Lack of Association of Interleukin-6 and Interleukin-8 Gene Polymorphisms in Chinese Patients With Systemic Lupus Erythematosus

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The purpose of this study was to determine whether interleukin (IL)-6 and IL-8 gene polymorphisms were markers of susceptibility to or severity of systemic lupus erythematosus (SLE) in Chinese patients. The study included 150 Chinese patients with SLE. A total of 130 unrelated healthy individuals living in central Taiwan served as control subjects. Polymorphisms of the IL-6 and IL-8 gene were typed from genomic DNA. The genotypes, allelic frequencies, and carriage rates were compared between SLE patients and control subjects. The relationship between allelic frequencies and clinical manifestations of 135 SLE patients was evaluated. There

were no statistically significant differences in IL-6 and IL-8 gene polymorphisms between the SLE and control groups (chi-squared test, $P=0.53$, $\chi^2=1.27$ and $P=0.44$, $\chi^2=1.62$, respectively). In addition, there was no significant association between the two groups in allelic frequency of IL-6 and IL-8 ($P=0.89$ and $P=0.26$, respectively). We also did not detect any association between the IL-6 and IL-8 genotype and clinical or laboratory profiles in SLE patients. The results suggest that the IL-6 and IL-8 gene polymorphisms are not related to SLE. *J. Clin. Lab. Anal.* 20:255–259, 2006. © 2006 Wiley-Liss, Inc.

Key words: IL-6; IL-8; SLE; gene polymorphism

INTRODUCTION

Systemic lupus erythematosus (SLE) is a prototypic immune complex-mediated disease with a broad spectrum of autoimmune phenomena and clinical symptoms. Many immunologic abnormalities have been found in SLE patients, including impaired T cell responses and dysregulation of B cell activation, leading to B cell hyperactivity and overproduction of autoantibodies (1,2). Cytokines are also known to be involved in the pathogenesis of SLE. It has been shown that alternation in cytokine production and cytokine networks contribute to tolerance defects, B-cell hyperactivity, and impaired cellular immunity in SLE.

Proinflammatory cytokines such as interleukin-1 (IL-1), IL-6, IL-8, and tumor necrosis factor- α (TNF- α) are produced primarily by activated macrophages and monocytes. IL-6, a multifunctional cytokine, plays

an important role in the regulation of acute phase response (3,4). Finck et al. (5) found that IL-6 accelerated the disease course of autoimmune New Zealand black/New Zealand white (NZB/NZW) F1 mice. Moreover, two studies demonstrated that serum levels of IL-6 are elevated in patients with SLE (6,7). The human IL-6 gene is located on chromosome 7p21. There are four naturally occurring haplotype polymorphisms in the IL-6 promoter (–174G→C, –373A (n)T(n), –572G→C, –597G→A) (8). One study suggested that the –373A(n)T(n) polymorphism influences rheumatoid

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arthritis (RA) disease progression (9), while another study found an association between the -174 G→C polymorphism and juvenile chronic arthritis (10). Furthermore, a study of postmenopausal women found that the -572G→C polymorphism influences levels of circulating C-reactive protein and bone resorption markers (11). Therefore, the -572G→C IL-6 gene polymorphism may be a potential genetic susceptibility factor that could be further evaluated with regard to the risk of SLE.

IL-8, a member of the CXC chemokine family, is a strong chemoattractant for neutrophils and lymphocytes (12,13). IL-8 is upregulated in IgA nephropathy, lupus nephritis, and antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (14–16). Furthermore, Rovin et al. (17) found that IL-8 -845C polymorphism is associated with severe lupus nephritis in African Americans. The human IL-8 gene is located on chromosome 4q12–q21. It contains four exons and three introns (18,19). The 3'-untranslated region (UTR) of the IL-8 gene has been suggested to contribute to its posttranscription. We hypothesized that genetic variation in IL-6 and IL-8 might be associated with the risk of developing SLE. Therefore, we studied the -572G/C polymorphism in the IL-6 gene and the A/G polymorphism in the 3'UTR of the IL-8 gene by comparing allelic and genotypic frequencies between 150 Chinese patients with SLE and 130 healthy individuals living in Taiwan. In addition, we also studied the association between IL-6 and IL-8 and SLE susceptibility and severity by focusing on single nucleotide polymorphisms (SNPs).

