Influence of CTLA-4 gene polymorphism in autoimmune and infectious diseases

Mercedes Fernández-Mestre a, Karen Sánchez a, Omar Balbás a, Ketevan Gendzekhadze a,b, Violeta Ogando a, Mayra Cabrera c, Zulay Layrisse a,∗

a Laboratorio de Fisiopatología, Centro de Medicina Experimental “Miguel Layrisse,” Instituto Venezolano de Investigaciones Científicas, Caracas, Venezuela
b Department of Structural Biology, Stanford University School of Medicine, Sherman Fairchild Building, Stanford, CA, USA

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ABSTRACT

Cell-mediated immunity requires costimulatory activity to initiate or inhibit antigen-specific T-cell responses. CTLA-4 is an inhibitory receptor expressed by activated and regulatory T cells. The single nucleotide polymorphism (SNP) +49 A/G of the CTLA-4 gene alters intracellular distribution of CTLA-4, interleukin-2 production, and, as a consequence, T-cell proliferation. The aim of this study was to analyze the only coding SNP CTLA-4 +49 A/G polymorphism in patients with either infectious (Chagas’s, Dengue, and American cutaneous leishmaniasis) or autoimmune diseases (myasthenia gravis, pemphigus, and psoriasis). No statistically significant differences were reported when all patients of each disease group were compared with healthy individuals. However, the +49 G/G genotype was moderately increased in pemphigus and myasthenia gravis. Patients with diffuse cutaneous leishmaniasis (DCL) exhibited an increased frequency of the A/G +49 genotype compared with patients with localized cutaneous leishmaniasis (LCL; p = 0.009; odds ratio [OR] = 4.25; 95% confidence interval [CI] = 1.245–14.501) and intermediate cutaneous leishmaniasis (ICL; p = 0.027; OR = 4.44; 95% CI = 1.273–15.516), indicating that the heterozygous genotype, associated with overactivation of T-cell proliferation, could confer susceptibility to the development of the more severe clinical form of cutaneous leishmaniasis. The A/A +49 genotype was increased in LCL patients compared with DCL patients (p = 0.019; OR = 0.25; 95% CI = 0.067–0.953), indicating that this genotype, which has been associated with normal proliferation of T cells, could confer protection to the development of DCL. The results indicate that the polymorphism of CTLA-4 is an important genetic factor associated with risk or protection for the development of diffuse cutaneous leishmaniasis and has influence in the pathogenesis of autoimmune diseases. However, other closely linked candidate genes in linkage disequilibrium with CTLA4, such as CD28 and ICOS, could be associated with the development of autoimmune and infectious disease.

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1. Introduction

CTLA-4 plays an essential role in immunologic homeostasis as a negative regulator of T-cell activation; however, little is known about the regulatory mechanism for CTLA-4 expression. This inhibitory signal not only determines whether T cells become activated, but also affects the clonal representation in an immune response [1]. In addition, CTLA-4 is highly expressed by T regulatory cells and could play an important role in their function [2]. Polymorphisms at certain locations of the CTLA-4 might conceivably affect its expression [1]. About 16 single nucleotide polymorphisms (SNP) and 1 microsatellite were reported at the CTLA-4 locus [3]; however, only 3 of them (SNP +49, –318, and the microsatellite) have been widely studied. The SNP +49 A/G is located at position +49 in the first exon of the CTLA-4 gene, leading to a threonine to alanine change in amino acid position 17 of the leader peptide. Recently, it has been reported that the +49 A/G polymorphism of the CTLA-4 gene alters intracellular distribution of CTLA-4 and interleukin (IL)-2 production, and, as a result, alters T-cell proliferation [4,5]. In humans, several reports have established an association between specific polymorphisms of the CTLA-4 gene and autoimmune diseases. The potential explanation is that the CTLA-4:B7 interaction plays a critical role in regulating self-tolerance and hence susceptibility to autoimmune disease [6]. Likewise, in infectious diseases where T cells are fundamental components of the mechanisms of defense of the host versus pathogens, the polymorphism of the CTLA-4 gene could play a role in the regulation of the immune response. We selected just one SNP for study because a direct correlation between the +49 A/G polymorphism of the CTLA4 gene and T-cell function, as well as that between the CTLA-4 genotype and the function of the CTLA-4 protein in T cells, has been reported. Therefore, CTLA-4 exerted less profound inhibitory effects on T-cell proliferation in patients bearing the G/G genotype than in patients bearing the A/A genotype. A CTLA-4 gene polymorphism in the leader sequence (exon 1) may influence the level or the pattern of
expression of the protein. Alternatively, the trafficking properties of CTLA-4 may be altered, such that the total level of CTLA-4 may be similar but the CTLA-4 levels responsible for the negative signaling capacity of the molecule may be reduced. Differences in the leader sequence of the gene may result in altered rates of endocytosis or surface trafficking [4].

