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Interleukin-10 (IL-10) gene polymorphism as a potential host susceptibility factor in tuberculosis

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Abstract

Several genes encoding for different cytokines may play crucial roles in host susceptibility to tuberculosis (TB), since the cytokine production capacity varies among individuals and depends on the cytokine gene polymorphism. The association of the cytokine gene polymorphisms with the development of TB was investigated in this study. DNA samples were obtained from a Turkish population of 81 patients with the different clinical forms of TB, and 50 healthy control subjects. All genotyping (IL-6, IL-10, IFN-γ, TGF-β and TNF-α) experiments were performed using sequence-specific primers PCR (PCR-SSP). Analysis of allele frequencies showed that IL-10 −1082 G allele frequency was significantly more common in TB patients than healthy controls (37.7% vs 23.0%, *p*: 0.014). No statistically significant differences were observed betwe\en the different clinical forms of the disease. These results suggest that the polymorphisms in IL-10 gene may affect susceptibility to TB and increase risk of developing the disease. To confirm the biological significance of our results, further studies should be performed on other population groups.

Keywords: Cytokine; Genotyping; PCR-SSP; Tuberculosis

1. Introduction

Tuberculosis (TB) remains to be a leading cause of morbidity and mortality in developing countries. However, the incidence of disease increases in developed countries [1]. One third of the world's population is infected by *Mycobacterium tuberculosis* but only about 5% of the infected people develop disease within the first year of infection (primary TB) and other 5% develop the disease later in life (reactivation TB). At present it is impossible to predict in whom the disease will develop and progress through several stages from mild to severe. There are several reports demonstrating that host genetic factors play significant roles in susceptibility to TB [2]. Therefore, the identification of host genes responsible for susceptibility and resistance to TB should provide a significant contribution for understanding of the pathogenesis and may lead to the development of new prophylaxis and treatment strategies.

Cytokines produced at the site of disease after interactions between T lymphocytes and infected macrophages are essential for the pathogenesis of TB [3]. The course of *M. tuberculosis* infection is regulated by two distinct T cell cytokine patterns. T helper 1 (Th1) cytokines, IL-2 and IFN-γ, are associated with resistance to infection, whereas Th2 cytokines, IL-4 and IL-13, are associated with progressive disease [4]. In addition, IL-10, one of the T regulatory cytokines, seem to play a pivotal role during the chronic/latent stage of pulmonary TB, with increased production playing a potentially central role in

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promoting reactivation of TB [5]. Another T regulatory cytokine TGF-β, mainly produced by Th3 cells, may be beneficial or detrimental by inducing fibrosis or by contributing cavity formation, respectively [6]. Of fundamental immunologic importance are the factors, that influence the nature of cytokine response, such as polymorphisms of cytokine genes. Polymorphisms in several cytokine genes have been described and demonstrated to influence gene transcription, leading to interindividual variations in cytokine production [7,8]. Cytokine gene polymorphisms have been shown to be involved in the susceptibility, severity and clinical outcome of several diseases including infectious ones [7–10].

The aim of this work was to determine whether there is any association between cytokine gene polymorphisms and susceptibility to TB. We performed a study in Turkish patients affected with different clinical forms of TB and healthy control subjects to determine the influence of single nucleotide polymorphisms in five cytokines (IL-6, IL-10, IFN-γ, TGF-β and TNF-α) on susceptibility and disease expression.

2. Materials and methods

2.1. Patients and controls

Blood samples, collected in ethylenediamine tetraacetate sterile tubes, were obtained from 81 Turkish patients affected by different forms of TB (Table 1). TB was diagnosed on the basis of radiographic and clinical presentation, positive special staining and cultures for *M. tuberculosis* and, in some cases, the finding of caseating granulomas in biopsies. A control group was composed of 50 healthy organ donors, matched for age and sex, ethnicity and from the same geographical area as the patients. The study was approved by the Ethical Committee of Uludağ University, Bursa, Turkey, and all subjects gave written informed consent.

