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Role of the IFNG +874T/A polymorphism in Chagas disease in a Colombian population

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ABSTRACT
Genetic susceptibility to Trypanosoma cruzi infection and the development of cardiomyopathy is complex, heterogeneous, and likely involves several genes. Previous studies have implicated cytokine and chemokine genes in susceptibility to Chagas disease. Here we investigated the association between the interferon-gamma gene (IFNG) +874T/A polymorphism and Chagas disease, focusing on susceptibility and severity. This study included 236 chagasic patients (asymptomatic, n = 116; cardiomyopathic, n = 120) and 282 healthy controls from a Colombian population where T. cruzi is highly endemic. Individuals were genotyped for functional single nucleotide polymorphism (SNP; rs2430561; A/T) of the IFNG gene by amplification refractory mutational system PCR (ARMS-PCR). Moreover, clinical manifestations of Chagas in patients were analyzed. We found a significant difference in the distribution of the IFNG +874 “A” allele between patients and healthy controls (P = 0.003; OR = 1.46, 95% CI, 1.13–1.89). The frequency of the IFNG +874 genotype A/A, which is associated with reduced production of interferon-gamma, was increased in the patients relative to controls (38.1% vs. 26.6%). We compared the frequencies of IFNG alleles and genotypes between asymptomatic patients and those with chagasic cardiomyopathy and found no significant difference. Our data suggest that the IFNG +874T/A genetic polymorphism may be involved in susceptibility but not in the progression of Chagas disease in this Colombian population.

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1. Introduction
Chagas disease, also known as American Trypanosomiasis, is caused by infection with the protozoan parasite Trypanosoma cruzi (WHO, 1991). More than 10 million people carry the protozoan organism T. cruzi, which multiplies inside cells, particularly of heart and smooth muscle (WHO, 2002a,b). Chagas disease has a broad spectrum of clinical presentations, ranging from asymptomatic infections to life-threatening cardiac and digestive disease. The type of clinical presentation varies by geographical region (Prata, 2001). Following the acute phase, patients enter the chronic phase. Up to 20 years after the initial infection, ~35% of infected people develop pathological signs characteristic of Chagas disease. Autoimmunity, granulocytic cell activation, tissue damage caused by T. cruzi, neurogenic factors, and microvascular disturbance have been reported in association with the development of the chronic features of the disease (Kierszenbaum, 1999). The mechanisms responsible for the susceptibility to infection and the clinical heterogeneity observed among infected individuals are not well understood, but substantial evidence suggests that differences in the expression of genes related to the immune response may be involved.

Previous studies have implicated cytokine and chemokine genes in determining increased susceptibility and further development of chagasic heart disease (Calzada et al., 2001, 2009; Ramasawmy et al., 2006; Torres et al., 2009). Nevertheless, genetic susceptibility to T. cruzi infection and the development of cardiomyopathy is complex, heterogeneous, and likely involves several genes (Nieto et al., 2000).

Interferon-gamma (IFN-γ) is a multifunctional cytokine, which is produced by effector T and natural killer cells. IFN-γ controls the development of T helper 1 (Th1) cells and is critical for host defense against a variety of intracellular pathogens, including T. cruzi infection (Silva et al., 1992; Torrico et al., 1991). The human IFNG
gene on chromosome 12q24.1 spans 5.4 kb and contains four exons that encode a 146-aa protein. Several polymorphisms within the IFNG non-coding regions, such as +874A/T, CA repeat microsatellite and −179T/G, have been implicated in numerous autoimmune and chronic inflammatory conditions (Chong et al., 2006; Pacheco et al., 2008; Pravica et al., 2000). A single nucleotide polymorphism (SNP) located in the first intron of the human IFNG gene at the 5′ end, adjacent to a CA repeat region (+874T/A polymorphism rs2430561), can influence the secretion of IFN-γ (Pravica et al., 2000). Analysis of the biological role of this SNP suggested that +874A allele carriers are low IFN-γ producers (Lopez-Maderuelo et al., 2003). Susceptibility to other infectious diseases like severe acute respiratory syndrome (SARS) have also been described (Chong et al., 2006) suggesting that variability in IFN-γ production linked to this SNP is possibly playing a major role in susceptibility to infectious diseases, especially intracellular pathogens. Due to this, we selected the +874T/A polymorphism of IFNG to assess the potential association of this SNP in the susceptibility and/or clinical features of Chagas disease in a Colombian population from an endemic area.

