

RESEARCH ARTICLE

Genetic association of CCR5 promoter single nucleotide polymorphism in seronegative and seropositive rheumatoid arthritis

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ABSTRACT. The aim of this study was to investigate the possible role of the CCR5 59029 A→G promoter point mutation polymorphism in determining the susceptibility to rheumatoid factor-positive and rheumatoid factor-negative rheumatoid arthritis. This polymorphism was assessed in 85 seropositive and 39 seronegative rheumatoid arthritis patients and in 126 healthy individuals of the same geographic and ethnic origin. We found an increase in the genetic frequency of the A allele in the 59029 A→G promoter region of the CCR5 receptor in patients with rheumatoid arthritis compared with healthy controls ($p = 0.01$; OR = 1.5, 95% CI (1.0-2.2)). Likewise, the homozygous state for the A allele was found to be more frequent in rheumatoid arthritis patients, again when compared with healthy controls ($p = 0.03$; OR = 1.8, 95% CI 1.0-3.0). The increased frequency of the A allele was more evident in the more benign, seronegative rheumatoid arthritis group when compared with controls ($p = 0.003$; OR 2.4 95% CI 1.3-4.4), and when combining the A homozygous and the AG heterozygous patients compared with healthy subjects. These results suggest that this CCR5 promoter polymorphism seems to play an important role in determining different clinical courses in both forms of rheumatoid arthritis.

Key words: seronegative rheumatoid arthritis, seropositive rheumatoid arthritis, CCR5 polymorphism

Rheumatoid arthritis (RA) is a chronic, systemic, inflammatory joint disease that leads to bone and cartilage destruction, as well as to a wide variety of extra articular manifestations [1]. Similar to other autoimmune conditions, RA is a heterogeneous disease with variable combinations of several genes polymorphisms that contribute to susceptibility to the disease [2]. Most of the genes implicated in the predisposition to RA are found within the HLA-DR loci [3], however these loci are not the only contributors.

Since the early 1960s, the presence of rheumatoid factor-negative RA patients has been widely recognized as a disease with a differential clinical and immunogenetic profile [4]. In term of prognosis, it has been shown that seronegative RA is characterized by a more benign course, with significantly less erosive joint damage and functional disability [5]. Moreover, extra-articular manifestations of the disease *e.g.*, rheumatoid nodules, cytopenias, are less prevalent in seronegative RA patients [6]. The evidence that rituximab has more significant therapeutic effects in seropositive RA patients suggest that the mechanism of disease in seropositive RA patients differs from that in seronegative patients [7]. The concept that seronegative RA is different is further supported by genetic studies,

which showed that a group of seronegative RA patients who did not have the characteristic shared HLA epitope found in seropositive RA patients, possessed instead a separate apoptosis-associated gene (PD-1.3 A) [8]. Other candidate genes that could be used to differentiate seronegative from seropositive RA include those for chemokines and their receptors, as chemokines are important mediators of the inflammatory response and play an important role in the pathophysiology of joint inflammation and destruction in RA [9].

The CCR5 chemokine receptor has been shown to be important in the transmission and pathogenesis of HIV-1 [10]. A number of polymorphic variants of the CCR5 gene have been described, some of which have functional significance. Of interest is a 32-base pair deletion in the coding region of CCR5 (CCR5 Δ32) that results in a non-functional receptor that is not expressed on the cell surface [11]. Homozygosity for this deletion protects almost completely against HIV-1 infection, while heterozygosity correlates with a delayed progression to acquired immune deficiency syndrome (AIDS) [12].

We have previously reported that the CCR5 Δ32 deletion is not common in the Mexican Mestizo population or in Mexican Mestizo RA patients [13]. Another genetic

polymorphism of CCR5 located in the promoter region 59029 A→G was also shown to affect the level of CCR5 expression and the rate of progression to AIDS in HIV-1-infected patients [14].

We hypothesized that the CCR5 59029 A→G polymorphism may have the potential to influence susceptibility and clinical outcome in seronegative and seropositive RA patients.

