Organization of the Canine Major Histocompatibility Complex: Current Perspectives

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The dog is a valuable model for studying several human diseases as well as one of the most important models for organ transplantation. Important to understanding the pathophysiology or development of some of these diseases is an understanding of the canine major histocompatibility complex (MHC) or dog leukocyte antigen (DLA). Initial characterization of the DLA involved primarily cellular, serological, and biochemical analyses. Later a molecular analysis of the DLA region was begun. There are at least four complete class I genes: DLA-88, DLA-12, DLA-64, and DLA-79. DLA-88 is highly polymorphic, with more than 40 alleles obtained from an examination of 50 mixed breed dogs. The other class I loci are less polymorphic, with fewer than 12 alleles each. In the class II region there is one complete DRB gene called DLA-DRB1 with at least 24 alleles and one full-length DQB gene, DLA-DQB1, with 20 alleles characterized to date. DLA-DQA is less polymorphic with nine alleles and DLA-DRA appears monomorphic. Two highly polymorphic canine microsatellite markers, one located in the class I region and one located in the class II region, can be used to identify DLA-matched and -mismatched dogs within families for organ transplantation experiments. Future projects include mapping the DLA region by pulsed-field gel electrophoresis and using a recently constructed canine bacterial artificial chromosome (BAC) library to search for new genes within the DLA. The dog has been a useful model for understanding several human diseases such as gluten-sensitive enteropathy (Hall and Batt 1990), rheumatoid arthritis (Halliwell et al. 1972), narcolepsy (Tafti et al. 1996), and systemic lupus erythematosus (Lewis and Schwartz 1971, Teichner et al. 1990), as well as an important model for solid organ and hematopoietic stem cell transplantation (Storb and Deeg 1985). Much of the impetus behind efforts to characterize the canine MHC comes from its importance in transplantation. In spite of the dog's importance in studying human disease and in immunology, molecular analysis of the DLA has lagged behind that of the mouse and human as well as several agricultural animals.

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An understanding of the general structure and function of the MHC is helpful in order to comprehend its importance in transplantation and disease. The MHC is a linked cluster of genes and gene families and is one of the most extensively studied regions of the genome in several species, including humans and mice. In normal physiology, MHC gene products interact with bound peptide ligands and with products of rearranged T-cell receptor (TCR) genes in the thymus. This results in positive and negative selection of the peripheral T-cell repertoire. In other words, this region of tightly linked genes is responsible for the presentation of self and nonself antigens to the immune system and thus is fundamental in the recognition and regulation of immune response. MHC molecules perform these roles by binding and presenting peptide antigens to T cells. This antigen presentation can lead to several events including elimination of infected cells or cellular rejection of transplanted organs.

The genes within the MHC are divided into at least three types: class I, class II, and class III. The class I and class II molecules are the cell surface glycoproteins of similar structure involved in antigen presentation to T cells. Class III molecules are structurally unrelated to class I and class II molecules and are not relevant to antigen presentation. Class I antigens are expressed on all somatic cells, whereas class II molecules are expressed on antigen-pre-

senting cells such as macrophages. Of interest, in contrast to mice and humans, canine class II gene products are present on almost all lymphocytes (Doxiadis et al. 1989).

In the HLA region some loci are quite polymorphic. Most of this polymorphism occurs in or around the peptide binding site. Histocompatibility typing or determining which alleles are present is important not only for organ transplantation but also for studying disease association. In dogs, DLA typing may also be helpful in trying to diversify relatively inbred populations in certain breeds.

