The Possible Protective Role of the Il6^{-174}GC Genotype in Dengue Fever

S.T. Moreira[1], D.M. Cardoso[1], J.E. Visentainer[1], U.J.V. Fonzar[2] and R.A. Moliterno[1,*]

[1]*Immunogenetics Lab, Clinical Analysis Department, Maringá State University, Maringá, Paraná, Brazil and* [2]*Maringá's Health Secretariat Epidemiological Vigilance, Maringá, Paraná, Brazil*

Abstract: Dengue is the most important viral disease transmitted by arthropod vectors. At present, it is known four types of virus serotypes, DEN 1, 2, 3 and 4. Differences in the host susceptibility to infection as well as the severity of disease can not be due only to viral virulence. Variations in immune response as a consequence of polymorphisms in regulatory regions of cytokine genes may have influence on the disease outcome. The aim of this study was to verify the occurrence of associations between cytokine gene polymorphisms and Dengue Fever. Two hundred patients from Paraná State and 313 control individuals from Southern and Southeastern Brazil were genotyped for single nucleotide polymorphisms (SNPs) of TNF^{-308}, IFNG^{+874}, IL6^{-174}, IL10$^{-1082,-819,-592}$ and TGFB1$^{+869,+915}$ by PCR-SSP (kits One Lambda, CA, USA). Phenotype, genotype and allele frequencies were compared by chi-square test, with Yates' correction or Fisher's exact test. There was a negative association between IL6^{-174}GC genotype and Dengue Fever (27.9% vs. 38.3%; P=0.015; OR=0.62; CI=0.42 - 0.91). Significant statistical differences with cytokine production phenotypes or alleles were not observed. These results suggest a protective role of IL6^{-174}GC genotype for Dengue Fever.

Key Words: Cytokine, dengue fever, genetic association, IL-6, polymorphism.

INTRODUCTION

Dengue is an acute infectious disease of viral etiology, transmitted to human beings by the mosquito *Aedes aegypti* and less frequently by *A. albopictus* and *A. scutellaris* mosquitoes [1]. The dengue virus is classified as a one strand RNA-virus of the *Flavivirus* (Flaviviridae) genre [2]. At present there are four known virus serotypes DEN 1, 2, 3 and 4, which are antigenically strongly related [3]. Dengue has become the most important disease in tropical areas, and the most important among the ones of viral ethiology transmitted by mosquito`s bite [4]. It is estimated that the incidence of the disease has increased thirty times in the last fifty years [5]. In 2007, there were more than 890,000 reported cases of dengue in the Americas, of which 26,000 cases were Dengue Hemorrhagic Fever (DHF) [6].

Different responses to dengue virus infection in distinct individuals of the same population have been verified, ranging from sub-clinical manifestations to patient's death. However, host susceptibility to infection as well as disease severity can not be due to viral virulence solely [7].

TH1 cytokine pattern produced by the patient in response to the virus has been related to the infection resolution while the TH2 cytokine pattern seems to promote a more severe symptomatology [8].

Several cytokine genes show polymorphisms in regulating regions, directly affecting gene transcription, and consequently the cytokine production profile [9]. Recent studies show that immune response variations from polymorphisms in regulatory regions of cytokine genes seem to influence the produced cytokine level and consequently the outbreak of different infectious disease, including Dengue Hemorrhagic Fever [10-15].

Thus, the aim of the present study was to analyze single nucleotide polymorphisms (SNPs) of *TNF*$^{-308}$, *IFNG*$^{+874}$, *IL6*$^{-174}$, *IL10*$^{-1082,-819,-592}$ and *TGFB1*$^{+869,+915}$, and verify the occurrence of possible associations with susceptibility or resistance to Dengue Fever (DF), characterized by self-limited fever, associated with myalgia, headache, and thrombocytopenia, but no signs of hemorrhage or plasma leakage shock.

MATERIALS AND METHODOLOGY

Two hundred mixed-origin Brazilian patients from Paraná state (87.6% self-reporting white and 12.4% mixed race), with clinical and laboratory positive diagnosis for DF, and three hundred and thirteen mixed-origin Brazilian individuals (84.7% self-reporting white and 15.3% mixed race), paired with patients by gender, age and racial group participated in this study.

