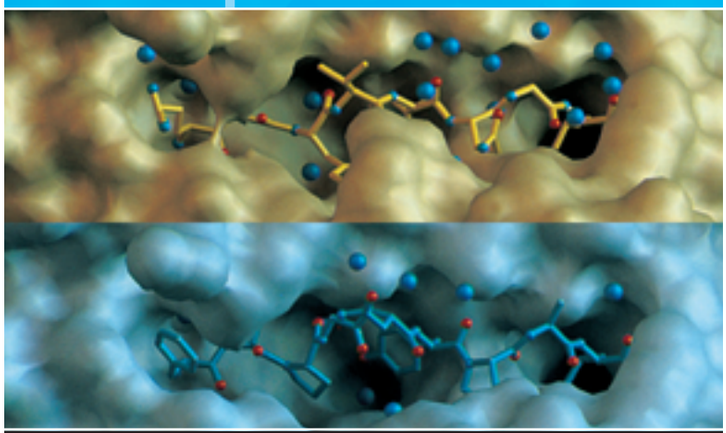
chapter 7

Major Histocompatibility Complex



Presentation of Vesicular Stomatitis Virus Peptide (top) and Sendai Virus Nucleoprotein Peptide by Mouse MHC Class I Molecule H-2K^b

Every mammalian species studied to date possesses a tightly linked cluster of genes, the **major histocompatibility complex (MHC),** whose products play roles in intercellular recognition and in discrimination between self and nonself. The MHC participates in the development of both humoral and cell-mediated immune responses. While antibodies may react with antigens alone, most T cells recognize antigen only when it is combined with an MHC molecule. Furthermore, because MHC molecules act as antigen-presenting structures, the particular set of MHC molecules expressed by an individual influences the repertoire of antigens to which that individual's T_H and T_C cells can respond. For this reason, the MHC partly determines the response of an individual to antigens of infectious organisms, and it has therefore been implicated in the susceptibility to disease and in the development of autoimmunity. The recent understanding that natural killer cells express receptors for MHC class I antigens and the fact that the receptor–MHC interaction may lead to inhibition or activation expands the known role of this gene family (see Chapter 14). The present chapter examines the organization and inheritance of MHC genes, the structure of the MHC molecules, and the central function that these molecules play in producing an immune response.

General Organization and Inheritance of the MHC

The concept that the rejection of foreign tissue is the result of an immune response to cell-surface molecules, now called **histocompatibility antigens,** originated from the work of Peter Gorer in the mid-1930s. Gorer was using inbred strains of mice to identify blood-group antigens. In the course of these studies, he identified four groups of genes, designated I through IV, that encoded blood-cell antigens. Work carried out in the 1940s and 1950s by Gorer and George Snell established that antigens encoded by the genes in the group designated II took part in the rejection of transplanted tumors and other tissue. Snell called these genes "histocompatibility genes"; their current designation as histocompatibility-2 (H-2) genes was in reference to Gorer's group II blood-group antigens. Although Gorer died before his contributions were recognized fully, Snell was awarded the Nobel prize in 1980 for this work.

The MHC Encodes Three Major Classes of Molecules

The major histocompatibility complex is a collection of genes arrayed within a long continuous stretch of DNA on chromosome 6 in humans and on chromosome 17 in mice. The MHC is referred to as the **HLA complex** in humans and as the **H-2 complex** in mice. Although the arrangement of genes is somewhat different, in both cases the MHC genes are organized into regions encoding three classes of molecules (Figure 7-1):

- **Class I MHC genes** encode glycoproteins expressed on the surface of nearly all nucleated cells; the major function of the class I gene products is presentation of peptide antigens to T_C cells.

VISUALIZING CONCEPTS

Mouse H-2 complex

Complex	H-2						
MHC class	I	II		III		I	
Region	K	IA	IE	S		D	
Gene products	H-2K	IA αβ	IE αβ	C′ proteins	TNF-α TNF-β	H-2D	H-2L

Human HLA complex

Complex	HLA							
MHC class	II			III		I		
Region	DP	DQ	DR	C4, C2, BF		B	C	A
Gene products	DP αβ	DQ αβ	DR αβ	C′ proteins	TNF-α TNF-β	HLA-B	HLA-C	HLA-A

FIGURE 7-1 Simplified organization of the major histocompatibility complex (MHC) in the mouse and human. The MHC is referred to as the H-2 complex in mice and as the HLA complex in humans. In both species the MHC is organized into a number of regions encoding class I (pink), class II (blue), and class III (green) gene products. The class I and class II gene products shown in this figure are considered to be the classical MHC molecules. The class III gene products include complement (C′) proteins and the tumor necrosis factors (TNF-α and TNF-β).

- **Class II MHC genes** encode glycoproteins expressed primarily on antigen-presenting cells (macrophages, dendritic cells, and B cells), where they present processed antigenic peptides to T_H cells.

- **Class III MHC genes** encode, in addition to other products, various secreted proteins that have immune functions, including components of the complement system and molecules involved in inflammation.

Class I MHC molecules encoded by the K and D regions in mice and by the A, B, and C loci in humans were the first discovered, and they are expressed in the widest range of cell types. These are referred to as *classical class I molecules.* Additional genes or groups of genes within the H-2 or HLA complexes also encode class I molecules; these genes are designated *nonclassical class I genes.* Expression of the nonclassical gene products is limited to certain specific cell types. Although functions are not known for all of these gene products, some may have highly specialized roles in immunity. For example, the expression of the class I HLA-G molecules on cytotrophoblasts at the fetal-maternal interface has been implicated in protection of the fetus from being recognized as foreign (this may occur when paternal antigens begin to appear) and from being rejected by maternal T_C cells.

The two chains of the class II MHC molecules are encoded by the IA and IE regions in mice and by the DP, DQ, and DR regions in humans. The terminology is somewhat confusing, since the D region in mice encodes class I MHC molecules, whereas the D region (DR, DQ, DP) in humans refers to genes encoding class II MHC molecules! Fortunately, the designation D for the general chromosomal location encoding the human class II molecules is seldom used today; the sequence of the entire MHC region is available so the more imprecise reference to region is seldom necessary. As with the class I loci, additional class II molecules encoded within this region have specialized functions in the immune process.

The class I and class II MHC molecules have common structural features and both have roles in antigen processing. By contrast, the class III MHC region, which is flanked by the class I and II regions, encodes molecules that are critical to immune function but have little in common with class I or II molecules. Class III products include the complement components C4, C2, BF (see Chapter 13), and inflammatory cytokines, including tumor necrosis factor (TNF) and heat-shock proteins (see Chapter 12).

Allelic Forms of MHC Genes Are Inherited in Linked Groups Called Haplotypes

As described in more detail later, the loci constituting the MHC are highly **polymorphic;** that is, many alternative forms of the gene, or **alleles,** exist at each locus among the population. The genes of the MHC loci lie close together; for example, the recombination frequency within the H-2 complex (i.e., the frequency of chromosome crossover events during mitosis, indicative of the distance between given gene segments) is only 0.5%—crossover occurs only once in every 200 mitotic cycles. For this reason, most individuals inherit the alleles encoded by these closely linked loci as two sets, one from each parent. Each set of alleles is referred to as a **haplotype.** An individual inherits one haplotype from the mother and one haplotype from the father. In outbred populations, the offspring are generally heterozygous at many loci and will express both maternal and paternal MHC alleles. The alleles are *codominantly expressed;* that is, both maternal and paternal gene products are expressed in the same cells. If mice are inbred (that is, have identical alleles at all loci), each H-2 locus will be homozygous because the maternal and paternal haplotypes are identical, and all offspring therefore express identical haplotypes.

Certain inbred mouse strains have been designated as prototype strains, and the MHC haplotype expressed by these strains is designated by an arbitrary italic superscript (e.g., H-2^a, H-2^b). These designations refer to the entire set of inherited H-2 alleles within a strain without having to list each allele individually (Table 7-1). Different inbred strains may have the same set of alleles, that is the same MHC haplotype, as the prototype strain. For example, the CBA, AKR, and C3H strains all have the same MHC haplotype (H-2^k). The three strains differ, however, in genes outside the H-2 complex.

If two mice from inbred strains having different MHC haplotypes are bred to one another, the F_1 generation inherits haplotypes from both parental strains and therefore expresses both parental alleles at each MHC locus. For example, if an H-2^b strain is crossed with an H-2^k, then the F_1 inherits both parental sets of alleles and is said to be H-2$^{b/k}$ (Figure 7-2a). Because such an F_1 expresses the MHC proteins of both parental strains on its cells, it is histocompatible with both strains and able to accept grafts from either parental strain (see example in Figure 7-2b). However, neither of the inbred parental strains can accept a graft from the F_1 mice because half of the MHC molecules will be foreign to the parent.

The inheritance of HLA haplotypes from heterozygous human parents is illustrated in Figure 7-2c. In an outbred population, each individual is generally heterozygous at each locus. The human HLA complex is highly polymorphic and multiple alleles of each class I and class II gene exist. However, as with mice, the human MHC loci are closely linked and usually inherited as a haplotype. When the father and mother have different haplotypes, as in the example shown (Figure 7-2c) there is a one-in-four chance that siblings will inherit the same paternal and maternal haplotypes and therefore be histocompatible with each other; none of the offspring will be histocompatible with the parents.

Although the rate of recombination by crossover is low within the HLA, it still contributes significantly to the diversity of the loci in human populations. Genetic recombination generates new allelic combinations (Figure 7-2d), and the high number of intervening generations since the appearance of humans as a species has allowed extensive recombination, so that it is rare for any two unrelated individuals to have identical sets of HLA genes.

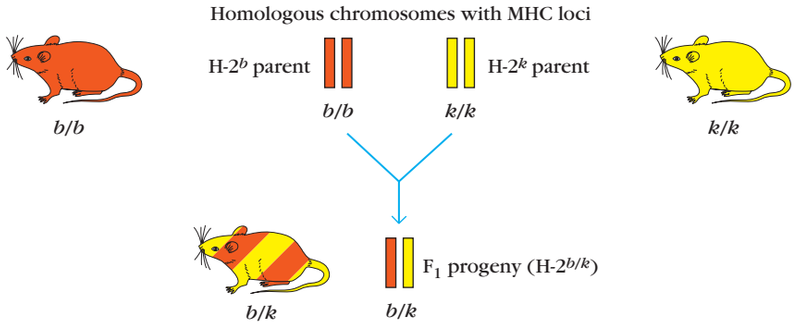
MHC Congenic Mouse Strains Are Identical at All Loci Except the MHC

Detailed analysis of the H-2 complex in mice was made possible by the development of congenic mouse strains. Inbred mouse strains are **syngeneic** or identical at all genetic loci. Two strains are **congenic** if they are genetically identical

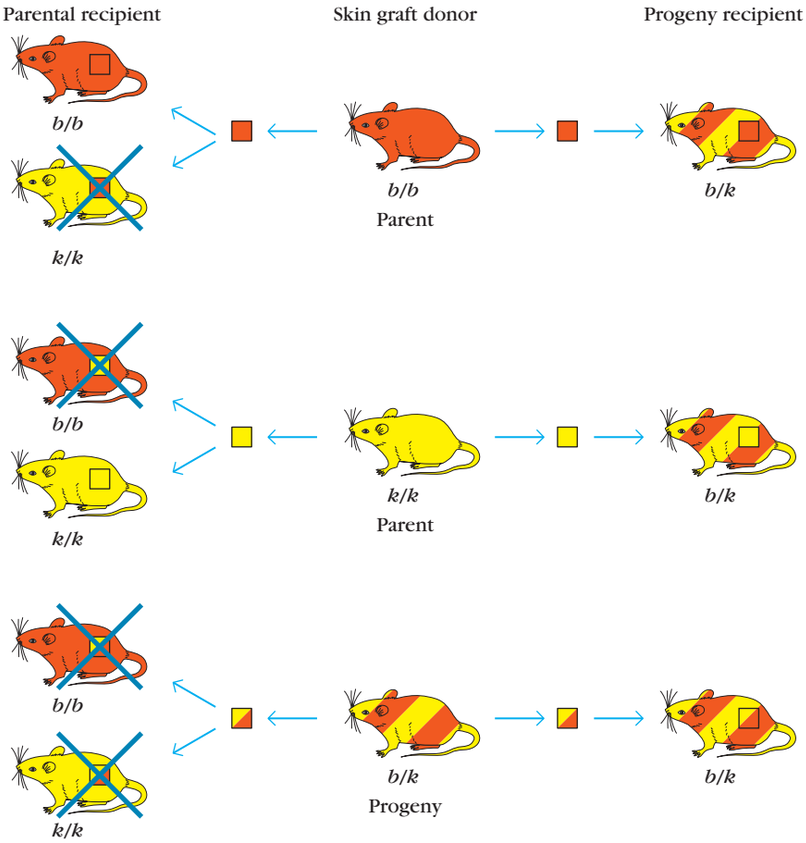
TABLE 7-1 H-2 Haplotypes of some mouse strains

			H-2 ALLELES				
Prototype strain	Other strains with the same haplotype	Haplotype	*K*	*IA*	*IE*	*S*	*D*
CBA	AKR, C3H, B10.BR, C57BR	*k*	*k*	*k*	*k*	*k*	*k*
DBA/2	BALB/c, NZB, SEA, YBR	*d*	*d*	*d*	*d*	*d*	*d*
C57BL/10 (B10)	C57BL/6, C57L, C3H.SW, LP, 129	*b*	*b*	*b*	*b*	*b*	*b*
A	A/He, A/Sn, A/Wy, B10.A	*a*	*k*	*k*	*k*	*d*	*d*
A.SW	B10.S, SJL	*s*	*s*	*s*	*s*	*s*	*s*
A.TL		*t1*	*s*	*k*	*k*	*k*	*d*
DBA/1	STOLI, B10.Q, BDP	*q*	*q*	*q*	*q*	*q*	*q*

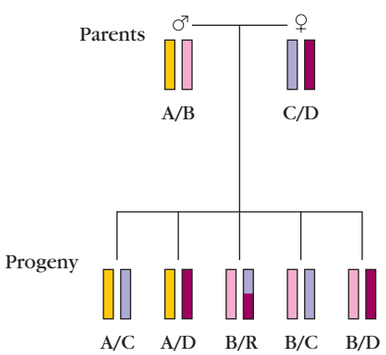
(a) Mating of inbred mouse strains with different MHC haplotypes



(b) Skin transplantation between inbred mouse strains with same or different MHC haplotypes



(c) Inheritance of HLA haplotypes in a typical human family



(d) A new haplotype (R) arises from recombination of maternal haplotypes

	HLA Alleles					
	A	B	C	DR	DQ	DP
A	1	7	w3	2	1	1
B	2	8	w2	3	2	2
Haplotypes C	3	44	w4	4	1	3
D	11	35	w1	7	3	4
R	3	44	w4	7	3	4

FIGURE 7-2 (a) Illustration of inheritance of MHC haplotypes in inbred mouse strains. The letters *b/b* designate a mouse homozygous for the H-2^b MHC haplotype, *k/k* homozygous for the H-2^k haplotype, and *b/k* a heterozygote. Because the MHC loci are closely linked and inherited as a set, the MHC haplotype of F1 progeny from the mating of two different inbred strains can be predicted easily. (b) Acceptance or rejection of skin grafts is controlled by the MHC type of the inbred mice. The progeny of the cross between two inbred strains with different MHC haplotypes (H-2^b and H-2^k) will express both haplotypes (H-2$^{b/k}$) and will accept grafts from either parent and from one another. Neither parent strain will accept grafts from the offspring. (c) Inheritance of HLA haplotypes in a hypothetical human family. In humans, the paternal HLA haplotypes are arbitrarily designated A and B, maternal C and D. Because humans are an outbred species and there are many alleles at each HLA locus, the alleles comprising the haplotypes must be determined by typing parents and progeny. (d) The genes that make up each parental haplotype in the hypothetical family in (c) are shown along with a new haplotype that arose from recombination (R) of maternal haplotypes.

