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Lack of association with interleukin 1 receptor antagonist and interleukin-1β gene polymorphisms in sarcoidosis patients

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Interleukin-1β (IL-1β) and its endogenous antagonist, the interleukin-1 receptor antagonist (IL-1ra), play important roles in immune responses. In sarcoidosis, IL-1β is reported to be increased whereas IL-1ra is decreased. It has recently been shown that polymorphisms in the IL-1ra and IL-1β genes may account for variation in the two proteins. These polymorphisms are also reported to be associated with several autoimmune diseases. Since this might be expected to affect sarcoidosis, an investigation of 108 sarcoidosis patients and 113 healthy control subjects was performed.

The IL-1ra genotype was determined using the polymerase chain reaction (PCR), and the IL-1β genotype by PCR restriction fragment length polymorphism.

We found no significant differences in IL-ra and IL-1β genotypes between sarcoidosis patients and healthy controls. Furthermore, there was no association between the IL-1β genotype and the roentgenographic stage, disappearance of chest X-ray shadows or organ involvement.

In conclusion, there is no bias in the IL-1ra and IL-1β genotype in Japanese sarcoidosis patients.

Key words: interleukin-1 receptor antagonist; interleukin-1β gene; polymorphism; sarcoidosis

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Introduction

Interleukin-1β (IL-1β) has been reported as an important mediator of the immune response. Previous study has shown that it activates lymphocytes and induces production of several cytokines by immune cells in the lung, including interleukin-6, interleukin-8, tumour necrosis factor-alpha (TNFα) and monocyte chemoattractant peptide 1. An endogenous antagonist, interleukin-1 receptor antagonist (IL-1ra), was isolated that inhibits IL-1β bioactivity competitive binding to the IL-1β receptor, thus suppressing the IL-1β induction of cytokines. Both IL-1β and IL-1ra may play important roles in inflammatory reactions (1–4).

The gene for IL-1β is located on the long arm of human chromosome 2, in close linkage with another gene of the IL-1 gene family, that encoding IL-1ra. Polymorphisms have recently been described for both IL-1ra and IL-1β (4–6). The polymorphism in human IL-1ra gene intron 2 is caused by variable numbers of an 86-bp tandem repeat. Allele 2 was reported to be associated with enhanced production of IL-1ra (4,7,8) and IL-1β proteins (4,9). Within the IL-1β gene, a Taq I restriction fragment length polymorphism in exon 5 has also been reported to influence the production of IL-1β protein. The allele 2 represents an IL-1β high-secretor phenotype (4,6).

Recently, the IL-1ra gene allele 2 was reported to be associated with inflammatory bowel disease (4,10,11), multiple sclerosis (4,12), systemic lupus erythematosis (4,13), lichen sclerosis (4,14) and Sjögren's syndrome (15). With regard to the IL-1β gene polymorphism, some investigators have found allele 2 to be linked to myasthenia gravis (4,16) and insulin-dependent diabetes mellitus (4,6).

Sarcoidosis is a systematic granulomatous disorder of unknown aetiology. Previous investigations demonstrated that IL-1ra and IL-1β both play a role in the immune response of sarcoidosis patients: IL-1β production by alveolar macrophages being increased and generation of IL-1ra decreased (17,18). IL-1β and IL-1ra may function as immunomodulators of sarcoid granulomas, so that allelic variation of IL-1β and IL-1ra gene might be expected to have an impact on the disease. Since this has so far not received attention to our knowledge, the present study was conducted with the aim of ascertaining whether IL-1β and IL-1ra might affect sarcoidosis.

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Materials and methods

SUBJECTS

The 108 sarcoidosis patients (88 females and 20 males) studied were all inhabitants of central Japan. Sarcoidosis was diagnosed based on the clinical picture and the presence of epithelioid cell granulomas in biopsy specimens from lung, skin or lymph nodes. They had a mean age of 55·7 ± 15·1 (mean ± SD) years. All but 14 had chest roentgenologic evidence of sarcoidosis, 78 with stage I, 11 with stage II and five with stage III disease.

As healthy controls, 113 unrelated healthy subjects (58 females and 55 males) living in the same area of Japan were selected. They had a mean age of 40·0 ± 11·8 years with no past history of pulmonary disease or any abnormalities on physical examination, chest radiography, ECG, urinalysis and routine laboratory blood testing. None of the controls was receiving medication at the time of the evaluation. Informed consent was obtained from all patients and healthy controls.

DETERMINATION OF IL-1ra GENOTYPES

DNA was extracted from peripheral leukocytes with standard techniques. Specific oligonucleotide primers 5′-CTCAGCAACACTCCTAT-3′ and 5′-TCCTGGTCTGCA GGTAA-3′ were utilized in PCR to amplify a fragment of the IL-1ra gene, with denaturation at 94°C for 1 min, followed by 35 cycles at 94°C for 1 min, 60°C for 1 min and 70°C for 1·5 min and a final extension at 72°C for 5 min (DNA Thermal Cycler 2400, Perkin Elmer-Cetus, Norwalk, CT, U.S.A.). PCR products were analysed on 2% ethidium bromide agarose gels. In the second intron of this gene five alleles are defined by different numbers of a 86-bp segment repeat. Genotypes were determined by comparing the size of the bands with a molecular weight ladder, with separation into 410 bp (allele 1), 240 bp (allele 2), 500 bp (allele 3), 325 bp (allele 4) and 595 bp (allele 5) (Fig. 1).

