

CC chemokine receptor 5 and interleukin-1 receptor antagonist gene polymorphisms in patients with primary Sjögren's syndrome

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ABSTRACT

Objective. *Interleukin-1 receptor antagonist (IL-1Ra) and also mononuclear cell-attractant chemokines CCL3, CCL4 and CCL5 have been implicated in the immunopathogenesis of primary Sjögren syndrome (pSS). Both the gene coding for receptor CCR5 binding the aforementioned CCL ligands and the gene for IL-1Ra are polymorphic. We have therefore in a case control study assessed the putative role of these "candidate" polymorphic genes in the inflammatory process in Sjögren syndrome.*

Methods. *DNA was obtained from 39 unrelated patients with primary Sjögren's syndrome and 76 unrelated healthy controls; all subjects were Caucasians of Slovak origin. CCR5Δ32 and IL-1Ra VNTR polymorphisms were genotyped by PCR-SSP.*

Results. *The frequencies of CCR5Δ32 in patients with pSS were different from that in control subjects: there was an apparent decrease of the mutant allele in the patient group. CCR5Δ32/CCR5 heterozygosity was associated with a reduced relative risk of pSS (OR 0.35, p = 0.043). There was no difference in the distribution of the alleles of the IL-1Ra VNTR polymorphism between the groups of pSS patients and control subjects.*

Conclusion. *In this population of patients with Sjögren's syndrome, the frequency of CCR5Δ32/CCR5 genotype is significantly decreased. The data suggests that carrier status for the CCR5 Δ32 allele may contribute to protection from the development of primary Sjögren's syndrome. In contrast, IL-1Ra VNTR polymorphism does not confer susceptibility to primary Sjögren's syndrome in Slovak Caucasians.*

Introduction

Sjögren's syndrome (SS) is a chronic autoimmune disease characterised by CD4+ T-lymphocyte infiltration of the exocrine glands and B cell hyperreactivity with production of autoantibodies (1). The disease may occur either alone (primary SS) or in association with other autoimmune rheumatic disorders (secondary SS). The clinical manifestation of SS includes except for the most

frequent xerostomia and keratoconjunctivitis sicca also systemic complications (including interstitial lung disease) in approximately one-third of patients. Genetic studies in SS imply a multigenic pattern of inheritance, which acts in conjunction with environmental factors in triggering the disease (2).

The pattern and kinetics of inflammatory cell infiltration in affected tissues may be controlled by chemotactic cytokines - chemokines (3). Indeed, CC chemokines MIP-1α (CCL3), MIP-1β (CCL4) and RANTES (CCL5) have recently been identified in ductal epithelial cells from biopsied minor salivary glands of patients with primary Sjögren's syndrome (pSS) while they were not detected in control biopsies (4). CC chemokine receptor (CCR) 5, which binds the aforementioned mononuclear attractant chemokines, is characterised by a gene polymorphism. A 32-bp deletion in the CCR5 gene (CCR5Δ32) results in a non-functional surface receptor molecule unable to bind its CC chemokine ligands (3).

The role of proinflammatory cytokine interleukin-1 (IL-1) in the pathophysiology of SS has been also demonstrated. Conjunctival and salivary gland epithelial cells in SS express increased levels of IL-1α RNA transcripts (5, 6). The local overexpression of IL-1β in murine autoimmune sialadenitis appears to be one of the factors promoting the organ-localised autoimmunity (7). The biological activity of IL-1 is counteracted by naturally occurring IL-1 receptor antagonist (IL-1Ra). Elevation of serum IL-1Ra, but decrease of salivary IL-1Ra levels has been reported in patients with SS (8). The IL-1Ra gene is polymorphic within intron 2, which contains variable numbers of 86-bp tandem repeat sequence (9).

The IL-1Ra VNTR polymorphism participates in the regulation of IL-1Ra protein production and may therefore be relevant *in vivo* for development of aberrant immune reaction in immune-mediated diseases (10). To clarify if the polymorphisms in the aforementioned "candidate" genes are relevant for the inflammatory process in Sjögren syndrome we have investigated the CCR5 Δ32 and IL-1Ra VNTR polymorphisms

in patients with pSS in comparison with normal control population.