2.2. DNA isolation and cytokine genotyping

Genomic DNA was extracted from blood samples by using Puregene Genomic DNA isolation kit (Gentra Systems, Minneapolis, USA) according to the manufacturer's instructions.

Table 1
Distribution of patients with clinical forms of TB

Clinical forms	Number of patients (%)
Pulmonary TB	29 (35.8)
TB pleurisy	22 (27.2)
Renal TB	9 (11.1)
TB lymphadenitis	9 (11.1)
Central nervus system TB	8 (9.9)
Genital system TB	2 (2.5)
TB thyroiditis	1 (1.2)
TB peritonitis	1 (1.2)

Single nucleotid polymorphisms were analyzed in five cytokines (IL-6, IL-10, IFN-γ, TGF-β and TNF-α) for genotype assignment. A single nucleotide polymorphism at position −174 of the promoter region was analyzed for the IL-6. Three different polymorphisms were examined for the IL-10 promoter region: position −1082 (G *vs* A), position −819 (C *vs* T), and position −592 (A *vs* C). The presence of a single nucleotide modification at position +874 was examined for IFN-γ. Two single nucleotide mutations in coding region were analyzed for TGF-β: codon 10 can be either T or C, and codon 25, either C or G. A single nucleotide polymorphism at position −308 of the promoter region (either A or C for both) were surveyed for TNF-α.

All genotypes were determined with the use of PCR-sequence specific primers (PCR-SSP) method by a commercially available kit (One lambda, Inc., Canoga Park, CA, USA) in accordance with the manufacturer's instructions. This kit contains specific primers to detect the polymorphisms of several cytokines, cytokine receptors and receptor antagonsits mentioned above. The DNA extractions and PCR amplifications were performed by a technician blinded to the study groups.

2.3. Statistical analysis

Statistical analysis was performed by Epi Info Software Version 3.2.2 (CDC, Atlanta GA, USA). The distribution of cytokine genes polymorphisms were compared between patients with TB and healthy controls by the χ^2 or Fisher's exact test. P values smaller than 0.05 were considered significant. Odds ratios (OR) and 95% confidence intervals (CI) were also calculated in case of that χ^2 or Fisher's exact test was significant. The data were analysed for appropriateness between the observed and expected genotype values and their fit to Hardy–Weinberg equilibrium, Arlequin Software v. 2000, (University of Geneve, Switzerland). Also, significant probability values obtained were corrected for multiple testing (Bonferroni correction; *Pc*).

3. Results

We evaluated the frequencies of cytokine gene polymorphisms in patients affected by different clinical forms of TB (Table 1) and in healthy volunteers.

The frequency of IL-10 (−1082, −819, −592) GCC/ACC *genotype* was significantly higher in TB patients when compared with those of healthy controls (37.0% *vs* 20.0%, *p*: 0.04), whereas significantly lower frequencies of IL-10 ACC/ATA genotype was observed in the patient group (14.8% *vs* 32.0%, *p*: 0.02). Statistical significant differences for both polymorphisms, however, were lost after Bonferroni correction of the *p* values (*Pc*) (Table 2).

Analysis of allele frequencies of IL-10 polymorphisms at positions −1082, −819, −592 demonstrated that IL-10 −1082 G allele frequency were significantly more common in TB patients than those of healthy controls (37.7% vs 23.0%, *p*: 0.014). Statistical significant difference was

