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Common interleukin 10 polymorphism associated with decreased risk of tuberculosis

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Abbreviations: IL10, interleukin10; SNP, single nucleotide polymorphism; TB, tuberculosis

Abstract

Interleukin 10 (IL10) is a powerful TH2-cell cytokine that inhibits lymphocyte replication and secretion of inflammatory cytokines. The genetic associations of polymorphisms in IL10 with clinical manifestations of tuberculosis (TB) were examined in a large number of patients with clinical TB infection (n = 459) and normal controls (n = 871). One common promoter SNP (IL10 -592 A>C) was found to be significantly associated with decreased risk of TB manifestation. The frequency of the "C"-bearing genotype was higher in normal controls than in patients with clinical TB infection (P = 0.005, OR = 0.69). A summary of the genetic effect of IL10 -1082 A>G, the other nearby promoter SNP, in other ethnic groups is also presented.

Keywords: IL10; polymorphism; tuberculosis

Introduction

Tuberculosis (TB) is a major public health problem globally (Nunn, 2001). Approximately one-third of the world's population is infected with the bacterium, *Mycobacterium tuberculosis*, that causes TB. This makes TB a significant cause of morbidity/mortality, as it results in approximately 2 million deaths annually (Dye *et al.,* 1999). However, only 10% of those infected are estimated to progress to active TB disease. Host genetic factors are important determinants of susceptibility to tuberculosis (Casanova and Abel, 2002). The doubly high risk of disease in identical twins compared with nonidentical twins (Comstock, 1978) indicates a host genetic component in susceptibility. Understanding the molecular mechanisms underlying protective immunity is a prerequisite for the development of improved therapies and vaccines for tuberculosis.

It is likely that host susceptibility to TB is at least partly under polygenic control. Previous genetic studies have reported that the major histocompatibility complex (MHC) genes have a role in influencing resistance and susceptibility to TB in human populations (Thursz *et al.,* 1995, Ghosh *et al.,* 2004). More recent reports have also identified a number of non-MHC gene polymorphisms that are associated with resistance or susceptibility to TB, including the natural resistance associated with the macrophage protein (*NRAMP1*) and the vitamin D receptor genes (*VDR*) (Bellamy *et al.,* 1998; 1999).

IL10 is a powerful TH2-cell cytokine produced by lymphoid cells that exerts its function by inhibiting macrophage/monocyte and T-cell lymphocyte replication and secretion of inflammatory cytokines (Redpath *et al.,* 2001; Choi *et al.,* 2003). Two SNPs in the promoter region, *IL10 -1082 A>G* and *-592 A>C*, are particularly interesting, because several previous reports have demonstrated quantitative differences in IL10 transcription and/or expression mediated by alternative alleles or haplotypes. Differences in nuclear-binding activity and IL10 production mediated by the *IL10 -592 A>C* polymorphism (Rosenwasser and Borish, 1997; Shin *et al.,* 2000) have also been reported.

In this study, one promoter SNP in IL10 is shown to be associated with the decreased risk of TB through examination of a large number of patients with clinical TB infection (n = 459) and normal controls (n = 871) recruited from the Korean population.

Materials and Methods

Study subjects

A total of 459 patients with clinical pulmonary tuberculosis (mean age, 46.9 years; range, 18-86 years) were recruited from the Clinical Research Center for Tuberculosis, National Masan Tuberculosis Hospital, Korea. The diagnosis of pulmonary tuberculosis was confirmed by the isolation of acid fast bacilli (AFB) from sputum or bronchoalveolar lavage fluid. TB patients who had a family history of the

disease were also excluded to eliminate the additional risk factors of exposure to TB. A total of 871 healthy controls (mean age, 56.1 years; range, 50-81 years) were simultaneously recruited from an unselected population who had come in for routine health check-ups in the same regional area. Only subjects whose ages were greater than 50 were included in normal controls to exclude the possibility of TB infection among young individuals (TB may subsequently develop in a proportion of the controls). Individuals with other apparent disease such as HIV, hepatitis (mainly chronic hepatitis B infection), autoimmune diseases, diabetes, alcoholism, and cancers were excluded from the study (cases and controls). Ethnicity of all patients and controls was Korean. Informed consents were obtained from all subjects before drawing blood. The study protocol was approved by the Institutional Review Board of National Masan Hospital. Written informed consent was obtained from each subject.

