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LECTURE 05: B-Cell GENERATION, ACTIVATION

The developmental process that results in the production of plasma cells and memory B cells can be divided into three broad stages: generation of mature, immunocompetent B cells (maturation), activation of mature B cells when they interact with antigen, and differentiation of activated B cells into plasma cells and memory B cells. In many vertebrates, including humans and mice, the bone marrow generates B cells. This process is an orderly sequence of Ig-gene rearrangements, which progresses in the absence of antigen. This is the antigen independent phase of B-cell development.

A mature B cell leaves the bone marrow expressing membrane-bound immunoglobulin (mIgM and mIgD) with a single antigenic specificity. These naive B cells, which have not encountered antigen, circulate in the blood and lymph and are carried to the secondary lymphoid organs, most notably the spleen and lymph nodes. If a B cell is activated by the antigen specific to its membrane-bound antibody, the cell proliferates (clonal expansion) and differentiates to generate a population of antibody-secreting plasma cells and memory B cells. In this activation stage, affinity maturation is the progressive increase in the average affinity of the antibodies produced and class switching is the change in the isotype of the antibody produced by the B cell from μ to γ, α, or ε. Since B cell activation and differentiation in the periphery require antigen, this stage comprises the antigen dependent phase of B-cell development. Many B cells are produced in the bone marrow throughout life, but very few of these cells mature.

Overview of B-cell development

During the antigen-independent maturation phase, immunocompetent B cells expressing membrane IgM and IgD are generated in the bone marrow. Only about 10% of the potential B cells reach maturity and exit the bone marrow. Naive B cells in the periphery die within a few days unless they encounter soluble protein antigen and activated TH cells. Once activated, B cells proliferate within secondary lymphoid organs. Those bearing high-affinity mIg differentiate into plasma cells and memory B cells, which may express different isotypes because of class switching. The numbers cited refer to B-cell development in the mouse, but the overall principles apply to humans as well.

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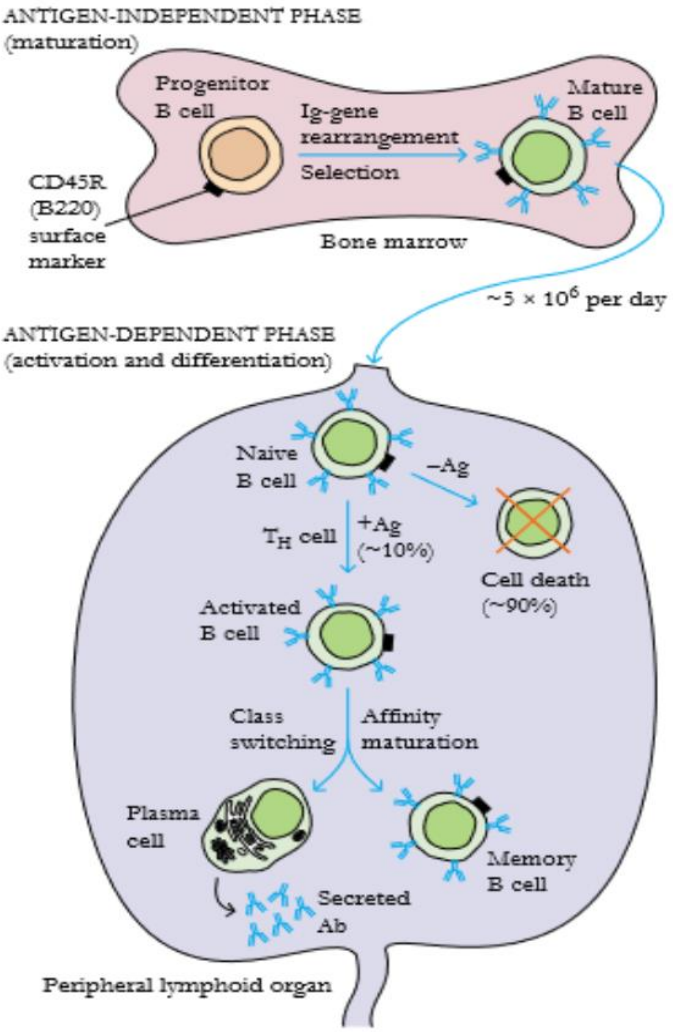


