Immunogenetics

THE IMMUNE RESPONSE: BASIC CONCEPTS

The Innate Immune System

- When a pathogenic microorganism is encountered, the body's first line of defense includes **phagocytes** (a type of cell that engulfs and destroys the microorganism) and the **complement system.**

- The complement proteins can destroy microbes directly by perforating their cell membranes, and they can also attract phagocytes and other immune system agents to microbes by coating the microbial surface (it is because of this assisting role that the term *complement* originated).

- **Natural killer cells,** a specific type of lymphocyte, can respond to certain viral infections and some tumor cells.

- Phagocytes, complement, and natural killer cells are all part of the **innate immune system,** which is capable of responding very rapidly to pathogens.

The Adaptive Immune System

- Key components of the adaptive immune response (Fig. 9-1) include **T lymphocytes** (or **T cells**) and **B lymphocytes** (or **B cells**). These cells develop in the body's primary lymphoid organs (bone marrow for B cells and the thymus for T cells).

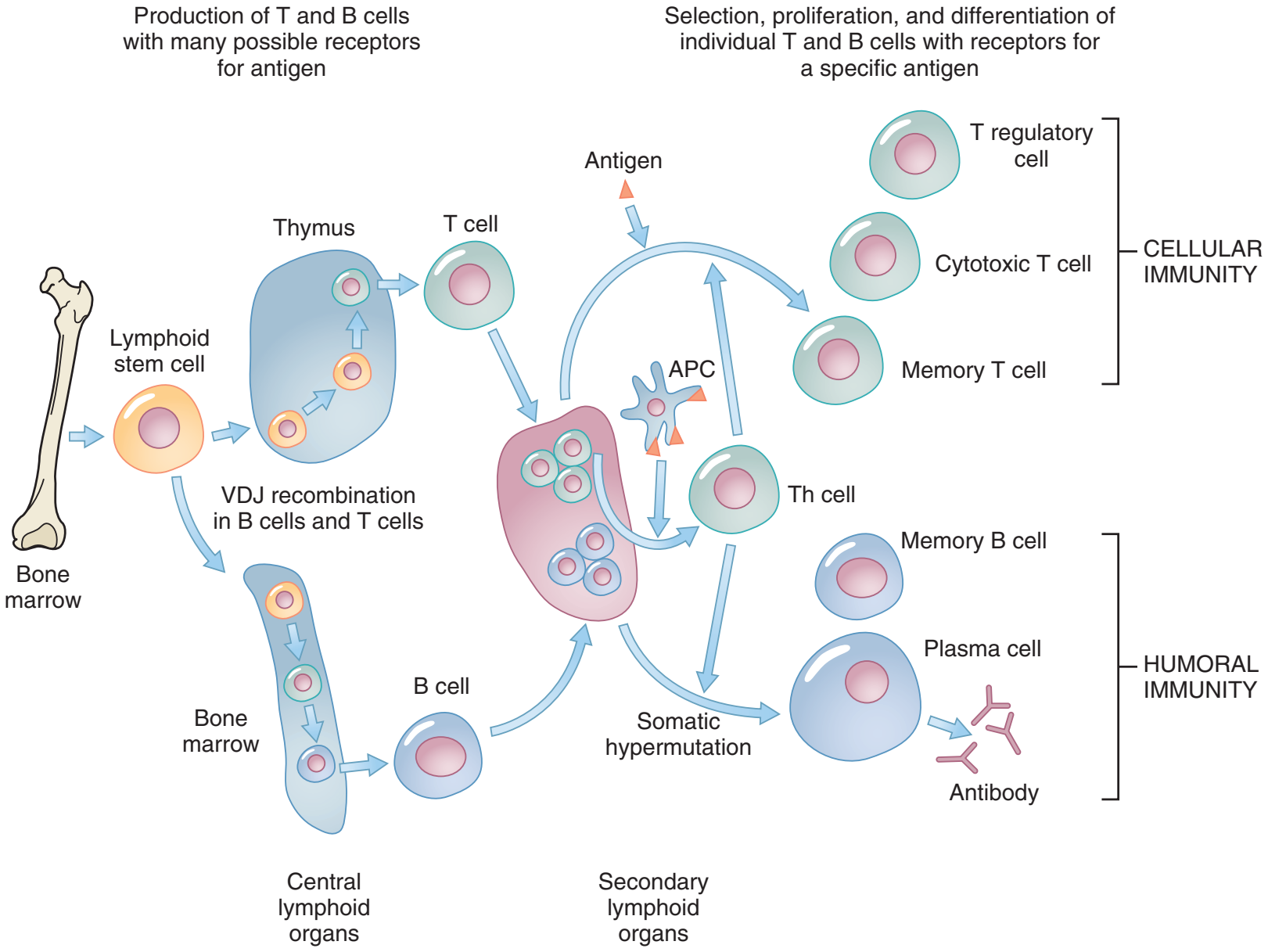
- In the thymus, developing T cells are exposed to a wide variety of the body's peptides. Those that can recognize and tolerate the body's own peptides are selected, and those that would attack the body's peptides are eliminated.

- The B and T cells progress to secondary lymphoid tissues, such as the lymph nodes, spleen, and tonsils, where they encounter disease-causing microorganisms.

- Mature B lymphocytes secrete circulating **antibodies,** which combat infections. The B lymphocyte component of the immune system is some- times called the **humoral immune system** because it produces antibodies that circulate in the blood stream.

- **Helper T lymphocytes** stimulate B lymphocytes and other types of T lymphocytes to respond to infections more effectively, and **cytotoxic T lymphocytes** can directly kill infected cells.

- Because of this direct interaction with infected cells, the T-cell component of the immune system is sometimes called the **cellular immune system.** It is estimated that the body contains several trillion B and T cells.


Production of T and B cells with many possible receptors for antigen
Selection, proliferation, and differentiation of individual T and B cells with receptors for a specific antigen
Bone marrow
Lymphoid stem cell
Thymus
T cell
VDJ recombination in B cells and T cells
Bone marrow
B cell
Central lymphoid organs
Antigen
APC
Th cell
Somatic hypermutation
Secondary lymphoid organs
T regulatory cell
Cytotoxic T cell
Memory T cell
CELLULAR IMMUNITY
Memory B cell
Plasma cell
Antibody
HUMORAL IMMUNITY