Based on HLA polymorphism, the white population of Paraná is mostly from European origin (80.6%), with significant contribution of genes of African origin (12.5%) and Amerindian (7%). The mulatto population is mainly from African origin (49.5%), with important European (41.8%) and Amerindian contribution (8.7%) [16].

The disease clinical diagnosis was accomplished according to the established criterion by the World Health Organization (WHO 1997) and confirmed by serological technique (IgM capture ELISA Kit) and/or virus culture by the Paraná's Infectious Disease Central Lab. The patients devel-

*Address correspondence to this author at the Immunogenetics Lab, Clinical Analysis Department, Maringá State University, Maringá, Paraná, Brazil; E-mail: ramoliterno@uem.br and ramoliterno11@yahoo.com.br

1874-3153/08 2008 Bentham Open

oped the disease during three epidemics caused by dengue virus 1 serotype: two in Maringá city in 1995 (N=46) and in 2002/03 (N=136) and one in Paranavaí city in 1999 (N=18).

Patients and controls signed a written consent for participating in the study, being approved by Maringá State University Permanent Ethics Committee in Research with Human Beings (Resolution nº 190/2004).

Genomic DNA from each collected blood sample was extracted and purified through EZ-DNA kit (Biological Industries, Israel) or NEOISOColumn kit (Biosystems, Middletown, CT), according to the manufactor's instructions.

There were evaluated the SNPs of *TNF*$^{-308}$G>A, *TGFB1*$^{+869,+915}$T>C, C>G, *IL10*$^{-1082,-819,-592}$A>G, T>C, A>C, *IL6*$^{-174}$G>C and *IFNG*$^{+874}$T>A, through polymerase chain reaction PCR-SSP (kits One Lambda, CA, USA). These SNPs were chosen because of their significant influence on the development of acquired immune response and possibly the course of dengue virus infection.

The genotypic, allelic and phenotypic frequencies for each SNP were obtained by direct counting and compared by the chi-square test with Yates correction or by the Fisher Exact Test. *P* values equal to or lower than 0.05 were considered significant. The strength of association was evaluated by the odds ratio (OR) according to Woolf's formula [17].

RESULTS

The patient's average age was 48.8±1.17 years, where 61.2% were women and 38.8% men.

The genotypic and allelic frequencies of cytokine regulatory gene polymorphisms (*TNF*$^{-308}$, *IFNG*$^{+874}$, *IL10*$^{-1082,-819,-592}$, *IL-6*$^{-174}$ and *TGFB1*$^{+869,+915}$) are shown in Tables **1** and **2**, respectively. As observed in Table **1**, it was verified only one negative association between the genotype *IL6*$^{-174}$GC and DF (27.86% vs. 38.26% *P* = 0.015; OR = 0.62; CI = 0.42 – 0.91).

Statistically significant differences could not be seen when comparing allelic (Table **2**) or phenotypic frequencies and DF (data not shown). All of the frequencies were in Hardy-Weinberg equilibrium.

DISCUSSION

Polymorphisms in cytokine regulatory gene regions have been described in literature. Some of these polymorphisms seem to be correlated to its production [18-25], and potentially conferring flexibility to immune response. The presence of certain genotypes may influence the course of both viral and bacterial infections [13, 26-29], as well as be associated to susceptibility or resistance to auto-immune diseases [30, 31], or influence both solid organ post-transplant rejection processes [32, 33], and graft versus host disease post bone-marrow transplantation [34, 35].

Variations at the levels of different cytokines, as TNF-α, IL-4, IL-6, IL-10, MIF, have been related to DF disease severity as well [36, 37]. As the production of these cytokines is genetically determined, cytokine regulatory gene polymorphisms may be correlated to DF susceptibility or resistance.