Material and methods

Subjects

In the study were included 39 patients with primary Sjögren's syndrome and 76 healthy controls. Both patients and controls were unrelated Slovak (western Slavonic) Caucasians. The diagnosis of pSS was based on the examination of salivary glands and diagnosis of keratoconjunctivitis sicca, positive labial gland biopsy and the presence in serum of autoantibodies to Ro (SSA) or La (SSB) or both (11). Healthy control subjects were recruited from ethnically matched volunteers in whom presence of any autoimmune disease was excluded. All subjects were informed about the purpose of the study and gave their consent.

Genetic analysis

Genomic DNA was extracted from 10 ml EDTA-anticoagulated peripheral blood by a salting-out method. For genotyping both investigated polymorphisms the polymerase chain reaction with sequence specific primers (PCR-SSP) was used. The PCR products were electrophoretically separated on 2% agarose gel stained with ethidium-bromide.

CCR5 genotyping. Sense primer nucleotide sequence was 5'-CTT CAT TAC ACC TgC AgC TCT-3', antisense primer sequence 5'-CAC AgC CCT gTg CCT CTT CTT C-3'. The polymerase chain reaction was run under the following conditions: 0.05 µg of DNA was amplified in 10 ml final volume containing: 0.2 mM dNTPs, 1.5 mM MgCl₂, 1 µM primers, 17 mU/ml Taq; 67 mM TrisCl pH 8.8; 16.6 mM (NH₄)₂SO₄ and 0.1% Tween. After initial denaturation (94°C for 2 min), the PCR was run using a touch-down principle: the annealing temperature was lowered after each cycle of the first ten cycles by 1°C. The cycling conditions were as follows: 94°C for 10 sec, 67°C -1°C per cycle for 50 sec, 72°C for 30 sec. The next 20 cycles were run at constant annealing temperature of 58°C as follows: 94°C for 10 sec, 58°C for 50 sec, 72°C for 30 sec. The PCR

Table I. Genotype and allele frequencies of CC chemokine receptor (CCR) 5 in patients with primary Sjögren's syndrome (pSS) and healthy controls.

CCR5		pSS n=39	Controls n=76	χ ²	P	OR
Genotypes	wt/wt	89.7% (35)	72.4% (55)	3.963	0.043	0.349*
	wt/mt	10.3% (4)	27.6% (21)			
	mt/mt	0% (0)	0% (0)			
Alleles	wt	94.9% (74)	86.2% (131)	3.422	0.061	0.369†
	mt	5.1% (4)	13.8% (21)			

OR: odds ratio; wt: wild type allele; mt: mutant type allele; n.s.: non-significant.
CI: confidence interval; *95% C.I.: 0.109 - 0.983; †95% C.I.: 0.129 - 1.061.

was finished by incubation at 72°C for 2 min followed by 25°C for 1 sec. The PCR products were separated by electrophoresis on 2% agarose gel in a Tris-borate buffer at 5.6 V/cm for 50 min.

IL-1RN genotyping. For amplification of the region containing VNTR primers 5' CTC AgC AAC ACT CCT AT and 5' TCC Tgg TCT gCA ggT AA were used (9). 0.05 µg DNA was amplified in 10 µl final volume containing 0.2 mM dNTPs, 1.5 mM MgCl₂, 5.0 mM primers; 25 mU/ml Taq polymerase; 67 mM TrisCl pH 8.8; 16.6 mM (NH₄)₂SO₄ and 0.1% Tween. After initial denaturation at 94°C for 2 min, the cycling conditions were as follows: 35 cycles at 95° for 10 sec, 55° for 30 sec, 72° for 45 sec, and finally 25° for 1 sec. The alleles were identified electrophoretically (see above) according to their size: allele 1 - 410 base pair (bp), allele 2 - 240 bp, allele 3 - 500 bp, allele 4 - 320 bp, allele 5 - 590 bp.