B-Cell Maturation

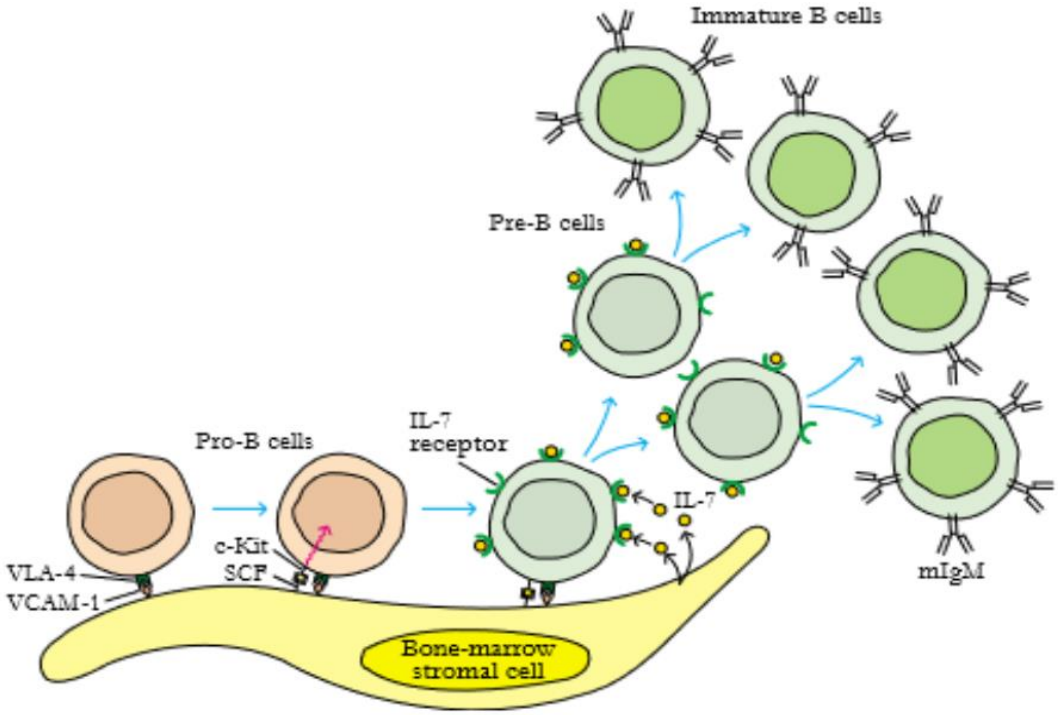
The generation of mature B cells first occurs in the embryo and continues throughout life. Before birth, the yolk sac, fetal liver, and fetal bone marrow are the major sites of B-cell maturation; after birth, generation of mature B cells occurs in the bone marrow.

Progenitor B Cells Proliferate in Bone Marrow

B-cell development begins as lymphoid stem cells differentiate into the earliest distinctive B-lineage cell—the progenitor B cell (pro-B cell)—which expresses a transmembrane tyrosine phosphatase called CD45R (sometimes called B220 in mice).Pro-B cells proliferate within the bone marrow, filling the extravascular spaces between large sinusoids in the shaft of a bone. Proliferation and differentiation of pro-B cells into precursor B cells (pre-B cells) requires the microenvironment provided by the bone-marrow stromal cells. If pro-B cells are removed from the bone marrow and cultured in vitro, they will not progress to more mature B-cell stages unless stromal cells are

present. The stromal cells play two important roles: they interact directly with pro-B and pre-B cells, and they secrete various cytokines, notably IL-7, that supports the developmental process.

At the earliest developmental stage, pro-B cells require direct contact with stromal cells in the bone marrow. This interaction is mediated by several cell-adhesion molecules, including VLA-4 on the pro-B cell and its ligand, VCAM-1, on the stromal cell (see Figure).



After initial contact is made, a receptor on the pro-B cell called c-Kit interacts with a stromal-cell surface molecule known as stem-cell factor (SCF). This interaction activates c-Kit, which is a tyrosine kinase, and the pro-B cell begins to divide and differentiate into a pre-B cell and begins expressing a receptor for IL-7. The IL-7 secreted by the stromal cells drives the maturation process, eventually inducing down-regulation of the adhesion molecules on the pre-B cells, so that the proliferating cells can detach from the stromal cells. At this stage, pre-B cells no longer require direct contact with stromal cells but continue to require IL-7 for growth and maturation.

B-Cell Activation and Proliferation

After export of B cells from the bone marrow, activation, proliferation, and differentiation occur in the periphery and require antigen. Antigen-driven activation and clonal selection of naive B cells leads to generation of plasma cells and memory B cells. In the absence of antigen-induced activation, naive B cells in the periphery have a short life span, dying within a few weeks by apoptosis.

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Thymus-Dependent and Thymus Independent Antigen Have Different Requirements for Response. Depending on the nature of the antigen, B-cell activation proceeds by two different routes, one dependent upon T_H cells, the other not. The B-cell response to thymus-dependent (TD) antigens requires direct contact with T_H cells, not simply exposure to T_H-derived cytokines. Antigens that can activate B cells in the absence of this kind of direct participation by T_H cells are known as thymus-independent (TI) antigens. TI antigens are divided into types 1 and 2, and they activate B cells by different mechanisms.

Some bacterial cell-wall components, including lipopolysaccharide (LPS), function as type 1 thymus-independent (TI-1) antigens. Type 2 thymus-independent (TI-2) antigens are highly repetitious molecules such as polymeric proteins (e.g., bacterial flagellin) or bacterial cell-wall polysaccharides with repeating polysaccharide units. Most TI-1 antigens are polyclonal B-cell activators (mitogens); that is, they are able to activate B cells regardless of their antigenic specificity. At high concentrations, some TI-1 antigens will stimulate proliferation and antibody secretion by as many as one third of all B cells. The mechanism by which TI-1 antigens activate B cells is not well understood.

