Polymorphisms of IL-1ß Gene in Japanese Patients with Sjögren's Syndrome and Systemic Lupus Erythematosus

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ABSTRACT. ***Objective.*** Interleukin (IL)-1ß is a proinflammatory cytokine involved in various immune responses. Five polymorphisms in the IL-1ß gene have been described, and relationships between these polymorphisms and some autoimmune diseases have been reported. Evidence suggests that IL-1ß may be involved in the destruction of salivary and lacrimal glands in Sjögren's syndrome (SS). We evaluated the significance of IL-1ß gene polymorphisms in SS.

Methods. Blood samples were taken from 101 patients with SS, 103 patients with systemic lupus erythematosus (SLE, excluding those with secondary SS), and 106 healthy volunteers. Each polymorphism of the IL-1ß gene was analyzed by polymerase chain reaction (PCR) amplification of the polymorphic site, followed by site-specific restriction digestion. Genotype frequencies of each polymorphism in SS patients were compared with those of the controls and SLE patients, and differences between primary and secondary SS patients were also compared.

Results. Genotypes CC, TT, and AA in positions –511, –31, and 3877, respectively, were significantly less frequent in SS patients than controls or patients with SLE. No significant differences were found in genotype frequencies of any of the polymorphisms between patients with primary SS and secondary SS.

Conclusion. IL-1ß gene polymorphisms may affect susceptibility to SS, but not SLE. (J Rheumatol 2004;31:720–5)

Key Indexing Terms:
INTERLEUKIN 1ß
SJÖGREN'S SYNDROME
GENE POLYMORPHISM
SYSTEMIC LUPUS ERYTHEMATOSUS

Interleukin (IL)-1ß is a proinflammatory cytokine involved in various immune responses, including autoimmune diseases[1]. The gene is located on chromosome 2q 13[2], and 5 polymorphisms have been described in the promoter regions (at –511: C/T)[3,4], TATA Box (at –31: T/C)[3,5], exon 4 (at +3263: C/T)[3,6], intron 4 (at +3877: A/G)[3,5], and exon 5 (at +3953: C/T)[3,7]. At least 3 of these, –511[8], –31[9], and +3953[7], are possibly related to IL-1ß production. It has been shown that the presence of the T allele at +3953 has a stimulatory effect on lipopolysaccharide (LPS)-induced IL-1ß protein production *in vitro*[7]. Santtila, *et al*[8] found that mononuclear cells derived from individuals with the T allele in the polymorphism at –511 and those without T allele in the polymorphism at 3953 showed slightly higher production of IL-1ß than other carriers, when stimulated by phorbol dibutyrate. The T allele at –31 of the IL-1ß gene comprises a TATA box-related gene structure, suggesting a role in gene expression[9].

Recent studies have described the relationships between these polymorphisms and various diseases such as rheumatoid arthritis (RA)[10-13], osteoporosis[6], osteoarthritis[14], inflammatory bowel diseases[15], gastric cancer[9], gastritis caused by *Helicobacter pylori*[16], multiple sclerosis (MS)[17], and alcoholic liver disease[18]. In autoimmune diseases, Buchs, *et al*[11] showed that patients with the T allele at +3953 show aggressive destructive arthritis compared to those without the T allele. On the other hand, Niino, *et al*[17] reported the lack of significant differences in the distribution of polymorphisms between Japanese patients with MS

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and controls. In addition, in their study, no association was observed between IL-1ß or IL-1ra gene polymorphisms and clinical characteristics. Huang, *et al*[19] also reported that the polymorphisms on promoter and exon 5 regions of the IL-1ß gene are not related to SLE in Taiwanese patients.

