Polymorphisms in the IL4 and IL4RA Genes in Colombian Patients with Rheumatoid Arthritis

OLGA MORENO, CLARA ISABEL GONZÁLEZ, DIEGO LUIS SAAIBI, WILLIAM OTERO, REYNALDO BADILLO, JAVIER MARTÍN, and GERARDO RAMÍREZ

ABSTRACT. ***Objective.*** Rheumatoid arthritis (RA) is considered a Th1-driven disease. Interleukin 4 (IL-4) binds to its receptor, promoting Th2 differentiation and limiting Th1 responses, but its role in the pathogenesis of RA is conflicting. We analyzed 2 polymorphisms of the *IL4* gene and 4 polymorphisms of the *IL4RA* gene in patients with RA and in a control population, as well as rheumatoid factor (RF) seropositivity, titers of RF, and history of replacement joint surgery among patients with RA.

Methods. The study population consisted of 102 patients with RA and 102 matched healthy controls. Genotyping of *IL4* –590, *IL4RA* +148, +1124, +1218, and +1902 was determined by restriction fragment length polymorphism-polymerase chain reaction (PCR) and sequence-specific primer-PCR. *IL4* variable number tandem repeat polymorphism was determined by direct amplification.

Results. The *IL4* –590TT genotype was significantly more frequent in patients with RA than in controls (p = 0.018, OR 3.34, 95% CI 1.08–11.04). The *IL4RA* +148A allele was significantly associated with the presence of RF (p = 0.0019, OR 2.55, 95% CI 1.55–4.86) and a history of articular joint replacement (p = 0.024, OR 2.08, 95% CI 1.04–4.18). The *IL4RA* +1902G allele was more frequently seen in patients with RA and high RF titers (p = 0.00067, OR 4, 95% CI 1.64–9.93).

Conclusion. Highly complex pathways lead to the development of RA and may not be similar in all patients. Our findings of higher frequency of *IL4* and *IL4RA* genotypes and alleles with RA, presence of RF, RF titers, and history of articular joint replacement support the polygenic expression of RA and the likely role of IL-4 in influencing its initiation and development. (First Release Dec 1 2006; J Rheumatol 2007;34:36–42)

Key Indexing Terms:
RHEUMATOID ARTHRITIS INTERLEUKIN 4 GENETIC PREDISPOSITION

Rheumatoid arthritis (RA) is a systemic inflammatory polyarthritis that affects 0.5–1% of the general population with high personal, social, and economic costs. It is a multifactorial disease, with genetic and enviromental factors contributing to the pathogenesis[1,2]. It is considered that the contribution of the genes localized at the HLA complex explains 30–50% of the genetic component of RA[3,4]. Besides MHC genes, cytokine genes are of particular interest, providing active participation in the inflammatory process of RA[5].

It is considered that RA is orchestrated by Th1 CD4+ cells that stimulate monocytes, macrophages, synovial fibroblasts, and other cells to produce cytokines such as tumor necrosis factor-α (TNF-α), interleukin 1 (IL-1), IL-6, IL-15, IL-17, and metalloproteinases that produce tissue damage[6]. In response to this proinflammatory reaction, antiinflammatory mechanisms are activated in an attempt to control the autoreactivity. These mechanisms include the production of IL-10, IL-13, IL-4, and transforming growth factor-ß, as well as antagonists of the mediators of inflammation such as the IL-1 receptor antagonist (IL-1Ra) and soluble receptors of TNF-α[6,7].

IL-4 is produced by Th2 cells, basophils, eosinophils, and mastocytes and promotes the change of immunoglobulin production of B cells from IgM to IgE and IgG4[8]. It has stimulatory and inhibitory effects, inducing differentiation of Th0 to Th2 cells, and among its antiinflammatory processes are downregulation of proinflammatory cytokines such as TNF-α, IL-1ß, IL-6, and interferon-γ (IFN-γ), inhibition of the proliferation of fibroblastic cells of the articu-

From the Laboratorio de Inmunología y Biología Molecular, Grupo de Inmunología y Epidemiología Molecular, Facultad de Salud, Universidad Industrial de Santander, Bucaramanga; Clínica Carlos Ardila Lulle, Floridablanca, Santander; and Departamento de Medicina Interna, Facultad de Salud, Universidad Industrial de Santander, Bucaramanga, Colombia; and the Instituto de Parasitología y Biomedicina López-Neyra, Granada, Spain.

Supported by grant 1102-04-12905 from Colciencias and Universidad Industrial de Santander.

O. Moreno, MSc; C.I. González, PhD, Laboratorio de Inmunología y Biología Molecular, Grupo de Inmunología y Epidemiología Molecular, Facultad de Salud, Universidad Industrial de Santander; D.L. Saaibi, MD, Clínica Carlos Ardila Lulle; W. Otero, MD; R. Badillo, MD, Departamento de Medicina Interna, Facultad de Salud, Universidad Industrial de Santander; J. Martin, MD, PhD, Instituto de Parasitología y Biomedicina López-Neyra; G. Ramírez, MD, Laboratorio de Inmunología y Biología Molecular, Grupo de Inmunología y Epidemiología Molecular, Facultad de Salud, Universidad Industrial de Santander, Clínica Carlos Ardila Lulle.