MATERIALS AND METHODS

Patient Selection

We enrolled 150 patients (136 women and 14 men) with definite SLE according to the 1982 revised American College of Rheumatology (ACR) criteria (20), and 130 sex-, age-matched healthy individuals (120 women and 10 men) living in central Taiwan served as the control subjects. The age of the SLE patients was 17–68 (mean 28.5 ± 2.9) years. The age of the volunteers was 18–71 (mean 29.3 ± 3.1) years. The duration of disease in SLE patients was 1–15 (mean 4.5 ± 1.4) years. Informed consent was obtained from each patient and control subject involved.

Polymerase Chain Reaction

The genomic DNA was prepared from peripheral blood by a Genomic DNA isolation reagent kit (Genomaker, Taiwan). Polymerase chain reaction (PCR) was

used to identify the genotypes of the IL-6 gene promoter region and IL-8 gene 3'-UTR polymorphisms. PCR of the polymorphisms was performed in a total volume of 50 μ L, containing genomic DNA (2–6 pmol of each primer), 1 $\times$ Taq polymerase buffer (1.5 mM $MgCl_2$), and 0.25 units of AmpliTaq DNA polymerase (Perkin Elmer; Foster City, CA). For the IL-6 gene promoter region, the primers used were upstream 5'-GCAAAGTCCTCACTGGGAGGA-3' and downstream 5'-TCTGACTCCATCGCAGCCC-3'. For the IL-8 gene 3'-UTR, the primers used were upstream 5'-CTTTAGTGTTTTATGTGCTCTCCA-3' and downstream 5'-GCAAATATGCTTAGGCTTTAACC-3'. The primers were selected according to the studies of Chung et al. (21), and Amoli et al. (22). PCR amplification was performed in a programmable PCR thermal cycler (GeneAmp PCR System 2400, Perkin Elmer). The PCR cycling conditions for IL-6 promoter -572 G/C polymorphism examination were as follows: 35 cycles at 95°C for 30 sec, 60°C for 30 sec, and 72°C for 45 sec; they were then left to stand at 72°C, then held at 4°C. The PCR cycling conditions for examination of the -2767A/G polymorphism in the 3'-UTR of the IL-8 gene were as follows: 35 cycles at 95°C for 30 sec, 55°C for 30 sec, and 72°C for 45 sec; they were then left to stand at 72°C, then held at 4°C.

The polymorphisms of the respective genes of interest were discerned by digestion with *Bsr*BI and *Apa*LI, respectively. The PCR products were mixed separately using the aforementioned enzymes and reaction buffer according to the manufacturer's instructions; both reactions were incubated for 3 hr at 37°C. After this, 10 μ L of each product was loaded into a 3% agarose gel containing ethidium bromide and subjected to electrophoresis. The resultant genotypes were classified into the categories of nonexcisable homozygote allele (CC and GG for IL-6 and IL-8 genes, respectively), excisable homozygote allele (GG and AA for IL-6 and IL-8 genes, respectively), and heterozygote allele (CG and AG for IL-6 and IL-8 genes, respectively).

Statistical Analysis

The genotype distributions, allelic frequencies, and carriage rates for the IL-6 gene and IL-8 gene polymorphisms for SLE patients and controls were compared using the chi-squared test. Results were considered statistically significant when the probability of findings occurring by chance was less than 5% ($P < 0.05$). The odds ratios (OR) were calculated from genotypic frequency and allelic frequency with a 95% confidence interval (CI) for the IL-6 gene and IL-8 gene polymorphisms.

