2002-285-1

Lack of Association Between –384 and 114 *IL-2* Gene Polymorphisms and Rheumatoid Arthritis

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ABSTRACT. ***Objective***. To investigate the association of 2 single nucleotide polymorphisms (SNP) at positions –384 and 114 in the human interleukin 2 (*IL-2*) gene with susceptibility to and severity of rheumatoid arthritis (RA).

Methods. Genotyping for these *IL-2* variants was performed by a polymerase chain reaction restriction fragment length polymorphism method in 174 RA patients and 153 control individuals.

Results. No statistically significant differences were observed when the –384 and 114 *IL-2* genotype distributions between RA patients and healthy controls were compared. In addition, no association was found between the *IL-2* genotypes with any demographic and clinical variables tested.

Conclusion. Our results provide no evidence for genetic association conferred by the –384 and 114 *IL-2* SNP with respect to susceptibility and severity of RA. (J Rheumatol 2003;30:435–7)

Key Indexing Terms:
INTERLEUKIN 2 GENE PROMOTER SUSCEPTIBILITY
SINGLE NUCLEOTIDE POLYMORPHISM RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is an autoimmune disease of unknown etiology characterized by chronic synovial inflammation in multiple joints with progressive loss of articular cartilage and bone destruction. Twin and family studies provide evidence to support the involvement of both genetic and environmental factors in the etiopathogenesis of RA[1]. So far, HLA class II genes on chromosome 6 are the only loci that have been confidently proved to be associated with RA susceptibility. Despite all these associations, HLA genes account for only about one-third of the genetic predisposition to the disease, indicating that genes outside the HLA region also contribute to the disease[2].

Attractive candidates for additional susceptibility or progression factors are cytokine genes. Cytokines are important mediators of the immune and inflammatory response and play an important role in the pathophysiology of joint inflammation and destruction in RA[3].

Interleukin 2 (IL-2) is produced by activated T cells and has a powerful immunoregulatory effect on a variety of immune cells[4]. Dysregulation of the IL-2/IL-2 receptor system could lead to functional or pathological alterations in the immune system including autoimmunity[5]. Although great discrepancies in IL-2 mRNA and protein measurements have been observed between different studies[6,7], it is generally accepted that spontaneous IL-2 mRNA expression is decreased in peripheral blood and synovial tissue and in fluid of RA patients compared with healthy controls[8,9]. One possible explanation for the differences observed in IL-2 concentrations between RA patients and controls is variation in the 5' promoter region of the *IL-2* gene.

The occurrence of common features of autoimmune diseases and the co-association of multiple autoimmune diseases in the same individual or family suggests the existence of common genetic factors that predispose to autoimmunity[10]. Moreover, analyzing the results of 23 autoimmune or immune mediated disease genome-wide scans revealed non-random clustering of susceptibility loci between different human autoimmune diseases[11]. Further, studies in the IDDM and EAE NOD mouse model showed evidence of linkage to *IL-2*; *Idd3* diabetes resistant gene was co-localized with the EAE-resistant gene in a genetic interval less than 0.15 cM containing *IL-2* gene[12].

These considerations suggest that *IL-2* is a strong candidate in autoimmune diseases. We investigated the contribution of the 2 recently described variations in the *IL-2* locus, one of them located in the promoter region of the gene, to the susceptibility and/or severity of RA.

MATERIALS AND METHODS

Study participants. The subjects enrolled in this study included 174 Spanish patients with RA and 153 healthy volunteer blood donors from the Granada area (southern Spain). The patients were from the Rheumatology Department of the Virgen de las Nieves Hospital in Granada, Spain. All patients were

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Supported by grants SAF00-213 from CICYT, FIS01/3149 and PM 99/0101 from MCYT. Dr. Fedetz is the recipient of an AECI Fellowship.

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Submitted March 28, 2002; revision accepted August 30, 2002.

diagnosed according to the revised criteria of the American College of Rheumatology[13]. Information on their clinical and demographic variables and on HLA typing has been published[14].