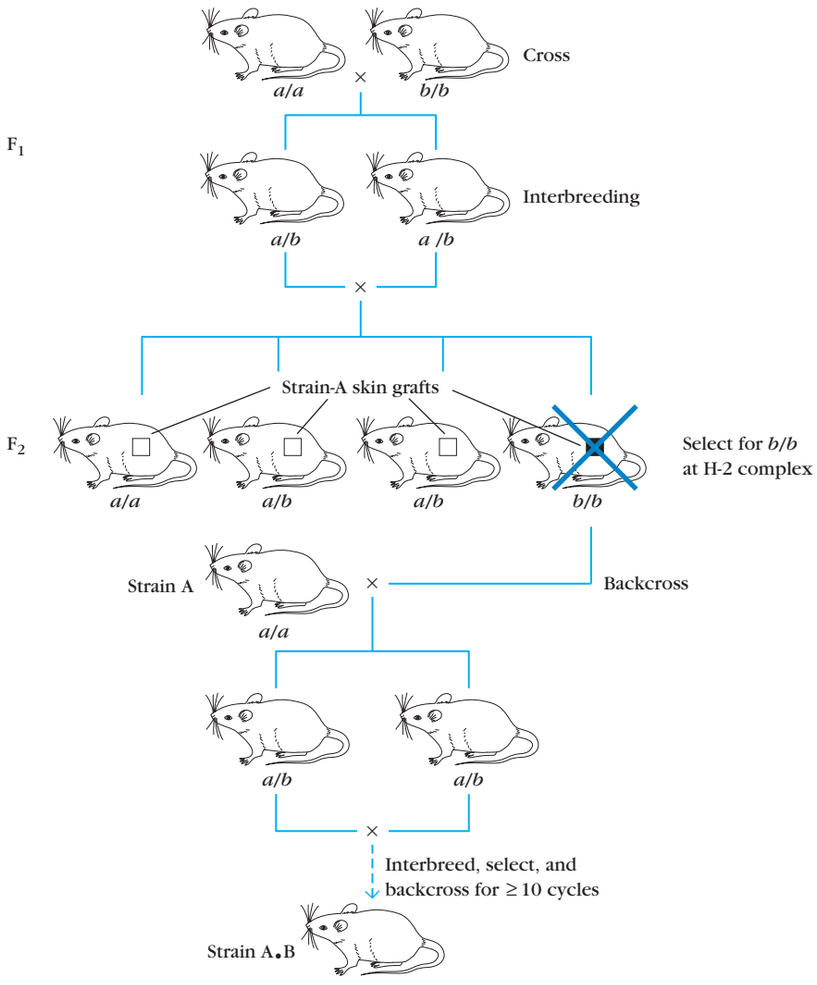


FIGURE 7-3 Production of congenic mouse strain A.B, which has the genetic background of parental strain A but the H-2 complex of strain B. Crossing inbred strain A (H-2^a) with strain B (H-2^b) generates F_1 progeny that are heterozygous (*a/b*) at all H-2 loci. The F_1 progeny are interbred to produce an F_2 generation, which includes *a/a, a/b,* and *b/b* individuals. The F_2 progeny homozygous for the B-strain H-2 complex are selected by their ability to reject a skin graft from strain A; any progeny that accept an A-strain graft are eliminated from future breeding. The selected *b/b* homozygous mice are then backcrossed to strain A; the resulting progeny are again interbred and their offspring are again selected for *b/b* homozygosity at the H-2 complex. This process of backcrossing to strain A, intercrossing, and selection for ability to reject an A-strain graft is repeated for at least 12 generations. In this way A-strain homozygosity is restored at all loci except the H-2 locus, which is homozygous for the B strain.

except at a single genetic locus or region. Any phenotypic differences that can be detected between congenic strains are related to the genetic region that distinguishes the strains. Congenic strains that are identical with each other except at the MHC can be produced by a series of crosses, backcrosses, and selections. Figure 7-3 outlines the steps by which the H-2 complex of homozygous strain B can be introduced into the background genes of homozygous strain A to generate a congenic strain, denoted A.B. The first letter in a congenic strain designation refers to the strain providing the genetic background and the second letter to the strain providing the genetically different MHC region. Thus, strain A.B will be genetically identical to strain A except for the MHC locus or loci contributed by strain B.

During production of congenic mouse strains, a crossover event sometimes occurs within the H-2 complex, yielding a recombinant strain that differs from the parental strains or the congenic strain at one or a few loci within the H-2 complex. Figure 7-4 depicts haplotypes present in several **recombinant congenic** strains that were obtained during production of a B10.A congenic strain. Such recombinant strains have been extremely useful in analyzing the MHC because they permit comparisons of functional differences

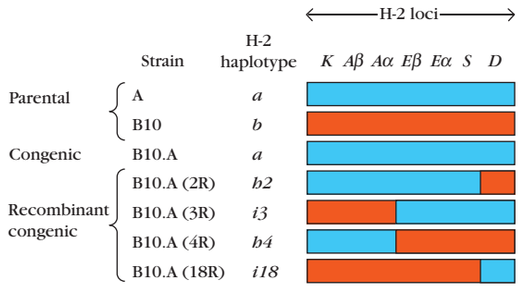


FIGURE 7-4 Examples of recombinant congenic mouse strains generated during production of the B10.A strain from parental strain B10 (H-2^b) and parental strain A (H-2^a). Crossover events within the H-2 complex produce recombinant strains, which have *a*-haplotype alleles (blue) at some H-2 loci and *b*-haplotype alleles (orange) at other loci.

between strains that differ in only a few genes within the MHC. Furthermore, the generation of new H-2 haplotypes under the experimental conditions of congenic strain development provides an excellent illustration of the means by which the MHC continues to maintain heterogeneity even in populations with limited diversity.

MHC Molecules and Genes

Class I and class II MHC molecules are membrane-bound glycoproteins that are closely related in both structure and function. Both class I and class II MHC molecules have been isolated and purified and the three-dimensional structures of their extracellular domains have been determined by x-ray crystallography. Both types of membrane glycoproteins function as highly specialized antigen-presenting molecules that form unusually stable complexes with antigenic peptides, displaying them on the cell surface for recognition by T cells. In contrast, class III MHC molecules are a group of unrelated proteins that do not share structural similarity and common function with class I and II molecules. The class III molecules will be examined in more detail in later chapters.

Class I Molecules Have a Glycoprotein Heavy Chain and a Small Protein Light Chain

Class I MHC molecules contain a 45-kilodalton (kDa) α chain associated noncovalently with a 12-kDa **β_2-microglobulin** molecule (see Figure 7-5). The α chain is a transmembrane glycoprotein encoded by polymorphic genes within the A, B, and C regions of the human HLA complex and within the K and D/L regions of the mouse H-2 complex (see Figure 7-1). β_2-Microglobulin is a protein encoded by a highly conserved gene located on a different chromosome. Association of the α chain with β_2-microglobulin is required for expression of class I molecules on cell membranes. The α chain is anchored in the plasma membrane by its hydrophobic transmembrane segment and hydrophilic cytoplasmic tail.

Structural analyses have revealed that the α chain of class I MHC molecules is organized into three external domains (α1, α2, and α3), each containing approximately 90 amino acids; a transmembrane domain of about 25 hydrophobic amino acids followed by a short stretch of charged (hydrophilic) amino acids; and a cytoplasmic anchor segment of 30 amino acids. The β_2-microglobulin is similar in size and organization to the α3 domain; it does not contain a transmembrane region and is noncovalently bound to the class I glycoprotein. Sequence data reveal homology between the α3

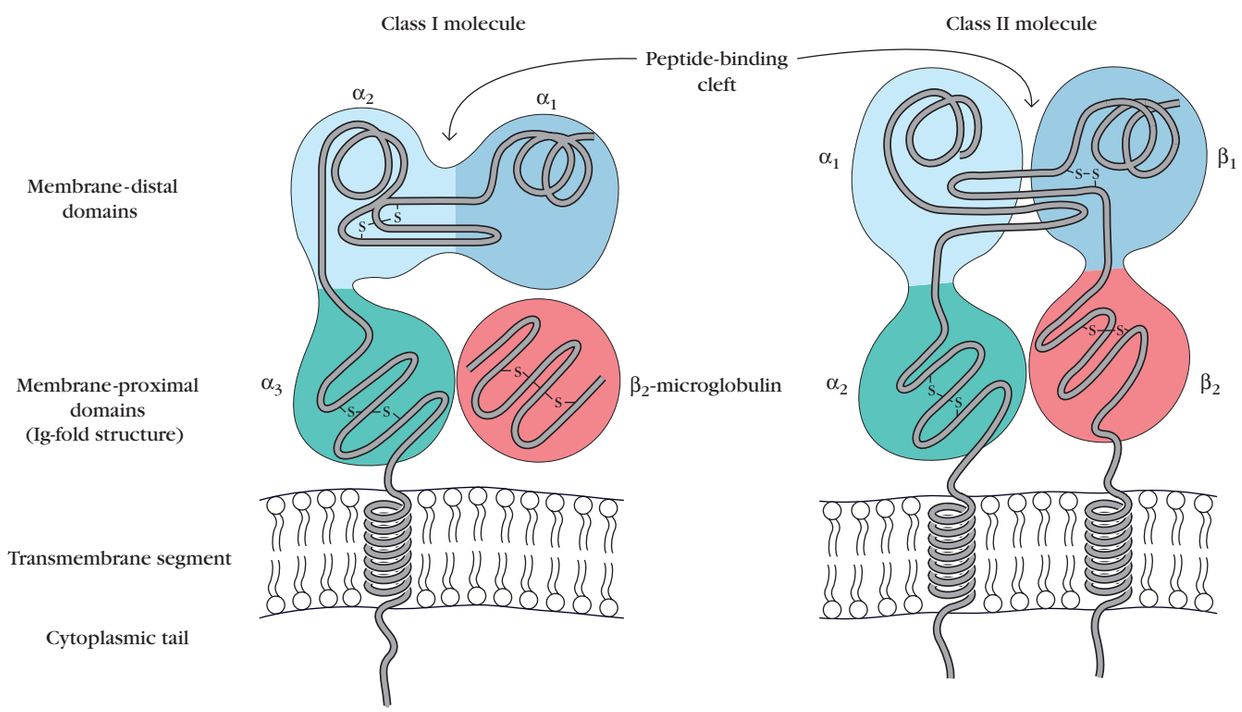


FIGURE 7-5 Schematic diagrams of a class I and a class II MHC molecule showing the external domains, transmembrane segment, and cytoplasmic tail. The peptide-binding cleft is formed by the membrane-distal domains in both class I and class II molecules. The membrane-proximal domains possess the basic immunoglobulin-fold structure; thus, class I and class II MHC molecules are classified as members of the immunoglobulin superfamily.