Table 1. Genotypic Frequency Distribution of Cytokine Regulatory Gene Polymorphisms in Both Patients with Dengue Fever (DF) and Control Individuals (CT) of a Southern Brazilian Population

Polymorphism	DF n (%)	CT n (%)	P
***TNF* (-308)**	**n = 200**	**n = 309**	
GG	157 (78.5)	230 (74.4)	0.345
GA	40 (20.0)	77 (24.9)	0.238
AA	3 (1.5)	2 (0.7)	0.386
***IL6* (-174)**	**n = 201**	**n = 311**	
CC	24 (11.9)	31 (10.0)	0.577
GC	56 (27.9)	119 (38.3)	0.015*
GG	121 (60.2)	161 (51.7)	0.076
***IFNG* (+874)**	**n = 201**	**n = 310**	
AA	73 (36.3)	102 (32.9)	0.484
TA	96 (47.8)	163 (52.6)	0.330
TT	32 (15.9)	45 (14.5)	0.759
***TGFB1* (+869)**	**n = 199**	**n = 308**	
TT	49 (24.6)	91 (29.6)	0.267
TC	114 (57.3)	163 (52.9)	0.383
CC	36 (18.1)	54 (17.5)	0.967
***TGFB1* (+915)**	**n = 199**	**n = 308**	
GG	163 (81.9)	265 (86.0)	0.260
GC	33 (16.6)	42 (13.7)	0.433
CC	3 (1.5)	1 (0.3)	0.158
***IL10* (-1082)**	**n = 200**	**n = 310**	
GG	24 (12.0)	46 (14.9)	0.437
GA	95 (47.5)	143 (46.1)	0.832
AA	81 (40.5)	121 (39.0)	0.812
***IL10* (-819)**	**n = 200**	**n = 310**	
CC	83 (41.5)	140 (45.2)	0.470
CT	88 (44.0)	139 (44.8)	0.924
TT	29 (14.5)	31 (10.0)	0.162
***IL10* (-592)**	**n = 200**	**n = 310**	
CC	83 (41.5)	140 (45.2)	0.470
CA	88 (44.0)	139 (44.8)	0.924
AA	29 (14.5)	31 (10.0)	0.162

*OR=0.62; IC=0.42-0.93.
P value obtained through chi-square test.

Table 2. Allelic Frequency Distribution of Cytokine Regulatory Gene Polymorphisms in Both Patients with Dengue Fever (DF) and Control Individuals (CT) of a Southern Brazilian Population

Polymorphism	DF n (%)	CT n (%)	P
***TNF* (-308)**	**n = 200**	**n = 309**	
G	354 (88.5)	537 (86.9)	0.509
A	46 (11.5)	81 (13.1)	
***IL6* (-174)**	**n = 201**	**n = 311**	
C	104 (25.9)	181 (29.1)	0.292
G	298 (74.1)	441 (70.9)	
***IFNG* (+874)**	**n = 201**	**n = 310**	
A	242 (60.2)	367 (59.2)	0.799
T	160 (39.8)	253 (40.8)	
***TGFB1* (+869)**	**n = 199**	**n = 308**	
T	212 (53.3)	345 (56.0)	0.428
C	186 (46.7)	271 (44.0)	
***TGFB1* (+915)**	**n = 199**	**n = 308**	
G	359 (90.2)	572 (92.9)	0.165
C	39 (9.8)	44 (7.1)	
***IL10* (-1082)**	**n = 200**	**n = 310**	
G	143 (35.7)	235 (37.9)	0.529
A	257 (64.3)	385 (62.1)	
***IL10* (-819)**	**n = 200**	**n = 310**	
C	254 (63.5)	419 (67.6)	0.202
T	146 (36.5)	201 (32.4)	
***IL10* (-592)**	**n = 200**	**n = 310**	
C	254 (63.5)	419 (67.6)	0.202
A	146 (36.5)	201 (32.4)	

P obtained through the chi-square test.

The present study analyzed SNPs of some cytokine genes that may direct the immune response to TH1/TH2, in order to verify the occurrence of possible associations among them and the susceptibility or resistance to DF. Our results suggest a protective role of the genotype *IL6*$^{-174}$GC for DF, and this genotype is associated by some authors with high IL-6 production [38].

