HLA haplotypes are associated with differential susceptibility to *Trypanosoma cruzi* infection

**Abstract:** We explored a possible role of HLA class II genes in determining the susceptibility to *Trypanosoma cruzi* infection as well as in the development of chagasic heart disease in a rural mestizo population from Arequipa (Southern Peru). HLA-DRB1 and DQB1 polymorphisms were determined in 85 seropositive (asymptomatic, *n*=52; cardiomyopathic, *n*=33) and 87 seronegative individuals. We observed that the DRB1*14-DQB1*0301 haplotype correlates with not having *T. cruzi* infection in a highly endemic area (OR = 0.26 (0.12–0.63); *P*=0.01). This protective association is a dominant trait. We found no differences in the allelic or haplotypic distributions we examined between asymptomatic and cardiomyopathic patients in this population. Our data offer indirect but compelling evidence that polymorphism in HLA region is involved in a differential susceptibility to *T. cruzi* chronic infection.

*Trypanosoma cruzi* is the causal agent of Chagas' disease infecting 16–18 million people in Latin America. Infection occurs primarily in those living under poor housing conditions in rural settlements although rural-urban migration has led to an urbanization of the originally rural disease (1). Primoinfection occurs mainly during childhood and is generally accepted that the acute phase is followed by an state of latent chronic infection with usually no evidence, other than positive serology, of exposure; spontaneous cure with negative serology has been observed, but only on rare occasions (2). Years or even decades after *T. cruzi* infection is acquired a proportion of patients suffer chronic heart disease possibly as a result of autoimmune attack (3).

It is worth noting that uninfected individuals are found in all reported studies of endemic areas. Although this has been ascribed to epidemiological circumstances, it has been demonstrated in humans that more than half of the variation in seropositivity is attributable to genetic factors (4). HLA genes are extremely polymorphic and play a central role in the (auto)immune response making them attractive candidates for influencing the differential outcomes of *T. cruzi* infection as suggested by murine models (5).

**Key words:** Chagas’ disease; HLA haplotypes; *Trypanosoma cruzi*

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It is hypothesized that parasites are a major force driving major histocompatibility complex (MHC) evolution (6). However, convincing demonstrations of associations between infectious diseases and MHC polymorphisms are scarce in spite of extensive investigation. A general problem is adequately matching a control group to the cases. This is generally easier in rural populations in developing countries than in admixed urban populations of modern cities (7). It has been argued that it may be more rewarding to study parasites living in harmony with their hosts rather than to study virulent parasites, for in the former cases the MHC could be responsible for co-adaptation from the hosts’ side (6).

We investigated the role of HLA class II polymorphism in chronic T. cruzi infection and Chagas’ disease related cardiomyopathy in a mestizo population in a highly endemic, rural of Southern Peru, where genetic admixture is limited to primitive Amerindians and Spaniards, and where human-T. cruzi interactions probably date back to several thousands of years (8).

Material and methods

Subjects

This study included 172 unrelated subjects native and resident of rural settlements in the district of Arequipa, Peru. Ninety-seven percent recognized the presence of Triatoma infestans in their dwellings. All the subjects were older than 15 years (range 15–74), who are believed to have experienced uniformly high levels of exposure to the vector. Clinical history, physical examination, and resting ECG were carried out.

Serological screening

Because of the low specificity of serological tests for Chagas’ disease, it is generally recommended that a serum specimen test positive in at least two different assays before it is accepted as positive (1). Therefore, sera were tested blindly using ELISA (Chagas’ IGG ELISA; Gull Laboratories, Salt Lake City, UT, USA) and indirect immunofluorescence. When discordant results were obtained the indirect hemagglutination assay was used. Individuals were then divided into seropositive (n = 85) and seronegative (n = 87) groups. The mean age of the seropositive group was 35 (SD18) years, and that of the seronegative group 36 (SD15) years. Seropositive subjects with cardiac symptoms and/or ECG compatible with chagasic cardiomyopathy (9) were ascribed to the cardiomyopathic group (n = 33); the rest of seropositives made up the asymptomatic group (n = 52).