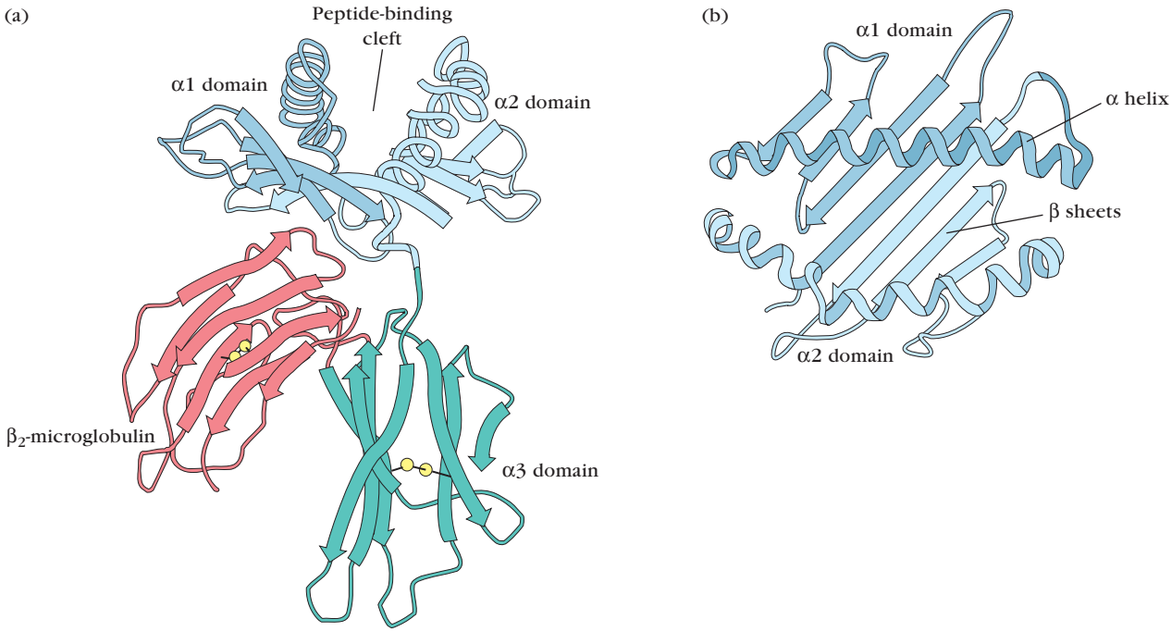


FIGURE 7-6 Representations of the three-dimensional structure of the external domains of a human class I MHC molecule based on x-ray crystallographic analysis. (a) Side view in which the β strands are depicted as thick arrows and the α helices as spiral ribbons. Disulfide bonds are shown as two interconnected spheres. The α1 and α2 domains interact to form the peptide-binding cleft. Note the immunoglobulin-fold structure of the α3 domain and β_2-microglobulin. (b) The α1 and α2 domains as viewed from the top, showing the peptide-binding cleft consisting of a base of antiparallel β strands and sides of α helices. This cleft in class I molecules can accommodate peptides containing 8–10 residues.

domain, β_2-microglobulin, and the constant-region domains in immunoglobulins. The enzyme papain cleaves the α chain just 13 residues proximal to its transmembrane domain, releasing the extracellular portion of the molecule, consisting of α1, α2, α3, and β_2-microglobulin. Purification and crystallization of the extracellular portion revealed two pairs of interacting domains: a membrane-distal pair made up of the α1 and α2 domains and a membrane-proximal pair composed of the α3 domain and β_2-microglobulin (Figure 7-6a).

The α1 and α2 domains interact to form a platform of eight antiparallel β strands spanned by two long α-helical regions. The structure forms a deep groove, or cleft, approximately 25 Å × 10 Å × 11 Å, with the long α helices as sides and the β strands of the β sheet as the bottom (Figure 7-6b). This *peptide-binding cleft* is located on the top surface of the class I MHC molecule, and it is large enough to bind a peptide of 8–10 amino acids. The great surprise in the x-ray crystallographic analysis of class I molecules was the finding of small peptides in the cleft that had cocrystallized with the protein. These peptides are, in fact, processed antigen and self-peptides bound to the α1 and α2 domains in this deep groove.

The α3 domain and β_2-microglobulin are organized into two β pleated sheets each formed by antiparallel β strands of amino acids. As described in Chapter 4, this structure, known as the immunoglobulin fold, is characteristic of immunoglobulin domains. Because of this structural similarity, which is not surprising given the considerable sequence similarity with the immunoglobulin constant regions, class I MHC molecules and β_2-microglobulin are classified as members of the immunoglobulin superfamily (see Figure 4-20). The α3 domain appears to be highly conserved among class I MHC molecules and contains a sequence that interacts with the CD8 membrane molecule present on T_C cells.

β_2-Microglobulin interacts extensively with the α3 domain and also interacts with amino acids of the α1 and α2 domains. The interaction of β_2-microglobulin and a peptide with a class I α chain is essential for the class I molecule to reach its fully folded conformation. As described in detail in Chapter 8, assembly of class I molecules is believed to occur by the initial interaction of β_2-microglobulin with the folding class I α chain. This metastable "empty" dimer is then stabilized by the binding of an appropriate peptide to form the native trimeric class I structure consisting of the class I α chain, β_2-microglobulin, and a peptide. This complete molecular complex is ultimately transported to the cell surface.

In the absence of β_2-microglobulin, the class I MHC α chain is not expressed on the cell membrane. This is illustrated by Daudi tumor cells, which are unable to synthesize β_2-microglobulin. These tumor cells produce class I MHC α chains, but do not express them on the membrane. However, if Daudi cells are transfected with a functional gene encoding β_2-microglobulin, class I molecules appear on the membrane.

Class II Molecules Have Two Nonidentical Glycoprotein Chains

Class II MHC molecules contain two different polypeptide chains, a 33-kDa α chain and a 28-kDa β chain, which associate by noncovalent interactions (see Figure 7-5b). Like class I α chains, class II MHC molecules are membrane-bound glycoproteins that contain external domains, a transmembrane segment, and a cytoplasmic anchor segment. Each chain in a class II molecule contains two external domains: α1 and α2 domains in one chain and β1 and β2 domains in the other. The membrane-proximal α2 and β2 domains, like the membrane-proximal $\alpha 3/\beta_2$-microglobulin domains of class I MHC molecules, bear sequence similarity to the immunoglobulin-fold structure; for this reason, class II MHC molecules also are classified in the immunoglobulin superfamily. The membrane-distal portion of a class II molecule is composed of the α1 and β1 domains and forms the antigen-binding cleft for processed antigen.

X-ray crystallographic analysis reveals the similarity of class II and class I molecules, strikingly apparent when the molecules are superimposed (Figure 7-7). The peptide-binding cleft of HLA-DR1, like that in class I molecules, is composed of a floor of eight antiparallel β strands and sides of antiparallel α helices. However, the class II molecule lacks the conserved residues that bind to the terminal residues of short peptides and forms instead an open pocket; class I presents more of a socket, class II an open-ended groove. These functional consequences of these differences in fine structure will be explored below.

An unexpected difference between crystallized class I and class II molecules was observed for human DR1 in that the latter occurred as a dimer of αβ heterodimers, a "dimer of dimers" (Figure 7-8). The dimer is oriented so that the two peptide-binding clefts face in opposite directions. While it has not yet been determined whether this dimeric form exists in vivo, the presence of CD4 binding sites on opposite sides of the class II molecule suggests that it does. These two sites on the α2 and β2 domains are adjacent in the dimer form and a CD4 molecule binding to them may stabilize class II dimers.

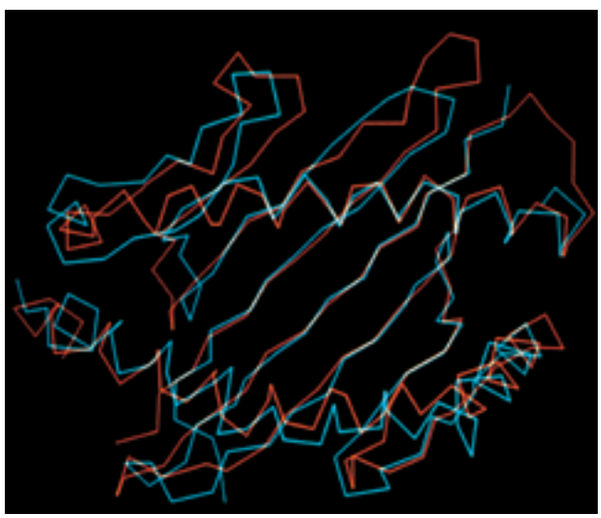


FIGURE 7-7 The membrane-distal, peptide-binding cleft of a human class II MHC molecule, HLA-DR1 (blue), superimposed over the corresponding regions of a human class I MHC molecule, HLA-A2 (red). *[From J. H. Brown et al., 1993,* Nature ***364:****33.]*

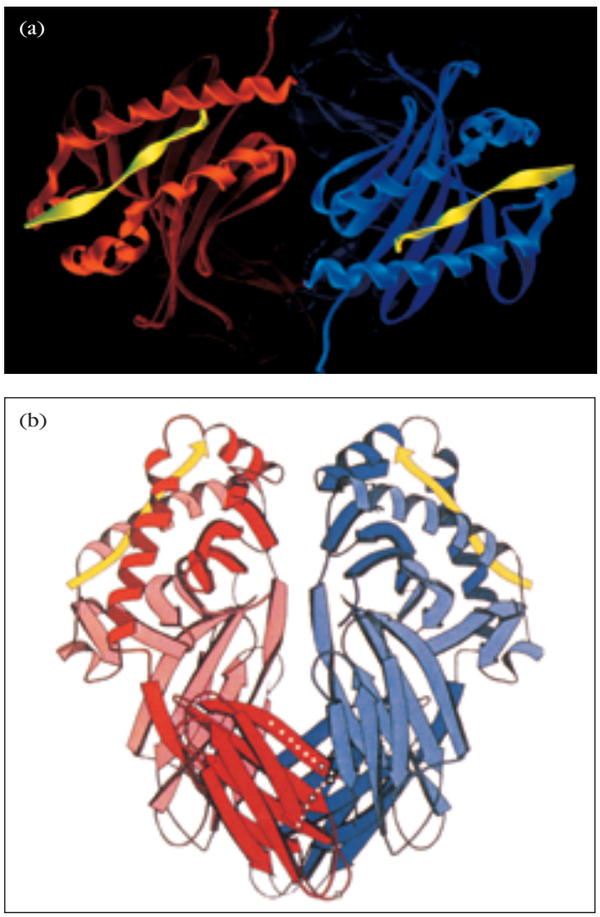


FIGURE 7-8 Antigen-binding cleft of dimeric class II DR1 molecule in (a) top view and (b) side view. This molecule crystallized as a dimer of the αβ heterodimer. The crystallized dimer is shown with one DR1 molecule in red and the other DR1 molecule in blue. The bound peptides are yellow. The two peptide-binding clefts in the dimeric molecule face in opposite directions. *[From J. H. Brown et al., 1993,* Nature ***364:****33.]*

The Exon/Intron Arrangement of Class I and II Genes Reflects Their Domain Structure

Separate exons encode each region of the class I and II proteins (Figure 7-9). Each of the mouse and human class I genes has a 5′ leader exon encoding a short signal peptide

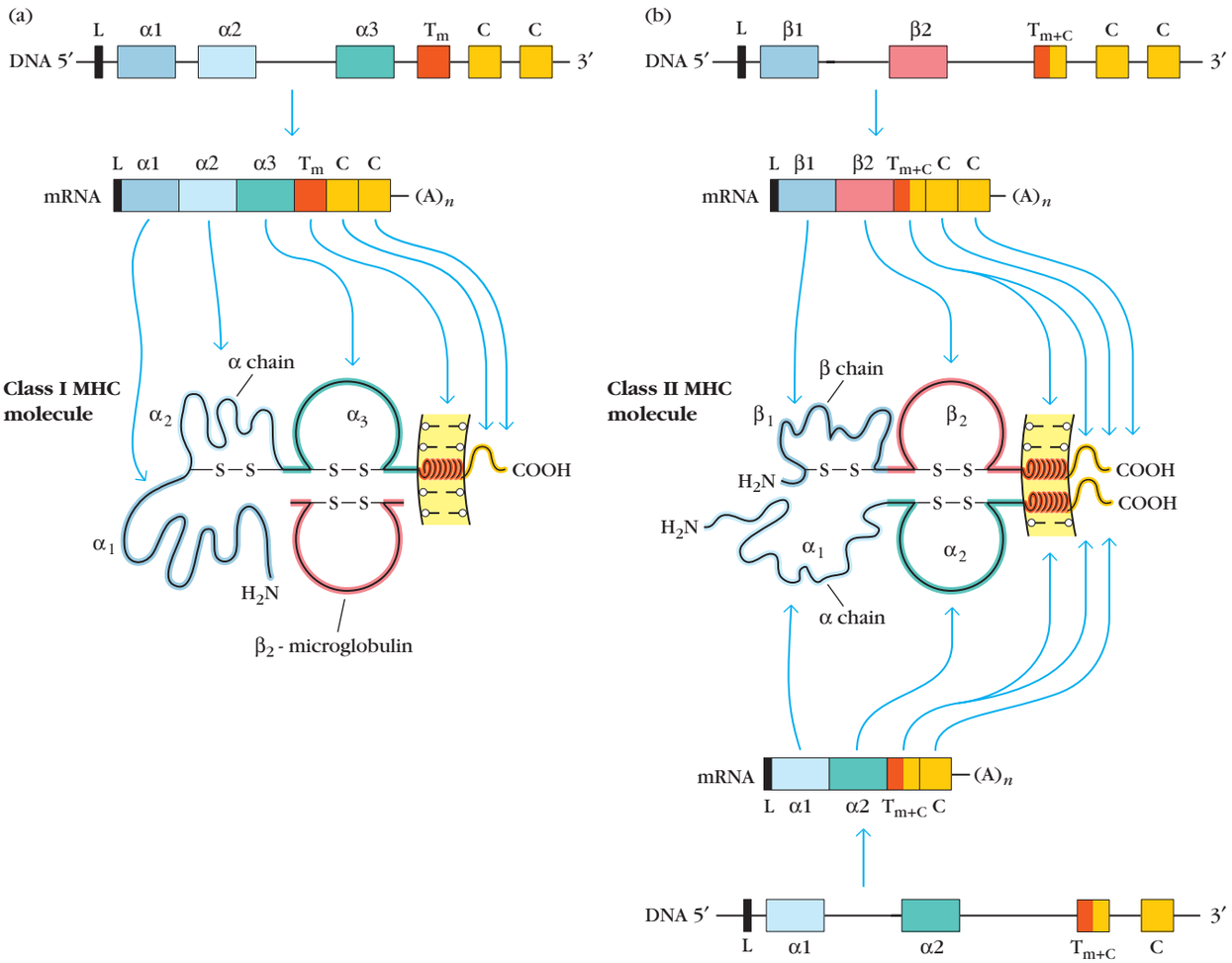


FIGURE 7-9 Schematic diagram of (a) class I and (b) class II MHC genes, mRNA transcripts, and protein molecules. There is correspondence between exons and the domains in the gene products; note that the mRNA transcripts are spliced to remove the intron sequences. Each exon, with the exception of the leader (L) exon, encodes a separate domain of the MHC molecule. The leader peptides are removed in a post-translational reaction before the molecules are expressed on the cell surface. The gene encoding β_2-microglobulin is located on a different chromosome. T_m = transmembrane; C = cytoplasmic.

followed by five or six exons encoding the α chain of the class I molecule (see Figure 7-9a). The signal peptide serves to facilitate insertion of the α chain into the endoplasmic reticulum and is removed by proteolytic enzymes in the endoplasmic reticulum after translation is completed. The next three exons encode the extracellular α1, α2, and α3 domains, and the following downstream exon encodes the transmembrane (T_m) region; finally, one or two 3′-terminal exons encode the cytoplasmic domains (C).

