An intragenic recombinant class I gene: H-2D<sup>dx</sup>

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Received January 22, 1991; revised version received March 11, 1991

H-2<sup>dx</sup> was first identified in the GRS mouse (Zacharova et al. 1975) and subsequently observed in the C3H.LG line (Ivanyi and Demant 1982). This haplotype is a natural recombinant encoding the K<sup>d</sup> allele and a unique allele in the D region characterized by a private specificity designated H-2.63 (Zacharova et al. 1975; Snoek et al. 1979). The I region of the dx haplotype is related serologically to the H-2<sup>f</sup> (Okuda et al. 1978), although restriction fragment length polymorphism (RFLP) and DNA sequence studies indicate that this is a cross-reaction and does not signify structural identity (Donovan et al. 1988). Capping and sequential immunoprecipitation experiments suggested the presence of H-2.1 positive, H-2.63 negative molecules in the products of the D region of H-2<sup>a</sup> (Snoek et al. 1979; Ivanyi and Demant 1982). This observation is the basis of the hypothesis that the H-2<sup>a</sup> D region encodes more than one expressed class I molecule that functions in antigen presentation, as seen in the H-2<sup>q</sup> and H-2<sup>q</sup> haplotypes. Similar studies suggested complexity in the D regions of H-2<sup>d</sup> and H-2<sup>k</sup> (Ivanyi et al. 1979). In the case of H-2<sup>d</sup> two gene products were ultimately identified on the cell surface (Hansen et al. 1983). However, the H-2<sup>k</sup> haplotype is now known to encode only a single D region product (Stephan et al. 1986) raising the possibility that capping and sequential precipitation studies may be distinguishing between post transcriptionally modified products derived from the D<sup>k</sup> locus. The exact structure and number of genes encoded by the H-2<sup>dx</sup> D region is unknown.

In the current study, the cDNA of H-2D<sup>dx</sup> was cloned using a locus specific approach. Total cellular RNA was prepared from spleen cells of B10.LG/DV mice from the immunogenetic colony at the Mayo Clinic. Twenty micrograms of the total cellular RNA was used for 5' RACE (rapid amplification of cDNA ends). Locus specific amplification of the D region gene was accomplished with a pair of D region specific primers using polymerase chain reaction (PCR; Cai and Pease 1990). The double strand cDNA was cloned into the vector pUC 18. The DNA sequences of three clones were determined throughout both strands and another seven clones were analyzed in the structurally diverse region encompassing the antigen binding domain. The sequences of all ten clones were identical except for occasional PCR associated point mutations that were confined to individual clones. The single identified sequence represented a typical D region gene and was designated H-2D<sup>dx</sup>. The cDNA sequence of the D<sup>dx</sup> gene and its comparison with the D<sup>p</sup> and D<sup>d</sup> genes are shown in Figure 1. The cDNA is 1405 base pairs (bp) long and has two ATG sites at the 5' end of the gene. It also has two D/L genes associated characteristics, a SINE 2 insertion in the 3' untranslated region and the short form of exon 8, resulting from alternative splicing (Handy et al. 1988). The cDNA also has a 9 bp insertion at the region encoding for the transmembrane region of the protein, a motif also found in the D<sup>d</sup>, D<sup>f</sup>, and D/L<sup>v</sup> alleles (Pease et al. 1991). The sequences coding for the leader peptide and the first 48 amino acids of the α1 domain are identical to the D<sup>p</sup> gene (Schepart et al. 1986). The coding regions of the gene 3' of the exon encoding the α2 domain and the 3' untranslated region are the same as those found in the D<sup>d</sup> gene with the exception of two silent nucleotide differences. The sequence coding for the carboxy-terminus of the α1 domain and the entire α2 domain are different from all other known class I genes.

The observation that the putative D<sup>dx</sup> glycoprotein expressed by B10.LG/DV mice is structurally identical to D<sup>d</sup>, carboxyterminal to the α2 region, indicated that the D<sup>dx</sup> specific mAb 34-2-12 (reactive with the α3 region) should react with the encoded D<sup>dx</sup> glycoprotein. Peripheral mononuclear cells from B10.GSR/DV (congenic for H-2 from GRS and B10.LG mice were analyzed by