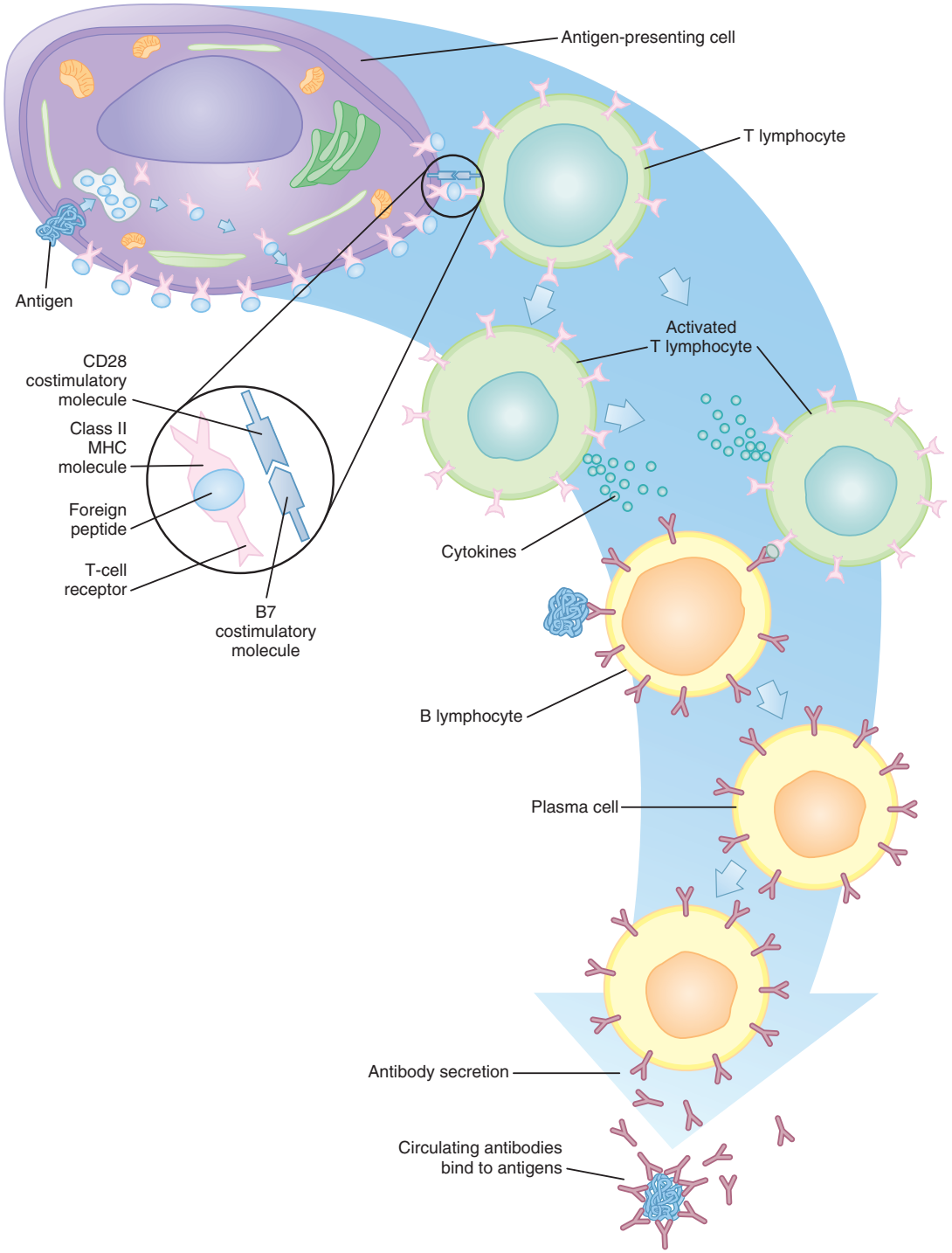
The B-Cell Response: Humoral Immune System

- A major element of the adaptive immune response begins when specialized types of phagocytes, which are part of the innate immune system, engulf invading microbes and then present peptides derived from these microbes on their cell surfaces.

- These cells, which include **macrophages** and **dendritic cells,** are termed **antigen-presenting cells (APCs).** B cells are also capable of engulfing microbes and presenting foreign peptides on their cell surfaces.

- The APCs alert the adaptive immune system to the presence of pathogens in two ways. First, the foreign peptide is transported to the surface of the APC by a **class II major histocompatibility complex (MHC)** molecule, which carries the foreign peptide in a specialized groove (Fig. 9-2).

- This complex, which projects into the extracellular environment, is recognized by T lymphocytes, which have receptors on their surfaces that are capable of binding to the MHC-peptide complex.


Antigen-presenting cell
T lymphocyte
Antigen
Activated
T lymphocyte
CD28
costimulatory
molecule
Class II
MHC
molecule
Foreign
peptide
T-cell
receptor
B7
costimulatory
molecule
Cytokines
B lymphocyte
Plasma cell
Antibody secretion
Circulating antibodies
bind to antigens

- In addition, the APCs, upon encountering a pathogen, display **costimulatory molecules** on their cell surfaces as a signal that foreign pathogens have been encountered (Fig. 9-3).

- Binding to the MHC-peptide complex stimulates the helper T lymphocyte to secrete **cytokines,** which are signaling proteins that mediate communication between cells.

- In particular, these cytokines help to stimulate the subset of B lymphocytes whose cell surface receptors, termed **immunoglobulins,** can bind to the invading microorganism's peptides (see Fig. 9-3).

- The immunoglobulin's capacity to bind a specific foreign peptide (i.e., its **affinity** for the peptide) is determined by its shape and other characteristics.