The aim of this study was to analyze the +49 A/G polymorphism of the CTLA-4 gene in Venezuelan patients with organ-specific autoimmune diseases (myasthenia gravis [MG], pemphigus [PE], and psoriasis [PS]) and patients with infectious diseases (Chagas’s [CH], dengue [D], and American cutaneous leishmaniasis [L]).

2. Subjects and methods

2.1. Patient and control populations

Whole blood was collected from 405 patients grouped in two series: patients with either infectious or autoimmune diseases. Patients with autoimmune diseases included 150 individuals with three organ-specific autoimmune diseases: MG, PE, and PV. The numbers of patients with infectious diseases were as follows: 77 with D, 105 with CH, and 73 with L.

2.2. CTLA-4 SNP +49 typing

Genomic DNA was extracted from blood samples using a modified salting-out procedure [10]. Polymerase chain reaction-based restriction fragment length polymorphism (PCR-RFLP) was used to establish the CTLA-4 genotypes as previously described [4,11,12], with some modifications. Briefly, two pairs of primers were used to amplify the appropriate segment of the CTLA-4 gene containing SNP +49. The PCR product was incubated with BbvI (Gibco, Rockville, MD) for 16 hours at 37°C and analyzed on 4% agarose gels.

3. Results

3.1. The SNP +49 (A/G) of the CTLA-4 gene in two series of patients and healthy individuals

The distribution of CTLA-4 genotype frequencies in control subjects and patients with infectious and autoimmune diseases was in Hardy–Weinberg equilibrium (Table 1). No statistically significant differences were reported when the control group was compared with all patients in each series. The A/G +49 genotype was the most common genotype in every group (healthy controls and patient groups). However, the frequency of the +49A allele was increased among patients with infectious diseases (D + CH + L) compared with patients with autoimmune diseases (MG + PE). Although PS is considered an autoimmune disease, the CTLA-4 genotype frequencies in these patients was not very different from the frequencies observed in patients with infectious diseases.

3.2. The SNP +49 (A/G) of the CTLA-4 gene in patients with infectious or autoimmune diseases grouped clinically

The distribution of CTLA-4 genotype frequencies in the different groups of patients with L exhibited an increased frequency of the A/G +49 genotype in patients with DCL compared with patients with LCL (71.42% vs. 37.03%; p = 0.009; OR = 4.25; 95% CI = 1.245–14.501) and ICL (71.42% vs. 36%; p = 0.027; OR = 4.44; 95% CI = 1.273–15.156). The A/A +49 genotype frequency was increased in patients with LCL compared with patients with DCL (48.14% vs. 19.04%; p = 0.019; OR = 0.25; 95% CI = 0.067–0.953) (Table 2). No statistically significant differences existed between patients with NF and patients with DHF. However, the frequency of the A/A +49 genotype associated with normal expression levels of CTLA-4 is moderately decreased in DHF patients compared with DF patients (28.57% vs. 40.9%); The distribution of CTLA-4 genotype frequencies in the different groups of serologically positive for Trypanosoma cruzi patients, (asymptomatic, arrhythmic, and cardiomyopathic) demonstrated no association between the CTLA-4 (SNP +49) polymorphism and different clinical forms of CH.

