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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.

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 defence against a variety of intracellular pathogens, including *T. cruzi* infection (Silva et al., 1992; Torrico et al., 1991). The human *IFNG*

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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 immunosorbent 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′-TCAACAAAGCTGATACTCCA-3′ (consensus primer), 5′-TTCTTACAACACAAAATCAAATCA-3′ (A allele specific), 5′-TTCTTACAACACAAAATCAAATCT-3′ (T allele specific). Amplification yielded a 263-bp PCR product. Primers amplifying human growth hormone (F: 5′-GCCTTCCCAACCATTCCCTTA-3′ and R: 5′-TCACGGATTTCTGTTGTGTTTC-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 heredity 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 χ^2 test. Odds ratios and 95% confidence intervals were calculated according to Woolf's method. The software Statcalc EpiInfo 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

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 $n=282$ (%)	χ^2	*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 (1.50–2.51)
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 $n=116$ (%)	χ^2	*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)

significant difference was observed in the distribution of alleles or genotypes of the *IFNG* +874A/T polymorphism among cardiac and 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., 2002; Zafra et al., 2007). 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 a 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).

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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References

Calzada, J.E., Beraun, Y., Gonzalez, C.I., Martin, J., 2009. Transforming growth factor beta 1 (TGFbeta1) gene polymorphisms and Chagas disease susceptibility in Peruvian and Colombian patients. Cytokine 45, 149–153.

Calzada, J.E., Nieto, A., Beraun, Y., Martin, J., 2001. Chemokine receptor CCR5 polymorphisms and Chagas' disease cardiomyopathy. Tissue Antigens 58, 154–158.

Cardillo, F., Voltarelli, J.C., Reed, S.G., Silva, J.S., 1996. Regulation of *Trypanosoma cruzi* infection in mice by gamma interferon and interleukin 10: role of NK cells. Infect. Immun. 64, 128–134.

Chong, W.P., Ip, W.K., Tso, G.H., Ng, M.W., Wong, W.H., Law, H.K., Yung, R.W., Chow, E.Y., Au, K.L., Chan, E.Y., Lim, W., Peiris, J.S., Lau, Y.L., 2006. The interferon gamma gene polymorphism +874 A/T is associated with severe acute respiratory syndrome. BMC Infect. Dis. 6, 82.

D'Avila, D.A., Guedes, P.M., Castro, A.M., Gontijo, E.D., Chiari, E., Galvao, L.M., 2009. Immunological imbalance between IFN-gamma and IL-10 levels in the sera of patients with the cardiac form of Chagas disease. Mem. Inst. Oswaldo Cruz 104, 100–105.

Excoffier, L., Laval, G., Schneider, S., 2005. Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evol. Bioinform. Online 1, 47–50.

Florez, O., Zafra, G., Morillo, C., Martin, J., Gonzalez, C.I., 2006. Interleukin-1 gene cluster polymorphism in chagas disease in a Colombian case-control study. Hum. Immunol. 67, 741–748.

Gomes, J.A., Bahia-Oliveira, L.M., Rocha, M.O., Martins-Filho, O.A., Gazzinelli, G., Correa-Oliveira, R., 2003. Evidence that development of severe cardiomyopathy in human Chagas' disease is due to a Th1-specific immune response. Infect. Immun. 71, 1185–1193.

Gutierrez, R., Angulo, V.M., Tarazona, Z., Britto, C., Fernandes, O., 2004. Comparison of four serological tests for the diagnosis of Chagas disease in a Colombian endemic area. Parasitology 129, 439–444.

Karplus, T.M., Jeronimo, S.M., Chang, H., Helms, B.K., Burns, T.L., Murray, J.C., Mitchell, A.A., Pugh, E.W., Braz, R.F., Bezerra, F.L., Wilson, M.E., 2002. Association between the tumor necrosis factor locus and the clinical outcome of Leishmania chagasi infection. Infect. Immun. 70, 6919–6925.

Kierszenbaum, F., 1999. Chagas' disease and the autoimmunity hypothesis. Clin. Microbiol. Rev. 12, 210–223.

Kumar, S., Tarleton, R.L., 1998. The relative contribution of antibody production and CD8+ T cell function to immune control of *Trypanosoma cruzi*. Parasite Immunol. 20, 207–216.

Lopez-Maderuelo, D., Arnalich, F., Serantes, R., Gonzalez, A., Codoceo, R., Madero, R., Vazquez, J.J., Montiel, C., 2003. Interferon-gamma and interleukin-10 gene polymorphisms in pulmonary tuberculosis. Am. J. Respir. Crit. Care Med. 167, 970–975.

Martin, D.L., Postan, M., Lucas, P., Gress, R., Tarleton, R.L., 2007. TGF-beta regulates pathology but not tissue CD8+ T cell dysfunction during experimental *Trypanosoma cruzi* infection. Eur. J. Immunol. 37, 2764–2771.

