Clinical and Experimental Rheumatology 2003; 21: 351-354.

Biological relevance of the polymorphism in the CCR5 gene in refractory and non-refractory rheumatoid arthritis in Mexicans

J.A. Zúñiga[1], C. Villarreal-Garza[2], E. Flores[1], R. Barquera[2], N. Pérez-Hernández[3], J.V. Montes de Oca[4], M.H. Cardiel[2], G. Vargas-Alarcón[3], J. Granados[2]

[1]Molecular Biology and Immunogenetics Laboratory, Instituto Nacional de Enfermedades Respiratorias, Mexico City; [2]Department of Immunology and Rheumatology, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City; [3]Cellular Biology Section, Physiology Department, Instituto Nacional de Cardiología, Ignacio Chávez, Mexico City; [4]Virgen Macarena Hospital, Seville, Spain.

Joaquín A. Zúñiga, PhD; Cynthia Villarreal-Garza, MD; Encarnación Flores, BS; Rodrigo Barquera, BS; Nonatzi Pérez-Hernández, BS; José V. Montes de Oca, MD; Mario H. Cardiel, MD; Gilberto Vargas-Alarcón, PhD; Julio Granados, MD.

Please address correspondence and reprint requests to: Joaquín Zúñiga, PhD, Molecular Biology and Immunogenetics Laboratory, Infectious Diseases Unit, Instituto Nacional de Enfermedades Respiratorias, Calzada Tlalpan 4502, Tlalpan 14080, Mexico, D.F. E-mail: joazu@yahoo.com

Received on August 9, 2002; accepted in revised form on January 10, 2003.

ABSTRACT

Objective. *The aim of this study was to analyze the frequencies of the CCR5 Δ32 deletion and HLA class II alleles in Mexican Amerindian populations and its relevance in the development and severity of RA.*

Methods. *We studied 212 Mexican Mestizo subjects (40 patients with refractory RA, 102 patients with non-refractory RA and 70 healthy individuals). At the same time, to evaluate the ethnicity of the CCR5Δ32 deletion we also studied 192 individuals from three Mexican Amerindian populations (70 Mayo (Capomo) individuals, 61 Teenek individuals, and 61 Mazatecan Indians). The Δ32 deletion in the CCR5 structural gene and HLA-DRB1 were determined by a PCR-SSP and a PCR-SSO procedure, respectively.*

Results. *In the non-refractory RA group the CCR5Δ32 gene frequency was 0.019 and the following genotype frequencies were observed: CCR5/ CCR5 =98.0%, CCR5/CCR5Δ32=1.9% and CCR5Δ32/CCR5Δ=1.0%. In the refractory RA group the CCR5Δ32 gene frequency was 0.025 and the genotype distribution was similar to that in the non-refractory RA group. The deletion was not detected in the Mexican Mestizo healthy group, or among the Teenek and Mayo Amerindians, all being individuals homozygous for the wild type allele. In the Mazatecan group the deletion frequency was 1.6% (g.f. = 0.016). We observed a significant increase in the frequency of the DRB1*07 allele in severe RA patients in relation to the non-severe RA group (p = 0.02, OR = 5.65, 95% CI = 0.95-43.05).*

Conclusion. *Our results suggest that the CCR5Δ32 deletion is not common in Mexican Amerindian populations and this study does not support an important role of CCR5Δ32 in the pathogenesis of RA or a severe form of the disease in Mexicans.*

Introduction

In the last six years, the β chemokine receptor gene CCR5 has been an important research topic in human immunodeficiency virus type 1 (HIV-1) infection and acquired immunodeficiency syndrome (AIDS) pathogenesis (1, 2), and its role in the pathophysiology of rheumatic diseases is now being studied. A 32 base pair deletion has been described, which generates a non-functional receptor in the cell surface (CCR5Δ32) (3). Regarding HIV-1 infection and the development of AIDS, several studies sustain that the CCR5-Δ32 deletion carriers show a significant resistance to HIV-1 infection (3, 4). Moreover, some studies have suggested that in HIV infected patients, the deletion correlates with lower levels of circulating viral load, with a slower decline on peripheral CD4+ T cells and, subsequently, with a retarded progression to AIDS (4, 5).