Biochemical Analyses

Early understanding of the canine MHC involved primarily cellular, serological, and immunochemical analyses. The DLA is divided into three serologically defined antigens DLA-A (with five specificities), DLA-B (with four specificities), and DLA-C (with three specificities) (Bull et al. 1987). A fourth antigen, DLA-D (with 10 specificities), is defined by mixed leukocyte culture (Deeg et al. 1986). These serological specificities depend on the reactivity of canine lymphocytes with various class I or class II antisera in a standardized microlymphocytotoxicity assay (Bull et al. 1987). Different class I or class II proteins on the cell surface (based on the allelic polymorphism) will react with different antisera. The DLA-A antigens are characterized as class I molecules by their association with β_2 microglobulin (Krumbacher et al. 1986). An immunochemical analysis of glycosylated and nonglycosylated DLA molecules suggests that the products of one predominant DLA class I locus are present on the surface of peripheral blood leukocytes, and DLA-C antigens are thought to be weakly expressed class I antigens (Doxiadis et al. 1986; Van der Feltz and Ploegh 1984). Conversely, DLA-B antigens when studied by two-dimensional gel electrophoresis and lysostrip experiments exhibit typical class II properties, with a high level of serological polymorphism in the beta chain and no serological polymorphism in the alpha chain (Doxiadis et al. 1989). DLA-A and DLA-B gene products have been defined by one-dimensional isoelectric focusing and immunoblotting, and there is a high degree of correlation between the biochemically defined antigens and the serological specificities (Kubens et al. 1995).

Molecular Analyses—Genes and Polymorphism

Class I molecules such as HLA-A, -B, and -C are heterodimeric glycoproteins that were initially defined by antisera. The molecules contain a polymorphic membrane-bound α chain (45 kD) that is noncovalently associated with β_2 microglobulin (a nonpolymorphic product of a non-MHC-linked gene).

The structure of class II genes is well conserved among mammalian species, and orthologous relationships exist, for example, between canine and human class II genes. This is in contrast to class I genes, where gene families within each mammalian order have arisen independently. Therefore class II genes in each mammalian species may have the same basic name, such as DRB, whereas class I genes do not have the same names outside each mammalian order. Class II molecules in humans, such as HLA-DR, -DQ, -DM, and -DP, exist as $\alpha\beta$ heterodimers composed of an α chain (MW 34 kD) and a β chain (MW 29 kD).

Beginning in the late 1980s a molecular analysis of the canine MHC began. Using an HLA-B7 (a human class I) cDNA probe and by studying the patterns on Southern analysis from 40 dogs, Sarmiento and Storb (1989) concluded that there are approximately eight canine class I genes. They used a similar approach with various human class II probes to study the number of class IIA and IIB genes (Sarmiento and Storb 1988a,b). Using reverse transcriptase-polymerase chain reaction (RT-PCR), Sarmiento and coworkers found that at least three class II loci—DRB, DQA, and DQB—are polymorphic (Sarmiento et al. 1990, 1992, 1993). The DRA locus appears to be monomorphic (Wagner et al. 1995).

Recently a better understanding of the location and organization of the canine MHC has emerged. Using the technique of fluorescence in situ hybridization (FISH), the DLA has been localized to chromosome 12 (Dutra et al. 1996). The probes for the FISH study were made from genomic clones of class I and class II loci. These genomic clones were isolated using canine cDNA probes that had been previously isolated using HLA probes. In the case of the class I loci, a canine cDNA library was screened with an HLA-B7 probe and only one clone was isolated, designated I16 (Sarmiento and Storb 1990a). Using I16 as a probe, seven distinct canine class I loci have been isolated by screening a genomic library (Burnett et al. 1997; Burnett and Geraghty 1995). One locus designated DLA-79 has shown limited polymorphism and relatively low mRNA expression (in a wide variety of tissues), and thus has been designated a class Ib gene (Burnett and Geraghty 1995). Classical class I (Ia) loci, in contradistinction to Ib loci, also tend to be transcribed at higher levels in more tissues and tend to be more polymorphic than class Ib loci. Class Ia loci are more important in transplantation biology because of their greater contribution to alloreactivity and organ rejection.

Three other class I loci—DLA-88, DLA-12, and DLA-64—appear to be complete genes by sequence analysis, and all three are transcribed in canine peripheral blood leukocytes (Burnett et al. 1997). DLA-88 appears to be more polymorphic than DLA-12, DLA-79, or DLA-64 (Graumann et al. 1998). Two other genes, termed DLA-53 and DLA-12a, are truncated class I pseudogenes (Burnett et al. 1997). C1pg-26 is a processed gene located outside the DLA (Burnett et al. 1997). Neither the tissue expression nor the function of any of the class I genes are known at present, although one could infer by analogy from other species that DLA class I gene products could serve as cytotoxic T-lymphocyte targets.

