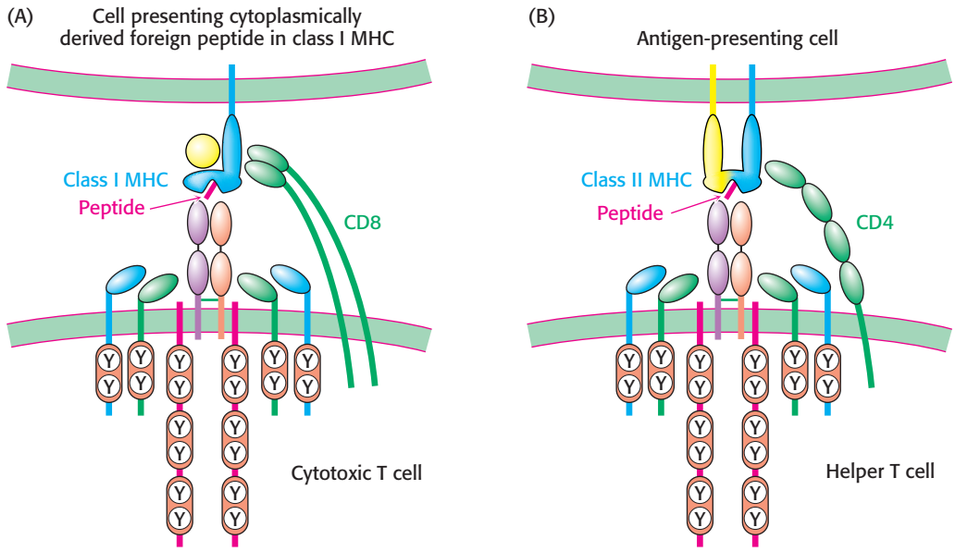

FIGURE 33.35 Variations on a theme. (A) Cytotoxic T cells recognize foreign peptides presented in class I MHC proteins with the aid of the coreceptor CD8. (B) Helper T cells recognize peptides presented in class II MHC proteins by specialized antigen-presenting cells with the aid of the coreceptor CD4.

When a helper T cell binds to an antigen-presenting cell expressing an appropriate class II MHC–peptide complex, signaling pathways analogous to those in cytotoxic T cells are initiated by the action of the kinase Lck on ITAMs in the CD3 molecules associated with the T-cell receptor. However, rather than triggering events leading to the death of the attached cell, *these signaling pathways result in the secretion of cytokines from the helper cell*. Cytokines are a family of molecules that include, among others, interleukin-2 and interferon-γ. Cytokines bind to specific receptors on the antigen-presenting cell and stimulate growth, differentiation, and in regard to plasma cells, which are derived from B cells, antibody secretion (Figure 33.36). Thus, the internalization and presentation of parts of a foreign pathogen help to generate a local environment in which cells taking part in the defense against this pathogen can flourish through the action of helper T cells.

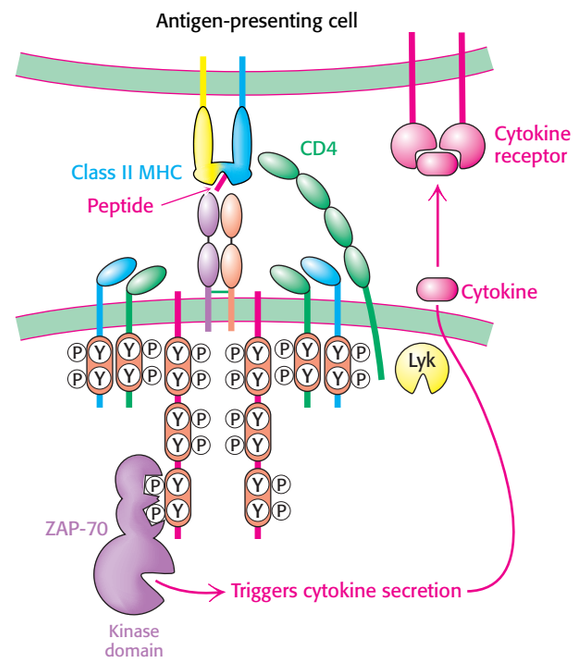

FIGURE 33.36 Helper T cell action. The engagement of the T-cell receptor in helper T cells results in the secretion of cytokines. These cytokines bind to cytokine receptors expressed on the surface of the antigen-presenting cell, stimulating cell growth, differentiation, and, in regard to a B cell, antibody secretion.