On the other hand, the inability of beta chemokines (RANTES, MIP-1α, MIP-1β) to bind to the non-functional CCR5 causes significant defects in CCR5 and CC chemokine mediated chemotaxis (6). Therefore, the precise role of CCR5 could be relevant in the pathophysiology of inflammatory and immunologic related diseases. Recently, a protective role of CCR5Δ32 in asthma (7), rheumatoid arthritis (8), multiple sclerosis (9), and renal allograft survival (10) has been described.

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by the infiltration of lymphocytes, inflammation and destruction of several joints (11). The etiology of RA is unknown but genetic factors play an important role in its pathogenesis (12). Recently, the protective role of the CCR5Δ32 deletion has been described in the Caucasian population (13, 14). Furthermore, this polymorphism has been presumed to be a genetic marker related to the severity of RA (15). The importance of this fact resides in the therapeutic implications for these pathologies. Since it has been postulated that CCR5 and CXC3-expressing cells are determinant in the pathogenesis of inflammatory chronic diseases, the depletion of this CCR5+ cells could be a new strategy for the treatment of RA (16-18).

However, it is important to consider that there is a significant variation in the global distribution of CCR5Δ32 (19), with the highest allele frequency being reported in Ashkenazi Jews (20.93%) (20). In addition, other au-

thors suggest that the ethnic origin of the deletion is markedly related to Caucasian populations (21). Therefore, the geographical distribution of the CCR5 Δ32 allele could help to elucidate the relevance of the deletion in the pathogenesis of RA.

Additionally, MHC class II alleles have been also associated with RA (22). Some HLA alleles encode a conserved amino acid sequence (QKRAA, QRRAA, or RRRAA) at position 70-74 in the third hypervariable region of the DRβ chain "shared epitope" (SE) (23). Several studies, however, have associated the SE HLA-DRB1 alleles with RA severity and progression rather than with susceptibility (24). The aim of this study was to analyze the frequencies of the CCR5Δ32 deletion and HLA class II alleles in Mexican Amerindian populations and its relevance in the development and severity of RA.

Patients and methods

Sample

We studied a sample of 212 Mexican Mestizo subjects (40 patients with refractory RA, 102 patients with non-refractory RA and 70 healthy individuals). The RA patients were diagnosed according to the American College of Rheumatology (ACR) criteria in the Department of Immunology and Rheumatology of the Instituto Nacional de Ciencias Médicas y de la Nutrición Salvador Zubirán. Table I shows demographics, treatment, and disease severity markers in refractory and non-refractory RA patients.

The control group comprised 70 healthy, non-related Mexican Mestizo individuals with no family history of rheumatic diseases. All of them were living in Mexico City and since Mexico City is an important destination for immigration, it can be considered as representative of the rest of the Mexican population. Each individual was asked about his birthplace as well as that of his parents and maternal and paternal grandparents. A Mexican Mestizo is defined as someone born in Mexico who is a descendant of the original autochthonous inhabitants of the region and of individuals,mainly Spaniards, Caucasians and/or blacks, who came to America during the 16th century. We considered Mexican Mestizos only those individuals who were born in Mexico, and both of whose parents were born in Mexico as well.

On the other hand, to evaluate the ethnicity of the CCR5Δ32 deletion, we also studied 192 individuals from three Mexican Amerindian populations. These Amerindian groups included 70 Mayo (Capomo) individuals belonging to the Macro-Yuma linguistic family located in the Northeast of Mexico, 61 Teenek individuals belonging to a linguistically unclassified group located in the Huasteca region of San Luis Potosi State, and 61 Mazatecan Indians who inhabit the north of Oaxaca State. In order to define the genetic background of these three populations, they had been previously characterized by using genetic markers from several chromosomes (blood group, serum haptoglobin, albumin, B factor, HLA, and HSP70-2 genes). Results from these studies have shown that the proportion of Indian, Caucasian, and Negroid genes are 56%, 40%, and 4%, respectively (25-27).