Genotyping

The sequences of amplifying and extension primers for IL10 SNP genotyping of *IL10 -1082 A>G, IL10 -819 T>C, IL10 -592 A>C*, and *IL10 +117 T>C* are the following: forward-5'-ccaactggctccccttaccttctac-3', reverse-5'-caggattccatggaggctgg-3', extension-5'-actttcctcttacctatccctacttcccc-3'; forward-5'-gggtgaggaaaccaaattctcag-3', reverse-5'-ggtagtgctcaccatgacccc-3', extension-5'-gtaccttgtacaggtgatgtaa-3'; forward-5'-ggtggaaacatgtgcctgag-3', reverse-5'-ctcaaagttcccaagcagcc-3', extension-5'-ttccattttactttccagagactggcttcctacag-3'; forward-5'-atagctgacccagcccctt-3', reverse-5'-aaatcgttcacagagaagctcagt-3', and extension-5'-gctcagtaaataaatagaaatgggggttgaggtatcagaggtaataaatattctat-3', respectively. Single-base extension methods were used for genotyping of SNPs in *IL10* as described previously (Makridakis and Reichardt, 2001). Genotyping quality control was performed in 10% of samples by duplicate checking (rate of concordance in duplicates was > 99%).

Statistics

Haplotypes of each individual were inferred using the algorithm developed by Stephens *et al.* (2001) (PHASE). Logistic regression models were used for calculating odds ratios (95% confidential interval) and corresponding *P*-values, controlling for age (continuous value), sex (male = 0, female = 1), and smoking status (non-smoker = 0, ex-smoker/smoker = 1) as covariates.

Results

The frequencies of minor alleles of four *IL10* SNPs were 0.07 (*IL10 -1082 A>G*), 0.31 (*IL10 -819 T>C*), 0.31 (*IL10 -592 A>C*), and 0.03 (*IL10 +117 T>C*), respectively, in the Korean population studied (n = 1,330). *IL10 -819 T>C* was excluded from analysis because it was in absolute LD with *IL10 -592 A>C*. Genotype distributions in all loci were in Hardy-Weinberg equilibrium ($P > 0.05$) (data not shown).

Table 1. Analyses of association of IL10 gene polymorphisms with the risk of tuberculosis in the Korean population.

