HLA-DQ RFLP variants of five HLA-DQw2-bearing major histocompatibility complex extended haplotypes

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Abstract. We have analyzed genomic DNA in a large number of independent examples of five HLA-DQw2-bearing extended haplotypes for their associated subtypes by restriction fragment length polymorphism (RFLP) using DRB, DQA, and DQB probes after Taq I and Pst I digestion and Southern blotting. In addition to three previously described HLA-DQw2 subtypes, DQw2a, DQw2b, and DQw2c, we observed a fourth subtype, HLA-DQw2d, characterized by 5.8 kilobase (kb) DRB/Taq I, 2.4, 2.3, and 1.8 kb DQB/Taq I, and 8.0 and 2.3 kb DQA/Pst I fragments. All 22 independent examples of the extended haplotype [HLA-B8,SCO1,DR3] carried DQw2a and all 11 independent examples of [HLA-B18,F1C30,DR3] carried DQw2b. In addition, all independent examples (21 and 4, respectively) of two DR7-carrying extended haplotypes, [HLA-B44,FC31,DR7] and [HLA-Bw47,FC91,0,DR7,DRw53,DQw2], and [HLA-Bw57,SC61,DR7,DRw53,DQw2] (Alper et al. 1985).

It has also been shown that some of these extended haplotypes are markers in autoimmune diseases. For example, the [HLA-B8,SC01,DR3,DRw52,DQw2] extended haplotype was increased among patients with insulin-dependent diabetes mellitus (IDDM) and gluten-sensitive enteropathy, while the [HLA-B18,F1C30,DR3,DRw52,DQw2] extended haplotype was found to be increased only among IDDM patients. HLA-DR3 on nonextended haplotypes was found not to be a disease marker (Raum et al. 1984).

We present evidence that only one of the four HLA-DQw2 subtypes is characteristic of each of the HLA-DR3,DQw2 or HLA-DR7,DQw2 extended haplotypes.

Introduction

The human major histocompatibility complex (MHC) encodes class I (HLA-A, B, and C) and class II (HLA-DR, DQ, and DP) products (Klein 1977; Dausset 1981). There is a group of genes located within the MHC that encodes four complement proteins (C2, BF, C4A, and C4B; Carroll et al. 1984) and an expressed gene and a pseudogene for the adrenal enzyme steroid 21-hydroxylase (White et al. 1985). From previous family studies, we found that about 30% of Caucasian MHC haplotypes show nonrandom association of alleles over the HLA-B/DR interval. Significant linkage disequilibrium among specific alleles of HLA-B, DR, and the four complement genes as a single genetic unit was observed. These particular haploietic combinations were named extended haplotypes and it was postulated that independent instances of each had a similar structure and DNA sequence (Alper et al. 1982). Among the extended haplotypes recognized, two with HLA-DR3 and three with HLA-DR7 carry the HLA-DQw2 specificity: [HLA-B8,SC01,DR3,DRw52,DQw2], [HLA-B18,F1C30,DR3,DRw52,DQw2], [HLA-B44,FC31,DR7,DRw53,DQw2], [HLA-Bw47,FC91,0,DR7,DRw53,DQw2], and [HLA-Bw57,SC61,DR7,DRw53,DQw2] (Alper et al. 1985).

We present evidence that only one of the four HLA-DQw2 subtypes is characteristic of each of the HLA-DR3,DQw2 or HLA-DR7,DQw2 extended haplotypes.

Materials and methods

Cell lines. Epstein-Barr virus-transformed cell lines homozygous or heterozygous for MHC alleles were obtained from the core of the Tenth International Histocompatibility Workshop (Yang et al. 1989), or were generated in our laboratory as has been described elsewhere (Spiro et al. 1979). In addition, two families carrying the [HLA-B8,SC01,DR3,Dw52,DQw2] extended haplotype, two families carrying the [HLA-B44,FC31,DR7,DRw53,DQw2] extended haplotype, and one family...
carrying the \([\text{HLA-Bw57,SC61,DR7,DRw53,DQw2}]\) extended haplotype were analyzed. Cell lines were maintained in RPMI 1640 containing 10\% heat-inactivated fetal calf serum, penicillin 50 units/ml, streptomycin 50 µg/ml, and 1 mM L-glutamine (all from BRL/Gibco, Gaithersburg, Maryland).

