Interleukin 10 (IL-10) Influences Autoimmune Response in Primary Sjögren's Syndrome and Is Linked to IL-10 Gene Polymorphism

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ABSTRACT. ***Objective.*** To investigate the association between serum levels of interleukin 10 (IL-10), the synthesis of autoantibodies, salivary gland disease activity, clinical manifestations, and IL-10 microsatellite polymorphism in patients with primary Sjögren's syndrome (pSS).

Methods. Serum IL-10 and autoantibody levels [IgG anti-Ro and anti-La, total and IgA rheumatoid factor (RF)] were measured by ELISA. A minor salivary gland (MSG) biopsy was performed in all patients and the focus score was determined as a measure of salivary gland disease activity. In addition, IL-10 microsatellite typing was performed by polymerase chain reaction technique.

Results. IL-10 concentration was higher in patients (n = 39) than in controls (n = 15) (21.4 ± 6.7 vs 2.5 ± 3.5 pg/ml; p = 0.001). We found a significant positive correlation between IL-10 levels and titers of IgA RF, anti-Ro, and anti-La antibodies, as well as focus score. In comparison with patients with low IL-10 production (< 9.5 pg/ml), patients producing high IL-10 had significantly more episodes of cutaneous vasculitis and a higher proportion of them carried the IL-10.G9 allele.

Conclusion. Autoimmune response in pSS patients as well as salivary gland disease activity and cutaneous involvement appears to be mediated by IL-10 levels; in turn, there is a linkage with IL-10 gene polymorphism. (J Rheumatol 2002;29:1874–6)

Key Indexing Terms:
SJÖGREN'S SYNDROME INTERLEUKIN 10 ANTI-RO ANTIBODIES
ANTI-LA ANTIBODIES RHEUMATOID FACTOR POLYMORPHISM GENETICS

In primary Sjögren's syndrome (pSS) overexpression of interleukin 10 (IL-10) has a critical role in the pathogenesis of disease[1-13]. IL-10 is a multifunctional cytokine with diverse effects on most hemopoietic cell types. It regulates growth and/or differentiation of B cells and the secretion of immunoglobulins, and also plays a key role in the function of some T cells[14]. In pSS patients, IL-10 is released by T cells from salivary glands and by peripheral blood mononuclear cells[2,7,8]. Further, IL-10 messenger RNA expression is elevated in the salivary glands of SS patients[1,3,6,9,10]. In a transgenic murine model, IL-10 induced both destruction of glandular tissues through apoptosis and lymphocytic infiltration consisting primarily of Fas-ligand+ CD4+ T cells[15]. This has also been reported in human disease.

The IL-10 gene is located on the long arm of chromosome 1 at 1q31-32 and is highly polymorphic. Two areas of multiple (CA)n repeat microsatellite polymorphisms and at least 7 linked point mutations have been recorded[16]. The ability to secrete IL-10 varies according to the genetic composition of the IL-10 locus[17]. We examined the association between serum IL-10 levels and autoantibody production as well as salivary gland disease activity, and determined IL-10 microsatellite polymorphism in patients with pSS.

MATERIALS AND METHODS

Study population. Thirty-nine patients fulfilling at least 4 of the European classification criteria for pSS, including a positive minor salivary gland (MSG) biopsy, were included[18]. The mean age was 48 ± 14 years, with a mean duration of disease of 5.2 ± 6.3 years. The clinical manifestations observed during the course of the disease were recorded and classified according to Oxholm, *et al*[19]. Fifteen healthy controls unrelated to the patients, without inflammatory or autoimmune disease, were matched to patients by age (± 5 yrs), sex, and geography.

Salivary gland disease activity. We considered the focus score on MSG biopsy as a measure of salivary gland disease activity. Focal lymphocytic sialadenitis (FLS), characteristic of SS, corresponds to aggregates of 50 or more lymphocytes (one foci) in perivascular or periductal locations, adjacent to normal appearing acini in gland lobules[18]. The FLS was graded by focus score, meaning the number of foci/4 mm^2 of MSG, and determined by semiautomatic analysis.