33.5.6 MHC Proteins Are Highly Diverse

MHC class I and II proteins, the presenters of peptides to T cells, were discovered because of their role in *transplantation rejection*. A tissue transplanted from one person to another or from one mouse to another is usually rejected by the immune system. In contrast, tissues transplanted from one identical twin to another or between mice of an inbred strain are accepted. Genetic analyses revealed that rejection occurs when tissues are transplanted between individuals having different genes in the major histocompatibility complex, a cluster of more than 75 genes playing key roles in immunity. The 3500-kb span of the MHC is nearly the length of the

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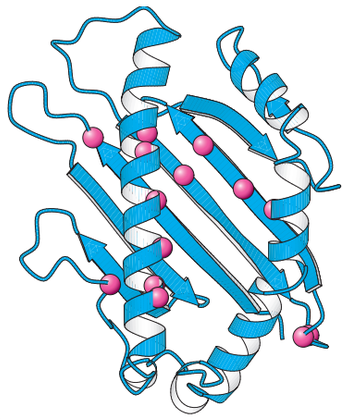

FIGURE 33.37 Polymorphism in class I MHC proteins. The positions of sites with a high degree of polymorphism in the human population are displayed as red spheres on the structure of the amino-terminal part of a class I MHC protein.

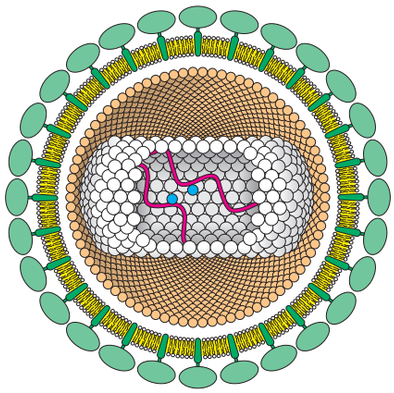

FIGURE 33.38 Human immunodeficiency virus. A schematic diagram of HIV reveals its proteins and nucleic acid components. The membrane-envelope glycoproteins gp41 and gp120 are shown in dark and light green. The viral RNA is shown in red, and molecules of reverse transcriptase are shown in blue. [After R. C. Gallo. The AIDS virus. Copyright © 1987 by Scientific American, Inc. All rights reserved.]

entire *E. coli* chromosome. The MHC encodes class I proteins (presenters to cytotoxic T cells) and class II proteins (presenters to helper T cells), as well as class III proteins (components of the complement cascade) and many other proteins that play key roles in immunity.

Human beings express six different class I genes (three from each parent) and six different class II genes. The three loci for class I genes are called HLA-A, -B, and -C; those for class II genes are called HLA-DP, -DQ, and -DR. These loci are *highly polymorphic*: many alleles of each are present in the population. For example, more than 50 each of HLA-A, -B, and -C alleles are known; the numbers discovered increase each year. Hence, the likelihood that two unrelated persons have identical class I and II proteins is very small ($<10^{-4}$), accounting for transplantation rejection unless the genotypes of donor and acceptor are closely matched in advance.

Differences between class I proteins are located mainly in the α_1 and α_2 domains, which form the peptide-binding site (Figure 33.37). The α_3 domain, which interacts with a constant β_2-microglobulin is largely conserved. Similarly, the differences between class II proteins cluster near the peptide-binding groove. Why are MHC proteins so highly variable? *Their diversity makes possible the presentation of a very wide range of peptides to T cells*. A *particular* class I or class II molecule may not be able to bind any of the peptide fragments of a viral protein. The likelihood of a fit is markedly increased by having several kinds (usually six) of each class of presenters in each individual. If all members of a species had identical class I or class II molecules, the population would be much more vulnerable to devastation by a pathogen that had evolved to evade presentation. The evolution of the diverse human MHC repertoire has been driven by the selection for individual members of the species who resist infections to which other members of the population may be susceptible.

