0.004[b]
CCL4/MIP-1β	0.006	0.005	1.006 (0.996-1.016)	0.217
CCL2/MCP-1	0.005	0.002	1.005 (1.002-1.009)	0.005[b]
Log IL-1Ra	-1.375	0.463	0.253 (0.102-0.626)	0.003[b]

a: models were chosen according to their best Akaike information criterion; *b*: < 0.01; CI: confidence interval; IL-1Ra: interleukin-1 receptor antagonist; IP-10: inducible protein-10; MCP-1: monocyte chemotactic protein-1; MIP-1β: macrophage inflammatory protein-1 beta; OR: odds ratio; SE: standard error.

DISCUSSION

Several cytokines and chemokines have been reported to be induced as a response to DENV in vitro and in vivo, but few detailed studies on B19V infection have been performed. The experimental approach used here was the analysis of the circulating proteins IL-1Ra, CXCL10/IP-10, CCL4/MIP-1β and CCL2/MCP-1 in clinical samples from DENV or B19V-infected patients exhibiting characteristic exanthemas during the acute phase of the disease. Moreover, considering the importance of both viruses in the paediatric cohort, we evaluated this panel of circulating proteins in children and adult patients. The results presented here show higher serum levels of CXCL10/IP-10 and IL-1Ra in DENV-infected patients compared with B19V-infected patients, while higher serum levels of CCL2/MCP-1 were detected in acute B19V infections. There were no differences in the levels of proteins between age groups.

Pro-inflammatory cytokines with short half-lives are rapidly cleared from circulation. In contrast, soluble inhibitors have much longer circulating half-lives and thus may reflect more accurately the production of the inflammatory cytokines, giving us useful information about how to monitor these diseases. In this study, other inflammatory cytokines were tested in the same multiplex biometric immunoassay; however, we obtained only detectable levels of a few chemokines and IL-1Ra. Among the cytokines found, TNF-α and IL-1β serve as important mediators in the evolution of inflammatory diseases by stimulating other pro-inflammatory mediators, such as IL-6 and IL-8. Several endogenous mechanisms limit the systemic activity of recently produced TNF and IL-1 (Dinarello 1995). IL-1 regulation is mediated by the balance between its specific IL-1Ra and soluble IL-1 receptors (Arend 1993, 2002, Firestein et al. 1994). An earlier report on another cohort of Brazilian DF patients showed that only 16% had altered IL-1Ra levels (Pinto et al. 1999). Here, significantly elevated levels of the anti-inflammatory cytokine IL-1Ra were detected in DENV patients. This was not true for B19V patients and controls. Another interesting observation is that fever with either one of these exanthematic diseases is positively associated with IL-1Ra levels. It is well known that IL-1β, a major mediator of lipopolysaccharide-induced fever, elicits febrile responses via cyclooxygenase-2-dependent production of prostaglandin E_2 in the brain (Kluger 1991, Li et al. 2001). The IL-1-induced fever can be attenuated by the central administration of neutralising anti-IL-1β (Klir et al. 1994, Gourine et al. 1998) or naturally occurring IL-1Ra (Luheshi et al. 1996, Miller et al. 1997). This observation raises the possibility that circulating IL-1Ra may exert its antipyretic action and may be produced as a feedback mechanism in an effort to counteract the early increase of IL-1β levels in DF.

CCL2/MCP-1 is a potent chemotactic factor for monocytes/macrophages (Gu et al. 1997), both major sources of CCL2 (Yoshimura et al. 1989). In addition, CCL2 regulates the migration and tissue infiltration of memory T lymphocytes and natural killer (NK) cells. CCL2 is produced by many cell types, including endothelial, fibroblast, epithelial, smooth muscle, mesangial, astrocyte and microglial cells (Cushing et al. 1990, Standiford et al. 1991, Brown et al. 1992, Barna et al. 1994). These cells are important for the anti-viral immune responses in the peripheral circulation and tissues. Accordingly, the study of Kerr et al. (2004) on B19V infection found a significant number of acutely infected patients with high CCL2/MCP-1 levels. The authors also demonstrated a trend towards lower CCL2/MCP-1 levels in acute B19V-associated arthritis and a negative association with rheumatoid factor production. Our data confirmed a direct association between CCL2/MCP-1 levels and B19V infection. However, no significant association was observed between CCL2/MCP-1

