Polymorphism in Promoter Region of IL10 Gene Is Associated with Rheumatoid Arthritis in Women

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ABSTRACT. ***Objective***. Rheumatoid arthritis (RA) is a genetically complex disease with many possible phenotypes. We investigated IL10 and TNFA gene polymorphisms in a group of Swedish women and men with RA compared with healthy individuals to estimate combinations of alleles specific for the disease.

Methods. We analyzed 264 patients with RA and 286 healthy controls for biallelic single-nucleotide polymorphisms in the –308 position of the TNFA and in the –1087 position of the IL10 gene by polymerase chain reaction with restriction endonuclease mapping.

Results. The frequencies of the –308 TNFA genotypes were not different in women and men with RA in comparison to the controls. In contrast, frequencies of the GG, AG, and AA –1087 IL10 genotypes were significantly different in women in the investigated groups: 26%, 58%, and 15% for RA patients and 24%, 54%, and 28% for the controls (chi-square = 8.18, $p < 0.02$). We confirmed this finding in a separate dataset of female patients and controls. The frequencies of the IL10 genotypes in men were similar in the patients and controls. We found no differences in the distribution of the TNFA or IL10 genotypes in relation to rheumatoid factor in the patients.

Conclusion. On the basis of IL10 polymorphism, female patients with RA seem to represent a separate disease subgroup. (J Rheumatol 2004;31:422–5)

Key Indexing Terms:
RHEUMATOID ARTHRITIS GENE POLYMORPHISM SEX
CYTOKINES INTERLEUKIN 10 TUMOR NECROSIS FACTOR

During the last decade abnormalities in the regulation of production of cytokines in rheumatoid arthritis (RA) have been under detailed investigation. A large body of evidence suggests an effect of the Th1 type of cytokine production on the course of RA. As well, macrophage products including tumor necrosis factor (TNF) and interleukin 1ß (IL-1ß) seem to play a central role in joint inflammation[1]. On the other hand, the influence of antiinflammatory cytokines like IL-10 might be important for downregulation of inflammatory responses during RA. It has been shown that the regulation of cytokine production (e.g., TNF, IL-1ß, IL-4, IL-6, and IL-10) is related to polymorphism in the promoter region of the respective genes, possibly influencing the susceptibility to certain diseases[2-7]. Data related to association of gene polymorphisms with RA are controversial and differ in various populations. Our previous study showed dramatic dissimilarity in the frequency of certain cytokine gene polymorphisms in groups of healthy Caucasian and Chinese individuals[8]. RA is known as a "female" disease, with a 1 to 3–3.5 ratio between the incidence in men and women, implying that sex differences could influence susceptibility to disease. Nevertheless, there are only scarce data related to factors involved in such different phenotypic outcomes. We analyzed the allele frequency for the TNFA and IL10 genes in a cohort of Swedish men and women with RA and investigated the relationship of genotypes to the clinical appearance of the disease.

MATERIALS AND METHODS

Patients and controls. Altogether 264 patients (192 women, 72 men) were selected from several Swedish hospitals; 286 healthy blood donors (159 women, 127 men) from the same geographical regions were included as controls. RA was diagnosed according to American College of Rheumatology criteria. Part of the collected data was from the EIRA Study (Environmental and Genetic Factors in RA). Rheumatoid factor (RF) concentrations (IgG, IgM, and IgA isotypes) were measured by routine ELISA methods used in hospital settings to assess seropositivity. In a separate dataset, 235 female controls and 463 female patients with RA were genotyped.

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DNA extraction. DNA was isolated by modification of the method described earlier[9]. Briefly, EDTA blood was centrifuged to obtain blood cells, and the cell suspension was lysed by Tris-EDTA buffer (Sigma Chemical Co., St. Louis, MO, USA) and washed by centrifugation. The wet pellet was resuspended in proteinase K buffer solution without sodium dodecyl sulfate and incubated 2 h at 55°C. Proteinase K was inactivated by incubation at 95°C for 10 min. Final preparations were stored at –20°C until used as template DNA for polymerase chain reaction (PCR) analysis. The material was extracted by the "salting-out" method.