RESULTS

The frequencies of the genotypes of IL-6 in the SLE and control groups are shown in Table 1. In the SLE group, 83 patients (55.3%) had the CC IL-6 genotype, 61 patients (40.7%) had the C/G genotype, and six patients (4.0%) had the GG genotype. In the control group, 76 people (58.5%) had the CC IL-6 genotype, 46 (35.4%) had the C/G IL-6 genotype, and eight (6.1%) had the GG IL-6 genotype. There were no significant differences in the distribution of the IL-6 gene C/G polymorphism between the SLE and control groups ($\chi^2 = 1.27$; $P = 0.53$). We also analyzed the relationship between the two groups in allelic frequencies and carriage rate of IL-6 (Table 1). There was no significant association in frequency ($P = 0.89$) and carriage rate ($P = 0.82$) between SLE patients and normal subjects.

Similarly, there was no significant difference in the genotype ($P = 0.44$), allelic frequency ($P = 0.26$) and carriage rate ($P = 0.52$) of IL-8 between SLE patients and normal subjects (Table 2).

TABLE 1. Comparison of IL-6 genotype distributions, allelic frequencies, and carriage rates observed in SLE patients and healthy control subjects*

IL-6	SLE patients n = 150 (%)	Controls n = 130 (%)	<i>P</i>	OR (95% CI)
Genotype				
CC	83 (55.3)	76 (58.5)	0.53	
C/G	61 (40.7)	46 (35.4)		
GG	6 (4.0)	8 (6.1)		
Allelic frequencies				
C	227 (75.7)	198 (76.2)	0.89	0.97 (0.67–1.42)
G	73 (24.3)	62 (23.8)		1.03 (0.70–1.49)
Carriage rate				
C	144 (96.0)	122 (93.8)	0.82	0.95 (0.63–1.44)
G	67 (44.7)	54 (41.5)		1.05 (0.69–1.59)

*All *P*-values represent chi-squared test results.

The clinical manifestations and laboratory findings of the SLE patients, given as ACR criteria, are shown in Tables 3 and 4. The association of IL-6 and IL-8 with particular clinical features of SLE was examined in the 135 patients. We did not find any relationship between IL-6 and IL-8 and severity of SLE (i.e., no association with renal or neurologic involvement) in the Chinese patients in this study.

DISCUSSION

The etiology of SLE is unknown, but both environmental and genetic factors are believed to be involved in the onset of the disease. It has been postulated that genetic polymorphisms affecting cytokine transcription exist in the regulatory regions of proinflammatory cytokine genes, and that such polymorphisms may control the level of inflammation.

Our study was undertaken to identify SNPs in the proinflammatory cytokine IL-6 and IL-8 genes. Very few studies of the relationship between IL-6 and IL-8

TABLE 2. Comparison of IL-8 genotype distributions, allelic frequencies and carriage rates observed in SLE patients and healthy control subjects*

IL-8	SLE patients n = 150 (%)	Controls n = 130 (%)	<i>P</i>	OR (95% CI)
Genotype				
AA	13 (8.7)	17 (13.1)	0.44	
A/G	70 (46.7)	61 (46.9)		
GG	67 (44.6)	52 (40.0)		
Allelic frequencies				
A	96 (32.0)	95 (36.5)	0.26	0.82 (0.58~1.15)
G	204 (68.0)	165 (63.5)		1.22 (0.87~1.73)
Carriage rate				
A	83 (55.3)	78 (60.0)	0.52	0.88 (0.59~1.29)
G	137 (91.3)	113 (86.9)		1.14 (0.77~1.68)

*All *P*-values represent chi-squared test results.

TABLE 3. Relationship between the IL-6 genotype and clinical signs and findings in patients with SLE

	CC n = 75 (%)	C/G n = 55 (%)	GG n = 5 (%)	Total n = 135 (%)	P
Malar rash	40 (53.3)	30 (55.6)	4 (80.0)	74 (54.8)	0.51
Discoid lupus	14 (18.7)	6 (11.1)	1 (20.0)	21 (15.6)	0.46
Photosensitivity	38 (50.7)	30 (55.6)	4 (80.0)	72 (53.3)	0.43
Mucosal ulcer	27 (36.0)	14 (25.9)	2 (40.0)	43 (31.9)	0.41
Arthritis	38 (50.7)	31 (57.4)	4 (80.0)	73 (54.1)	0.40
Serositis	17 (22.7)	9 (16.7)	1 (20.0)	27 (20.0)	0.67
Glomerulonephritis	30 (40.0)	18 (33.3)	2 (40.0)	50 (37.0)	0.69
Neuropsychiatric	11 (14.7)	4 (7.4)	1 (20.0)	16 (11.9)	0.37
Hematological	36 (48.0)	29 (53.7)	1 (20.0)	66 (48.9)	0.36
Immunology	61 (81.3)	42 (77.3)	3 (60.0)	106 (78.5)	0.47
ANA	73 (97.3)	54 (99.9)	5 (96.8)	132 (97.8)	0.89

