Genetic mapping of the LMP2 proteasome subunit gene to the BoLA class IIb region

Recent identification of four tightly-linked genes within the class II region of the major histocompatibility complex (MHC) in humans and rodents has led to a better understanding of class I antigen processing mechanisms (recent reviews include Monaco 1992; Barber and Parham 1993; Momburg et al. 1994). Two of these genes, LMP2 and LMP7, encode subunits of a low molecular mass polypeptide (LMP) complex (Monaco 1992; Howard and Seelig 1993). Several observations suggest that the LMP complex may be the proteolytic system responsible for generating the size-restricted peptides required for MHC class I assembly. For example, the LMP complex is a large cytoplasmic structure that is antigenically and biochemically related to the proteasome, a proteolytic complex that mediates degradation of ubiquitinated substrates (Driscoll and Goldberg 1990; Brown et al. 1991). Data regarding proteolytic specificity indicates that the LMP complex may specifically produce nonamers, the appropriate peptide size for class I binding (Driscoll et al. 1993). In addition, similar to all components of the class I assembly process, intra-MHC LMP genes are regulated by IFNγ (Yang et al. 1992; Gaczynska et al. 1993).

As polymorphism of intra-MHC antigen processing genes has been shown to alter the antigenic and biochemical properties of cell surface class I molecules (Barber and Parham 1993; Momburg et al. 1994), these loci might also be important determinants of MHC-associated disease susceptibility. As an initial step to understanding possible relationships between genetic variants of the antigen processing genes and disease resistance in cattle, the present study was undertaken to determine the map location of cattle LMP2 and to investigate structural polymorphism of this gene among cattle breeds.

As an initial screen for LMP2 polymorphism, DNA from nine cattle breeds (Ayshire, Brown Swiss, Gelbvieh, Guernsey, Holstein, Jersey, Red Angus, Salers, Simmental) was digested with the restriction endonucleases DraI, EcoRI, HaeIII, HhaI, HindIII, PstI, and TaqI, and analyzed by standard Southern blot procedures, using an 806 base pair mouse Lmp2 probe (Martinez and Monaco 1991). A two-allele EcoRI polymorphism consisting of two variable LMP2 fragments and one monomorphic fragment was revealed (Fig. 1). The smallest variable fragment was 6.5 kilobases (kb) and the larger fragment was 10.6 kb. Alleles were designated LMP2*A1 for the fastest migrating fragment (6.5 kb) and LMP2*A2 for the slower migrating fragment (10.6 kb). The frequency of LMP2*A1 and LMP2*A2 among a population of 78 dams of random breeds was 0.378 and 0.622, respectively.

Following identification of the Eco RI-LMP2 polymorphism, four sires of the Illinois Reference/Resource Families (IRRF), a collection of nine paternal half-sib families consisting of 459 offspring and 393 of their respective dams (purebred and crossbred) were screened by Southern blot analysis. These families, belonging to four beef cattle breeds (Angus, Devon, Gelbvieh, Simmental), were assembled to construct a high-resolution male-specific linkage map of the cattle genome (Lewin et al. 1994). Two sires heterozygous for LMP2 were identified. In addition to Southern blot analysis of LMP2 restriction fragment length polymorphisms (RFLPs), all nine IRRF family sires were screened for the microsatellites BM1258, BM47 (Bishop et al. 1994), and for DRB3, DYA, and PRL polymorphism with 8, 7, 8, 1 and 2 sires found to be heterozygous for each locus, respectively. Microsatellites were distinguished by polymerase chain reaction (PCR) according to a modified hot-start protocol (Beever et al. 1994) and DRB3 typing was performed by PCR-RFLP analysis as described previously (van Eijk et al. 1992b). PCR-RFLP analysis of PRL and DYA was performed, using 30 ng genomic DNA in 20 µl reactions with amplification conditions similar to those described previously (van Eijk et al. 1992a; van Eijk et al. 1993). Alleles were distinguished by Rsal or HincII digestion of PCR products for PRL and DYA, respectively.
Offspring of the LMP2 heterozygous sires and available dams were then typed for DRB3, DYA, LMP2, and PRL; offspring were also typed for the microsatellite markers BM1258 and BM47. Two-point LOD scores for LMP2 and the above BTA23 genetic markers are shown in Table 1. Linkage of LMP2 with DRB3 and BM1258 allows unequivocal assignment of LMP2 to the BoLA region on BTA23. Three loci, DRB3, BM1258, and BM47 contributed the informative meioses needed for ordering LMP2. No recombinants were observed between LMP2 and BM47 and between BM47 and DYA. A multipoint analysis of six BTA23-encoded loci, BM1258, BM47, DRB3, DYA, LMP2, and PRL, was performed. The most favored order is (BM47 – LMP2 – DYA) – BM1258 – DRB3 – PRL with map distances of 12.3, 6.3, and 6.8 cM, respectively (Fig. 2). This order was favored by odds of 2 x 10^5:1 over the next most likely order which placed the cluster containing BM47, LMP2, and DYA between BM1258 and DRB3.

Previously, a recombination rate (\(\theta\)) of 0.15 between DYA and DRB3 was determined by sperm typing (van Eijk et al. 1993), and gene-centromere mapping of BTA23 has placed DYA centromeric to both DRB3 and PRL (V. L. Jarrell, Y. Da, H. A. Lewin and M. B. Wheeler, submitted). Consolidating the above evidence with the results of the present study, \(\theta_{\text{LMP2-DRB3}} = 0.15\), \(\theta_{\text{LMP2-BM47}} = 0.00\) and \(\theta_{\text{DYA-BM47}} = 0.00\), shows that LMP2 is located centromeric to BM1258 and DRB3. Recombination distances between BM1258, DRB3, and PRL that contribute to the mapping of LMP2 in this study agree with the map proposed by Bishop and co-workers (1994), but are notably different from those reported by Barendse and co-workers (1994).