Address reprint requests to Dr. G. Ramírez, Laboratorio de Inmunología y Biología Molecular, Facultad de Salud, Carrera 32 # 29-31, Universidad Industrial de Santander, Bucaramanga, Colombia AA 678. E-mail: gemensu@yahoo.com

Accepted for publication September 20, 2006.

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2007. All rights reserved.

Downloaded on November 15, 2026 from www.jrheum.org

lar synovium, and diminution of bone resorption[9]. It has been established that synovial concentrations of IL-4 are low or absent in patients with RA[10].

The *IL4* gene is located at the long arm of chromosome 5 (q23–31), closely linked to other cytokines responsible for allergic reactions mediated by Th2, such as IL-5 and IL-13. The gene has several polymorphisms — the promoter polymorphism –590 and a 70 bp tandem repeat (variable number tandem repeat, VNTR) located in the third intron being the most widely studied[11].

IL-4 binds to its receptor at the surface of the target cell. IL-4 receptor is composed of 2 subunits; the binding to a subunit (*IL4RA*) is of high affinity, whereas the function of the γ_c subunit is to amplify the signal transduced by the IL-4Ra[8]. *IL4RA* is located at the short arm of chromosome 16p12.1. Several polymorphisms have been described in the codifying and in non-codifying regions of the gene[12]. It has been established that polymorphisms located in the +148 codifying region (transition A/G, Ile50Val) of the extracellular domain and in the +1902 codifying region (transition A/G, Gln576Arg) of the intracellular domain affect the binding of its ligand or the intracellular signaling, respectively[8,13].

The role of IL-4 in the pathogenesis of RA is conflicting. For example, in experimental models of arthritis in mice, gene therapy with IL-4 prevented articular damage and bone erosion, although it did not diminish inflammation[14]. High levels of expression of intraarticular IL-4 by retroviral transduction in an arthritis model induced clinical and radiological improvement of the arthritis[15]. Additionally, administration of monoclonal antibodies against IL-4 in a mouse model of collagen-induced arthritis resulted in greater severity of disease, also suggesting a protective role of IL-4 in this model[16]. However, the administration of recombinant IL-4 (rIL-4), in a randomized, double-blind, placebo controlled study did not show encouraging results, despite good rIL-4 tolerance[17]. Further, recent information suggests that IL-4 could play an important inflammatory function in the early phases of RA[18,19].

Polymorphic variations of the cytokine genes or their receptors have received attention as potential markers of susceptibility, severity, and/or protection in RA. Given that RA is a chronic inflammatory process with variable presentation and development, it is possible that these differences are related to individual variations at the level of cytokine production as a result of polymorphic variations of their genes[20].

We investigated 6 polymorphisms, 2 in the *IL4* and 4 in the *IL4RA* genes, and compared allele frequencies in a normal healthy population with those of patients with RA, as well as with some clinical features such as RF seropositivity, titer of RF, and history of replacement joint surgery among patients with RA.

MATERIALS AND METHODS

Patients and controls. We enrolled 102 patients with RA and 102 healthy local controls of similar age and sex. All patients met the 1987 revised American College of Rheumatology (ACR) criteria for RA[21]. The study was approved by the Ethical Committee of the Industrial University of Santander Faculty of Health, and all participants gave signed informed consent. Only individuals older than 16 years, who had not received blood transfusions in the last 6 months before sampling, were enrolled for study.

Clinical data. A patient questionnaire at the time of enrollment recorded demographic data that included age, sex, age at presentation of RA, duration of disease, history of major joint replacement surgery, and a Health Assessment Questionnaire (HAQ), besides the ACR criteria. RF titers were determined in all patients and controls.

DNA isolation. Genomic DNA was isolated from anticoagulated peripheral blood using the salting-out method[22].

Typing for IL4 and IL4RA polymorphism. A restriction fragment length polymorphism-polymerase chain reaction was used to identify the polymorphisms of *IL4* –590(C/T), *IL4RA* +148(A/G), *IL4RA* +1124(A/C), and *IL4RA* +1218(T/C)[11,23-26]. The polymorphism of *IL4RA* +1902 was determined by PCR-sequence-specific primers[27]. *IL4* VNTR polymorphisms were identified by direct amplification[11]. Primers, polymerase chain reactions, and restriction enzyme conditions required to determine polymorphisms are shown in Table 1.

Rheumatoid factor (RF) determination. RF was measured from a serum sample by latex agglutination (Humantex RF, Germany). Titers ≥ 20 IU/ml were considered positive.

Severity of disease. A history of major joint replacement surgery, usually knee or hip, was taken as a criterion of severity.

