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Interleukin-12B & interleukin-10 gene polymorphisms in pulmonary tuberculosis

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***Background & objectives*: Cytokines play an important role in anti-tuberculosis immune response. Skewing of immunity from protective to pathogenic may involve a shift in Th1-Th2 paradigm. Cytokine gene polymorphism is known to be associated with functional differences in cytokine regulation and altered clinical performance in a variety of diseases. The aim of this study was to know whether Interleukin-12B 3' UTR (Taq1) (A/C) and Interleukin-10 (-1082 G/A) gene polymorphisms were associated with susceptibility to pulmonary tuberculosis.**

***Methods*: IL -10 (-1,082 G/A) and IL-12B gene polymorphisms were studied in132 pulmonary TB (PTB) patients and 143 normal healthy subjects (NHS), using DNA based polymerase chain reaction (PCR) with sequence specific primers and restriction digestion.**

***Results*: The allelic as well as genotypic frequencies of Interleukin -10 (-1082) and Interleukin -12B (3'UTR Taq 1) did not differ significantly between the patients and controls.**

***Interpretation & conclusion*: Our findings suggested that IL -10 (-1082 G/A) and IL -12B 3'UTR (Taq I) (A/C) gene polymorphisms were not associated either with susceptibility or resistance to pulmonary tuberculosis in the south Indian population.**

Key words Cytokine - IL-10 - IL-12B - polymorphism

Mycobacterium tuberculosis is responsible for at least 1.5 million deaths per year worldwide. One third of the world's population is infected with *M. tuberculosis*[1]. But development of tuberculosis depends on complex environmental factors and host genetic factors. Case control studies have found significant associations between tuberculosis and gene polymorphisms like human leukocyte antigen (HLA)[2] and non-HLA genes such as natural resistance associated macrophage protein (NRAMP1)[3], vitamin D receptor (VDR)[4], mannose binding lectin (MBL)[5], interleukin-1 (IL-1)[6], IL-10[7] and IL-12 receptor[8]. Interaction between the pathogen and host immune response probably determines the development or resistance to tuberculosis. Moreover, cytokines play an important role in anti-tuberculosis immune response[9]. Studies on mouse models have equated Th1 response to protective cell-mediated immunity and Th2 response to disease status[10,11]. IL-12 is a regulatory cytokine which connects the innate and

adaptive host response to mycobacterium and exerts its effects mainly through induction of interferon gamma (IFN-γ)[12]. Deleterious mutations in genes of IL-12B encoding IL-12p40, a subunit of IL-12 and IL-12R have been shown to be associated with susceptibility to infections caused by poorly pathogenic mycobacterium[13]. Studies also have shown that IL-10, a Th2 cytokine is a potent inhibitor of T cell functions, Major histocompatibility complex (MHC) class II expression, antigen specific proliferation and IFN-γ synthesis[14]. Moreover, IL-10 is inversely correlated in human tuberculosis[15]. Hence allelic variations in the coding as well as regulatory regions of IL-10 and IL-12B genes that are associated with differential cytokine levels might be expected to have an impact on susceptibility to tuberculosis. In this study, we therefore investigated IL-10 (-1082 G/A) and IL-12B (3'UTR, Taq1 A/C) gene polymorphisms in pulmonary tuberculosis (PTB) patients and normal healthy subjects in south India.

Material & Methods

Patients attending Tuberculosis Research Centre (TRC), Chennai, from January 2004 to December 2005, with respiratory symptoms and radiographic abnormalities suggestive of PTB and sputum positive for *M. tuberculosis* by both smear and culture were included. Normal healthy subjects (NHS) included were staffs, trainees and students of TRC who volunteered to donate blood, were clinically normal at the time of blood collection. The study group consisted of 132 PTB patients (mean age ± SD: 35.5 ± 12.3 yr) and 143 NHS (mean age ± SD: 29.7 ± 9.5 yr). Among the 132 patients, 75 were males and in 143 NHS, 84 were males. The patients and the healthy normal subjects were south Indian Tamil speaking population of Dravidian descent living in and around Chennai, Tamil Nadu. Blood samples were collected after an informed consent was obtained. The study was approved by the ethical committee of the institute.