Studies on association of DF with cytokine gene polymorphisms are rare in literature. Fernandez-Mestre *et al*. [12], when studying the SNPs *TNF*$^{-308}$G>A, *TGFB1*$^{+869,+915}$T>C, C>G, *IL10*$^{-1082,-819,-592}$A>G, T>C, A>C, *IL6*$^{-174}$G>C and *IFNG*$^{+874}$T>A, suggested the allele *TNF*-308 A as a possible risk factor to hemorrhagic manifestations in dengue patients. Conversely to our results, these authors did not observe associations with DF.

The frequency of the genotype *IL6*$^{-174}$GC in the patients with DF presented by the authors was similar to the one found in our study (26.8% vs. 27.9%). However, the frequency of this genotype in controls was significantly lower than ours (21.7% vs 38.3% personal communication), possibly because of a difference in the racial components formation of the two populations. It is important to note that the *IL-6*^{174}GC frequency observed in our controls was similar to the one found by Pieroni *et al*. [39] in a population sample of São Paulo, Brazil.

IL-6 is a pleiotropic cytokine, produced during innate and adaptive immune response by T and B lymphocytes, macrophages, monocytes, fibroblasts and endothelial cells, besides some tumoral cells [40, 41]. It is not constitutively expressed, but induced in response to various inflammatory stimuli as IL-1, TNF-α and viral infection [42, 43]. Its biological activities include participation in immunological responses, hematopoiesis, and acute phase reactions [44]. The deregulated expression of IL-6 has been associated to diseases as plasmacytoma, myeloma and proliferative chronic inflammatory diseases [44].

This cytokine also seems to increase in DF patients [45], and may seem to have an action in viral eradication by stimulating a TH1 immune response [46]. Moreover, IL-6 promotes the decreasing of consequential symptoms to infection, since it inhibits the acute phase of the inflammatory response through induction of pro-inflammatory cytokine antagonists as IL1ra and sTNFR p55 [47]. This way, the association of these two biological activities may promote protection against DF clinical symptoms development in patients expressing the IL-6 high producer genotype: *IL6*$^{-174}$GC.

Alternatively, there is low consensus about the relation between polymorphisms and cytokine production in general [48], and IL-6 production, more specifically [49, 50]. Kilpinen *et al*. [51] observed an association between polymorphism and the production of IL-6 both *in vivo* or *in vitro* only in truly "naive" newborns, whilst in adults the previous contact with hexogen antigens would modify its response.

Moreover, the influence of SNP *IL6*$^{-174}$G>C in gene transcription may be more complex and involve various polymorphic sites. The SNP *IL6*$^{-174}$G>C is in truth part of an haplotype of SNPs, genetically and functionally linked, including the positions -634G>C, -597G>A, -572G>C, -373A_nT_n [42, 52]. Thus, a specific polymorphism of this haplotype may exert influence in IL-6 transcription, but each SNP would not act independently from others. A complex interaction may occur between the effects of each SNP, influencing the final phenotype produced by the haplotype. Therefore, the study of SNP *IL6*$^{-174}$G>C solely may not correspond to the IL-6 high or low producer phenotype, unless the other IL-6 haplotype SNPs do not affect significantly the gene expression.

Anyway, SNP *IL6*$^{-174}$G>C has been related to various infectious disease [25, 53]; although the mechanism through which it influences the manifestation of the disease is unknown. More recently, Castellucci *et al*. [54] associated it to

mucosal/ cutaneous leishmaniasis susceptibility; Budak *et al*. [55] found it as a potential factor of susceptibility to brucellosis; Buraczynska *et al.* [56] emphasized it as a possible risk factor for quick progression of chronic glomerulonephritis to final stage renal disease, and Moreira *et al.* [57] associated it to periodontal disease severity.

CONCLUSION

The *IL6*$^{-174}$GC genotype was observed at the present study as a resistance marker to DF, although the mechanism through which it would exert protective action against the disease is still a matter of debate.

ACKNOWLEDGEMENTS

Thanks to Maringá's Health Secretariat Epidemiologic Vigilance for the access to the patients' records; to the patients who participated in the study, to both Karen Machado and Fábio Machado for helping with sample collection, and to Maria Christina da Rocha Moliterno for paper revising.

