Interleukin 1α Single-Nucleotide Polymorphism Associated with Systemic Sclerosis

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ABSTRACT. ***Objective.*** In systemic sclerosis (SSc), constitutive expression of the proinflammatory and fibrogenic cytokine interleukin 1α (IL-1α) by dermal fibroblasts from the affected skin has been observed. We investigated the association of a single-nucleotide polymorphism at position –889 in the IL-1α gene in patients with SSc.

Methods. Genotyping of IL-1α –889 polymorphism was performed in 46 patients with SSc and in 150 healthy controls by polymerase chain reaction with sequence-specific primers. All subjects were unrelated Slovak Caucasians.

Results. In SSc patients, carriers of the IL-1α –889 T allele were significantly overrepresented in comparison with controls (63.0% vs 42.0%; p = 0.01, OR 2.3, 95% CI 1.2–4.6). The frequency of the IL-1α –889 T allele was increased in SSc patients (38.0%) in comparison with controls (25.7%; p = 0.02).

Conclusion. The IL-1α –889 polymorphism, previously shown to predispose to increased IL-1 protein expression, may be involved in susceptibility to SSc. (J Rheumatol 2004;31:81–4)

Key Indexing Terms:
SYSTEMIC SCLEROSIS INTERLEUKIN 1 SINGLE-NUCLEOTIDE POLYMORPHISM
CASE-CONTROL STUDY GENETIC SUSCEPTIBILITY

Scleroderma is an autoimmune connective tissue disease of unknown origin with extensive deposition of fibrous tissue in skin, blood vessels, and visceral organs[1]. Scleroderma may occur in systemic form as systemic sclerosis (SSc) or in localized form, which is limited to the skin and subcutaneous tissue without multiorgan involvement. Pulmonary fibrosis in SSc, the prevalence of which is the highest among the connective tissue diseases, is the main cause of death in SSc[2]. The familial occurrence and association with HLA antigens and gene polymorphisms of proteins involved in the inflammatory response and regulation of connective tissue-matrix synthesis support the concept of the participation of genetic factors in the pathogenesis of scleroderma[3-6].

The pleiotropic cytokine interleukin 1 (IL-1) is one of the key regulators of the inflammatory response. IL-1 is also involved in regulating connective tissue remodeling and cellular differentiation of epithelial and ectodermal cells[7]. There are 3 major members of the IL-1 family: IL-1α, IL-1ß, and IL-1 receptor antagonist. IL-1α is mainly present as the cell-associated precursor pro-IL-1α, which in contrast to inactive pro-IL-1ß expresses biological activity and acts in an autocrine manner. The gene encoding IL-1α, located within the IL-1 gene cluster on chromosome 2q13–21, is polymorphic at position –889 from the transcription start site[8,9]. IL-1α –889 is a single-nucleotide polymorphism (SNP) with a cytosine–thymine substitution, which has been shown to influence IL-1 protein production[10]. The role of IL-1 in SSc has been implicated in several reports[11,12]. It has been shown that dermal fibroblasts from affected scleroderma skin express IL-1α mRNA and protein constitutively, while normal fibroblasts lack constitutive production and synthesize IL-1α only after stimulation[11].

On the basis of previous studies we hypothesized that the IL-1α –889 polymorphism might be a genetic factor contributing to the predisposition to SSc due to its ability to affect protein expression. Thus we investigated the distribution of the IL-1α –889 polymorphism in patients with SSc and compared them with a healthy control population.

MATERIALS AND METHODS

Patients and controls. A case-control study was performed in 46 patients with SSc and 150 healthy control subjects. All 46 patients with SSc fulfilled

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the American College of Rheumatology criteria[13]. Patients' clinical characteristics are shown in Table 1. The control population consisted of healthy volunteers, in which the presence of any autoimmune disease was excluded by health questionnaire and interview. All patients and controls were unrelated Caucasians of Western Slavonic ancestry living in the Slovak Republic. Informed consent was obtained from all subjects enrolled in the study.

