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Chemokine receptor CCR5 polymorphisms and Chagas' disease cardiomyopathy

Key words:
CCR5; polymorphism; *Trypanosoma cruzi*; Chagas' disease

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Abstract: In this study we investigated the possible role of two CCR5 gene polymorphisms, CCR5Δ32 deletion and CCR5 59029 A→G promoter point mutation, in determining the susceptibility to *Trypanosoma cruzi* infection as well as in the development of chagasic heart disease. These CCR5 polymorphisms were assessed in 85 seropositive (asymptomatic, $n=53$; cardiomyopathic, $n=32$) and 87 seronegative individuals. The extremely low frequency (0.009) of the CCR5Δ32 allele in our population did not allow us to analyse its possible influence on *T. cruzi* infection. We found no differences in the distribution of CCR5 59029 promoter genotype or phenotype frequencies between total chagasic patients and controls. However, we observed that the CCR5 59029-A/G genotype was significantly increased in asymptomatic with respect to cardiomyopathic patients ($P=0.02$; OR=0.33, 95% CI 0.10–0.94). In addition, the presence of the CCR5 59029-G allele was also increased in asymptomatics when compared with cardiomyopathics ($P=0.02$; OR=0.35, 95% CI 0.12–0.96). Our data suggest that the CCR5 59029 promoter polymorphism may be involved in a differential susceptibility to chagasic cardiomyopathy.

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Trypanosoma cruzi is an intracellular protozoan parasite that causes Chagas' disease or American trypanosomiasis, a disease considered to be the most serious parasitic disease of the Americas with an important social and economical impact (1), which has been recently considered as an opportunistic infection of HIV-1-infected individuals in the United States (2). Although the precise mechanisms of the anti-parasitic response remain to be characterized, it is clear that immune and inflammatory molecules are important in the clearance of *T. cruzi*.

Chemokines are small secreted polypeptides involved in the migration and activation of immune cells at sites of antigenic challenge, playing important roles in the inflammatory process occurring in response to infection and autoimmune diseases (3–5). The biological activities of chemokines are mediated by interactions with their cell-surface receptors that belong to the structurally re-

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lated seven-transmembrane domain superfamily of proteins (6). The chemokine receptor-5 (CCR5) mediates chemotaxis by the CC-chemokines RANTES, MIP-1α and MIP-1β; which have been recently proposed to enhance *T. cruzi* trypomastigote uptake by human macrophages and induce trypanocidal activity of these cells via nitric oxide (NO) (7–9). Besides their physiologic functions chemokine receptors have themselves been used as gateways by several intracellular pathogens for mediating their cellular entry (10).

The CCR5 chemokine receptor, which is present on T lymphocytes and on monocytes/macrophages, has been shown to be important in the transmission and pathogenesis of HIV-1 (11). A number of polymorphic variants have been described in the CCR5 gene, some of which have functional significance. Of interest, is a 32-base pair (bp) deletion in the coding region of CCR5 (CCR5Δ32) that results in a non-functional receptor that is not expressed on the cell surface (12). Homozygosity for this deletion protects almost completely against HIV-1 infection while heterozygocity, which is associated with a reduced cell surface expression of CCR5, correlates with a delayed progression to acquired immune deficiency syndrome (AIDS) (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 CCR5 genetic variants with functional consequences may have the potential to influence the susceptibility and clinical outcome of *T.cruzi* infection. To address this, we analyzed the distribution of CCR5Δ32 and CCR5 59029 A→G polymorphisms in a group of chagasic patients and control subjects from the same geographic and ethnic origin.

Material and methods

Study participants

The study included 172 unrelated individuals from a rural settlement in the district of Arequipa, Perú, where *T. cruzi* infection is highly endemic. Almost all the study participants (97%) recognized the presence of the local parasite vector, *Triatoma infestans*, around their dwellings, who are believed to have experienced uniformly high levels of exposure to the vector. All the individuals were of Amerindian origin and they were older than 15 years (range 15–74). Clinical history, physical examination, and resting ECG were carried out. Based on serological screening (ELISA, hemagglutination and indirect immunofluorescence) individuals were divided into seropositive patients (n=85) and seronegative healthy controls (n=87). Patients serologically positive for *T. cruzi* infection were further classified according to the absence (asymptomatics, n=53) or presence (cardiomyopathics, n=32) of cardiac symptoms, which were assessed by clinical and electrocardiographic (ECG) characteristics compatible with chagasic cardiomyopathy. The mean age of the seropositive group was 35 (SD±18) years, and that of the seronegative group 36 (SD±15) years. The groups were matched for age and sex.