Like class I MHC genes, the class II genes are organized into a series of exons and introns mirroring the domain structure of the α and β chains (see Figure 7-9b). Both the α and the β genes encoding mouse and human class II MHC molecules have a leader exon, an α1 or β1 exon, an α2 or β2 exon, a transmembrane exon, and one or more cytoplasmic exons.

Class I and II Molecules Exhibit Polymorphism in the Region That Binds to Peptides

Several hundred different allelic variants of class I and II MHC molecules have been identified in humans. Any one individual, however, expresses only a small number of these molecules—up to 6 different class I molecules and up to 12 different class II molecules. Yet this limited number of MHC molecules must be able to present an enormous array of different antigenic peptides to T cells, permitting the immune system to respond specifically to a wide variety of antigenic challenges. Thus, peptide binding by class I and II molecules does not exhibit the fine specificity characteristic of antigen binding by antibodies and T-cell receptors. Instead, a given MHC molecule can bind

TABLE 7-2 Peptide binding by class I and class II MHC molecules

	Class I molecules	Class II molecules
Peptide-binding domain	$\alpha1/\alpha2$	$\alpha1/\beta1$
Nature of peptide-binding cleft	Closed at both ends	Open at both ends
General size of bound peptides	8–10 amino acids	13–18 amino acids
Peptide motifs involved in binding to MHC molecule	Anchor residues at both ends of peptide; generally hydrophobic carboxyl-terminal anchor	Anchor residues distributed along the length of the peptide
Nature of bound peptide	Extended structure in which both ends interact with MHC cleft but middle arches up away from MHC molecule	Extended structure that is held at a constant elevation above the floor of MHC cleft

numerous different peptides, and some peptides can bind to several different MHC molecules. Because of this broad specificity, the binding between a peptide and an MHC molecule is often referred to as "promiscuous."

Given the similarities in the structure of the peptide-binding cleft in class I and II MHC molecules, it is not surprising that they exhibit some common peptide-binding features (Table 7-2). In both types of MHC molecules, peptide ligands are held in a largely extended conformation that runs the length of the cleft. The peptide-binding cleft in class I molecules is blocked at both ends, whereas the cleft is open in class II molecules (Figure 7-10). As a result of this difference, class I molecules bind peptides that typically contain 8–10 amino acid residues, while the open groove of class II molecules accommodates slightly longer peptides of 13–18 amino acids. Another difference, explained in more detail below, is that class I binding requires that the peptide contain specific amino acid residues near the N and C termini; there is no such requirement for class II peptide binding.

The peptide–MHC molecule association is very stable ($K_d \sim 10^{-6}$) under physiologic conditions; thus, most of the MHC molecules expressed on the membrane of a cell will be associated with a peptide of self or nonself origin.

CLASS I MHC–PEPTIDE INTERACTION

Class I MHC molecules bind peptides and present them to $CD8^+$ T cells. In general, these peptides are derived from endogenous intracellular proteins that are digested in the cytosol. The peptides are then transported from the cytosol into the cisternae of the endoplasmic reticulum, where they interact with class I MHC molecules. This process, known as the cytosolic or endogenous processing pathway, is discussed in detail in the next chapter.

Each type of class I MHC molecule (K, D, and L in mice or A, B, and C in humans) binds a unique set of peptides. In addition, each allelic variant of a class I MHC molecule (e.g., H-$2K^k$ and H-$2K^d$) also binds a distinct set of peptides. Because a single nucleated cell expresses about 10^5 copies of each class I molecule, many different peptides will be expressed simultaneously on the surface of a nucleated cell by class I MHC molecules.

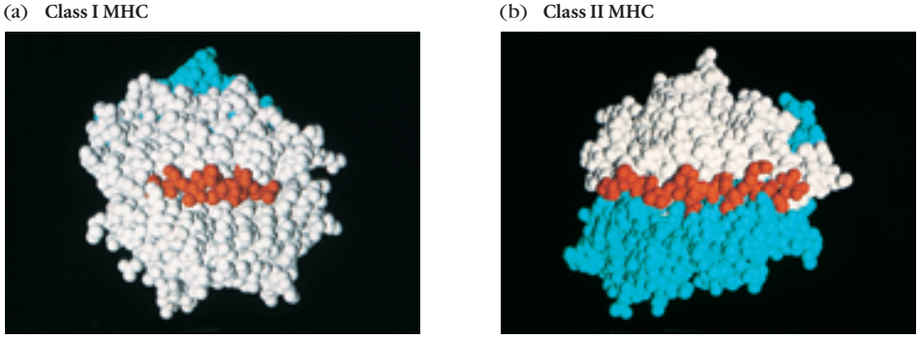


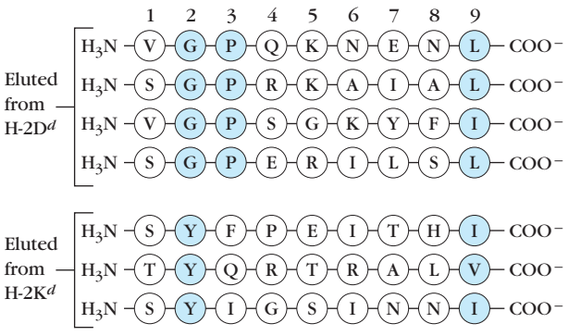
FIGURE 7-10 MHC class I and class II molecules with bound peptides. (a) Space-filling model of human class I molecule HLA-A2 (white) with peptide (red) from HIV reverse transcriptase (amino acid residues 309–317) in the binding groove. β_2-microglobulin is shown in blue. Residues above the peptide are from the $\alpha1$ domain, those below from $\alpha2$. (b) Space-filling model of human class II molecules HLA-DR1 with the DRα chain shown in white and the DRβ chain in blue. The peptide (red) in the binding groove is from influenza hemagglutinin (amino acid residues 306–318). *[From D. A. Vignali and J. Strominger, 1994,* The Immunologist ***2:****112.]*

In a critical study of peptide binding by MHC molecules, peptides bound by two allelic variants of a class I MHC molecule were released chemically and analyzed by HPLC mass spectrometry. More than 2000 distinct peptides were found among the peptide ligands released from these two class I MHC molecules. Since there are approximately 10^5 copies of each class I allelic variant per cell, it is estimated that each of the 2000 distinct peptides is presented with a frequency of 100–4000 copies per cell. Evidence suggests that as few as 100 peptide-MHC complexes are sufficient to target a cell for recognition and lysis by a cytotoxic T lymphocyte with a receptor specific for this target structure.

The bound peptides isolated from different class I molecules have been found to have two distinguishing features: they are eight to ten amino acids in length, most commonly nine, and they contain specific amino acid residues that appear to be essential for binding to a particular MHC molecule. Binding studies have shown that nonameric peptides bind to class I molecules with a 100- to 1000-fold higher affinity than do peptides that are either longer or shorter, suggesting that this peptide length is most compatible with the closed-ended peptide-binding cleft in class I molecules. The ability of an individual class I MHC molecule to bind to a diverse spectrum of peptides is due to the presence of the same or similar amino acid residues at several defined positions along the peptides (Figure 7-11). Because these amino acid residues anchor the peptide into the groove of the MHC molecule, they are called *anchor residues.* The side chains of the anchor residues in the peptide are complementary with surface features of the binding cleft of the class I MHC molecule. The amino acid residues lining the binding sites vary among different class I allelic variants and determine the identity of the anchor residues that can interact with the molecule.

All peptides examined to date that bind to class I molecules contain a carboxyl-terminal anchor. These anchors are generally hydrophobic residues (e.g., leucine, isoleucine), although a few charged amino acids have been reported. Besides the anchor residue found at the carboxyl terminus, another anchor is often found at the second or second and third positions at the amino-terminal end of the peptide (see Figure 7-11). In general, any peptide of correct length that contains the same or similar anchor residues will bind to the same class I MHC molecule. The discovery of conserved anchor residues in peptides that bind to various class I MHC molecules may permit prediction of which peptides in a complex antigen will bind to a particular MHC molecule, based on the presence or absence of these motifs.

X-ray crystallographic analyses of peptide–class I MHC complexes have revealed how the peptide-binding cleft in a given MHC molecule can interact stably with a broad spectrum of different peptides. The anchor residues at both ends of the peptide are buried within the binding cleft, thereby holding the peptide firmly in place (Figure 7-12). As noted already, nonameric peptides are bound preferentially; the main contacts between class I MHC molecules and peptides involve residue 2 at the amino-terminal end and residue 9 at the carboxyl terminus of the nonameric peptide. Between the anchors the peptide arches away from the floor of the cleft in the middle (Figure 7-13), allowing peptides that are slightly longer or shorter to be accommodated. Amino acids that arch away from the MHC molecule are more exposed and presumably can interact more directly with the T-cell receptor.



A = alanine	K = lysine	R = arginine
E = glutamic acid	L = leucine	S = serine
F = phenylalanine	N = asparagine	T = threonine
G = glycine	P = proline	V = valine
H = histidine	Q = glutamine	Y = tyrosine
I = isoleucine		

FIGURE 7-11 Examples of anchor residues (blue) in nonameric peptides eluted from two class I MHC molecules. Anchor residues that interact with the class I MHC molecule tend to be hydrophobic amino acids. *[Data from V. H. Engelhard, 1994,* Curr. Opin. Immunol. ***6:****13.]*

CLASS II MHC–PEPTIDE INTERACTION

Class II MHC molecules bind peptides and present these peptides to $CD4^+$ T cells. Like class I molecules, molecules of class II can bind a variety of peptides. In general, these peptides are derived from exogenous proteins (either self or nonself), which are degraded within the endocytic processing pathway (see Chapter 8). Most of the peptides associated with class II MHC molecules are derived from membrane-bound proteins or proteins associated with the vesicles of the endocytic processing pathway. The membrane-bound proteins presumably are internalized by phagocytosis or by receptor-mediated endocytosis and enter the endocytic processing pathway at this point. For instance, peptides derived from digestion of membrane-bound class I MHC molecules often are bound to class II MHC molecules.

Peptides recovered from class II MHC–peptide complexes generally contain 13–18 amino acid residues, somewhat longer than the nonameric peptides that most commonly bind to class I molecules. The peptide-binding cleft in class II molecules is open at both ends (see Figure 7-10b), allowing longer peptides to extend beyond the ends, like a long hot dog in a bun. Peptides bound to class II MHC molecules maintain a roughly constant elevation on the

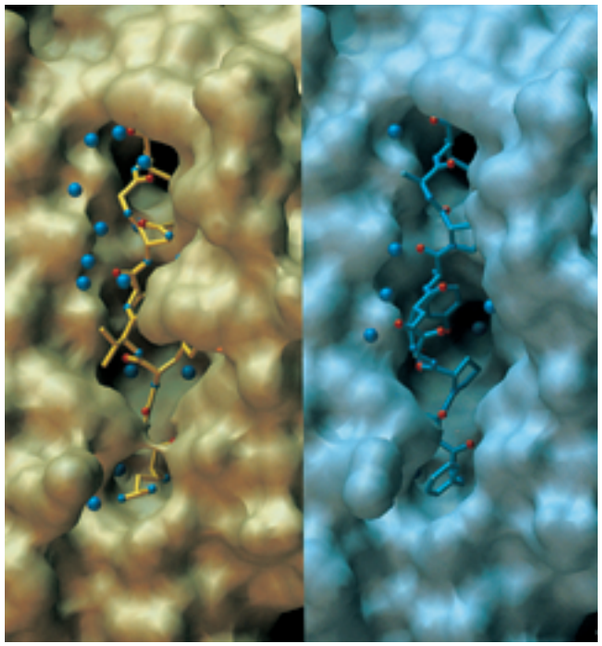


FIGURE 7-12 Model of the solvent-accessible area of class I H-2K^b, depicting the complex formed with a vesicular stomatitis virus (VSV-8) peptide (*left,* yellow backbone) and Sendai virus (SEV-9) nucleoprotein (*right,* blue backbone). Water molecules (blue spheres) interact with the bound peptides. The majority of the surface of both peptides is inaccessible for direct contact with T cells (VSV-8 is 83% buried; SEV-9 is 75% buried). The H-2K^b surface in the two complexes exhibits a small, but potentially significant, conformational variation, especially in the central region of the binding cleft on the right side of the peptides, which corresponds to the α helix in the α2 domain (see Figure 7-6b). *[From M. Matsumura et al., 1992,* Science ***257:****927; photographs courtesy of D. H. Fremont, M. Matsumura, M. Pique, and I. A. Watson.]*

floor of the binding cleft, another feature that distinguishes peptide binding to class I and class II molecules.

Peptide binding studies and structural data for class II molecules indicate that a central core of 13 amino acids determines the ability of a peptide to bind class II. Longer peptides may be accommodated within the class II cleft, but the binding characteristics are determined by the central 13 residues. The peptides that bind to a particular class II molecule often have internal conserved "motifs," but unlike class I–binding peptides, they lack conserved anchor residues. Instead, hydrogen bonds between the backbone of the peptide and the class II molecule are distributed throughout the binding site rather than being clustered predominantly at the ends of the site as for class I–bound peptides. Peptides that bind to class II MHC molecules contain an internal sequence comprising 7–10 amino acids that provide the major contact points. Generally, this sequence has an aromatic or hydrophobic residue at the amino terminus and three additional hydrophobic residues in the middle portion and carboxyl-terminal end of the peptide. In addition, over 30% of the peptides eluted from class II molecules contain a proline residue at position 2 and another cluster of prolines at the carboxyl-terminal end.