When B cells are exposed to lower concentrations of TI-1 antigens, only those B cells specific for epitopes of the antigen will be activated. These antigens can stimulate antibody production in nude mice (which lack a thymus and thus are greatly deficient in T cells), and the response is not greatly augmented by transferring T cells into these athymic mice, indicating that TI-1 antigens are truly T-cell independent. The prototypic TI-1 antigen is lipopolysaccharide (LPS), a major component of the cell walls of gram-negative bacteria. At low concentrations, LPS stimulates the production of antibodies specific for LPS. At high concentrations, it is a polyclonal B-cell activator.

TI-2 antigens activate B cells by extensively crosslinking the mIg receptor. However, TI-2 antigens differ from TI-1 antigens in three important respects. First, they are not B-cell mitogens and so do not act as polyclonal activators. Second, TI-1 antigens will activate both mature and immature B cells, but TI-2 antigens activate mature B cells and inactivate immature B cells. Third, although the B-cell response to TI-2 antigens does not require direct involvement of T_H cells, cytokines derived from T_H cells are required for efficient B-cell proliferation and for class switching to isotypes other than IgM.

The humoral response to thymus-independent antigens is different from the response to thymus-dependent antigens (Table 11-2).The response to TI antigens is generally weaker, no memory cells are formed, and IgM is the predominant antibody secreted, reflecting a low level of class switching. These differences highlight the important role played by T_H cells in generating memory B cells, affinity maturation, and class switching to other isotypes.

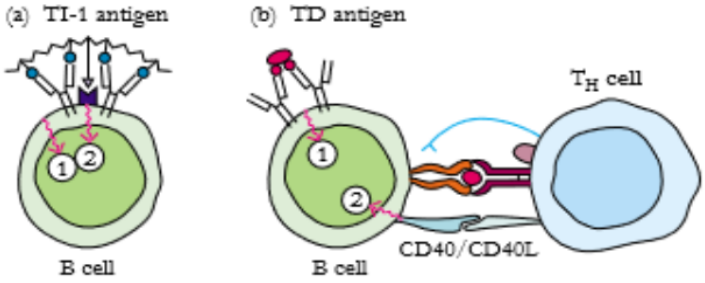
TABLE 11-2 Properties of thymus-dependent and thymus-independent antigens

		TI ANTIGENS	
Property	**TD antigens**	**Type 1**	**Type 2**
Chemical nature	Soluble protein	Bacterial cell-wall components (e.g., LPS)	Polymeric protein antigens; capsular polysaccharides
Humoral response			
Isotype switching	Yes	No	Limited
Affinity maturation	Yes	No	No
Immunologic memory	Yes	No	No
Polyclonal activation	No	Yes (high doses)	No

Two Types of Signals Drive B Cells into and Through the Cell Cycle

Naive, or resting, B cells are nondividing cells in the G0 stage of the cell cycle. Activation drives the resting cell into the cell cycle, progressing through G1 into the S phase, in which DNA is replicated. The transition from G1 to S is a critical restriction point in the cell cycle. Once a cell has reached S, it completes the cell cycle, moving through G2 and into mitosis (M). Analysis of the progression of lymphocytes from G0 to the S phase revealed similarities with the parallel sequence in fibroblast cells. These events could be grouped into two categories, competence signals and progression signals.

Competence signals drive the B cell from G0 into early G1, rendering the cell competent to receive the next level of signals. Progression signals then drive the cell from G1 into S and ultimately to cell division and differentiation. Competence is achieved by not one but two distinct signaling events, which are designated signal 1 and signal 2. These signaling events are generated by different pathways with thymus-independent and thymus-dependent antigens, but both pathways include signals generated when multivalent antigen binds and crosslinks mIg (see Figure).



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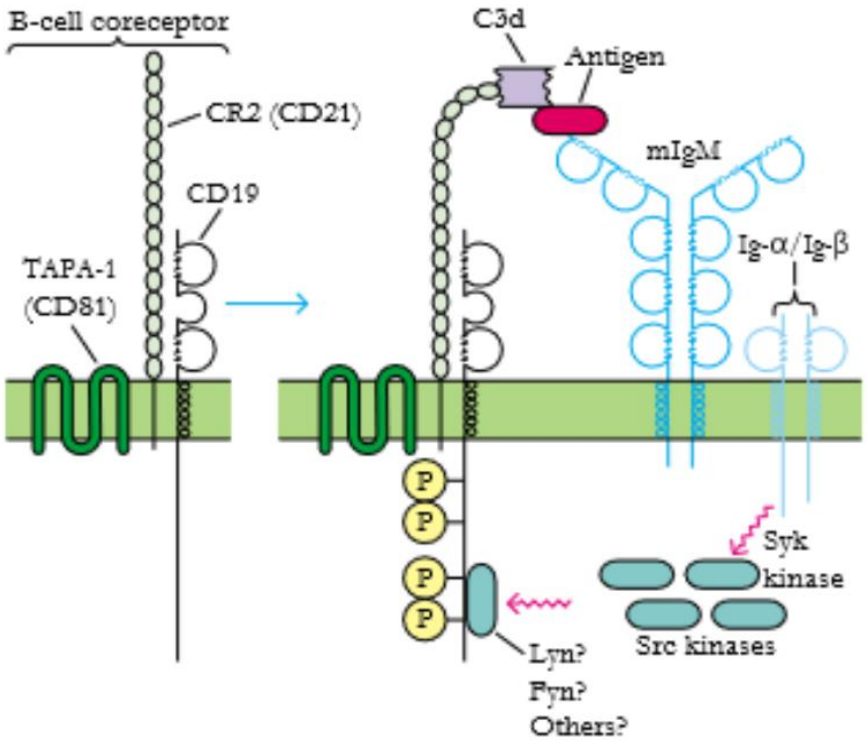
Once the B cell has acquired an effective competence signal in early activation, the interaction of cytokines and possibly other ligands with the B-cell membrane receptors provides progression signals.

