Functional IL-10 Gene Polymorphism Is Associated with Chagas Disease Cardiomyopathy

Germaino C. Costa,1 Manoel Otávio da Costa Rocha,2 Paula Rocha Moreira,1 Cristiane Alves Silva Menezes,1 Micena R. Silva,1 Kenneth J. Gollob,3 and Walderез O. Dutra1

1Laboratory of Cell-Cell Interactions, Department of Morphology, 2Laboratory of Lymphocyte Biology, Department of Biochemistry and Immunology, and 3Program of Tropical Medicine, School of Medicine, Federal University of Minas Gerais, Belo Horizonte, Brazil

This study was designed to determine whether the functional IL-10 gene polymorphism $-1082G/A$ is associated with the development of cardiomyopathy in individuals infected with Trypanosoma cruzi and whether interleukin (IL)–10 expression can be correlated with patients’ cardiac function. Our results demonstrated that the polymorphic allele, which correlates with lower expression of IL-10, was associated with the development of Chagas disease cardiomyopathy. Accordingly, correlative analysis showed that low IL-10 expression was associated with worse cardiac function, as determined by left-ventricular ejection fraction values. Therefore, the IL-10 gene polymorphism and IL-10 expression are important in determining susceptibility to chagasic cardiomyopathy.

Chagas disease is an infection caused by the protozoan Trypanosoma cruzi that affects approximately 16–18 million people in Latin America, with an estimated 120 million people at risk of infection. During the chronic phase of the disease, 70% of infected individuals remain asymptomatic for years, or even throughout life, and are clinically classified as indeterminate [1]. Between 20% and 30% of the chronic patients develop cardiac disease, the most severe and main cause of disability and mortality in patients with Chagas disease [2]. The search for risk factors associated with the development of cardiomyopathy is critical because it could reduce the morbidity and mortality associated with Chagas disease by allowing for the administration of early treatment.

It is currently accepted that the host’s immune response is essential in determining the course of infection. Cytotoxic cells and inflammatory cytokines, such as interferon (IFN)–γ and tumor necrosis factor (TNF)–α, while essential for protection during the acute phase of the disease, have been correlated with tissue damage and the severity of chronic disease [3–5]. Thus, controlling the inflammatory response seems to be important to avoid establishment of pathology. We have shown that high expression of interleukin (IL)–10 by monocytes was observed in patients with the indeterminate form, but not in patients with cardiac disease, suggesting a lack of immunological control in patients with the cardiac clinical form [5].

IL-10 is capable of down-modulating the immune response by indirect or direct affects on T cells. The IL-10 gene promoter region is highly polymorphic, and a correlation between the polymorphic allele A of the $-1082G/A$ polymorphism with low IL-10 production has been described [6]. Recently, a correlation between BAT, a putative anti-inflammatory molecule gene polymorphism, and chagasic cardiomyopathy was observed [7].

We hypothesize that low IL-10 expression is associated with the development of cardiomyopathy in chronic chagasic patients. Thus, this study was designed to investigate whether the functional IL-10 gene polymorphism $-1082G/A$ is a predisposing factor for the development of cardiomyopathy in individuals infected with T. cruzi and whether IL-10 expression can be correlated with cardiac function. The identification of genetic markers related to susceptibility and/or resistance is critical for identifying patients with greater potential to progress toward severe forms of disease and, therefore, to enable possible interventions to prevent disease development or improve treatment choices.

**Materials and methods.** This study employed a cross-sectional design that involved patients from Chagas-endemic areas in Minas Gerais, Brazil (under the medical care of M.O.C.R.). One hundred fifty-five patient volunteers with specific serological results positive for T. cruzi who were in the chronic phase of the disease were enrolled in this study. Detailed evaluations, including physical examinations, an electrocardiogram, chest radiographs, and an echocardiogram were performed to classify patients into different clinical groups, as we have described else-

© 2008 by the Infectious Diseases Society of America. All rights reserved.
0952-1899/2009/19903-0023$15.00
DOI: 10.1086/598061