CCR5 typing

Typing was performed directly on genomic DNA obtained by standard methods. CCR5Δ32 typing was carried out as described previously (13). Briefly, a 189-bp polymerase chain reaction (PCR) product was detected from the normal CCR5 allele and a 157-bp PCR product from the deletion allele. The CCR5 59029 A→G promoter polymorphism was analyzed with a PCR-restriction fragment length polymorphism (RFLP) 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

Genotype frequencies were determined by direct counting. Comparisons between seronegative controls and total seropositive patients as well as between asymptomatics and cardiomyopathics were calculated using 2×2 contingency tables and the Chi-square test and Fisher's exact test when appropriate. Odds ratio (OR) were calculated by Woolf's method with 95% confidence interval or by Haldane's modification.

Results

Frequency of the CCR5Δ32 polymorphism

The prevalence of the CCR5Δ32 deletion was investigated among 87 seronegative and 85 seropositive for *T. cruzi*. None of the 172 samples from our Peruvian population were homozygous for this deletion, and only three individuals were CCR5/CCR5Δ32 heterozygous: two cardiac patients and one control. The extremely low frequency (0.009) of the CCR5Δ32 allele observed in this study did not allow us to analyze its possible influence on *T. cruzi* infection. The CCR5Δ32 deletion is common in Caucasian populations where between 10–15% are heterozygous and approximately 1% are homozygous for the CCR5Δ32 allele and found at lower frequencies in Middle East and India. Our findings agree with previous epide-

Genotype and phenotype distribution of CCR5 59029 A/G polymorphism in chagasic patients and control subjects

	Controls n=87 (%)	Patients n=85 (%)	Asymptom. n=53 (%)	Cardiomyop. n=32 (%)
Genotypes				
59029-G/G	8 (9.2)	12 (14.1)	8 (15.1)	4 (12.5)
59029-A/G	33 (38.9)	28 (32.9)	22 (41.5)	6 (18.8)*
59029-A/A	46 (52.9)	45 (52.9)	23 (43.4)	22 (68.8)
Phenotypes				
59029-G	41 (47.1)	40 (47)	30 (56.6)	10 (31)*
59029-A	79 (90.8)	73 (85.8)	45 (85)	28 (87.5)

* 41.5% asymptom. vs. 18.8% cardiomyop.; P=0.02; OR=0.33, 95% CI 0.10–0.94
* 56.6% asymptom. vs. 31% cardiomyop.; P=0.02; OR=0.35, 95% CI 0.12–0.96

Table 1

miological data indicating that CCR5Δ32 is very rare in Amerindian, native Africans and East Asians (15).

Frequency of CCR5 promoter 59029 A→G polymorphism

The distribution of the CCR5 promoter 59029 genotypes in asymptomatic, cardiomyopathic and control individuals is shown in Table 1. Frequencies of CCR5 59029 G/G, 59029 A/G and 59029 A/A genotypes were 9.2%, 37.9%, and 52.9% in the controls and 14.1%, 32.9%, and 52.9% in the total patients group, respectively. The study population was found to be in Hardy-Weinberg equilibrium. No statistically significant differences were observed when the CCR5 59029 genotype distribution between total chagasic patients and healthy controls was compared, suggesting that this CCR5 promoter polymorphism does not influence the susceptibility to *T. cruzi* infection.

In order to investigate the possible influence of the CCR5 59029 A→G variant in the development of Chagas' disease cardiomyopathy, the genotype and phenotype frequencies between seropositive patients without manifestation of the disease (asymptomatics) and those with chagasic cardiomiopathy were compared. Interestingly, we found that the CCR5 59029-A/G genotype was significantly increased in asymptomatic with respect to cardiomyopathic patients (P=0.02; OR=0.33, CI 95% 0.10–0.94). In addition, we observed that the 59029-G allele was present at significantly higher frequency in asymptomatics as compared with cardiomyopathics (P=0.02; OR=0.35, 95% CI 0.12–0.96). Taken together, these results suggest that this CCR5 promoter polymorphism seems to play an important role in Chagas' disease outcome, or is tightly linked to a polymorphism that is important.