Class I and Class II Molecules Exhibit Diversity Within a Species and Multiple Forms Occur in an Individual

An enormous diversity is exhibited by the MHC molecules within a species and within individuals. This variability echoes the diversity of antibodies and T-cell receptors, but the source of diversity for MHC molecules is not the same. Antibodies and T-cell receptors are generated by several somatic processes, including gene rearrangement and somatic mutation of rearranged genes (see Table 5-2). Thus, the generation of T and B cell receptors is dynamic, changing over time within an individual. By contrast, the MHC molecules expressed by an individual are fixed in the genes and do not change over time. The diversity of the MHC within a species stems from polymorphism, the presence of multiple alleles at a given genetic locus within the species. Diversity of MHC molecules in an individual results not only from having different alleles of each gene but also from the presence of duplicated genes with similar or overlapping functions, not unlike the isotypes of immunoglobulins. Because it includes genes with similar, but not identical structure and function (for example, HLA-A, -B, and -C), the MHC may be said to be **polygenic.**

The MHC possesses an extraordinarily large number of different alleles at each locus and is one of the most polymorphic genetic complexes known in higher vertebrates. These alleles differ in their DNA sequences from one individual to another by 5% to 10%. The number of amino acid differences between MHC alleles can be quite significant, with up to 20 amino acid residues contributing to the unique structural nature of each allele. Analysis of human HLA class I genes has revealed, as of early 2002, approximately 240 A alleles, 470 B alleles, and 110 C alleles. In mice, the polymorphism is similarly enormous. The human class II genes are also highly polymorphic and, in some cases, there are different gene numbers in different individuals. The number of HLA-DR beta-chain genes may vary from 2 to 9 in different haplotypes, and approximately 350 alleles of DRB genes have been reported. Interestingly, the DRA chain is highly conserved, with only 2 different alleles reported. Current estimates of actual polymorphism in the human MHC are probably on the low side because the most detailed data were obtained from populations of European descent. The fact that many non-European population groups cannot be typed using the MHC serologic typing reagents available indicates that the worldwide diversity of the MHC genes is far greater. Now that MHC genes can be sequenced directly, it is expected that many additional alleles will be detected.

This enormous polymorphism results in a tremendous diversity of MHC molecules within a species. Using the numbers given above for the allelic forms of human HLA-A, -B,

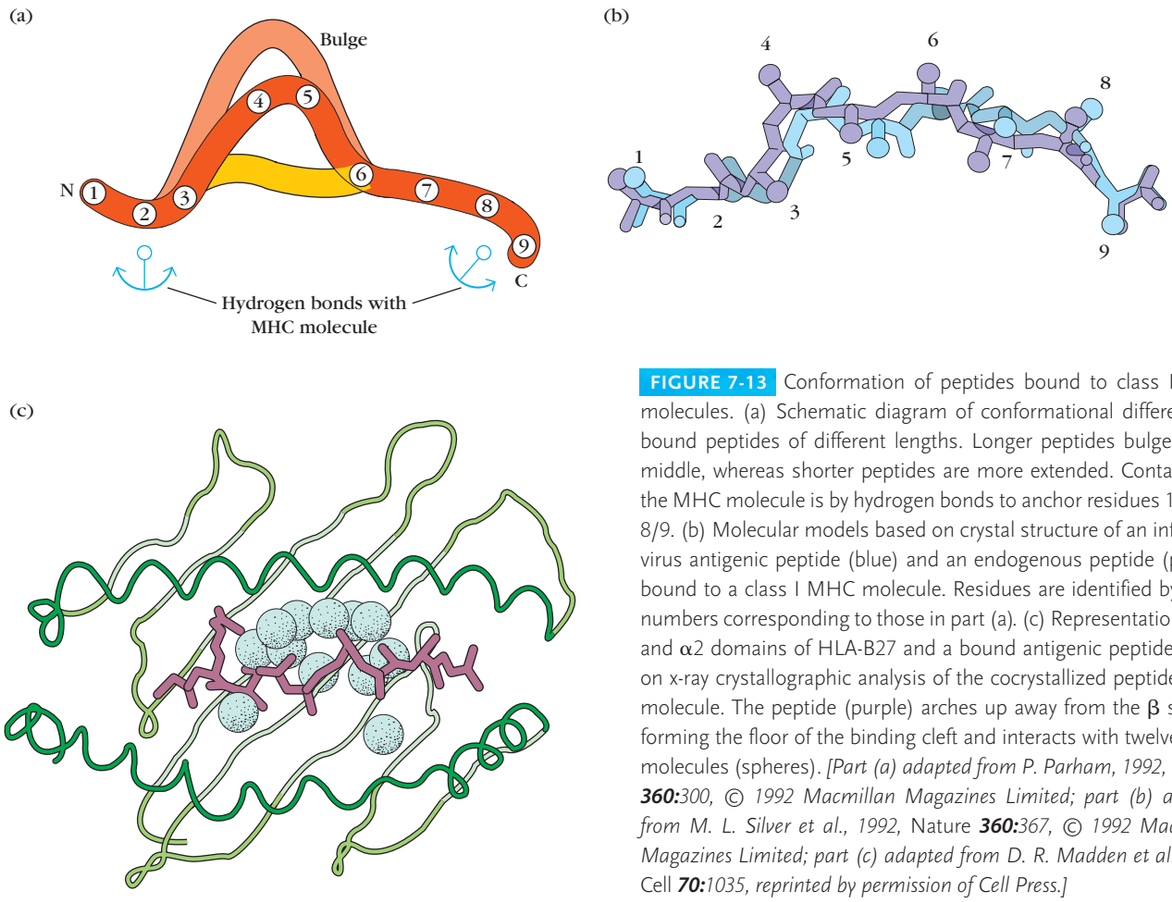


FIGURE 7-13 Conformation of peptides bound to class I MHC molecules. (a) Schematic diagram of conformational difference in bound peptides of different lengths. Longer peptides bulge in the middle, whereas shorter peptides are more extended. Contact with the MHC molecule is by hydrogen bonds to anchor residues 1/2 and 8/9. (b) Molecular models based on crystal structure of an influenza virus antigenic peptide (blue) and an endogenous peptide (purple) bound to a class I MHC molecule. Residues are identified by small numbers corresponding to those in part (a). (c) Representation of α1 and α2 domains of HLA-B27 and a bound antigenic peptide based on x-ray crystallographic analysis of the cocrystallized peptide–HLA molecule. The peptide (purple) arches up away from the β strands forming the floor of the binding cleft and interacts with twelve water molecules (spheres). *[Part (a) adapted from P. Parham, 1992,* Nature ***360:****300, © 1992 Macmillan Magazines Limited; part (b) adapted from M. L. Silver et al., 1992,* Nature ***360:****367, © 1992 Macmillan Magazines Limited; part (c) adapted from D. R. Madden et al., 1992,* Cell ***70:****1035, reprinted by permission of Cell Press.]*

and -C, we can calculate the theoretical number of combinations that can exist by multiplying $240 \times 470 \times 110$, yielding upwards of 12 million different class I haplotypes possible in the population. If class II loci are considered, the 5 DRB genes B1 through B5 have 304, 1, 35, 11, and 15 alleles respectively, DQA1 and B1 contribute 22 and 49 alleles, respectively and, DPB1 96 alleles; this allows approximately 1.8×10^{11} different class II combinations. Because each haplotype contains both class I and class II genes, the numbers are multiplied to give a total of 2.25×10^{18} possible combinations of these class I and II alleles.

LINKAGE DISEQUILIBRIUM

The calculation of theoretical diversity in the previous paragraph assumes completely random combinations of alleles. The actual diversity is known to be less, because certain allelic combinations occur more frequently in HLA haplotypes than predicted by random combination, a state referred to as *linkage disequilibrium.* Briefly, linkage disequilibrium is the difference between the frequency observed for a particular combination of alleles and that *expected* from the frequencies of the individual alleles. The expected frequency for the combination may be calculated by multiplying the frequencies of the two alleles. For example, if HLA-A1 occurs in 16% of individuals in a population (frequency = 0.16) and HLA-B8 in 9% of that group (frequency = 0.09) it is expected that about 1.4% of the group should have both alleles ($0.16 \times 0.09 = 0.014$). However, the data show that HLA-A1 and HLA-B8 are found together in 8.8% of individuals studied. This difference is a measure of the linkage disequilibrium between these alleles of class I MHC genes.

Several explanations have been advanced to explain linkage disequilibrium. The simplest is that too few generations have elapsed to allow the number of crossovers necessary to reach equilibrium among the alleles present in founders of the population. The haplotypes that are over-represented in the population today would then reflect the combinations of alleles present in the founders. Alternatively, selective effects could also result in the higher frequency of certain allelic combinations. For example, certain combinations of alleles might produce resistance to certain diseases, causing them to be selected for and over-represented, or they might generate harmful effects, such as susceptibility to autoimmune disorders, and undergo negative selection. A third hypothesis is that crossovers are more frequent in certain DNA sequence regions, and the presence or absence of regions prone to crossover (hotspots) between alleles can dictate the

frequency of allelic association. Data in support of this was found in mouse breeding studies that generated new recombinant H-2 types. The points of crossover in the new MHC haplotypes were not randomly distributed throughout the complex. Instead, the same regions of crossover were found in more than one recombinant haplotype. This suggests that hotspots of recombination do exist that would influence linkage disequilibrium in populations.

Despite linkage disequilibrium, there is still enormous polymorphism in the human MHC, and it remains very difficult to match donor and acceptor MHC types for successful organ transplants. The consequences of this major obstacle to the therapeutic use of transplantation are described in Chapter 21.

FUNCTIONAL RELEVANCE OF MHC POLYMORPHISM

Sequence divergence among alleles of the MHC within a species is very high, as great as the divergence observed for the genes encoding some enzymes across species. Also of interest is that the sequence variation among MHC molecules is not randomly distributed along the entire polypeptide chain but instead is clustered in short stretches, largely within the membrane-distal α1 and α2 domains of class I molecules (Figure 7-14a). Similar patterns of diversity are observed in the α1 and β2 domains of class II molecules.

Progress has been made in locating the polymorphic residues within the three-dimensional structure of the membrane-distal domains in class I and class II MHC molecules and in relating allelic differences to functional differences (Figure 7-14b). For example, of 17 amino acids previously shown to display significant polymorphism in the HLA-A2 molecule, 15 were shown by x-ray crystallographic analysis to be in the peptide-binding cleft of this molecule. The location of so many polymorphic amino acids within the binding site for processed antigen strongly suggests that allelic differences contribute to the observed differences in the ability of MHC molecules to interact with a given antigenic peptide.

Detailed Genomic Map of MHC Genes

The MHC spans some 2000 kb of mouse DNA and some 4000 kb of human DNA. The recently completed human genome sequence shows this region to be densely packed