The B-Cell–Coreceptor Complex Can Enhance B-Cell Responses

Stimulation through antigen receptors can be modified significantly by signals through coreceptors. Recall that costimulation through CD28 is an essential feature of effective positive stimulation of T lymphocytes, while signaling through CTLA-4 inhibits the response of the T cell. In B cells a component of the B-cell membrane, called the B-cell coreceptor, provides stimulatory modifying signals. The B-cell coreceptor is a complex of three proteins: CD19, CR2 (CD21), and TAPA-1 (CD81) (Figure 11-9).



CD19, a member of the immunoglobulin superfamily, has a long cytoplasmic tail and three extracellular domains. The CR2 component is a receptor of C3d, a breakdown product of the complement system, which is an important effector mechanism for destroying invaders; note that the involvement of C3d in the pathway for coreceptor activity reveals different arms of the immune system interacting with each other. CR2 also functions as a receptor for a membrane molecule and the transmembrane protein TAPA-1. In addition to the stimulatory coreceptor, another molecule, CD22, which is constitutively associated with the B-cell receptor in resting B cells, delivers a negative signal that makes B-cells more difficult to activate.

The CR2 component of the coreceptor complex binds to complement-coated antigen that has been captured by the mIg on the B cell. This crosslinks the coreceptor to the BCR and allows the CD19 component of the coreceptor to interact with the Ig-α / Ig-β component of the BCR.CD19 contains six tyrosine residues in its long cytoplasmic tail and is a major substrate of the protein tyrosine kinase activity that is mediated by cross linkage of the BCR. Phosphorylation of CD19 permits it to bind a number of signaling molecules, including the protein tyrosine kinase Lyn.

The delivery of these signaling molecules to the BCR complex contributes to the activation process, and the coreceptor complex serves to amplify the activating signal transmitted through the BCR. In one experimental in vitro system, for example, 104 molecules of mIgM had to be engaged by antigen for B-cell activation to occur when the coreceptor was not involved. But when CD19/CD2/TAPA-1 coreceptor was crosslinked to the BCR, only 102 molecules of mIgM had to be engaged for B-cell activation.

Another striking experiment highlights the role played by the B-cell coreceptor. Mice were immunized with either unmodified lysozyme or a hybrid protein in which genetic engineering was used to join hen's egg lysozyme to C3d.The fusion protein bearing 2 or 3 copies of C3d produced anti-lysozyme responses that were 1000 to 10,000 times greater than those to lysozyme alone. Perhaps coreceptor phenomena such as these explain how naive B cells that often express mIg with low affinity for antigen are able to respond to low concentrations of antigen in a primary response. Such responses, even though initially of low affinity, can play a significant role in the ultimate generation of high-affinity antibody. As described later in this chapter, response to an antigen can lead to affinity maturation, resulting in higher average affinity of the B-cell population.

Finally, two experimental observations indicate that the CD19 component of the B-cell coreceptor can play a role independent of CR2, the complement receptor. In normal mice, artificially crosslinking the BCR with anti-BCR antibodies results in the stimulation of some of the signal-transduction pathways characteristic of B-cell activation. On the other hand, treatment of B cells from mice in which CD19 has been knocked out with anti-BCR antibody fails to induce these pathways. Furthermore, CD19 knockout mice make greatly diminished antibody response to most antigens.

T_H Cells Play Essential Roles in Most B-Cell Responses

As noted already, activation of B cells by soluble protein antigens requires the involvement of T_H cells. Binding of antigen to B-cell mIg does not itself induce an effective competence signal

without additional interaction with membrane molecules on the T_H cell. In addition, a cytokine-mediated progression is required for B-cell proliferation.

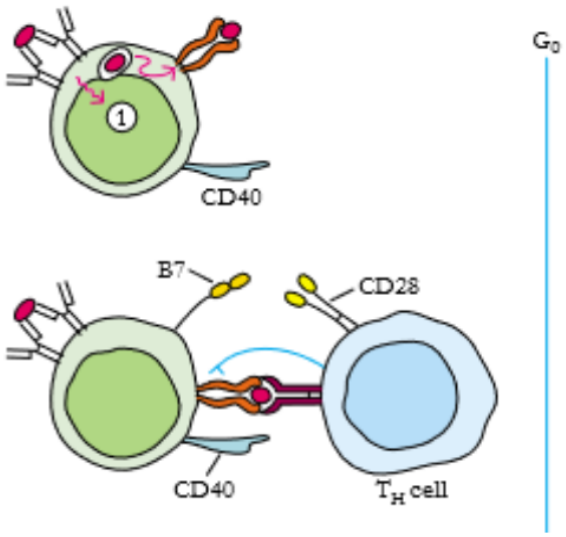
Figure 11-10 outlines the probable sequence of events in B-cell activation by a thymus-dependent (TD) antigen. This process is considerably more complex than activation induced by thymus independent (TI) antigens.

