Arthur G. Johnson

HIGH-YIELD™ IMMUNOLOGY

High-Yield™ Immunology is designed to:

- Provide a succinct review of immunology
- Help equip you for the immunology questions on the USMLE Step 1
- Clarify difficult concepts

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The Science of Review™

High-Yield Immunology

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Preface

High-Yield Immunology is a compendium of the knowledge considered necessary by the National Board of Medical Examiners (NBME) to achieve competence in immunology for the United States Medical Licensing Examination (USMLE). Immunology is a science that pervades most medical disciplines; this volume spans the breadth of the discipline and includes basic science, as well as clinical, information. I based my selection of what subject areas to include on the experience I have gleaned from years of teaching medical and graduate students. Topics, which include the following, are presented in a concise, "narrative outline" format:

- Development and function of the organs and cells involved in the immune response
- Properties of antigens and antibodies and the way they interact with each other
- Synthesis, genetic control, and regulation of antibody
- Cell-mediated immunity (CMI) and inflammation
- Immunologic disorders, including hypersensitivity disorders, immunodeficiency disorders, and autoimmune diseases
- The role of the immune system in transplantation and cancer
- Currently available vaccines and recommended immunization schedules

Numerous tables and illustrations round out the presentation by summarizing information and clarifying difficult concepts.

This book is not meant to replace the many excellent textbooks of immunology. Rather, it is my hope that the concise presentation of information in *High-Yield Immunology* will assist the reader in the quick recall of the facts considered essential for understanding this exciting and rapidly changing science.

1

Overview

I. SYNOPSIS. The immune system is a complex system composed of several types of sessile and mobile cells that interact in lymphoid tissue dispersed throughout the body. This system is stimulated by the introduction of foreign material **(antigen)** into the host; its function is the elimination of this material.

A. Organs

1. **Central lymphoid organs** (where immunocompetent cells are developed)
a. Thymus
b. Bone marrow

2. **Peripheral lymphoid organs** (where immunocompetency is expressed)
a. Spleen
b. Lymph nodes
c. Tonsils
d. Intestinal Peyer's patches
e. Mucosa

B. Cells. **Antigen-presenting cells (APC), thymus-derived (T) cells,** and **bone marrow-derived (B) cells** interact in the organs to produce two types of immunity.

1. **Humoral immunity** is mediated by proteins called **antibodies**, which neutralize microorganisms and toxins, and remove antigens in the body fluids by **amplifying phagocytosis or lysis.**

2. **Cellular (cell-mediated) immunity (CMI)** is mediated by **T cytotoxic cells, natural killer (NK) cells,** and **macrophages** and is responsible for **eradicating microorganisms residing within body cells,** as well as the **killing of aberrant host cells.**

II. CONCERNS IN MEDICINE include:

A. The immune system's role in protection against infectious diseases and cancer

B. Immune-mediated complications of organ transplantation

C. The immune system's role in allergic disorders

D. The immune system's role in autoimmune disorders

E. The development of specific, sensitive assays for the diagnosis of disease

III. HOST-PARASITE EQUILIBRIUM is largely in favor of the host. Whether the host or parasite prevails depends on whether the parasite's pathogenicity can overcome the host's immunity.

A. Attributes of the parasite

1. Communicability: the transfer of microorganisms between individuals or via vectors

2. Penetrability: the ability of the microorganism to gain access to the host

3. Invasiveness: the ability of the microorganism to enter the tissues

4. Toxigenicity: the ability of the microorganism to inflict damage on the host

B. Attributes of the host

1. Native (innate) immunity is nonspecific and encompasses factors present in an individual independent of antigenic stimulus (e.g., **skin, mucous membranes, sebaceous secretions, pinocytosis, phagocytosis**).

2. Acquired immunity (specific antibody and CMI)

a. Actively acquired by:

(1) Infection

(2) Vaccination

b. Passively acquired by:

(1) Placental transfer of antibody

(2) Injection of specific antibody

IV. DEVELOPMENT OF THE IMMUNE SYSTEM

A. Multipotential stem cells originate in the **fetal liver and bone marrow.**

1. T cells. When stem cells migrate to the fetal thymus, they acquire the phenotypic characteristics of T cells under the influence of thymic hormones (Figure 1-1).

a. Clusters of differentiation (CD). These phenotypic markers appear on the T cell membrane as proteins at different stages of differentiation in the thymus.

(1) CD2 **and** CD3 are major markers retained on all peripheral T cells.

(2) CD4 defines a **T helper cell (Th) subset,** which differentiates in the thymus into **Th1** and **Th2 cells** based on differences in the molecules they secrete (known as **cytokines**).

(a) **Th1 cells** secrete mainly **interleukin-2 (IL-2), interferon-γ (IFN-γ),** and **tumor necrosis factor-α (TNF-α).**

(b) **Th2 cells secrete** mainly **IL-4, IL-5, IL-6, IL-10,** and **IL-13.**

(3) CD8 defines a **T cytotoxic–suppressor cell (Tc or Ts) subset.**

b. The T cell antigenic receptor (TCR) is **epitope specific** and exists on the T cell membrane as two types, designated **α:βTCR** and **γ:δTCR.**

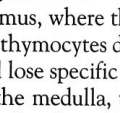
c. A T cell–dependent **homing area** exists periarteriolarly in the spleen, in the paracortical and deep cortical regions in the lymph nodes, and in the gastrointestinal-associated and bronchus-associated tissues.

d. Approximately 1%–2% of T cells leave the thymus and enter the peripheral tissues; the remaining T cells die in **apoptosis,** characterized by condensation and fragmentation of nuclei and membrane blebbing.

2. B cells

a. If stem cells remain in the bone marrow, they acquire the phenotypic CD markers characteristic of the stages of **B cell differentiation** (Figure 1-2).

b. A **membrane-bound, epitope-specific, antigenic receptor** that is a **monomeric immunoglobulin M (IgM) antibody** distinguishes the B cell antigenic receptor from that of the T cell.