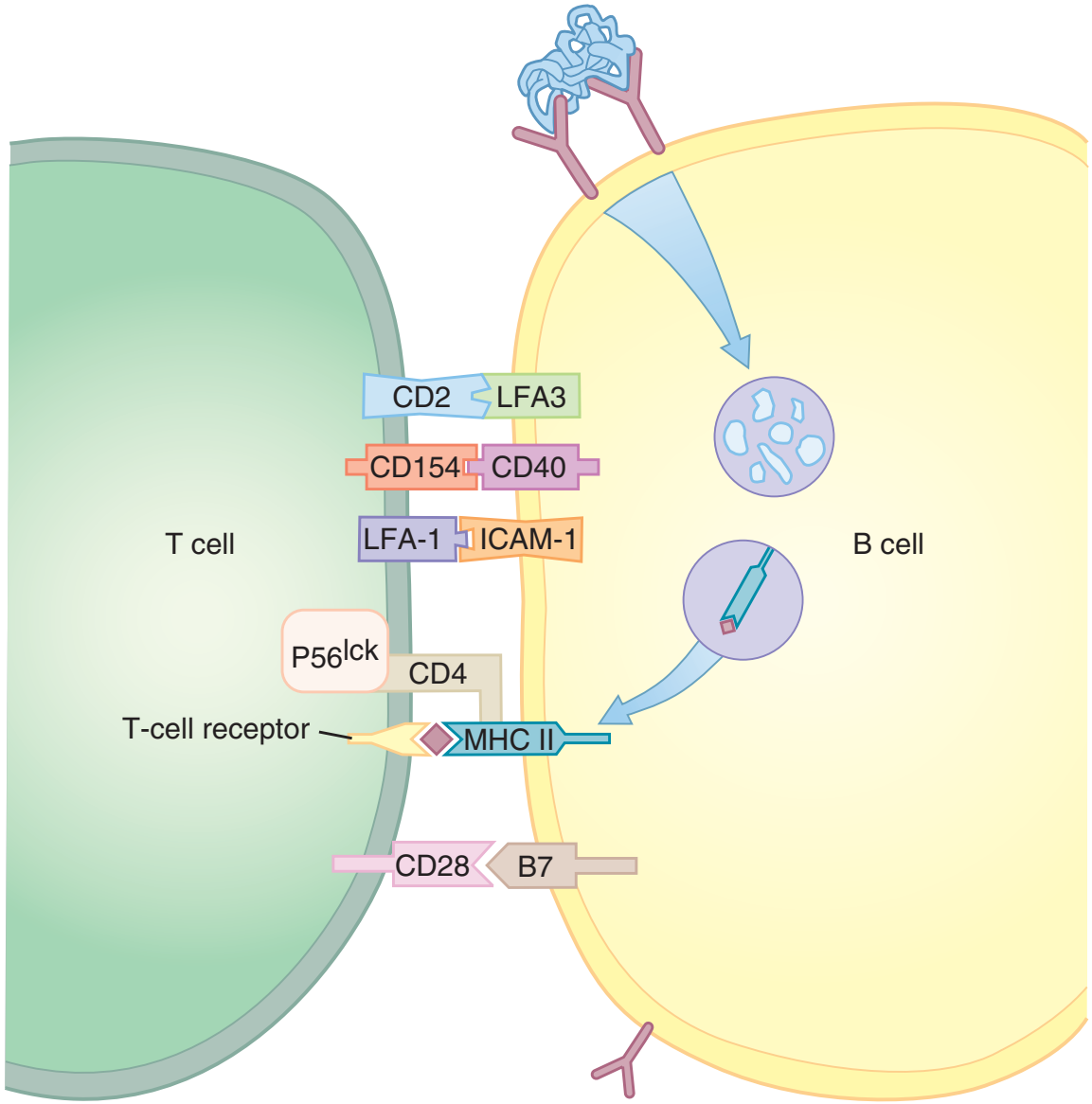


FIG 9-3 A detailed view of the binding between a helper T cell and a B cell. In addition to the binding of the T-cell receptor to the MHC-peptide complex, a number of other molecules interact with one another, such as the costimulatory B7-CD28 complex. MHC, major histocompatibility complex. (Modified from Roitt I, Brostoff J, Male D. *Immunology.* 6th ed. St. Louis: Mosby; 2001.)

- It is estimated that upon initial exposure to a foreign microbe, as few as 1 in every 1 million B lymphocytes happens to produce cell-surface receptors capable of binding to the microbe. This number is too small to fight an infection effectively and the receptor's binding affinity is likely to be relatively poor.

- Once this relatively small population of B lymphocytes is stimulated, they begin an adaptive process in which additional DNA sequence variation is generated via the process of **somatic hypermutation**.

- These DNA mutations, which are confined to the genes that encode the cell-surface receptors, in turn produce alterations in the receptors' binding characteristics (e.g., the shape of the protein). Some of these variant receptors possess a higher level of binding affinity for the microorganism.

- The B cells that produce these receptors are favorably selected because they bind the pathogen for a longer period of time. They thus proliferate rapidly.

- These B cells then become **plasma cells,** which secrete their cell-surface receptors, or immunoglobulins, into the blood stream. The secreted molecules, which are structurally identical to the receptors on the B cell's surface, are antibodies.

- After initial stimulation by the disease pathogen, the process of B-cell differentiation and maturation into antibody-producing plasma cells requires about 5 to 7 days for completion.

- Each plasma cell is capable of secreting approximately 10 million antibody molecules per hour. Anti- bodies bind to the pathogen's surface **antigens** and may neutralize the microorganism directly. More often, the antibody tags the pathogen for destruction by other components of the immune system, such as complement proteins and phagocytes.

- Another important activity of the humoral immune response is the creation of **memory B cells,** a subset of high-affinity-binding B cells that persist in the body after the infection has subsided.

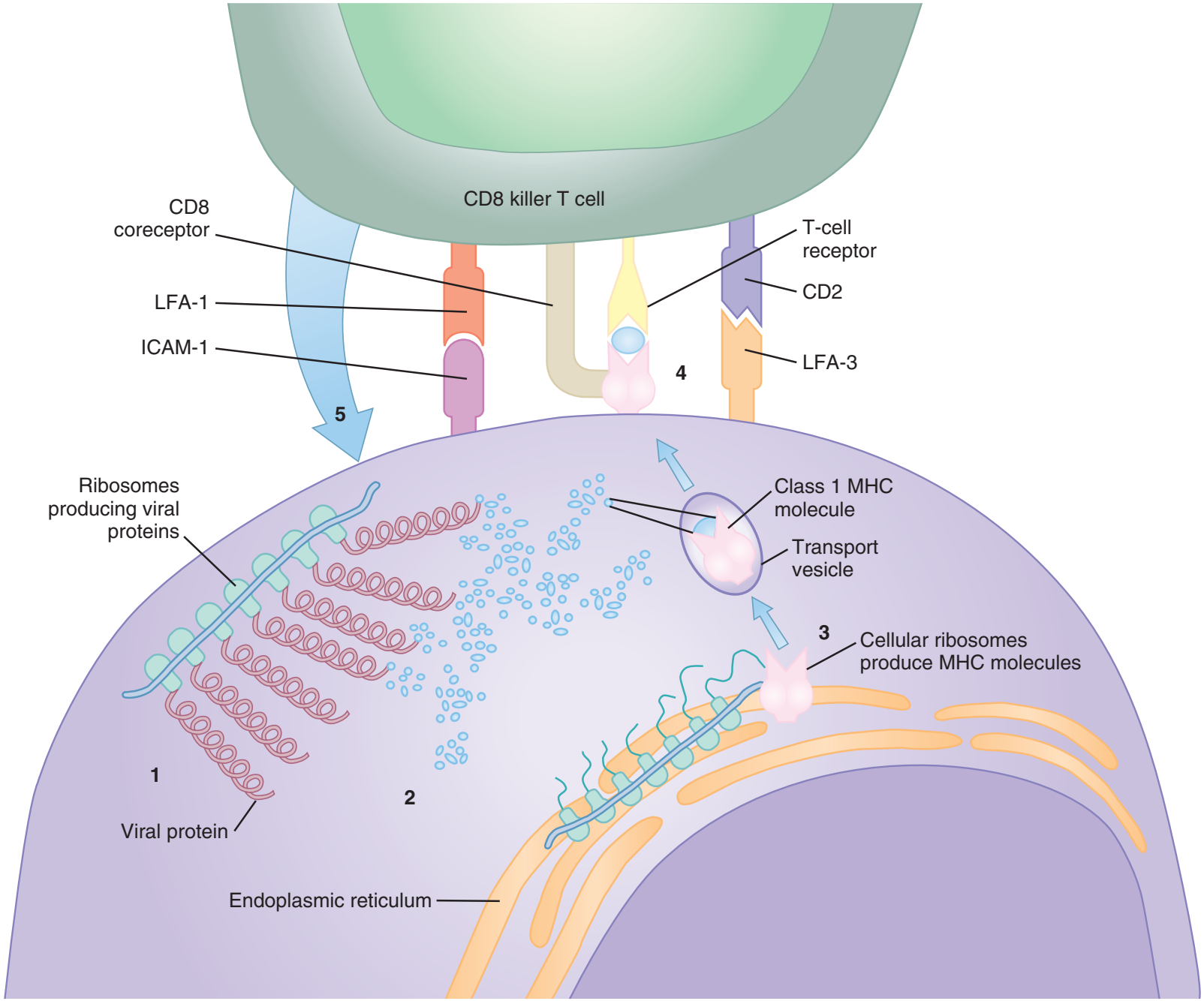
- These cells, which have already been highly selected for response to the pathogen, provide a more rapid response should the pathogen be encountered again later in the individual's life.