FORMATION OF T-B CONJUGATE

After binding of antigen by mIg on B cells, the antigen is internalized by receptor-mediated endocytosis and processed within the endocytic pathway into peptides. Antigen binding also initiates signaling through the BCR that induces the B cell to up-regulate a number of cell-membrane molecules; including class II MHC molecules and the co-stimulatory ligand B7 (see Figure 11-10a).

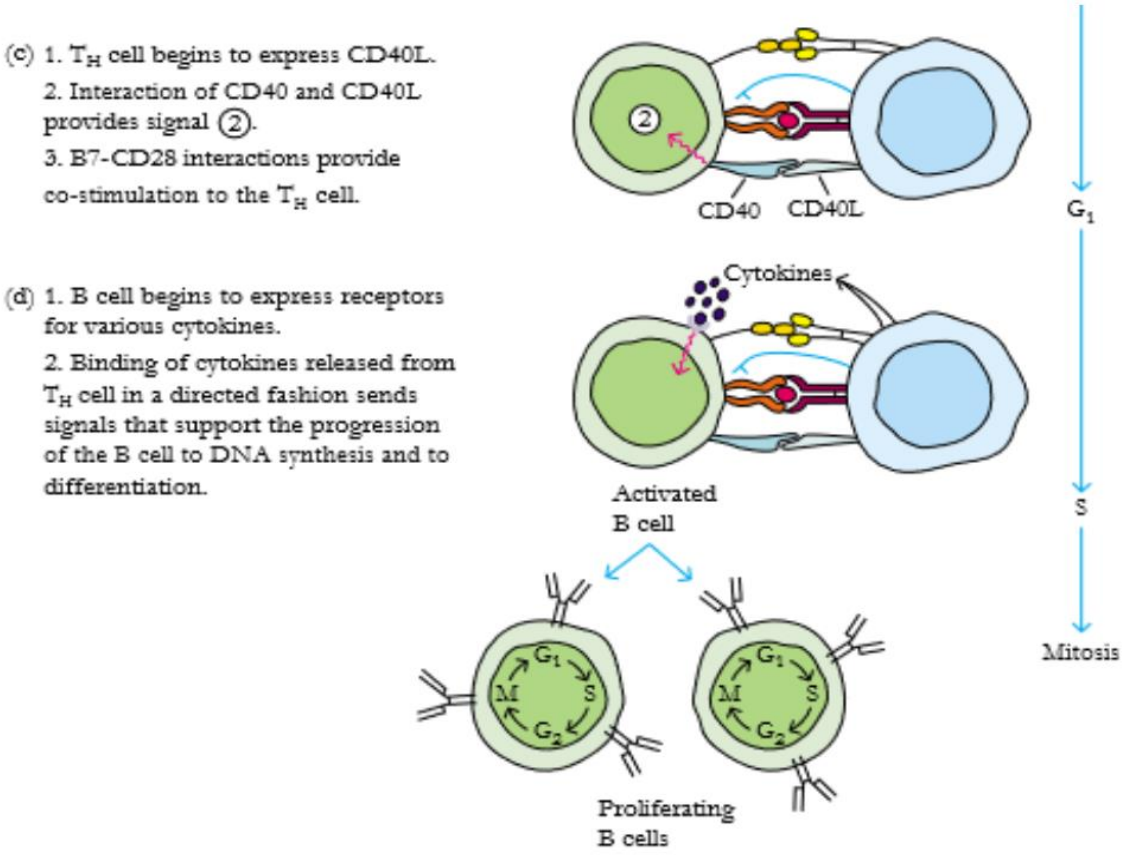
(a) Antigen crosslinks mIg, generating signal (1), which leads to increased expression of class II MHC and co-stimulatory B7. Antigen–antibody complexes are internalized by receptor-mediated endocytosis and degraded to peptides, some of which are bound by class II MHC and presented on the membrane as peptide–MHC complexes.

(b) T_H cell recognizes antigen–class II MHC on B-cell membrane. This plus co-stimulatory signal activates T_H cell.



Increased expression of both of these membrane proteins enhances the ability of the B cell to function as an antigen-presenting cell in T_H-cell activation. B-cells could be regarded as helping their helpers because the antigenic peptides produced within the endocytic processing pathway associate with class II MHC molecules and are presented on the B-cell membrane to the TH cell, inducing its activation. It generally takes 30–60 min after internalization of antigen for processed antigenic peptides to be displayed on the B-cell membrane in association with class II MHC molecules. Because a B cell recognizes and internalizes antigen specifically, by way of its membrane-bound Ig, a B cell is able to present antigen to TH cells at antigen concentrations that are 100 to 10,000 times lower than what is required for presentation by macrophages or dendritic cells.