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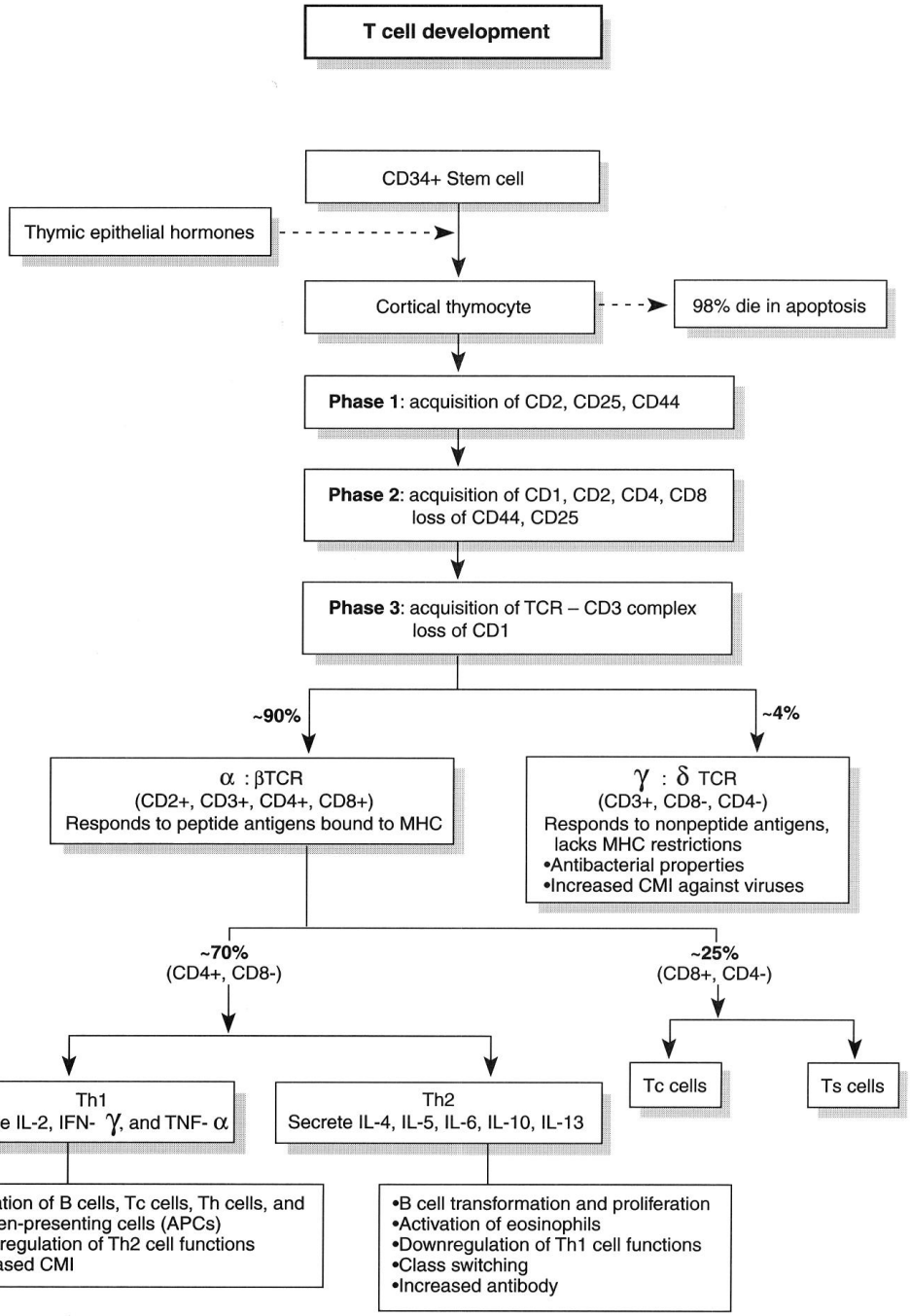

Figure 1-1. T cell development. Stem cells from bone marrow bearing a CD34 marker migrate to the fetal thymus, where they become cortical thymocytes under the influence of epithelial hormones. Most of the cortical thymocytes die; the surviving 1%–2% pass through three phases of development, during which they acquire and lose specific membrane markers [clusters of differentiation *(CD)*]. During the final phase, which takes place in the medulla, the thymocytes acquire the T cell antigenic receptor *(TCR)* and the CD3 signaling complex, and they lose the CD1 marker. The T cells can have one of two types of TCR (α:βTCR or γ:δTCR); the types are differentiated according to the amino acids in the two peptide chains that form the receptor. The α:βTCR T cells respond to peptide antigens bound to the major histocompatibility complex *(MHC)*, while the γ:δTCR T cells respond to nonpeptide antigens. There are two populations of αβ:TCR T cells, Th1 cells and Th2 cells. These cells secrete different cytokines and therefore have different functions. *CMI* = cell-mediated immunity; *IL* = interleukin; *IFN* = interferon; *Tc* = cytotoxic T (cell); *Th* = T helper (cell); *Ts* = T suppressor (cell).

