Inherited predisposition to iridocyclitis with juvenile rheumatoid arthritis: Selectivity among HLA-DR5 haplotypes
(early-onset pauciarticular/linkage/HLA-Bw44, HLA-Bw35)

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ABSTRACT  HLA-DR5 is associated with a chronic iridocyclitis and juvenile rheumatoid arthritis with onset in early childhood. Previously published data provided indirect evidence for selective linkage between two HLA-B alleles and HLA-DR5. To test this observation further, 38 families, the probands of which have chronic iridocyclitis and juvenile rheumatoid arthritis, were HLA typed so that haplotypes associated with disease could be established. HLA-DR5 was linked to HLA-Bw44 or to HLA-Bw35 and to HLA-Cw4 in the majority of haplotypes obtained in the probands. Both HLA-Bw44, -DR5 and HLA-Bw35, -Cw4, -DR5 occurred more commonly in the proband haplotypes than in the control haplotypes. The frequency of the haplotype HLA-Bw44, -DR5 was 0.133 compared with 0.007 (χ² = 27.04, P < 10⁻⁴) and for HLA-Bw35, -Cw4, -DR5, it was 0.093 compared with 0.11 (χ² = 13.83, P = 0.0002). The relative risks were 20.77 and 9.23, respectively, versus 3.52 for HLA-DR5 alone. HLA-Bw44 occurred much more commonly in proband HLA-DR5 haplotypes (0.455) compared with control HLA-DR5 haplotypes (0.067) (χ² = 8.26, P < 0.01). Not all HLA-DR5-bearing haplotypes predisposed equally to chronic iridocyclitis and early-onset pauciarticular juvenile rheumatoid arthritis.

Juvenile rheumatoid arthritis (JRA) is associated with a range of HLA antigens that are in part distinct from those found in chronic arthropathies in adults (1, 2). These HLA associations include antigens HLA-DR5, HLA-DR8, and HLA-B27 and reflect the different forms of JRA that can be distinguished clinically. HLA-DR5 is associated with one form that occurs in children, predominately females, with limited joint disease at onset (pauciarticular). This form develops in early childhood and is associated with antinuclear antibody in serum (3–7). In at least half of the instances, these children have a chronic iridocyclitis from which the long-term morbidity is often substantially greater than that from arthritis alone (8, 9). In an initial report of the association between the disease and HLA-DR5, indirect evidence for selective linkage between two HLA-B series antigens, HLA-B12 and HLA-Bw35, and HLA-DR5 was obtained (3). These data, obtained through study of probands alone, were based on estimates of linkage disequilibrium, the excess of observed over expected numbers of individuals sharing particular HLA antigens.

In the present investigation the necessary family studies were performed to examine directly the linkage between HLA-A, -B, and -C genes and HLA-DR5 in a group of patients with JRA and chronic iridocyclitis.11

METHODS

Subjects. Thirty-eight North American Caucasian patients (35 females and 3 males) with early-onset pauciarticular JRA with iridocyclitis and 239 family members were studied. All were drawn from communities throughout the New England area and reflect the ethnic variation of the region. Patients of both Northern and Southern European extraction were included. Eighteen of the probands were included in a previous study (3). All children met the criteria for pauciarticular onset JRA as defined by the Criteria Committee of the American Rheumatism Association (10). Additional study criteria included onset of arthritis before the age of 8 yr and the presence of iridocyclitis observed by either of two ophthalmologists experienced with the eye disease associated with JRA. Evidence either of active inflammation or of previous inflammatory eye disease, including synechiae or band keratopathy in the absence of active inflammation, was acceptable. Patients with posterior uveitis were not studied. Finally, the availability and consent of sufficient family members to establish genotypes for the HLA region in the probands was required.

Probands and family members were typed for HLA-A, -B, -C, -DR, DC(MB), and MT antigens in the Tissue Typing Laboratory of the Department of Rheumatology and Immunology, The Brigham and Women’s Hospital.

Family trees were constructed and haplotype assignments were made for each locus when an informative assortment of alleles was demonstrated. If an assignment could not be made for a particular polymorphism, that haplotype was not considered in calculating the gene frequencies for the locus. The denominator used to calculate gene frequencies varied therefore between polymorphisms being lower for some, especially the DC(MB) and MT genes, than for HLA-A, -B, -C, and -DR.