Sjögren's syndrome (SS) is an autoimmune disease characterized by multisystem inflammation mainly affecting the exocrine glands. There are common features between SS and systemic lupus erythematosus (SLE), but compared to SLE, primary SS (pSS) is a more organ-specific disease. Infiltrating mononuclear inflammatory cells inhibit the function of glandular cells in the salivary and/or lacrimal gland tissues. The typical symptoms of SS, dry eye (keratoconjunctivitis sicca) and dry mouth occur following destruction of these glands[20]. IL-1ß and tumor necrosis factor (TNF)-α are proinflammatory cytokines and seem to be involved in the destruction of acinar structure in human salivary glands[21,22]. In addition, IL-1ß may play a key role in the pathogenesis of keratoconjunctivitis sicca[23]. Although several genetic studies of autoimmune diseases including studies on cytokine polymorphisms, have already been reported[24], the relationships between IL-1ß gene polymorphisms and occurrence of SS have not been examined, and only one study is reported for SLE[19]. To investigate the effect of IL-1 gene polymorphisms on the susceptibility to SS, we analyzed the gene polymorphisms of IL-1ß by the polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) method, and compared the genotype frequencies of each polymorphism between SS and controls, SLE and controls, or SS and SLE. In addition, detailed analysis evaluated whether these polymorphisms affect the phenotypes of SS. Finally, we studied the relationships between gene polymorphisms and characteristics of these diseases.

MATERIALS AND METHODS

SS patients, SLE patients and healthy controls. Approval for this study was obtained from the local ethics committee and written informed consent was obtained from all patients and volunteers who participated in this study. Blood samples were collected from 101 Japanese patients with SS and 103 Japanese patients with SLE followed at the Department of Internal Medicine, University of Tsukuba Hospital. All SS patients [43 with primary SS, 58 with secondary SS including 21 with rheumatoid arthritis, 20 with SLE, 11 with SSC, 4 with mixed connective tissue disease (MCTD), and 2 with Hashimoto's disease] satisfied the Japanese Ministry of Health criteria for the classification of SS[25], and all SLE patients (no patients with secondary SS, 10 men and 93 women) satisfied the 1997 revised American College of Rheumatology criteria for SLE[26]. As healthy controls, 106 Japanese volunteers (41 men, 65 women) were recruited from our institute.

IL-1ß polymorphism typing. Genomic DNA was isolated from 0.5 ml of anti-coagulated peripheral blood by using DNA QuickII DNA purification kit (Dainippon Pharmaceuticals, Osaka, Japan) and the instructions supplied with the kit. Five polymorphisms of the IL-1ß gene were analyzed by PCR-RFLP[4-6,13]. Briefly, 4 sets of PCR reactions were performed, and the amplified products were digested with appropriate restriction enzymes that cut one of the alleles in each polymorphism. The primers and restriction enzymes for each polymorphism were as follows. For –511: 5′-TGGCATTGATCTGGTTCATC-3′, 5′-GTTTAGGAATCTTCCCACTT-3′, and *Ava* I. For –31: 5′-TCATAGTTTGCTACTCCTTGC-3′, 5′-CAAAAAGCTGAGAGAGGAGG-3′, and *Alu* I. For +3263: 5′-TTTGAGAGGCAGGCTGTTTG -3′, 5′-CTTGGTTGTCTTCCTACTATG-3′ and *Mbo* II. For +3877 and +3953: 5′-GTTGTCATCAGACTTTGACC-3′, 5′-TTCAGTTCATATGGACCAG-3′, *BsoF* I for +3877 and *Taq* I for +3953. Digested fragments were run on 2.5% agarose gels and visualized with ethidium bromide (Figure 1).

Serum levels of IL-1ß. IL-1ß levels were measured with human IL-1ß US ELISA kit (BioSource International, Inc. Camarillo, CA, USA), using the instructions provided by the manufacturer.

Statistical analyses. Linkage disequilibrium analysis of these polymorphic sites in the IL-1ß gene was performed using Graphical Overview of Linkage Disequilibrium (GOLD) software package[27]. Chi-square analysis was used to compare genotypes or frequencies of alleles and phenotypes between patients and control groups. Fisher's exact test was used where appropriate. A p value of 0.05 was considered significant. For multiple comparisons (SS or pSS, healthy controls, SLE), a p value of 0.016 was considered as significant. Odds ratios were also calculated for disease susceptibility in carriers of a specific genotype. Correlations between each genotype in each polymorphism and clinical and biological variables were evaluated by analysis of variance or chi-square analysis.

RESULTS

Validation of the RFLP analyses. Fragments of predicted sizes were obtained for all polymorphisms (Figure 1), except for position +3263, where all samples were digested by *Mbo* II, indicating the absence or extreme rarity of the T allele in our Japanese population. Several randomly selected samples were sequenced to further confirm the accuracy of the RFLP analysis (data not shown).