Venous blood (20 ml) was collected, defibrinated and the peripheral blood mononuclear cells (PBMC) were separated by Ficoll-Hypaque density gradient centrifugation[16]. DNA was extracted from a portion of lymphocytes as described earlier[17] and the concentration and purity of DNA estimated spectrophotometrically. IL-10 genotyping was studied using amplification refractory mutation system-polymerase chain reaction (PCR) method as described earlier[18]. Single nucleotide polymorphism (SNP) at the IL-12B 3'UTR (Taq I) was determined using PCR-RFLP as described previously[19].

Allele and genotype frequencies were calculated by direct allele and genotypic counts and expressed as percentage. Comparison between patients and controls was done using 2x2 contingency tables and χ^2 tests with Yates correction (Stat calc programme, Epi Info Version 6.04, CDC, Atlanta, GA, USA). The allele frequencies were also tested for Hardy-Weinberg equilibrium by calculating allele frequencies using Pearson's χ^2 tests with one degree of freedom[20].

Results & Discussion

The allele and genotype frequencies of IL-12B (A/C) polymorphism did not differ significantly between normal healthy subjects (NHS) and pulmonary tuberculosis patients (PTB) (*P*=0.98, 0.95 and 0.59 for genotypes CC, CA and AA respectively). Similarly, the allele and genotype frequencies of IL-10-1082 (G/A) polymorphism did not vary significantly between NHS

Table. Allele and genotype frequencies of IL-12 and IL-10 in normal healthy subjects (NHS) and pulmonary tuberculosis patients (PTB)

IL-12 and IL-10 polymorphisms	Allele frequencies		Genotypes	Genotype frequency % (n)	
	NHS (n=143)	PTB (n=132)		NHS (n=143)	PTB (n=132)
IL-12 p40 3'UTR	C - 0.42	0.43	CC	19.60 (28)	18.94 (25)
(A/C)	A - 0.58	0.57	CA	44.00 (63)	48.48 (64)
			AA	36.40 (52)	32.58 (43)
IL-10 (-1082 G/A)*	A - 0.74	0.77	AA	52.14 (73)	56.06 (74)
	G - 0.26	0.23	AG	43.57 (61)	41.67 (55)
			GG	4.29 (6)	2.27 (3)

n = number of subjects

Number in parentheses represents number of subjects positive in each group

* In case of IL-10 polymorphism, n=140, 3 failed PCR (NHS)

Differences in genotype frequency between PTB and NHS were not significant

and PTB (P=0.51, 0.84 and 0.55 for genotypes AA, AG and GG respectively) (Table). The genotypes of both the cytokine polymorphism were within Hardy-Weinberg equilibrium.

Candidate gene-based case-control studies found several single nucleotide polymorphisms (SNPs) with a moderate effect on risk of tuberculosis[21]. We studied the associations of single nucleotide polymorphisms present in two vital cytokine genes namely IL-12B (3'UTR Taq 1) and IL-10 (-1082). The genotypes of IL-12B (3'UTR Taq 1) and IL-10 (-1082) did not differ significantly between patient group and controls. Our results suggested a lack of association of these single nucleotide polymorphisms with tuberculosis. The results were in concordance with a study wherein no association between IL-12B 3'UTR A/C polymorphism and tuberculosis was observed both in whites as well as African American populations[22]. Similarly, no association between IL-10 (-1082) and tuberculosis in Spanish population has been reported[18]. Contrary to our results, association between IL-12B intron 2-repeat marker (ATT) as well as haplotypes composed of IL-12B promoter, intron 2, intron 4 and 3'UTR alleles with tuberculosis has been reported in Hong Kong Chinese population[23]. Moreover, heterozygosity for the -1082 polymorphism of the IL-10 promoter has been shown to be associated with tuberculosis susceptibility in a Cambodian population[7]. The apparent inconsistency between these studies could be due to ethnic-specific genetic variations greatly influencing host immunity to tuberculosis, causing differential susceptibility to tuberculosis.

Since human susceptibility to tuberculosis is a complicated polygenic trait, interactive effects between the polymorphism studied and other SNPs in human genes (including those in IFN-γ network), need to be evaluated.

