Association of Human Leukocyte Class II Antigens With Rheumatic Fever or Rheumatic Heart Disease in a Brazilian Population

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Background. The incidence of rheumatic heart disease is great in Brazil. We analyzed the distribution of human leukocyte (HLA) antigens in a Brazilian population sample with rheumatic fever or rheumatic heart disease, with the aim of better understanding the mechanisms involved.

Methods and Results. HLA class I (A, B, and C) and class II (DR and DQ) antigen distribution was studied in 40 patients with diagnosis of rheumatic fever or rheumatic heart disease and compared with a control group of 617 healthy individuals for class I typing, from which 118 were drawn for class II typing. A strong correlation between rheumatic fever and rheumatic heart disease and HLA-DRw53 (72.9% in the disease group versus 39% in the control group: p = 0.00061, relative risk, 4.2; etiologic fraction, 0.43) was found. We also found an increase in the frequency of HLA-DR7 (57.5% in the disease group versus 26.3% in control group: p = 0.00715; relative risk, 3.8; etiologic fraction, 0.56). HLA class I and HLA-DQ typing did not point to any association with these diseases.

Conclusions. HLA-DR7 and HLA-DRw53 are markers for susceptibility to rheumatic fever and rheumatic heart disease in Brazil. These results could be explained by genetic differences resulting from racial or geographical diversity. (Circulation 1991;83:1995–1998)

Rheumatic fever (RF) consists of nonsuppurative sequelae of infection by group A \( \beta \) hemolytic streptococci. Determination of a genetic pattern of susceptibility to RF and rheumatic heart disease (RHD) has been sought for more than a century. An increased susceptibility to RF or RHD was assigned by Cheadle in 1889.1 Many studies have been conducted in this area of research, with an aim of defining the pattern of inheritance responsible for the observed susceptibility to RF. Some researchers2 have assumed an autosomal recessive model; others3 have dismissed a mendelian pattern of inheritance. Observation of RF or RHD in identical twins4 suggests that if a mendelian pattern is present, penetrance must be incomplete.

Recent studies have tried to uncover specific markers for RF susceptibility. Correlation with blood groups or secretor status of patients with RF was observed, with a higher incidence of a nonsecretor pattern in affected subjects as well as a reduction of blood group O frequency in rheumatic children.5

Other studies have analyzed human leukocyte (HLA) class I antigens, but no consistent association of these antigens with RF was found.6–15 Subsequent studies of class II antigens have disclosed an association with different HLA-DR alleles according to the population analyzed.16–20

Our HLA phenotyping of the Brazilian population with RF or RHD was motivated by both the great incidence of the disease in Brazil and the fact that our population presents considerable interracial mixing (i.e., most individuals are not exclusively caucasian, black, or of indigenous origin). We believe that in a highly mixed population such as that of Brazil, with more diversified haplotypes, the presence of a specific HLA allele implicated in susceptibility to RF and RHD would be more easily apparent. Furthermore, the discordant data from regions outside of Brazil might be clarified.

Methods

Patients

We studied 40 patients with RF or RHD who were selected by a pediatric rheumatologist or cardiologist

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According to Jones modified criteria. The patients were from different regions of Brazil and were 5–53 years old (mean age, 11 years). There were 15 males and 27 females. The average length of patient follow-up was 5 years. We studied 27 patients with pure carditis, four with pure chorea, and nine with chorea plus carditis. Presence or absence of active disease was not taken into account.

The patients with RF or RHD were characterized as white or light mulatto.

The control group comprised 617 individuals geographically similar to the patient group. Based on racial characteristics, the antigen frequencies for the control group showed values intermediate between those of caucasians and those of blacks, confirming their typically Brazilian profile of white or light mulatto individuals. All control individuals were used for HLA class I analysis; of these, 118 were used for HLA-DR and HLA-DQ analysis, and 78 were used for HLA-DQ analysis.

**HLA Typing**

Mononuclear cells were isolated from peripheral blood according to the method of Boyum and stored frozen in liquid nitrogen. After thawing, cell viability was tested with Trypan blue, and the HLA class I phenotype was determined by the microlymphocytotoxicity test using 120 specific antisera (Pel-Freez and local sera) according to the method of Amos and Pool. Class II antigens were defined using 60 specific antisera (Pel-Freez) and the same technique on peripheral blood B cells purified by adherence to nylon wool columns according to the method of Danilovs et al.

**Statistical Analysis**

The frequencies of HLA class I and II antigens were compared using the $\chi^2$ test and Yates’s correction when the number of individuals with a specified antigen was less than five. Probability values were corrected for the number of HLA-DR antigens analyzed ($n=11$). When statistical significance was achieved, the relative risk (RR) was calculated according to the method of Svejgaard et al.

When the RR was more than 1, we calculated the etiologic fraction that indicates the proportional value of the HLA class II marker in the induction of the disease according to Ryder et al. and Bengtsson and Thomson.

**Results**

**Class I Antigens**

We tested for 16 locus A and 26 locus B antigens in 40 patients. The control group comprised 617 healthy, unrelated individuals, as previously defined in “Methods.” No significant association of HLA class I antigens with presence of RF or RHD could be observed.