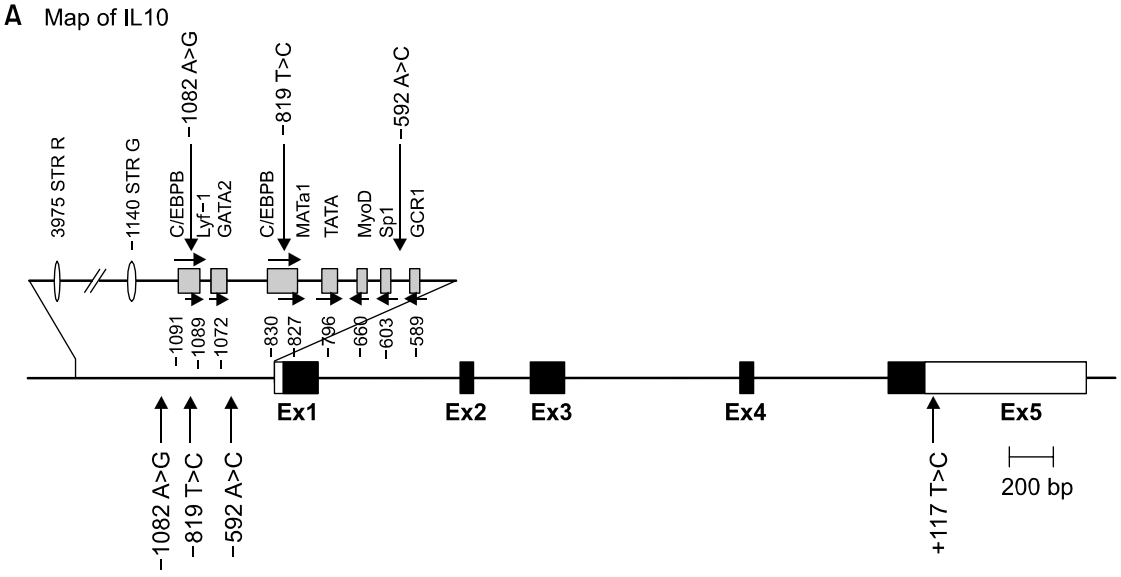
Locus	Genotype	Diagnosis				Additive analysis		Dominant analysis**	
		TB patients (n = 459)	Freq.*	Normal controls (n = 871)	Freq.*	OR (95% CI)	*P*	OR (95% CI)	*P*
-1082	A	394 (87.8%)		718 (84.4%)					
A>G	AG	53 (11.8%)	0.06	124 (14.6%)	0.08	0.78 (0.54-1.11)	0.16	0.78 (0.53-1.15)	0.22
	G	2 (0.5%)		9 (1.1%)					
-592	A	238 (52.9%)		376 (44.2%)					
A>C	AC	173 (38.4%)	0.28	384 (45.1%)	0.33	0.78 (0.63-0.95)	0.02	0.69 (0.53-0.89)	0.005
	C	39 (8.7%)		91 (10.7%)					
+117	T	433 (94.8%)		801 (92.7%)					
T>C	CT	23 (5.0%)	0.03	60 (6.9%)	0.04	0.72 (0.42-1.23)	0.23	0.70 (0.40-1.23)	0.22
	C	1 (0.2%)		3 (0.4%)					
ht2	-/-	275 (62.5%)		464 (56.0%)					
	ht2/-	144 (32.7%)	0.21	319 (38.5%)	0.25	0.81 (0.64-1.02)	0.07	0.75 (0.57-0.98)	0.04
	ht2/ht2	21 (4.8%)		46 (5.5%)					

Note: Logistic regression models were used for calculating odds ratios (95% confidential interval), corresponding *P*-values for SNP sites and haplotypes, controlling for age, sex and smoking status as covariates. *P*-values of additive and dominant analyses are also given. *IL10 -819T>C* was excluded in this further analysis because it was in absolute LD with *IL10 -592A>C*. Only one haplotype (ht2) was analyzed because of equivalence of haplotypes 1 and 3 with *IL10 -592* and *IL10 +117*, as well as relatively low frequency (ht4, freq = 0.038) (Figure 1B). Linkage disequilibrium analyses (|D| and r^2) among normal controls showed that all SNPs were tightly linked.
*Minor allele frequencies, **Dominant analysis: homozygotes for the major allele *vs.* heterozygotes+homozygotes for the minor allele.

Table 2. Comparison of the genetic effect of IL10 -1082A>G on the risk of TB infection across previous studies.

Reference	Population	TB patients					Controls					*P*
		AA	AG	GG	Freq.*	HWE**	AA	AG	GG	Freq.*	HWE**	
Delgado *et al.*	Cambodian	86	259	11	0.39	<0.00001	39	64	3	0.33	0.0008	0.03
Lopez-Maderuelo *et al.*	Spanish (White)	33	47	33	0.50	0.20	21	50	29	0.54	0.99	NS
Scola *et al.*	Italian (Sicilian)	17	22	6	0.38	0.97	24	77	13	0.45	0.0005	0.05
Bellamy *et al.*	Gambian	165	185	51	0.36	0.99	179	184	45	0.34	0.98	NS
This study	Korean	394	53	2	0.06	0.99	718	124	9	0.08	0.39	NS

*Frequencies of the G allele of *IL10 -1082 A>G*, **P - values of deviation from Hardy-Weinberg equilibrium.