Table I. Main demographic and clinical characteristics in refractory versus non-refractory rheumatoid arthritis patients.

Variable	Refractory RA	Non-refractory RA	p value
Age in years (mean/SD)	49/15	48/14	0.45
Formal education in years (mean/SD)	9/4.9	9/5	0.7
HAQ-Di (mean/SD)	1.4/0.6	0.7/0.5	0.001
Prednisone (mean/SD)	8.6/5.4	6.2/2.4	0.04
Erosions (%)	97	24	0.0001
Rheumatoid factor (%)	85	71	0.08
Extra-articular manifestations (%)	85	41	0.0001

HLA typing

Genomic DNA was isolated from peripheral blood EDTA anticoagulated samples by using standard techniques (28). HLA-DRB1, DRB3, DRB4, DRB5 amplification was done by polymerase chain reaction using *Taq* DNA polymerase and biotin labeled primers. A PCR-SSO reverse dot blot using the Dynal PCR-SSO Kit (Dynal Biotech Ltd., UK) was performed.

CCR5 genotyping

DNA was amplified by a PCR procedure using 5'- TCAAAAAGAAGGTC TTCATTACACC-3' (sense) and 5'-AGCCCAGAAGAGAAAA-TAAA-CAATC-3' (antisense) primers spanning the region of the Δ32 deletion (10). The size of the wild type PCR product was 241 base pairs and the CCR5Δ32 deletion product was 209 base pairs. To confirm the CCR5 polymorphism we employed a previously described PCR-RFLP procedure (1) using the *Eco* RI restriction endonuclease.

Statistical analysis

Allele and genotype frequencies of CCR5 were obtained by direct counting. The Hardy-Weinberg equilibrium was tested using the ARLEQUIN program. The differences among groups were evaluated by the Mantel-Haenszel, chi-square test that combined the 2 x 2 contingency tables using the EPI-INFO statistical program. If the number in any cell was < 5, Fisher's exact test was used. The p values were corrected by the Bonferroni method multiplying the p value for the number of comparisons. Odds ratios with 95% confidence intervals (OR, 95% CI) were calculated.

Results

The results are shown in Table II. In the non-refractory RA group (N=102) the CCR5Δ32 gene frequency was 0.019 and the following genotype frequencies were observed: CCR5/CCR5 = 97.0%, CCR5/CCR5Δ32 = 1.9% and CCR5Δ32/CCR5Δ32 = 1.0%. In this group the genotype distribution was not in Hardy-Weinberg equilibrium (Chi-square = 6.35, p = 0.01).

The deletion was uncommon in the

Table II. Allele and genotype frequencies of CCR5-Δ32 deletion in Mexican-Mestizo individuals.

Genotype	Refractory RA (N=40) n (%).	RA* (N= 102) n (%)	Controls (N=70) n (%)	Teenek (N=61) n (%)	Capomo (N=70) n (%)	Mazatecan (N=61) n (%)
CCR5/CCR5	38 (95.0)	99 (97.0)	70 (100)	61 (100)	70 (100)	59 (96.7)
CCR5/CCR5-Δ32	2 (5.0)	2 (1.9)	0 (0)	0 (0)	0 (0)	2 (3.2)
CCR5-Δ32/CCR5-Δ32	0 (0)	1 (0.9)	0 (0)	0 (0)	0 (0)	0 (0)
Allele	n g.f.	n (g.f.)	n (g.f.)	n (g.f.)	n (g.f.)	n (g.f.)
CCR5 (wild type)	78 (0.975)	200 (0.980)	140 (1.0)	122 (1.0)	140 (1.0)	120 (0.983)
CCR5-Δ32 (deletion)	2 (0.025)	4 (0.019)	0 (0)	0 (0)	0 (0)	2 (0.016)