The Cellular Immune System

- A key member of the cellular immune response is the **class I MHC molecule,** which is found on the surfaces of nearly all of the body's cells.
- In a normal cell, the class I MHC molecule binds with small peptides (8 to 10 amino acids long) derived from the interior of the cell. It migrates to the cell's surface, carrying the peptide with it and displaying it outside the cell. Because this is one of the body's own peptides, no immune response is elicited.

- In an infected cell, however, the class I MHC molecule can bind to small peptides that are derived from the infecting organism. Cell-surface presentation of foreign peptides by the class I MHC molecule alerts the immune system, T cells in particular.
- The MHC- peptide complex binds to receptors on the appropriate T cell's surface, which prompts the T cell to emit a chemical that destroys the infected cell (Fig. 9-4).

- Because of their ability to destroy cells in this way, these T lymphocytes are termed **cytotoxic T lymphocytes** or **killer T lymphocytes.*** Each cytotoxic T lymphocyte can destroy one infected cell every 1 to 5 minutes.


CD8 killer T cell
CD8
coreceptor
LFA-1
ICAM-1
5
T-cell
receptor
CD2
LFA-3
4
Ribosomes
producing viral
proteins
Class 1 MHC
molecule
Transport
vesicle
3
Cellular ribosomes
produce MHC molecules
1
2
Viral protein
Endoplasmic reticulum

- In contrast to cytotoxic T cells, which destroy infected cells directly, helper T (TH) cells respond to the presence of pathogens by secreting **cytokines.**
- These molecules in turn stimulate the development of other components of the immune system, such as B cells and cytotoxic T cells.

- TH cells are classified into subsets, depending on which cytokines they secrete. For example, TH1 cells, which are involved primarily in combatting intracellular pathogens, secrete interleukin-2 (IL-2), interferon-γ, and tumor necrosis factor β.
- TH2 cells, which secrete IL-4, IL-5, IL-6, and IL-13, help to fight multicellular parasites and are involved in allergic responses.
- Other helper T-cell subsets include TH17 cells, which secrete IL-17, and TH22 cells, which secrete IL-22.

- As in the B-cell component of the adaptive immune system, a subset of long-lived T cells is retained **(memory T cells)** to quickly respond to a foreign pathogen should it be encountered again in the future. Yet another type of T cell, the **regulatory T cell,** helps to regulate the immune system so that self peptides are not inadvertently attacked.

The Innate, Humoral, and Cellular Immune Systems: A Comparison

- The innate system, because it recognizes general features of pathogens, can react very quickly to foreign elements. While doing so, it signals the adaptive immune system to initiate a fine-tuned response to the pathogen.
- Without this signal, the adaptive immune system is incapable of responding to an infection. After several days during which the adaptive system "learns" the characteristics of the pathogen, it can launch a massive, specialized response.

- Through the creation of memory B and T cells, the adaptive immune system allows the organism to respond quickly and effectively to a pathogen should it be encountered again. No such memory cells exist for the innate immune system.

- The humoral immune system is specialized to combat extracellular infections, such as circulating bacteria and viruses. The cellular immune system combats intracellular infections, such as parasites and viruses within cells. However, this division of labor is not strict, and there is again a great deal of interaction between the humoral and cellular components of the immune system.

IMMUNE RESPONSE PROTEINS: GENETIC BASIS OF STRUCTURE AND DIVERSITY

Immunoglobulin Molecules and Genes

- As illustrated in Figure 9-5, each **antibody** (or immunoglobulin) molecule is composed of four chains: an identical pair of longer **heavy chains** and an identical pair of shorter **light chains,** which are linked together by disulfide bonds.

- There are five different types of heavy chains (termed γ, μ, α, δ, and ε) and two types of light chains (κ and λ). The five types of heavy chains determine the major **class** (or **isotype**) to which an immunoglobulin (Ig) molecule belongs: γ, μ, α, δ, and ε correspond to the immunoglobulin isotypes IgG, IgM, IgA, IgD, and IgE, respectively.

- Immature B lymphocytes produce only IgM, but as they mature, a rearrangement of heavy chain genes called **class switching** occurs. This produces the other four major classes of immunoglobulins, each of which differs in amino acid composition, charge, size, and carbohydrate content.