Genetic analysis. Genomic DNA was extracted from 10 ml samples of EDTA-anticoagulated peripheral blood by the salting-out method[14]. The polymorphic region of the IL-1α gene (IL1A) was amplified by polymerase chain reaction with sequence-specific primers (PCR-SSP). For determining the IL-1α –889 genotype, 2 reactions were used with primer mixes specific to C or T allele (consensus primer 5'AAg TAg CCC TCT ACC AAg gA, specific primer CTT TAA TAA TAg TAA CCA ggC AAC A C/T) with a final volume of 13 µl. The PCR conditions were established according to a described phototyping method[15]. The PCR products were analyzed on 2% agarose gel stained with ethidium-bromide.

Statistical analysis. The genotype and allele frequencies in the disease and control populations were determined. The data sets were compared using a standard 2 × 2 chi-square test with SIGTEST, a program that uses Woolf-Haldane correction in cases of small numbers. This program has a facility for calculation of chi-square statistics, significance value, and the relative risk (odds ratio, OR). A p value < 0.05 was considered significant. The populations were tested for conformity to Hardy-Weinberg equilibrium using a 2 × 2 chi-square test between observed and expected numbers.

RESULTS

The distribution of IL-1α –889 genotypes and alleles is shown in Table 2. In SSc patients, the genotype IL-1α –889 C/C was less frequent than in controls (37.0% vs 58.0%; p = 0.01), whereas the C/T genotype was overrepresented (50.0% vs 32.7%; p = 0.03). In comparison with controls there were significantly more carriers of the IL-1α –889 T allele among patients with SSc (63.0% vs 42%; p = 0.01), whose relative risk was 2.3 (95% CI 1.2–4.6). The frequency of the IL-1α –889 T allele was significantly increased in the SSc group (38.0%) compared with controls (25.7%; p = 0.02). The distribution of genotypes in the populations of patients and controls was consistent with Hardy-Weinberg equilibrium, with nonsignificant differences between the expected and observed numbers.

Table 1. Clinical characteristics of 46 patients with systemic sclerosis.

Male:Female	4:42
Diffuse SSc, n	10
Limited SSc, n	36
Lung involvement	33
GI tract involvement	19
Kidney involvement	10
Acral ulcers	14
Myositis	5
Sjögren's syndrome	6
Acroosteolysis	14
Anti-DNA-topo I antibodies	18
Anticentromere antibodies	4

DISCUSSION

We investigated the relationship between the IL-1α –889 polymorphism and SSc. We report an association of the IL-1α –889 polymorphism with susceptibility to SSc with significant overrepresentation of the IL-1α –889 T allele carriers among SSc patients. Patients with IL-1α –889 C/T or T/T genotypes have an increased relative risk of 2.3–fold of developing SSc in comparison with healthy controls.

In the etiology of SSc 3 main pathogenic mechanisms are considered: fibroblast activation, abnormal immune response, and vascular/endothelial pathology[1,3]. Fibroblasts from patients with SSc display aberrant regulation of growth and production of excessive amounts of collagen[3]. IL-1 plays an important role in connective tissue remodeling: it modulates both degradation and synthesis of extracellular matrix[7,16]. The data for the direct effect of IL-1 on collagen production and fibroblast proliferation *in vitro* are controversial, depending on cell type, and appear to be influenced by IL-1 induced prostaglandins, which attenuate the profibrotic effects of IL-1[16-18]. However, IL-1 has been shown to stimulate the expression of a range of profibrotic growth factors and cytokines *in vitro* and *in vivo*[7]. Kolb, *et al* have reported that overexpression of IL-1ß in rat lungs induces production of platelet derived growth factor (PDGF), transforming growth factor-ß1, and IL-6, which are important stimulators of extracellular matrix production, and results in severe pulmonary fibrosis[19]. Human renal fibroblasts derived from a kidney with interstitial fibrosis display spontaneous IL-6 and IL-8 synthesis dependent on the intrinsic release of IL-1[20].

A similar pathway leading to extensive collagen accumulation may be present in SSc. In SSc dermal fibroblasts, constitutive production of IL-1α, not present in normal fibroblasts, has been described[11]. This constitutive IL-1α expression, together with high responsiveness of SSc fibroblasts to exogenous IL-1 due to increased expression of IL-1 receptors, indicates that IL-1α may play a role in the

Table 2. Genotype and allele frequencies of IL-1α –889.