B Haplotypes in IL10

Haplotype	−1082	−819	−592	+117	Frequencies
ht 1	A	T	A	T	0.703
ht 2	A	C	C	T	0.230
ht 3	G	C	C	C	0.029
ht 4	G	C	C	T	0.038

C LDs among SNPs in IL10

		|D'|			
	SNPs	−1082	−819	−592	+117
r^2	−1082	−	1	1	1
	−819	0.16	−	1	1
	−592	0.16	1	−	1
	117	0.40	0.07	0.07	−

Figure 1. Map and haplotype of the IL10 gene on chromosome 1q31-32. (A) Coding exons are marked by shaded blocks and 5' and 3'UTR by white blocks. Putative transcription factor sites are indicated (molsun1.cbrc.aist.go.jp/research/db/TFSEARCH.html, putative score > 0.9). The positions were calculated from the transcriptional start site (*IL10 -1082, IL10 -819 and IL10 -592*) and from the first nucleotide (+1) of 5'UTR (*IL10 +117 T > C*). (B) IL10 haplotypes constructed from four SNPs and their frequencies in the Korean population (n = 1,330). Genotype distributions in all loci were in Hardy-Weinberg equilibrium ($P > 0.05$). Haplotypes 1 and 3 are equivalent with *IL10 -592* and *IL10 +117*, respectively. The *IL10* haplotypes were constructed using PHASE haplotype inference software [12]. (C) Analysis of linkage disequilibrium; |D'| and r^2 among four SNPs in *IL10*. Lewontins D' (|D'|) and linkage disequilibrium (LD) coefficient r^2 between all pairs of biallelic loci were calculated.

Four haplotypes were identified without any ambiguous phasing due to LDs among SNPs (Figure 1B). Only one haplotype (ht2) was analyzed due to the equivalence of haplotypes 1 and 3 with *IL10 -592* and *IL10 +117*, respectively, as well as low frequency (ht4, freq = 0.038) (Figure 1B). Linkage disequilibrium

analyses (|D| and r^2) among normal controls showed that all SNPs were tightly linked (Figure 1).

Logistic regression analyses controlling for age (continuous value), sex (male = 0, female = 1), and smoking status (non-smoker = 0, ex-smoker/smoker = 1) as covariates revealed significant associations of *IL10 -592 A>C* and one common (freq. = 0.24) haplotype (ht2[A-C-C-T]) with decreased risk of clinical tuberculosis disease. The frequency of *-592C*-bearing genotypes (AC or CC of IL10 *-592 A>C*) was higher in normal controls (55.8%) than in patients (47.1%) (P = 0.005, OR = 0.69). Similar genetic effects from IL10-ht2, which was constructed from a combination of *IL10 -1082 A>G* and *-592 A>C* (Figure 1B), were also observed (P = 0.04, OR = 0.75) (Table 2).

In order to evaluate the genetic effect of *IL10 -1082 A>G*, a nearby SNP in the promoter region, which has been controversial, we have compared the results of previous studies. We found that the frequencies of *IL10 -1082 A>G* were much higher in Caucasian than in Asian (Korean) populations. Marginal associations had been reported in Cambodian (Delgado *et al.,* 2002) and Italian populations (Scola *et al.,* 2003), whereas no significant associations in Spanish (Lopez-Maderuelo *et al.,* 2003), Gambian (Bellamy *et al.,* 1998), and Korean populations (this study) have been found.

Discussion

The World Health Organization (WHO) has estimated the incidence of TB (the number of new cases arising each year) to be more than 8.7 million, and that 2 million deaths resulted from TB in 2002 alone. HIV and TB form a lethal combination, each speeding the other's progress. HIV weakens the immune system; thus, someone who is HIV-positive and infected with TB is many times more likely to become sick with TB than someone infected with TB who is HIV-negative. TB is a leading cause of death among people who are HIV-positive. It accounts for about 13% of AIDS deaths worldwide. In Africa, HIV is the single most important factor contributing to the increased incidence of TB in the past 10 years (Please see http://www.who.int/mediacentre/factsheets/fs104/en/).