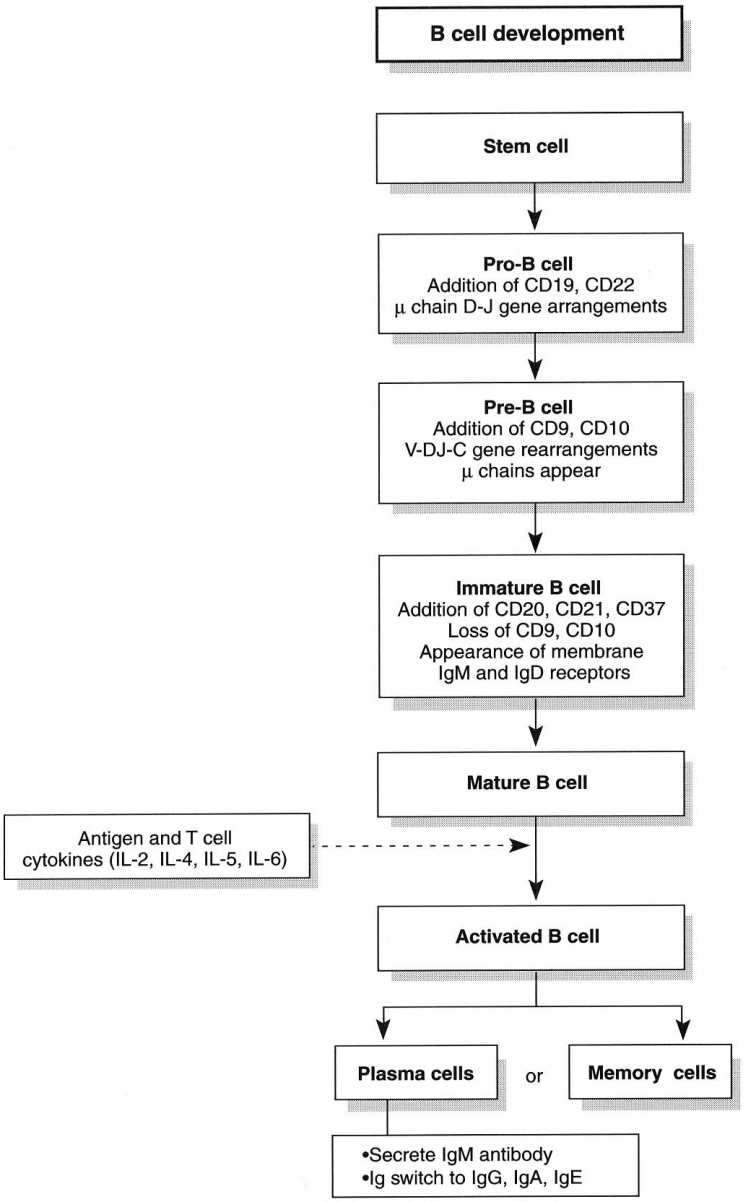

Figure 1-2. B cell development. Stem cells differentiate in the bone marrow and pass through several stages of development before becoming mature B cells. Random selection by each B cell from a variety of germ line genes results in a large number of possible structures for the epitope-binding regions of the immunoglobulins. At the pro-B cell stage, a joining *(J)* region gene links with a diversity *(D)* segment gene. At the pre-B cell stage, the DJ complex links with a variable *(V)* region gene, and then the VDJ complex links to the μ constant *(C)* region gene. At the immature B cell stage, the appearance of membrane IgM and IgD receptors defines B cell clones. Activation of the mature B cells by antigen and T cell cytokines leads to differentiation and division of the B cells. *CD* = cluster of differentiation.

c. B cell homing areas exist primarily in the splenic follicles and red pulp, the lymph nodes, and mucosal-associated tissues.

B. Partial maturation in the thymus and bone marrow in utero is followed by migration to and seeding of the peripheral lymphoid tissues. After birth, the T and B cells differentiate further and gain immunocompetency under antigenic stimulus.

V. CLONAL SELECTION THEORY. Expression of immunity is governed conceptually by the clonal selection theory: the total population of T cells as well as the total population of B cells in the body is made up of millions of individual clones of cells, each clone defined by the occurrence of a **specific receptor for a particular antigen epitope**. On entry, antigen is modified by APCs and selects T cells, B cells, or both possessing the membrane-bound receptor specific for its epitope. Cytokine-induced transformation and expansion of only that clone follows through division. Thus, only T and B cell clones specific for the particular organism responsible for the patient's disease increase to adequate numbers and function to eradicate the pathogen.

2

Antigens

I. DEFINITIONS

A. **Antigen** is a foreign substance that induces antibody or CMI after binding to the specific antigenic receptor on T and B cell clones.

B. **Epitope (antigenic determinant, ligand).** An epitope is the short sequence of amino acids or sugars in an antigen molecule that combines with a hypervariable reactive site on the antibody molecule. The sequence is usually repeated several times, and the number of repeats is referred to as the **valence.**

C. **Hapten.** A hapten is the portion of the antigen molecule that contains the epitope. This area reacts specifically with an antibody but is incapable of inducing antibody synthesis without a carrier molecule.

D. **Superantigen.** Certain retroviral proteins and bacterial toxins (e.g., staphylococcal enterotoxins, toxic shock syndrome toxin 1) can link multiple T cells—via particular T-cell receptor (TCR) Vβ regions—to the major histocompatibility complex (MHC) of antigen-presenting cells (APCs). Because this linking occurs at regions independent of the specific peptide-binding sites, many T cells and APCs are activated, secreting extraordinary amounts of cytokines (e.g., IL-2, IL-1).

E. **Thymus-independent antigens** activate B cells without T helper cell (Th) involvement. Most thymus-independent antigens possess multiple branched polysaccharide repeating units (e.g., lipopolysaccharides from Gram-negative bacteria) and activate B cells polyclonally, without regard to B cell specificity (B cell mitogens).

II. FACTORS DETERMINING ANTIGENICITY.

Antigens are usually protein or polysaccharide; lipids are poorly antigenic. Factors that determine antigenicity include:

A. Degree of "foreignness" and host background

B. Size, shape, chemical composition, and exposure (amount, route, and frequency of exposure)

III. EXAMPLES OF ANTIGENS

A. Microorganisms

1. Frequently, **bacterial antigens** can be components of virulence of the organism. Therefore, **attenuated or killed vaccines** must retain the antigens that are important for virulence.

2. **Antigenic mosaics** are the basis for **serologic classification.**

a. **Streptococcal antigens** are exemplified by group-specific carbohydrates, type-specific M proteins, streptolysin O, erythrogenic toxins, and multiple enzymes.

b. ***Salmonella*** **antigens** are exemplified by O antigen (lipopolysaccharide), H (flagellar) antigen, virulence (Vi) antigen, pili, and several exotoxins.
(1) Terminal sugars with varying optical isomerism determine different species.
(2) Cross-reactions can occur with closely related epitopes.

c. **Human immunodeficiency virus (HIV) antigens** are exemplified by glycoproteins (e.g., gp160, gp120, gp41) and enzymes (e.g., reverse transcriptase). The numerical designation of the glycoproteins refers to the molecular weight.

B. **Human tissue antigens**

1. Blood-group antigens

2. Organ-specific antigens

3. Individual-specific leukocytic antigens [e.g., human leukocyte antigens (HLA)]

3

Antibodies

I. GENERAL PROPERTIES

A. Definition. Antibodies are mucoproteins that are found in the γ-globulin fraction of serum on electrophoresis. These mucoproteins are called **immunoglobulins (Ig).**

B. Heterogeneity

1. Five classes. When injected into animals, human immunoglobulin becomes antigenic. The resulting antihuman immunoglobulin antibodies are grouped into five classes: **IgG, IgA, IgM, IgE, and IgD.**

2. Structure. The basic structural unit for each class is a four-chain protein with two heavy (H) and two light (L) chain polypeptides linked by disulphide bonds (Figure 3-1).

a. Amino acid sequences

(1) H chains. A specific amino acid sequence on the H chains differentiates the classes (i.e., IgG, IgA, IgM, IgE, and IgD). These H-chain differences are called **isotypes** and are designated by the Greek letters **gamma (γ), alpha (α), mu (μ), epsilon (ε),** and **delta (δ),** respectively. Isotypes are genetic variations that all normal humans possess.

(2) L chains

(a) All five classes have an amino acid sequence in common on the L chains. Thus, they can be classified together as immunoglobulins.

(b) In addition, two L-chain isotypes, designated **kappa (κ)** and **lambda (λ)** exist for all five classes.

b. Amino acid composition. Both H and L chains are divided into **constant region domains** (designated **CH** and **CL**) and **variable region domains** (designated **VH** and **VL**).

(1) Constant region domains. The amino acid sequence in the constant regions of both the H and L chains is similar for all antibody molecules within each class.