Relationship between IL-1ß genotypes and SS and SLE. Comparison of SS group with other groups showed no evident differences in allele frequencies at any of polymorphisms (data not shown; can be calculated from Table 1A). In contrast, comparison of SS patients with other groups showed significant differences in genotype distribution at positions –511, –31, and 3877 (–511CC/CT/TT: SS vs controls and SLE: p = 0.053 and p = 0.028; –31 TT/TC/CC: SS vs SLE: p = 0.027; 3877 AA/AG/GG: SS vs controls and SLE: p = 0.039 and p = 0.054) (Table 1A). Specifically, the frequencies of genotypes CC, TT, and AA at positions –511, –31, and 3877, respectively, were lower in SS patients compared with the controls and the SLE group (–511, –31, 3877: SS/HC/SLE:18.8, 19.6, 22.8%; 32.1, 29.2, 36.8%; 34.0, 32.4, 35.9%). These differences were also evident when patients were categorized according to the presence or absence of these genotypes (–511CC/CT+TT: SS vs control and SLE: p = 0.029 and p = 0.014; –31 TT/TC+CC: SS vs control and SLE: p = 0.111 and p = 0.041: 3877 AA/AG+GG: SS vs control and SLE: p = 0.028 and p = 0.039, respectively, Table 1B). After adjustment for multiple comparisons according to Bonferroni, only the difference at 511 between SS and SLE remained significant. No significant differences were present between the SLE patients and controls (Table 1A-B). No significant differences in genotype frequencies were observed between primary and secondary SS (Table 1A). In agreement with previous

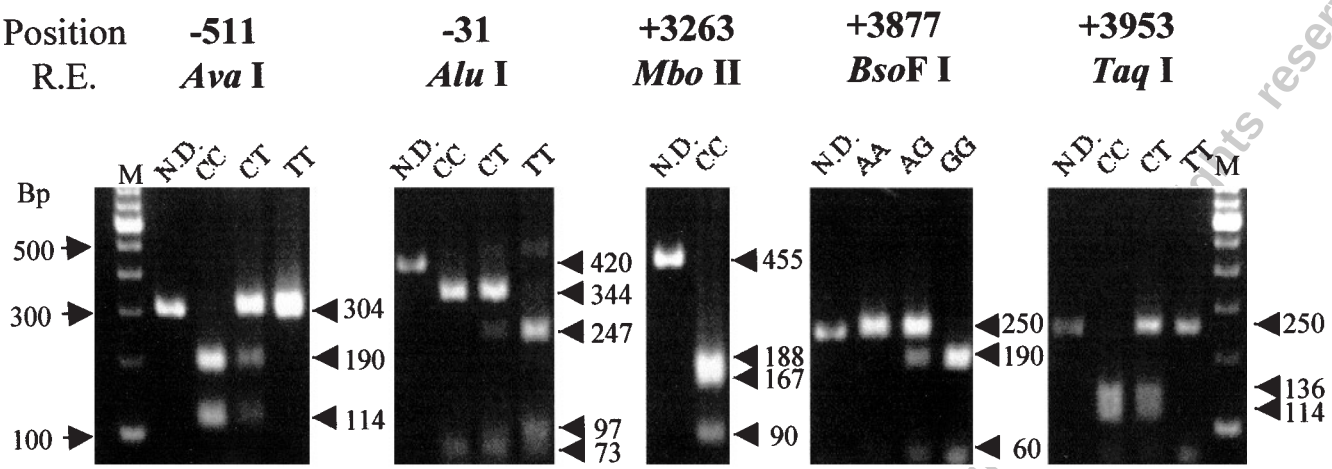

Figure 1. Polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) analysis of the 5 polymorphisms of the IL-1ß gene. cDNA was amplified with primers indicated in the text, and restriction digested by appropriate enzymes. Digested PCR products were run on agarose gels and visualized with ethidium bromide. RE: restriction enzyme. M: 100 bp marker. ND: non-digested PCR product.

Table 1A. Genotype distribution of IL-1ß gene polymorphisms in patients with SS and SLE and healthy controls.