	SSc, % (n = 46)	Controls, % (n = 150)	p	OR	95% CI
Genotype					
C/C	37.0 (17)	58.0 (87)	0.01	0.43	0.22–0.85
C/T	50.0 (23)	32.7 (49)	0.03	2.05	1.05–3.99
T/T	13.0 (6)	9.3 (14)	0.58	—	—
Allele					
C	62.0 (57)	74.3 (223)	0.02	0.56	0.34–0.92
T	38.0 (35)	25.7 (77)	0.02	1.78	1.09–2.91

known differential behavior of SSc fibroblasts[11]. The cell-associated IL-1α appears to act in SSc-affected dermal fibroblasts in an autocrine manner and its effect on collagen synthesis and fibroblast activity has been shown to be partially mediated by the IL-1-inducible factors IL-6 and PDGF[21]. Another possible IL-1 induced mediator participating in the pathogenesis of SSc is nitric oxide (NO); it was reported NO production increased in peripheral blood mononuclear cells from SSc patients in response to IL-1[22]. Dysregulation of NO production has been implicated in autoimmune disorders and wound healing[22].

A regulatory effect of IL-1α –889 polymorphism on IL-1 protein expression has been suggested. The IL-1α –889 T allele has been related to elevated IL-1ß plasma levels in healthy subjects and increased IL-1α concentrations in gingival crevicular fluid of individuals with severe periodontal disease[10,23].

In a recent study, Kawaguchi, *et al*[24] have reported the association of the CTG haplotype of the IL1A gene (single-nucleotide polymorphisms at –889, +4729, and +4845) with susceptibility to SSc and presence of interstitial lung disease in SSc, as an indicator of disease severity, in a Japanese population. In Japanese patients, SSc was associated with IL-1α –889 C allele in contrast with our observation in a Slovak population (genotype IL-1α -889 C/C was 90%, C/T was 10%, and T/T was 0%), whereas the genotype and allele frequencies in healthy controls were similar to those of our Slovak control population. Previously, juvenile rheumatoid arthritis, periodontitis, and Alzheimer's disease have been associated with IL-1α –889 T allele similarly to our findings in this study[9,25,26]. Kawaguchi, *et al* speculated that association of SSc in a Japanese population and the aforementioned chronic inflammatory diseases with the opposite IL-1α –889 allele is related to the influence of IL-1α gene variants on pro-IL-1α cleavage[24]. In SSc, fibroblast accumulation of pro-IL-1α in the cell nucleus has been described, whereas in inflammatory diseases the mature IL-1α protein may be involved[24]. However, the mechanism of interaction between IL-1α –889 polymorphism and the level of protein synthesis has not been elucidated yet. It is still unclear whether IL-1α –889 polymorphism has a direct influence on protein expression or is in linkage disequilibrium with another functionally significant DNA variant. The contradictory results in Japanese and our Slovak patients with SSc may be caused by a different degree of linkage disequilibrium within the IL-1 cluster in these populations. Differences between ethnic groups have also been observed in associations between ulcerative colitis and IL-1 receptor antagonist variable-number tandem-repeat polymorphism[27].

In our previous study we reported association of IL-1α –889 polymorphism with sarcoidosis[28]. In contrast with SSc, which is associated with carriage of IL-1α –889 T allele, in sarcoidosis there was increased frequency of the genotype IL-1α –889 C/C. This disparity might be related to different functional T cell polarization in the 2 diseases. Sarcoidosis is generally considered to develop Th1 immune response, with predominant secretion of interferon-γ and IL-2[29]. In SSc peripheral blood, T lymphocytes show both Th1 and Th2 activation, whereas skin-infiltrating CD4+ T cells display a Th2 cytokine profile with production of IL-4[30-33]. The T cell receptor-triggered proliferation of Th2 cells requires an additional signal mediated by IL-1, and an IL-4-independent pathway of Th2 cell proliferation resulting from the autocrine IL-1α loop has been identified[34]. Therefore we speculate that the IL-1α gene polymorphism may affect the polarized T cell activation in sarcoidosis and SSc through different levels of IL-1 protein production.

Another discrepancy between our previous and the current study is present in IL-1α –889 genotype and allele frequencies differing between the Czech and Slovak control populations, which are both of Western Slavonic ancestry[28]. These differences may be attributed to population stratification. Allele frequencies are known to vary widely within and between populations, irrespective of disease status[35]. The frequencies of IL-1α –889 polymorphism in our Czech and Slovak control groups are both within the interval of frequencies of IL-1α –889 genotypes and alleles existing in European Caucasian populations[36]. To minimize the risk of a population stratification problem in this case-control study we chose a Slovak control population from the same geographic region of the Slovak Republic as the patients with SSc.