Statistical analysis. Allelic frequencies were calculated in the RA and control groups by direct count. Hardy-Weinberg equilibrium (HWE) for population allelic frequencies of the different loci was determined. Linkage disequilibrium analysis was performed to establish linkage among the *IL4RA* polymorphisms. The Arlequin software package v. 2000 was utilized for HWE and linkage disequilibrium analysis[28]. Chi-square test was used for comparisons of allelic frequencies between patients and controls. Fisher's exact test was used when values in the 2×2 tables were < 5. The association of allelic polymorphisms with age of onset (early onset, before 45 yrs; late onset, after 45 yrs), RF positivity, RF titer (low titers, ≥ 20 IU/ml to < 192 IU/ml; high titers, ≥ 192 IU/ml), and joint replacement surgery was also determined by chi-square test and Fisher's exact test as appropriate. The 95% confidence intervals for OR were determined; p values < 0.05 were considered statistically significant[29]. The software used was Statcalc EpiInfo 6 (Centers for Disease Control and Prevention, Atlanta, GA, USA). For the haplotype analysis, pairwise linkage disequilibrium measures were investigated and haplotypes constructed by the expectation-maximization algorithm implemented in Unphased software[30].

RESULTS

Clinical features. Patients' clinical characteristics are shown in Table 2. There was no significant difference between the age of the control group (50.3 ± 12.5 yrs) and that of patients (51 ± 12.8 yrs).

Association between IL4 and IL4RA alleles and RA. The distributions of allelic frequencies in patients and controls are shown in Table 3 and were found to be in Hardy-Weinberg equilibrium. Fst variation coefficient showed low structure of the population (Fst = 0.06047, range 0.005–0.15, moderate structure), which indicates the population from both groups was homogeneous. There was linkage disequilibrium (LD) between *IL4RA* +1124, +1218, and

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2007. All rights reserved.

Downloaded on November 15, 2026 from www.jrheum.org

Table 1. Primers, polymerase chain reactions, and restriction enzyme conditions required to determine polymorphisms.

Polymorphism	Primers	PCR conditions	Restriction Enzyme/ bp fragments
IL4 –590 C/T	F5'-TAAACTTGGGAGAACATGGT-3' R5'-TGGGGAAAGATAGAGTAATA-3'	94ºC 30s 57ºC 30s 72ºC 30s 40 cycles	***Ava II*** 196 pb C: 177+18bp T: 196bp (Noguchi et al. /98
IL4 VNTR 70bp	F5'-AGGCTGAAAGGGGGAAAGC-3' R5'-CTGTTCACCTCAACTGCTCC-3'	95ºC 30s 66ºC 40s 72ºC 40s 30 cycles	RP1: 183bp RP2: 253bp (Cantagrel et al./99)
IL4RA 148 A/G (I50V)	F5'-GGCAGGTGTGAGGAGCATCC-3' R5'-GCCTCCGTTGTTCTCAGGTA-3'	93ºC 60s 62ºC 60s 72ºC 60s 30 cycles	***Rsa I*** 273 bp A: 273bp G: 254+19bp (Noguchi et al 1999)
IL4RA 1124 A/C (E375A)	F5'TTAGCCGGGCCACAAAGGCC3' R5'TGGAGATCAGCAAGACAGTC3'	94ºC 45s 64ºC 45s 72ºC 45s 35 cycles	***Stu* I** 189pb A: 171+18bp G: 189bp (Novack et al./02)
IL4RA 1218 T/C (C406R)	F5'GCTCTGGAAAATCAGCATGG3' R5'ACTTGGGAACTCATCCCAGG3'	94ºC 45s 62ºC 45s 72ºC 45s 35 cycles	***Tsp45* I** 347pb T: 347bp C: 283+64bp (Mujica-López et al./02)
IL4RA 1902 A/G (Q551R)	F5'-CAGTCCTCTGGCCAGAGAG-3' R5'-CACCGCATGTACAAACTCCT-3' R5'-CACCGCATGTACAAACTCCC-3'	96ºC 25s 70ºC 45s 72ºC 45s, 5 cycles; 96ºC 25s 65ºC 50s 72ºC 45s, 21 cycles; 96ºC 25s 55ºC 60s 72ºC 120s, 4 cycles	701 bp (Hunt et al/00)
HGH	F5'-TGCCAAGTG GAGCACCCAA-3' R5'-GCCTTCCCAACCATTCCCTT-3'	Same as *IL4RA* 1902	450bp (Mullighan *et al.* /99)

HGH: Human growth hormone

Table 2. Clinical characteristics of patients with rheumatoid arthritis.