Polymorphism	Genotype	HC n = 106 (%)	SS n = 101 (%)	pSS n = 43 (%)	sSS n = 58 (%)	SLE n = 103 (%)	p (chi-square)
	CC	34 (32.1)	19 (18.8)	7 (16.3)	12 (20.7)	35 (34.0)	
–511	CT	49 (46.2)	62 (61.4)	26 (60.5)	36 (62.1)	46 (44.7)	0.053
	TT	23 (21.7)	20 (19.8)	10 (23.3)	10 (17.2)	22 (21.4)	0.028*
	TT	31 (29.2)	19 (19.6)	7 (17.5)	12 (21.1)	33 (32.4)	
–31+	TC	52 (49.1)	57 (58.8)	23 (57.5)	34 (59.6)	41 (40.2)	0.246
	CC	23 (21.7)	21 (21.6)	10 (25.0)	11 (19.3)	28 (27.5)	0.027*
	AA	39 (36.8)	23 (22.8)	10 (23.3)	13 (22.4)	37 (35.9)	
3877	AG	48 (45.3)	63 (62.4)	24 (55.8)	39 (67.2)	50 (48.5)	0.039
	GG	19 (17.9)	15 (14.9)	9 (20.9)	6 (10.3)	16 (15.5)	0.054*
	CC	98 (92.5)	92 (91.1)	40 (93.0)	52 (89.7)	93 (90.3)	
3953	CT	8 (7.5)	9 (8.9)	3 (7.0)	6 (10.3)	9 (8.7)	
	TT	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.0)	

SS: Sjögren's syndrome; SLE: systemic lupus erythematosus; HC: healthy control; pSS: primary SS; sSS: secondary SS; +: 4 SS patients and 1 SLE patient were not genotyped. p: SS vs HC; *: SS vs SLE. p values of ≤ 0.016 were considered significant after Bonferroni corrections.

Table 1B. Genotype skewing of IL-1ß gene polymorphisms in patients with SS and SLE and healthy controls.

Polymorphism	Phenotype	HC n = 106 (%)	SS n = 101 (%)	pSS n = 43 (%)	SLE n = 103 (%)	p (chi-square)	p (Fisher's probability test)	OR (95% CI)
						0.029‡	0.038	0.49 (0.26–0.93)
–511	CC	34 (32.1)	19 (18.8)	7 (16.3)	35 (34.0)	0.053*	0.068*	0.41 (0.17–1.02)
	CT/TT	72 (67.9)	82 (81.2)	36 (83.7)	68 (66.0)	0.014**	0.017**	0.45 (0.24–0.86)
						0.031***	0.044***	0.38 (0.15–0.94)
	TT	31 (29.2)	19 (19.6)	7 (17.5)	33 (32.4)	0.111	0.142	0.59 (0.31–1.13)
–31+	TC/CC	75 (70.8)	78 (80.4)	33 (82.5)	69 (67.6)	0.041**	0.053**	0.51 (0.27–0.98)
	AA	39 (36.8)	23 (22.8)	10 (23.3)	37 (35.9)	0.028	0.034	0.51 (0.28–0.93)
3877	AG/GG	67 (63.2)	78 (77.2)	33 (76.7)	66 (64.1)	0.039**	0.046**	0.53 (0.28–0.97)
	CC	98 (92.5)	92 (91.1)	40 (93.0)	93 (90.3)			
3953	CT/TT	8 (7.5)	9 (8.9)	3 (7.0)	10 (9.7)			

SS: Sjögren's syndrome; SLE: systemic lupus erythematosus; HC: healthy control; pSS: primary SS; OR: odds ratio; 95% CI: 95% confidence interval; +: 4 SS patients and 1 SLE patient were not genotyped; ‡ SS vs HC; * pSS vs HC; ** SS vs SLE; *** pSS vs SLE. p values of ≤ 0.016 were considered significant after Bonferroni corrections.

reports[9,16], –511 and –31 polymorphisms were in almost complete linkage disequilibrium (Table 1A-B). Detailed analysis of linkage disequilibrium among the 4 polymorphisms examined in this study showed that these polymorphisms are indeed in linkage disequilibrium (D′= 0.622~1.000), and are not independent of each other.