Control data consisted of 314 haplotypes established in members of the probands’ families but excluded haplotypes inherited by a proband. Inclusion of all available family members ensured adequate numbers of control haplotypes. No haplotype was counted twice and one potential control haplotype in recombinant with a proband’s haplotype was excluded. This method of establishing control gene frequency and control haplotype frequencies by using the proband’s family’s has been compared with haplotype frequencies derived from families without an affected member. Very comparable results have been obtained between the two methods (11). The control gene frequencies were, in addition, com-

Abbreviations: JRA, juvenile rheumatoid arthritis; MNL, mononuclear leukocyte(s); CDC, complement-dependent cytotoxicity.

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1Alternative terms to describe the same population include juvenile arthritis and juvenile chronic polyarthritis.
pared with data for North American Caucasians from the Eighth International Histocompatibility Workshop (Table 2).

Cells. Peripheral blood mononuclear leukocytes (MNL) were obtained by density gradient centrifugation through Ficoll-Paque (Pharmacia).

HLA-A, -B, -C, -DR Typing. HLA-A, -B, -C typing for 39 specificities was carried out by microdroplet complement-dependent cytotoxicity (CDC) test. HLA-A, -B, -C antisera were kindly supplied by the National Institute of Allergy and Infectious Diseases (Bethesda, MD).

HLA-DR Typing. HLA-DR specificities were typed by CDC on a nylon wool B-cell-enriched MNL population.

All preparations studied were evaluated in CDC with a polyvalent rabbit antisera (1) serum kindly supplied by J. Strominger (Biological Laboratories, Harvard University, Boston) and were found to contain 70% B-bearing MNL.

The CDC assays were performed as follows: MNL with antisera were incubated at room temperature for 1 hr and then incubated for a further 2 hr (14) with rabbit complement (Pel-Freez).

Typing antisera, including sera derived by the Eighth Histocompatibility Workshop (15), covered 9 DR specificities, were obtained locally, or were gifts from other laboratories.

DC(MB)/MT Typing. The MNL population was collected and the CDC assay was performed as for the HLA-DR specificities. Typing antisera defined a DC(MB)/MT1 specificity and 4 additional specificities, 2 for DC(MB) and 2 for MT (16, 17).

Statistical Methods. HLA gene frequencies and selected HLA haplotypes were compared to controls by $\chi^2$ analysis. Relative risks were calculated as in Woolf (18).

The HLA haplotype data, in addition to direct $\chi^2$ testing, were analyzed by multivariate techniques (19) similar to methods recently applied to HLA data (20). This allows one to control for observed differences in gene frequencies between probands and controls, after which the data were retested by $\chi^2$ analysis for differential linkage between probands and controls.

Corrections of $P$ values (Table 4) for the numbers of statistical tests run have not been made in view of the data suggesting linkage disequilibrium between HLA-DP and both HLA-Bw35 and HLA-Bw44 in independently conducted population studies (21, 22).

RESULTS

The mean age of onset of joint disease in the 38 probands was 2.5 yr (range, 10 months to 6 yr). The mean age at first detection of eye disease was 5.0 yr (range, 11 months to 17 yr). Antinuclear antibody is present in serum in 76% of those tested (19/25). The mean length of follow-up from first presentation was 8.6 yr (range, 10 months to 24 yr).

Gene frequencies of the HLA-A, -B, -C, -DR, -DQ, -DC(MB), and -MT polymorphisms for the locally established control haplotypes did not differ significantly from the gene frequencies reported from the Eighth Histocompatibility Workshop (15) ($\chi^2 = 8.75$, 9 df, $P = 0.46$ for the HLA-DR gene frequencies). Thirty of the 314 control haplotypes were shown to include HLA-DR5.

Gene Frequencies. HLA-A, -B, and -C loci. The frequencies of HLA-A2, HLA-B44, HLA-Bw16, HLA-Bw35, and HLA-Cw4 were found to be increased in the proband compared with control haplotypes (Table 1).

Genes for the HLA-A locus could be assigned for 75 proband haplotypes and 291 control haplotypes. The frequency of HLA-A2 was 0.453 versus 0.278, and the $\chi^2$ comparing the frequencies of HLA-A2 equaled 6.14 ($P < 0.01$). Assignments for HLA-B alleles were possible for 75 proband haplotypes and 293 control haplotypes. The HLA-Bw44 frequency was 0.280 (21/75) compared with 0.130 (38/293), for which the $\chi^2$ was 8.43, yielding $P < 0.01$. HLA-Bw16 was found with a frequency of 0.120/0.75 compared with 0.020 (6/293); the $\chi^2$ was 14.49 ($P < 0.001$). Additionally, the frequency of HLA-Bw35 was 0.200 (15/75) versus 0.102 (30/293). The $\chi^2$ from the comparison of HLA-Bw35 frequencies equaled 4.66 ($P < 0.02$).