33.5.7 Human Immunodeficiency Viruses Subvert the Immune System by Destroying Helper T Cells

In 1981, the first cases of a new disease now called *acquired immune deficiency syndrome (AIDS)* were recognized. The victims died of rare infections because their immune systems were crippled. The cause was identified two years later by Luc Montagnier and coworkers. AIDS is produced by *human immunodeficiency virus (HIV)*, of which two major classes are known: HIV-1 and the much less common HIV-2. Like other *retroviruses*, HIV contains a single-stranded RNA genome that is replicated through a double-stranded DNA intermediate. This viral DNA becomes integrated into the genome of the host cell. In fact, viral genes are transcribed only when they are integrated into the host DNA.

The HIV virion is enveloped by a lipid bilayer membrane containing two glycoproteins: gp41 spans the membrane and is associated with gp120, which is located on the external face (Figure 33.38). The core of the virus contains two copies of the RNA genome and associated transfer RNAs, and several molecules of reverse transcriptase. They are surrounded by many copies of two proteins called p18 and p24. *The host cell for HIV is the helper T cell*. The gp120 molecules on the membrane of HIV bind to CD4 molecules on the surface of the helper T cell (Figure 33.39). This interaction allows the associated viral gp41 to insert its amino-terminal head into the host-cell membrane. The viral membrane and the helper-cell membrane fuse, and the viral core is released directly into the cytosol. Infection by HIV leads to the destruction of helper T cells because the permeability of the host plasma membrane is markedly increased by the insertion of viral

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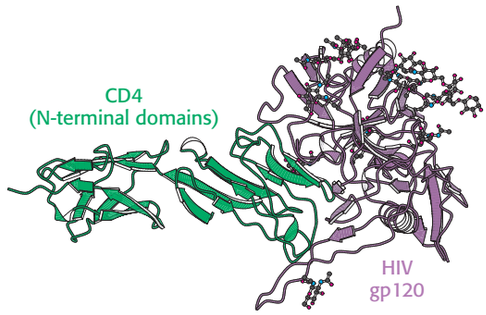


FIGURE 33.39 HIV receptor. A complex between a modified form of the envelope glycoprotein gp120 from HIV and a peptide corresponding to the two amino-terminal domains from the helper T-cell protein CD4 reveals how viral infection of helper T cells is initiated.

glycoproteins and the budding of virus particles. The influx of ions and water disrupts the ionic balance, causing osmotic lysis.

The development of an effective AIDS vaccine is difficult owing to the antigenic diversity of HIV strains. Because its mechanism for replication is quite error prone, a population of HIV presents an ever-changing array of coat proteins. Indeed, the mutation rate of HIV is more than 65 times as high as that of influenza virus. A major aim now is to define relatively conserved sequences in these HIV proteins and use them as immunogens.

33.6 IMMUNE RESPONSES AGAINST SELF-ANTIGENS ARE SUPPRESSED

The primary function of the immune system is to protect the host from invasion by foreign organisms. But how does the immune system avoid mounting attacks against the host organism? In other words, how does the immune system distinguish between self and nonself? Clearly, proteins from the organism itself do not bear some special tag identifying them. Instead, selection processes early in the developmental pathways for immune cells kill or suppress those immune cells that react strongly with self-antigens. The evolutionary paradigm still applies; immune cells that recognize self-antigens are generated, but selective mechanisms eliminate such cells in the course of development.

33.6.1 T Cells Are Subject to Positive and Negative Selection in the Thymus

T cells derive their name from the location of their production—the thymus, a small organ situated just above the heart. Examination of the developmental pathways leading to the production of mature cytotoxic and helper T cells reveals the selection mechanisms that are crucial for distinguishing self from nonself. These selection criteria are quite stringent; approximately 98% of the thymocytes, the precursors of T cells, die before the completion of the maturation process.