© 2008 by the Infectious Diseases Society of America. All rights reserved.
from all individuals, and the study was approved by the ethical
dysfunction parameter. Informed written consent was obtained
left-ventricular ejection fraction (LVEF) was used as a systolic
characteristics of the clinical groups as well as the genotype and allele
rheumatic or autoimmune diseases. The demographic charac-
atives), as we described elsewhere [10]. Cultures used 2
were obtained by Ficoll-Hypaque gradient (Amersham Biosci-
ulation with parasite antigens. PBMCs from chagasic patients
by silver-stained 10% acrylamide gel.
Approximately 20 mL of blood was collected from each of the
chagasic patients to evaluate expression levels of IL-10 by
by PCR amplification, followed by digestion with re-
assessed by PCR amplification, followed by digestion with re-
swab procedure performed with a sterile plastic spatula. DNA
extraction was performed by the silica method, as we described
swab procedure performed with a sterile plastic spatula. DNA
extraction was performed by the silica method, as we described
where [2]. These clinical groups were as follows: indeterminate pa-
tients (Ind; n = 58), who were asymptomatic and presented no
branch blockage and different degrees of conductive functional
alterations but no heart dilation; and dilated cardiac patients
(©); n = 53), who presented severe cardiomyopathy with heart
enlargement. We also included in our analysis individuals with-
Chagas disease (N; n = 43), as determined by specific neg-
serological test results. The exclusion criteria were as fol-
presence of diabetes mellitus, thyroid dysfunction, renal
sufficiency, chronic obstructive pulmonary disease, and/or rheumatic or autoimmune diseases. The demographic charac-
istics of the clinical groups as well as the genotype and allele
frequency of the IL-10 −1082G/A polymorphism are summa-
Table 1. Demographic characteristics and genotype and allele frequency of the IL-10 −1082G/A polymorphism in Brazilian study subjects.

<table>
<thead>
<tr>
<th>Clinical group</th>
<th>Indeterminate (n = 58)</th>
<th>Nondilated cardiac (n = 44)</th>
<th>Dilated cardiac (n = 53)</th>
<th>Uninfected (n = 43)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographic characteristic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>27 (46.5)</td>
<td>16 (36.4)</td>
<td>32 (60.4)</td>
<td>18 (41.9)</td>
</tr>
<tr>
<td>Female</td>
<td>31 (53.5)</td>
<td>28 (63.6)</td>
<td>21 (39.6)</td>
<td>25 (58.1)</td>
</tr>
<tr>
<td>Age, years</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>27–73</td>
<td>31–67</td>
<td>21–73</td>
<td>20–77</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>44.4 ± 9.8</td>
<td>45.5 ± 9.6</td>
<td>50.2 ± 11.3</td>
<td>31.4 ± 11.82</td>
</tr>
<tr>
<td>Genotypeb</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG (%)</td>
<td>8 (14)</td>
<td>1 (2)</td>
<td>5 (9)</td>
<td>7 (16)</td>
</tr>
<tr>
<td>GA (%)</td>
<td>35 (60)</td>
<td>18 (41)</td>
<td>28 (53)</td>
<td>20 (46)</td>
</tr>
<tr>
<td>AA (%)</td>
<td>15 (26)</td>
<td>25 (57)</td>
<td>20 (38)</td>
<td>16 (38)</td>
</tr>
<tr>
<td>G+</td>
<td>43 (74)</td>
<td>19 (43)</td>
<td>33 (62)</td>
<td>27 (62)</td>
</tr>
<tr>
<td>G−</td>
<td>15 (26)</td>
<td>25 (57)</td>
<td>20 (38)</td>
<td>16 (38)</td>
</tr>
<tr>
<td>Allelec</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>51 (44)</td>
<td>20 (23)</td>
<td>38 (36)</td>
<td>34 (40)</td>
</tr>
<tr>
<td>A</td>
<td>65 (56)</td>
<td>68 (77)</td>
<td>68 (64)</td>
<td>52 (60)</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. (%) of subjects, unless otherwise indicated. χ^2_2_2_2_2_2_2 contingency table (comparison among different clinical groups, degrees of freedom; df = 2) and 4 × 3 contingency table (comparison among nonchagasic and different clinical groups; df = 3). CI, confidence interval; OR, odds ratio.