2. Materials and methods

2.1. Study subjects

This study included 518 patients from the province of Santander, Colombia, divided into 282 serologically negative and 236 positive for T. cruzi antigens. Both seropositive and seronegative patients were from rural area of an endemic region in Northeastern Colombia, the samples were collected directly in the same villages, where approximately 50% of individuals are seropositive for T. cruzi infection (Gutierrez et al., 2004).

All participants were older than 28 years. The mean age of the seronegative group was 41 years, the mean age of the asymptomatic group was 48.7 years, and the mean age of the cardiomyopathic group was 55.2 years. A total of 69% of asymptomatic and 59% of cardiomyopathic were female. The serological diagnosis was based on results of two independent tests, enzyme-linked immunosorbert assay and indirect hemagglutination test (WHO, 2002a,b). Patients were classified according to clinical and electrocardiographic characteristics. Those without cardiac symptoms (n = 116) and with a normal electrocardiogram (ECG) were classified as asymptomatic. Patients that by clinical evaluation, ECG, Holter monitoring (24 h) and echocardiogram showed conduction alterations and/or structural cardiomyopathy were included in the cardiomyopathic or symptomatic group (n = 120) as follows: CC II (n = 20, radiology indicative of light heart hypertrophy or minor ECG alterations), CC III (n = 80, moderate heart hypertrophy and considerable ECG alterations, mainly advanced conduction abnormalities) and CC IV (n = 20, severe cardiomegaly and marked ECG alterations, predominantly frequent and/or complex forms of ventricular arrhythmia) (Rocha et al., 2003). All the individuals are from the same geographic region and have been living there for more than 10 years and they shared the same environmental and socioeconomic living conditions. The population from this region is homogeneous and there is not concentration of ethnical groups such as indigenous or black population. The population’s structuring was determined by the Arlequin 3 program (Excoffier et al., 2005). All the subjects were included in this study after written informed consent. We obtained approval for the study from all local ethical committees.

2.2. Genotyping

Genomic DNA was isolated from 7 ml of EDTA-anticoagulated blood sample using the standard salting-out technique (Miller et al., 1998). IFNG +874A/T (rs2430561) polymorphism was determined by amplification refractory mutational system (ARMS) PCR method followed by gel electrophoretic analysis as described previously (Pravica et al., 2000). The following primers were used for amplification: 5′-TCAAAAGCTGATACTCCA-3′ (consensus primer), 5′-TTCTTACAACACAAAATCAATCTA-3′ (A allele specific), 5′-TTCTTACAACACAAAATCAATCT-3′ (T allele specific). Amplification yielded a 263-bp PCR product. Primers amplifying human growth hormone (F: 5′-GGTTTTACACATTCTCTTTA-3′ and R: 5′-TACCGATTCCGTGTTGTT-3′), yielding a 408-bp PCR product, were utilised as an internal control. The PCR conditions consisted of an initial denaturation step at 95 °C for 2 min, 10 cycles of incubation at 95 °C for 15 s, 62 °C for 50 s and 72 °C for 40 s, followed by 20 cycles of incubation at 95 °C for 20 s, 56 °C for 50 s and 72 °C for 50 s, with a final extension at 72 °C for 5 min. The amplified products were visualised by electrophoresis using 2% agarose gels containing ethidium bromide.

2.3. Sample size calculations

The power of the sample size was calculated using the Quanto software, version 1.1. using an unmatched (1:0.84) case-control design, and a gene only hypothesis. We calculated power for analyzed SNP to confirm the effect. In our case-control study, we had a power of 0.8467 to detect a modest effect sizes (OR = 1.5), assuming a two-sided α-level of 0.05 and a dominant heridity pattern.

2.4. Statistical analyses

Allele and genotype frequencies were obtained by direct counting. We assessed the quality of the genotype data by testing for Hardy–Weinberg equilibrium in the case and control samples, using Fisher’s exact test (P > 0.05). Differences between allele and genotype frequencies were determined using a χ² test. Odds ratios and 95% confidence intervals were calculated according to Woolf’s method. The software Statcalc Epilinfo 2002 (Centers for Disease Control and Prevention, Atlanta, GA) was used for statistical analyses. A P-value < 0.05 was considered statistically significant.