Chromosomal assignment of HLA-C alleles could be made for 69 proband haplotypes, of which 0.261 (18/69) were HLA-Cw4, and for 245 control haplotypes, with 0.106 (26/245) being HLA-Cw4. The difference was significant ($\chi^2 = 10.70, P < 0.005$).

HLA-DR locus. Seventy-six HLA-DR alleles could be assigned to the proband haplotypes and 273 in the control haplotypes. The frequencies of two genes were increased: HLA-DR5, 23/76 compared with 30/273 ($\chi^2 = 14.55, P < 0.001$), and HLA-DRw8, 9/76 compared with 9/273 ($\chi^2 = 8.42, P < 0.01$), whereas the frequencies of three genes were decreased: HLA-DR1 ($\chi^2 = 5.30, P < 0.02$), HLA-DR4 ($\chi^2 = 7.05, P < 0.01$), and HLA-DR7 ($\chi^2 = 5.56, P < 0.02$) (Table 2).

DC(MB)/MT. The DC(MB)/MT polymorphisms were informative for 54 alleles in proband haplotypes and 216 in control haplotypes (Table 3). Proband and control haplotypes had similar frequency distributions with regard to the DC(MB) genes but differed with regard to the MT genes. In particular, MT2 was more frequent ($\chi^2 = 8.71, P < 0.01$) and MT3 was less frequent ($\chi^2 = 6.45, P < 0.02$).

HLA-DR5 Haplotypes. The distribution of 3 alleles, HLA-Bw44, HLA-Bw35, and HLA-Cw4, observed to be in linkage

Table 2. Frequency of HLA-DR genes in iridocyclitis and early-onset pauciarticular JRA

<table>
<thead>
<tr>
<th>HLA specific</th>
<th>Haplotype</th>
<th>Proband (n = 76)</th>
<th>Control (n = 273)</th>
<th>Workshop* $\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DR1</strong></td>
<td>0.026</td>
<td>0.121</td>
<td>0.106</td>
<td>5.30</td>
</tr>
<tr>
<td><strong>DR2</strong></td>
<td>0.105</td>
<td>0.161</td>
<td>0.136</td>
<td>1.25</td>
</tr>
<tr>
<td><strong>DR3</strong></td>
<td>0.145</td>
<td>0.117</td>
<td>0.118</td>
<td>0.36</td>
</tr>
<tr>
<td><strong>DR4</strong></td>
<td>0.039</td>
<td>0.168</td>
<td>0.148</td>
<td>7.05</td>
</tr>
<tr>
<td><strong>DR5</strong></td>
<td>0.303</td>
<td>0.110</td>
<td>0.102</td>
<td>14.55†</td>
</tr>
<tr>
<td><strong>DRw6</strong></td>
<td>0.079</td>
<td>0.033</td>
<td>0.037</td>
<td>2.92</td>
</tr>
<tr>
<td><strong>DRw7</strong></td>
<td>0.026</td>
<td>0.125</td>
<td>0.126</td>
<td>5.56</td>
</tr>
<tr>
<td><strong>DRw8</strong></td>
<td>0.118</td>
<td>0.033</td>
<td>0.027</td>
<td>8.42‡</td>
</tr>
<tr>
<td><strong>DRw9</strong></td>
<td>0.013</td>
<td>0.004</td>
<td>0.015</td>
<td>0.94</td>
</tr>
<tr>
<td><strong>DRX</strong></td>
<td>0.145</td>
<td>0.128</td>
<td>0.185</td>
<td>0.12</td>
</tr>
</tbody>
</table>

*Eighth International Histocompatibility Workshop.
†Relative risk, 3.52.
‡Relative risk, 3.94.
§Not detected by presently available serology.

**HLA-Bw44 is a serological split of HLA-B12.
Table 3. Frequency of DC(MB)/MT genes in iridocyclitis and early-onset pauciarticular JRA

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>DC(MB)/MT specificity</th>
<th>Proband (n = 54)</th>
<th>Control (n = 216)</th>
<th>χ²</th>
</tr>
</thead>
<tbody>
<tr>
<td>MB2</td>
<td></td>
<td>0.259</td>
<td>0.241</td>
<td>0.06</td>
</tr>
<tr>
<td>MB3</td>
<td></td>
<td>0.259</td>
<td>0.245</td>
<td>0.03</td>
</tr>
<tr>
<td>MT2</td>
<td></td>
<td>0.556*</td>
<td>0.292</td>
<td>8.71</td>
</tr>
<tr>
<td>MT3</td>
<td></td>
<td>0.074</td>
<td>0.255</td>
<td>6.45</td>
</tr>
</tbody>
</table>

*Relative risk, 3.04.

disequilibrium with HLA-DR5 in earlier studies of phenotypes (3), was compared in proband and in control haplotypes (Table 4). HLA-Bw44, -DR5 was present with a frequency of 0.133 (10/75) compared with 0.007 (2/273) (χ² = 27.04, P < 10⁻⁶). The HLA-Bw35, -Cw4, -DR5 haplotype also differed, 0.093 (7/75) versus 0.011 (3/273), the χ² being 13.83 (P = 0.0002).