Thymocytes produced in the bone marrow do not express the T-cell-receptor complex, CD4, or CD8. On relocation to the thymus and rearrangement of the T-cell-receptor genes, the immature thymocyte expresses all of these molecules. These cells are first subjected to *positive selection*

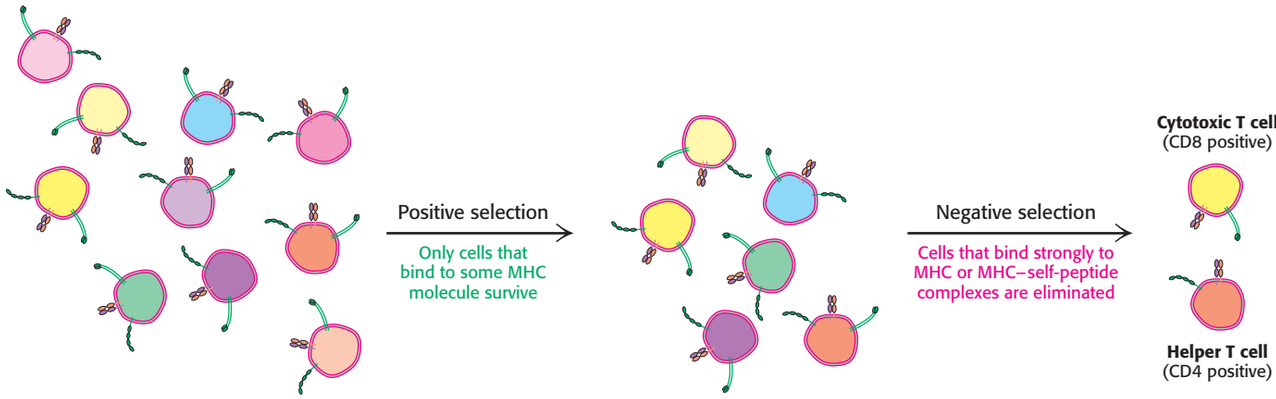
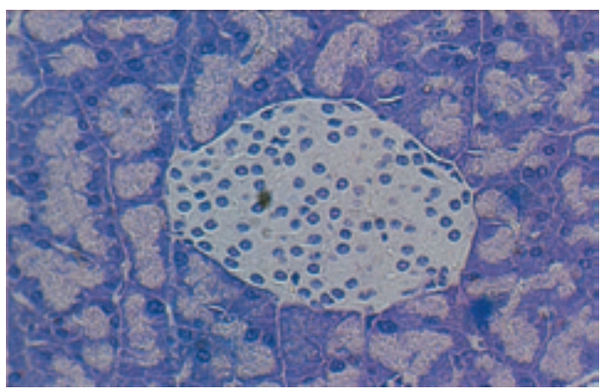


FIGURE 33.40 T-cell selection. A population of thymocytes is subjected first to positive selection to remove cells that express T-cell receptors that will not bind to MHC proteins expressed by the individual organism. The surviving cells are then subjected to negative selection to remove cells that bind strongly to MHC complexes bound to self-peptides.

(Figure 33.40). Cells for which the T-cell receptor can bind with reasonable affinity to either class I or class II MHC molecules survive this selection; those for which the T-cell receptor does not participate in such an interaction undergo apoptosis and die. The affinities of interaction required to pass this selection are relatively modest, and so contacts between the T-cell receptor and the MHC molecules themselves are sufficient without any significant contribution from the bound peptides (which will be derived from proteins in the thymus). *The role of the positive selection step is to prevent the production of T cells that will not bind to any MHC complex present, regardless of the peptide bound.*

The cell population that survives positive selection is subjected to a second step, *negative selection*. Here, T cells that bind with high affinity to MHC complexes bound to self-peptides expressed on the surfaces of antigen-presenting cells in the thymus undergo apoptosis or are otherwise suppressed. Those that do not bind too avidly to any such MHC complex complete development and become mature cytotoxic T cells (which express only CD8) or helper T cells (which express only CD4). The negative selection step leads to *self tolerance*; cells that bind an MHC–self-peptide complex are removed from the T-cell population. Similar mechanisms apply to developing B cells, suppressing B cells that express antibodies that interact strongly with self-antigens.