Finally, both groups of patients with PE exhibited a similar frequency of the A/G +49 genotype; however, the frequency of the A/A +49 genotype was increased in patients with PF compared with patients with PV (36.36% vs. 20.51%; OR = 2.21; 95% CI =

Table 1

<table>
<thead>
<tr>
<th>Genotype/allele</th>
<th>MG (n = 37)</th>
<th>PE (n = 48)</th>
<th>PS (n = 54)</th>
<th>D (n = 77)</th>
<th>CH (n = 105)</th>
<th>L (n = 69)</th>
<th>Control (n = 98)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>29.7 (11)</td>
<td>22.9 (11)</td>
<td>38.9 (21)</td>
<td>35.06 (27)</td>
<td>37.14 (39)</td>
<td>37.68 (26)</td>
<td>32.65 (32)</td>
</tr>
<tr>
<td>AG</td>
<td>46.0 (17)</td>
<td>43.7 (21)</td>
<td>48.1 (26)</td>
<td>45.45 (35)</td>
<td>47.61 (50)</td>
<td>47.61 (50)</td>
<td>47.95 (47)</td>
</tr>
<tr>
<td>GG</td>
<td>24.3 (9)</td>
<td>33.3 (16)</td>
<td>13.0 (7)</td>
<td>19.48 (15)</td>
<td>15.24 (16)</td>
<td>17.39 (12)</td>
<td>19.38 (19)</td>
</tr>
<tr>
<td>A</td>
<td>52.7 (39)</td>
<td>44.8 (43)</td>
<td>63.0 (68)</td>
<td>57.79 (89)</td>
<td>60.95 (128)</td>
<td>66.14 (83)</td>
<td>56.63 (111)</td>
</tr>
<tr>
<td>G</td>
<td>47.3 (35)</td>
<td>55.2 (53)</td>
<td>37.0 (40)</td>
<td>42.21 (65)</td>
<td>39.05 (82)</td>
<td>39.85 (53)</td>
<td>43.37 (85)</td>
</tr>
</tbody>
</table>

Entries are percentages and numbers (in parentheses) of individuals or chromosomes. Organ-specific autoimmune diseases: MG—myasthenia gravis; PE—pemphigus; PS—psoriasis. Infectious diseases: D—Dengue; CH—Chagas’s disease; L—leishmaniasis; CG—control group (healthy individuals).

2.3. Statistical analysis

Allele, genotype, and haplotype frequencies were determined by direct counting. The statistical significance of allele frequency differences between patients and controls was estimated using the Mantel–Haenszel $\chi^2$ test (because of the small sample size) using 2 × 2 contingency tables; p values were corrected by multiplying the number of comparisons made (Bonferroni correction) and considered significant when p < 0.05 [13]. Relative risks with corresponding 95% confidence intervals (95% CI) were calculated as odds ratios (OR) according to Woolf’s formula [14] or by the modified method described by Haldane [15] when one element of the equation was zero.
Our study of the (A/G) polymorphism of the CTLA-4 gene in two series of patients, those with autoimmune and infectious diseases, indicated no significant genotype frequency difference when all patients in each group were compared with healthy individuals. However the +49 G/G genotype was moderately increased in patients with PE and MG. This genotype is associated with a reduced control of T-cell activation and in this way probably contributes to the pathogenesis in autoimmune diseases. Accumulating evidence verifies the importance of CTLA-4 in the pathogenesis of MG. Anti-CTLA-4 antibody treatment triggers determinant spreading and enhances murine MG [17]. Several studies have confirmed that CTLA-4 polymorphism is associated with MG [18-20]. Recently, Pavoni et al. studied the influence of CTLA4 –318 and +49 polymorphisms in the pathogenesis of PE; however, no statistically significant association between PF and variants of the CTLA4 gene was observed [21], similar to our results. On the other hand, in our study the frequency of the G/G +49 genotype, associated with a reduced control of T-cell activation and in this way probably contributing to the pathogenesis, was increased in PV patients compared with healthy individuals. The potential explanation for the association between specific genetic polymorphisms of the CTLA-4 gene and autoimmune diseases is that such genetic polymorphism may affect the expression or function of the CTLA receptor, thus resulting in autoreactivity [22].