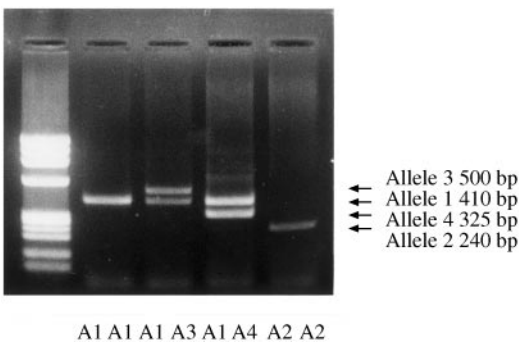

FIG. 1. Interleukin-1 receptor antagonist genotypes obtained in this study. Panel shows a representative 2% agarose gel stained with ethidium bromide and photographed under ultraviolet transillumination after PCR amplification. The bands show allele 3 (500 bp), allele 1 (410 bp), allele 4 (325 bp) and allele 2 (240 bp). Four genotypes, A1A1, A1A3, A1A4 and A2A2 are evident. Allele 5 (595 bp) was not found in this study. The left lane contains markers.

DETERMINATION OF IL-1β GENOTYPES

The region containing the IL-1β polymorphic site was amplified with the primers 5′-GTTGTCATCAGACTTT-GACC-3′ and 5′-TTCAGTTCATATGGACCAGA-3′. The PCR conditions were 95°C for 5 min, followed by five cycles at 95°C for 1 min, 51°C for 1 min and 74°C for 1 min, then 30 cycles at 95°C for 0·5 min, 51°C for 0·5 min and 74°C for 0·5 min. The final extension was carried out at 74°C for 10 min. Aliquots of the PCR products was analysed on 2% agarose gels stained with ethidium bromide before digestion to control for correct amplification. Taq I digestion (1 unit) of the 249 bp fragments at 65°C for 90 min resulted in fragments that either remained intact or were cut in two fragments of 135 bp and 114 bp, respectively. The samples were then analysed by electrophoresis on a 3% agalose gel stained with ethidium bromide and the genotypes were determined. The allele with a recognition sequence for the Taq I has been named allele 1, and the other, which is not cut by Taq I, allele 2. There are three genotypes, homozygous for the digestive allele A2A2, homozygous for undigestive allele A1A1 and heterozygous for A1A2 (Fig. 2).

STATISTICAL ANALYSIS

The allele ratio and genotype distribution of sarcoidosis patients and healthy control subjects, and clinical manifestation of the disease among the genotypes were analysed with the Fisher's exact test. A P-value $< 0{\cdot}05$ was considered significant.

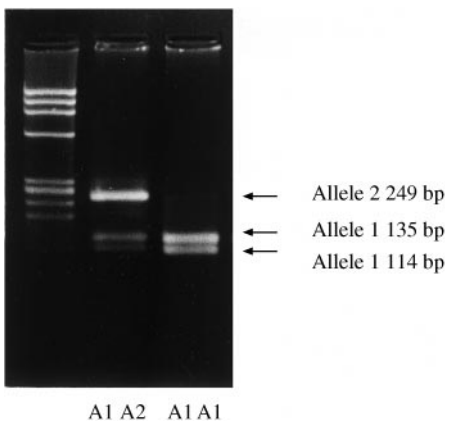

FIG. 2. Determination of interleukin-1β genotypes. Panel shows a representative 3% agarose gel stained with ethidium bromide after PCR amplification and digestion by Taq I. The upper single band is the allele 2 fragment (249 bp) and the lower double bands are allele 1 fragments (135 bp and 114 bp). The A1A1 type and the A1A2 type are shown. We found no A2A2 type in this study. The left lane contains markers.

Results

IL-1ra GENOTYPE DISTRIBUTION

Of the 113 healthy control subjects, 111 had the A1A1 genotype, one the A1A3 genotype and one the A1A4 genotype. Of the 108 sarcoidosis patients, 105 had the A1A1 genotype, two had A1A3 and one had A2A2. Since there were few subjects other than A1A1 type we grouped other genotypes together for comparison. When we compared A1A1 with other types, we found no difference in IL-1ra genotype distribution between healthy subjects and sarcoidosis patients (Table 1).

IL-1β GENOTYPE DISTRIBUTION

Of the 113 healthy control subjects, 104 had the A1A1 genotype and nine the A1A2 type; no A2A2 type individuals were included. The A1 allele/A2 allele ratio was 0·960/0·040. Of the 108 sarcoidosis patients, 94 were A1A1 type, 14 were A1A2 and none were A2A2. The A1/A2 allele ratio was 0·935/0·065. There was no significant difference in the genotype distribution between healthy controls and sarcoidosis patients, and no variation in the frequency of the allele was observed (Table 1).