* Hardy-Weinberg equilibrium value in the non-refractory RA group, Chi square = 6.35; p = 0.01.
N = number of patients

refractory RA group (N= 40) g.f.= 0.025. 95% of refractory RA patients were CCR5/CCR5 homozygous and the last 5 % were CCR5/CCR5Δ32 heterozygous. The deletion was not observed in the healthy Mexican Mestizo group nor in the Teenek and Mayo Amerindians, all of whom were individuals homozygous for the wild type allele. In the Mazatecan group the deletion frequency was 1.6%.

HLA-DRB1 typing revealed that the most common alleles were DRB1*04, 07 and 08 in severe RA patients with gene frequencies of 0.350, 0.150 and 0.125, respectively. We observed a significant increase in the frequency of the DRB1*07 allele in severe RA patients in comparison to the non-severe RA group (p = 0.02, OR = 5.65, 95% CI = 0.95 - 43.05). We also observed a decreased frequency of the DRB1*13 and *03 alleles in the severe RA group (data not shown).

Discussion

Several studies have implicated the CCR5 β chemokine receptor in the pathogenesis of rheumatoid arthritis (13, 14). In addition, other studies have described the highest frequency of the CCR5Δ32 deletion in Caucasian populations, suggesting its ethnic origin (29). This is the first study done in Mexico to analyze the frequency of the CCR5Δ32 deletion in genetically well defined ethnic groups, including three Amerindian populations, healthy Mestizos, and diseased individuals from different geographical areas of the country (30-32).

The CCR5Δ32 deletion was not found in the Mexican Amerindians nor in the healthy Mexican Mestizo subjects. The absence of this allele was also previously reported in indigenous Brazilian populations (33). Similarly, in our Mexican Mestizo and RA patients, the frequency of the deletion was uncommon, being 1.9% in non-refractory RA patients and 2.5% in the refractory group. Interestingly, the frequency of CCR5Δ32 was not in Hardy-Weinberg equilibrium in the non-severe RA group, suggesting the action of natural selection in the frequency of this gene in this population. Several studies have associated the HLA-DRB1 gene with RA severity and disease progression in several populations, including Mexicans (34, 35).

Moreover, the association with disease severity and the presence of the SE varies among different ethnic groups (36). In this study, we observed an increase in HLA-DRB1*04 allele frequency in the severe RA group (unpublished data), although the statistical analysis did not reveal a significant p value. This fact could be due to the small sample size or the high frequency of the DRB1*04 allele in the general Mexican population (37).

In conclusion,the CCR5Δ32 deletion is not common in Mexican Amerindian populations and confirms the hypothesis of its Caucasoid origin. This study does not support an important role of CCR5Δ32 in the pathogenesis of RA and did not show a relationship between the allele and a severe form of the disease in Mexicans.