Statistical analysis

Genotype and allele frequencies in cases and controls were determined by direct counting. The data sets were compared using a standard 2 x 2 χ² analysis by SIGTEST, a computer program that uses a Woolf-Haldane correction in cases of small numbers. Relative risk (odds ratio, OR) was also calculated. Conformity to the Hardy-Weinberg equilibrium was tested by 2 x 2 χ² test comparing the expected and observed numbers.

Results

The investigated population groups were found to be in Hardy-Weinberg equilibrium with regard to the distribution of studied genotypes. The distribu-

Table II. Genotype and allele frequencies of interleukin-1 receptor antagonist (IL-1Ra) in patients with primary Sjögren's syndrome (pSS) and healthy controls.

IL-1Ra		pSS n=39	Controls n=76
Geno- types	1,1	61.5% (24)	54.0% (41)
	1,2	23.1% (9)	32.9% (25)
	1,3	5.1% (2)	6.6% (5)
	1,4	0% (0)	1.3% (1)
	2,2	5.1% (2)	3.9% (3)
	2,3	2.6% (1)	1.3% (1)
Alleles	1	75.7% (59)	74.3% (113)
	2	19.2% (15)	21.1% (32)
	3	3.8% (3)	3.9% (6)
	4	0% (0)	0.7% (1)
	5	1.3% (1)	0% (0)

The differences in genotype and allele frequencies between pSS and controls were non-significant.

tion of gene and genotype frequencies of CCR5Δ32 and IL-1Ra VNTR polymorphism in patients with pSS and control subjects is shown in Tables I and II, respectively. Frequency of the CCR5Δ32 allele was decreased in patients with primary Sjögren's syndrome in comparison to control population. Strikingly, in the patient group there were only 4 heterozygote (CCR5Δ32/CCR5) individuals, while according to the data from normal population the number of heterozygous patients should be expected to be nearly three times higher. The difference in the genotype frequencies between patients and control group was significant. Regarding IL-1Ra VNTR polymorphisms, no statistically significant differences have been found comparing the patients and controls.

Discussion

In the present association study we have evaluated the distribution of two polymorphic candidate genes CCR5 and IL-1Ra in patients with primary Sjögren's syndrome in comparison with ethnically matched control population. We report that frequency of CCR5Δ32/CCR5 genotype is decreased in patients with pSS.

Our finding that individuals carrying the CCR5Δ32 allele are at reduced risk of pSS (OR < 0.37) is the first suggestion of implicating a role for a chemokine receptor polymorphism in Sjögren's syndrome. In agreement with rules of interpretation of association studies (12) it would be appropriate if the results of this study can be independently replicated which, due to the disease incidence, requires study by new investigators outside our geographic region. In the meantime, it should be investigated, if CCR5 gene polymorphism affects clinical course of SS, especially with regard to the frequent lung involvement in this disease (13). In this context it is worth to mention that CCR5Δ32 has been previously found in lower frequency in individuals with asthma (14). On the contrary, the deletion mutation was increased in patients with pulmonary sarcoidosis and importantly, it was associated with the more advanced disease (15). These two conditions differ in the localisation of pulmonary inflammatory process and by a different T-helper (Th) cell profile: while in asthma (a Th2 airway disease), CCR5Δ32 is a protective factor, in sarcoidosis (Th1 alveolitis) it is a factor of susceptibility (15).

No association between pSS and IL-1Ra VNTR polymorphism was found in our study. In the report of Perrier *et al.* (8) the allele 2 of this polymorphism (IL-1RN*2) has been described as a factor of severity, but not susceptibility to pSS. The IL-1RN* 2 was increased only in the severe form of pSS, i. e. in the definite SS, but not in patients with possible and definite pSS taken together as a group. Our study performed in groups of patients of different ethnicity confirms that the IL-1RN polymorphism does not represent a susceptibility marker for pSS.

Further investigations could answer the question if there is a common CCR5-based link between the aforementioned immune (T-cell) mediated conditions and Sjögren's syndrome, a disease with not yet precisely defined Th-cell profile.

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