REFERENCES

[1] Figueiredo LTM, Fonseca BAL. Dengue. In: Veronesi R, Focaccia R, Eds. Tratado de Infectologia. São Paulo, SP: Atheneu 1996; 201-14.

[2] Henchal EA, Putnak JR. The dengue viruses. Clin Microbiol Rev 1990; 3: 376-96.

[3] Rothman AL, Ennis FA. Immunopathogenesis of Dengue Hemorrhagic Fever. Virology 1999; 257: 1-6.

[4] Rigau-Perez JG, Clark GG, Gubler DJ, *et al.* Dengue and dengue Hemorrhagic fever. Lancet 1998; 352: 971-7.

[5] World Health Organization. Strengthening implementation of the global strategy for Dengue fever and Dengue haemorrhagic fever, prevention and control. Report in the informal consultation. Geneva: WHO HQ 1999: 18-20.

[6] World Health Organization [homepage on the Internet]. Geneva, Switzerland: World Health Organization [updated 2008 Aug 01; cited 2008 Aug 05]. Available from: http://www.who.int/mediacentre/factsheets/fs117/en/

[7] Chaturvedi UC, Nagar R, Shrivastava R. Dengue and dengue haemorrhagic fever: implications of host genetics. FEMS Immunol Med Microbiol 2006; 47: 155-66.

[8] Chaturvedi UC, Agarwal R, Elbishbishi EA, Mustafa AS. Cytokine cascade in dengue hemorrhagic fever: implications for pathogenesis. FEMS Immunol Med Microbiol 2000; 28: 183-8.

[9] Reynard MP, Turner D, Navarrete CV. Allele frequencies of polymorphisms of the tumour necrosis factor-alpha, interleukin-10, interferon-gamma and interleukin-2 genes in a North European Caucasoid group from the UK. Eur J Immunogenet 2000 27: 241-9.

[10] Mira JP, Cariou A, Grall F, *et al.* Association of TNF2, a TNF-alpha promoter polymorphism, with septic shock susceptibility and mortality: a multicenter study. JAMA 1999; 282: 561-8.

[11] Read RC, Camp NJ, Di Giovine FS, *et al.* An interleukin-1 genotype is associated with fatal outcome of meneingococcal disease. J Infect Dis 2000; 182: 1557-60.

[12] Fernández-Mestre MT, Gendzekhadze K, Rivas-Vetencourt P, Layrisse Z. TNF-alpha-308A allele, a possible severity risk factor of hemorrhagic manifestation in dengue fever patients. Tissue Antigens 2004; 64: 469-72.

[13] Chan HL, Tse AM, Chim AM, *et al.* Association of cytokine gene polymorphisms and liver fibrosis in chronic hepatitis B. J Gastroenterol Hepatol 2008; 23: 783-9.

[14] Chen TY, Hsieh YS, Wu TT, *et al.* Impact of serum levels and gene polymorphism of cytokines on chronic hepatitis C infection. Transl Res 2007; 150: 116-21.

[15] Ribeiro CS, Visentainer JE, Moliterno RA. Association of cytokine genetic polymorphism with hepatites B infection evolution in adult patients. Mem Inst Oswaldo Cruz 2007; 102: 435-40.

[16] Probst CM, Bompeixe EP, Pereira NF, *et al.* HLA polymorphism and evaluation of European, African, and Amerindian contribution to the white and mulatto populations from Paraná, Brazil. Hum Biol 2000; 72: 597-617.

[17] Woolf B. On estimating the relation between blood group and disease. Ann Hum Genet 1995; 19: 251-3.

[18] Turner DM, Williams DM, Sankaran D, Lazarus M, Sinnott PJ, Hutchinson IV. An investigation of polymorphism in the interleukin-10 gene promoter. Eur J Immunogenet 1997; 24: 1-8.

[19] Fishman D, Faulds G, Jeffry R, *et al.* The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. J Clin Invest 1998; 102: 1369-76.

[20] Awad MR, El-Gamel A, Hasleton P, Turner DM, Sinnott PJ, Hutchinson IV. Genotypic variation in the transforming growth factor-β1 gene. Transplantation 1998; 66: 1014-20.