The observed differences in HLA-DR5 haplotypes were not simply a consequence of the difference in frequency of the HLA-DR5 genes between the two groups. HLA-B alleles linked to HLA-DR5 had different frequencies in the 22 proband HLA-DR5 (+) haplotypes compared to the 30 control HLA-DR5 (+) haplotypes (Table 5). HLA-Bw44 was linked to HLA-DR5 much more frequently in proband haplotypes compared with control haplotypes, 0.455 (10/22) versus 0.067 (2/30) (χ² = 8.26, P < 0.01). The frequency of HLA-Bw35, -Cw4 linked to HLA-DR5 was 0.318 (7/22) versus 0.100 (3/30) (χ² = 3.15, P < 0.1). Proband and control HLA-DR5 (-) haplotypes differed in HLA-Bw35, -Cw4 genes (χ² = 4.92, P < 0.05).

Multivariate analysis of the data given in Table 5 indicated some difference in the relative frequencies of HLA-B locus genes, between probands and controls, depending on the presence or absence of HLA-DR5 in the haplotype. The χ² statistic for an excess of Bw44 in DR5 (+) haplotypes was 4.18 (P < 0.05).

Twelve of the proband’s HLA-DR5 haplotypes were also informative for the DC(MB) and MT polymorphisms; 11 carried the MB3 and 10 carried the MT2 alleles. The remaining alleles, 1 at the MB locus and 2 at the MT locus, were not serologically defined (MBX or MTX). Of the 30 control HLA-DR5 haplotypes, 16 were informative at the MB and 20 were informative at the MT loci. Fifteen of the MB alleles were MB3 and 1 was MBX. Eighteen of the MT alleles were MT2, 1 was MT1, and 1 was MTX.

**DISCUSSION**

The gene frequencies of 8 HLA alleles from 5 loci have been shown to be increased in 38 patients with chronic iridocyclitis and early-onset pauciarticular JRA. The alleles HLA-A2, HLA-Bw44, HLA-Bw35, HLA-Bw16, HLA-Cw4, HLA-DR5, HLA-DRw8, and MT2 were found more frequently than expected in the disease group. This increased risk, albeit of a fairly modest degree, was greater for the HLA-DR alleles than for those of other loci, a finding common to many diseases with an immunologic component in their pathogenesis.

Table 4. Frequency of selected HLA-B, -DR5 haplotypes

<table>
<thead>
<tr>
<th>HLA allele</th>
<th>Haplotype</th>
<th>Proband (n = 75)</th>
<th>Control (n = 273)</th>
<th>Relative risk</th>
<th>χ²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bw44, DR5</td>
<td></td>
<td>0.133</td>
<td>0.007</td>
<td>20.77</td>
<td>27.04</td>
</tr>
<tr>
<td>Bw35, Cw4, DR5</td>
<td></td>
<td>0.093</td>
<td>0.011</td>
<td>9.23</td>
<td>13.83</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td>0.773</td>
<td>0.982</td>
<td>0.06</td>
<td>2.65</td>
</tr>
</tbody>
</table>

Table 5. HLA-B alleles linked to HLA-DR alleles

<table>
<thead>
<tr>
<th>HLA allele</th>
<th>Haplotype</th>
<th>Proband</th>
<th>Control</th>
<th>Relative risk</th>
<th>χ²</th>
</tr>
</thead>
<tbody>
<tr>
<td>DR5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bw44</td>
<td></td>
<td>0.455</td>
<td>0.067</td>
<td>11.67</td>
<td>8.26</td>
</tr>
<tr>
<td>Bw35, Cw4</td>
<td></td>
<td>0.318</td>
<td>0.100</td>
<td>4.20</td>
<td>3.15</td>
</tr>
<tr>
<td>B other</td>
<td></td>
<td>0.227</td>
<td>0.833</td>
<td>0.06</td>
<td>8.09</td>
</tr>
<tr>
<td>Non-DR5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bw44</td>
<td></td>
<td>0.170</td>
<td>0.128</td>
<td>1.39</td>
<td>0.56</td>
</tr>
<tr>
<td>Bw35, Cw4</td>
<td></td>
<td>0.113</td>
<td>0.037</td>
<td>3.30</td>
<td>4.92</td>
</tr>
<tr>
<td>B other</td>
<td></td>
<td>0.717</td>
<td>0.835</td>
<td>0.50</td>
<td>0.76</td>
</tr>
</tbody>
</table>

n values are given in parentheses.