HLA typing

Genomic DNA was extracted by standard methodology. HLA-DRB1 alleles were determined at a low resolution level using polymerase chain reaction amplification followed by digestion with restriction enzyme (PCR-RFLP), using amplification-created restriction sites (ACRS) (10). HLA-DQB1 alleles were typed using a commercially available kit (Inno-Lipa HLA-DQB, Innogenetics, Zwijndrecht, Belgium) based on reverse hybridization with sequence-specific oligonucleotides following PCR amplification (reverse PCR-SSO).

Statistical analysis

DRB1 and DQB1 allele frequencies and DRB1-DQB1 haplotype frequencies were determined by direct counting. Eleven HLA-DRB1 and 14 HLA-DQB1 alleles were observed. DRB1-DQB1 haplotypes were unequivocally determined by homozygosity or inferred from established linkage disequilibrium (11). Two-locus linkage disequilibrium was measured with the normalized disequilibrium parameter D’ as described (12). The significance of the disequilibrium calculated for individual haplotypes was measured with Fisher’s exact test. The comparisons of HLA frequencies between the groups under study were done by the Chi-square test (Mantel-Haenzsel) or Fisher’s exact test (two-tails). P-values were corrected for the number of comparisons made (13). Odds ratio (OR) were calculated by Woolf’s method with 95% confidence interval or Haldane’s modification.

Results

Comparison of HLA frequencies between seronegatives and seropositives for T. cruzi

Among DRB1 alleles, DRB1*14 showed a highly significant increased frequency in seronegatives compared with seropositives (P = 0.01) (Table 1). DRB1*14 was found in two different haplotypes, DRB1*14-DQB1*0301 (D’ = 0.84; P = 10^-5) and DRB1*14-DQB1*0503 (D’ = 0.83; P = 5.5 x 10^-5). Interestingly, analysis by haplotypes showed that DRB1*14-DQB1*0301, which is characteristic and frequent in many Amerindian populations, accounted for all the DRB1*14 association given that DRB1*14-DQB1*0503, which is of Caucasoid origin, was found at equal rates in both seropositives and seronegatives (Table 1). No other DQB1*0301 bearing haplotypes, individually or as a whole, were associated with the disease. This suggests that DRB1*14 in haplotypic combination with DQB1*0301 or another factor linked to them are involved in resistance to chronic infection by T. cruzi.
Phenotypic distribution of DRB1*14 and its haplotypes

<table>
<thead>
<tr>
<th>DRB1*14</th>
<th>Seronegative</th>
<th>Seropositive</th>
<th>OR (CI 95%)</th>
<th>P</th>
<th>P_e</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=87 (36.7%)</td>
<td>n=85 (14.1%)</td>
<td>0.28 (0.12–0.63)</td>
<td>0.00068</td>
<td>0.01</td>
</tr>
<tr>
<td>DRB1<em>14-DQB1</em>0301</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>12</td>
<td>0.26 (0.11–0.63)</td>
<td>0.0007</td>
<td>0.01</td>
</tr>
<tr>
<td>Homoz.</td>
<td>4</td>
<td>1</td>
<td>0.25 (0.01–2.58)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heteroz.</td>
<td>25</td>
<td>9</td>
<td>0.29 (0.12–0.72)</td>
<td>0.0028</td>
<td></td>
</tr>
<tr>
<td>DRB1<em>14-DQB1</em>0503</td>
<td>3 (3.4%)</td>
<td>3* (3.5%)</td>
<td>1.02 (0.13–7.87)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* One individual was DRB1*14–DQB1*0301/DRB1*14-DQB1*0503 heterozygote

Table 1

Subsequent stratification of DRB1*14-DQB1*0301 individuals in homozygotes and heterozygotes (Table 1) showed that this haplotype confers similar protection in both cases (OR=0.25 vs. 0.29). Although association in homozygotes did not reach statistical significance, most probably due to the low number of homozygotes sampled, a trend towards protection is clear. Therefore, resistance against chronic infection by *T. cruzi* afforded by DRB1*14-DQB1*0301 is a dominant trait.