(A)



(B)

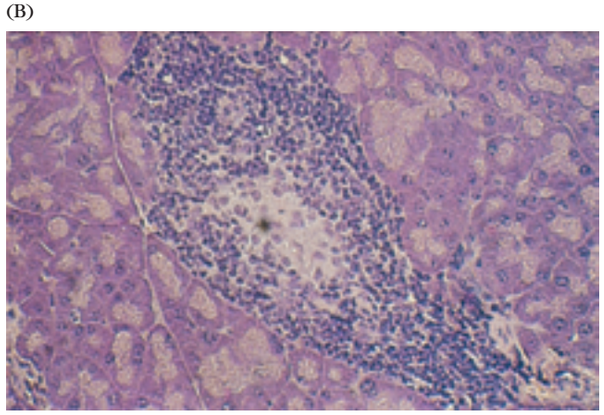


FIGURE 33.41 Consequences of autoimmunity. Photomicrographs of an islet of Langerhans (A) in the pancreas of a normal mouse and (B) in the pancreas of a mouse with an immune response against pancreatic β cells, which results in a disease resembling insulin-dependent diabetes mellitus in human beings. [From M. A. Atkinson and N. K. Maclaren. What causes diabetes? Copyright © 1990 by Scientific American, Inc. All rights reserved.]

33.6.2 Autoimmune Diseases Result from the Generation of Immune Responses Against Self-Antigens

Although thymic selection is remarkably efficient in suppressing the immune response to self-antigens, failures do occur. Such failures results in *autoimmune diseases*. These diseases include relatively common illnesses such as insulin-dependent diabetes mellitus, multiple sclerosis, and rheumatoid arthritis. In these illnesses, immune responses against self-antigens result in damage to selective tissues that express the antigen (Figure 33.41).

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In many cases, the cause of the generation of self-reactive antibodies or T cells in unclear. However, in other cases, infectious organisms such as bacteria or viruses may play a role. Infection leads to the generation of antibodies and T cells that react with many different epitopes from the infectious organism. If one of these antigens closely resembles a self-antigen, an autoimmune response can result. For example, *Streptococcus* infections sometimes lead to rheumatic fever owing to the production of antibodies to streptococcal antigens that cross-react with exposed molecules in heart muscle.

33.6.3 The Immune System Plays a Role in Cancer Prevention

The development of immune responses against proteins encoded by our own genomes can be beneficial under some circumstances. Cancer cells have undergone significant changes that often result in the expression of proteins that are not normally expressed. For example, the mutation of genes can generate proteins that do not correspond in amino acid sequence to any normal protein. Such proteins may be recognized as foreign, and an immune response will be generated specifically against the cancer cell. Alternatively, cancer cells often produce proteins that are expressed during embryonic development but are not expressed or are expressed at very low levels after birth. For example, a membrane glycoprotein protein called *carcinoembryonic antigen (CEA)* appears in the gasterointestinal cells of developing fetuses but is not normally expressed at significant levels after birth. More than 50% of patients with colorectal cancer have elevated serum levels of CEA. Immune cells recognizing epitopes from such proteins will not be subject to negative selection and, hence, will be present in the adult immune repertoire. These cells may play a cancer surveillance role, killing cells that overexpress antigens such as CEA and preventing genetically damaged cells from developing into tumors.

SUMMARY

- To respond effectively to a vast array of pathogens, the immune system must be tremendously adaptable. Adaptation by the immune system follows the principles of evolution: an enormously diverse set of potentially useful proteins is generated; these proteins are then subjected to intense selection so that only cells that express useful proteins flourish and continue development, until an effective immune response to a specific invader is generated.

