The single $DR_b$ gene of the $DRw8$ haplotype is closely related to the $DR_b3III$ gene encoding $DRw52$

Göran Andersson¹*, Bertil Lindblom², Leif Andersson³, Jack Gorski⁴, Bernard Mach⁴, and Lars Rask¹

¹ Department of Cell Research, Uppsala University and Swedish University of Agricultural Sciences, Biomedical Center, Uppsala, Sweden
² State Institute for Blood Group Serology, University Hospital, Linköping, Sweden
³ Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Biomedical Center, Uppsala, Sweden
⁴ Department of Microbiology, University of Geneva Medical School, CH-1211 Geneva, Switzerland

Abstract. In most individuals two HLA-DR$_b$ genes are expressed from each chromosome. One of these genes encodes one of the classical DR specificities, while the other encodes either of the supertypic DRw52/DRw53 specificities. In addition to these genes usually one or two DR$_b$ pseudogenes are present. In contrast, the DRw8 chromosomal region only contains a single DR$_b$ gene. To determine the relationship of this single gene to the multiple DR$_b$ genes of other DR specificities, comparisons of Southern genomic blots were carried out. In this analysis genomic clones for each individual DR$_b$ gene locus were included. The DR$_b$w8 gene was indistinguishable from the DR$_b$III gene of DR3 cells (encoding DRw52), suggesting that it is closely related to the latter gene. The functional implications of this finding are discussed.

Introduction

Class II histocompatibility antigens in man are encoded by the HLA-D region. Serological, cellular, and, more recently, molecular cloning analyses have shown that this region is composed of three major subregions, HLA-DR, -DQ, and -DP. From each subregion at least one class II molecule consisting of an $\alpha$ and a $\beta$ chain is expressed (Albert et al. 1985). The class II antigens are extremely polymorphic (Bach 1985). A large number of serologically defined DR specificities are known. By the use of cellular methods several of these specificities have been further split. These specificities correspond to allelic series of DR$_b$ chains, while the DR$_b$ chain is invariant. Two separate allelic series of DR specificities, the classical DR and DRw52/DRw53, have been established by serological studies. The DR series involves a large number of alleles, while the DRw52/DRw53 polymorphism is much more restricted (reviewed in Mach et al. 1986). The DRw52 specificity is carried by DR3, 5, w6, and w8 individuals, whereas those with DR specificities 4, 7, and w9 have the DRw53 specificity. More than one DR$_b$ chain is expressed from each chromosome for most of the DR specificities (Rollini et al. 1985, Giles and Capra 1985). Epitopes corresponding to the supertypic specificities DRw52 and DRw53 are present on $\beta$ chains separate from those displaying the classical DR specificities (Tanigaki and Tosi 1982).

In addition to the sequence variabilty between DR alleles there is a variability in the number of DR$_b$ genes (Böhme et al. 1985). Molecular cloning and genomic hybridizations have shown that three DR$_b$ genes, designated I, II, and III, are present in the DR3, 5, and w6 haplotypes (Rollini et al. 1985). DR$_b$I encodes the DR3 specificity, DR$_b$III corresponds to the DRw52 epitope (Gorski et al. 1985), while DR$_b$II is a pseudogene. In the DR4 haplotype four DR$_b$ genes exist, two of which are pseudogenes, while the other two encode the DR4 and DRw53 specificities. Hybridization experiments have shown that the DR7 and DRw9 haplotypes have a gene organization similar to DR4 (Böhme et al. 1985, Andersson et al. 1987). These studies also indicated that the DR2 haplotype contains three and the DR1 haplotype two DR$_b$ genes. In the DRw8 haplotype a single DR$_b$ gene has been found (Böhme et al. 1985). The present study was undertaken to clarify the relationship of this single gene to the multiple genes encountered in the DR3 and DR4 haplotypes. Since detailed molecular information is available from the cloned DR$_b$ genes of the DR haplotypes DR3 and DR4 it is possible to assign every single restriction fragment hybridizing with a DR$_b$ probe in Southern hybridizations of restricted DNA of these haplotypes to a specific gene. To minimize allelic restriction site polymorphism which might make such a comparison impossible, we decided to use hybridization probes corresponding
to the reasonably well-conserved 3' portions of the $DR_\beta$ genes.