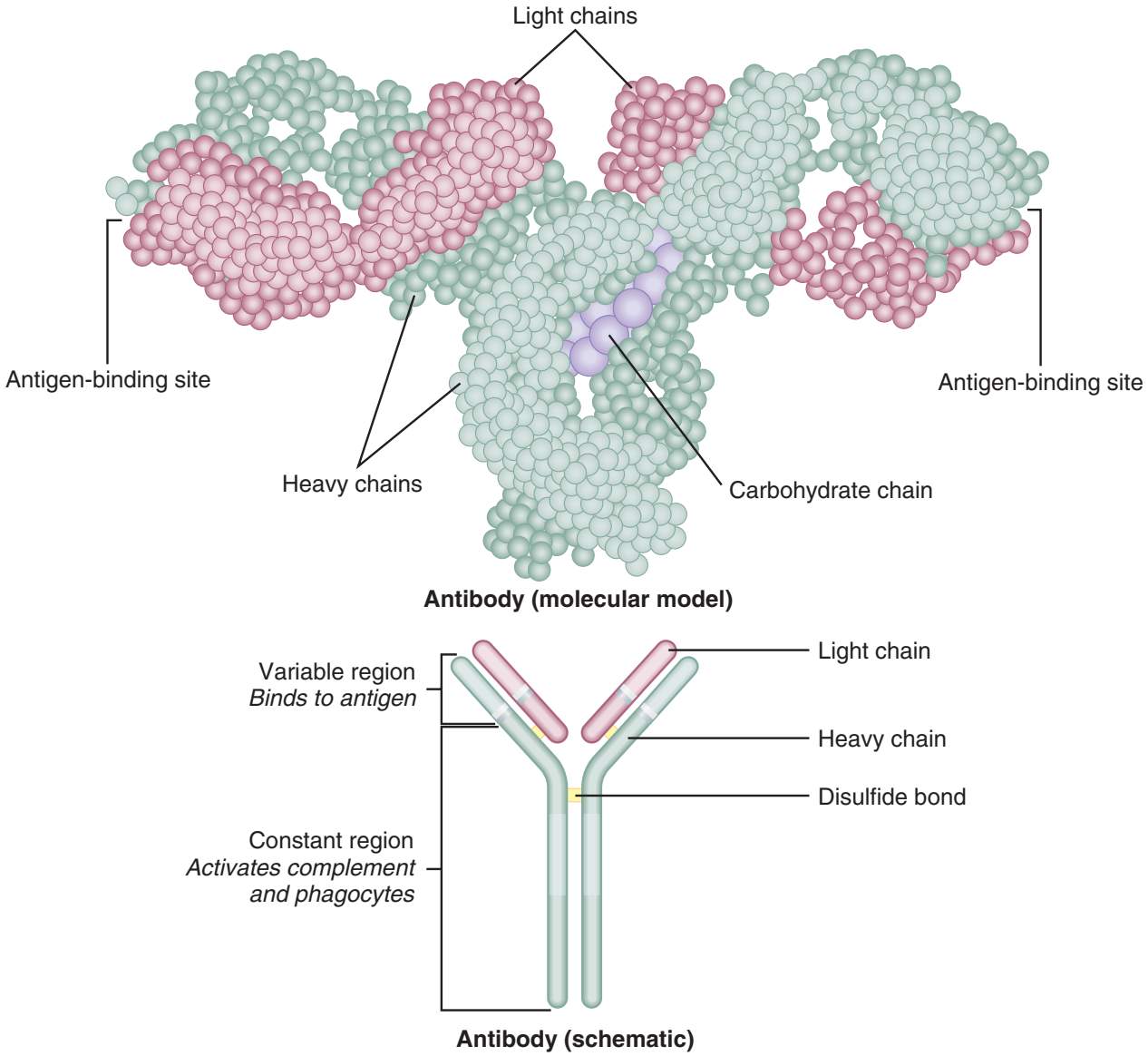


FIG 9-5 An antibody molecule consists of two identical light chains and two identical heavy chains. The light chain includes variable, joining, and constant regions; the heavy chain includes these regions as well as a diversity region located between its variable and joining regions. The *upper portion* of the figure depicts a molecular model of antibody structure.

- The heavy and light chains both contain a **constant** and a **variable** region, which are located at the carboxyl (C)-terminal and amino (N)-terminal ends of the chains, respectively.

- The arrangement of genes encoding the constant region determines the major class of the Ig molecule (e.g., IgA, IgE).
- The variable region is responsible for antigen recognition and binding and thus varies within immunoglobulin classes.

- Three distinct gene segments encode the light chains: C for the constant region, V for the variable region, and J for the region that joins the constant and variable regions. Four gene segments encode the heavy chains, with C, V, and J coding again for the constant, variable, and joining regions, respectively, and a “diversity” (D) region located between the joining and variable regions.

The Genetic Basis of Antibody Diversity

- Because the immune system cannot "know" in advance which types of microbes it will encounter, the system must contain a huge reservoir of structurally diverse immune cells so that at least a few cells can respond (i.e., bind) to any invading microbe.

- Indeed, the humoral immune system is capable of generating approximately 100 billion structurally distinct antibodies.
- At one time, it was thought that because each antibody has a unique amino acid sequence, each must be encoded by a different gene. However, this one gene–one antibody hypothesis could not possibly be correct, because the human genome has only 20,000 to 25,000 protein-coding genes.

- Further study has shown that several mechanisms are responsible for generating antibody diversity in somatic cells:

1. Multiple Germline Immunoglobulin Genes

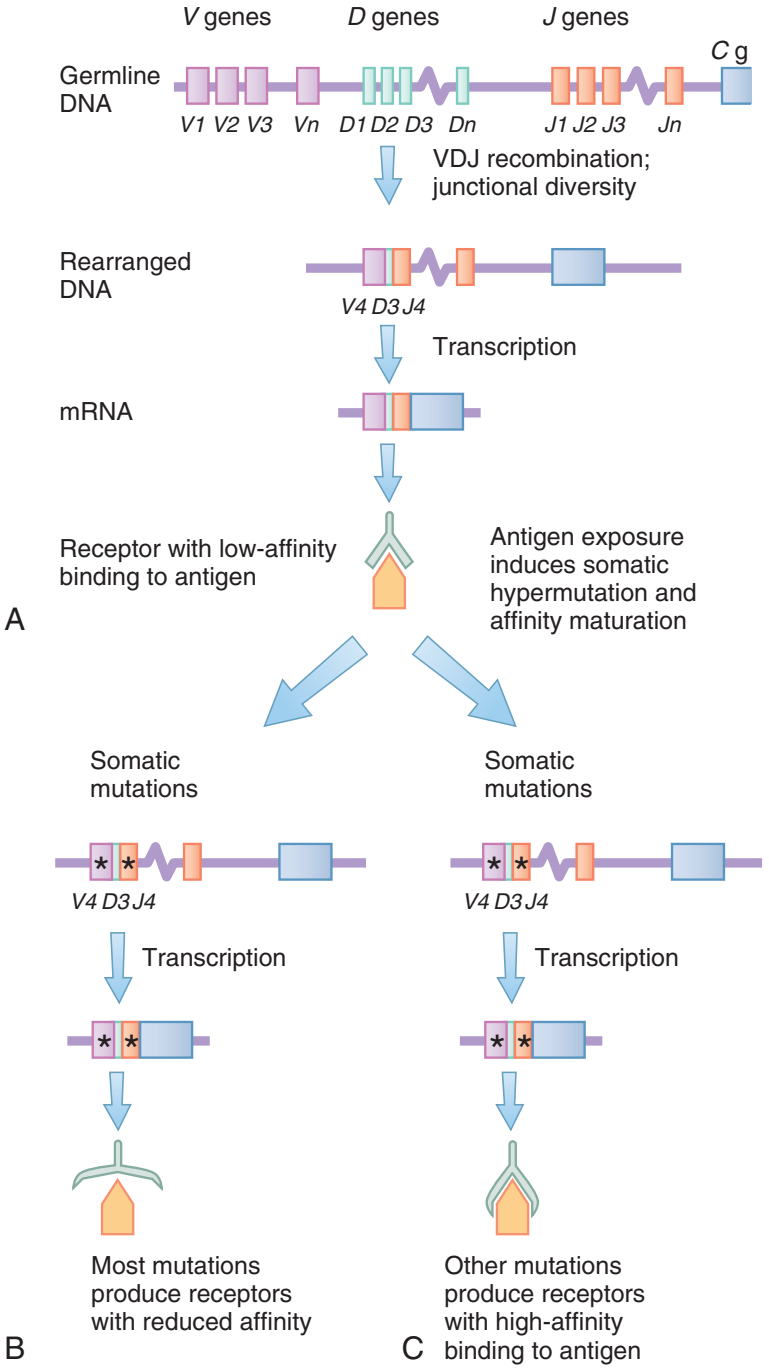
- Molecular genetic studies (cloning and DNA sequencing) have shown that for each heavy and light chain, an individual has more than 80 different V segments located contiguously in his or her germline and six different J segments. There are at least 30 D segments in the heavy chain region.

2. Somatic Recombination (VDJ Recombination)

- As immunoglobulin molecules are formed during B lymphocyte maturation, a specific combination of single V and J segments is selected for the light chain, and another combination of V, D, and J segments is selected for the heavy chain.
- This is accomplished by deleting the DNA sequences separating the single V, J, and D segments before they are transcribed into mRNA (Fig. 9-6). The deletion process is carried out in part by **recombinases** (encoded by the *RAG1* and *RAG2* genes), which initiate double-strand DNA breaks at specific DNA sequences that flank the V and D gene segments.
- After the deletion of all but one V, D, and J segment, the nondeleted segments are joined by ligases. This cutting-and-pasting process is known as **somatic recombination** (in contrast to the germline recombination that takes place during meiosis).