3. Results

The IFNG +874A/T genotype and allele frequencies for Chagas patients and healthy controls as well as for cardiac and asymptomatic patients are listed in Tables 1 and 2, respectively. The genotype frequencies of the polymorphism studied were not found to be significantly different from those predicted by the Hardy–Weinberg equilibrium among healthy controls or patients. To ensure the absence of population substructure we estimated the Fst using approximately 40 different markers to IFN-γ, we found that the population from this region is a homogeneous mixture and there is not concentration of ethnical groups (Fst 0.0013).

We found a statistically significant difference in the distribution of the A/A genotype (low production of IFN-γ) and the A allele at the IFNG polymorphism between Chagas patient and control groups. These findings suggest a genetic influence of this polymorphism on T. cruzi infection susceptibility. The A/A genotype among individuals with the IFNG +874A/T polymorphism was significantly more prevalent in Chagas patients than in controls (P = 0.005; OR = 1.70, 95% CI = 1.50–2.51) (Table 1). In addition, the IFNG A allele showed evidence of association with Chagas disease (P = 0.003; OR = 1.46, 95% CI, 1.13–1.89).

To investigate the possible influence of the IFNG +874A/T polymorphism on the development of cardiomyopathy, IFNG genotype and allele frequencies between asymptomatic patients and those with chagasic cardiomyopathy were compared. No
The A allele with lower IFN-g gene expression that this allele may be a risk factor for genetic susceptibility to Chagas disease. Resistance to acute infection with the amastigote form of the parasite (Torrico et al., 1991). In addition, producing nitric oxide (NO) and killing the obligate intracellular parasite (Torrico et al., 1991). Asymptomatic individuals, indicating no influence of this polymorphism on Chagas disease progression (Table 2).

4. Discussion

A significant amount of evidence indicates that susceptibility to Chagas disease or other infectious diseases may be related to genetic variability at cytokine loci (Florez et al., 2006; Karplus et al., 2005). Control of Chagas infection requires both humoral and cell-mediated immunity directed by a type 1 cytokine response (Kumar and Tarleton, 1998). Endogenous IFN-γ and TNF-α play critical roles in the control of the infection through an immune mechanism including release of free radicals (Silva et al., 1995).

In this work, we genotyped a SNP located within the first intron of the human IFNG gene at the 5’ end, adjacent to a CA repeat region (+874T/A). The location of this polymorphism coincides with a putative NF-κB binding site, which might have functional consequences on the transcription of the human IFNG gene (Pravica et al., 2000). Indeed, the T allele at the IFNG gene was shown to be associated with higher IFN-γ protein production and the A allele with lower IFN-γ protein production in healthy individuals (Lopez-Maderuelo et al., 2003; Pravica et al., 1999).

In this study, the frequency of the A allele or A/A genotype, coding for low production of IFN-γ, was found to be higher in patients with Chagas disease than in healthy individuals, indicating that this allele may be a risk factor for genetic susceptibility to Chagas disease. Resistance to acute infection with T. cruzi has been shown to be dependent on IFN-γ, which activates macrophages to produce nitric oxide (NO) and kill the obligate intracellular amastigote form of the parasite (Torrico et al., 1991). In addition, TNF-α provides a second signal that stimulates NO production and anti-T. cruzi activity in IFN-γ-activated macrophages (Silva et al., 1992). This mechanism would explain the higher susceptibility to T. cruzi infection among individuals carrying the A allele compared with individuals carrying the “T” allele. Similar results have been reported for other infectious diseases, such as pulmonary tuberculosis and severe acute respiratory syndrome (Chong et al., 2006; Lopez-Maderuelo et al., 2003).

Previous studies have shown an association between IFNG genetic polymorphisms and severity or progression of diseases, including diseases of severe acute respiratory syndrome and hepatitis B infection (Chong et al., 2006; Ribeiro et al., 2007). Contrary to expectations, no significant differences were observed in the distribution of alleles or genotypes of the IFNG +874T/A polymorphism between cardiomyopathic and asymptomatic patients with Chagas disease, indicating no influence of this polymorphism on Chagas disease progression.