(2) Variable region domains. The amino acid sequence of the variable regions on both H and L chains varies with the epitope toward which the particular antibody is directed.

(a) Amino acids that show marked differences between antibodies of different specificities form the **hypervariable region** within each variable region.

(b) The hypervariable regions of the H and L chains associate to form the **epitope binding region,** known as the antibody **idiotype,** of which there are two.

(3) A **hinge region** also exists between the CH_1 and CH_2 domains, permitting flexibility in the movement of the two antigen-binding sites.

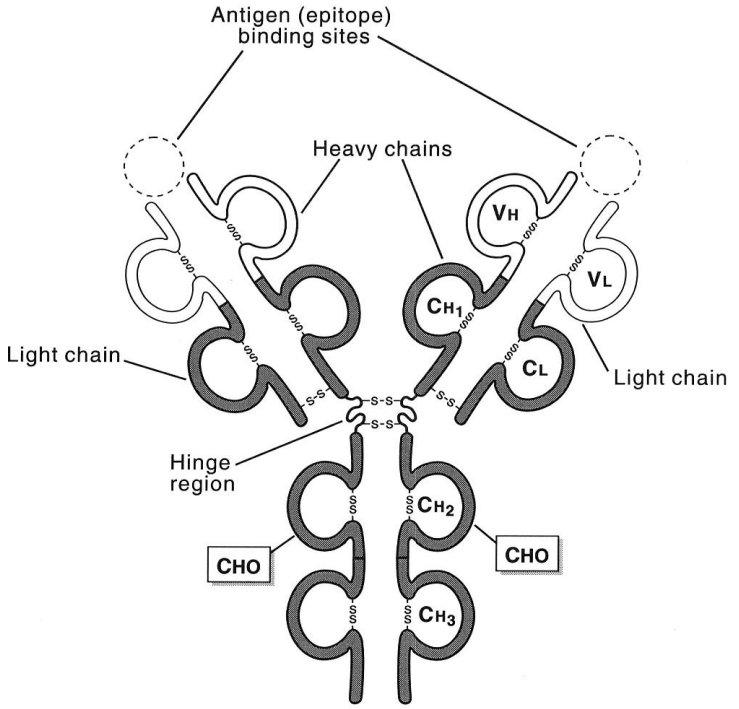

Figure 3-1. The basic four-peptide structure of immunoglobulins is illustrated by this IgG pattern. Three heavy chain constant region domains (CH_1, CH_2, CH_3) and one light chain constant domain (CL)are shown in *black*. The variable region of both the light and heavy chains (VL and VH respectively; in *white*) associate to form the specific epitope binding site. Several of the most critical disulfide bonds are shown (—*ss*—). *CHO* indicates where the carbohydrate is attached. (Redrawn with permission from Eisen HN: *General Immunology*. Philadelphia, Lippincott-Raven, 1990, p 48.)

C. Monoclonal antibodies. Most antigenic preparations give rise to a mixture of antibodies. However, antibodies of a single specificity are highly desirable for many purposes, including specific diagnostic tests and immunotherapy. Monoclonal antibodies can be made routinely.

1. Single B cells from an immunized animal fuse with malignant (immortal) plasma cells, forming a **hybridoma.**
2. Following screening, the **hybrid clone** making the desired antibody is isolated, expanded, and the secreted monoclonal antibody removed.

II. IgG

A. Structural properties (Table 3-1)

1. **Molecular weight.** IgG is composed of two L chains (each with a molecular weight of 22,000 d) and two H chains (each with a molecular weight of 53,000 d). The total molecular weight is 150,000 d.
2. The **structural designation** is ($\gamma_2\kappa_2$) or ($\gamma_2\lambda_2$), with the γ-marker indicating the IgG H-chain isotype and the κ- or λ-marker indicating the L-chain isotype.
3. **Four subclasses** exist: γ_1, γ_2, γ_3, and γ_4. These subclasses are differentiated by slight changes in the amino acid sequences on the γ H chain.

Table 3-1.
Structural Properties of Human Immunoglobulins

Property	IgG	IgM	IgA	IgE	IgD
H-chain isotype	γ	μ	α	ε	δ
H-chain subclass	γ_1, γ_2, γ_3, γ_4	. . .	α_1, α_2	. . .	. . .
L-chain isotype	κ or λ	κ or λ	κ or λ	κ or λ	κ or λ
Associated chains	. . .	J chain	J chain, SP	. . .	. . .
Structural designation	$\gamma_2\kappa_2$ or $\gamma_2\lambda_2$	$(\mu_2\kappa_2)_5$ or $(\mu_2\lambda_2)_5$	**Serum:** $\alpha\kappa_2$ or $\alpha\gamma_2$ **Mucosa:** $(\alpha_2\kappa_2)_2$ J, SP or $(\alpha_2\gamma_2)_2$ J, SP	$\varepsilon_2\kappa_2$ or $\varepsilon_2\lambda_2$	$\delta_2\kappa_2$ or $\delta_2\lambda_2$
Percent carbohydrate	4	15	10	18	18
Molecular weight (daltons)	150,000	**Monomer:** 180,000 **Pentamer:** 950,000	**Monomer:** 160,000 **Dimer:** 318,000 **Dimer + SP:** 380,000	188,000	184,000

J = J chain; SP = secretory piece.

4. Enzymatic cleavage (Figure 3-2)

a. Papain splits IgG into three fragments.

(1) Two of these fragments **(Fab; fragment, antigen binding)** are similar, with each containing only one of the reactive sites for the epitope. Because Fab is monovalent, it can bind to but cannot enter into lattice formation and precipitate or agglutinate antigen.

(2) A third fragment **(Fc; crystallizable)** activates complement, controls catabolism of IgG, fixes IgG to tissues or cells via an Fc receptor, and mediates placental transfer.

b. Pepsin splits behind the disulphide bond joining the two H chains, permitting the two Fab fragments to remain joined. Consequently, this fragment is termed **F(ab′)2.**

(1) Because F(ab′)2 is bivalent, it is capable of lattice formation and aggregation of antigens.

(2) F(ab′)2 is removed more rapidly from the circulation than the intact IgG.

(3) The Fc fragment is extensively degraded.

B. Functional properties (Table 3-2)

1. Serum and half-life. IgG has the highest serum concentration of all immunoglobulins (700–1500 mg%) and a serum half-life of 18–25 days.

2. Functions

a. IgG adheres to cells that possess a receptor for the Fc fragment from IgG (Fcγ).

b. IgG fixes complement (i.e., a series of enzymes resulting in cell lysis).

c. IgG mediates placental passage of maternal antibody to the fetus.