Ind × DC × C: χ^2 = 5.6, P = .018; OR, 1.58 (CI, 0.16–0.85). C, nondilated cardiac; DC, dilated cardiac; Ind, indeterminate; N, uninfected.

Ind × DC × C: χ^2 = 12.35, P = .002; G^+: Ind × C: χ^2 = 12.35, P = .002; G^−: Ind × C: P = .001, OR, 1.6 (95% CI, 1.63–8.71).

Individuals with the G allele express higher levels of interleukin (IL)–10 than those with the A allele. Ind × DC × C: χ^2 = 10.25, P = .006; Ind × C: P = .001; OR, 0.84 (95% CI, 1.44–4.95).

Committee of Universidade Federal de Minas Gerais (COEP-UFMG–ETIC006/05).

Cells from 198 individuals were obtained by use of an oral swab procedure performed with a sterile plastic spatula. DNA extraction was performed by the silica method, as we described previously [8]. The IL-10 −1082G/A polymorphism was assessed by PCR amplification, followed by digestion with restriction enzyme. PCR primers sequences were 5’CCAAGACAAACACTACTAAGGGCTCCTTT3’ and 5’GCTTCTTATATGCTAGTCAGGTA3’, with an expected PCR product size of 377 bp, as described elsewhere [9]. Products were digested with 5 units of restriction enzyme EcoNI (New England Biolabs) at 37°C for 4 h. Digestion products of 280 + 97 bp and 253 + 97 + 27 bp were obtained for A and G alleles, visualized by silver-stained 10% acrylamide gel.

Approximately 20 mL of blood was collected from each of the 10 chagasic patients to evaluate expression levels of IL-10 by peripheral blood mononuclear cells (PBMCs) after in vitro stimu-

**Table 1.** Demographic characteristics and genotype and allele frequency of the IL-10 −1082G/A polymorphism in Brazilian study subjects.
in the Ind group (with lower expression of IL-10) was higher in the C group than our data by grouping the patients as Gmozygous and heterozygous genotypes [11], we also analyzed 1). Because differences in IL-10 levels were associated with ho-
group (AA, with lower expres-
with each of the other 2 symptomatic forms (the DC and C
type distribution between patients in the Ind group and patients
ality and death in human Chagas disease, with prognosis de-
ted by ascertaining the severity of heart lesions, which are
Correlation analysis was performed for IL-10 expression intensity and the left-ventricular ejection fraction (LVEF), as a systolic function marker, in patients with Chagas disease. LVEF values were taken by echocardiographic exam. The intensity of IL-10 expression by peripheral blood mononuclear cells stimulated with trypomastigote-soluble antigen was analyzed by flow cytometry, as described in the Materials and Methods section. A positive correlation between higher intensity of IL-10 expression and improved cardiac function as indicated by higher LVEF values was observed. The P value was calculated by using Spearman’s rank correlation test. P < .05 was considered statistically significant.

Results. Table 1 shows age, sex, and clinical group, as well as genotype and allele distribution for all individuals analyzed in this study. An association between the occurrence of dilated cardiomyopathy and male sex was observed (comparison of C × DC: \( \chi^2 = 5.6; P = .018 \); OR, 1.58; [95% CI, 1.16–2.10]). IL-10 polymorphism analysis showed that genotype distribution was nonrandom among the 3 clinical groups evaluated in this study (\( \chi^2 = 12.38; P = .015 \) (table 1). Comparison of geno-
type distribution between patients in the Ind group and patients with each of the other 2 symptomatic forms (the DC and C groups) showed association between C × Ind (\( \chi^2 = 12.35; P < .01 \)). The frequency of the polymorphic allele A (associated with lower expression of IL-10) was higher in the C group than in the Ind group (\( P < .01; OR, 0.84 \) [95% CI, 1.14–4.95]) (table 1). Because differences in IL-10 levels were associated with homozygous and heterozygous genotypes [11], we also analyzed our data by grouping the patients as G⁺ (GG or GA genotypes, with higher expression of IL-10) and G⁻ (AA, with lower expres-
sion of IL-10). These analyses showed an association between the occurrence of the G⁻ genotype and the C group, when compared with the Ind group (\( P < .01; OR, 1.6 \) [95% CI, 1.63–8.71]). Comparisons between the Ind and DC groups or the C and DC groups did not show association with any genotype or allele frequency.