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References

1. World Health Organization (WHO). Tuberculosis Fact Sheet No. 104-Global and regional incidence. Geneva: World Health Organization; 2006.
2. Uma S, Selvaraj P, Kurain SM, Reetha AM, Narayanan PR. HLA-DR2 subtypes and immune responses in pulmonary tuberculosis. *Indian J Med Res* 2001; *113* : 117-24.
3. Bellamy R, Ruwende C, Corrah T, McAdam KP, Whittle HC, Hill AV. Variations in the NRAMP1 gene and susceptibility to tuberculosis in West Africans. *N Engl J Med* 1988; *338* : 640-4.
4. Selvaraj P, Chandra G, Kurain SM, Reetha AM, Narayanan PR. Association of Vitamin D receptor gene variants of Bsml, Apal and Fokl polymorphisms with susceptibility or resistance to pulmonary tuberculosis. *Curr Sci* 2003; *84* : 1564-68.
5. Selvaraj P, Narayanan PR, Reetha AM. Association of functional mutant homozygotes of the mannose binding protein gene with susceptibility to pulmonary tuberculosis in India. *Tuber Lung Dis* 1999; *79* : 221-7.
6. Bellamy R, Ruwende C, Corrah T, McAdam KP, Whittle HC, Hill AV. Assessment of the interleukin 1 gene cluster and other candidate gene polymorphisms in host susceptibility to tuberculosis. *Tuber Lung Dis* 1998; *79* : 83-9.
7. Delgado JC, Baena A, Thim S, Goldfeld AE. Ethnic-specific genetic associations with pulmonary tuberculosis. *J Infect Dis* 2002; *186* : 1463-8.
8. Akahoshi M, Nakashima H, Miyake K, *et al*. Influence of interleukin-12 receptor b1 polymorphism on tuberculosis. *Hum Genet* 2003; *112* : 237-43.
9. Flynn J, Chan J. Immunology of tuberculosis. *Annu Rev Immunol* 2002; *22* : 345-52.
10. Andersen P, Herron L. Specificity of a protective memory immune response against *Mycobacterium tuberculosis*. *Infect Immun* 1993; *61* : 844-51.
11. Orme IM, Roberts AD, Griffin JP, Abrams JS, Cytokine secretion by CD4 T lymphocytes acquired in response to *Mycobacterium tuberculosis* infection. *J Immunol* 1993; *151* : 518-25.
12. Reinout van Crevel, Tom HM, Ottenhoff, Jos WM van der Meer. Innate immunity to *Mycobacterium tuberculosis*. *Clin Micro Rev* 2002; *15* : 294-309.
13. Tom HM, Ottenhoff, Frank AW Verreck, Marieke A Hoeve, Esther Van De Vosse. Control of human host immunity to mycobacteria. *Tuberculosis* 2005; *85* : 53-64.
14. Howard M, Garra AO. Biological properties of IL-10. *Immunol Today* 1992; *13* : 198-80.
15. Torres M, Herrera T, Villareal H, Rich EA, Sada E. Cytokine profiles for peripheral blood lymphocytes from patients with active pulmonary tuberculosis and healthy household contacts in response to the 30-kilodalton antigen of *Mycobacterium tuberculosis*. *Infect Immun* 1998; *66* : 176-80.
16. Boyum A. Separation of leucocytes from blood and bone marrow. Introduction. *Scand J Clin Lab Invest Supplement* 1968; *97* : 7.
17. Miller S, Dykes D, Polesky H. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988; *16* : 1215.
18. Dolores Lopez-Maderuleo, Francisco Arnalich, Racio Serantes, Alicia Gonzalez, Rosa Codoceo, Rosario Madero, *et al*. Interferon gama and interleukin-10 gene polymorphisms in pulmonary tuberculosis. *Am J Respir Crit Care Med* 2003; *167* : 970-5.

19. Huang D, Cancilla MR, Morahan G. Complete primary structure, chromosomal localization and definition of polymorphisms of the gene encoding the human interleukin-12 p40 subunit. *Genes Immunity* 2000; *1* : 515-20.

20. Pak Sham. *Statistics in human genetics*. Great Britain: Arnold; 1998 p. 39-41.

21. Bellamy R. Identifying genetic susceptibility factor for tuberculosis in African: a combined approach using candidate gene study and a genome-wide screen. *Clin Sci* 2000; *98* : 245-50.

22. Xin Ma, Robert A Reich, Omar Gonzalez, I Pan, Amanda K Fothergill, Jeffery R Starke, *et al*. No evidence for association between the polymorphism in the 3' untranslated region of interleukin-12B and human susceptibility to tuberculosis. *J Intect Dis* 2003; *188* : 1116-8.

23. Hoi W Tso, Yu Lung Lau, Cheuk M Tam, Hing S Wong, and Alan KS. Chiang. Associations between IL12B Polymorphisms and tuberculosis in the Hong Kong Chinese Population. *J Infect Dis* 2004; *190* : 913-9.

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