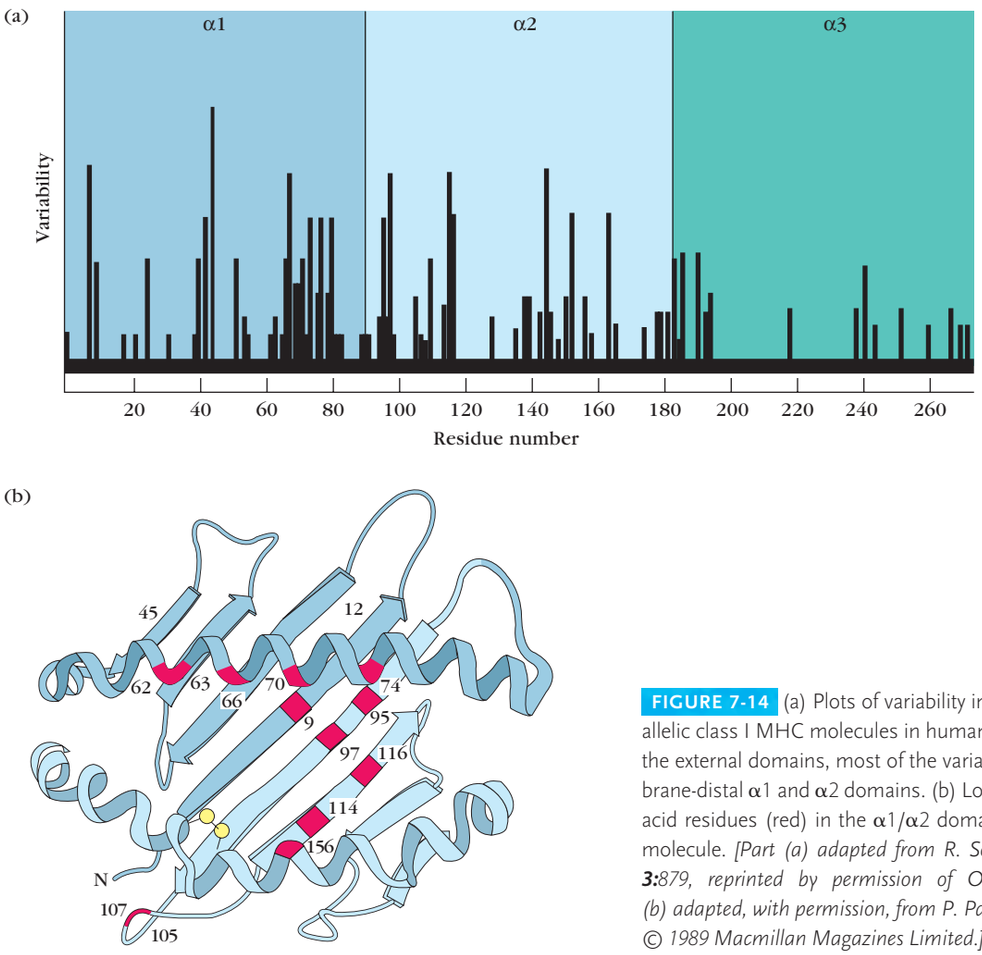
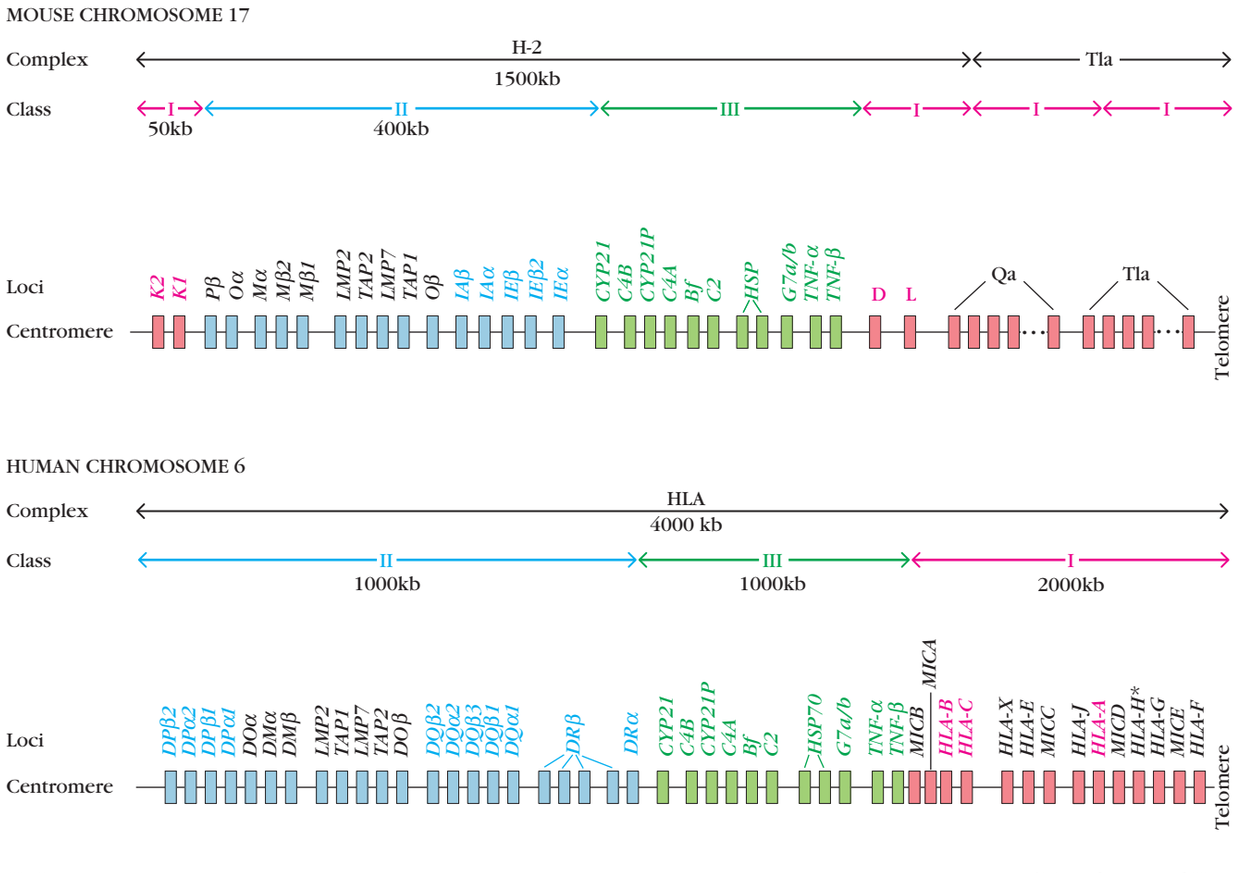


FIGURE 7-14 (a) Plots of variability in the amino acid sequence of allelic class I MHC molecules in humans versus residue position. In the external domains, most of the variable residues are in the membrane-distal α1 and α2 domains. (b) Location of polymorphic amino acid residues (red) in the α1/α2 domain of a human class I MHC molecule. *[Part (a) adapted from R. Sodoyer et al., 1984,* EMBO J. ***3:****879, reprinted by permission of Oxford University Press; part (b) adapted, with permission, from P. Parham, 1989,* Nature ***342:****617,* © *1989 Macmillan Magazines Limited.]*



KEY

Gene	Encoded protein
C2, C4A, C4B, Bf	Complement components
CYP21,CYP21P	Steroid 21-hydroxylases
G7a/b	Valyl-tRNA synthetase
HSP	Heat-shock protein
LMP2, LMP7	Proteasome-like subunits
TAP1, TAP2	Peptide-transporter subunits
TNF-α, TNF-β	Tumor necrosis factors α and β

*Now designated HFE

FIGURE 7-15 Detailed genomic map of the mouse and human MHC, including genes encoding classical and nonclassical MHC molecules. The class I MHC genes are colored red, MHC II genes are colored blue, and genes in MHC III are colored green. Classical class I genes are labeled in red, class II in blue, and the nonclassical MHC genes are labeled in black. The concept of classical and nonclassical does not apply to class III. The functions for certain proteins encoded by the nonclassical class I genes are known. In the mouse, there are nonclassical genes located downstream from Tla that are not shown.

with genes, most of which have known functions. Our current understanding of the genomic organization of mouse and human MHC genes is diagrammed in Figure 7-15.

The Human Class I Region Spans about 2000 kb at the Telomeric End of the HLA Complex

In humans, the class I MHC region is about 2000 kb long and contains approximately 20 genes. In mice, the class I MHC consists of two regions separated by the intervening class II and class III regions. Included within the class I region are the genes encoding the well-characterized classical class I MHC molecules designated HLA-A, HLA-B, and HLA-C in humans and H-2K, H-2D, and H-2L in mice. Many nonclassical class I genes, identified by molecular mapping, also are present in both the mouse and human MHC. In mice, the nonclassical class I genes are located in three regions (*H-2Q, T,* and *M*) downstream from the H-2 complex (*M* is not shown in Figure 7-15). In humans, the nonclassical class I genes include the *HLA-E, HLA-F, HLA-G, HFE, HLA-J,* and *HLA-X* loci as well as a recently discovered family of genes called *MIC,* which includes *MICA* through *MICE.* Some of the nonclassical class I MHC genes are pseudogenes and do not encode a protein product, but others, such as *HLA-G* and *HFE,* encode class I–like products with highly specialized functions. The MIC family of class I genes has only 15%–30% sequence identity to classical class I, and those designated as MICA are highly polymorphic. The MIC gene products are expressed at low levels in epithelial cells and are induced by heat or other stimuli that influence heat shock proteins.

The functions of the nonclassical class I MHC molecules remain largely unknown, although a few studies suggest that some of these molecules, like the classical class I MHC molecules, may present peptides to T cells. One intriguing finding is that the mouse molecule encoded by the *H-2M* locus is able to bind a self-peptide derived from a subunit of NADH dehydrogenase, an enzyme encoded by the mitochondrial genome. This particular self-peptide contains an amino-terminal formylated methionine. What is interesting about this finding is that peptides derived from prokaryotic organisms often have formylated amino-terminal methionine residues. This *H-2M*–encoded class I molecule may thus be uniquely suited to present peptides from prokaryotic organisms that are able to grow intracellularly. Such organisms include *Mycobacterium tuberculosis, Listeria monocytogenes, Brucella abortus,* and *Salmonella typhimurium.*

Up to this point, all description of antigen presentation by class I and class II molecules has been confined to presentation of peptide antigens. As will be seen in the description of antigen presentation (Chapter 8), there are also molecules with structural similarity to class I molecules that present non-peptide antigens, such as glycolipids, to T cells. A major family of such molecules, designated CD1, has been shown to present lipid antigens derived from bacteria. The CD1 molecules are not encoded within the MHC but are located on chromosome 1.

The Class II MHC Genes Are Located at the Centromeric End of HLA

The class II MHC region contains the genes encoding the α and β chains of the classical class II MHC molecules designated HLA-DR, DP, and DQ in humans and H-2IA and -IE in mice. Molecular mapping of the class II MHC has revealed multiple β-chain genes in some regions in both mice and humans, as well as multiple α-chain genes in humans (see Figure 7-15). In the human DR region, for example, there are three or four functional β-chain genes. All of the β-chain gene products can be expressed together with the α-chain gene product in a given cell, thereby increasing the number of different antigen-presenting molecules on the cell. Although the human DR region contains just one α-chain gene, the DP and DQ regions each contains two.

Genes encoding nonclassical class II MHC molecules have also been identified in both humans and mice. In mice, several class II genes (*Oα, Oβ, Mα,* and *Mβ*) encode nonclassical MHC molecules that exhibit limited polymorphism and a different pattern of expression than the classical IA and IE class II molecules. In the human class II region, nonclassical genes designated *DM* and *DO* have been identified. The *DM* genes encode a class II–like molecule (HLA-DM) that facilitates the loading of antigenic peptides into the class II MHC molecules. Class II DO molecules, which are expressed only in the thymus and mature B cells, have been shown to serve as regulators of class II antigen processing. The functions of HLA-DM and HLA-DO will be described further in Chapter 8.

Human MHC Class III Genes Are Between Class I and II

The class III region of the MHC in humans and mice contains a heterogeneous collection of genes (see Figure 7-15). These genes encode several complement components, two steroid 21-hydroxylases, two heat-shock proteins, and two cytokines (TNF-α and TNF-β). Some of these class III MHC gene products play a role in certain diseases. For example, mutations in the genes encoding 21-hydroxylase have been linked to congenital adrenal hyperplasia. Interestingly, the presence of a linked class III gene cluster is conserved in all species with an MHC region.

Cellular Distribution of MHC Molecules

In general, the classical class I MHC molecules are expressed on most nucleated cells, but the level of expression differs among different cell types. The highest levels of class I molecules are expressed by lymphocytes, where they constitute approximately 1% of the total plasma-membrane proteins, or some 5×10^5 molecules per cell. In contrast, fibroblasts, muscle cells, liver hepatocytes, and neural cells express very low levels of class I MHC molecules. The low level on liver cells may contribute to the considerable success of liver transplants by reducing the likelihood of graft recognition by T_c of the recipient. A few cell types (e.g., neurons and sperm cells at certain stages of differentiation) appear to lack class I MHC molecules altogether.

As noted earlier, any particular MHC molecule can bind many different peptides. Since the MHC alleles are codominantly expressed, a heterozygous individual expresses on its cells the gene products encoded by both alleles at each MHC locus. An F_1 mouse, for example, expresses the K, D, and L from each parent (six different class I MHC molecules) on each of its nucleated cells (Figure 7-16). A similar situation occurs in humans; that is, a heterozygous individual expresses the A, B, and C alleles from each parent (six different class I MHC molecules) on the membrane of each nucleated cell. The expression of so many class I MHC molecules allows each cell to display a large number of peptides in the peptide-binding clefts of its MHC molecules.

In normal, healthy cells, the class I molecules will display self-peptides resulting from normal turnover of self proteins. In cells infected by a virus, viral peptides, as well as self-peptides, will be displayed. A single virus-infected cell should be envisioned as having various class I molecules on its membrane, each displaying different sets of viral peptides. Because of individual allelic differences in the peptide-binding clefts of the class I MHC molecules, different individuals within a species will have the ability to bind different sets of viral peptides.

Unlike class I MHC molecules, class II molecules are expressed constitutively only by antigen-presenting cells, pri-

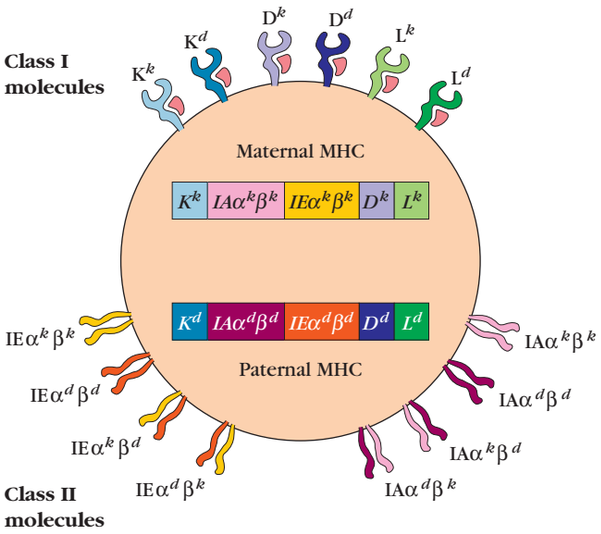


FIGURE 7-16 Diagram illustrating various MHC molecules expressed on antigen-presenting cells of a heterozygous H-$2^{k/d}$ mouse. Both the maternal and paternal MHC genes are expressed. Because the class II molecules are heterodimers, heterologous molecules containing one maternal-derived and one paternal-derived chain are produced. The β_2-microglobulin component of class I molecules (pink) is encoded by a gene on a separate chromosome and may be derived from either parent.

marily macrophages, dendritic cells, and B cells; thymic epithelial cells and some other cell types can be induced to express class II molecules and to function as antigen-presenting cells under certain conditions and under stimulation of some cytokines (see Chapter 8). Among the various cell types that express class II MHC molecules, marked differences in expression have been observed. In some cases, class II expression depends on the cell's differentiation stage. For example, class II molecules cannot be detected on pre-B cells but are expressed constitutively on the membrane of mature B cells. Similarly, monocytes and macrophages express only low levels of class II molecules until they are activated by interaction with an antigen, after which the level of expression increases significantly.

Because each of the classical class II MHC molecules is composed of two different polypeptide chains, which are encoded by different loci, a heterozygous individual expresses not only the parental class II molecules but also molecules containing α and β chains from different chromosomes. For example, an H-2^k mouse expresses IA^k and IE^k class II molecules; similarly, an H-2^d mouse expresses IA^d and IE^d molecules. The F_1 progeny resulting from crosses of mice with these two haplotypes express four parental class II molecules and four molecules containing one parent's α chain and the other parent's β chain (as shown in Figure 7-16). Since the human MHC contains three classical class II genes (*DP, DQ,* and *DR*), a heterozygous individual expresses six parental class II molecules and six molecules containing α and β chain combinations from either parent. The number of different class II molecules expressed by an individual is increased further by the presence of multiple β-chain genes in mice and humans, and in humans by multiple α-chain genes. The diversity generated by these mechanisms presumably increases the number of different antigenic peptides that can be presented and thus is advantageous to the organism.