Different mechanisms of action of CTLA-4 have been reported. One is delivery of a negative signal to T cells, with rapid inhibition of T-cell activation, and another is B7 (CD80, CD86) sequestration on antigen-presenting cells and induction of T-cell anergy [23]. A role for CTLA-4 in autoimmune disease is suggested by the observation that blockade of the B7-CTLA-4 interaction via administration of antibodies against CTLA-4 exacerbates autoimmune diseases in animal models, whereas stimulation via CTLA-4Ig ameliorates experimental autoimmune MG [reviewed in 20]; indeed, an exogenous recombinant CTLA-4Ig molecule infused to the patients ameliorated symptoms in several autoimmune diseases including PS [24,25]. On the other hand, sCTLA-4 may similarly inhibit the interaction of the cell membrane-anchored CTLA-4 molecule with CD80 and CD86, thus blocking the inhibition of T-cell activation [20]. In light of the findings mentioned above, it has been hypothesized that elevated sCTLA-4 concentration in PS results in its successful competition with activated T-cell membrane-anchored CTLA-4 for interaction with CD80/CD86 ligands, weakening the control over T-cell response and resulting in the aggravation of disease symptoms [26]. Although in our study CTLA-4 exon 1 polymorphism was not associated with susceptibility to PS, other variants such as T/C-1772, associated with levels of sCTLA-4 and SNP genotypes [16], could play an important role in this autoimmune disease.

Our data indicate that the +49A allele increases the risk of development of viral and parasitic diseases (D, CH, L), but confers resistance to autoimmune diseases (MG, PE). In contrast, the +49 G allele might be a risk factor for autoimmune disease (PE). Different mechanisms are postulated to explain the protective effect of infections on autoimmune diseases, such as competition, regulation, and stimulation of innate immunity [28]. The occurrence of overdominant selection in the population (a heterozygote advantage), in which homozygotes for a specific allele are resistant to one disease but susceptible to another, could explain genetically the inverse relationship between infectious and autoimmune diseases [29]. There could be opposing effects of the two homozygotes: while one of the three genotypes confers susceptibility to one type of disease (infectious disease), the other confers susceptibility to a different disease (autoimmunity).

The analysis among the different clinical groups of patients infected with Leishmania demonstrated an increased frequency of the A/G +49 genotype in patients with DCL compared with patients with LCL and ICL, indicating that the heterozygous genotype, associated...
associated with overactivation of T-cell proliferation, could confer susceptibility to the development of the more severe clinical form of cutaneous L. The A/A +49 genotype was increased in LCL patients compared with DCL patients, indicating that this genotype, which is associated with the normal proliferation of T cells, could confer protection to the development of DCL. Recently, Favalí et al. demonstrated that CD28:B7 is important in IFN-γ and TNF-α production by peripheral blood mononuclear cells from cutaneous L patients [30]. These cytokines are essential to control parasite multiplication because they influence macrophage activation, and the blockade of the CD28:B7 pathway leads to downmodulation of both proinflammatory cytokines. The three active forms of American tegumentary L are characterized by cytokine patterns in lesions: LCL by a Th1 response, DCL by a Th2 response, and ICL by a mixed pattern of cytokines [31]. CTLA-4 engagement during differentiation inhibits polarization of naïve CD4+ cells to the Th2 but not the Th1 cell subset. Once polarized, CTLA-4 engagement inhibits cytokine production in both Th2 and Th1 effector cells. All together, these data indicate that CTLA-4 may interfere not only with the signaling involved in transcriptional activation of both Th1 and Th2 cells, but also with the development of one of the Th cell subsets [32]. CTLA-4 is a critical and potent inhibitor of Th2 differentiation. Thus, the B7-CD28/CTLA-4 pathway plays a critical role in regulation of Th2 differentiation in two ways: CD28 promotes Th2 differentiation, whereas CTLA-4 limits Th2 differentiation. Hence, reductions in CTLA-4 expression (GG or A/G +49 genotype) promote a Th2 response that favors the development of DCL. Although DCL may be Th2 mediated and less effective, CTLA-4 could play a role (+49 G). MG is also antibody mediated, yet there is no association with +49 G, possibly because only a subgroup of MG/thymoma patients has been associated with CTLA-4 gene polymorphism. Our group included patients with thymic hyperplasia and patients without thymoma or hyperplasia; only 4/46 patients had thymoma.

The results indicate that the polymorphism of CTLA-4 is an important genetic factor associated with the risk of or protection for development of diffuse cutaneous L, which is infrequent in Venezuela, with only 20 cases reported up to 1995 [33]. However, other closely linked candidate genes in linkage disequilibrium with CTLA4, such as CD28 and ICOS, could be associated with the development of autoimmune and infectious disease.

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