Characteristic	Values
No.	102
Sex ratio, FM (%)	86/16 (84.3/15.7)
Relation, F/M	5.4/1
Age, mean + SD yrs	51.05 ± 12.8
Age at onset, mean ± SD yrs	39.2 ± 13.8
Duration of disease, mean ± SD yrs	12.2 ± 9.9
HAQ, mean ± SD	0.71 ± 0.5
History of joint replacement surgery, n (%)	26 (25.5)
Rheumatoid factor, n (%)	
< 20 IU/ml (RF–) (%)	36 (35.3)
≥ 20 IU/ml (RF+) (%)	66 (64.7)
≥ 20 IU/ml < 192 IU/ml (%)	30 (45.5)
≥ 192 IU/ml (%)	36 (54.5)
Mean RF+, IU/ml (range)	304.3 (20–3072)

HAQ: Health Assessment Questionnaire; IU: International Units.

+1902 polymorphisms (D' = 0.98 and 0.95). The polymorphisms *IL4RA* +148, +1124, and +1218 showed moderate LD, with D' values of 0.4, and low LD with +1902 (D' = 0.1).

IL4 –590TT genotype was significantly associated with the development of RA (p = 0.018, OR 3.34, 95% CI 1.08–11.04), as well as the *IL4* –590T allele (95% CI 1.01–2.41, p = 0.03). No associations were observed between the *IL4* VNTR and the several *IL4RA* single-nucleotide polymorphisms analyzed with RA compared with the control group (Table 3). For the *IL4* –590T allele, the frequency was also increased in RA patients with early onset (41.3%) compared with late onset disease (31.8%), but the difference was not statistically significant (data not shown).

The frequency of allele *IL4RA* +148A was significantly higher in RF-positive RA patients (p = 0.0019, OR 2.55,

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2007. All rights reserved.

Downloaded on November 15, 2026 from www.jrheum.org

Table 3. Comparison of genotypic and allelic frequencies between RA patients and healthy controls.

SNP	Genotypes/Alleles	Controls, n = 102 (%)	RA Patients, n = 102 (%)
IL4–590	CC	49 (48.0)	39 (38.2)
Genotypes	CT	48 (47.1)	48 (47.1)
	TT	5 (4.9)	15 (14.7)*
Alleles	C	146 (71.5)	126 (61.7)
	T	58 (28.9)	78 (38.2)**
IL4 VNTR	RP1RP1	5 (4.9)	12 (11.8)
Genotypes	RP2RP2	48 (47.1)	41 (40.2)
	RP1RP2	49 (48.0)	48 (47.0)
	RP2RP3	0	1 (1.0)
Alleles	RP1	59 (28.9)	72 (35.3)
	RP2	145 (71.1)	131 (64.2)
	RP3	0	1 (0.5)
IL4RA +148	AA	28 (27.5)	20 (19.6)
Genotypes	AG	52 (51.0)	60 (58.8)
	GG	22 (21.5)	22 (21.6)
Alleles	A	108 (52.9)	100 (49)
	G	96 (47.1)	104 (51)
IL4RA +1124			
Genotypes	AA	65 (63.7)	72 (70.6)
	AC	31 (30.4)	26 (25.5)
	CC	6 (5.9)	4 (3.9)
Alleles	A	161 (78.9)	170 (83.3)
	C	43 (21.1)	34 (16.7)
IL4RA +1218	TT	69 (67.6)	78 (76.5)
Genotypes	CT	28 (27.5)	21 (20.6)
	CC	5 (4.9)	3 (2.9)
Alleles	T	166 (81.4)	177 (86.6)
	C	38 (18.6)	27 (13.2)
IL4RA +1902	AA	41 (40.2)	50 (49.0)
Genotypes	AG	52 (51.0)	45 (44.1)
	GG	9 (8.8)	7 (6.9)
Alleles	A	134 (65.7)	145 (71.1)
	G	70 (34.3)	59 (28.9)

* For genotype *IL4* -590 vs CC + CT, p = 0.018, OR 3.34, 95% CI 1.08–11.04. ** For allele *IL4* -590 T vs C, p = 0.035, OR 1.56, 95% CI 1.01–2.41. VNTR: variable number tandem repeat.

95% CI 1.55–4.86; Table 4), while the *IL4RA* +1902G allele showed significant association with high RF titers (p = 0.00067, OR 4, 95% CI 1.64–9.93; Table 4). The *IL4RA* +148A allele was also found in significantly higher frequency in RA patients who underwent articular joint replacement (p = 0.024, OR 2.08, 95% CI 1.04–4.18; Table 4).

The analysis of haplotypes of *IL4* and *IL4RA* polymorphism with RA susceptibility and/or severity did not give valid information due to the limited sample size and the low number of samples in each subgroup.

DISCUSSION

Despite better knowledge of the pathogenic mechanisms and immunogenetics of RA, given the complexity of cells, cytokines, chemokines, and adhesion molecules, we still do not have a clear picture of all the genetic mechanisms involved. As a polygenic disease, many different genes could be interacting and influencing susceptibility, chronicity, immune response or severity.