A puzzling feature of mycobacterial infection is that clinically evident disease occurs in only a small proportion of those who are infected. However, the mechanisms that distinguish a successful immune response from an ineffective response remain poorly understood. Familial clustering, racial differences in incidence, and twin studies suggest that genetic factors play a role in susceptibility (Comstock, 1978;, Fine, 1981).

IL10, produced mainly by macrophages, acts as a potent immunosuppressive cytokine by down-regulating the expression of Th1 cytokines and co-stimulatory molecules (Redpath *et al.,* 2001). Several positive associations of IL10 polymorphisms with various infectious diseases have previously been detected (Opdal, 2004). The genetic involvement of IL10 polymorphisms in infectious diseases, and TB in this study, suggest that *IL10* polymorphisms play a critical role in immunity and in inflammation progress.

By screening a large number of TB patients in the Korean population, we are able to suggest, for the first time, that one IL10 promoter SNP (*IL10 -592A>C*) and one common haplotype (*ht2*[A-C-C-T]) are significantly associated with decreased risk of clinical TB disease. When considering that *IL10 -ht2* is mostly (>93%) tagged by the C allele of *IL10 -592 A>C* (Figure 1B), it seems very likely that the genetic effects of IL10-ht2 come from *IL10 -592 A>C*. Although the protective mechanisms against clinical TB disease by increased IL10 are not fully understood, several clues were reported. Much higher serum IL10 was detected in patients with clinical TB (Barnes *et al.,* 1993), suggesting that IL10 plays a role in susceptibility to tuberculosis.

On the other hand, although the power to detect was relatively low (48% for *IL10 -1082A>G* [freq.= 0.067] and 31% for *+117 T>C* [freq = 0.038, equivalent with ht4] in the Korean population [459 patients and 871 controls]), we could not detect a significant association of *IL10 -1082 A>G* with the risk of TB in the Korean population (459 patients *vs.* 871 controls, Table 2). The associations of *IL10 -1082 A>G* have been examined previously in separate studies (Bellamy *et al.,* 1998; Delgado *et al.,* 2002; Lopez-Maderuelo *et al.,* 2003; Scola *et al.,* 2003). The previous researchers examined only the genetic effect of the *IL10 -1082 A>G* polymorphism, and discrepancies were observed among these studies. Specifically, two positive (elgado *et al.,* 2002; Scola *et al.,* 2003) and two negative (Bellamy *et al.,* 1998; Lopez-Maderuelo *et al.,* 2003) associations had been reported.

Although it is hard to decipher the discrepancies among studies on the effect of *IL10 -1082 A>G* on TB, one possible explanation might be the low sample sizes (cases or controls) and/or marginal significances in two studies in which positive associations were detected, *i.e.*, 462 subjects (356 cases *vs.* 106 controls, P = 0.03) (Delgado *et al.,* 2002) and 159 subjects (45 cases *vs.* 114 controls, P = 0.05) (Scola *et al.,* 2003). Another possible explanation might be the different genetic and/or environmental backgrounds among populations. It is also worth noting that 1) allele frequencies of *IL10 -1082G** were much higher in Caucasian (freq.>0.36) (Lopez-Maderuelo *et al.,* 2003; Scola *et al.,* 2003) than in Asian (Korean) populations (freq.= 0.07 in this study; results from Delgado *et al.* (2002) were excluded because severe deviation from HWE (<0.0001), even in normal controls, was observed), and 2) complete LD (|D|=1 and $r^2 \neq 1$) was observed between *IL10 -1082 A>G* and *-592 A>C*.

In summary, one common promoter SNP (*IL10 -592 A>C*) was found to have significant association with decreased risk of tuberculosis infection through screening of a large number of TB patients (n = 459) and normal controls (n = 871) recruited from the

Korean population. The genetic effects of *IL10 -1082 A>G*, which have been controversial, were also presented.

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