DRB1*02-DQB1*0602 was associated with a decreased susceptibility to chronic infection (8% seronegatives vs. 0% seropositives; \(P=0.013\)), although it did not reach statistical significance when the \(P\)-value was corrected. We also found an increased frequency of the DRB1*08-DQB1*0402 haplotype in seropositive individuals compared with seronegatives (27.6% vs. 43.5%, \(P=0.029\)), although again the corrected \(P\)-value was not significant.

Comparison of HLA frequencies between chronically infected asymptomatics and cardiomyopathics

We found no differences in the distribution of DRB1 and DQB1 alleles or DRB1-DQB1 haplotypes between seropositives without manifestations of the disease and those with chagasic cardiomyopathy (data not shown). These data suggest that the studied HLA class II polymorphism does not play a relevant role in the development of symptomatic disease.

Discussion

This study shows that individual genetic differences linked to the HLA are associated with differential susceptibility to become chronically infected with *T. cruzi*, as noted by seropositivity. Although we recognise that exposure to the parasite has not been formally demonstrated, the probability that these subjects have been in contact with the parasite, not once but many times, is very high as inferred by their age and the recognized presence of the vector insect in their dwellings. Therefore we believe it is unlikely that the observed differences are due to epidemiological circumstances only. In addition, our data are consistent with recent findings demonstrating that more than half of the variation in seropositivity to *T. cruzi* is attributable to genetic factors (4). We have found that part of these factors may reside in the HLA system.

The simplest explanation for HLA and infectious disease associations is that they relate to the antigen-presenting ability of HLA molecules (14). However, the strong linkage disequilibrium in the MHC region, as reflected by the existence of ancestral haplotypes (15), along with the complexity of host-parasite interactions make other possibilities conceivable. In this sense, we believe that the role of innate immunity has been underestimated. Innate immunity successfully prevents most infections from becoming established and this type of immunity also plays an essential part in inducing the subsequent adaptive response to those infections that overcome the first line of defence (16). It is worth noting that HLA class III region contains several known polymorphic gene families playing a prominent role in the innate immunity (factor B, C2, C4 and TNF). Thus, it is possible that polymorphisms in these and/or other genes (17), and in strong linkage with class II alleles determine the differential capabilities to clear *T. cruzi* upon early infection.

As an alternative to a direct effect to parasite killing, it is possible that such polymorphisms along with class II polymorphisms influence the type of adaptive immune response mounted (18, 19). Thus, it may be that DRB14-DQB1*0301 is prone to mount protective antigen-specific cell-mediated responses without serological evidence. The existence of such a kind of response is supported by very recent data showing that complete immunity against *T. cruzi*,
defined by a negative result in parasitological tests and lack of serological evidence of infection after an infective challenge with a virulent strain, can be obtained in a significant proportion of outbred Swiss mice pre-immunised with a low virulent *T. cruzi* strain (20). The infective challenge replicated natural, vector-delivered infection and, interestingly, resistance was associated both with delayed-type hypersensitivity reactions and high natural resistance.

These two explanations are consistent with an integrated view of the innate and adaptive immune responses against pathogens and makes biological sense with the well-established conservation of ancestral haplotypes (15). Two very recent studies have analyzed the HLA-class II polymorphism in *T. cruzi* infection. Deghaide et al. found a protective effect for DQB1*06 specificity and a susceptibility association with DQ1 (21). Fernández-Mestre et al. found a decreased frequency of DRB1*14 and DQB1*0303 in seropositives, suggesting an independent protective effect for these alleles were found (22). These results would also support the notion that HLA is involved in differential host-parasite interactions.

It has been reported that the presence of the class I haplotype B40-Cw4 in *T. cruzi* infected individuals is associated with a decreased susceptibility to develop chagasic cardiomyopathy in a Chilean population (23). However, we did not find any difference in the class II allelic or haplotypic distribution between asymptomatics and cardiomyopathics.

In conclusion, our data offer indirect but compelling evidence that polymorphism in HLA region is involved in a differential susceptibility to *T. cruzi* chronic infection and suggest that the studied class II polymorphisms are not associated with chagasic cardiomyopathy in this population.

References