Interleukin-10 gene polymorphisms are associated with the SLICC/ACR Damage Index in systemic lupus erythematosus

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Objective. Overproduction of interleukin-10 (IL-10) is a pivotal feature in the pathophysiology of systemic lupus erythematosus (SLE). We examined the IL10 genotype of Korean patients with SLE and normal controls to determine whether associations exist between the pattern of inherited IL10 genes and SLE susceptibility or the SLICC/ACR Damage Index (SDI).

Methods. A total of 350 Korean SLE patients and 330 healthy subjects were enrolled. Direct DNA sequencing and primer extension procedures were employed. Logistic regression analyses were performed to examine the genetic association with SLE and SDI.

Results. Eight sequence variants were identified by direct DNA sequencing in 24 Korean individuals. Five of the polymorphisms were selected for larger scale genotyping (n = 680) by considering their allele frequencies, haplotype-tagging status and linkage disequilibrium coefficients among polymorphisms. Haplotypes and allele distributions of the IL10 polymorphisms did not differ significantly between SLE patients and controls. Among identified SNPs, the rare C allele of IL10-592A→C was significantly associated with the SDI among SLE patients in the following three alternative models: codominant (P = 0.007, odds ratio = 1.70), dominant (P = 0.02, odds ratio = 1.85) and recessive (P = 0.05, odds ratio = 2.25). Similarly, IL10+955T→G and IL10-h2 were significantly associated with the SDI in the codominant and dominant models.

Conclusion. IL10 polymorphisms are not associated with disease susceptibility in Korean patients with SLE. However, IL10-592A→C, IL10+955T→G and IL10-h2 are significantly associated with the SDI, suggesting that IL10-592C, IL10+955G and IL10-h2 accelerate the damage induced by SLE.

Key words: IL10, Polymorphism, SLE, Damage.

Systemic lupus erythematosus (SLE) is a prototypic autoimmune disease with a diverse array of clinical manifestations, and it is characterized by the production of antibodies to components of the cell nuclei [1]. Most cases of SLE are sporadic without identifiable genetic predisposing factors. However, the concordances of the disease in identical and dizygotic twins are approximately 25 and 2%, respectively [2]. This suggests that genetic factors play an important role in the predisposition to SLE.

Interleukin-10 (IL-10) is a 36-kDa homodimeric cytokine produced by mononuclear cells. It inhibits T cells by suppressing the expression of Th1 cytokines and it enhances the survival, proliferation and differentiation of B cells. The role of IL-10 in the pathogenesis of SLE is unknown, but the increased production of IL-10 in sporadic cases of SLE [3, 4] and the clinical improvements in SLE patients resulting from the administration of an anti-IL-10 monoclonal antibody [5] support a central role for IL-10 in the pathogenesis of SLE. The IL10 gene has been mapped to human chromosome 1, between 1q31 and 1q32, and this region is known as a major SLE susceptibility locus (LOD = 3.79) [6]. However, the genetic studies of IL10 in SLE have produced contradictory results, some showing significant associations of IL10 promoter polymorphisms and microsatellites with SLE, but other studies indicate that these SNPs might be important in the development of certain clinical features, such as disease activity, renal involvement and the production of antibodies rather than susceptibility in SLE. Although these clinical factors might be correlated with irreversible damage in patients, no study has investigated the association between IL10 gene polymorphisms and such damage.

In the present study, therefore, we scrutinized sequence variations in the IL10 gene in the Korean population (n = 24), and then examined the IL10 genotype of Korean patients with SLE (n = 350) and normal controls (n = 330) to evaluate associations between the pattern of inherited IL10 genes and SLE susceptibility or damage.
Association of IL10 polymorphism with SLICC/ACR Damage Index

Patients and methods

Patients and normal subjects

A total of 350 Korean patients who fulfilled the 1997 ACR (American College of Rheumatology) criteria for SLE [7] were consecutively enrolled from the Hospital for Rheumatic Diseases, Hanyang University, Seoul, Korea. Among them, 335 (95.7%) were female and the mean age at baseline was 35.6 yr (10.5–72.7). The mean duration of disease since diagnosis was 29.1 months (6.0–63.7). As a control group, we included 330 healthy ethnically matched subjects in order to examine the genetic association with susceptibility to SLE. Written informed consent was obtained from each subject. The study was approved by the Institutional Review Board of Hanyang University Medical Center.