Polymorphism analysis. Detection of biallelic single-nucleotide polymorphisms in the –308 position of the TNFA and the –1087 position of the IL10 genes was performed as described[8]. Briefly, the PCR was performed with reagents from Applied Biosystems (Foster City, CA, USA). PCR products were tested by polyacrylamide gel electrophoresis (PAGE) before digestion by restriction endonucleases. Between 5 and 35 ng of DNA was used for the amplification and 2–4 µl of the nonpurified PCR mixture was used as substrate for the restriction endonucleases.

Digestion with the restriction endonucleases *Nco*I and *Eco*NI (New England Biolabs, Beverly, MA, USA) was performed according to the manufacturer's instructions in a volume of 25 µl, using 1–3 units of enzyme/probe and 90 min incubation time followed by inactivation at 65°C for 20 min. Introduction of the conservative recognition site for *Eco*NI in PCR product from IL10 gene provided a reliable internal control for the quality of digestion[8]. PAGE was performed in a Novex Xcell MiniCell (San Diego, CA, USA) in a 4–20% gradient for IL10 gene fragments, or 20% Tris-Borate-EDTA gels for TNFA gene fragments, with subsequent silver staining (PlusOne DNA silver staining kit, Amersham Pharmacia-Biotech, Uppsala, Sweden). A ϕX174 RF DNA-*Hae*III digest (GibcoBRL, Life Technologies, Gaithersburg, MD, USA) was used as size marker.

The 5' nuclease assay (TaqMan allelic discrimination) was used as an alternative method for genotyping of –1087 IL10 marker on an ABI Prism™ 7700 Sequence Detector according to the standard protocol. Sequences of primers and probes can be obtained on request from the corresponding author.

Statistical analysis. Chi-square analysis or Fisher exact test were used to determine random associations between the observed alleles in the groups. The power of analysis for categorical data was calculated accordingly to Altman[10].

RESULTS

Our modification of the method for genotyping provides clear and robust differentiation between genotypes for –308 TNFA and –1087 IL10 polymorphisms. Introduction of the conservative recognition site in IL10 PCR products provides a reliable internal control for restriction endonuclease activity for each analyzed sample. As shown in Tables 1 and 2 the frequencies of the TNFA in the female and male patients were not significantly different in comparison with the control group.

In contrast, the distribution of the –1087 IL10 genotypes was different in the female RA patients in comparison with the female controls (chi-square = 7.57, DF = 2, p = 0.02, alpha = 0.81 at p = 0.05). It seemed that the main contribution within this difference was due to the more prevalent AA genotype among women with RA in comparison with healthy women (Table 1). In contrast, the distribution of the frequency of the –1087 IL10 genotype in men was not different in the 2 analyzed groups (chi-square = 0.73, DF = 2 p = 0.69, alpha < 0.1 at p = 0.05) (Table 2).

In a separate independent dataset 235 female controls and 463 women with RA were genotyped for the –1087 IL10 marker; this resulted in a similar distribution of genotypes for both groups: 48/235 AA, 132/235 AG, 55/235 GG for controls, and 134/463 AA, 227/463 AG, and 102/463 GG for patients (chi-square = 6.01, DF = 2, p = 0.049), confirming our finding.

We also tested the association of the investigated genotypes with respect to the occurrence of serum RF in the RA patients. No significant associations between the appearance of RF and the genotype patterns were found, neither in the whole group nor in women and men separately.

DISCUSSION

A complex interplay between genetic and environmental factors leads to RA, and the influence of sex on the incidence of the disease is evident. We found that female, but not male patients have a different distribution of functionally important IL10 genotypes in comparison with healthy controls. Thus, the propensity to trigger and maintain IL-10 might influence the development of the disease.