Discussion

The immunogenetics of most infectious diseases is complex, and it involves interactions between pathogen and host susceptibility genes (16). Genes encoding chemokine receptors are attractive candidates as host genetics factors related to susceptibility and outcome of infectious diseases, as *T. cruzi* infection, due to their key role in inflammation, regulation of immune response and their exploitation by certain microorganism to initiate infection (3–5, 17). The results of the present study provide the first evidence that genetic polymorphism in the CCR5 promoter region may affect the development of Chagas' disease related cardiomyopathy.

None of the individuals analyzed in this study was homozygous for the CCR5Δ32 allele, which encodes for a non-functional CCR5 receptor. Thus, it was not possible to assess the importance of CCR5Δ32 deletion in *T. cruzi* cell infection. In addition, the low frequency of CCR5Δ32 allele observed in our population did not allow us to further investigate the possible influence on Chagas'disease outcome. On the other hand, the low distribution of the CCR5Δ32 allele in the Peruvian sample is consistent with its anthropological origin, which is a mestizo population with a strong Amerindian component (18). Similar to our findings various studies have confirmed the low prevalence of the Δ32 allele among Amerindian populations (15, 19, 20), and corroborate the hypothesis that the CCR5Δ32 allele has a European origin, and that its presence in South American populations is probably the result of immigration. HIV-1 has only recently become endemic in Europe to consider selective pressure mechanisms as responsible for the fixation of this CCR5 genetic variation in the population. It is possible that another ancestral and important parasite sharing the same HIV-1 entrance mechanism might be implicated in determining this selective advantage.

The CCR5 59029 A→G promoter polymorphism was frequently found in the studied Peruvian population. There were no significant differences in genotype or phenotype distribution among healthy controls and total patient group, suggesting a lack of effect on primary *T. cruzi* infection by this single nucleotide polymorphism (SNP). Interestingly, we found a significant higher frequency of the 59029-A/G genotype and the presence of the 59029-G allele in asymptomatic with respect to cardiomyopathic patients. Our data can be interpreted as showing a protective role of the CCR5 59029-G allele in the development of chagasic cardiomyopathy, although it is possible that this association could be due to linkage disequilibrium with other CCR5 promoter polymorphisms not studied here (21, 22). These results are consistent with the previously reported genetic associations of these CCR5 variants with AIDS progression in HIV-1-infected individuals (14, 23).

Recent studies have reported that the 59029-G allele had a lower promoter *in vitro* activity than the 59029-A allele and that the 59029 A/A genotype results in a higher CCR5 expression in CD4 T cells (14, 24). It is likely that the possible protective role of 59029-G allele may be the result of a reduced CCR5 membrane expression in individuals bearing this allele. The enhanced local production of the chemokines RANTES, MIP-1α and MIP-1β that occurs during *T. cruzi* macrophage infections may drive the selective migration toward affected tissues of immune cells preferentially expressing CCR5, such as Th1-like cells (25). Therefore, the membrane level of this CCR5 chemokine receptor could determine not only the amount and type of immune cells in the infected organs but also the degree of the inflammatory response in *T. cruzi* infection. It has been demonstrated that T-cell-mediated control of infection is almost inevitably accompanied by some degree of immunopathology. In fact, cardiac lesions observed during *T. cruzi* chronic infections have been associated with the local inflammatory response produced by the host's immune cells (26, 27). It is therefore likely that a reduced recruitment of Th1 cells into cardiac inflamed tissue due to a decreased CCR5 membrane expression may protect against the development of chagasic cardiomiopathy.

At present, there are no vaccines available or effective chemotherapies to control the chronic phase of *T. cruzi* infection. Considering that CCR5 seems to play a role in the clinical course of Chagas' disease it is possible that the treatment with chemokine receptor modulators could be a valuable therapy for retarding cardiac symptoms in *T. cruzi* chronic infections. With regards to this, novel CCR5 antagonists exist and have been proposed as antiviral drugs (28, 29).

In conclusion, this study provides the first evidence for a genetic association between CCR5 59029 promoter polymorphism and the development of chagasic cardiomyopathy. The CCR5 59029-A/G polymorphism may also prove to be a useful marker of severe Chagas' disease, and could highlight a group of patients who require a more aggressive therapy in the early stages of the disease. It is possible that this association is due to linkage disequilibrium between CCR5 and other susceptibility gene(s), therefore these findings require confirmation in a larger cohort of chagasic patients and in a different population.

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