IL-10 levels and microsatellite typing. Serum IL-10 was measured by ELISA

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(OptEIA™ Human IL-10 Kit; Pharmingen, San Diego, CA, USA). Genotyping at the IL-10.G and IL-10.R microsatellites was performed in genomic DNA using the polymerase chain reaction (PCR) technique as described[17].

Autoantibodies. Antinuclear antibodies were determined by indirect immunofluorescence on HEp-2 cells. IgG anti-DNA, anti-RNP, anti-Sm, anti-Ro and anti-La antibodies were determined by ELISA (Inova Diagnostics, San Diego, CA, USA). Total and IgA rheumatoid factor (RF) were measured by turbidimetry and ELISA, respectively (Immco Diagnostics, Buffalo, NY, USA). All these determinations were done simultaneously in serum at time of obtaining MSG biopsy.

Statistical analysis. Data are shown as means ± standard deviation (SD) and as percentages. Differences between means and proportions were established using the Mann-Whitney U test, the chi-square test, and Fisher's exact test as appropriate. Correlations were assessed by Spearman rank correlation test. A p value of < 0.05 was chosen as indicative of statistical significance.

RESULTS

Serum IL-10 levels. The mean IL-10 level in the control group was 2.5 ± 3.5 compared to 21.4 ± 6.7 pg/ml in the patient group ($p = 0.001$). IL-10 levels were undetectable in one patient and in 2 controls. In patients, the levels of IL-10 correlated with levels of anti-Ro antibodies ($r = 0.3$, $p = 0.04$), anti-La antibodies ($r = 0.4$, $p = 0.02$), IgA RF ($r = 0.7$, $p = 0.006$), and the focus score ($r = 0.4$, $p = 0.01$). No patient was positive for anti-DNA, anti-Sm, or anti-RNP antibodies.

Comparison between high and low IL-10 producers. Patients were compared according to IL-10 status. High IL-10 producers were defined as those with IL-10 levels > 9.5 pg/ml (normal range + 2 SD). High IL-10 producers had more episodes of cutaneous vasculitis and carried the IL-10.G9 allele in a higher proportion than patients with low levels of serum IL-10 (Table 1). There were no significant differences between the proportions of patients positive for autoantibodies according to IL-10 producer status.

DISCUSSION

In this study, an increased serum level of IL-10 was found in pSS patients correlating with autoantibody production and salivary gland disease activity. No significant difference in the percentages of patients positive for antibodies according to the IL-10 status was observed (Table 1). Our results indicate that IL-10 influences the titers but not the presence *per se* of autoantibodies. Besides IL-10, other cytokines such as IL-6 may also participate in the induction of antibodies by B cells[1]. IL-10 together with IL-6 plays a central role in the maturation of plasma cells and activation of immunoglobulin synthesis. The positive correlation between IL-10 and IgA RF but not total RF may be attributed to the influence of IL-10 in isotype switching[14].

Our results support those of a previous study showing a correlation between serum IL-10 levels and MSG lymphocytic infiltration[12], and indicate that IL-10 participates in the mononuclear cell recruitment observed in pSS patients. In pSS, IL-10 is produced mainly in MSG by CD4+ T lymphocytes and acinar cells adjacent to lymphoid focus[1,3,10].

High IL-10 producer patients had significantly more episodes of cutaneous vasculitis and a higher proportion of them carried the IL-10.G9 allele (Table 1). In Caucasians, the presence of the GCC haplotype or the GCC/ATA genotype and the absence of the ACC haplotype of the IL-10 gene was associated with an increased susceptibility to pSS[13]. Eskdale, *et al* described 4 major haplotype families at the human IL-10 locus[16], and found that not a single allele but rather the complete haplotype was associated with IL-10 synthesis. In particular, the highest levels of IL-10 were observed in the IL-10.R2-(IL-10.G)-A-C-C haplotype[16]. Due to the subtle nature of allelic effects, longitudinal and larger studies are needed to find phenotypic differences clearly associated with disease[13].