Table 2
Cytokine gene polymorphisms among TB patients and healthy controls

Cytokine gene polymorphisms	Genotype	Patients *n* (%)	Controls *n* (%)	*p* values (*Pc*)
TNF-α (−308)	G/G	63 (77.8)	33 (66.0)	>0.05
	G/A	18 (22.2)	14 (28.0)	>0.05
	A/A	0 (0.0)	3 (6.0)	>0.05
TGF-β1 (codon 10–25)	T/T–G/G	15 (18.5)	15 (30.0)	>0.05
	T/C–G/G	30 (37.0)	20 (40.0)	>0.05
	T/C–G/C	11 (13.6)	2 (4.0)	>0.05
	C/C–G/G	14 (17.3)	8 (16.0)	>0.05
	T/T–G/C	0 (0.0)	1 (2.0)	>0.05
	C/C–G/C	7 (8.6)	1 (2.0)	>0.05
	C/C–C/C	2 (2.5)	0 (0.0)	>0.05
	T/T–C/C	0 (0.0)	2 (4.0)	>0.05
	T/C–C/C	2 (2.5)	1 (2.0)	>0.05
IL-10 (−1082, −819, −592)	GCC/GCC	10 (12.3)	5 (10.0)	>0.05
	GCC/ACC	30 (37.0)	10 (20.0)	0.04 (0.22)
	GCC/ATA	11 (13.6)	3 (6.0)	>0.05
	ACC/ACC	8 (9.9)	9 (18.0)	>0.05
	ACC/ATA	12 (14.8)	16 (32.0)	0.02 (0.11)
	ATA/ATA	10 (12.3)	7 (14.0)	>0.05
IL-6 (−174)[a]	G/G	48 (59.3)	27 (55.1)	>0.05
	G/C	27 (33.3)	13 (26.5)	>0.05
	C/C	6 (7.4)	9 (18.4)	>0.05
IFN-γ (+874)	T/T	21 (25.9)	8 (16.0)	>0.05
	T/A	29 (35.8)	21 (42.0)	>0.05
	A/A	31 (38.3)	21 (42.0)	>0.05

[a] The genotyping results of 49 healthy controls were included due to a technical problem.

Table 3
The frequencies of IL-10 alleles at positions −1082, −819 and −592 in TB patients and healthy controls

Alleles	Patients (%)	Controls (%)	*p* value (*Pc*)	OR (95% CI)
IL-10 (−1082)				
G	61 (37.7)	23 (23.0)	0.014 (0.028)	2.02 (1.11–3.70)
A	101 (62.3)	77 (77.0)		
IL-10 (−819)				
C	119 (73.5)	67 (67.0)	>0.05	
T	43 (26.5)	33 (33.0)		
IL-10 (−592)				
C	119 (73.5)	67 (67.0)	>0.05	
A	43 (26.5)	33 (33.0)		

still kept after Bonferroni correction of the *p* values (*Pc*) (Table 3).

No statistically significant differences in the distribution of TNF-α, TGF-β, IL-6 and IFN-γ gene polymorphisms were observed between patients and controls (Table 3). Analysis of allele frequencies of TNF-α, TGF-β, IL-6 and IFN-γ genes also did not show any statistically significant differences between patients and controls (data not shown). In addition, further subgroup analysis was performed, and so no statistically significant differences in the distribution of any of cytokine alleles, genotypes or haplotypes were detected between the patients with different clinical forms of TB (data not shown).

4. Discussion

Single nucleotide polymorphisms in several candidate genes have been linked to relatively increased risk for TB [9]. In this polymorphism-association study, we investigated the significance of the relationship between several cytokine gene polymorphisms and susceptibility to TB.

Interleukin-10 is a multifunctional cytokine first described as cytokine synthesis inhibitory factor, which inhibits IFN-γ cytokine production by Th1 cells in mice [11,12]. It inhibits monocyte/macrophage function during inflammation by downregulating the production of pro-inflammatory cytokines, such as IL-12 and TNF-α, and suppressing the surface expression of major histocompatability class II [13,14]. IL-10 can also inhibit $CD4^+$ T cell chemotaxis towards IL-8 [11,15] and T cell apoptosis [16], by leading to Bcl-2 up-regulation. Interestingly, it can induce the proliferation of $CD8^+$ T cells [17]. The suppressor effect of IL-10 on T cells has been recently shown to be directed to block CD28 signaling cascade and subsequent phosphatidylinositol 3-kinase activation in T cells [15]. Since modulation of T cell responses by