In our study we show that the IL-1α –889 polymorphism could be one of genetic factors leading to the aberrant immune response and fibroblast activity in SSc and thus predisposing to the development of systemic sclerosis. Further studies with larger numbers of patients with this rare disease in populations of different ethnic background are required to confirm this association.

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REFERENCES

1. Haustein UF. Systemic sclerosis — scleroderma [review]. Dermatol Online J 2002;8:3.
2. Olivieri D, du Bois RM. Interstitial lung diseases. European Respiratory Monograph 2000;5 (Monograph 14):137-64.
3. Tan FK, Arnett FC. Genetic factors in the etiology of systemic sclerosis and Raynaud phenomenon. Curr Opin Rheumatol 2000;12:511-9.
4. Arnett FC, Cho M, Chatterjee S, Aguilar MB, Reveille J, Mayes MD. Familial occurrence frequencies and relative risks for systemic sclerosis (scleroderma) in three United States cohorts. Arthritis Rheum 2001;44:1359-62.
5. Renzoni E, Lympany P, Sestini P, et al. Distribution of novel polymorphisms of the interleukin-8 and CXC receptor 1 and 2 genes in systemic sclerosis and cryptogenic fibrosing alveolitis. Arthritis Rheum 2000;43:1633-40.

6. Avila JJ, Lympany PA, Pantelidis P, Welsh KI, Black CM, du Bois RM. Fibronectin gene polymorphism associated with fibrosing alveolitis in systemic sclerosis. Am J Respir Cell Mol Biol 1999;20:106-12.
7. Dinarello CA. Biologic basis for interleukin-1 in disease. Blood 1996;87:2095-147.
8. Nicklin MJ, Weith A, Duff GW. A physical map of the region encompassing the human interleukin-1 alpha, interleukin-1 beta, and interleukin-1 receptor antagonist genes. Genomics 1994;19:382-4.
9. McDowell TL, Symons JA, Ploski R, Forre O, Duff GW. A genetic association between juvenile rheumatoid arthritis and a novel interleukin-1α polymorphism. Arthritis Rheum 1995;38:221-8.
10. Hulkkonen J, Laippala P, Hurme M. A rare allele combination of the interleukin-1 gene complex is associated with high interleukin-1 beta plasma levels in healthy individuals. Eur Cytokine Netw 2000;11:251-5.
11. Kawaguchi Y. IL-1 alpha gene expression and protein production by fibroblasts from patients with systemic sclerosis. Clin Exp Immunol 1994;97:445-50.
12. Kantor TV, Friberg D, Medsger TA Jr, Buckingham RB, Whiteside TL. Cytokine production and serum levels in systemic sclerosis. Clin Immunol Immunopathol 1992;65:278-85.
13. Preliminary criteria for the classification of systemic sclerosis (scleroderma). Subcommittee for Scleroderma Criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. Arthritis Rheum 1980;23:581-90.
14. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucl Acids Res 1988;16:1215-8.
15. Bunce M, O'Neill CM, Barnardo MC, et al. Phototyping: comprehensive DNA typing for HLA-A, B, C, DRB3, DRB4, DRB5 and DQB1 by PCR with 144 primer mixes utilising sequence-specific primers (PCR-SSP). Tissue Antigens 1995;46:355-67.
16. Postlethwaite AE, Raghow R, Stricklin GP, Poppleton H, Seyer JM, Kang AH. Modulation of fibroblast functions by interleukin 1: increased steady-state accumulation of type I procollagen messenger RNAs and stimulation of other functions but not chemotaxis by human recombinant interleukin 1 alpha and beta. J Cell Biol 1988;106:311-8.
17. Riquet FB, Lai WF, Birkhead JR, Suen LF, Karsenty G, Goldring MB. Suppression of type I collagen gene expression by prostaglandins in fibroblasts is mediated at the transcription level. Mol Med 2000;6:705-19.