Relationships between IL-1ß genotypes and serum IL-1ß. Serum IL-1ß was measured in 84 healthy controls, 43 patients with SS, and 101 patients with SLE. In these groups, measurable amounts of serum IL-1ß were detected in 11 controls, 4 patients with SS, and 5 patients with SLE. IL-1ß concentration in other samples did not exceed the detection limit (0.3 pg/ml). IL-1ß genotypes in individuals with measurable serum IL-1ß did not show any skewing toward a particular genotype (data not shown).

Relationships between IL-1ß genotypes and disease characteristics of SS and SLE. Disease characteristics and variables were examined in the context of IL-1ß genotypes in SS and SLE patients. Presence of anti-SSA antibody was slightly increased in patients with genotype CC and genotype TT at positions –511 and –31, respectively, (p = 0.040 and p = 0.025, Table 2). The presence of antinuclear antibody or anti-SSB antibody was not related to any of the genotypes examined (Table 2). Age at onset and presence of arthritis were also not associated with any of the genotypes examined (data not shown). In SS patients, serum IgG or presence of sicca syndrome was not associated with any of the IL-1ß genotypes studied (data not shown). In SLE patients, no significant relationship was observed between IL-1ß genotypes and disease variables or presence of symptoms including SLE Disease Activity Index[28], anti-DNA antibody, C_3, C_4, CH50, central nervous system lupus, serositis, and glomerulonephritis (data not shown).

DISCUSSION

Our major finding was that the genotype frequencies and/or the genotype skewings of 3 (–511, –31, +3877) of the 5 known IL-1ß gene polymorphisms were significantly different between SS patients and controls or SLE subjects. No such difference was observed between SLE patients and controls, which is in accord with a report on Taiwanese SLE patients [19]. In fact, in our study, significant differences were observed between patients with SS and SLE. Two (–511, –31) of these 3 polymorphisms were on the promoter region of the IL-1ß gene, and thus may affect IL-1ß gene expression and subsequent cytokine production. The third (+3877) was on intron 4, and we cannot speculate whether this polymorphism influences IL-1ß production or some other immunological or inflammatory functions, although this polymorphism is suggested to be an informative marker for disease association studies[5]. Although many of the significant differences were lost after adjustment of the p value for multiple comparisons, our results may provide a rationale for further studies on the role of the IL-1ß and the pathogenesis of SS. Analysis of linkage disequilibrium among the 4 polymorphisms observed in this study showed that these polymorphisms are in linkage disequilibrium and are not independent, and therefore, adjustment for multiple comparison was performed for comparisons between patients and healthy controls.

In SS, salivary and lacrimal glands are the main target organs for autoimmune tissue damage. IL-1ß and TNF-α are proinflammatory cytokines, and affect the destruction of acinar structure in salivary glands[21]. Messenger RNA of both Th1 and Th2 cytokines are detected in salivary glands of patients with SS, and may have important roles in the onset and progression of SS[22,29,30]. Interferon-γ, IL-2, IL-6, IL-10, and transforming growth factor-ß play essential roles in the induction of SS, while IL-4 and IL-5 may be involved in the progression of disease process[29]. IL-1ß has multiple biologic effects, which include induction of lymphokine synthesis (IL-2, -3, -4, -5, -6, -7, -10, and -12), and devel-

Table 2. Comparison of genotype distribution between patients positive for autoantibodies (ANA, SSA, SSB) and negative in SS and SLE.

Polymorphism	Genotype	ANA				Anti-SSA				Anti-SSB			
		SS (n = 97)		SLE (n = 89)		SS (n = 81)		SLE (n = 86)		SS (n = 65)		SLE (n = 85)	
		+	–	+	–	+	–	+	–	+	–	+	–
	CC	16	3	26	5	15	1	6	23	0	15	0	28
–511	CT	43	16	33	6	29	18*	10	31	7	29	0	41
	TT	15	4	16	3	14	4	6	10	2	12	1	15
	TT	16	3	26	4	15	1	6	22	0	15	0	27
–31	TC	41	14	27	6	26	18**	9	26	6	28	0	35
	CC	15	5	21	4	14	4	7	15	2	12	1	21
	AA	16	7	28	2	14	4	5	23	0	14	0	27
3877	AG	47	13	35	9	33	17	11	32	6	33	1	42
	GG	11	3	12	3	11	2	6	9	3	9	0	15
	CC	67	21	69	12	52	22	19	59	8	50	1	76
3953	CT	7	2	6	1	6	1	2	5	1	6	0	7
	TT	0	0	0	1	0	0	1	0	0	0	0	1