IL-10 seems to influence the susceptibility of the host to TB infection [18], determining the polymorphisms in IL-10 genes may be beneficial for predicting the disease susceptibility. In fact, the −1082 polymorphism of IL-10 promoter was reported to be associated with TB susceptibility in the Cambodian population [19]. Similarly to our data, decrease in the frequency of IL-10 −1082 allele A suggested to be associated with the progression of lung TB [20]. In a recent study, IL-10 −1082 A/A genotype was demonstrated to be associated with TB pleurisy, which is a self-cured clinical form of TB [21]. In contrast, no differences in IL-10 genotype frequencies among TB patients were observed in some other studies [22,23].

IFN-γ is produced as a result of the activation of early immune response mechanisms and subsequently by antigen-specific T cells during the course of *M. tuberculosis* infection [24]. The critical role of IFN-γ and its receptor in TB have been demonstrated in both animal models and humans [25,26]. IFN-γ knock-out mice were found to be highly susceptible to *M. tuberculosis* infection [25]. Therefore, persons having a genotype associated with a low IFN-γ producer status would be expected to show increased susceptibility to TB. Indeed, Lio et al. observed that +874 T/T genotype, which has been suggested to be associated with high IFN-γ production [27], was significantly decreased in the patients with lung TB [28]. Another study demonstrated that the individuals with IFN-γ +874 A/A genotype had a 3.75-fold increased risk of developing pulmonary TB [22]. In contrary, our study demonstrated no association between the polymorphisms at position +874 of IFN-γ gene and disease susceptibility.

TNF-α is another cytokine which can be synergic with IFN-γ to active macrophages [29]. Mice deficient in TNF-α has been shown to fail to form organised granuloma, which resulted in widespread dissemination of *M. tuberculosis* and the rapid death of the infected animals [30]. Additionally, recent data obtained from the rheumatoid arthritis patients treated with TNF-α antagonists revealed that blocking the effect of TNF-α can lead to reactivation of TB [31]. Therefore, it can be concluded that TNF-α is essential cytokine for granuloma formation. In harmony with this, Scola et al. reported a significantly increased frequency of TNF-α −308 A "low producer" allele in chronic TB patients [20]. However, no association or linkage of TB with TNF-α gene polymorphisms was demonstrated in most studies [32,33]. Consistent with the latter, no association was also found between TNF-α gene polymorphism at position −308 and the susceptibility to TB in our study.

TGF-β is produced by mainly activated macrophages, in response to tissue injury [34]. It is a potent immunosuppressive cytokine, which inhibits macrophage activation and modulates T cell function [35]. Additionally, it plays a role in tissue fibrosis by leading to increase in the synthesis of extracellular matrix components [36,37]. The presence of TGF-β in TB granulomas may be beneficial since it can induce fibrosis and seal off TB lesions from surrounding healthy tissue. However, this effect may also be detrimental by seeding the potential for cavitation and subsequent reactivation [6]. To date, similarly to our data, no significant association between TGF-β gene polymorphisms and TB was reported.

The role of IL-6 in the immune response to *M. tuberculosis in vivo* is not well established. In previous studies, significant levels of IL-6 are produced in response to *M. tuberculosis* infection; human and murine macrophages secrete IL-6 in response to *M. tuberculosis in vitro* [38,39], and elevated concentrations of IL-6 are present in plasma of patients with TB [40]. It was also reported that induction of IL-6 activity by *M. tuberculosis* leads to inhibition of IFN-signaling and therefore evade eradication by a cellular immune response [41]. To date, there are no reports on association between IL-6 polymorphisms and TB [21]. In our study, no difference was shown in IL-6 −174 polymorphism between TB patients and healthy controls.

Our results demonstrate that the polymorphisms in IL-10 gene may affect susceptibility to TB and increase risk of developing the disease. The polymorphisms of IL-10 gene may be valuable markers to predict the risk for the development of TB.

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