Antinuclear antibodies were tested by indirect immunofluorescence using IT-1 cells, anti-dsDNA antibodies by the Crithidia luciliae assay, and anti-Sm, -SSA (Ro), -SSB (La), and -RNP (ribonuclear protein) antibodies by double immunodiffusion. The SLICC (Systemic Lupus International Collaborating Clinics)/ACR Damage Index (SDI) was used to quantify the accumulated damage in these patients [8, 9]. Early and no damage were defined as scores of ≥1 and 0 at the assessment, respectively.

Sequencing analysis of the IL10 gene

We sequenced the exons and their boundaries of the IL10 gene, including the promoter region (~1.0 kb), to discover genetic variants in 24 Korean DNA samples using a DNA analyser (ABI Prism 3700; Applied Biosystems, Foster City, CA, USA). The VariantSEQ™ Resequencing System (Applied Biosystems) and the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kits (Applied Biosystems) were used for comparative sequencing in accordance with the recommendations of the manufacturer. Information regarding primers is available on our website (http://www.snp-genetics.com/user/additional_list.asp). Sequence variants were verified by chromatograms.

Primer extension procedure

For genotyping of polymorphic sites, amplification and extension primers were designed for single-base extension (SBE) [10]. Primer extension reactions were performed with the SNPshot ddNTP Primer Extension Kit (Applied Biosystems). Information regarding primers is available on our website (http://www.snp-genetics.com/user/additional_list.asp). We performed a genotyping quality control in 10% of samples by duplicate checking. The rate of concordance in duplicates was →99%.

Statistics

The χ² test was used to compare the observed numbers of each genotype with those expected for a population in Hardy–Weinberg equilibrium (HWE). The following widely used measures of linkage disequilibrium (LD) were examined between all pairs of biallelic loci: Lewontin’s D’ (|D’|) and the measure r². Logistic regression models were used to calculate the odds ratios (ORs), 95% confidential intervals and the corresponding P values of codominant, dominant and recessive models whilst controlling for age and sex as covariates.

Results

Genetic variants in the IL10 gene

Eight sequence variants were identified: two in the promoter (~819T→C and −592A→C), four in introns (+955T→G, +1117A→G, +1568T→C, and +2700G→A) and two in the 3’ UTR region (+3871T→C and +3952T→C). The locations of nine SNPs (including −1082A→G, another well-known polymorphism in the promoter region) relative to the genomic structure of the IL10 gene are shown in Fig. 1A. Four polymorphisms, including two well-known polymorphisms in the promoter region (~819T→C and −592A→C) and two polymorphisms in introns (+1117A→G and +1583T→C), were in absolute LD (|D’| = 1 and r² = 1). A new polymorphism, an A→G transition in intron 1 at position +955, with a relatively high frequency (0.316), was identified. Other SNPs of the IL10 gene with low frequencies (+2700G→A, 0.025; +3871T→C, 0.05; and +3952T→C, 0.025) were also identified in our Korean population.

Five of these polymorphisms, including IL10-1082A→G, were selected for larger scale genotyping (n = 680) and statistical analysis based on their allele frequencies (>5%), haplotype-tagging status and LD coefficients among polymorphism. A well-known IL10 SNP, +3952T→C, was included in the statistical analysis despite its low frequency. In contrast, IL10-819T→C, IL10-1171A→G and IL10+1583T→C were excluded from further analyses because they were in absolute LD with IL10-592A→C.

Haplotypes and their frequencies were inferred using the algorithm developed by Stephens et al. [11]. Two major haplotypes account for more than 90% of the six haplotypes observed (Fig. 1B). Haplotypes either equivalent to a single polymorphism (IL10-h1t1 and IL10-h1t3) or with frequencies less than 3% (IL10-h5t and IL10-h6t) were excluded from further statistical analyses. Figure 1C lists the LD coefficients (|D’| and r²) for SNP loci in the IL10 genes in our Korean population.