18. Rezzonico R, Burger D, Dayer JM. Direct contact between T lymphocytes and human dermal fibroblasts or synoviocytes down-regulates types I and III collagen production via cell-associated cytokines. J Biol Chem 1998;273:18720-8.
19. Kolb M, Margetts PJ, Anthony DC, Pitossi F, Gauldie J. Transient expression of IL-1ß induces acute lung injury and chronic repair leading to pulmonary fibrosis. J Clin Invest 2001;107:1529-36.
20. Lonnemann G, Engler-Blum G, Müller GA, Koch KM, Dinarello CA. Cytokines in human renal interstitial fibrosis. II. Intrinsic interleukin (IL)-1 synthesis and IL-1-dependent production of IL-6 and IL-8 by cultured kidney fibroblasts. Kidney Int 1995;47:845-54.
21. Kawaguchi Y, Hara M, Wright TM. Endogenous IL-1α from systemic sclerosis fibroblasts induces IL-6 and PDGF-A. J Clin Invest 1999;103:1253-60.
22. Yamamoto T, Sawada Y, Katayama I, Nishioka K. Increased production of nitric oxide stimulated by interleukin-1ß in peripheral blood mononuclear cells in patients with systemic sclerosis. Br J Rheumatol 1998;37:1123-5.
23. Shirodaria S, Smith J, McKay IJ, Kennett CN, Hughes FJ. Polymorphisms in the IL-1A gene are correlated with levels of interleukin-1α protein in gingival crevicular fluid of teeth with severe periodontal disease. J Dent Res 2000;79:1864-9.
24. Kawaguchi Y, Tochimoto A, Ichikawa A, et al. Association of IL1A gene polymorphisms with susceptibility to and severity of systemic sclerosis in the Japanese population. Arthritis Rheum 2003;48:186-92.
25. Kornman KS, Crane A, Wang HY, et al. The interleukin-1 genotype as a severity factor in adult periodontal disease. J Clin Periodontol 1997;24:72-7.
26. Grimaldi LM, Casadei VM, Ferri C, et al. Association of early-onset Alzheimer's disease with an interleukin-1 alpha gene polymorphism. Ann Neurol 2000;47:361-5.
27. Tountas NA, Casini-Raggi V, Yang H, et al. Functional and ethnic association of allele 2 of the interleukin-1 receptor antagonist gene in ulcerative colitis. Gastroenterology 1999;117:806-13.
28. Hutyrova B, Pantelidis P, Drábek J, et al. Interleukin-1 gene cluster polymorphisms in sarcoidosis and idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2002;165:148-51.
29. Moller DR. Involvement of T cells and alterations in T cell receptors in sarcoidosis. Semin Respir Infect 1998;13:174-83.
30. Valentini G, Baroni A, Esposito K, et al. Peripheral blood T lymphocytes from systemic sclerosis patients show both Th1 and Th2 activation. J Clin Immunol 2001;21:210-7.
31. Tsuji-Yamada J, Nakazawa M, Minami M, Sasaki T. Increased frequency of interleukin 4 producing CD4+ and CD8+ cells in peripheral blood from patients with systemic sclerosis. J Rheumatol 2001;28:1252-8.
32. Fujii H, Hasegawa M, Takehara K, Mukaida A, Sato S. Abnormal expression of intracellular cytokines and chemokine receptors in peripheral blood T lymphocytes from patients with systemic sclerosis. Clin Exp Immunol 2002;130:548-56.
33. Mavalia C, Scaletti C, Romagnani P, et al. Type 2 helper T-cell predominance and high CD30 expression in systemic sclerosis. Am J Pathol 1997;151:1751-8.
34. Huber M, Beuscher HU, Rohwer P, Kurrle R, Röllinghoff M, Lohoff M. Costimulation via TCR and IL-1 receptor reveals a novel IL-1α-mediated autocrine pathway of Th2 cell proliferation. J Immunol 1998;160:4242-7.
35. Cardon LR, Palmer LJ. Population stratification and spurious allelic association. Lancet 2003;361:598-604.
36. Louie LG, Wallenstein E, Direskeneli GS, et al. Report of the Anthropology Group from the Cytokine Polymorphism Component, 13th IHWC. In: Hansen JA, Dupont B, editors. HLA 2002. Immunology of the human MHC. vol. 2. Seattle: IHWG Press; 2003 (in press).