Figure 3-2. Enzymatic cleavage of IgG by pepsin and papain. (*A*) Papain cleavage results in two unlinked antigen binding (Fab) fragments with the disulphide bonds (—*ss*—) remaining with the crystallizable (Fc) fragment. Because the fragments are univalent, they cannot precipitate or agglutinate antigens. (*B*) Pepsin cleavage results in retention of the disulphide bonds with the two Fab fragments linked as F(ab′)2. The Fc portion is degraded. CH_1, CH_2, CH_3 = heavy chain constant region domains; *VL*, *VH* = variable region of light and heavy chains, respectively. (Redrawn with permission from Abbas AK, Lichtman AH, Pober JS: *Cellular and Molecular Immunology*, 3rd ed. Philadelphia, WB Saunders, 1997, p 50.)

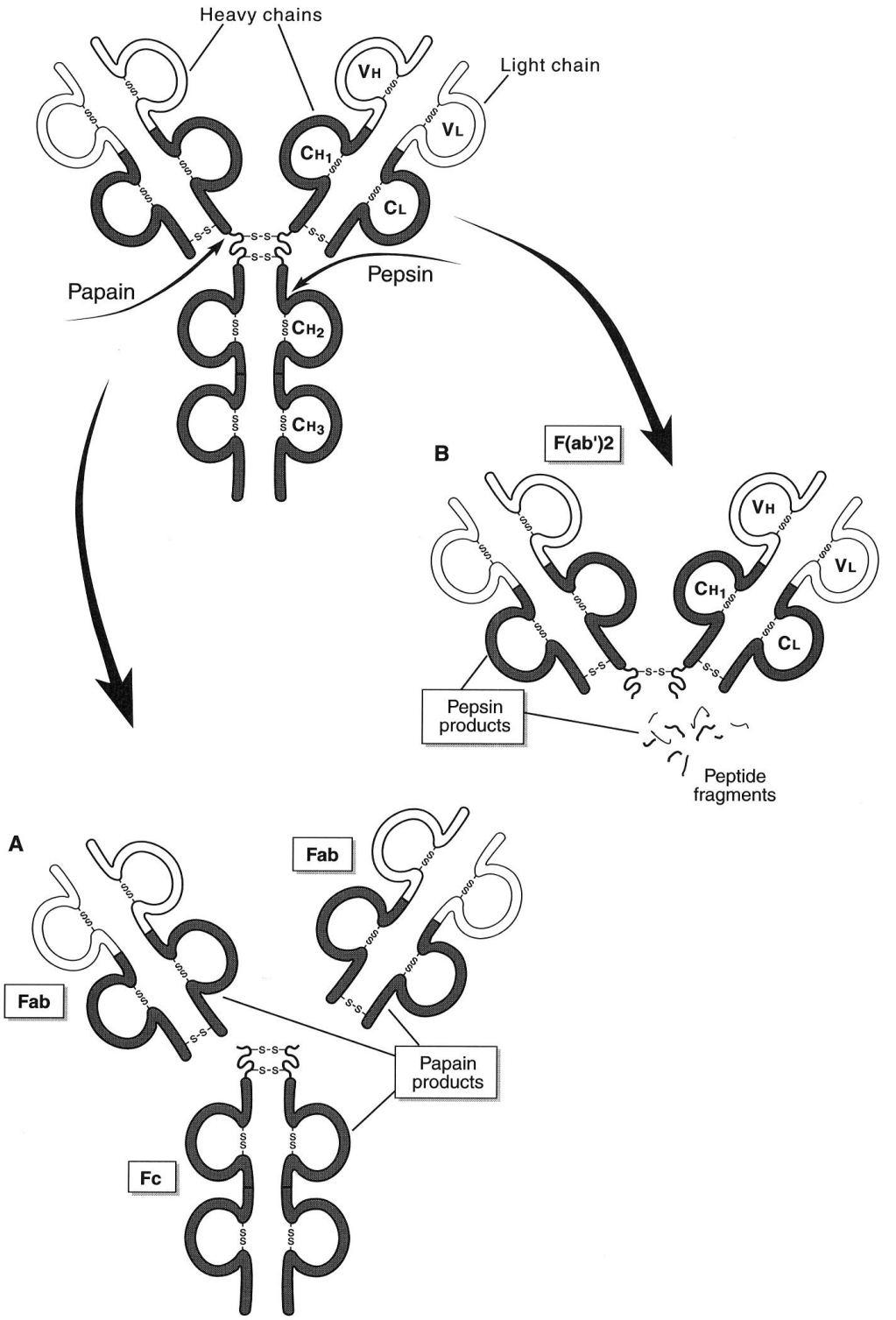

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Table 3-2.
Functional Properties of Human Immunoglobulins

Property	IgG				IgM	IgA		IgE	IgD
	γ_1	γ_2	γ_3	γ_4		α_1	α_2		
Average serum concentration (mg%)	900	300	100	50	150	300	50	0.03	3
Serum half-life (days)	23	23	8	23	5	6	6	3	2.5
Activates complement	+	±	++	–	+++	–	–	–	–
Binds to Fc receptor	+	±	++	+	+	–	–	+	–
Crosses placenta	+	±	+	+	–	–	–	–	–

III. IgM

A. Structural properties (see Table 3-1)

1. Form. IgM exists in two structural forms.

a. A **monomer** is synthesized by and retained on the membrane of B cells and is designated $\mu_2\kappa_2$ or $\mu_2\lambda_2$.

(1) A monomer serves as the B cell receptor specific for a single antigenic epitope.

(2) The hypervariable region of the monomer differs for each B cell clone.

b. Secreted IgM exists as a **pentamer** (i.e., five monomeric IgM molecules joined together by a J chain; Figure 3-3). The IgM pentamer is designated $(\mu_2\kappa_2)_5$ or $(\mu_2\lambda_2)_5$.

(1) The pentamer is secreted following antigen and cytokine activation of plasma cells, with the hypervariable regions on the pentamer the same as those on the membrane-bound monomeric receptor.

(2) Of the 10 possible epitope-binding sites on the pentamer, five are of high affinity and five are of low affinity.

2. Molecular weight. IgM has four constant domains on the H and L chains (in contrast with the three found on IgG, IgA, and IgD); therefore, its pentamer form has the highest molecular weight of the immunoglobulins.

B. Functional properties (see Table 3-2). IgM, the earliest antibody to appear after antigenic stimulus, fixes complement avidly.