IL10 gene polymorphisms and susceptibility to SLE

The genotype distributions of IL10 gene SNPs were compared between SLE patients and normal subjects with multiple logistic regression models. We excluded SNPs that showed significant deviation from HWE in a susceptibility analysis (~592A→C and +955T→G). No significant genetic associations were observed between other variants and the risk of SLE.

IL10 gene polymorphisms and SDI

An SDI of 0 was recorded in 236 of the 350 patients. The other 114 patients (32.6%) were assigned the following SDI values: 1 (n = 69), 2 (n = 34), 3 (n = 9), 4 (n = 1) and 5 (n = 1). Damage was primarily present in the renal, musculoskeletal and neuropsychiatric systems. Associations of the presence of damage with IL10 gene polymorphisms and haplotypes were analysed. Among identified SNPs, the rare C allele of IL10-592A→C was significantly associated with the SDI among SLE patients in all three alternative models: codominant (P = 0.007, OR = 1.70), dominant (P = 0.02, OR = 1.85) and recessive (P = 0.05, OR = 2.25). Similarly, IL10+955T→G was significantly associated with the SDI in the codominant (P = 0.02, OR = 1.58) and dominant (P = 0.05, OR = 1.67) models. IL10-h2t1, a haplotype including IL10-592C and IL10+955G, also showed significant associations with the SDI in the codominant (P = 0.02, OR = 1.71) and dominant (P = 0.02, OR = 1.87) models. However, other SNPs and haplotypes were not associated with the SDI (Table 1). To reduce the corticosteroid effects on organ damage [12], we analysed the associations of IL10 gene polymorphisms and haplotypes with the SDI excluding the items osteoporotic fracture (n = 1), symptomatic coronary artery disease (n = 2) and cataracts (n = 9). IL10-592A→C, IL10+955T→G and IL10-h2t1 still showed significant associations with the SDI (data not shown).
A. Map of IL10 (Interleukin 10) on chromosome 1q31-q32 (5kb)

B. Haplotypes in IL10

C. LDs among IL10 SNPs

Fig. 1. Gene maps and haplotypes of IL10. Coding exons are marked by black blocks and 5’ and 3’ UTR by white blocks. The first base of transcriptional start site was denoted as nucleotide +1. Asterisks (*) indicate polymorphisms genotyped in a larger population (n = 680). The frequencies of polymorphisms without larger scale genotyping were based on sequencing data (n = 24). (A) Map of IL10 gene on chromosome 1q31-q32 (Genome Seq. NT_021877.16). (B) Frequencies of IL10 gene haplotypes constructed from four SNPs and their frequencies in both SLE patients and controls (n = 680). (C) LD coefficients (D’ and r²) among IL10 SNPs.

Discussion

IL-10 is a pleiotropic cytokine with diverse effects on most haemopoietic cell types. The principal physiological function of IL-10 appears to be to limit inflammatory responses, but it also regulates growth and/or differentiation of B cells, natural killer cells, cytotoxic and helper T cells, mast cells and granulocytes [13]. Peripheral blood mononuclear cells (PBMCs) from SLE patients spontaneously produce large amounts of IL-10 in vitro as well as large amounts of IL-10 mRNA ex vivo.

There have been attempts to elucidate the molecular basis of increased production of IL-10 in SLE patients. Khoa et al. [14] demonstrated that the frequency of the G allele at IL10 promoter −1082 was higher in Vietnamese SLE patients than in healthy controls. Gibson et al. [15] also reported a positive association of SNPs in the IL10 promoter region in African–American SLE patients. However, these associations were not found in Mexican, British, and southern Chinese SLE patients. These ethnicity-related differences prompted us to determine the presence of SNPs using direct DNA sequencing and to analyse the association in the Korean population.