DONORS AND METHODS

Subjects

We selected from a large cohort of RA patients those who had been regularly followed up at our department for more than two years from the onset of RA. All patients met the American College of Rheumatology 1987 revised criteria for RA (15). A total of 134, unrelated, Mexican Mestizo patients with RA were included. Their ages ranged from 26 to 93 years (mean 54 years). Disease duration ranged between 2.5 and 50 years (mean 16 years). One hundred and twenty six (91.3%) of the patients were female and 96 (69.5 %) had a positive assay for rheumatoid factor. As controls, 126, healthy, Mexican Mestizo blood donors, age- and ethnically-matched, with no history of inflammatory arthritis were included in the study. A Mexican Mestizo is defined as an individual who was born in Mexico and is a descendant from the native inhabitants of the region, and from individuals, mainly Spaniards of white or African origin, or both, who came to America during the 16th century. The study was approved by the Institutional Committee of Biomedical Research. All patients and controls were informed about the objectives and methods of the study, and gave their written consent.

Genotyping

Genotyping was performed directly on genomic DNA obtained by standard methods. The CCR5 59029 A→G promoter polymorphism was analyzed using a polymerase chain reaction-restriction fragment length procedure, digesting the amplified fragment of 270 bp with the enzyme *Bsp*1286 I that recognizes the 59029-G allele as described (14).

Statistical analysis

The allele and genotyping frequencies of the polymorphisms studied in patients and healthy controls were

obtained by direct counting. Hardy-Weinberg equilibrium was tested using the goodness-of-fit χ^2 test to compare the allele frequencies observed with the expected frequencies determined from control subjects. The significance of the differences between groups was determined using Mantel-Haenszel X2 analysis, which was combined with the 2 × 2 contingency tables using the Epidemiology & Biostatistics Unit statistical (EPISTAT) program (version 5.0; USD Incorporated 1990, Stone Mountain, GA, USA). Fisher's exact test was used if the number in any cell of the 2 × 2 contingency table was <5. Relative risk at 95% confidence intervals (CI) was calculated as the odds ratio (OR).

RESULTS

The allele and genotype distribution of the CCR5 promoter 59029 in RA and control individuals is shown in *table 1*. The frequencies of the A and G alleles were 56.3% and 43.6% in the controls, and 66.8% and 33.2% in the patient group, respectively, the genetic frequency of the A allele being statistically increased in RA patients ($p = 0.01$; OR 1.5, 95% CI 1.0-2.2).

The frequencies of the CCR5 59029 A/A, 59029 A/G and 59029 G/G genotypes were 31.7%, 49.2% and 19.0% in the controls and 45.3%, 42.5% and 11.9% in the patient group. The study population was found to be in Hardy-Weinberg equilibrium. The CCR5 59029 A/A genotype was significantly increased in RA patients when compared to controls ($p = 0.03$; OR 1.8, 95% CI 1.0-3.0).

When we classified the patients according to RF positivity, the A allele was found to be significantly increased in seronegative RA patients when compared with controls ($p = 0.003$; OR 2.4, 95% CI 1.3-4.4) *table 2*. This was also evident when we combined the A/A and A/G genotypes into a single category, $p = 0.03$ seronegative *versus* seropositive RA patients, and $p = 0.02$ seronegative RA patients *versus* controls.

DISCUSSION

Chemokines are proinflammatory cytokines that play a major role in the inflammatory response by activating cellular chemotaxis. CCR5 is a receptor of β -chemokines, such as RANTES, macrophage inflammatory protein (MIP)-1 α , and MIP-1 β , that is mainly expressed in macrophages, T lymphocytes and dendritic cells [15].