ANA: antinuclear antibody; SS: Sjögren's syndrome; SLE: systemic lupus erythematosus; * p = 0.040; ** p = 0.025 (n = 78), by chi-square test.

opment of Th2 type T cells[1]. IL-1 is known to initiate transcription and stabilize the mRNA of a variety of genes. Messenger RNA expression of inflammatory cytokines, such as IL-6 and 8, TNF-α, and granulocyte-macrophage colony-stimulating factor genes are upregulated by IL-1[1,31,32]. In this study, we found that the genotype skewings of 3 polymorphisms of the IL-1ß gene were significantly different in SS patients compared to those with SLE or controls. While adjustment for multiple comparison leads to the loss of statistical significance in many of the differences observed, we still believe that our data are of interest, since our primary focus was on SS, which has evidence suggesting the importance of IL-1ß in its pathogenesis, and not on SLE, which has no such evidence and which was included in this study rather as a comparator disease. We speculate that these polymorphisms may have direct or indirect impact on the onset or progression of SS.

In previous reports on IL-1ß polymorphisms and IL-1ß production, the relationship between the polymorphism and IL-1ß gene expression or IL-1ß production is not fully determined[8,33]. In our study, although we tried to evaluate the relationship between these polymorphisms and serum IL-1ß we could not find any relationship between the 4 polymorphisms of IL-1ß gene and concentration of serum IL-1ß, since only 11 individuals in the control group, 4 in the SS group, and 5 in the SLE group had measurable amounts of serum IL-1ß. These results were not unexpected, since it is difficult to detect IL-1ß in serum or plasma without *in vitro* stimulation by mitogens such as LPS because circulating levels of IL-1ß are very low and plasma normally contains IL-1sRII (IL-1 soluble receptor II), α-2-macroglobulin and complement, which bind to IL-1ß[1]. In addition, in most of our patients, disease activity was adequately controlled at the time of sampling. Therefore, to assess whether these polymorphisms are indeed associated with IL-1ß production, either a study with a large number of untreated patients or an *in vitro* study using a promoter assay would be necessary.

Genotypes at –511 and –31 of the IL-1ß gene were in almost complete linkage disequilibrium as reported by others[9,16]. Therefore, SS patients showed similar significant decreases of CC homozygous genotype at –511 and of TT homozygous genotype at –31. Presence of the T allele at –31 would make a TATA box-related sequence, which speculatively may lead to increased IL-1ß production. However, the number of individuals who are TT homozygous at –31 was significantly decreased in the SS patient group. Since the presence of this genotype was tightly linked with the probable high IL-1ß producing allele at –511[9,16], the significance of TATA box formation at –31 cannot be determined at this stage.

Polymorphism at +3877 in intron 4 of the IL-1ß gene, originally reported by Guasch, *et al*[5] was proposed as an adequate marker for genotype-disease association studies, because of its heterogeneity and low linkage to other polymorphisms. However, Langdahl, *et al*[6] reported the lack of any difference in genotype distributions at +3877 of the IL-1ß gene, or at +11100 of the IL-1ra gene between healthy controls and patients with osteoporosis. Furthermore, they found no relationships between these polymorphisms and disease variables of osteoporosis such as bone mass and bone turnover. In our study, we found that the G allele carriers at +3877 of the IL-1ß gene are significantly more frequent among SS patients compared to healthy controls or SLE patients. Since there is no information on the relationship between this polymorphism and gene expression, the significance of this finding remains unclear at present.

We found no significant skewing of genotype distribution in any of the polymorphisms studied with any of the disease characteristics including age at onset, severity of sicca syndrome, presence or absence of arthritis, anti-SSB antibody, and antinuclear antibody in both SS and SLE patients. The CC homozygous genotype at –511 and TT homozygous genotype at –31 were slightly increased in anti-SSA antibody-positive patients, compared to negative patients. Although IL-1ß and anti-SSA antibody production may be indirectly associated in some way in the complex steps of autoantibody production, considering the number of comparisons made in this study, this weak association should be retested in future studies.