The frequencies of minor alleles of IL10 SNPs at the loci −1082A→G, −819T→C and −592A→C were 8.6, 34 and 34%, respectively, in the present study. These results are similar to those of the recent study of IL10 polymorphisms and the risk of tuberculosis in the Korean population (n = 1330), with frequencies at these loci of 7.3 and 31%, respectively [16]. Among them, especially, the frequency of the IL10-1082A→G allele differs greatly between Korean and other ethnic groups. Although IL10-1082G is a major allele in Europeans (50%), Africans (59.5%) and Hispanic Americans (67.4%), only 8.6% of Koreans in the present study carried IL10-1082G.

Although we observed no genetic association between SNPs, including −1082G, and the risk of SLE, we could not deduce any conclusion because the normal controls diverged from HWE for IL10-592C (P = 0.005) and IL10+955G (P = 0.01).

Several studies have yielded functional data showing that the level of IL-10 production is related to IL10 promoter haplotypes. Kurreeman et al. [17] suggest that IL10-592C and haplotype 2, containing ACC (−1082/−819/−592), are related to the high levels of IL-10 in SLE. Decreased production of IL-10 by PBMCs or whole blood is associated with the IL10-592A allele or its associated haplotype, ATA (−1082/−819/−592) [18]. Among the identified SNPs we examined, the C allele of IL10-592A→C was significantly associated with the SDI in SLE patients. Similarly, IL10+955T→G and IL10-h2 also showed associations with the SDI. The fact that IL10+955T→G was in almost absolute LD with IL10-592A→C and IL10-h2 containing IL10-592C and IL10+955G in our study suggests that IL10-592C is mainly related to the production of a large amount of IL-10 and, consequently, damage in SLE. However, the frequencies of IL10 gene polymorphisms and estimated haplotypes did not differ in patients in the presence of each antibody and renal involvement (data not shown).

The effects of IL10 polymorphisms on SDI were not dramatic in the present study. Therefore, it may be argued that Bonferroni correction should be applied to the P values obtained. If Bonferroni correction were strictly adopted, associated P values could not retain the significances [the threshold of significance would be 0.004 (six polymorphisms and two phenotypes)]. However, although there is a chance of type I error due to multiple comparisons, when considering the facts that the comparisons were not totally independent of each other due to tight LD among SNPs/haplotype, the significance of association
<table>
<thead>
<tr>
<th>Gene</th>
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Logistic regression models were used to calculate the ORs, 95% CIs and the corresponding P values of codominant (minor allele homozygotes vs heterozygotes vs major allele homozygotes), dominant (minor allele homozygotes plus heterozygotes vs major allele homozygotes), and recessive (minor allele homozygotes vs heterozygotes plus major allele homozygotes) models whilst controlling for age and sex as covariates. The common alleles were used as the reference genotype to the heterozygote and homozygote of the minor allele. P values of codominant, dominant and recessive models are also given. Significant associations are shown in bold.
might be noticeable, especially in $-592A \rightarrow C$ ($P = 0.007$, codominant model for SDI score).

The exact contribution of \( IL10\)-592C and haplotypes containing \( IL10\)-592C to irreversible damage in SLE (as quantified by the SDI) is unclear. However, it is in the anticipated direction with the finding that \( IL10\)-592C and haplotype 2 are known to be associated with the increased production of IL-10 as described before. In addition, a recent study on the progression of chronic hepatitis B to liver cirrhosis and hepatocellular carcinoma in Korean subjects suggests a similar result [19]. That study showed a significant association of disease progression in hepatitis B patients with certain \( IL10\) haplotypes ($-1082A/-819C/-592C/+117T$) containing \( IL10\)-592C, which suggests that \( IL10\)-592C and haplotypes containing ACC ($-1082A/-819C/-592C$) are related to irreversible changes in each disease. Therefore, future studies should investigate the association between IL-10 production and irreversible damage, such as fibrosis in SLE patients, and additional research is required to determine the contribution of IL-10 production to the pathogenesis of specific types of organ damage.

In conclusion, \( IL10\)-592A$\rightarrow$C, \( IL10\)+955T$\rightarrow$G and \( IL10\)-ht2 are significantly associated with the SDI, suggesting that \( IL10\)-592C, \( IL10\)+955G and \( IL10\)-ht2 accelerate the damage induced by SLE.

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