- Somatic recombination produces a distinctive result: unlike most other cells of the body, whose DNA sequences are identical to one another, mature B lymphocytes *vary* in terms of their rearranged immunoglobulin DNA sequences.

FIG 9-6 **A,** Somatic VDJ recombination in the formation of a heavy chain of an antibody molecule. The functional heavy chain is encoded by only one segment each from the multiple V, D, and J segments. This produces a subset of B cells whose receptors have low-affinity binding for a foreign antigen. Once the antigen is encountered in the secondary lymphoid tissue, somatic hypermutation **(B** and **C)** is initiated. Most of the mutated receptors have little binding affinity **(B),** but eventually somatic hypermutation produces a subset of receptors with high-affinity binding **(C).** The cells that contain these receptors become antibody-secreting plasma cells.



3. Junctional Diversity

- As the V, D, and J regions are assembled, slight variations occur in the position at which they are joined, and small numbers of nucleotides may be deleted or inserted at the junctions joining the regions. This creates even more variation in antibody amino acid sequence.

4. Somatic Hypermutation

- Typically, only a small subset of B cells has cell-surface receptors (immunoglobulins) that can bind to a specific foreign antigen, and their binding affinity is usually low.
- Once this subset of B cells is stimulated by a foreign antigen, they undergo an **affinity maturation** process characterized by somatic hypermutation of the V segments of immunoglobulin genes, as mentioned previously.

- An enzyme termed *activation-induced deaminase* causes cytosine bases to be replaced by uracil. Error-prone DNA polymerases are recruited, and DNA repair processes are modified so that mutations can persist in the DNA sequence.

5. Multiple Combinations of Heavy and Light Chains

- Further diversity is created by the random combination of different heavy and light chains in assembling the immunoglobulin molecule.
- Each of these mechanisms contributes to antibody diversity. Considering all of them together, it has been estimated that as many as 10^{11} distinct antibodies can potentially be produced.

T-Cell Receptors

- Like the immunoglobulins, T-cell receptors must be able to bind to a large variety of peptides derived from invading organisms.
- Unlike immunoglobulins, however, T-cell receptors are never secreted from the cell, and T-cell activation requires the presentation of foreign peptide along with an MHC molecule.

- Approximately 90% of T-cell receptors are heterodimers composed of an α and a β chain, and approximately 10% are heterodimers composed of a γ and a δ chain(Fig.9-7).

- A given T cell has a population of either α-β receptors or γ-δ receptors.

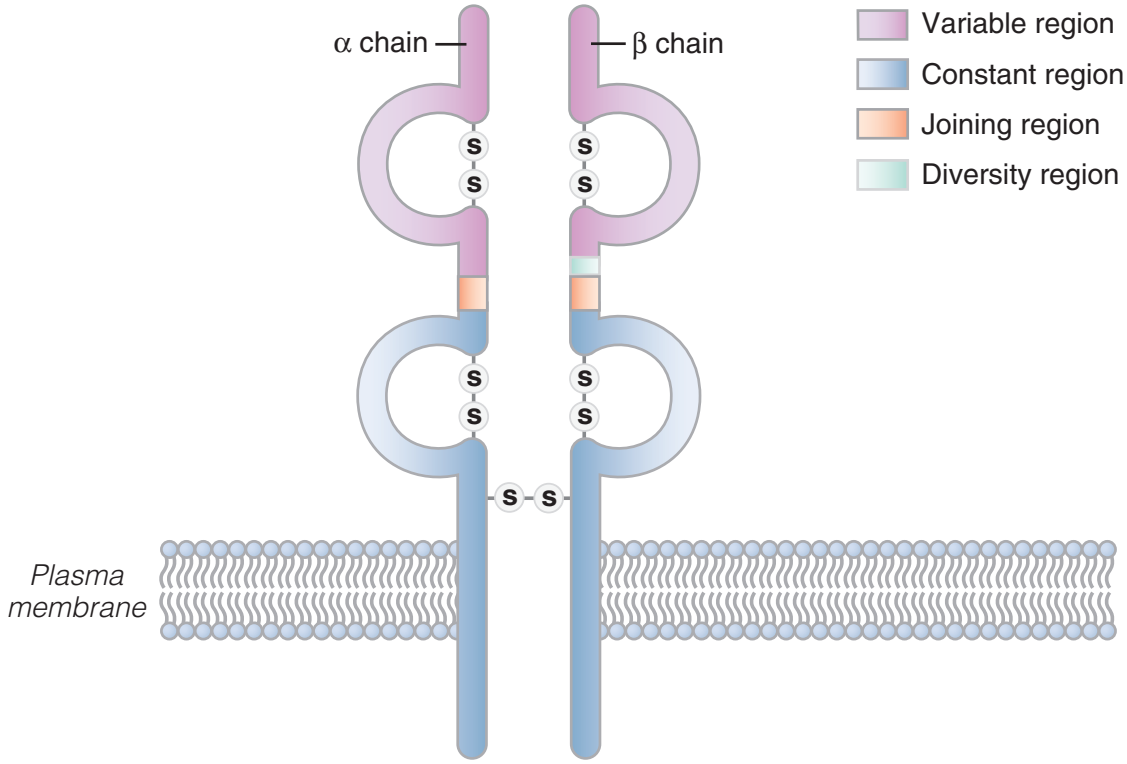


FIG 9-7 The T-cell receptor is a heterodimer that consists of either an α and a β chain or a γ and a δ chain. The complex of MHC molecule and antigen molecule is bound by the variable regions of the α and β chains. (Modified from Raven PH, Johnson GB. *Biology.* 3rd ed. St. Louis: Mosby; 1992.)

- Most of the mechanisms involved in generating immunoglobulin diversity—multiple germline gene segments, VDJ somatic recombination, and junctional diversity—are also important in generating T-cell receptor diversity.

- Somatic hypermutation does not occur in the genes that encode the T-cell receptors. This is thought to help avoid the generation of T cells that would react against the body's own cells

THE MAJOR HISTOCOMPATIBILITY COMPLEX

Class I, II, and III Genes

- The MHC, which includes a series of more than 200 genes that lie in a 4-Mb region on the short arm of chromosome 6 (Fig. 9-8), is commonly classified into three groups: class I, class II, and class III.
- The class I MHC molecule forms a complex with foreign peptides that is recognized by receptors on the surfaces of cytotoxic T lymphocytes.
- Class I presentation is thus essential for the cytotoxic T-cell response. Some viruses evade cytotoxic T-cell detection by down-regulating the expression of MHC class I genes in the cells they infect.