Finally, the IL-1ß gene allele frequencies at positions –511 and +3953 observed in our study controls were similar to those obtained in healthy Japanese subjects studied by Takamatsu, *et al*[18] and in healthy Taiwanese subjects studied by Huang, *et al*[19] , but were different from those obtained in healthy Caucasian subjects studied by Cantagrel, *et al*[13] because the allele T on +3953 is much more common in Caucasians than in the Japanese (frequency of TT homozygous healthy individuals was 0% in our study and in Takamatsu's[18], but was 4.7% in Cantagrel's[13]).

There are important steps, e.g., IL-1ß converting enzyme (ICE) digestion, competition with IL-1ra and IL-1a, and binding with the IL-1 receptor, before IL-1ß gene products exert their functions. Other cytokines and mediator molecules also affect this cascade. Therefore, the extent of IL-1ß function is dependent not only on IL-1ß gene expression. Nevertheless, our results showed significant differences in genotype distributions in 3 polymorphisms of the IL-1ß gene in SS patients compared with controls or patients with SLE. These findings imply that IL-1ß gene polymorphisms, and hence, IL-1ß function, may influence the onset and progression of SS. On the other hand, no such genotype skewing was observed in SLE patients, suggesting that this cytokine might not be an important factor in the onset of SLE.

REFERENCES

1. Dinarello CA. Biologic basis for interleukin-1 in disease. Blood 1996;87:2095-147.

2. Webb AC, Collins KL, Auron PE, et al. Intereukin-1 gene (IL1) assigned to long arm of human chromosome 2. Lymphokine Res 1986;5:77-85.
3. Bidwell JL. Cytokine gene polymorphisms in human disease: on-line database [Internet]. Available from http://bris.ac.uk/pathand-micro/services/GAI/polymorphisms.html
4. Di Giovine FS, Takhsh E, Blakemore AIF, Duff GW. Single base polymorphism at –511 in the human interleukin-1ß (IL1ß) gene. Hum Mol Genet 1992;1:450.
5. Guasch JF, Bertina RM, Reitsma PH. Five novel intragenic dimorphisms in the human interleukin-1 genes combine to high informativity. Cytokine 1996;8:598-602.
6. Langdahl BL, Løkke E, Carstens M, Stenkjær LL, Eriksen EF. Osteoporotic fractures are associated with an 86-base pair repeat polymorphism in the interleukin-1-receptor antagonist gene but not with polymorphisms in the interleukin-1ß gene. J Bone Miner Res 2000;15:402-14.
7. Pociot F, Mølvig J, Wogensen L, Worsaae H, Nerup J. A Taq I polymorphism in the human interleukin-1ß gene correlates with IL-1ß (IL1ß) secretion in vitro. Eur J Clin Invest 1992;22:396-402.
8. Santtila S, Savinainen K, Hurme M. Presence of the IL-1RA allele2 (IL1RN*2) is associated with enhanced IL-1ß production in vitro. Scand J Immunol 1998;47:195-8.
9. EI-Omar EM, Carrington M, Chow WH, et al. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. Nature 2000;404:398-402.
10. Cvetkovic JT, Wållberg-Jonsson S, Stegmayr B, Rantapää-Dahlqvists, Levert AK. Susceptibility for and clinical manifestations of rheumatoid arthritis are associated with polymorphisms of the TNF-a, IL-1ß, and IL-1Ra genes. J Rheumatol 2002; 29: 212-9.
11. Buchs N, Di Giovine FS, Silvestri T, Vannier E, Duff GW, Miossec P. IL-1ß and IL-1Ra gene polymorphisms and disease severity in rheumatoid arthritis: interaction with their plasma levels. Genes Immun 2001;2:222-8.
12. Huang CM, Tsai FJ, Wu JY, Wu MC. Interleukin-1ß and interleukin-1 receptor antagonist gene polymorphisms in rheumatoid arthritis. Scand J Rheumatol 2001;30:225-8.
13. Cantagrel A, Navaux F, Loubet-Lescoulié P, et al. Interleukin-1ß, interleukin-1 receptor antagonist, interleukin-4, and interleukin-10 gene polymorphisms. Arthritis Rheum 1999;42:1093-100.