IV. IgA

A. Structural properties (see Table 3-1)

1. Forms. IgA exists in three forms: a **monomer, a dimer** (in which a J chain joins two monomers; Figure 3-4), and **a dimer plus a secretory piece.**

a. The dimer is transported across respiratory and intestinal mucosal barriers into the lumen by the secretory piece, which is a receptor for the IgA Fc region **(FcαR)** on the mucosal epithelium.

b. The secretory piece also protects IgA from proteolysis.

2. The **structural designation** is $(\alpha_2\kappa_2)$ or $(\alpha_2\lambda_2)$ as the monomer, and $(\alpha_2\kappa_2)_2$ or $(\alpha_2\lambda_2)_2$ as the dimer.

3. Two subclasses exist: α_1 and α_2.

B. Functional properties (see Table 3-2)

1. Serum half-life and concentration. IgA is found in high concentrations in secretions; in serum, IgA exists mainly as a dimer with a half-life of 6 days.

2. Functions. IgA is located in and protects mucosal tissues, saliva, tears, and colostrum by blocking bacteria, viruses, and toxins from binding to host cells.

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Figure 3-4.
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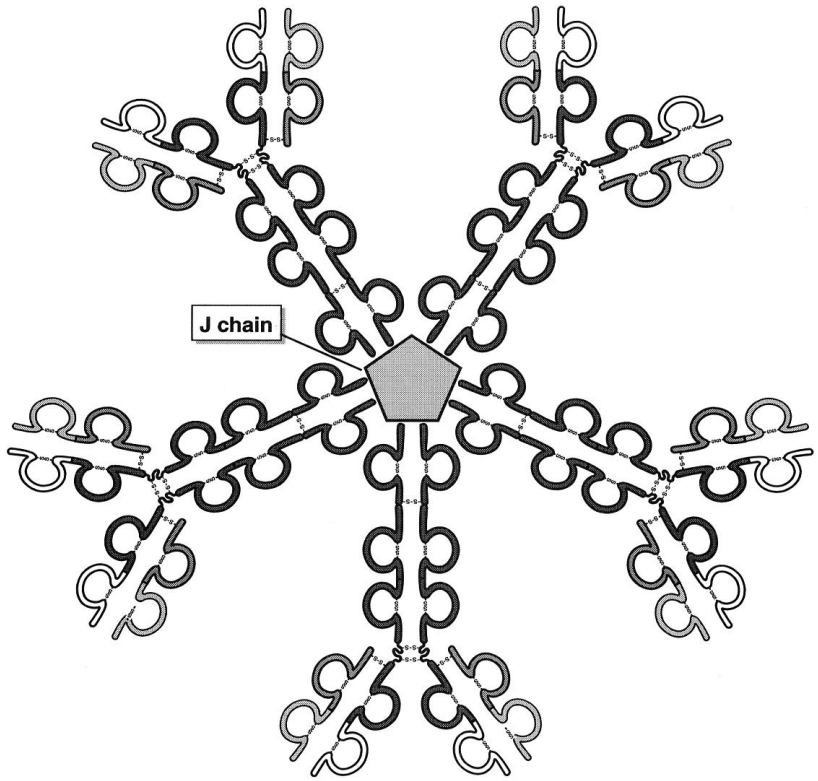


Figure 3-3. IgM pentamer. (Modified with permission from Abbas AK, Lichtman AH, Pober JS: *Cellular and Molecular Immunology*, 3rd ed. Philadelphia, WB Saunders, 1997, p 48.)

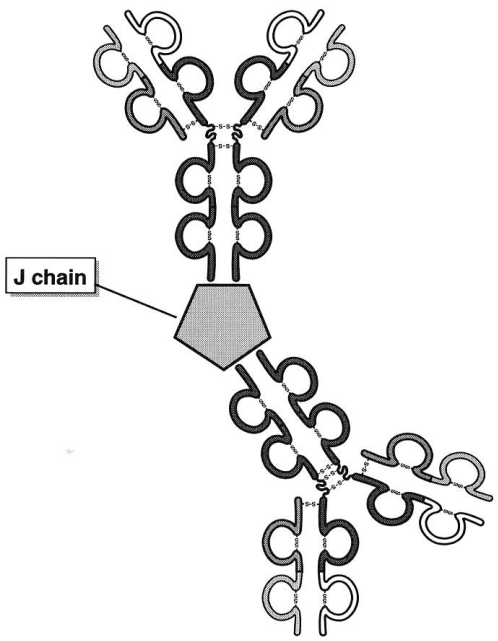


Figure 3-4. IgA dimer. (Modified with permission from Abbas AK, Lichtman AH, Pober JS: *Cellular and Molecular Immunology*, 3rd ed. Philadelphia, WB Saunders, 1997, p 48.)

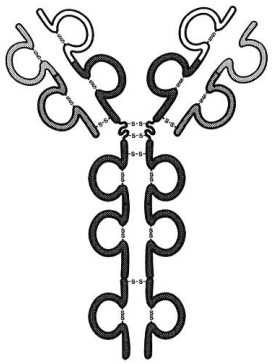


Figure 3-5. IgE. Note the four C domains. (Modified with permission from Abbas AK, Lichtman AH, Pober JS: *Cellular and Molecular Immunology*, 3rd ed. Philadelphia, WB Saunders, 1997, p 48.)

V. IgE

A. Structural properties (see Table 3-1)

1. Molecular weight. IgE has four C domains (Figure 3-5) and a carbohydrate content of 18%, resulting in a molecular weight of 188,000 d.

2. The **structural designation** for IgE is $\varepsilon_2\kappa_2$ or $\varepsilon_2\lambda_2$.

3. The IgE molecule is unstable at 56°C and is called **reagin.**

B. Functional properties (see Table 3-2)

1. Serum concentration and half-life. IgE has an extremely low serum concentration because its Fc region binds avidly to mast cells and basophils.

2. Functions

a. IgE adheres to tissue-bound mast cells and circulating basophils via Fcε receptors on these cells. The binding of antigen to these IgE-sensitized cells triggers the release of vasoactive amines (mainly histamine), resulting in **atopic disease** characterized by hives (a local reaction) and anaphylaxis (a systemic reaction).

b. IgE does not cross the placenta or fix complement by the conventional pathway.

VI. IgD

A. Structural properties (see Table 3-1). The structural formula for IgD is $\delta_2\kappa_2$ or $\delta_2\lambda_2$.

B. Functional properties (see Table 3-2)

1. Serum half-life and concentration. IgD is found on the membranes of 15% of newborns and 5% of adult peripheral blood lymphocytes in conjunction with IgM; serum levels are very low. The serum half-life is 2 to 3 days.