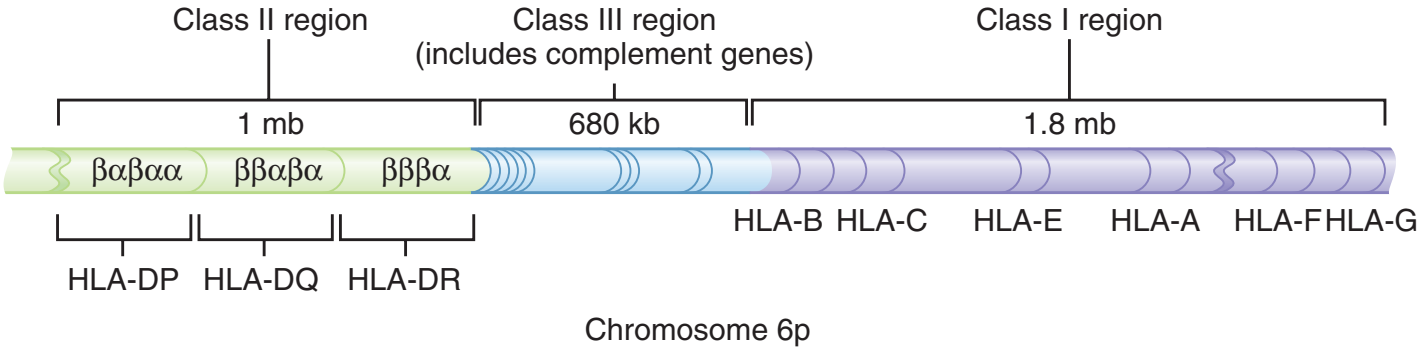


FIG 9-8 A map of the human major histocompatibility complex. The 4-Mb complex is divided into three regions: classes I, II, and III.

- Class I MHC molecules are composed of a single heavy glycoprotein chain and a single light chain called β2-microglobulin (Fig. 9-9A).

- The most important of the class I loci are labeled **human leukocyte antigens** A, B, and C **(HLA- A, -B,** and -**C).** Each of these loci has dozens or hundreds of alleles, resulting in a high degree of class I MHC variability among individuals.

- The class I region spans 1.8 Mb and includes a number of additional genes and **pseudogenes**.

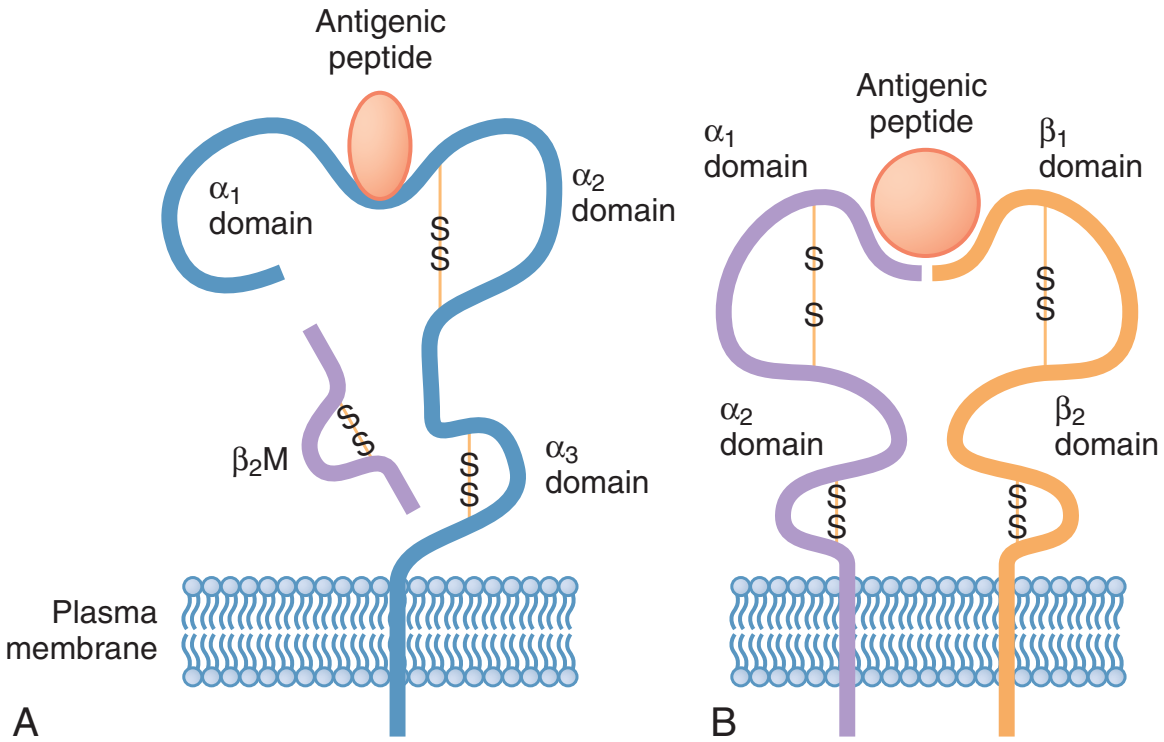


FIG 9-9 **A,** A class I major histocompatibility complex (MHC) molecule, showing the structure of the heavy chain, which consists of three extracellular domains (α_1, α_2, and α_3), a membrane-spanning domain, and a cytoplasmic domain. A groove formed by the α_1 and α_2 domains carries peptide for presentation to T-cell receptors. The α_3 domain associates closely with the β_2-microglobulin (β_2M) chain. **B,** A class II MHC molecule, showing the structure of the α and β chains. Each has two globular extracellular domains, a membrane-spanning domain, and a cytoplasmic domain. The α_1 and β_1 domains form a groove into which peptide nestles for presentation to T-cell receptors. (Modified from McCance KL, Huether SE. *Pathophysiology: the Biologic Basis for Disease in Adults and Children.* 7th ed. St. Louis: Elsevier; 2014:234.)

- The class I molecules were first discovered in the 1940s by scientists who were experimenting with tissue grafts in mice.

- When the class I alleles in donor and recipient mice differed, the grafts were rejected. This is the historical basis for the term ***major histocompatibility complex.***

- In humans, matching of the donor's and recipient's class I alleles increases the probability of graft or transplant tolerance.