Restriction fragment length polymorphism (RFLP). DNA was extracted from 5 x 10⁷ to 1 x 10⁸ cells as described elsewhere (Davis et al. 1986). Ten micrograms of digested DNA per sample was electrophoresed in a 0.8\% agarose-TAE (0.04 M Tris-acetate, 0.001 M ethylenediaminetetraacetate) gel. Gels were transferred to Nytran membranes (Schleicher and Schuell, Keene, New Hampshire), prehybridized, and hybridized according to the guidelines provided by the Tenth International Histocompatibility Workshop. The \(\text{DRB}\) (Long et al. 1982) and \(\text{DQA}\) (Auffray et al. 1982) cDNA probes were obtained from the Tenth International Histocompatibility Workshop. The \(\text{DQB}\) (pDQB7a) cDNA probe was kindly provided by J. Lee (Sloan-Kettering Memorial Center, New York, New York). Complementary DNA probes were labeled by the random primer method (Feinberg and Vogelstein 1984). The relative mass of the restriction fragments obtained in this study was determined with the aid of the DIGI-GEL computer program (DNASTAR, Madison, Wisconsin).

**Results**

Table 1 summarizes the results of analyzing genomic DNA from peripheral blood leukocytes or lymphoblastoid cell lines from homozygotes and families with independent examples of extended haplotypes carrying \(\text{HLA}\)-

<table>
<thead>
<tr>
<th>Extended haplotype</th>
<th>(\text{Taq I fragments in kb})</th>
<th>(\text{Pst I fragments in kb})</th>
<th>Number of independent examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>([\text{B8,SC01,DR3,DRw52,DQw2a}])</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\text{DRB})</td>
<td>10.1, 7.0</td>
<td>5.6, 5.0, 2.45</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>2.3, 2.0, 1.4, 1.3</td>
<td></td>
</tr>
<tr>
<td>(\text{DQB})</td>
<td>4.3, 2.6</td>
<td>8.2, 5.1, 2.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.6, 1.2</td>
<td>2.6, 2.1</td>
<td></td>
</tr>
<tr>
<td>(\text{DQA})</td>
<td>4.6, 2.2</td>
<td>8.0, 4.3</td>
<td></td>
</tr>
<tr>
<td>([\text{B18,F1C30,DR3,DRw52,DQw2b}])</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\text{DRB})</td>
<td>11.4*, 7.0</td>
<td>5.6, 5.0, 2.45</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>2.3, 2.0, 1.4</td>
<td></td>
</tr>
<tr>
<td>(\text{DQB})</td>
<td>4.3, 2.6</td>
<td>5.1, 4.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.6, 1.2</td>
<td>2.6, 2.1</td>
<td></td>
</tr>
<tr>
<td>(\text{DQA})</td>
<td>4.6, 2.1</td>
<td>7.2, 4.3</td>
<td></td>
</tr>
<tr>
<td>([\text{B44,FC31,DR7,DRw53,DQw2c}])</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>([\text{Bw47,FC91,0,DR7,DRw53,DQw2c}])</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\text{DRB})</td>
<td>13.1†, 7.0</td>
<td>5.6, 5.0, 2.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.0, 2.6</td>
<td>2.1, 1.4</td>
<td></td>
</tr>
<tr>
<td>(\text{DQB})</td>
<td>6.5, 4.3</td>
<td>5.1, 4.2, 2.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.6, 1.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\text{DQA})</td>
<td>5.4, 2.1</td>
<td>7.2, 2.3</td>
<td></td>
</tr>
<tr>
<td>([\text{Bw57,SC61,DR7,DRw53,DQw2}])</td>
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<tr>
<td>(\text{DRB})</td>
<td>13.1†, 5.8</td>
<td>5.6, 5.0, 2.6</td>
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<tr>
<td></td>
<td>4.0, 2.6</td>
<td>2.1, 1.4</td>
<td></td>
</tr>
<tr>
<td>(\text{DQB})</td>
<td>4.3, 2.6, 2.4</td>
<td>5.0, 4.2, 2.1</td>
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<tr>
<td></td>
<td>2.3, 1.8</td>
<td></td>
<td></td>
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<tr>
<td>(\text{DQA})</td>
<td>5.4, 2.1</td>
<td>8.0, 2.3</td>
<td></td>
</tr>
</tbody>
</table>

Underlined fragments were unique in defining \(\text{DR3,DQw2}\) and \(\text{DR7,DQw2}\) subtypes.

* \(\text{DRBI}^{III}\) fragment.

† \(\text{DRBIV}\) fragment.