2. Functions. IgD is a major receptor on B cells for antigen.

4

Immunologic Assays

I. OVERVIEW

A. Immunologic assays detect antigen–antibody (Ag–Ab) reactions *in vitro* (e.g., agglutination of bacteria or cells or precipitation of soluble antigens) or *in vivo*.

B. **Ag–Ab reactions.** The union of antigen with antibody is **specific** and **firm,** but **reversible;** multiple short-range forces are involved.

1. **Binding** occurs in seconds but is not visible until a **lattice** forms, which occurs more slowly. The composition of the lattice depends on the ratio of antigen to antibody.

a. **Affinity** measures the binding energy between an antibody and a univalent epitope.

b. **Avidity** is the total binding energy between an antibody and a multivalent antigen.

2. **Specificity** of Ag–Ab reactions is extreme. Changing the position of atoms, double bonds, or the composition of amino acids or sugars of the epitope changes specificity.

II. PROTECTION TESTS are used to determine the potency of vaccines.

A. **Active.** Following immunization with the vaccine that is being tested, the animal is challenged with increasing numbers of microorganisms. The lowest number of microorganisms lethal for 50% of the animals (i.e., the LD_{50}) is determined and compared to the LD_{50} in nonvaccinated animals in order to measure the protective power of the vaccine.

B. **Passive.** Graded amounts of serum from immunized individuals are transferred to normal animals, which are then challenged with the infectious agent. The highest dilution of serum effective at protecting 50% of the animals (i.e., ED_{50}) is determined as a measure of the efficacy of the vaccine.

III. AGGLUTINATION TESTS are used to detect antibody union with large, particulate antigens.

A. **Slide agglutination (qualitative)**

1. **Blood grouping.** Slide agglutination is used in blood grouping to determine whether the donor's cells or serum possess antigens or antibodies reactive with the recipient's serum or cells.

a. **Major crossmatch** uses the donor's cells plus the recipient's serum to determine whether anti–red blood cell (RBC) antibodies are present in the recipient's serum. Rapid clumping of the donor's cells will occur *in vivo* if anti-donor RBCs are present.

b. Minor crossmatch uses the donor's serum plus the recipient's cells. Agglutination of the recipient's cells occurs if anti–RBC antibodies are present in the donor's serum. However, a transfusion reaction under these conditions would be much less severe than one associated with a major crossmatch because the amount of antibodies transfused in the donor's serum is minimal relative to the number of RBCs in the recipient.

2. Rapid identification of bacteria. Bacteria can be identified by mixing a loopful of bacteria from the patient's culture with a battery of specific antibacterial antisera and noting which antiserum causes agglutination.

B. Tube agglutination (semi-quantitative). The microorganism suspected of causing the disease is added to dilutions of the patient's serum. The highest dilution that results in visible agglutination is called the **titer.** A **fourfold increase** in titer is necessary for diagnosis, owing to low levels of "natural" antibodies occurring in most normal human beings.

C. Hemagglutination

1. Viral. Myxoviruses (e.g., influenza, mumps, some pox viruses, and arboviruses) spontaneously agglutinate RBCs. This reaction is blocked in the presence of specific antiviral antibody. The patient's serum titer is determined by dilution.

2. Coombs' test. Weak or nonagglutinating anti–RBC antibody (generally Rh) can be detected by adding **antihuman immunoglobulin** to the RBC–anti-RBC complex.

a. Direct test. Nonagglutinating Rh antibody attached *in utero* to the fetal or newborn Rh+ RBCs can be revealed by adding antihuman immunoglobulin to the infant's RBCs.

b. Indirect test. Anti-Rh antibodies in the maternal circulation can be detected by adding the mother's serum to Rh+ RBCs *in vitro*. The addition of antihuman immunoglobulin results in agglutination of the sensitized RBCs.

3. Cold agglutination. IgM complement–fixing antibodies, which agglutinate RBCs at temperatures below 37°C, are detected by incubation at lower temperatures. These antibodies are frequently autoimmune in nature and occur commonly in patients with primary atypical pneumonia caused by *Mycoplasma pneumoniae*.

IV. PRECIPITATION REACTIONS are used to detect soluble proteins and polysaccharides.

A. Quantitative precipitin test. This test measures either antigen or antibody in serum with analytical precision.

1. Increasing amounts of antigen are added in separate tubes containing a constant amount of the patient's serum.

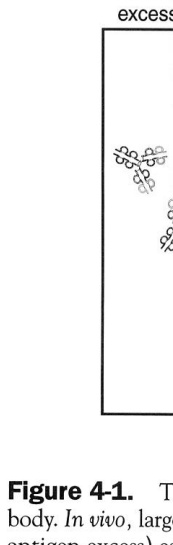
2. The resulting precipitate in each tube is washed and analyzed by micromethods, and the precipitated antibody is plotted as a function of antigen added. **Three zones** result: **antibody excess, equivalence, and antigen excess** (Figure 4-1).

B. Gel diffusion

1. Double diffusion (Ouchterlony test). This technique detects impurities and identifies antigens in a mixture.

a. Antigen and antibody diffuse toward each other from wells cut in 1% agar, forming a line of precipitate on contact.

b. The number of lines reflects the number of antigens with different diffusion coefficients in a mixture (Figure 4-2).



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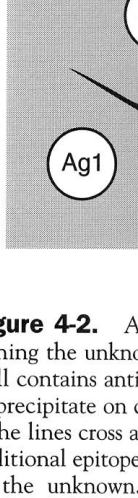

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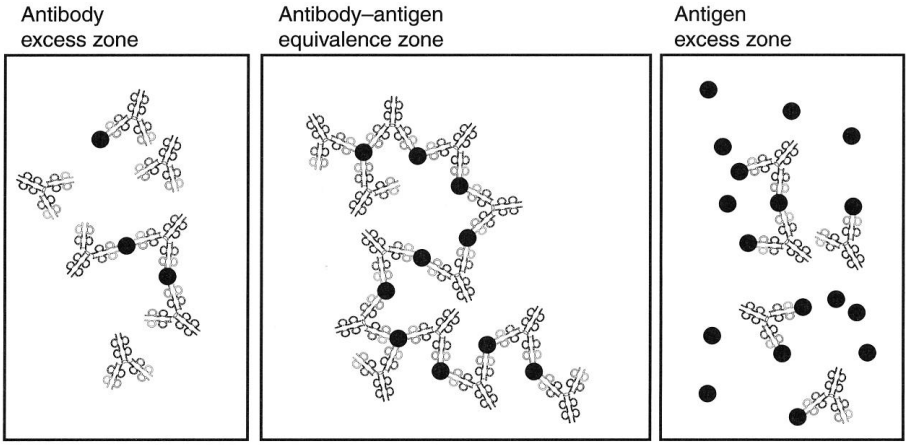


Figure 4-1. The size of antigen–antibody (Ag–Ab) complexes is determined by the ratio of antigen to antibody. *In vivo*, larger complexes (in antibody excess and at equivalence) are phagocytosed; smaller complexes (in antigen excess) escape and lodge in blood vessels and behind the renal basement membrane, causing vasculitis and glomerulonephritis.