It is recognized that the pathways of tissue damage and repair in RA are highly complex[31]. Recent information suggests the Th1/Th2 dichotomy to explain RA as a process driven by Th1 CD4+ cells is an oversimplification. IL-4 is best known for its role in promoting Th2 differentiation and limiting T–1 responses[32], but it can also direct Th1 responses[33-35]. The site and timing of IL-4 expression in an immune response may determine its ultimate effects on CD4+ T cells[36]. Epidemiological studies have shown that occurrences of Th1 and Th2-driven diseases are not mutually exclusive; on the contrary, they might have common risk factors and pathogenesis[37,38].

We observed a significantly higher frequency of the *IL4* –590TT genotype in RA patients compared with controls, and a lesser association with the *IL4* –590 allele. This could imply that the effect of the gene is dose-dependent. Other groups have also suggested association of this allele with RA and palindromic rheumatism, and with greater disease activity[39,40]. Additionally, there has been reported to be a

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2007. All rights reserved.

Downloaded on November 15, 2026 from www.jrheum.org

Table 4. Comparison of allelic frequencies in RF, RF titers, and history of joint replacement surgery among RA patients.

Alleles	RA Patients					
	RF-negative, n = 36 (%)	RF-positive, n = 66 (%)	RF (+) Low Titer, n = 30 (%)	RF (+) High Titer, n = 36 (%)	No Replacement Joint Surgery, n = 76 (%)	With Replacement Joint Surgery, n = 26 (%)
IL4 –590						
C	47 (65.3)	79 (59.8)	35 (58.3)	44 (61.1)	91 (59.2)	35 (67.3)
T	25 (34.7)	53 (40.2)	25 (41.7)	28 (38.9)	61 (40.8)	17 (32.7)
IL4 VNTR						
RP1	23 (31.9)	49 (37.1)	23 (38.3)	26 (36.1)	57 (37.5)	15 (28.8)
RP2	49 (68.1)	82 (62.1)	37 (61.7)	45 (62.5)	94 (61.3)	37 (71.2)
RP3	0	1 (0.8)	0	1 (1.4)	1 (0.7)	0
IL4RA 148						
A	24 (36.1)	74 (56.1)*	34 (56.7)	40 (55.5)	66 (44.7)	32 (61.5)**
G	48 (63.9)	58 (43.9)	26 (43.3)	32 (44.4)	86 (55.3)	20 (38.5)
IL4RA 1124						
A	61 (84.7)	109 (82.6)	54 (90)	55 (76.4)	125 (82.2)	45 (86.5)
C	11 (15.3)	23 (17.4)	6 (10)	17 (23.6)	27 (17.8)	7 (13.5)
IL4RA 1218						
T	63 (87.3)	114 (86.4)	63 (87.5)	59 (81.9)	131 (86.2)	46 (88.5)
C	9 (12.5)	18 (13.6)	9 (12.5)	13 (18.1)	21 (13.8)	6 (11.5)
IL4RA 1902						
A	54 (76.4)	90 (68.2)	50 (83.3)	40 (55.6)	107 (71)	37 (71.2)
G	18 (23.6)	42 (31.8)	10 (16.7)	32 (44.4)†	45 (28.9)	15 (28.8)

RF (+) low titer: ≥ 20 IU/ml to < 192 IU/ml; RF (+) high titer: ≥ 192 IU/ml. * For allele IL4RA 148 A vs G, p = 0.0019, OR 2.55, 95% CI 1.55–4.86. ** For allele IL4RA 148 A vs G, p = 0.024, OR 2.08, 95% CI 1.04–4.18. † For allele IL4RA 1902 G vs A, p = 0.00067, OR 4, 95% CI 1.64–9.93.

higher frequency of *IL4* –590T allele when it is associated simultaneously with the allele *IL4* VNTR RP1, a finding that we could not reproduce[11]. In our population, this allele was also found more frequently in patients with early onset RA, but this difference did not achieve significance. Of note, the *IL4* –590T allele has been correlated with enhanced IL-4 activity *in vitro*, with elevated serum IgE *in vivo*, and with allergic diseases[41,42] and subacute sclerosing panencephalitis[43].

Information derived from *in vitro* studies, animal models, and patients with RA suggests a role of IL-4 in the pathogenesis of RA. IL-4 protects synoviocytes against apoptosis induced by nitric oxide[44] that could be important in the development of the fibroblast hypertrophy seen in RA pannus[45], and contributes to the differentiation of local dendritic cells from blood monocytes[46]; and the addition of IL-4 to synovial fibroblasts dramatically modulates their gene expression, making them similar to fibroblasts in secondary lymphoid tissue[47]. This information suggests that IL-4 could participate in the development of changes in the synovial microenvironment that lead to the formation of tertiary lymph nodes so prominent in RA pannus. In some murine models of arthritis the increase of Th2 immune responses and production of IL-4 was related to the development of the arthritis[48-50]. In the K/BxN mouse model of arthritis, the articular damage is mediated by antibodies against glucose-6-phosphate isomerase, a glycolytic enzyme expresed by all cells[51]. In this model the development of arthritis is critically dependent on the presence of IL-4 in the early phases of the disease[52].