GENOTYPES AND CLINICAL CHARACTERISTICS OF DISEASE

Since genotypes other than A1A1 were very rare, it was difficult to examine any subgroup analysis of IL-1ra genotypes. Therefore we investigated the correlation between only the IL-1β genotype and clinical characteristics of sarcoidosis patients. Firstly, we examined IL-1β genotype distributions among the three roentgenographic stages of sarcoidosis patients. Of 94 subjects with roentgenographic findings, 80 were of A1A1 type: 66 in stage I, nine in stage II and five in stage III, while 14 were of A1A2 type: 12 stage I, two stage II and no stage III. There was no significant correlation between roentgenographic stage and the IL-1β genotype. We also examined the genotype distribution with respect to disappearance of shadows on chest radiography within 3 years. Of 108 sarcoidosis patients, 79 were available for 3-year follow-up without steroid therapy. In these cases, 69 cases were of A1A1 type with 14 disappearance cases, whereas 10 cases were of A1A2 type with two disappearance cases. The relative rates were not different. Cases of eye, skin, heart, and three or more organ involvement were examined, but the results again indicated no specific association with the genotype (Table 2).

Discussion

Sarcoidosis patients show different production patterns of cytokines compared with healthy subjects, with IL-1β being increased and IL-1ra decreased (17,18). Polymorphisms of both the IL-1ra and IL-1β genes have been reported to affect IL-1ra and IL-1β production (4). For example, allele 2 of IL-1ra and allele 2 of IL-1β are associated with increased *in-vitro* production of IL-1β protein (4,6,9). The IL-1ra allele 2 is also reported to be linked to elevated IL-1ra plasma levels (4,7) or production by activated peripheral blood mononuclear cells (4,8). The effect of the IL-1ra allele 2 on plasma IL-1ra levels could only be seen in persons who were also carriers of the IL-1β allele 2 (7). Therefore, alleles with high agonist and high antagonist generation might have enhanced homeostasis.

However, the present study did not demonstrate any difference in distribution of either IL-1ra or IL-1β genotypes between healthy control subjects and sarcoidosis patients, or variation in IL-1β with clinical characteristics. While IL-1ra allele 2 and IL-1β allele 2 have been reported to confer risk of chronic inflammatory and autoimmune disease (4,6,10–16), not all findings have been positive (4,19–23). Thus, an association between these alleles and disease has not been confirmed at the clinical level, in agreement with our results.

Our Japanese healthy control group and sarcoidosis patients contained few individuals with other than A1A1 type IL-1ra and A1A2 type IL-1β gene phenotypes, in contrast with previous Caucasian studies (4–14,19–23) and suggesting a racial bias. Even if the IL-1ra and IL-1β genes do affect the production of IL-1ra and IL-1β, we speculate the total influence might be weak and these alleles may not represent a risk factor for Japanese sarcoidosis patients.

However, the small size of the population of high-secretor allele carriers is a limitation of our study, precluding firm conclusions. In comparison with healthy control subjects, a slight increase of IL-1β A1A2 type was

TABLE 1. Genotype distributions of control subjects and sarcoidosis patients

		IL-1ra genotype		IL-1β genotype	
		A1A1 (%)	Others (%)	A1A1 (%)	A1A2 (%)
Healthy control subjects	n=113	111 (98·2)	2 (1·8)	104 (92·0)	9 (8·0)
Sarcoidosis patients	n=108	105 (97·2)	3 (2·8)	94 (87·0)	14 (13·0)

No significant differences in distributions in IL-1ra (P=0·68) and IL-1β (P=0·27) genotypes were observed between healthy control subjects and sarcoidosis patients.

TABLE 2. IL-1β genotype and clinical characteristics of disease

		A1A1 (%)	A1A2 (%)
Rentgenographic stage[*]			
I	n=78	66 (84·6)	12 (15·4)
II	n=11	9 (81·8)	2 (18·2)
III	n=5	5 (100·0)	–
Disappearance of shadow[†]			
Disappearance	n=16	14 (87·5)	2 (12·5)
Without disappearance	n=63	55 (87·3)	8 (12·7)
Organ involvement			
Eye[‡]	n=59	50 (84·7)	9 (15·3)
Skin[§]	n=23	22 (95·7)	1 (4·3)
Heart[‖]	n=7	5 (71·4)	2 (28·6)
More than three organs[¶]	n=27	24 (88·9)	3 (11·1)

No significant differences in genotype distributions were observed with respect to clinical characteristics.
[*]P=0·86; [†]P=1·00; [‡]P=0·57; [§]P=0·29; [‖]P=0·22; [¶]P=1·00.

found (13% *vs.* 8%) and, while this was not significant, further investigation of IL-ra and IL-1β gene polymorphisms in a larger series of sarcoidosis patients appears warranted, especially with Caucasians who are reported to be more likely to carry IL-1ra allele 2 or IL-1β allele 2.

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