14. Moos V, Rudwaleit M, Herzog V, Höling K, Sieper J, Müller B. Association of genotypes affecting the expression of interleukin-1ß or interleukin-1 receptor antagonist with osteoarthritis. Arthritis Rheum 2000;43:2417-22.
15. Mwantembe O, Gaillard MC, Barkhuizen M, et al. Ethnic differences in allelic associations of the interleukin-1 gene cluster in South Africa patients with inflammatory bowel disease (IBD) and in control individuals. Immunogenetics 2001;52:249-54.
16. Hamajima N, Matsuo K, Saito T, et al. Interleukin-1 polymorphisms, lifestyle factors, and Helicobacter pylori infection. Jpn J Cancer Res 2001; 92:383-9.
17. Niino M, Kikuchi S, Fukazawa T, Yabe I, Sasaki H, Tashiro K. Genetic polymorphisms of IL-1 beta and IL-1 receptor antagonist in association with multiple sclerosis in Japanese patients. J Neuroimmunol 2001;118:295-9.
18. Takamatsu M, Yamauchi M, Maezawa Y, Saito S, Maeyama S, Uchikoshi T. Genetic polymorphisms of interleukin-1ß in association with the development of alcoholic liver disease in Japanese patients. Am J Gastroenterol 2000;95:1305-11.
19. Huang CM, Wu MC, Wu JY, Tsai FJ. Lack of association of interleukin-1ß gene polymorphisms in Chinese patients with systemic lupus erythematosus. Reumatol Int 2002;21:173-5.
20. Jonsson R, Haga HJ, Gordon TP. Current concepts on diagnosis, autoantibodies and therapy in Sjögren's syndrome. Scand J Rheumatol 2000;29:341-8.
21. Azuma M, Motegi K, Aota K, Hayashi Y, Sato M. Role of cytokines in the destruction of acinar structure in Sjögren's syndrome salivary glands. Lab Invest 1997;77:269-80.
22. Fox RI, Kang HI, Ando D, Abrams J, Pisa E. Cytokine mRNA expression in salivary gland biopsies of Sjögren's syndrome. J Immunol 1994;152:5532-9.
23. Solomon A, Dursun D, Liu Z, Xie Y, Macri A, Pflugfelder SC. Pro- and anti-inflammatory forms of interleukin-1 in the tear fluid and conjunctiva of patients with dry-eye disease. Invest Ophthalmol Vis Sci 2001;42:2283-92.
24. Magusson V, Nakken B, Bolstad AI, Alarcón-Riquelme ME. Cytokine polymorphisms in systemic lupus erythematosus and Sjögren's syndrome. Scand J Immunol 2001;54:55-61.
25. Fujibayashi T, Sugai S, Miyasaka N, Tojo T, Miyawaki S, Ichikawa Y, Tubota K. Revised Japanese criteria for Sjögren's syndrome. Autoimmune Disease Research Committee Annual report of Ministry of the Health and Welfare. Tokyo: Ministry of the Health and Welfare; 1999:135-8.
26. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1997;40:1725.
27. Abecasis GR, Cookson WOC. GOLD. Graphical overview of linkage disequilibrium. Bioinformatics 2000;16:182-3.
28. Le Loet X. Evaluation of the clinical activity of systemic lupus erythematosus. Ann Med Interne Paris 1990;141:261-4.
29. Ohyama Y, Nakamura S, Matsuzaki G, et al. Cytokine messenger RNA expression in labial salivary glands of patients with Sjögren's syndrome. Arthritis Rheum 1996;39:1376-84
30. Matsumoto I, Okada S, Kuroda K, et al. Single cell analysis of T cells infiltrating labial salivary glands from patients with Sjögren's syndrome. Int J Mol Med 1999;4:519-27.
31. Chrousos GP. The hypothalamic-pituitary-adrenal axis and immune-mediated inflammation. N Engl J Med 1995;332:1351-62.
32. Fibbe WE, Daha MR, Hiemstra PS, et al. Interleukin 1 and poly(rI) poly(rC) induced production of granulocyte CSF, macrophage CSF, and granulocyte-macrophage CSF by human endothelial cells. Exp Hematol 1989;17:229-34.
33. Hulkkonen J, Laippala P, Hurme M. A rare allele combination of the interleukin-1 gene complex is associated with high interleukin-1ß plasma levels in healthy individuals. Eur Cytokine Netw 2000;11: 251-5.