Several human studies have shown that cytokines produced in the early phases of RA are different than those in established disease. Gerli, *et al* found that IL-4 and IL-10 were the main cytokines produced at the beginning phase, whereas IFN-γ and IL-2 were produced in long-lasting RA[18]. Aarvak, *et al* showed an initial Th2 dominance pattern in the synovium, with high production of IL-4 and IL-10. The Th2 profile was not permanent and changed to a Th0 and thereafter a Th1 profile in subsequent biopsies[53]. Recent work by Raza, *et al* also supports the potential role of IL-4 during the early phases of RA. A transitory cytokine profile with high IL-4 and other cytokines suggestive of a Th2 pattern was found in synovial fluid from patients with early RA, clearly different from that in patients who later developed short-term or nonrheumatoid persistent synovitis, and also different from patients with well established RA, osteoarthritis, or gout[19].

IgM RF is present in 60–80% of patients with RA and the titer correlates with disease severity and extraarticular manifestations[54]. Patients with high RF titers have articular damage more persistent and severe than those with low titers or negative RF[55]. Numerous patients who eventually develop RA have positive RF and other serological abnormalities many years before development of RA symptoms[56]. We found an association of allele *IL4RA* +148A (Ile50 variant) with the presence of RF in RA patients and with a history of articular joint replacement. Additionally, allele *IL4RA* +1902G (551R variant) was associated with high RF titers in patients with RA. The IL-4R α-chain is responsible for IL-4 binding, transduction of the signal into

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2007. All rights reserved.

Downloaded on November 15, 2026 from www.jrheum.org

the cell, and cellular response. Its locus is characterized by a large number of polymorphic sites, most located in the intracellular domain[12]. It is interesting that the Ile50 variant of the extracellular domain significantly upregulates the receptor response to IL-4, with resultant increased activation of STAT6, increased cell proliferation, and greater IgE production[13,57]. The intracellular *IL4RA* +1902G allele (551R variant) is also associated with enhanced signaling of the IL-4 receptor[58]. Considering the limited sample size of our study, our results need to be replicated in other populations.

Evidence is accumulating that IL-4 has a complex function in the development of innate and adaptive immunity and might play an important role in the preclinical or early phases of RA. Our results, showing the associations of *IL4* –590TT genotype with RA, *IL4RA* +148A allele with the presence of RF and higher frequency of joint replacement surgery, and *IL4RA* +1902G allele with high RF titers, illustrate the polygenic expression of the disease and the likely role of IL-4 in influencing its initiation and development. Also, the data suggest caution on the planned use of recombinant IL-4 in the treatment of RA.

ACKNOWLEDGMENT

We thank Carmen Cecilia Cabrales for technical assistance.