[21] Pravica V, Asderakis A, Perrey C, Hajeer A, Sinnott PJ, Hutchinson IV. *In vitro* production of IFN-gamma correlates with CA repeat polymorphism in the human IFN-gamma gene. Eur J Immunogenet 1999; 26: 1-3.

[22] Meenagh A, Williams F, Ross OA, *et al.* Frequency of cytokine polymorphisms in populations from Western Europe, Africa, Asia, the Middle East and South America. Hum Immunol 2002; 63: 1055-61.

[23] Warlé MC, Farhan A, Metselaar HJ, *et al.* Are cytokine gene polymorphism related to *in vitro* cytokine production profiles? Liver Transpl 2003; 9: 170-81.

[24] Yilmaz V, Yentür SP, Saruhan-Direskeneli G. IL-12 and IL-10 polymorphisms and their effects on cytokine production. Cytokine 2005; 30: 188-94.

[25] Haukim N, Bidwell JL, Smith AJP, *et al.* Cytokine gene polymorphism in human disease: on-line databases. Genes Immunol 2002; 3(6 Suppl 2): S313-30.

[26] Mira JP, Cariou A, Grall F, *et al.* Association of TNF2, a TNF-alpha promoter polymorphism, with septic shock susceptibility and mortality: a multicenter study. JAMA 1999; 282: 561-8.

[27] Read RC, Camp NJ, Di Giovine FS, *et al.* An interleukin-1 genotype is associated with fatal outcome of meningococcal disease. J Infect Dis 2000; 182: 1557-60.

[28] Ribeiro CS, Visentainer JE, Moliterno RA. Association of cytokine genetic polymorphism with hepatites B infection evolution in adult patients. Mem Inst Oswaldo Cruz 2007; 102: 435-40.

[29] Chen TY, Hsieh YS, Wu TT, *et al.* Impact of serum levels and gene polymorphism of cytokines on chronic hepatitis C infection. Transl Res 2007; 150: 116-21.

[30] Bidwell J, Keen L, Gallagher G, *et al.* Cytokine gene polymorphism in human disease: on-line databases. Genes Immun 1999; 1: 3-19.

[31] Rood MJ, van Krugten MV, Zanelli E, *et al.* TNF-308A and HLA-DR3 alleles contribute independently to susceptibility to systemic lupus erythematosus. Arthritis Rheum 2000; 43: 129-34.

[32] Marshal SE, Mclaren AJ, Mckinney EF, *et al.* Donor cytokine genotype influences the development of acute rejection after renal transplantation. Transplantation 2001; 71: 469-76.

[33] Fernandes H, Koneru B, Fernandes N, *et al.* Investigation of promoter polymorphisms in the tumor necrosis factor-α and interleukin-10 genes in liver transplant patients. Transplantation 2002; 73: 1886-91.

[34] Cavet J, Dickinson AM, Norden J, Taylor PR, Jackson GH, Middleton PG. IFN-γ and interleukin-6 gene polymorphisms associate with graft-versus-host disease in HLA-matched sibling bone marrow transplantation. Blood 2001; 98: 1594-600.

[35] Visentainer JEL, Lieber SR, Persoli LBL, *et al.* Relationship between cytokine gene polymorphisms and graft-versus-host disease after allogeneic stem cell transplantation in a Brazilian population. Cytokine 2005; 32: 171-7.

[36] Gagnon SJ, Mori M, Kurane I, *et al.* Cytokine gene expression and protein production in peripheral blood mononuclear cells of children with acute dengue virus infections. J Med Virol 2002; 67: 41-6.

[37] Chen LC, Lei HY, Liu CC, *et al.* Correlation of serum levels of macrophage migration inhibitory factor with disease severity and clinical outcome in dengue patients. Am J Trop Med Hyg 2006; 74: 142-7.

[38] Fernandez-Real JM, Broch M, Vendrell J, Richart C, Ricart W. Interleukin-6 gene polymorphism and lipid abnormalities in healthy subjects. J Clin Endocrinol Metab 2000; 85: 1334-9.