Using methods similar to those described above for class I loci, several class II loci have been characterized. Using a human DRB cDNA probe, a canine DRB clone called DRB5 was isolated (Sarmiento and Storb 1990b). This canine cDNA clone was used to screen a genomic library. From these experiments one highly polymorphic DRB gene, designated DLA-DRB1, and a pseudogene termed DLA-DRB2 were cloned and sequenced (Wagner et al. 1996c,d). Similar strategies were used for the DQ loci. There is one DQA gene with a limited amount of polymorphism (Wagner et al. 1996b). There is one polymorphic DQB gene and one DQB pseudogene (Wagner et al. 1998a). A summary of known DLA loci is shown in Table 1.

In all these situations random mixed breed dogs were used to determine the degree of polymorphism present at each locus. With the exception of DLA-64, almost all of these polymorphisms resulted in changes in the amino acid sequence, and the location of most of these substitutions was in the putative peptide binding site (Burnett et al. 1997; Burnett and Geraghty 1995; Graumann et al. 1998; Wagner et al. 1995; Wagner et al. 1996b,d, 1998a). The number of amino acid differences between alleles varied from one to five (Burnett et

Table 1. Known DLA loci and their polymorphism

Locus name	Gene type	Class	Number of alleles known	Number of dogs analyzed
DLA-DRA	Complete	II	1	15
DLA-DRB1	Complete	II	24	150
DLA-DRB2	Pseudo	II	—	—
DLA-DQA	Complete	II	9	60
DLA-DQB1	Complete	II	20	70
DLA-DQB2	Pseudo	II	—	—
DLA-88	Complete	I?a	44	50
DLA-79	Complete	Ib	11	40
DLA-12	Complete	I?a or b	3	20
DLA-64	Complete	I?a or b	3	20
DLA-53	Pseudo	I	—	—
DLA-12a	Pseudo	I	—	—

References: Burnett et al. 1997; Burnett and Geraghty 1995; Graumann et al. 1998; Wagner et al. 1995, 1996b,d, 1998a.

al. 1997; Burnett and Geraghty 1995a; Graumann et al. 1998; Wagner et al. 1995, 1996b,d, 1998a).

Histocompatibility Typing

Currently intrafamilial histocompatibility typing is done using two polymorphic satellite markers—one located in the class I region (C.2200) near DLA-53 and one located in the class II region (C.2202) near DLA-DRBB2 (Wagner et al. 1996a). Both of these markers are tetranucleotide repeats of $(GAAA)_n$. Both markers were identified during the sequencing of genomic clones. A polymorphism informational content (PIC) value of 0.804 (Burnett et al. 1995) was obtained for C.2200 and a value of 0.947 (Wagner et al. 1996a) was obtained for C.2202. Analysis of both markers in over 30 families has shown that each is stable for following Mendelian inheritance through multigeneration families. Because of the high polymorphic index of these markers and their stability, they are useful for determining the inheritance of DLA haplotypes within families. DLA matching of dogs within families determines which littermates are best suited for organ transplantation experiments.

Microsatellite markers are not always suitable for finding DLA-matched unrelated dogs for transplantation experiments because they do not identify the genotype of any loci. Genotyping unrelated dogs at each polymorphic locus is an area of current investigation. Francino et al. (1997) have recently used PCR-restriction fragment length polymorphism (RFLP) analysis for DLA-DRB1 genotyping.

Future Directions

Understanding of the canine MHC still is considerably behind that of the human and the mouse. Active research is now ongoing to determine the tissue distribution of the class I genes as well as their function. As part of this endeavor it will be important to correlate earlier immunochemical data with more recent molecular data, for example, determine which are the major class I loci expressed on peripheral blood leukocytes and what is the role of genes such as DLA-79 in immune function.

The availability of a canine bacterial artificial chromosome (BAC) library, the use of canine/rodent hybrid cell lines, and the use of pulsed-field gel electrophoresis will aid in the composition of a physical map of the canine MHC. The BAC library will also serve as a tool for characterizing new genes within the canine MHC. For histocompatibility typing, current efforts are focused on defining alleles in unrelated dogs (genotyping). PCR single-stranded conformational polymorphism combined with sequence-based typing (Wagner et al. 1998b) or sequence-specific oligonucleotide probes may prove to be useful techniques to achieve this goal. Other studies are in progress to determine the phenotype frequencies for various alleles within individual breeds or crossbreeds and the full extent of polymorphism at each locus.

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