When antigen concentrations are high, macrophages and dendritic cells are effective antigen-presenting cells, but, as antigen levels drop, B cells take over as the major presenter of antigen to T_H cells. Once a T_H cell recognizes a processed antigenic peptide displayed by a class II MHC molecule on the membrane of a B cell, the two cells interact to form a T-B conjugate (Figure 11-11). Micrographs of T-B conjugates reveal that the TH cells in antigen-specific conjugates have reorganized the Golgi apparatus and the microtubular-organizing center toward the junction with the B cell.This structural adjustment facilitates the release of cytokines toward the antigen-specific B cell.



The Humoral Response

Primary and Secondary Responses Differ Significantly

The kinetics and other characteristics of the humoral response differ considerably depending on whether the humoral response results from activation of naive lymphocytes (primary response) or memory lymphocytes (secondary response). In both cases, activation leads to production of secreted antibodies of various isotypes, which differ in their ability to mediate specific effector functions (see Table 4-2). The first contact of an exogenous antigen with an individual generates a primary humoral response, characterized by the production of antibody-secreting plasma cells and memory B cells. The kinetics of the primary response, as measured by serum antibody level, depend on the nature of the antigen, the route of antigen administration, the presence or absence of

adjuvants, and the species or strain being immunized. In all cases, however, a primary response to antigen is characterized by a lag phase, during which naive B cells undergo clonal selection, subsequent clonal expansion, and differentiation into memory cells or plasma cells (Figure 11-14).

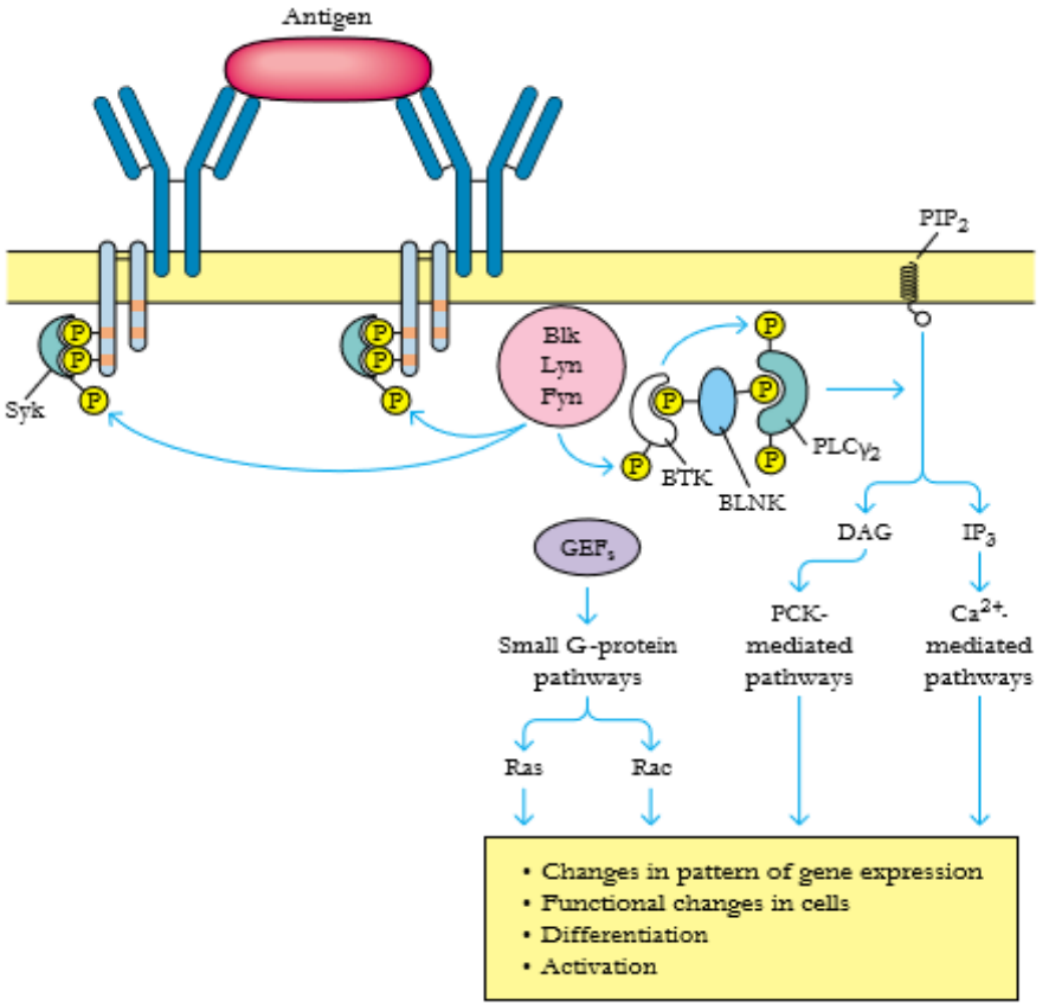
The lag phase is followed by a logarithmic increase in serum antibody level, which reaches a peak, plateaus for a variable time, and then declines. The duration of the lag phase varies with the nature of the antigen. Immunization of mice with an antigen such as sheep red blood cells (SRBCs) typically results in a lag phase of 3–4 days. Eight or nine successive cell divisions of activated B cells during days 4 and 5 then generate plasma and memory cells. Peak plasma-cell levels are attained at day 4–5; peak serum antibody levels are attained by around day 7–10. For soluble protein antigens, the lag phase is a little longer, often lasting about a week, peak plasma-cell levels are attained by 9–10 days, and peak serum titers are present by around 14 days.