- **Antibodies Possess Distinct Antigen-Binding and Effector Units**
  The major immunoglobulin in the serum is immunoglobulin G. An IgG protein is a heterotetramer with two heavy chains and two light chains. Treatment of IgG molecules with proteases such as papain produces three fragments: two F_{ab} fragments that retain antigen-binding activity and an F_c fragment that retains the ability to activate effector functions such as the initiation of the complement cascade. The F_{ab} fragments include the L chain and the amino-terminal half of the H chain; the F_c domain is a dimer consisting of the carboxyyl-terminal halves of two H chains. Five different classes of antibody—IgG, IgM, IgA, IgD, and IgE—differ in their heavy chains and, hence, in their effector functions.

- **The Immunoglobulin Fold Consists of a Beta-Sandwich Framework with Hypervariable Loops**
  One particular protein fold is found in many of the key proteins of the immune system. The immunoglobulin fold consists of a pair of β sheets

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that pack against one another, linked by a single disulfide bond. Loops projecting from one end of the structure form a binding surface that can be varied by changing the amino acid sequences within the loops. Domains with immunoglobulin folds are linked to form antibodies and other classes of proteins in the immune system including T-cell receptors.

Antibodies Bind Specific Molecules Through Their Hypervariable Loops

Two chains come together to form the binding surface of an antibody. Three loops from each domain, the complementarity-determining regions, form an essentially continuous surface that can vary tremendously in shape, charge, and other characteristics to allow particular antibodies to bind to molecules ranging from small molecules to large protein surfaces.

Diversity Is Generated by Gene Rearrangements

The tremendous diversity of the amino acid sequences of antibodies is generated by segmental rearrangements of genes. For antibody κ light chains, one of 40 variable regions is linked to one of five joining regions. The combined VJ unit is then linked to the constant region. Thousands of different genes can be generated in this manner. Similar arrays are rearranged to form the genes for the heavy chains, but an additional region called the diversity region lies between the V and the J regions. The combination L and H chains, each obtained through such rearranged genes, can produce more than 10^8 distinct antibodies. Different classes of antibodies are also generated by gene rearrangements that lead to class switching. Oligomerization of membrane-bound antibody molecules initiates a signal-transduction cascade inside B cells. Key steps in this signaling process include the phosphorylation of specific tyrosine residues in sequences termed immunoreceptor tyrosine-based activation motifs (ITAMs), present in proteins that associate with the membrane-bound antibodies.

Major-Histocompatibility-Complex Proteins Present Peptide Antigens on Cell Surfaces for Recognition by T-Cell Receptors

Intracellular pathogens such as viruses and mycobacteria cannot be easily detected. Intracellular proteins are constantly being cut into small peptides by proteasomes and displayed in class I major-histocompatibility-complex proteins on cell surfaces. Such peptides lie in a groove defined by two helices in the class I MHC proteins. The combination of MHC protein and peptide can be bound by an appropriate T-cell receptor. T-cell receptors resemble the antigen-binding domains of antibodies in structure, and diversity in T-cell-receptor sequence is generated by V(D)J gene rearrangements. The T-cell receptor recognizes features of both the peptide and the MHC molecule that presents it. Cytotoxic T cells initiate apoptosis in cells to which they bind through T-cell receptor–class I MHC-peptide interactions aided by interactions with the coreceptor molecule CD8. Helper T cells recognize peptides presented in class II MHC proteins, a distinct type of MHC protein expressed only on antigen-presenting cells such as B cells and macrophages. Helper T cells express the coreceptor CD4, rather than CD8. CD4 interacts with class II MHC proteins present on antigen-presenting cells. Signaling pathways, analogous to those in B cells, are initiated by interactions between MHC–peptide complexes and T-cell receptors and the CD8 and CD4 coreceptors. Human immunodeficiency virus damages the immune system by infecting cells that express CD4, such as helper T cells.

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Immune Responses Against Self-Antigens Are Suppressed

In principle, the immune system is capable of generating antibodies and T-cell receptors that bind to self-molecules; that is, molecules that are normally present in a healthy and uninfected individual organism. Selection mechanisms prevent such self-directed molecules from being expressed at high levels. The selection process includes both positive selection, to enrich the population of cells that express molecules that have the potential to bind foreign antigens in an appropriate context, and negative selection, which eliminates cells that express molecules with too high an affinity for self-antigens. Autoimmune diseases such as insulin-dependent diabetes mellitus can result from amplification of a response against a self-antigen.