Table 1
Allele and genotype frequencies of the CCR5-59029 A/G polymorphism in rheumatoid arthritis patients and control subjects

CCR5 59029	RA N = 134	Controls N = 126		
Allele	n (frequency)	n: (frequency)	p	Odds ratio (C.I. 95%)
A	179 (0.668)	142 (0.563)	0.01	1.5 (1.0-2.2)
G	89 (0.332)	110 (0.436)		
Genotypes				
A/A	61 (0.455)	40 (0.317)	0.03	1.8 (1.0-3.0)
A/G	57 (0.425)	62 (0.492)		
G/G	16 (0.119)	24 (0.190)		

Table 2
CCR5-59029 polymorphisms in seropositive and seronegative rheumatoid arthritis patients and healthy controls.

CCR5-59029		RF+	RF-	Healthy controls	RF+ Vs RF-	Odds ratio (C.I.95%)	RF- Vs HC	Odds ratio (C.I.95%)	
Allele	n: (frequency)	n: (frequency)	n: (frequency)	n: (frequency)	p	Odds Ratio (C.I. 95%)	p	Odds Ratio (C.I. 95%)	
A	120 (0.631)	59 (0.756)	142 (0.563)		0.06	0.55 (0.29-1.0)		0.003	2.4 (1.3-4.4)
G	70 (0.368)	19 (0.243)	110 (0.436)						
Genotypes									
A/A A/G+	80 (0.842)	38 (0.974)	102 (0.809)		0.03	0.14 (0.01-1.0)		0.02	8.9 (1.2-183.5)
GG	15 (0.157)	1 (0.025)	24 (0.190)						

RF: rheumatoid factor, HC: healthy controls

Recruitment of CCR5-positive cells into inflamed sites is a typical feature of a variety of autoimmune conditions including RA [16]. A potential association between some chemokine polymorphisms such as regulated on activation, normal T expressed and secreted protein (RANTES), and RA has previously been suggested [17, 18].

This study shows an increase in the genetic frequency of the A allele in the 59029 A→G promoter region of the CCR5 receptor in Mexican patients with RA, compared with healthy controls. Likewise, the homozygous state for the A allele was found to be increased in RA patients, again when compared with healthy controls. Interestingly, these data were more evident in the more benign, seronegative RA group both for the A allele and when combining the A homozygous and the AG heterozygous, and compared with healthy subjects.

These data suggest a direct role for CCR5 in the physiopathology of RA. The different sites of the promoter region in CCR5 influence the number of receptor copies, which increases the availability of receptor molecules that determines the magnitude of the immune response to the assaulting agent, so that the responding cells act accordingly and in proportion to the assault. Recently however, it has been suggested that CCR5 (and CCR2) are not critical for the migration of monocytes towards the synovial compartment in RA [19]. Moreover, it is also known that CCR5 is not only expressed on monocytes, but also on effector and memory T cells and dendritic cells where it has an important role in innate immunity, in addition to its more established role as a chemoattractant receptor for adaptive immune responses [20]. Thus, it is not unreasonable to suppose that the CCR5 promoter 59029 will also show functions other than inflammatory or cell recruitment, and this might suggest a differential role in seronegative and seropositive RA.

Be as it may, the role of polymorphisms in the physiopathology of RA, and the genotypic and allelic differences that distinguish patients from healthy controls in Mexican individuals, is probably due to natural selection mechanisms which have acted relatively recently in this geographical region, as a consequence of the addition of pathogens of Old World provenance to the indigenous American population. The great number of infectious epidemics that depopulated the American continent of its autochthonous population over the last 500 years, from the XVI century to our times, reduced the native Mexican population by nearly 90%. Survivors were positively selected through different genes that included HLA-DR,

TNF, the complement system [2], and probably also including chemokines and their receptors in such a way that the presence of chronic inflammatory diseases such as RA in Mexicans is evidence of their success, and survival in the face of pathogens.

That alleles and genotypes of chemokines receptors distinguish between patients with seronegative RA and seropositive RA as independent pathogenic entities is still mere speculation. We can however, consider the possibility that these genetic markers can determine different clinical courses in both forms of RA.

In conclusion, the study of genetic markers of chemokine receptors is useful in the analysis of the physiopathology of RA, and of the different clinical outcomes according to its different varieties.

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