Detection of IL-2 gene polymorphisms. The *IL-2* promoter –384 mutation and the 114 polymorphism were analyzed by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) as described[15]. Briefly, for the –384 polymorphism determination, a 131 bp fragment was amplified using the following primers: forward GTGATAGCTCTAATTCATGC and reverse ATTCACATGTTCAGTGTAGTTCT; the amplified product was digested with the enzyme *Bfa* I that recognizes the G allele. For detection of the 114 polymorphism a 262 bp fragment was obtained using the primers: forward ATGTACAGGATGCAACTCCT and reverse TGGTGAGTTTGGGATTCTTG; digestion of the PCR product with the restriction enzyme *Mwo* I produces 2 fragments of 111 and 151 bp from the 114-G allele.

Statistical analysis. For association studies, *p* values were calculated by chi-square method or Fisher's exact test when appropriate. SPSS Version 10.0 software was used to analyze the data. For nonparametric data analysis, Mann-Whitney U test was used for ordinal variables and Fisher's exact test for dichotomous variables.

RESULTS

The distribution of the –384 and 114 *IL-2* genotypes and alleles in RA patients and controls is shown in Table 1. The study population was found to be in Hardy-Weinberg equilibrium. As reported[15], the –384 and 114 *IL-2* variants were in linkage disequilibrium, since we found 6 different genotype combinations of the 9 theoretically possible. No statistically significant differences were observed when the –384 and 114 *IL-2* genotype distributions between RA patients and controls were compared, suggesting that these *IL-2* polymorphisms do not influence susceptibility to RA. Next, to investigate a possible association of the *IL-2* polymorphisms with disease severity, we analyzed demographic and clinical characteristics of RA patients according to their *IL-2* genotypes, and no associations were found with any of the variables tested (data not shown).

DISCUSSION

Many studies have examined the relationship between cytokine gene polymorphism and susceptibility to and clinical severity of diseases[16,17]. This is the first investigation of an association of *IL-2* polymorphisms at position –384 and 114 with RA, and our results provide no evidence for genetic association conferred by these single nucleotide polymorphisms with respect to susceptibility and severity of RA.

Significant differences in allele frequencies have been reported between ethnically diverse populations regarding cytokine gene polymorphism[18,19], suggesting that polymorphisms within cytokine genes may be responsible for the ethnic-based differences in the susceptibility to a variety of diseases, although this theory has not been confirmed. Similarly, the –384 *IL-2* genotype frequencies in our control population show few or no differences from the reported distribution among Caucasians and were different from African-American subjects[19,20]. These differences in the *IL-2* genotype frequencies observed in different populations may exist as a result of the selective characteristics of different infectious diseases.

In addition to the *IL-2* polymorphism analyzed in this study, several other *IL-2* polymorphisms have been reported that are located in different regions of the *IL-2* locus[21,22]. The dinucleotide repeat polymorphism in the 3' untranslated portion of the *IL-2* gene has been reported to be associated with a subset of RA patients and with ulcerative colitis, but not with juvenile rheumatoid arthritis[23,24]. It is worth noting that a recent genome-wide screen in multiplex RA revealed that the microsatellite marker D4S1647 had the most significant linkage with RA outside the HLA region[25]. Of interest, the microsatellite D4S1647 is situated in the chromosome 4q25, whereas *IL-2* gene is located in the region 4q26–27. Therefore, it would be of interest to investigate the possible existence of other polymorphisms in both the promoter and the coding region of the *IL-2* gene and to test them for potential involvement in the genetic predisposition to RA. On the other hand, the biology of IL-2 is also modulated by the IL-2 receptor, and it is possible that *IL-2* receptor polymorphism, individually or in combination with *IL-2* gene polymorphism, may also be important in the pathogenesis of RA.

Our results appear to rule out the relevance of –384 and 114 *IL-2* polymorphisms in the susceptibility to or severity of RA, although the participation of IL-2 protein in the immunopathogenesis of RA is not brought into question by these data.

Table 1. Distribution of the IL-2 –384 and 114 genotypes and alleles in RA patients and healthy controls.

IL-2	RA Patients, n = 174		Controls, n = 153	
	n	%	n	%
–384 Genotypes				
G/G	19	10.9	14	9.1
G/T	65	37.4	50	32.7
T/T	90	51.7	89	58.2
–384 Alleles				
G	103	29.6	78	25.5
T	245	70.4	228	74.5
114 Genotypes				
G/G	74	42.5	71	46.4
G/T	83	47.7	63	41.2
T/T	17	9.8	19	12.4
114 Alleles				
G	231	66.4	205	67
T	117	33.6	101	33

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