ANA, antinuclear antibody.

TABLE 4. Relationship between the IL-8 genotype and clinical signs and findings in patients with SLE

	AA n = 11 (%)	A/G n = 63 (%)	GG n = 61 (%)	Total n = 135 (%)	P
Malar rash	5 (45.5)	38 (60.3)	31(50.8)	74 (54.8)	0.46
Discoid lupus	1 (9.1)	14 (22.2)	6 (9.8)	21 (15.6)	0.14
Photosensitivity	5 (45.5)	37 (58.7)	30 (49.2)	72 (53.3)	0.49
Mucosal ulcer	2 (18.2)	21 (33.3)	20 (32.8)	43 (31.9)	0.59
Arthritis	5 (45.5)	30 (47.6)	38 (62.3)	73 (54.1)	0.22
Serositis	4 (36.4)	9 (14.3)	14 (23.0)	27 (20.0)	0.18
Glomerulonephritis	7 (63.6)	21 (33.3)	22 (36.1)	50 (37.0)	0.15
Neuropsychiatric	1 (9.1)	9 (14.3)	6 (9.8)	16 (11.9)	0.71
Hematological	4 (36.4)	32 (50.8)	30 (49.2)	66 (48.9)	0.68
Immunology	10 (90.9)	49(77.8)	47 (77.0)	106 (78.5)	0.58
ANA	10 (90.9)	61 (96.8)	61 (100)	132 (97.8)	0.13

ANA, antinuclear antibody.

gene polymorphisms and SLE have been reported (17,23,24). Fugger et al. (23) demonstrated that there were no significant differences between healthy control groups and three disease groups [RA (n = 24), juvenile rheumatoid arthritis (JRA) (n = 48), and SLE (n = 30)]. Although the number of SLE patients was rather small in that study, the result was similar to that found in our present study (n = 150). Linker-Israeli et al. (24) showed that the 3' minisatellite alleles of the IL-6 gene have biologic significance in SLE patients. However, there was no statistical association between SLE patients (n = 146) and the alleles in the 5' promoter region (at -174) of the IL-6 gene. Rovin et al. (17) found that the IL-8 -854C polymorphism might predispose African Americans with SLE nephritis to more severe renal injury, perhaps by influencing IL-8 expression. In this study, we examined the relationship between the IL-6 promoter -572 G/C polymorphism and the -2767A/G polymorphism in the 3'-UTR of IL-8 gene and Chinese patients with SLE in Taiwan. In our present study, there were no significant differences in the distribution of IL-6 and IL-8 gene polymorphisms between subjects in the healthy control group and patients in the SLE group. There was also no significant association between the two groups in allelic frequency and carriage rate. Similarly, we did not find any relationship between IL-6 and IL-8 and severity of SLE (i.e., no association with renal or neurologic involvement) in Chinese patients.

There are two possible reasons for the lack of association between IL-6 and IL-8 gene SNP and SLE found in the present study that are worth further discussion. First, the number of patients (n = 150) and controls (n = 130) in the present study was relatively small, which may have caused a type II error. Therefore, a similar study involving more patients and controls should be conducted. Second, the cooperative influence of genetic polymorphisms on disease development should also be considered. Therefore, investigation of IL-6 and IL-8 gene SNPs alone may have been

inadequate to reveal an association. This argument is supported by a previous study that found that the base differences at distinct polymorphic sites influence each other and do not act independently (25). Further studies of the transcriptional control region as a whole in haplotype polymorphisms may give more information on the genetic basis of disease.

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