References

1. SAMSON M, LIBERT F, DORANZ BJ *et al.*: Resistance to HIV-1 infection in Caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. *Nature* 1996; 382: 722-25.
2. DEAN M, CARRINGTON M, WINKLER C *et al.*: Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CKR5 structural gene. *Science* 1996; 273: 1856-62.
3. DE RODA HUSMAN AM, KOOT M, CORNELISSEN M *et al.*: Association between CCR5 genotype and the clinical course of HIV-1 infection. *Ann Intern Med* 1997; 127: 882-90.
4. BERGER EA, MURPHY PM, FARBER JM: Chemokine receptors as HIV-1 coreceptors: roles in viral entry, tropism, and disease. *Annu Rev Immunol*. 1999; 17: 657-700.
5. WLLI R, REINHART B, LUCKLOW B *et al.*: HIV-1 infected long term slow progressors heterozygous for delta 32-CCR5 show significantly lower plasma viral load than wild-type slow progressors. *J Acquir Immune Defic Syndr Hum Retrovirol* 1998; 18: 229-33.
6. BLANPLAIN C, MIGEOTTE I, LEE B *et al.*: CCR5 binds multiple CC-chemokines:MCP-3 acts as a natural antagonist. *Blood* 1999; 94: 1899-905.
7. HALL IP, WHEATLEY A, CHRISTIE G, MCDOUGALL C, HUBBARD R, HELMS PJ: Association of CCR5 delta 32 with reduced risk of asthma. *Lancet* 1999; 354: 1264-65.
8. COOKE SP, FORREST G, VENABLES PJ, HAJEER A: The delta32 deletion of CCR5 receptor in rheumatoid arthritis. *Arthritis Rheum* 1998; 41: 1135-6.
9. SELLEBJERG F, MADSEN HO, JENSEN CV, JENSEN J, GARRED P: CCR5 delta32, matrix metalloproteinase-9 and disease activity in multiple sclerosis. *J Neuroimmunol* 2000; 102: 98-106.
10. FISCHEREDER M, LUCKOW B, HOCHER B *et al.*: CC chemokine receptor 5 and renal-transplant survival. *Lancet* 2001; 357: 1758-61.
11. ARNETT FC, EDWORTHY SM, BLOCH DA *et al.*: The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988; 31: 315-24.