and DENV infection. The forward stepwise logistic regression analysis showed a significant association of arthritis/arthralgias with patient age ≥ 15 years independent of the virus. It is possible that studies with a higher number of patients would be more accurate with respect to this association, but also may indicate that both diseases behave similarly in this aspect.

The induction of several chemokines in DENV infection has been reported. Elevated levels of CXCL8/IL-8 (Raghupathy et al. 1998, Juffrie et al. 2000), CCL2/MCP-1 (Lee et al. 2006) and CXCL10/IP-10 (Fink et al. 2007) have been observed in dengue patients, mainly in more severe cases. Increased CCL4/MIP-1β levels were detected in patients with mild dengue when compared with the more severe forms of the disease. In addition, CCL2/MCP-1 levels were associated with marked thrombocytopenia and hypotension (Bozza et al. 2008). Recently, Becerra et al. (2009) showed higher circulating CCL8/MCP-2 and CXCL10/IP-10 levels during the febrile course of DF. Another interesting study from Chau et al. (2008) evaluated circulating levels of chemokines during acute DF. Plasma CXCL10/IP-10 levels were significantly higher in infants with dengue and significantly correlated with DENV viral loads in the same sample. Moreover, their data showed that CXCL10/IP-10 is associated with viremia during acute primary dengue infections in infants. Dejnirattisai et al. (2008) found that CXCL10 levels were higher in DHF than in DF patients before defervescence, but showed no significant difference at the defervescence day. Our data confirm a direct association between CXCL10/IP-10 levels and DENV infection that was not observed during B19V infection.

CXCL10, also known as IFN-γ-IP-10, is a chemoattractant for activated T and NK cells bearing CXCR3, the chemokine receptor for CXCL9, CXCL10 and CXCL11 (Loetscher et al. 1996). Hsieh et al. (2006) showed that both CXCR3 and CXCL10 deficient (CXCR3-/- and CXCL10-/-) mice presented higher mortality than wild-type (WT) mice following DENV infection. Moreover, CXCR3-/- mice developed increased mortality accompanied by increased viral titres and reduced T cell infiltration in infected brain tissue as compared to WT mice, suggesting that CXCR3 plays a critical role in host defence by mobilising T cells into DENV-infected brain tissue for viral clearance. Recently, Ip and Liao (2010) investigated CXCL10 antiviral activity in DENV infection. Unexpectedly, the recruitment of effector cells into sites of infection was not impaired in CXCL10-/- mice. Furthermore, primary neuronal cells infected with DENV produced CXCL10, but not CXCL9 and CXCL10 inhibited viral infection in neuronal cells by competing with DENV for binding to heparan sulphate, a co-receptor for DENV entry. Their data support an unappreciated role for CXCL10 in innate host defence against DENV infection, reducing mortality during DENV infection. According to our results, CXCL10/IP-10 was confirmed as a strong pro-inflammatory marker in acute DENV disease, explaining perhaps the quicker virus clearance and recovery in these patients. In the future, it would be interesting to conduct ex vivo studies evaluating peripheral mononuclear cells obtained from DENV patients for chemokine receptor induction on immune cells and their impact on the regulatory immune response during infection.

The present data confirm and extend previous observations that unregulated production of chemokines/cytokines are found in the circulation of patients with acute symptomatic infections with B19V or DENV as compared with non-infected individuals. In short, our data are a distinctive and comparative profile of some circulating proteins between DF and parvovirosis. We conclude that circulating IL-1Ra and CXCL10/IP-10 seem to prevail during DF, while CCL2/MCP-1 is most prevalent in B19V.

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