Given the observed association between IL-10 polymorphism and the cardiac form of Chagas disease, we assessed the mean fluorescence intensity of IL-10 produced by TRP-stimulated PBMCs. We then evaluated the correlation between IL-10 intensity and the LVEF as an assessment of cardiac function, as described above. We observed a correlation between low levels of IL-10 expression and low LVEF values (figure 1). Conversely, higher levels of IL-10 were correlated with high LVEF values, which were related to better prognosis for Chagas disease.

Discussion. Chronic myocarditis is the main cause of disability and death in human Chagas disease, with prognosis determined by ascertaining the severity of heart lesions, which are correlated with the myocardial inflammatory process [1, 2]. The goal of this study was to evaluate the influence of host genetic factors, specifically of the −1082G/A IL-10 polymorphism, on the development of chagasic cardiomyopathy. Our results demonstrated an association between the low-expressing A allele of the IL-10 gene and the cardiac form of Chagas disease. Additionally, we showed that the lower the expression of IL-10, the worse the heart function of chagasic patients, as measured by LVEF. Moreover, an association between male sex and dilated instead of nondilated cardiomyopathy was observed (table 1). A Brazilian study that evaluated prognostic factors for mortality in human Chagas disease also showed male sex as a risk factor for worse disease outcome [12].

The IL-10 −1082G/A polymorphism has also been associated with the outcome of autoimmune and/or inflammatory diseases [9, 11]. Our data showed that genotypic and allelic frequencies for this locus are in accordance with those observed in another study performed previously among the Brazilian population [13]. However, the G allele, as well as the GG genotype, were more frequent in white populations [9] than in Brazilians. In the present study, we did not group Brazilians into ethnic groups on
the basis of skin colors and other phenotypic characteristics because another study [14] clearly demonstrated that in highly miscegenated populations, such as Brazilians, this type of stratification does not correlate with genotype.

Comparison between uninfected individuals and chagasic patients with respect to the genotype and allele frequency for the IL-10 polymorphism did not show any differences. However, since the uninfected individuals were never exposed to the parasite, the best measure of the polymorphisms’ association with Chagas disease pathology is the comparison between asymptomatic individuals (the Ind group) and those who developed cardiac disease after T. cruzi infection. The single-nucleotide polymorphism at the −1082 promoter region of the IL-10 gene follows a nonrandom distribution among Chagas disease patients. A difference was found between the C and Ind groups with respect to the occurrence of the G− genotype and the A allele, putative low producers of IL-10 (table 1). No difference was found between the DC and Ind groups. On the basis of this evidence, we suggest that the pathogenesis of the nondilated and dilated cardiac forms may involve distinct mechanisms, although additional studies should be undertaken to test this hypothesis. Others have also shown associations between gene polymorphisms and susceptibility to cardiomyopathy in Chagas disease [7, 15, 16]. These findings suggest that the human genetic background is a critical factor that could influence susceptibility and the outcome of cardiomyopathy in human Chagas disease.

Phenotypic IL-10 expression levels were correlated with LVEF values, a clinical criterion that measures heart function. Patients selected for this analysis displayed a G+ genotype for IL-10, with a potential for high production of this cytokine. We showed that the higher the expression of IL-10, the better the heart function as evaluated by LVEF levels, suggesting a protective role for IL-10 in Chagas disease pathology. In conclusion, IL-10 is associated with cardiac function, and the IL-10 gene polymorphism predisposes individuals infected with T. cruzi to cardiac disease.

References