Regulation of MHC Expression

Research on the regulatory mechanisms that control the differential expression of MHC genes in different cell types is still in its infancy, but much has been learned. The publication of the complete genomic map of the MHC complex is expected to greatly accelerate the identification and investigation of coding and regulatory sequences, leading to new directions in research on how the system is controlled.

Both class I and class II MHC genes are flanked by 5′ promoter sequences, which bind sequence-specific transcription factors. The promoter motifs and transcription factors that bind to these motifs have been identified for a number of MHC genes. Transcriptional regulation of the MHC is mediated by both positive and negative elements. For example, an MHC II transactivator, called *CIITA,* and another transcription factor, called *RFX,* both have been shown to bind to the promoter region of class II MHC genes. Defects in these transcription factors cause one form of *bare lymphocyte syndrome* (see the Clinical Focus box in Chapter 8). Patients with this disorder lack class II MHC molecules on their cells and as a result suffer a severe immunodeficiency due to the central role of class II MHC molecules in T-cell maturation and activation.

The expression of MHC molecules is also regulated by various cytokines. The interferons (alpha, beta, and gamma) and tumor necrosis factor have each been shown to increase expression of class I MHC molecules on cells. Interferon gamma (IFN-γ), for example, appears to induce the formation of a specific transcription factor that binds to the promoter sequence flanking the class I MHC genes. Binding of this transcription factor to the promoter sequence appears to coordinate the up-regulation of transcription of the genes encoding the class I α chain, β_2-microglobulin, the proteasome subunits (LMP), and the transporter subunits (TAP). IFN-γ also has been shown to induce expression of the class II transactivator (CIITA), thereby indirectly increasing expression of class II MHC molecules on a variety of cells, including non-antigen-presenting cells (e.g., skin keratinocytes, intestinal epithelial cells, vascular endothelium, placental cells, and pancreatic beta cells). Other cytokines influence MHC expression only in certain cell types; for example, IL-4 increases expression of class II molecules by resting B cells. Expression of class II molecules by B cells is down-regulated by IFN-γ; corticosteroids and prostaglandins also decrease expression of class II molecules.

MHC expression is decreased by infection with certain viruses, including human cytomegalovirus (CMV), hepatitis

B virus (HBV), and adenovirus 12 (Ad12). In some cases, reduced expression of class I MHC molecules on cell surfaces is due to decreased levels of a component needed for peptide transport or MHC class I assembly rather than in transcription. In cytomegalovirus infection, for example, a viral protein binds to β_2-microglobulin, preventing assembly of class I MHC molecules and their transport to the plasma membrane. Adenovirus 12 infection causes a pronounced decrease in transcription of the transporter genes (*TAP1* and *TAP2*). As the next chapter describes, the TAP gene products play an important role in peptide transport from the cytoplasm into the rough endoplasmic reticulum. Blocking of *TAP* gene expression inhibits peptide transport; as a result, class I MHC molecules cannot assemble with β_2-microglobulin or be transported to the cell membrane. Decreased expression of class I MHC molecules, by whatever mechanism, is likely to help viruses evade the immune response by reducing the likelihood that virus-infected cells can display MHC–viral peptide complexes and become targets for CTL-mediated destruction.

MHC and Immune Responsiveness

Early studies by B. Benacerraf in which guinea pigs were immunized with simple synthetic antigens were the first to show that the ability of an animal to mount an immune response, as measured by the production of serum antibodies, is determined by its MHC haplotype. Later experiments by H. McDevitt, M. Sela, and their colleagues used congenic and recombinant congenic mouse strains to map the control of *immune responsiveness* to class II MHC genes. In early reports, the genes responsible for this phenotype were designated *Ir* or immune response genes, and for this reason mouse class II products are called IA and IE. We now know that the dependence of immune responsiveness on the class II MHC reflects the central role of class II MHC molecules in presenting antigen to T_H cells.

Two explanations have been proposed to account for the variability in immune responsiveness observed among different haplotypes. According to the *determinant-selection model,* different class II MHC molecules differ in their ability to bind processed antigen. According to the alternative *holes-in-the-repertoire model,* T cells bearing receptors that recognize foreign antigens closely resembling self-antigens may be eliminated during thymic processing. Since the T-cell response to an antigen involves a trimolecular complex of the T cell's receptor, an antigenic peptide, and an MHC molecule (see Figure 3-8), both models may be correct. That is, the absence of an MHC molecule that can bind and present a given peptide, or the absence of T-cell receptors that can recognize a given peptide–MHC molecule complex, could result in the absence of immune responsiveness and so account for the observed relationship between

TABLE 7-3 Differential binding of peptides to mouse class II MHC molecules and correlation with MHC restriction

		PERCENTAGE OF LABELED PEPTIDE BOUND TO‡			
Labeled peptide*	MHC restriction of responders†	IA^d	IE^d	IA^k	IE^k
Ovalbumin (323–339)	IA^d	**11.8**	0.1	0.2	0.1
Influenza hemagglutinin (130–142)	IA^d	**18.9**	0.6	7.1	0.3
Hen egg-white lysozyme (46–61)	IA^k	0.0	0.0	**35.2**	0.5
Hen egg-white lysozyme (74–86)	IA^k	2.0	2.3	**2.9**	1.7
Hen egg-white lysozyme (81–96)	IE^k	0.4	0.2	0.7	**1.1**
Myoglobin (132–153)	IE^d	0.8	**6.3**	0.5	0.7
Pigeon cytochrome *c* (88–104)	IE^k	0.6	1.2	1.7	**8.7**
λ repressor (12–26)§	$IA^d + IE^k$	1.6	**8.9**	0.3	2.3

*Amino acid residues included in each peptide are indicated by the numbers in parentheses.

†Refers to class II molecule (IA or IE) and haplotype associated with a good response to the indicated peptides.

‡Binding determined by equilibrium dialysis. Bold-faced values indicate binding was significantly greater ($p < 0.05$) than that of the other three class II molecules tested.

§The λ repressor is an exception to the rule that high binding correlates with the MHC restriction of high-responder strains. In this case, the T_H cell specific for the λ peptide–IE^d complex has been deleted; this is an example of the hole-in-the-repertoire mechanism.

SOURCE: Adapted from S. Buus et al., 1987, *Science* **235:**1353.

MHC haplotype and immune responsiveness to exogenous antigens.

According to the determinant-selection model, the MHC polymorphism within a species will generate a diversity of binding specificities, and thus different patterns of responsiveness to antigens. If this model is correct, then class II MHC molecules from mouse strains that respond to a particular antigen and those that do not should show differential binding of that antigen. Table 7-3 presents data on the binding of various radiolabeled peptides to class II IA and IE molecules with the H-2^d or H-2^k haplotype. Each of the listed peptides binds significantly to only one of the IA or IE molecules. Furthermore, in all but one case, the haplotype of the class II molecule showing the highest affinity for a particular peptide is the same as the haplotype of responder strains for that peptide, as the determinant-selection model predicts.

The single exception to the general pattern in Table 7-3 (residues 12–26 of the λ repressor protein) gives evidence that the influence on immune responsiveness can also be caused by absence of functional T cells (holes-in-the-repertoire model) capable of recognizing a given antigen–MHC molecule complex. The λ repressor peptide binds best in vitro to IE^d, yet the MHC restriction for response to this peptide is known to be associated not with IE^d but instead with IA^d and IE^k. This suggests that T cells recognizing this repressor peptide in association with IE^d may have been eliminated by negative selection in the thymus, leaving a hole in the T-cell repertoire.

MHC and Disease Susceptibility

Some HLA alleles occur at a much higher frequency in those suffering from certain diseases than in the general population. The diseases associated with particular MHC alleles include autoimmune disorders, certain viral diseases, disorders of the complement system, some neurologic disorders, and several different allergies. The association between HLA alleles and a given disease may be quantified by determining the frequency of the HLA alleles expressed by individuals afflicted with the disease, then comparing these data with the frequency of the same alleles in the general population. Such a comparison allows calculation of **relative risk** (see Table 7-4). A relative risk value of 1 means that the HLA allele is expressed with the same frequency in the patient and general populations, indicating that the allele confers no increased risk for the disease. A relative risk value substantially

TABLE 7-4 Some significant associations of HLA alleles with increased risk for various diseases

Disease	Associated HLA allele	Relative risk*
Ankylosing spondylitis	*B27*	90
Goodpasture's syndrome	*DR2*	16
Gluten-sensitive enteropathy	*DR3*	12
Hereditary hemochromatosis	*A3*	9.3
	B14	2.3
	A3/B14	90
Insulin-dependent diabetes mellitus	*DR4/DR3*	20
Multiple sclerosis	*DR2*	5
Myasthenia gravis	*DR3*	10
Narcolepsy	*DR2*	130
Reactive arthritis (*Yersinia, Salmonella, Gonococcus*)	*B27*	18
Reiter's syndrome	*B27*	37
Rheumatoid arthritis	*DR4*	10
Sjogren's syndrome	*Dw3*	6
Systemic lupus erythematosus	*DR3*	5

*Relative risk is calculated by dividing the frequency of the HLA allele in the patient population by the frequency in the general population:

$$RR = \frac{(Ag^+/Ag^-)\ \text{disease}}{(Ag^+/Ag^-)\ \text{control}}$$

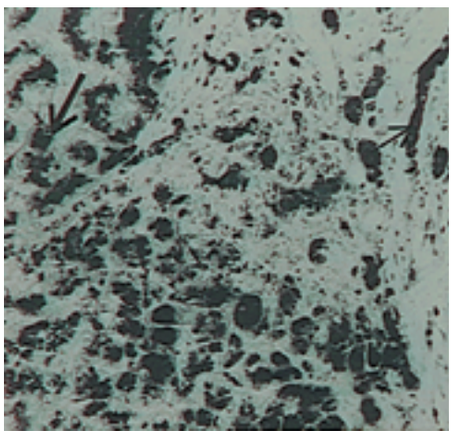
SOURCE: Data from *SAM CD: A Comprehensive Knowledge Base of Internal Medicine,* D. C. Dale and D. D. Federman, eds., 1997, Scientific American, New York.

CLINICAL FOCUS

HFE and Hereditary Hemochromatosis

Hereditary hemochromatosis (HH) is a disease in which defective regulation of dietary iron absorption leads to increased levels of iron. HH (which in earlier reports may be referred to as *idiopathic* or *primary* hemochromatosis) is the most common known autosomal recessive genetic disorder in North Americans of European descent, with a frequency of 3–4 cases per 1000 persons. Recent studies show that this disease is associated with a mutation in the nonclassical class I gene *HFE* (formerly designated *HLA-H*), which lies to the telomeric side of *HLA-A*. The association of the *HFE* gene with HH is an example of how potentially lifesaving clinical information can be obtained by studying the connection of HLA genes with disease.

The total iron content of a normal human adult is 3 to 4 grams; the average dietary intake of iron is about 10 to 20 milligrams per day; of this, only 1 to 2 mg is absorbed. The iron balance is maintained by control of its absorption from digested food in the intestinal tract. The primary defect in HH is increased gastrointestinal uptake of iron and, as a result of this, patients with HH may throughout their lives accumulate 15 to 35 grams of iron instead of the normal 3 to 4 grams. The iron overload results in pathologic accumulation of iron in cells of many organs, including the heart and liver. Although a severe form of HH may result in heart disease in children, the clinical manifestations of the disease are not usually seen until 40 to 50 years of age. Males are affected eight times more frequently than females. Early symptoms of HH are rather nonspecific and include weakness, lethargy, abdominal pain, diabetes, impotence, and severe joint pain. Physical examination of HH sufferers reveals liver damage, skin pigmentation, arthritis, en-



High-magnification iron stain of liver cells from HH patient. The stain confirms the presence of iron in both parenchymal cells (thick arrow) and bile duct cells (thin arrow). This woman with hemochromatosis required removal of 72 units (about 36 liters or 9 gallons) of blood during one and a half years to render her liver free of excess iron. [SAM CD: A Comprehensive Knowledge Base of Internal Medicine, *D. C. Dale and D. D. Federman, eds., 1997, Scientific American, New York.*]

above 1 indicates an association between the HLA allele and the disease. As Table 7-4 shows, individuals with the HLA-B27 allele have a 90 times greater likelihood (relative risk of 90) of developing the autoimmune disease ankylosing spondylitis, an inflammatory disease of vertebral joints characterized by destruction of cartilage, than do individuals with a different HLA-B allele.

The existence of an association between an MHC allele and a disease should not be interpreted to imply that the expression of the allele has caused the disease—the relationship between MHC alleles and development of disease is complex. In the case of ankylosing spondylitis, for example, it has been suggested that because of the close linkage of the TNF-α and TNF-β genes with the HLA-B locus, these cytokines may be involved in the destruction of cartilage. An association of HLA class I genes with the disease hereditary hemochromatosis is discussed in the Clinical Focus box in this chapter.

When the associations between MHC alleles and disease are weak, reflected by low relative risk values, it is likely that multiple genes influence susceptibility, of which only one is in the MHC. That these diseases are not inherited by simple Mendelian segregation of MHC alleles can be seen in identical twins; both inherit the MHC risk factor, but it is by no means certain that both will develop the disease. This finding suggests that multiple genetic and environmental factors have roles in the development of disease, especially autoimmune diseases, with the MHC playing an important but not exclusive role. An additional difficulty in associating a particular MHC product with disease is the genetic phenomenon of linkage disequilibrium, which was described above. The fact that some of the class I MHC alleles are in linkage disequilibrium with the class II MHC alleles makes their contribution to disease susceptibility appear more pronounced than it actually is. If, for example, DR4 contributes to risk of a disease, and if it occurs frequently in combination with A3 because of linkage disequilibrium, then A3 would incorrectly appear to be associated with the disease. Improved genomic mapping techniques make it possible to analyze the linkage between the MHC and various diseases more fully and to assess the contributions from other loci.

larged spleen, jaundice, and peripheral edema. If untreated, HH results in hepatic cancer, liver failure, severe diabetes, and heart disease. Exactly how the increase in iron content results in these diseases is not known, but repeated phlebotomy (taking blood) is an effective treatment if the disease is recognized before there is extensive damage to organs. Phlebotomy does not reverse damage already done. Phlebotomy (also called blood-letting) was used as treatment for many conditions in former times; HH may be one of the rare instances in which the treatment had a positive rather than a harmful effect on the patient.