[39] Pieroni F, Lourenço DM, Morelli VM, Maffei FH, Zago MA, Franco RF. Cytokine gene variants and venous thrombotic risk in the bratros (Brazilian thrombosis study). Thromb Res 2007; 120: 221-9.
[40] Van Snick J. Interleukin-6: an overview. Annu Rev Immunol 1990; 8: 253-78.
[41] Matsuda T, Hirano T. Interleukin 6 (IL-6). Biotherapy 1990; 2: 363-73.
[42] Terry CF, Loukaci V, Green FR. Cooperative influence of genetic polymorphisms on interleukin 6 transcriptional regulation. J Biol Chem 2000; 275: 18138-44.
[43] Juffrie M, Meer GM, Hack CE, *et al*. Inflammatory mediators in dengue virus infection in children: interleukin-6 and its relation to C-reactive protein and secretory phospholipase A2. Am J Trop Med Hyg 2001; 65: 70-5.
[44] Naka T, Nishimoto N, Kishimoto T. The paradigm of IL-6: from basic science to medicine. Arthritis Res 2002; 4: S233-42.
[45] Pinto LM, Oliveira SA, Braga EL, Nogueira RM, Kubelka CF. Increased pro-inflammatory cytokines (TNF-alpha and IL-6) and anti-inflammatory compounds (sTNFRp55 and sTNFRp75) in Brazilian patients during exanthematic dengue fever. Mem Inst Oswaldo Cruz 1999; 94: 387-94.
[46] Romani L, Mencacci A, Cenci E, *et al.* Impaired neutrophil response and CD4+ T helper cell 1 development in interleukin 6 deficient mice infected with Candida albicans. J Exp Med 1996; 183: 1345-55.
[47] Tilg H, Dinarello CA, Mier JW. IL-6 and APPs: anti-inflammatory and immunossupressive mediators. Immunol Today 1997; 18(9): 428-32.
[48] Warlé MC, Farhan A, Metselaar HJ, *et al*. Are cytokine gene polymorphisms related to *in vitro* cytokine production profiles? Liver Transpl 2003; 9: 170-81.
[49] Endler G, Marsik C, Joukhadar C, *et al.* The interleukin-6 G(-174)C promoter polymorphism does not determine plasma interleukin-6 concentrations in experimental endotoxemia in humans. Clin Chem 2004; 50: 195-200.
[50] Hegedus CM, Skibola CF, Bracci P, Holly EA, Smith MT. Screening the human serum proteome for genotype-phenotype associations: an analysis of the IL-6 – 174G>C polymorphism. Proteomics 2007; 7: 548-57.
[51] Kilpinen S, Hulkkonen J, Wang XY, Hurme M. The promoter polymorphism of the interleukin-6 gene regulates interleukin-6 production in neonates but not in adults. Eur Cytokine Netw 2001; 12: 62-8.
[52] Rivera-Chavez F, Peters-Hybki DL, Barber RC, O'Keefe GE. Interleukin-6 promoter haplotypes and interleukin-6 cytokine responses. Shock 2003; 20: 218-23.
[53] Hollegaard MV, Bidwell JL. Cytokine gene polymorphism in human disease: on-line databases, supplement 3. Genes Immun 2006; 7(4 Suppl 3): S269-76.
[54] Castellucci L, Menezes E, Oliveira J, *et al*. IL6 -174 G/C promoter polymorphism influences susceptibility to mucosal but not localized cutaneous leishmaniasis in Brazil. J Infect Dis 2006; 194: 519-27.
[55] Budak F, Göral G, Heper Y, *et al.* IL-10 and IL-6 gene polymorphisms as potential host susceptibility factors in Brucellosis. Cytokine 2007; 38: 32-6.
[56] Buraczynska M, Jozwiak L, Ksiazek P, Borowicz E, Mierzicki P. Interleukin-6 gene polymorphism and faster progression to end-stage renal failure in chronic glomerulonephritis. Transl Res 2007; 150: 101-5.
[57] Moreira PR, Lima PM, Sathler KO, *et al*. Interleukin-6 expression and gene polymorphism are associated with severity of periodontal disease in a sample of Brazilian individuals. Clin Exp Immunol 2007; 148: 119-26.

Received: August 8, 2008 Revised: October 23, 2008 Accepted: October 23, 2008