REFERENCES

1. Silman AJ, Pearson J. Epidemiology and genetics of rheumatoid arthritis. Arthritis Res 2002;4 Suppl 3:S265-S272.
2. Nepom GT. The role of the DR4 shared epitope in selection and commitment of autoreactive T cells in rheumatoid arthritis. Rheum Dis Clin North Am 2001;27:305-15.
3. Newton JL, Harney SMJ, Wordsworth BP, Brown MA. A review of the MHC genetics of rheumatoid arthritis. Genes Immun 2004;5:151-7.
4. Deighton CM, Walter DJ, Griffiths ID, Robertos DF. The contribution of HLA to rheumatoid arthritis. Clin Genet 1989;36:178-82.
5. Choy EH, Panayi GS. Cytokine pathways and joint inflammation in rheumatoid arthritis. N Engl J Med 2001;344:907-16.
6. Firestein GS. Evolving concepts of rheumathoid arthritis. Nature 2003;423:356-61.
7. Smith JB, Haynes MK. Rheumatoid arthritis. A molecular understanding. Ann Intern Med 2002;36:908-22.
8. Nelms K, Keegan A, Zamorano J, Ryan J, Paul W. The IL-4-receptor: Signaling mechanisms and biologic functions. Annu Rev Immunol 1999;17:701-38.
9. Morita Y, Yamamura M, Kawashima M, et al. Differential in vitro effects of IL-4, IL-10, and IL-13 on proinflamatory cytokine production and fibroblast proliferation in rheumatoid synovium. Rheumatol Int 2001;20:49-54.
10. Miossec P, Navilliat M, Dupuy d'Angeac VJ, Sany J, Banchereau J. Low levels of interleukin-4 and high levels of trasforming growth factor ß in rheumatoid synovitis. Arthritis Rheum 1990;33:1180-7.
11. Cantagrel A, Navaux F, Loubet-Lescoulié P, et al. Interleukin-1ß, interleukin-1 receptor antagonist, interleukin-4 and interleukin-10 gene polymorphisms. Relationship to occurrence and severity of rheumatoid arthritis. Arthritis Rheum 1999;42:1093-100.
12. Deichmann K, Bardutsky J, Forster J, Heinzmann A, Kuehr J. Common polymorphisms in the coding part of the IL-4 receptor gene. Biochem Biophys Res Commun 1997;231:696-7.
13. Mitsuyasu H, Izuhara K, Mao X-Q, et al. Ile50Val variant of IL-4R upregulates IfE synthesis and associates with atopic asthma. Nature Genetics 1998;19:119-20.
14. Lubberts E, Joosten LAB, Chabaud M, et al. IL-4 gene therapy for collagen arthritis suppresses synovial IL-17 and osteoprotegerin ligand and prevents bone erosion. J Clin Invest 2000;105:1697-710.
15. Boyle DL, Nguyen KHY, Zhuang S, et al. Intra-articular IL-4 gene therapy in arthritis: anti-inflammatory effect and enhanced th2 activity. Gene Ther 1999;6:1911-8.
16. Yoshino S. Effects of a monoclonal antibody against interleukin-4 on collagen-induced arthritis in mice. Br J Pharmacol 1988;123:237-42.
17. Van der Hosch F, Russel A, Keystone EC, et al. rHUIL-4 in subjects with active rheumatoid arthritis: a phase I dose escalating safety study [abstract]. Arthritis Rheum 1998;41 Suppl:S56.
18. Gerli R, Bistoni O, Russano A, et al. In vivo activated T cells in rheumatoid synovitis. Analysis of Th1- and Th2-type cytokine production at clonal level in different stages of disease. Clin Exp Immunol 2002;129:549-55.
19. Raza K, Falciani F, Curnow SJ, et al. Early rheumatoid arthritis is characterized by a distinct and transient synovial fluid cytokine profile of T cell and stromal cell origin. Arthritis Res Ther 2005;7:R784-R795.
20. Lee DM, Weinblatt ME. Rheumatoid arthritis. Lancet 2001;358:903-11.
21. Arnett FC, Edworthy SM, Bloch DA, et al. American Rheumatism Association 1987 revised criteria for classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-24.
22. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16:1215.
23. Noguchi E, Shibasaki M, Arinami T, et al. Association of asthma and the interleukin-4 promoter gene in Japanese. Clin Exp Allergy 1998;28:449-53.
24. Noguchi E, Shibasaki M, Arinami T, et al. No association between atopy/asthma and the ile50val polymorphism of IL-4 receptor. Am J Respir Crit Care Med 1999;160:342-5.
25. Novak N, Kruse S, Kraft S, et al. Dichotomic nature of atopic dermatitis reflected by combined analysis of monocytes immunophenotyping and single nucleotide polymorphisms of the interleukin-4/interleukin 13 receptor gene: the dichotomy of extrinsin and intrinsic atopic dermatitis. J Invest Dermatol 2002;119:870-5.
26. Mujica-Lopez KI, Florez-Martínez SE, Ramos-Zepeda R, et al. Association analysis of polymorphisms in the interleukin-4 receptor gene with atopic asthma in patients from western Mexico. Eur J Immunogenet 2002;29:375-8.
27. Hunt PJ, Marshall SE, Weetman AP, Bell JI, Wass JAH, Welsh KI. Cytokine gene polymorphisms in autoimmune thyroid disease. J Clin Endocrinol Metab 2000;85:1984-8.
28. Emery AEH. Methodology in medical genetics. An introduction to statistical methods. London: Churchill Livingstone; 1976:3-10.
29. Daniel WW. Bioestadística. Base para el análisis de las ciencias de la salud. 4th ed. Mexico City: Limusa Wiley DF; 2002:571-657.
30. Dudbridge F. Pedigree disequilibrium tests for multilocus haplotypes. Genet Epidemiol 2003;25:115-21.
31. Weyand CM, Goronzy JJ. Pathomechanisms in rheumatoid arthritis — time for a string theory? J Clin Invest 2006;116:869-71.
32. Abbas AK, Murphy KM, Sher A. Functional diversity of helper lymphocytes. Nature 1996;383:787.
33. Hochrein H, O'Keeffe M, Luft T, et al. Interleukin (IL)-4 is a major regulatory cytokine governing bioactive IL-12 production by mouse and human dendritic cells. J Exp Med 2000;192:823-33.

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2007. All rights reserved.