**Class II Antigens**

We typed 40 patients for HLA-DR; of these, 37 were typed for HLA-DRw52/53 and HLA-DQ. These patients were compared with the control groups of 118 and 78 unrelated individuals, respectively, for HLA-DR, HLA-DRw52/53, and HLA-DQ, all stemming from the original group of 617 individuals.

In our sample, 23 of 40 patients (57.5%) typed HLA-DR7 compared with 26.3% in the control group. Calculated $\chi^2$ was 11.59 with a probability of 0.00065 and a corrected probability of 0.00715 when 11 HLA-DR antigens were considered. Furthermore, the data indicated an RR value of 3.8 and an etiologic fraction of 0.43. The results are shown in Table 1.

The analysis of 37 patients for HLA-DRw52/53 disclosed an increased percentage of HLA-DRw53-positive patients (72.9% compared with 39.0% of the control group; $\chi^2$, 11.73; $p=0.00061$; RR, 4.2; etiologic fraction, 0.56). The data are given in Table 2.

HLA-DQ typing of 37 patients and 78 unrelated controls did not disclose an association with RF or RHD (see Table 3).

### Table 1. Frequency of HLA-DR Antigens

<table>
<thead>
<tr>
<th>HLA</th>
<th>Patients ($n=40$) (%)</th>
<th>Controls ($n=118$) (%)</th>
<th>$\chi^2$</th>
<th>p</th>
<th>Corrected p</th>
<th>RR</th>
<th>EF</th>
</tr>
</thead>
<tbody>
<tr>
<td>DR1</td>
<td>5.0</td>
<td>22.0</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DR2</td>
<td>15.0</td>
<td>26.0</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DR3</td>
<td>12.5</td>
<td>24.0</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DR4</td>
<td>15.0</td>
<td>23.0</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DR5</td>
<td>22.5</td>
<td>38.0</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DRw6</td>
<td>22.5</td>
<td>18.0</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DR7</td>
<td>57.5</td>
<td>26.3</td>
<td>11.59</td>
<td>0.00065</td>
<td>0.00715</td>
<td>3.8</td>
<td>0.43</td>
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<tr>
<td>DRw8</td>
<td>12.5</td>
<td>2.5</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
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<tr>
<td>DRw9</td>
<td>2.5</td>
<td>0.0</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drw10</td>
<td>0.0</td>
<td>0.9</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DR blank</td>
<td>35.0</td>
<td>27.5</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HLA, human leukocyte; RR, relative risk; EF, etiologic fraction.
Table 2. Frequency of HLA-DRw52 and HLA-DRw53 Antigens

<table>
<thead>
<tr>
<th>HLA</th>
<th>Patients (n=37) (%)</th>
<th>Controls (n=118) (%)</th>
<th>$\chi^2$</th>
<th>$p$</th>
<th>RR</th>
<th>EF</th>
</tr>
</thead>
<tbody>
<tr>
<td>DRw52</td>
<td>62.16</td>
<td>68.00</td>
<td>NS</td>
<td>. .</td>
<td>. .</td>
<td>. .</td>
</tr>
<tr>
<td>DRw53</td>
<td>72.90</td>
<td>39.00</td>
<td>11.73</td>
<td>0.00061</td>
<td>4.2</td>
<td>0.56</td>
</tr>
</tbody>
</table>

HLA, human leukocyte; RR, relative risk; ER, etiologic fraction.

Table 3. Frequency of HLA-DQ Antigens

<table>
<thead>
<tr>
<th>HLA</th>
<th>Patients (n=37) (%)</th>
<th>Controls (n=78) (%)</th>
<th>$\chi^2$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>DQw1</td>
<td>56.8</td>
<td>66.0</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>DQw2</td>
<td>59.5</td>
<td>41.8</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>DQw3</td>
<td>45.9</td>
<td>45.8</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>DQ blank</td>
<td>35.1</td>
<td>39.8</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

HLA, human leukocyte.

Discussion

The first immunogenetic studies of RF and RHD done in the 1970s analyzed only the HLA class I antigens. The pioneer study,\(^6\) which characterized 17 class I antigens, demonstrated a reduction of the HLA-A3 frequency in patients with RF. Further studies indicated increased frequencies of HLA-A29, HLA-A30, and HLA-A31 in RHD,\(^7\) HLA-A3 and HLA-B8, B8, and HLA-Bw22\(^9\) and HLA-Bw35 and HLA-B18 in the acute form of the disease.\(^10\)

In vitro studies by Greenberg et al.\(^11\) have shown an increase of lymphocyte proliferation in patients with RF who bear the HLA-B5 antigen. Yoshinoya and Pope\(^12\) demonstrated an association between the presence of elevated immune complex levels and the positivity for the antigen HLA-B5 in rheumatic patients; however, this antigen was reduced in rheumatic individuals' families.\(^13\) In contrast, reduced frequencies of HLA-A10 were described.\(^9\) Nevertheless, no other authors have observed an association between RF and the HLA class I phenotype.\(^11,12\)

HLA class II typing performed in the 1980s has also led to conflicting results. Jhinghan et al.\(^16\) studied an Indian population and described a positive association of RF with HLA-DR3 and a negative association with HLA-DR2.