During a primary humoral response, IgM is secreted initially, often followed by a switch to an increasing proportion of IgG. Depending on the persistence of the antigen, a primary response can last for various periods, from only a few days to several weeks. The memory B cells formed during a primary response stop dividing and enter the G0 phase of the cell cycle. These cells have variable life spans, with some persisting for the life of the individual. The capacity to develop a secondary humoral response (see Figure 11-14) depends on the existence of this population of memory B cells as well as memory T cells. Activation of memory cells by antigen results in a secondary antibody response that can be distinguished from the primary response in several ways (Table 11-4). The secondary response has a shorter lag period, reaches a greater magnitude, and lasts longer.

The secondary response is also characterized by secretion of antibody with a higher affinity for the antigen, and isotypes other than IgM predominate. A major factor in the more rapid onset and greater magnitude of secondary responses is the fact that the population of memory B cells specific for a given antigen is considerably larger than the population of corresponding naive B cells. Furthermore, memory cells are more easily activated than naive B cells. The processes of affinity maturation and class switching are responsible for the higher affinity and different isotypes exhibited in a secondary response. The higher levels of antibody coupled with the overall higher affinity provide an effective host defense against reinfection. The change in isotype provides antibodies whose effector functions are particularly suited to a given pathogen. The existence of long-lived memory B cells accounts for a phenomenon called “original antigenic sin,” which was first observed when the antibody response to influenza vaccines was monitored in adults.

Monitoring revealed that immunization with an influenza vaccine of one strain elicited an antibody response to that strain but, paradoxically, also elicited an antibody response of greater magnitude to another influenza strain that the individual had been exposed to during childhood. It was as if the memory of the first antigen exposure had left a life-long imprint on the immune system. This phenomenon can be explained by the presence of a memory-cell population, elicited by the influenza strain encountered in childhood that is activated by cross-reacting epitopes on the vaccine strain encountered later. This process then generates a secondary response, characterized by antibodies with higher affinity for the earlier viral strain.

Some of the many signal-transduction pathways activated by the BCR



In one pathway, Syk activates PLCγ_2 by tyrosine phosphorylation. PLCγ_2 then hydrolyzes PIP_2, a membrane phospholipid, to produce the second messengers DAG and IP_3. DAG and Ca2+ released by the action of IP_3 collaboratively activate the PKC, which induces additional signal-transduction pathways. The activated receptor complex also generates signals that activate the Ras

pathway. Activated Ras initiates a cascade of phosphorylations that culminates in the activation of transcription factors that up-regulate the expression of many genes.

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Major Histocompatibility Complex (MHC)

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.

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):

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**.

2. **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**.
3. **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**.

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

Simplified organization of the major histocompatibility complex (MHC) in the mouse and human

→ 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.

→ 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!

→ 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

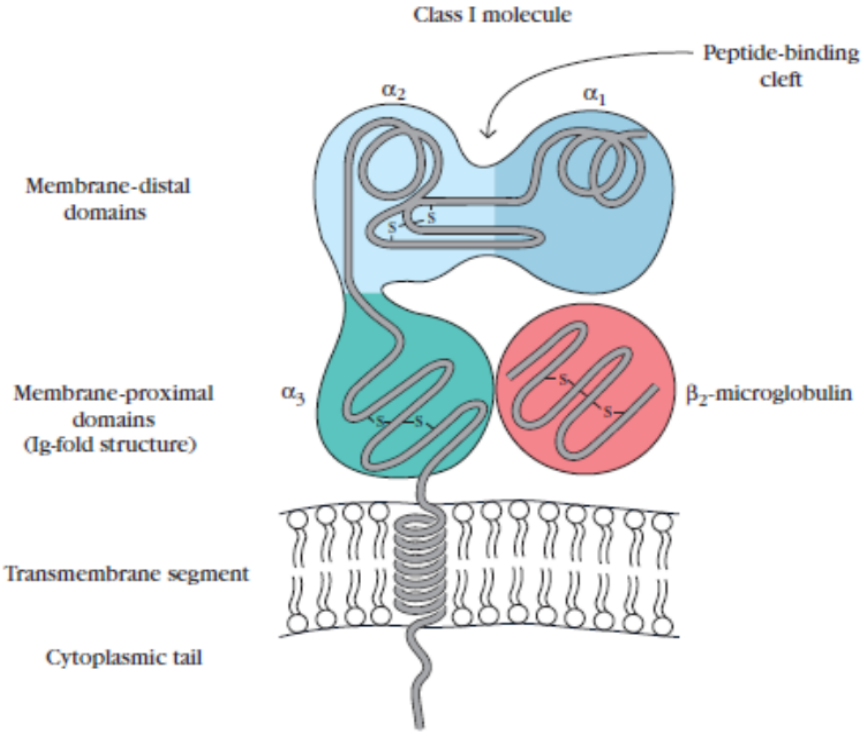
regions, encodes molecules that are critical to immune function but have little in common with class I or II molecules. Class III products include the complement components C4, C2, BF, and inflammatory cytokines, including tumor necrosis factor (TNF) and heat-shock proteins.