12. REVIELLE JD: The genetic contribution to the pathogenesis of rheumatoid arthritis. *Curr Opin Rheumatol* 1998; 10: 187-200.
13. GARRED P, MADSEN HO, PETERSEN J *et al*.: CC chemokine receptor 5 polymorphism in rheumatoid arthritis. *J Rheumatol* 1998; 25: 1462-5.
14. GOMEZ-REINO JJ, PABLOS JL, CARREIRA PE *et al*.: Association of rheumatoid artritis with a functional chemokine receptor, CCR5. *Arthritis Rheum* 1999; 42: 989-92.
15. ZAPICO I, COTO E, RODRIGUEZ A, ALVAREZ C, TORRE JC, ALVAREZ V: CCR5 (chemokine receptor-5) DNA-polymorphism influences the severity of rheumatoid artritis. *Genes Immun* 2000; 1: 288-9.
16. QIN S, ROTTHMAN JB, MYERS P *et al*.: The chemokine receptors CXCR3 and CCR5 mark subsets of T cells associated with certain inflammatory reactions. *J Clin Invest* 1998; 101: 746-54.
17. BONECCHI R, BIANCHI G, BORDINGNON PP *et al*.: Differential expression of chemokine receptors and chemotactic responsiveness of type 1 T helper cells (Th1s) and Th2s. *J Exp Med* 1998; 187: 129-34.
18. BRUHL H, CIHAK J, STANGASSINGER M, SCHLONDORF D, MACK M: Depletion of CCR5-expressing cells with bispecific antibodies and chemokine toxins: A new strategy in the treatment of chronic inflammatory diseases and HIV. *J Immunol* 2001; 166: 2420-6.
19. MARTINSON JJ, CHAPMAN NH, REES DC, LIU YT, CLEEG JB: Global distribution of the CCR5 gene 32-base pari deletion. *Nat Genet* 1997; 16: 100-3.
20. KANTOR R, GERSHIONI JM: Distribution of the CCR5 gene 32-base pair deletion in Israeli ethnic groups. *J Acquir Immune Defic Syndr Hum Retrovirol* 1999; 20: 81-4.
21. LUCOTTE G: Distribution of the CCR5 gene 32-base pair deletion in West Europe. A hypothesis about the possible dispersion of the mutation by the Vikings in historical times. *Hum Immunol* 2001; 62: 933-6.
22. JAWAHEER D, GREGERSEN PK: Rheumatoid arthritis. The genetic components. *Rheum Dis Clin North Am* 2002;28:1-15.
23. JAWAHEER D, THOMSON W, MACGREGOR AJ, CARTHY D, DAVIDSON J, DYER PA *et al*.:"Homozygosity" for the HLA-DR shared epitope contributes the highest risk for rheumatoid arthritis concordance in identical twins. *Arthritis Rheum* 1994; 37: 681-6.
24. MATTEY DL, HASSELL AB, DAWES PT *et al*.: Independent association of rheumatoid factor and the HLA-DRB1 shared epitope with radiographic outcome in rheumatoid arthritis. *Arthritis Rheum* 2001; 44: 1529-1533.
25. LISKER R, PÉREZ-BRICEÑO R, GRANADOS J *et al*.:Gene frequencies and admixture estimates in a Mexican City population. *Am J Physical Anthropol* 1986; 71: 203-7.
26. LISKER R, PÉREZ-BRICEÑO R, GRANADOS J, BABINSKY V: Gene frequencies and admixture estimates in the State of Puebla, Mexico. *Am J Physical Anthropol* 1988; 76: 331-5.
27. LISKER R, RAMIREZ E, PÉREZ-BRICEÑO R, GRANADOS J: Gene frequencies and admixture estimates in four Mexican urban centers. *Hum Biology* 1990; 62: 791-801.
28. DAVIS RW, THOMAS M, CAMERON J *et al*.: Rapid DNA isolation for enzymatic and hybridization analysis. M*ethods Enzymol* 1980: 65: 404-11.
29. STEPHENS JC,REICH DE, GOLDSTEIN DB *et al*.: Dating the origin of the CCR5-Delta32 AIDS resistance allele by the coalescence of haplotypes. *Am J Hum Genet* 1998; 62: 1507-15.
30. ARNAIZ-VILLENA A, VARGAS-ALARCÓN G, GRANADOS J *et al*.: HLA genes in Mexican Mazatecans, the peopling of the Americas and the uniqueness of Amerindians. *Tissue Antigens* 2000; 56: 405-16.
31. VARGAS-ALARCÓN G, GOMEZ-CASADO E, MARTINEZ-LASO J *et al*.: Description of a new HLA-B40 allele (B*4011) found in a Mexican individual of Nahua (Aztec) descent. *Immunogenetics* 1997; 46: 359-60.
32. ZÚÑIGA J, VARGAS-ALARCÓN G, HERNÁNDEZ-PACHECO G, PORTAL-CELHAY C, YAMAMOTO-FURUSHO JK, GRANADOS J: Tumor necrosis factor-alpha promoter polymorphisms in Mexican patients with systemic lupus erythematosus (SLE). *Genes Immun* 2001; 2: 363-7.
33. LEBOUTE AP, DE CAVALHO MW, SIMOES AL: Absence of the deltaCCR5 mutation in indigenous populations of the Brazilian Amazon. *Human Genet* 1999; 105: 442-3.
34. GORMAN JD, CRISWELL LA: The shared epitope and severity of rheumatoid arthritis. *Rheum Dis Clin North Am* 2002; 28: 59-78.
35. DEL RINCON I, ESCALANTE A: HLA-DRB1 alleles associated with susceptibility and resistance to rheumatoid arthritis, articular deformities,and disability in Mexican Americans. *Arthritis Rheum* 1999; 42: 1329-38.
36. CONSTANTIN A, LAUWERS-CANCES V, NAVAUX F *et al*.: Stromelysin 1 (matrix metalloproteinase 3) and HLA-DRB1 gene polymorphisms: Association with severity and progression of rheumatoid arthritis in a prospective study. *Arthritis Rheum* 2002; 46: 1754-62.
37. VARGAS-ALARCÓN G, GAMBOA R, ZÚÑIGA J *et al*.: HLA-DR4 allele frequencies on Indian and Mestizo population from Mexico. *Human Immunol* 2000; 61: 341-4.