Miller 3rd, C.A., Martinat, M.A., Hyman, L.E., 1998. Assessment of aryl hydrocarbon receptor complex interactions using pBEVY plasmids: expressionvectors with bi-directional promoters for use in Saccharomyces cerevisiae. Nucleic Acids Res. 26, 3577–3583.

Nieto, A., Beraun, Y., Collado, M.D., Caballero, A., Alonso, A., Gonzalez, A., Martin, J., 2000. HLA haplotypes are associated with differential susceptibility to *Trypanosoma cruzi* infection. Tissue Antigens 55, 195–198.

Pacheco, A.G., Cardoso, C.C., Moraes, M.O., 2008. IFNG +874T/A, IL10-1082G/A and TNF −308G/A polymorphisms in association with tuberculosis susceptibility: a meta-analysis study. Hum. Genet. 123, 477–484.

Prata, A., 2001. Clinical and epidemiological aspects of Chagas disease. Lancet Infect. Dis. 1, 92–100.

Pravica, V., Asderakis, A., Perrey, C., Hajeer, A., Sinnott, P.J., Hutchinson, I.V., 1999. In vitro production of IFN-gamma correlates with CA repeat polymorphism in the human IFN-gamma gene. Eur. J. Immunogenet. 26, 1–3.

Pravica, V., Perrey, C., Stevens, A., Lee, J.H., Hutchinson, I.V., 2000. A single nucleotide polymorphism in the first intron of the human IFN-gamma gene: absolute correlation with a polymorphic CA microsatellite marker of high IFN-gamma production. Hum. Immunol. 61, 863–866.

Ramasawmy, R., Cunha-Neto, E., Fae, K.C., Martello, F.G., Muller, N.G., Cavalcanti, V.L., Ianni, B., Mady, C., Kalil, J., Goldberg, A.C., 2006. The monocyte chemoattractant protein-1 gene polymorphism is associated with cardiomyopathy in human chagas disease. Clin. Infect. Dis. 43, 305–311.

Ribeiro, C.S., Visentainer, J.E., Moliterno, R.A., 2007. Association of cytokine genetic polymorphism with hepatitis B infection evolution in adult patients. Mem. Inst. Oswaldo Cruz 102, 435–440.

Rocha, M.O., Ribeiro, A.L., Teixeira, M.M., 2003. Clinical management of chronic Chagas cardiomyopathy. Front. Biosci. 8, e44–54.

Rodriguez-Perez, J.M., Cruz-Robles, D., Hernandez-Pacheco, G., Perez-Hernandez, N., Murguia, L.E., Granados, J., Reyes, P.A., Vargas-Alarcon, G., 2005. Tumor necrosis factor-alpha promoter polymorphism in Mexican patients with Chagas' disease. Immunol. Lett. 98, 97–102.

Silva, J.S., Morrissey, P.J., Grabstein, K.H., Mohler, K.M., Anderson, D., Reed, S.G., 1992. Interleukin 10 and interferon gamma regulation of experimental *Trypanosoma cruzi* infection. J. Exp. Med. 175, 169–174.

Silva, J.S., Vespa, G.N., Cardoso, M.A., Aliberti, J.C., Cunha, F.Q., 1995. Tumor necrosis factor alpha mediates resistance to *Trypanosoma cruzi* infection in mice by inducing nitric oxide production in infected gamma interferon-activated macrophages. Infect. Immun. 63, 4862–4867.

Torres, O.A., Calzada, J.E., Beraun, Y., Morillo, C.A., Gonzalez, C.I., Gonzalez, A., Martin, J., 2009. Association of the macrophage migration inhibitory factor −173G/C polymorphism with Chagas disease. Hum. Immunol. 70, 543–546.

Torrico, F., Heremans, H., Rivera, M.T., Van Marck, E., Billiau, A., Carlier, Y., 1991. Endogenous IFN-gamma is required for resistance to acute *Trypanosoma cruzi* infection in mice. J. Immunol. 146, 3626–3632.

WHO, 1991. Control of Chagas disease. Report of a WHO Expert Committee. World Health Organ Tech Rep Ser. 811, 1–95.

WHO, 2002a. Control of Chagas disease. World Health Organ Tech Rep Ser. 905, i–vi, 1–109, back cover.

WHO, 2002b. WHO expert committee on specifications for pharmaceutical preparations. World Health Organ Tech Rep Ser. 902, i–vii, 1–208.

Zafra, G., Morillo, C., Martin, J., Gonzalez, A., Gonzalez, C.I., 2007. Polymorphism in the 3′ UTR of the IL12B gene is associated with Chagas' disease cardiomyopathy. Microbes Infect. 9, 1049–1052.