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.

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



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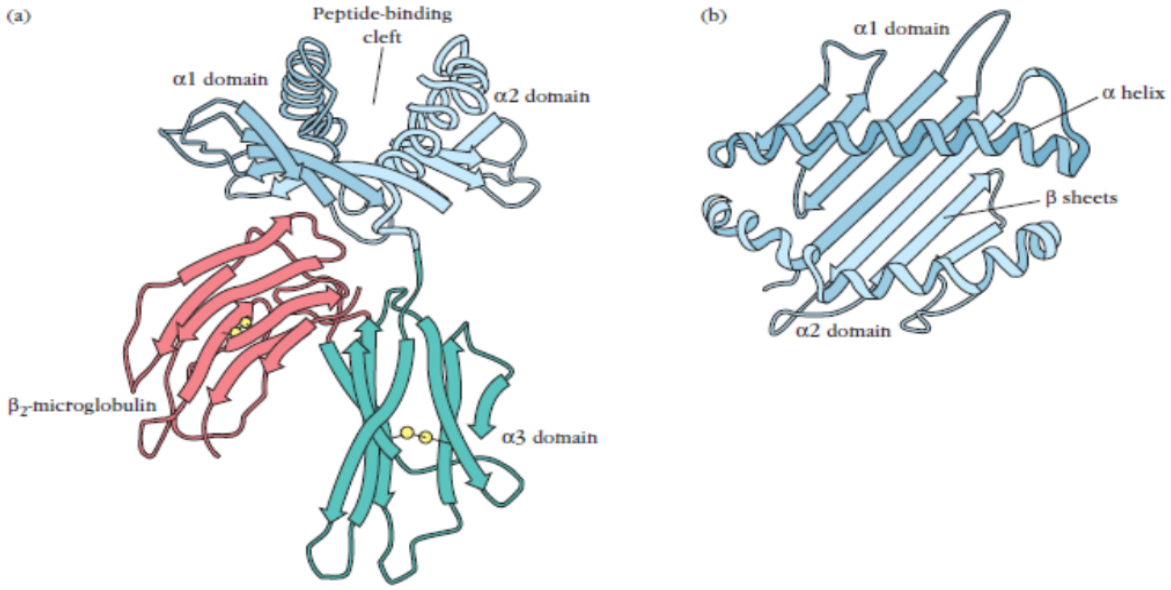
Class I MHC molecules contain a 45-kilodalton (kDa) α chain associated noncovalently with a 12-kDa β_2-microglobulin molecule. The α chain is a transmembrane glycoprotein encoded by polymorphic genes within the A, B, and C regions of the human HLA complex and within the K and D/L regions of the mouse H-2 complex.

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. 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 a). 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 X 10 Å X 11 Å, with the long α helices as sides and the β strands of the β sheet as the bottom (Figure b).

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.

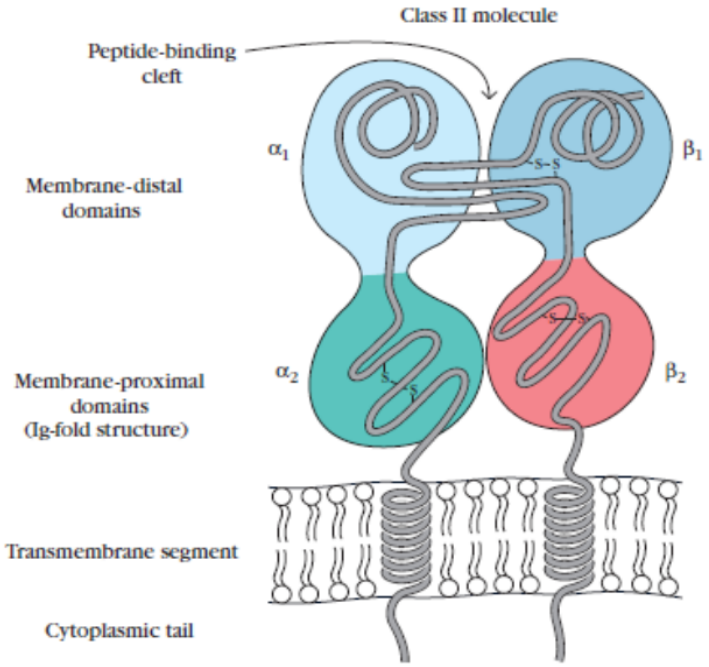
The α3 domain and β_2-microglobulin are organized into two β pleated sheets each formed by antiparallel β strands of amino acids. Because of this structural similarity, which is not surprising given the considerable sequence similarity with the immunoglobulin constant regions, class I MHC molecules and β_2-microglobulin are classified as members of the immunoglobulin superfamily. 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 TC 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.

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.

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). 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 α3/β_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 surperimposed. 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.

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 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 (see Table).

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

	Class I molecules	Class II molecules
Peptide-binding domain	α1/α2	α1/β1
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

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 (Kd ~ 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.