A larger sample size may be required in order to establish whether a cause-effect association exists between this polymorphism and to development of cardiomyopathy. Consistent with our result, D’Avila et al. (2009) found no difference in IFN-γ production between cardiac and asymptomatic patients. Complex interactions take place following parasite infection, predicting that the clinical course of the disease cannot be explained by a single mechanism. Consistent with this prediction, interleukin IL-10 and TGF-β are associated with susceptibility to infection (Cardillo et al., 1996) by inhibiting IFN-γ-mediated macrophage activation. Therefore, not only the presence of IFN-γ, per se, but also the secretion levels of others cytokines (e.g., IL-4, IL-10, TGF-β, and TNF-α) constitute key factors in the immunoregulation of the host–parasite relationship (Gomes et al., 2003; Martin et al., 2007; Rodriguez-Perez et al., 2005).

Table 1 Genotype and allele frequencies of the rs2430561 IFNG +874T/A between Chagas’ patients and healthy controls.

| IFNG +874T/A rs2430561 | Patients n = 236 (%) | Controls n = 282 (%) | χ² | P value | OR (95% CI) |
|------------------------|---------------------|---------------------|----|--------|-------------|
| Genotype               |                     |                     |    |        |             |
| AA                     | 90 (38.1)           | 75 (26.6)           |    |        |             |
| TA                     | 119 (50.4)          | 156 (55.3)          |    |        |             |
| TT                     | 27 (11.4)           | 51 (18.1)           |    |        |             |
| Genotype comparison    |                     |                     |    |        |             |
| AA vs. TA plus TT      | 7.87                | 0.005               | 1.70 | 0.50–2.51 |
| AA plus TT vs. TC      | 1.05                | 0.321               | 0.84 | 0.59–1.21 |
| AA plus TA vs. TT      | 4.43                | 0.035               | 0.59 | 0.34–0.99 |
| Allele                 |                     |                     |    |        |             |
| A                      | 299 (63.3)          | 306 (54.3)          |    |        |             |
| T                      | 173 (36.7)          | 258 (45.7)          |    |        |             |
| Allele comparison, A vs. T | 8.73          | 0.003               | 1.46 | 1.13–1.89 |

Table 2 Genotype and allele frequencies of the rs2430561 IFNG +874T/A between cardiomyopathic and asymptomatic patients.

| IFNG +874T/Ars2430561 | Cardiac n = 120 (%) | Asymptomatic n = 116 (%) | χ² | P value | OR (95% CI) |
|-----------------------|--------------------|-------------------------|----|--------|-------------|
| Genotype              |                    |                         |    |        |             |
| AA                    | 46 (38.7)          | 44 (37.6)               |    |        |             |
| TA                    | 60 (50.4)          | 59 (50.4)               |    |        |             |
| TT                    | 13 (10.4)          | 14 (12.0)               |    |        |             |
| Genotype comparison   |                    |                         |    |        |             |
| AA vs. TA plus TT     | 0.03               | 0.86                    | 1.05 | 0.60–1.83 |
| AA plus TT vs. TA     | 0.00               | 0.99                    | 1.00 | 0.58–1.72 |
| AA plus TA vs. TT     | 0.06               | 0.80                    | 0.90 | 0.38–2.16 |
| Allele                |                    |                         |    |        |             |
| A                     | 152 (63.9)         | 147 (62.8)              |    |        |             |
| T                     | 86 (36.1)          | 87 (37.2)               |    |        |             |
| Allele comparison, A vs. T | 0.02            | 0.81                    | 1.05 | 0.71–1.55 |
In conclusion, our data suggest that the *IFNG* +874T/A genetic polymorphism may be involved in susceptibility to *T. cruzi* infection in the South American population studied here. However, the association between polymorphisms and disease progression is still unclear. Given the crucial role of IFN-γ in the inflammatory response, further studies on other functional polymorphisms of *IFNG* and the genes coding for the IFN-γ receptors are required to clarify the role of IFN-γ in the pathogenesis of Chagas disease.

**Conflict of interest**

None.

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