KEY TERMS

humoral immune response (p. 921)
B lymphocyte (B cell) (p. 922)
antigen (p. 922)
antigenic determinant (epitope) (p. 922)
cellular immune response (p. 922)
cytotoxic T lymphocyte (killer T cell) (p. 922)
helper T lymphocyte (p. 922)
immunoglobulin G (p. 923)
F_{ab} (p. 923)
F_c (p. 923)
light chain (p. 923)
heavy chain (p. 923)
segmental flexibility (p. 924)
immunoglobulin M (p. 924)
immunoglobulin A (p. 924)
immunoglobulin D (p. 924)
immunoglobulin E (p. 924)
variable region (p. 925)
constant region (p. 925)
immunoglobulin fold (p. 926)
hypervariable loop (p. 926)
complementarity-determining region (CDR) (p. 926)
V(D)J recombination (p. 931)
immunoreceptor tyrosine-based activation motif (ITAM) (p. 932)
cyclosporin (p. 933)
hapten (p. 933)
class switching (p. 934)
T cell (p. 935)
major histocompatibility complex (MHC) (p. 935)
class I MHC protein (p. 935)
human leukocyte antigen (HLA) (p. 935)
β_2-microglobulin (p. 935)
T-cell receptor (p. 937)
CD8 (p. 937)
perforin (p. 939)
granzymes (p. 939)
helper T cell (p. 939)
class II MHC protein (p. 939)
CD4 (p. 940)
human immunodeficiency virus (HIV) (p. 940)
positive selection (p. 943)
negative selection (p. 944)
autoimmune disease (p. 944)
carcinoembryonic antigen (CEA) (p. 945)

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PROBLEMS

1. *Energetics and kinetics*. Suppose that the dissociation constant of an F_{ab}–hapten complex is 3×10^{-7} M at 25°C.

(a) What is the standard free energy of binding?
(b) Immunologists often speak of affinity (K_a), the reciprocal of the dissociation constant, in comparing antibodies. What is the affinity of this F_{ab}?
(c) The rate constant of release of hapten from the complex is 120 s^{-1}. What is the rate constant for association? What does the magnitude of this value imply about the extent of structural change in the antibody on binding hapten?

2. *Sugar niche*. An antibody specific for dextran, a polysaccharide of glucose residues, was tested for its binding of glucose oligomers. Maximal binding affinity was obtained when the oligomer contained six glucose residues. How does the size of this site compare with that expected for the binding site on the surface of an antibody?

3. *A brilliant emitter*. Certain naphthalene derivatives exhibit a weak yellow fluorescence when they are in a highly polar environment (such as water) and an intense blue fluorescence when they are in a markedly nonpolar environment (such as hexane). The binding of ε-dansyl-lysine to specific antibody is accompanied by a marked increase in its fluorescence intensity and a shift in color from yellow to blue. What does this finding reveal about the hapten–antibody complex?

4. *Avidity versus affinity*. The standard free energy of binding of F_{ab} derived from an antiviral IgG is -7 kcal mol^{-1} (-29 kJ mol^{-1}) at 25°C.

(a) Calculate the dissociation constant of this interaction.
(b) Predict the dissociation constant of the intact IgG, assuming that both combining sites of the antibody can interact with viral epitopes and that the free-energy cost of assuming a favorable hinge angle is $+3$ kcal mol^{-1} (12.6 kJ mol^{-1}).

5. *Miniantibody*. The F_{ab} fragment of an antibody molecule has essentially the same affinity for a monovalent hapten as does intact IgG.

(a) What is the smallest unit of an antibody that can retain the specificity and binding affinity of the whole protein?
(b) Design a compact single-chain protein that is likely to specifically bind antigen with high affinity.