Previous genetic analysis of susceptibility to RA was done in relation to DRB1 genes. It was found that there was a stronger genetic susceptibility in Finnish male patients compared to female patients with familial RA, as defined by the shared-epitope genes background[11]. Analysis of another population (American Caucasians) also suggested that sex-related factors influenced RA penetrance by their association with the DRB1 genotypes[12]. Nevertheless, the influence of genes outside the MHC has not been investigated and remains unclear.

The regulatory role of gene polymorphisms with respect to the production of several cytokines, including TNF, transforming growth factor-ß1, IL-1ß, IL-4, IL-6, and IL-10 has been reported[3,4,6,7,13,14]. However, despite all efforts, the role of these polymorphisms in the development of RA remains unclear[15]. Possibly, one of the main reasons for the difficulties is involvement of several physically non-linked genes in the pathogenesis of RA. Some of these genes that influence the penetrance of the disease might not be absolutely essential for the development of disease, but in combination with other environmental and/or genetic factors (including sex differences) could contribute to the initiation and/or progression of RA. Indeed, our data show that the homozygotic AA –1087 IL10 genotype is significantly more prevalent in female patients with RA in comparison with healthy women. This suggests that women with the AA genotype may have a higher risk of developing RA than individuals with any other IL10 genotype. The functional background of this process might be connected with a decreased production of IL-10, known to occur in individuals with the IL10 AA genotype[14], although the importance of other genes closely linked to the IL10 gene should not be ignored. IL-10 is an antiinflammatory cytokine with multiple influences on immune reactions, especially downregulation of the produc-

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Table 1. Frequency of the –308 TNFA and –1087 IL10 genotypes in women (%).

	–308 TNF Alleles*		–1087 IL10 Genotype**		
	TNF1/TNF1	TNF2 Carriage	GG	AG	AA
Controls	116 (73.0)	43 (27)	42 (26.4)	93 (58.5)	24 (15.1)
Patients	138 (71.9)	54 (28.1)	47 (24.5)	92 (47.9)	53 (27.6)

* Not significant. ** Chi-square = 7.57, $p < 0.05$. Odds ratio for AA genotype against AG together with GG = 2.07 (95% CI 1.18–3.65, $p < 0.01$).

Table 2. Frequency of the –308 TNFA and –1087 IL10 genotypes in men (%).

	–308 TNF Alleles*		–1087 IL10 Genotype*		
	TNF1/TNF1	TNF2 Carriage	GG	AG	AA
Controls	81 (63.8)	46 (36.2)	31 (24.4)	69 (54.3)	27 (21.3)
Patients	44 (61.1)	28 (38.9)	18 (25.0)	35 (48.6)	19 (26.4)

* Not significant.

tion of proinflammatory cytokines. Therefore, decreased production of IL-10 may result in enhanced inflammatory and autoimmune responses including increased MHC class II expression[16]. This may cause excessive presentation of the potential autoantigens and thus development of autoaggressive T and B cells that are characteristic for RA[17,18]. Indeed, a recent study suggested an association of nonerosive disease with a high level of IL10 mRNA in synovial biopsies from women with RA[19].

The absence of association of the –308 TNFA gene polymorphism with RA in this and other studies[20] contradicts our knowledge about the importance of this cytokine for the development of arthritis during RA[21]. There are several possible explanations for this. First, it is still not clear what type of polymorphism may play the major regulatory role in TNF production[20]. The expression of both soluble and cell-associated forms of TNF and several types of receptors indicates the existence of complicated mechanisms regulating TNF production, not only at the level of DNA sequence consensus. Considering these findings, it is understandable that a simple association of the –308 TNFA gene polymorphism with RA cannot easily be found.

We found no association between seropositivity and the analyzed gene polymorphisms in patients with RA. This is not surprising, since many different genes influence susceptibility and severity of experimental arthritis[22].

We found that the AA –1087 IL10 genotype was more frequently associated with RA in women. The relevance of cytokine gene polymorphism with respect to different clinical manifestations of RA should be further analyzed in distinctly different groups of patients with this disease.

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