**Materials and methods**

**DNA sources.** DNA was prepared from the following homozygous typing cells that were selected from the typing panels used at the State Institute for Blood Group Serology, University Hospital, Linköping, Sweden: PH, DR1/1; IML, DR2/2; DL, DR3/3; SA, DR5/5; and IH, DR7/7.

Cell lines SPL, DRw8/w8 and KOZ, DRw9/DRw9 were obtained from Dr. J. Bodmer of the Tissue Antigen Laboratory, Imperial Cancer Research Fund Laboratories, London, England. Additional DNA was prepared from the cells used in our earlier work: BO, DR4/4 (Andersson et al. 1987) and GP, DRw6/w6 (Böhme et al. 1985).

**Hybridization probes.** Two $DR_\beta$-specific probes corresponding to (i) the cytoplasmic exon with flanking introns and (ii) the $DR_\beta$ 3'-untranslated exon were used (Table 1). After restriction enzyme digestions of the clones, the probe fragments were isolated by preparative agarose gel electrophoresis. Fragments were radioactively labeled to high specific activity with $\alpha$-32P deoxycytidine triphosphate by either nick-translation (Rigby et al. 1977) or random priming (Feinberg and Vogelstein 1984).

**DNA preparation and Southern blot analysis.** DNA was isolated from either 5 ml of peripheral blood according to the method of Böhme and co-workers (1983) or 500 µl of pelleted cultured cells as described by Grosveld and co-workers (1982). Samples of 10 µg of human DNA were digested to completion with restriction enzymes according to the manufacturer’s recommendations. The digests were separated by agarose gel electrophoresis in 0.7 and 1.5% gels, in 1 x TBE (89 mM Tris-borate buffer, pH 8.3, 2.5 mM ethylenediaminetetraacetate). Southern transfer of restricted DNA to Biodyne hybridization membranes (Pall) was made overnight with 1 x SSC (0.015 M sodium citrate buffer, pH 7.0, 0.15 M NaCl). Hybridizations were carried out in 40% formamide at 42°C. Washings were performed at high stringency (0.2 x SSC, 0.25% sodium dodecyl sulfate) at 58°C for 1 h.

**Results and Discussion**

To define the relationship of the $DR_\beta$ gene carried by DRw8 individuals to those of the DR3 and DR4 haplotypes, genomic DNA samples from homozygous typing cells of DR specificities 1 to w9 were separately digested with Pst I, Bam HI, and Hind III and hybridized to a probe corresponding to exon 5 of $DR_\beta$ genes (Table 1). Hybridization to Hind III-digested DNA is shown in Figure 1. In all cases a single hybridizing fragment was found for the DRw8 cells, which is in agreement with the proposal that this haplotype contains only a single $DR_\beta$ gene. A single $DR_\beta$ fragment in DRw8 cells was also obtained when genomic DNA digested with Pvu II was

**Table 1. Documentation of hybridization probes**

<table>
<thead>
<tr>
<th>Probe corresponding to</th>
<th>Type of clone</th>
<th>Designation of clone</th>
<th>Restriction sites employed</th>
<th>Size of probe fragment (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$DR_\beta$ exon 5 and intron</td>
<td>Genomic</td>
<td>p801-2</td>
<td>Bgl II</td>
<td>376</td>
<td>Larhammar et al. 1985</td>
</tr>
<tr>
<td>$DR_\beta$ exon 6</td>
<td>cDNA</td>
<td>pII-β-4</td>
<td>Pst I</td>
<td>220</td>
<td>Gustafsson et al. 1984</td>
</tr>
</tbody>
</table>

**Fig. 1.** Hybridization with the $DR_\beta$ exon 5-specific probe to Hind III-restricted human genomic DNA corresponding to DR specificities 1 to w9. The sizes of restriction fragments are given in kilobases

**Fig. 2.** Restriction fragment patterns of human genomic DNA, representing the same individuals as in Figure 1, digested with Pvu II and hybridized with the $DR_\beta$ 3'-untranslated probe. The sizes of restriction fragments are given in kilobases