The significant role played by IL-10 in pSS has been confirmed in most studies (Table 2). However, Boumba, *et al* did not detect IL-10 mRNA in MSG from patients or controls[20]. Hagiwara, *et al* observed no change in the number of peripheral cells spontaneously secreting IL-10 compared with controls[21]. García-Carrasco, *et al* observed almost identical mean levels of serum IL-10 in pSS patients and in controls[22]. However, when their patients were compared according to the presence of extraglandular manifestations, the mean IL-10 levels were higher in 5 patients with liver involvement[22].

IL-10 gene homologs have been found in some viruses including Epstein-Barr (EBV)[14]. It is not clear if IL-10 posi-

Table 1. Characteristics of patients with pSS according to IL-10 production.

Characteristic	High IL-10 n = 20 (%)	Low IL-10 n = 19 (%)
Age, yrs	48.8 ± 16.4	47.5 ± 12
Age at onset, yrs	39 ± 15.5	41.4 ± 12.8
Antinuclear antibodies	17 (85)	15 (79)
Anti-Ro antibodies*	13 (65)	12 (63)
Anti-La antibodies*	7 (35)	8 (42)
Total RF**	14 (70)	12 (63)
IgA RF***	10 (50)	7 (37)
Internal organ — exocrine disease	8 (40)	4 (19)
Inflammatory — vascular	15 (75)	13 (68)
Skin	10 (50)	3 (16)†
Raynaud's phenomenon	4 (20)	5 (26)
Mediator-induced	2 (10)	5 (26)
IL-10 Microsatellites		
IL-10.R2.G14	4 (20)	6 (32)
IL-10.R3	3 (15)	7 (37)
IL-10.R2	19 (95)	19 (100)
IL-10.R2.G13	12 (60)	7 (37)
IL-10.G9.13	7 (35)	2 (11)
IL-10.G13	10 (50)	8 (42)
IL-10.G9	16 (80)	8 (42)‡

* Positive > 20 U/ml (by ELISA); ** positive > 40 U/ml (by turbidimetry); *** positive > 20 U/ml (by ELISA); † OR: 5.3, 95% CI: 1.2–24, $p = 0.04$, ‡ OR: 5.5, 95% CI: 1.3–22, $p = 0.02$. High IL-10 levels > 9.5 pg/ml. Clinical disease manifestations were classified according to Oxholm, *et al*[19]. Internal organ exocrine disease included pulmonary, pancreatic, gastrointestinal, renal or hepatic involvement. Inflammatory vascular disease included skin, musculoskeletal or neurologic involvement. Skin manifestations corresponded to cutaneous vasculitis (purpura or urticarial vasculitis).

Table 2. Evidence favoring an important pathogenic role of IL-10 in pSS.

	Reference
Local production (MSG)	
Increased production by lymphocytes	3
Increased production by CD4+ T cells	1
Involved in the progression towards B cell clonality	4
Increased mRNA expression in MSG than in PBMC	6
Increased levels in saliva correlated with MSG mRNA levels	1
Increased production by salivary gland-derived CD4+ clones than blood-derived CD4+ clones	7
Strong expression on mRNA in MSG correlated with high serum autoantibodies in 2 patients	9
Higher mRNA expression by lymphoid and acinar cells adjacent to a lymphoid focus	10
Peripheral production	
Increased production by B cells and monocytes	2,8
Spontaneous mRNA expression in T cells	5
Higher number of PBMC secreting IL-10	11
Increased serum levels correlated with increased IgG1 and lymphocytic infiltrate	12
Increased serum levels correlated with autoantibody levels and focus score on MSG, and were associated with cutaneous vasculitis and IL-10.G9 allele	Present study
Genetic influence of IL-10 locus on pSS	13
IL-10 transgenic murine model resembling human SS	15

MSG: minor salivary glands, PBMC: peripheral blood mononuclear cells.

tive results in pSS patients correspond to human IL-10 or to a previous infection with EBV.

In summary, our results and literature review (Table 2) indicate that IL-10, produced mainly at the inflammatory site in MSG, plays a key role in B cell function, different isotype antibody production, salivary gland disease activity, and clinical expression of pSS. The IL-10 locus may also participate in the immunopathogenesis of disease. Further investigation on the possible viral origin of IL-10 in pSS, and therapeutic blockade of this cytokine in pSS patients, will help to elucidate its role in this disease.

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