6. *Turning on B cells*. B lymphocytes, the precursors of plasma cells, are triggered to proliferate by the binding of multivalent antigens to receptors on their surfaces. The cell-surface receptors are transmembrane immunoglobulins. Univalent antigens, in contrast, do not activate B cells.

(a) What do these findings reveal about the mechanism of B-cell activation?
(b) How might antibodies be used to activate B cells?

7. *An ingenious cloning strategy*. In the cloning of the gene for the α chain of the T-cell receptor, T-cell cDNAs were hybridized with B-cell mRNAs. What was the purpose of this hybridization step? Can the principle be applied generally?

8. *Instruction*. Before the mechanism for generating antibody diversity had been established, a mechanism based on protein folding around an antigen was proposed, primarily by Linus Pauling. In this model, antibodies that had different specificities had the same amino acid sequence but were folded in different ways. Propose a test of this model.

9. *Dealing with nonsense*. Cells, including immune cells, degrade mRNA molecules in which no long open reading frame is present. The process is called nonsense-mediated RNA decay. Suggest a role for this process in immune cells.

10. *Crystallization*. The proteolytic digestion of a population of IgG molecules isolated from human serum results in the generation of F_{ab} and F_c fragments. Why do F_c fragments crystallize more easily than F_{ab} fragments generated from such a population?

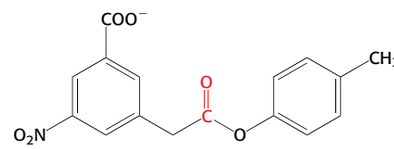
11. *Presentation*. The amino acid sequence of a small protein is:

MSRLASKNLIRSDHAGGLLQATYSAVSS-
IKNTMSFGAWSNAALNDSRDA

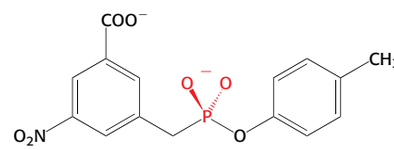
Predict the most likely peptide to be presented by the class I MHC molecule HLA-A2.

Mechanism Problem

12. *Catalytic antibody*. Antibody is generated against a transition state for the hydrolysis of the following ester.



Ester



Transition-state analog

Some of these antibodies catalyze the hydrolysis of the ester. What amino acid residue might you expect to find in the binding site on the antibody?

Chapter Integration Problem

13. *Signaling*. Protein tyrosine phosphatases, such as the molecule CD45 expressed in both B cells and T cells, play important roles in activating such protein tyrosine kinases as Fyn and Lck, which are quite similar to Src. Suggest a mechanism for the activation of such protein kinases by the removal of a phosphate from a phosphotyrosine residue.

Data Interpretation Problem

14. *Affinity maturation*. A mouse is immunized with an oligomeric human protein. Shortly after immunization, a cell line

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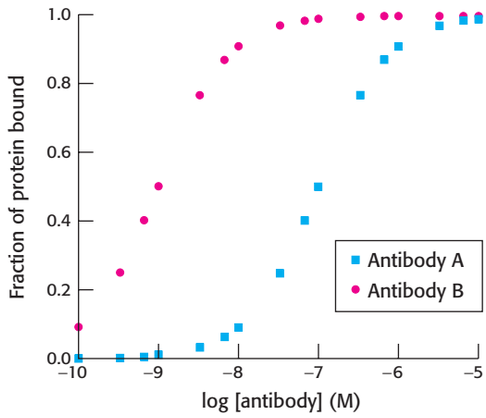

that expresses a single type of antibody molecule (antibody A) is derived. The ability of antibody A to bind the human protein is assayed with the results shown in the adjoining graph. After repeated immunizations with the same protein, another cell line is derived that expresses a different antibody (antibody B). The results of analyzing the binding of antibody B to the protein also are shown. From these data, estimate

(a) the dissociation constant (K_d) for the complex between the protein and antibody A.
(b) the dissociation constant for the complex between the protein and antibody B.

Comparison of the amino acid sequences of antibody A and antibody B reveals them to be identical except for a single amino acid. What does this finding suggest about the mechanism by which the gene encoding antibody B was generated?