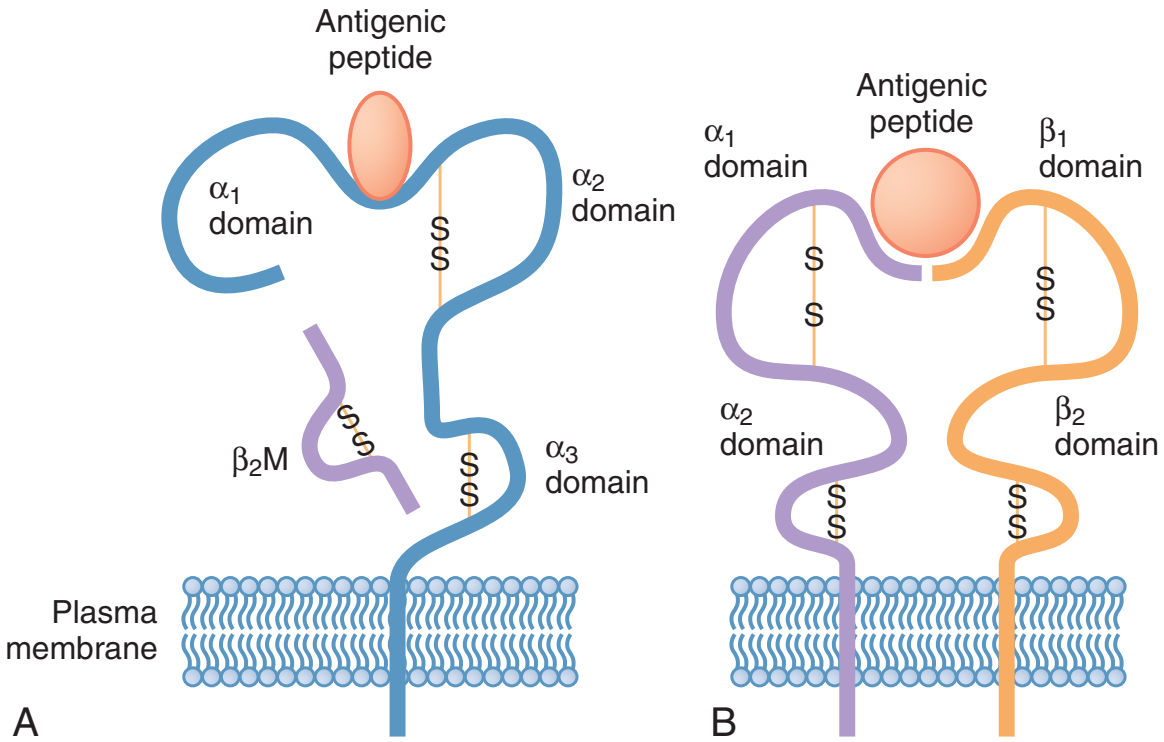
- Because grafts and transplants are a relatively new phenomenon in human history, the MHC obviously did not evolve to effect transplant rejection. Instead, T cells, when confronted with foreign MHC molecules on donor cells, interpret these as foreign peptides and attack the cells.

- Whereas the class I MHC molecules are found on the surfaces of nearly all cells and can bind with cytotoxic T-cell receptors, the class II MHC molecules ordinarily are found only on the surfaces of the immune system's APCs.

- When associated with foreign peptides, they stimulate helper T cell activity after binding to the T cells' receptors, as described previously.

- The class II molecules are heterodimers consisting of an α and a β chain, each of which is encoded by a different gene located on chromosome 6 (see Fig. 9-9B).

- In addition to the genes in the major class II groups *(HLA-DP, -DQ,* and *-DR),* this region includes genes that encode peptide transporter proteins *(TAP1* and *TAP2)* that help to transport peptides into the endoplasmic reticulum, where they initially form complexes with class I molecules before migrating to the cell surface.



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- Like the major class I MHC loci, the major class II loci are highly polymorphic, expressing hundreds of different alleles. Indeed, the MHC loci are, as a class, the most polymorphic loci known in humans.

- Each MHC allele encodes a molecule with slightly different binding properties: some variants bind peptide from a given pathogen more effectively than others do. Consequently, a person who expresses a greater variety of MHC molecules has a better chance of dealing effectively with diverse infectious organisms.

- For example, someone who is homozygous for each of the major class I loci *(A, B,* and *C)* expresses only three different class I MHC molecules in each cell, whereas someone who is heterozygous for each of these loci expresses six different class I MHC molecules in each cell and can cope more successfully with pathogenic diversity (many thousands of MHC molecules are expressed on a typical cell's surface).

- A higher degree of polymorphism in the general population increases the chance that any individual in the population is heterozygous.

- For example, HIV- infected persons who are heterozygous for the *HLA-A, HLA-B,* and/or *HLA-C* loci have longer survival times than those who are homozygous at these loci.

- In addition, greater MHC polymorphism in a population decreases the chance that an infectious pathogen can spread easily through the population. Thus, the high degree of polymorphism in MHC genes is thought to be the result of natural selection for allelic variation.

- In some cases, specific MHC alleles are known to produce proteins that are effective against specific pathogens. For example, the *HLA-B53* allele was shown to have a strong protective effect against severe malaria in the population of Gambia, and the *HLA-DRB1*13:02* allele protects against hepatitis B infection in the same population. These alleles produce MHC molecules that have higher-affinity binding of the infectious agents.

- Both class I and class II MHC molecules guide T-cell receptors (cytotoxic and helper, respectively) to specific cells.

- T-cell receptors recognize peptides only in combination with MHC molecules on cell surfaces, a phenomenon known as **MHC restriction.** Not all components of the immune system are MHC restricted.

- Some virus-infected cells and tumor cells take advantage of MHC restriction: they suppress the expression of MHC molecules on their surfaces in an attempt to evade detection by T cells.
- Fortunately, natural killer cells are activated by the absence, rather than the presence, of MHC molecules on cell surfaces. This activation is mediated by an important and diverse family of receptors found on the surfaces of natural killer cells, **killer cell immunoglobulin-like receptors (KIR).**

- These receptors inhibit natural killer cells when they bind to MHC class I molecules on the surfaces of normal cells but activate them when MHC class I molecules are absent.

- The class III MHC region spans 680 kb and contains at least 36 genes, only some of which are involved in the immune response. Among the most important of these are the genes encoding the complement proteins.

- The genes encoding the immunoglobulins, the T-cell receptors, KIR, and the class I and class II MHC proteins all share similar DNA sequences and structural features. Thus, they are members of a gene family, like the globin genes, the color vision genes, and the collagen genes.

- It is important to emphasize that the class I and class II MHC molecules differ greatly *among* individuals, but each cell within an individual has the same class I and class II molecules (this uniformity is necessary for recognition by T cells).
- In contrast, after VDJ recombination the T-cell receptors and immunoglobulins differ from cell to cell *within* individuals, allowing the body to respond to a large variety of different infectious agents.

TABLE 9-1 Chromosome Location and Function of Major Immune Response Genes

GENE SYSTEM	CHROMOSOME LOCATION	GENE PRODUCT FUNCTION
Immunoglobulin heavy chain (*C, V, D,* and *J* genes)	14q32	Heavy chain, the first part of antibody molecule, which binds foreign antigens
Immunoglobulin κ light chain (*C, V,* and *J* genes)	2p13	Light chain, the second part of antibody molecule
Immunoglobulin λ light chain (*C, V,* and *J* genes)	22q11	Light chain, the second part of antibody molecule (either κ or λ may be used)
T-cell receptor α	14q11	One chain of the α-β T-cell receptor, which recognizes antigen with MHC molecule
T-cell receptor β	7q35	The second chain of the α-β T-cell receptor
T-cell receptor γ	7p15	One chain of the γ-δ T-cell receptor
T-cell receptor δ	14q11	The second chain of the γ-δ T-cell receptor
MHC (classes I, II, and III); includes *TAP1* and *TAP2*	6p21	Cell-surface molecules that present peptides to T-cell receptors. *TAP1* and *TAP2* are transporter molecules that process foreign peptides and carry them to the endoplasmic reticulum.
β_2-microglobulin	15q21-22	Forms second chain of the class I MHC molecule
RAG1, RAG2	11p13	Recombinases that participate in VDJ somatic recombination