Anastasiou-Nana et al.\(^17\) described a higher frequency of HLA-DR4 and a lower frequency of HLA-DRw6 in US caucasian patients with RHD.

Ayoub et al.\(^18\) disclosed an association of RF with HLA-DR2 in black patients and with HLA-DR4 in caucasian patients. Furthermore, in caucasian, they suggested an association between RF and HLA-DRw9. This association, however, may not be significant because of the very low-control antigen frequencies observed, which give minimal estimates for this allele.

Rajapakse et al.\(^19\) defined HLA-DR4 as a genetic marker of RHD in a Saudi Arabian population. Maharaj et al.\(^20\) disclosed higher frequencies of HLA-DR1 and DRw6 in black patients with chronic heart disease.

Patarroyo et al.\(^29\) noted the existence of a surface marker on peripheral blood mononuclear cells stimulated with pokeweed mitogen, defined by the alloantiserum 883, which reacted with 75% of the patients with RF. The authors were not able to assign a HLA-D-related specificity to this serum. They suggested, however, that the 883 reagent could be recognizing a second Ia locus or, alternatively, a totally different antigen, because this marker was often present as a third specificity in addition to the conventional non-cross-reactive HLA-DR antigens.

Zabriskie et al.\(^30\) produced a monoclonal antibody, D8/17, that is capable of defining a surface marker on B cells of the majority of patients with RF or RHD. These data suggest that alloantigens are expressed on B cells of patients with RF. Taneja et al.\(^31\) recently proposed an association with HLA-DQw2 in a study of Indian patients positive for the D8/17 susceptibility marker. However, correlation of these markers with the HLA-DR system has not been clearly established.

We analyzed HLA class I and II antigen distribution in our study population with RF or RHD to characterize an association between RF or RHD and HLA. Our results did not disclose an association of class I antigens with RF or RHD (data not shown).

On the other hand, a very strong correlation of HLA-DR7 with HLA-DRw53 was demonstrated. The correlation with HLA-DR4, which has been found in other populations, was not apparent in the present study, but HLA-DR7, which is included in the HLA-DRw53 group, was abnormally high and significantly correlated with RF and RHD. HLA-DRw9 was underrepresented in our patient population and absent in the controls; therefore, association analysis was impossible.

No association with HLA-DQ was found, not even with HLA-DQw2, which is the most common antigen in linkage disequilibrium with HLA-DR7. Most HLA-DQ blank individuals (35.1%) were HLA-DR7/DR blank, HLA-DQw2/DQ blank (nine of 14), and probably homozygous for HLA-DR7 and HLA-DQw2. The remaining HLA-DQ blank subjects were HLA-DRw8 (four patients), that is, in linkage disequilibrium with HLA-DQw4 not tested by us.

Our calculated etiologic fractions indicate that the presence of the antigen is responsible for 43% (for HLA-DR7) and 56% (for HLA-DRw53) of the factors involved in the pathogenesis of the disease in a susceptible individual.

Recently, Khana et al.\(^32\) studied the D8/17 marker present on B lymphocytes and did not observe a correlation with HLA antigens. A closer analysis of the authors' results, however, points to an increased number of HLA-DRw53-positive patients (six of eight). In addition, in the two RF pedigrees analyzed, both HLA-DR7 and HLA-DRw53 were present. It is possible that the HLA-DRw53 antigen or a gene close to it may be involved in an abnormal immune response directed against streptococcal antigens, leading to the clinical picture of RF.
We conclude that HLA-DR7 (or HLA-DRw53) is a marker for susceptibility to RF and RHD and that other genetic factors might have roles in determining this susceptibility. The diversity of clinical forms suggests that multiple factors are involved; multiple genetic systems may be interacting to define this susceptibility. In the present study, no preferential association of HLA with any of the classic forms of the disease, such as chorea or carditis, was seen. HLA-DR7 was identified in 10 of 21 patients with carditis, in two of five with active carditis, in two of four with chorea, and in seven of 10 with both chorea and carditis (data not shown). A similar profile could be observed for HLA-DRw53-positive patients. On the other hand, we cannot underestimate the role of nongenetic factors such as social and economic factors in the analysis of RF and RHD as well as different streptococcal strains that may elicit different patterns of immune response.

Conclusion
Based on our studies and those of others, genetic differences resulting from racial and geographical variations must have a role in susceptibility to RF and RHD, but a major influence must be a susceptibility gene situated in or near the HLA-DR locus.

To more accurately define an association between HLA and RF, we are approaching this issue through the use of restriction fragment length polymorphism studies in our patients. Preliminary studies with this technique have already identified specific fragments associated with susceptibility to the disease.

References

KEY WORDS • human leukocyte antigens • genetics • surface markers • rheumatic fever • rheumatic heart disease
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