Downloaded on November 15, 2026 from www.jrheum.org

34. Yao Y, Li W, Kaplan MH, Chang CH. Interleukin (IL)-4 inhibits IL-10 to promote IL-12 production by dendritic cells. J Exp Med 2005;201:1899-903.
35. Biederman T, Himmelrich H, Gumy A, et al. IL-4 instructs TH1 responses and resistance to Leishmaniasis major in susceptible BALB/c mice. Nat Immunol 2001;2:1054-60.
36. Gregory GD, Raju SS, Winandy S, Brown MA. Mast cell IL-4 expression is regulated by Ikaros and influences encephalitogenic Th1 responses in EAE. J Clin Invest 2006;116:1327-36.
37. Edwards CJ, Cooper C. Early environmental factors and rheumatoid arthritis. Clin Exp Immunol 2006;143:1-5.
38. Simpson CR, Anderson WJ, Helms PJ, et al. Coincidence of immune-mediated diseases driven by Th1 and Th2 subsets suggests a common aetiology. A population-based study using computerized general practice data. Clin Exp Allergy 2002;32:37-42.
39. Maksymowych WP, Suarez-Almazor M, Buenviaje H, et al. HLA and cytokine gene polymorphisms in relation to occurrence of palindromic rheumatism and its progression to rheumatoid arthritis. J Rheumatol 2002;29:2319-26.
40. Pawlik A, Wrzesniewska J, Florezak M, Ostanek L, Gawronska B, Herczynska M. The –590 IL-4 promoter polymorphism in patients with rheumatoid arthritis. Rheumatol Int 2005;26:48-51.
41. Rosenwasser LJ. Structure/function variants as candidates genes in asthma: linkage vs association for relevance. Clin Exp Allergy 1998;28 Suppl 1:90-2.
42. Kawashima T, Noguchi E, Arinami T, et al. Linkage and association of an interleukin 4 gene polymorphism with atopic dermatitis in Japanese families. J Med Genet 1998;35:502-4.
43. Inoue T, Kira R, Nakao F, et al. Contribution of the interleukin 4 gene to susceptibility to subacute sclerosing panencephalitis. Arch Neurol 2002;59:822-7.
44. Relic B, Guicheux J, Mezin F, et al. IL-4 and IL-13, but not IL-10, protect human synoviocytes from apoptosis. J Immunol 2001;166:2775-82.
45. Salmon M, Pilling D, Borthwick NJ, Akbar AN. Inhibition of T cell apoptosis: a mechanism for persistence in chronic inflammation. The Immunologist 1997;5:87-92.
46. Zhou LJ, Tedder TF. CD14+ blood monocytes can differentiate into functionally mature CD83+ dendritic cells. Proc Natl Acad Sci USA 1996;93:2588-92.
47. Parsonage G, Falciani F, Burman A, et al. Global gene expression profiles in fibroblasts from synovial, skin and lymphoid tissue reveals distinct cytokine and chemokine expression patterns. Thromb Haemost 2003;90:688-97.
48. Chen Y, Rosloniec E, Goral MJ, Boothby M, Chen J. Redirection of T cell IL-2Rß/IL-4Ra chimeric cytokine receptor transgene. J Immunol 2001;166:4163-9.
49. Mauri C, Williams RO, Walmsley M, Feldmann M. Relationship between Th1/Th2 cytokine patterns and the arthritogenic response in collagen induced arthritis. Eur J Immunol 1996;26:1511-8.
50. Schimmer RC, Schrier DJ, Flory CM, et al. Streptococcal cell wall-induced arthritis: requirements for IL-4, IL-10, IFN-γ, and monocyte chemoattractant protein 1. J Immunol 1998;160:1466-71.
51. Hirano T. Revival of the autoantibody model in rheumatoid arthritis. Nature Immunol 2002;3:342-4.
52. Ohmura K, Nguyen LT, Locksley RM, Mathis D, Benoist C. Interleukin-4 can be a positive regulator of inflammatory arthritis. Arthritis Rheum 2005;52:1866-75.
53. Aarvak T, Chabaud M, Thoen J, Miossec P, Natvig JB. Changes in the Th1 or Th2 cytokine dominance in the synovium of rheumatoid arthritis (RA): a kinetic study of the Th subsets in one unusual RA patient. Rheumatology Oxford 2000;39:513-22.
54. Saaibi DL. Factor reumatoideo y otros auotanticuerpos en artritis reumatoide. In: Ramírez LA, Anaya J-M, editors. Artritis reumatoide. 2 ed. Bogotá: Editorial Edimeco SA; 2004:117-25.
55. Bukhari M, Lunt M, Harrison BJ, Scott DGI, Symmons DPM, Silman AJ. Rheumatoid factor is the major predictor of increasing severity of radiographic erosions in rheumatoid arthritis. Arthritis Rheum 2002;46:906-12.
56. Markus M, Nielen J, van Schaardenburg D, et al. Specific autoantibodies precede the symptoms of rheumatoid arthritis: A study of serial measurements in blood donors. Arthritis Rheumatism 2004;50:380-6.
57. Stephenson L, Johns MH, Woodward E, Mora AL, Boothby M. An IL-4Ra allelic variant, I50, acts as a gain-of-function variant relative to V50 for STAT6, but not Th2 differentiation. J Immunol 2004;173:4523-8.
58. Hershey GK, Friedrich MF, Esswein LA, Thomas ML, Chatila TA. The association of atopy with a gain-of-function mutation in the alpha subunit of the interleukin-4 receptor. New Engl J Med 1997;337:1720-5.

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2007. All rights reserved.

Downloaded on November 15, 2026 from www.jrheum.org