Prior to appearance of the recognized signs of the disease, such as the characteristic skin pigmentation or liver dysfunction, diagnosis is difficult unless for some reason (such as family history of the disease) HH is suspected and specific tests for iron metabolism are performed. A reliable genetic test for HH would allow treatment to commence prior to disease manifestation and irreversible organ damage.

Because it is a common disease, the association of HH with HLA was studied; initially a significant association with the *HLA-A3* allele was found (RR of 9.3). This association is well documented, but the relatively high frequency of the *HLA-A3* allele (present in 20% of the North American population) makes this an inadequate marker; the majority of individuals with *HLA-A3* will not have HH. Further studies showed a greatly increased relative risk in individuals with the combination of *HLA-A3* and *HLA-B14;* homozygotes for these two alleles carried a relative risk for HH of 90. Detailed studies of several populations in the US and France with high incidence of HH revealed a mutation in the nonclassical HLA class I gene *HFE* in 83%–100% of patients with HH. *HFE,* which lies close to the *HLA-A* locus, was shown in several independent studies to carry a characteristic mutation at position 283 in HH patients, with substitution of a tyrosine residue for the cysteine normally found at this position. The substitution precludes formation of the disulfide link between cysteines in the $\alpha 3$ domain, which is necessary for association of the MHC α chain with β_2-microglobulin and for expression on the cell surface. HFE molecules are normally expressed on the surface of cells in the stomach, intestines, and liver. There is evidence showing that HFE plays a role in the ability of these organs to regulate iron uptake from the circulation. The mechanism by which HFE functions involves binding to the transferrin receptor, which reduces the affinity of the receptor for iron-loaded transferrin. This lowers the uptake of iron by the cell. Mutations that interfere with the ability of HFE to form a complex with transferrin and its receptor can lead to increased iron absorption and HH.

There are several possible reasons for why this defect continues to be so common in our population. Factors that favor the spread of the defective *HFE* gene would include the fact that it is a recessive trait, so only homozygotes are affected; the gene is silent in carriers. In addition, even in most homozygotes affected with HH, the disease does not manifest itself until later in life and so may have minimal influence on the breeding success of the HH sufferer.

Studies of knockout mice that lack the gene for β_2-microglobulin demonstrate that MHC class I products on cell surfaces are necessary for the maintenance of normal iron metabolism. These mice, which are unable to express any of their class I molecules on the cell surfaces, suffer from iron overload with disease consequences similar to HH.

A number of hypotheses have been offered to account for the role of the MHC in disease susceptibility. As noted earlier, allelic differences may yield differences in immune responsiveness arising from variation in the ability to present processed antigen or the ability of T cells to recognize presented antigen. Allelic forms of MHC genes may also encode molecules that are recognized as receptors by viruses or bacterial toxins. As will be explained in Chapter 16, the genetic analysis of disease must consider the possibility that genes at multiple loci may be involved and that complex interactions among them may be needed to trigger disease.

Some evidence suggests that a reduction in MHC polymorphism within a species may predispose that species to infectious disease. Cheetahs and certain other wild cats, such as Florida panthers, that have been shown to be highly susceptible to viral disease have very limited MHC polymorphism. It is postulated that the present cheetah population (Figure 7-17) arose from a limited breeding stock, causing a loss of MHC diversity. The increased susceptibility of cheetahs to various viral diseases may result from a reduction in

FIGURE 7-17 Cheetah female with two nearly full grown cubs. Polymorphism in MHC genes of the cheetah is very limited, presumably because of a bottleneck in breeding that occurred in the not too distant past. It is assumed that all cheetahs alive today are descendants of a very small breeding pool. [*Photograph taken in the Okavango Delta, Botswana, by T. J. Kindt.*]

the number of different MHC molecules available to the species as a whole and a corresponding limitation on the range of processed antigens with which these MHC molecules can interact. Thus, the high level of MHC polymorphism that has been observed in various species may provide the advantage of a broad range of antigen-presenting MHC molecules. Although some individuals within a species probably will not be able to develop an immune response to any given pathogen and therefore will be susceptible to infection by it, extreme polymorphism ensures that at least some members of a species will be able to respond and will be resistant. In this way, MHC diversity appears to protect a species from a wide range of infectious diseases.

SUMMARY

- The major histocompatibility complex (MHC) comprises a stretch of tightly linked genes that encode proteins associated with intercellular recognition and antigen presentation to T lymphocytes.
- A group of linked MHC genes is generally inherited as a unit from parents; these linked groups are called haplotypes.
- MHC genes are polymorphic in that there are large numbers of alleles for each gene, and they are polygenic in that there are a number of different MHC genes.
- Class I MHC molecules consist of a large glycoprotein chain with 3 extracellular domains and a transmembrane segment, and β_2-microglobulin, a protein with a single domain.
- Class II MHC molecules are composed of two noncovalently associated glycoproteins, the α and β chain, encoded by separate MHC genes.
- X-ray crystallographic analyses reveal peptide-binding clefts in the membrane-distal regions of both class I and class II MHC molecules.
- Both class I and class II MHC molecules present antigen to T cells. Class I molecules present processed endogenous antigen to CD8 T cells. Class II molecules present processed exogenous antigen to CD4 T cells.
- Certain conserved motifs in peptides influence their ability to interact with the membrane-distal regions of class I and class II MHC molecules.
- Class I molecules are expressed on most nucleated cells; class II antigens are restricted to B cells, macrophages, and dendritic cells.
- The class III region of the MHC encodes molecules that include a diverse group of proteins that play no role in antigen presentation.
- Detailed maps of the human and mouse MHC reveal the presence of genes involved in antigen processing, including proteasomes and transporters.
- Studies with mouse strains have shown that MHC haplotype influences immune responsiveness and the ability to present antigen.
- Increased susceptibility to a number of diseases, predominantly, but not exclusively, of an autoimmune nature, has been linked to certain MHC alleles.

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Review and quiz of key terms

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USEFUL WEB SITES

http://www.bioscience.org/knockout/b2micrgl.htm
for beta-2 microglobulin KO

http://www.bioscience.org/knockout/mhci.htm
for MHC class I KO

http://www.bioscience.org/knockout/mhcii.htm
for KO of an MHC class II chain

http://www.bioscience.org/knockout/mhc2inva.htm
for KO of the invariant chain

This series of destinations in the Bioscience Web site provides updated information on studies of the consequences of targeted disruption of MHC molecules and other component molecules including β_2 microglobulin and the class II invariant chain.

http://www.bshi.org.uk/

British Society for Histocompatibility and Immunogenetics home page contains information on tissue typing, transplantation, and links to worldwide sites concerned with MHC.

http://www.ebi.ac.uk/imgt/hla/

The International ImMunoGeneTics (IMGT) database section contains links concerned with HLA gene structure and genetics. It also contains up-to-date listings and sequences for all HLA alleles officially recognized by the World Health Organization HLA nomenclature committee.

Study Questions

CLINICAL FOCUS QUESTION Almost 90% of Caucasians homozygous for a mutation in position 283 of the HFE gene have clinical signs of hemochromatosis. The fact that 10% of those with the mutation are not affected causes a critic of the work to state that the HFE is not involved with HH. She contends that this association is just a result of linkage disequilibrium. How would you answer her? Can you design an experiment to shed further light on this association?

1. Indicate whether each of the following statements is true or false. If you think a statement is false, explain why.
   a. A monoclonal antibody specific for β_2-microglobulin can be used to detect both class I MHC K and D molecules on the surface of cells.
   b. Antigen-presenting cells express both class I and class II MHC molecules on their membranes.
   c. Class III MHC genes encode membrane-bound proteins.
   d. In outbred populations, an individual is more likely to be histocompatible with one of its parents than with its siblings.
   e. Class II MHC molecules typically bind to longer peptides than do class I molecules.
   f. All cells express class I MHC molecules.
   g. The majority of the peptides displayed by class I and class II MHC molecules on cells are derived from self-proteins.
2. You wish to produce a syngeneic and a congenic mouse strain. Indicate whether each of the following characteristics applies to production of syngeneic (S), congenic (C), or both (S and C) mice.
   a. Requires the greatest number of generations
   b. Requires backcrosses
   c. Yields mice that are genetically identical
   d. Requires selection for homozygosity
   e. Requires sibling crosses
   f. Can be started with outbred mice
   g. Yields progeny that are genetically identical to the parent except for a single genetic region
3. You have generated a congenic A.B mouse strain that has been selected for its MHC haplotype. The haplotype of strain A was *a/a* and of strain B was *b/b*.
   a. Which strain provides the genetic background of this mouse?
   b. Which strain provides the haplotype of the MHC of this mouse?
   c. To produce this congenic strain, the F1 progeny are always backcrossed to which strain?
   d. Why was backcrossing to one of the parents performed?
   e. Why was interbreeding of the F_1 and F_2 progeny performed?
   f. Why was selection necessary and what kind of selection was performed?
4. You cross a BALB/c (H-2^d) mouse with a CBA (H-2^k) mouse. What MHC molecules will the F_1 progeny express on (a) its liver cells and (b) its macrophages?
5. To carry out studies on the structure and function of the class I MHC molecule K^b and the class II MHC molecule IA^b, you decide to transfect the genes encoding these proteins into a mouse fibroblast cell line (L cell) derived from the C3H strain (H-2^k). L cells do not normally function as antigen-presenting cells. In the following table, indicate which of the listed MHC molecules will (+) or will not (−) be expressed on the membrane of the transfected L cells.

	MHC molecules expressed on the membrane of the transfected L cells					
Transfected gene	D^k	D^b	K^k	K^b	IA^k	IA^b
None						
K^b						
$IA\alpha^b$						
$IA\beta^b$						
$IA\alpha^b$ and $IA\beta^b$						

6. The SJL mouse strain, which has the H-2^k haplotype, has a deletion of the *IE*α locus.
   a. List the classical MHC molecules that are expressed on the membrane of macrophages from SJL mice.
   b. If the class II *IE*α and *IE*β genes from an H-2^s strain are transfected into SJL macrophages, what additional classical MHC molecules would be expressed on the transfected macrophages?
7. Draw diagrams illustrating the general structure, including the domains, of class I MHC molecules, class II MHC molecules, and membrane-bound antibody on B cells. Label each

chain and the domains within it, the antigen-binding regions, and regions that have the immunoglobulin-fold structure.

8. One of the characteristic features of the MHC is the large number of different alleles at each locus.

a. Where are most of the polymorphic amino acid residues located in MHC molecules? What is the significance of this location?
b. How is MHC polymorphism thought to be generated?

9. As a student in an immunology laboratory class, you have been given spleen cells from a mouse immunized with the LCM virus. You determine the antigen-specific functional activity of these cells with two different assays. In assay 1, the spleen cells are incubated with macrophages that have been briefly exposed to the LCM virus; the production of interleukin 2 (IL-2) is a positive response. In assay 2, the spleen cells are incubated with LCM-infected target cells; lysis of the target cells represents a positive response in this assay. The results of the assays using macrophages and target cells of different haplotypes are presented in the table below. Note that the experiment has been set up in a way to exclude alloreactive responses (reactions against nonself MHC molecules).

a. The activity of which cell population is detected in each of the two assays?
b. The functional activity of which MHC molecules is detected in each of the two assays?
c. From the results of this experiment, which MHC molecules are required, in addition to the LCM virus, for specific reactivity of the spleen cells in each of the two assays?
d. What additional experiments could you perform to unambiguously confirm the MHC molecules required for antigen-specific reactivity of the spleen cells?
e. Which of the mouse strains listed in the table below could have been the source of the immunized spleen cells tested in the functional assays? Give your reasons.

10. A T_C-cell clone recognizes a particular measles virus peptide when it is presented by H-2D^b. Another MHC molecule has a peptide-binding cleft identical to the one in H-2D^b but differs from H-2D^b at several other amino acids in the $\alpha 1\beta 1$ domain. Predict whether the second MHC molecule could present this measles virus peptide to the T_C-cell clone. Briefly explain your answer.

11. How can you determine if two different inbred mouse strains have identical MHC haplotypes?

12. Human red blood cells are not nucleated and do not express any MHC molecules. Why is this property fortuitous for blood transfusions?

13. The hypothetical allelic combination *HLA-A99* and *HLA-B276* carries a relative risk of 200 for a rare, and yet unnamed, disease that is fatal to pre-adolescent children.

a. Will every individual with *A99/B276* contract the disease?
b. Will everyone with the disease have the *A99/B276* combination?
c. How frequently will the *A99/B276* allelic combination be observed in the general population? Do you think that this combination will be more or less frequent than predicted by the frequency of the two individual alleles? Why?

For use with Question 9.

Mouse strain used as source of macrophages and target cells	MHC haplotype of macrophages and virus-infected target cells				Response of spleen cells	
	K	IA	IE	D	IL-2 production in response to LCM-pulsed macrophages (assay 1)	Lysis of LCM-infected cells (assay 2)
C3H	*k*	*k*	*k*	*k*	+	–
BALB/c	*d*	*d*	*d*	*d*	–	+
(BALB/c × B10.A)F_1	*d/k*	*d/k*	*d/k*	*d/d*	+	+
A.TL	*s*	*k*	*k*	*d*	+	+
B10.A (3R)	*b*	*b*	*b*	*d*	–	+
B10.A (4R)	*k*	*k*	–	*b*	+	–