Four of these observations, those relating to HLA-Bw35, -Cw4, -Dw5, and -DRw8, agree with earlier findings of increased antigen frequencies in several studies of probands alone (2, 3). In an earlier investigation, although HLA-Bw44 was not increased in frequency overall, it was observed to be in linkage disequilibrium with HLA-DR5 in the study population (3). HLA-A2 has been reported to be associated with JRA in two independent investigations (23, 24). To the best of our knowledge, the associations of HLA-Bw16 and MT2 with the disease have not been documented hitherto. That MT2 is predictable given the established relationship between HLA-DR5, HLA-DRw8, and MT2 (17).

The tissue typing of family members allowed us to make chromosomal assignments and thereby establish haplotypes for the HLA region. Comparisons could be made between haplotypes in probands and controls. Sufficient HLA-DR5 haplotypes were documented among the 314 control haplotypes to allow this comparison for HLA-DR5 but not for the other relevant but less common HLA-DR allele, HLA-DRw8. HLA-DR5 was linked to HLA-Bw44 or to HLA-Bw35 and HLA-Cw4 more commonly in the probands than HLA-DR5 in the control haplotypes (Table 5). HLA-DR5 was shown to be linked to either HLA-Bw44 or HLA-Bw35 and HLA-Cw4 in 77% of instances in the probands compared with 16.6% of the control HLA-DR5 haplotypes. There was little evidence for an effect of the HLA-Bw44 gene independent of that from HLA-DR5, although HLA-Bw44 was commoner in the haplotypes established among the HLA-DR5 (-) probands; this increase did not reach a level of statistical significance. In contrast, the increase in the HLA-Bw35, -Cw4 haplotype in the HLA DR5 (+) probands may not be dependent entirely on the HLA-DR5 linkage, as HLA-Bw35, -Cw4 haplotypes did occur more commonly than expected in the HLA-DR5 (-) probands. These data demonstrate that two haplotypes, HLA-Bw44, -DR5, MT2, MB3 and HLA-Bw35, -Cw4, -DR5, MT2, MB3, predispose an individual to early-onset pauciarticular JRA with iridocyclitis more strongly than does the inheritance of chromosomes bearing the HLA-DR5 gene with other linkages.

The relative risks for these haplotypes were 20.77 for the HLA-DR5, HLA-Bw44 haplotype and 9.23 for the HLA-DR5, HLA-Bw35, and HLA-Cw4 haplotype. Both are higher than the relative risk for single HLA allele and disease associations, even of the HLA-D locus.

The two HLA-DR5 haplotypes associated with JRA and chronic iridocyclitis are not unique to the disease population. Both occurred in the control population, albeit infrequently. In addition, there is some evidence that HLA-Bw44, HLA-Bw35 with HLA-Cw4 and HLA-DR5 are in linkage disequilibrium in unselected populations (21, 22) as well as in this disease situation.

Why an HLA disease association should be stronger for a haplotype that shows linkage disequilibrium rather than one without a characteristic linkage group is not known. Other disease associations with haplotypes have been reported, in-
cluding juvenile-onset diabetes mellitus, 21-hydroxalase deficiency, and drug toxicity (25, 26).

Studies limited to probands suggest yet other diseases will have strong haplotype associations when the appropriate family studies are done (27–29).

Some 20–30% of all HLA haplotypes show linkage disequilibrium that generally extends between HLA-B and -DR and includes alleles of the four HLA-linked complement loci (22, 29, 30). Occasionally, the linkage group involves an HLA-A allele and a glyoxalase allele.†† the latter being mapped centromeric to HLA-D. Several mechanisms have been suggested to account for the phenomenon of linkage disequilibrium. These include a relatively recent mutation, natural selection, and migration. Evidence for yet another mechanism has been recently presented. This mechanism would involve r-loci equivalents, loci concerned with organogenesis, which are likely to be linked to HLA (22). If these loci in man have the property of suppressing chromosomal recombination, as is the case in the mouse model, the trapping of disease-associated genes on a chromosome coding for characteristic linkage groups could occur (31). This explanation is consistent with the data presented above and is likely to be applicable to other HLA-associated diseases.


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