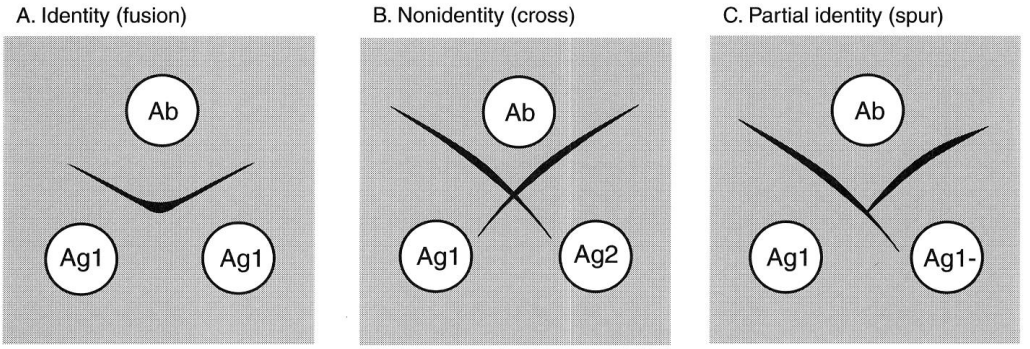


Figure 4-2. Agar-gel diffusion (Ouchterlony technique). To identify an unknown antigen, a sample containing the unknown is placed in a well adjacent to a well containing the suspected known antigen. A third well contains antibodies against each. As the antigens and antibodies diffuse through the gel, they form lines of precipitate on contact. Should the lines fuse as in *(A)*, the unknown antigen is identified with the known; if the lines cross as in *(B)*, the two are not identical; and if partial fusion occurs as in *(C)*, one antigen has an additional epitope. The presence of additional lines of precipitation in the agar indicates a mixture of antigens in the unknown. *Ab* = antibody; *Ag* = antigen. (Redrawn with permission from Sell S: *Immunology, Immunopathology and Immunity*, 5th ed. Stamford, CT, Appleton & Lange, 1996, p 111.)

2. **Immunoelectrophoresis** is used to identify immunologic disorders. Components of an antigen mixture are separated in agar first by migration in an electric field, followed by diffusion and subsequent precipitation with specific antibody diffusing from an overhead trough (Figure 4-3).

C. Radioimmunoassay (RIA) is based on the displacement of a known, radiolabeled antigen from an Ag–Ab complex by an unknown, unlabeled antigen (e.g., hormone) in a patient's body fluids. The extent of loss of the labeled antigen from the Ag–Ab complex can be measured and is a function of the concentration of the unknown antigen in the patient's fluid. Sensitivity is less than 0.001 μg.

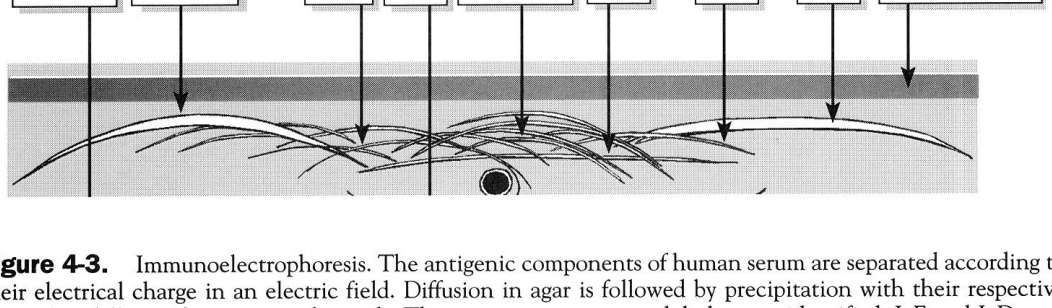


Figure 4-3. Immunoelectrophoresis. The antigenic components of human serum are separated according to their electrical charge in an electric field. Diffusion in agar is followed by precipitation with their respective antibodies diffusing from a central trough. The component immunoglobulins are identified. IgE and IgD concentrations are too low to be detected by this technique.

D. Enzyme-linked immunosorbent assay (ELISA)

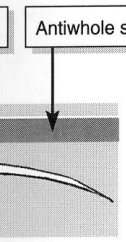
1. **Antibody detection** is useful in detecting antibodies in a patient's serum (e.g., HIV).
   - **a.** Dilutions of the test antibody solution are added to antigen adsorbed onto plastic wells. The complex is washed, and an enzyme-conjugated, anti-isotype antibody is added.
   - **b.** After washing, the enzyme substrate is added.
   - **c.** The resulting color is measured using a spectrophotometer. The titer is recorded as the highest dilution of antibody giving a color above the background.
2. **Antigen detection** is useful for measuring nanogram (ng) amounts of hormones, drugs, and serum proteins.
   - **a.** Dilutions of antigen are added to antibody that is adsorbed onto plastic wells. The resulting complex is washed, and an enzyme-conjugated antibody specific for a different epitope on the test antigen is added.
   - **b.** After washing, the enzyme substrate is added, and the colored reaction is measured using a spectrophotometer. The titer is recorded as the highest dilution of antigen giving a color above background.

V. COMPLEMENT FIXATION

A. Complement system.

Complement (C′) fixation results in **cell lysis** and requires **nine major factors (C′1–C′9),** which have enzymatic functions. Complement is fixed via **two pathways** (Figure 4-4).

1. **Classic pathway.** Binding of the proenzyme C′1 to an Ag–Ab complex triggers a sequential reaction that results in cell lysis.
   - **a.** IgM or a doublet of IgG bound to a cell surface antigen activates C′1qrs, which cleaves C′4 and C′2.
   - **b.** Fragments C′4b and C′2b bind to the cell surface as C′4b2b, becoming a C′3 convertase that cleaves C′3 into two fragments, C′3a and C′3b.
   - **c.** C′3b complexes with C′4b2b to become a C′5 convertase, which cleaves C′5 to C′5a and C′5b.
   - **d.** C′5b combines with C′6 and C′7 and inserts into the cell membrane.
   - **e.** C′8 and C′9 combine with the C′5b, 6, 7 complex to form the **membrane attack complex (MAC),** resulting in cell lysis.



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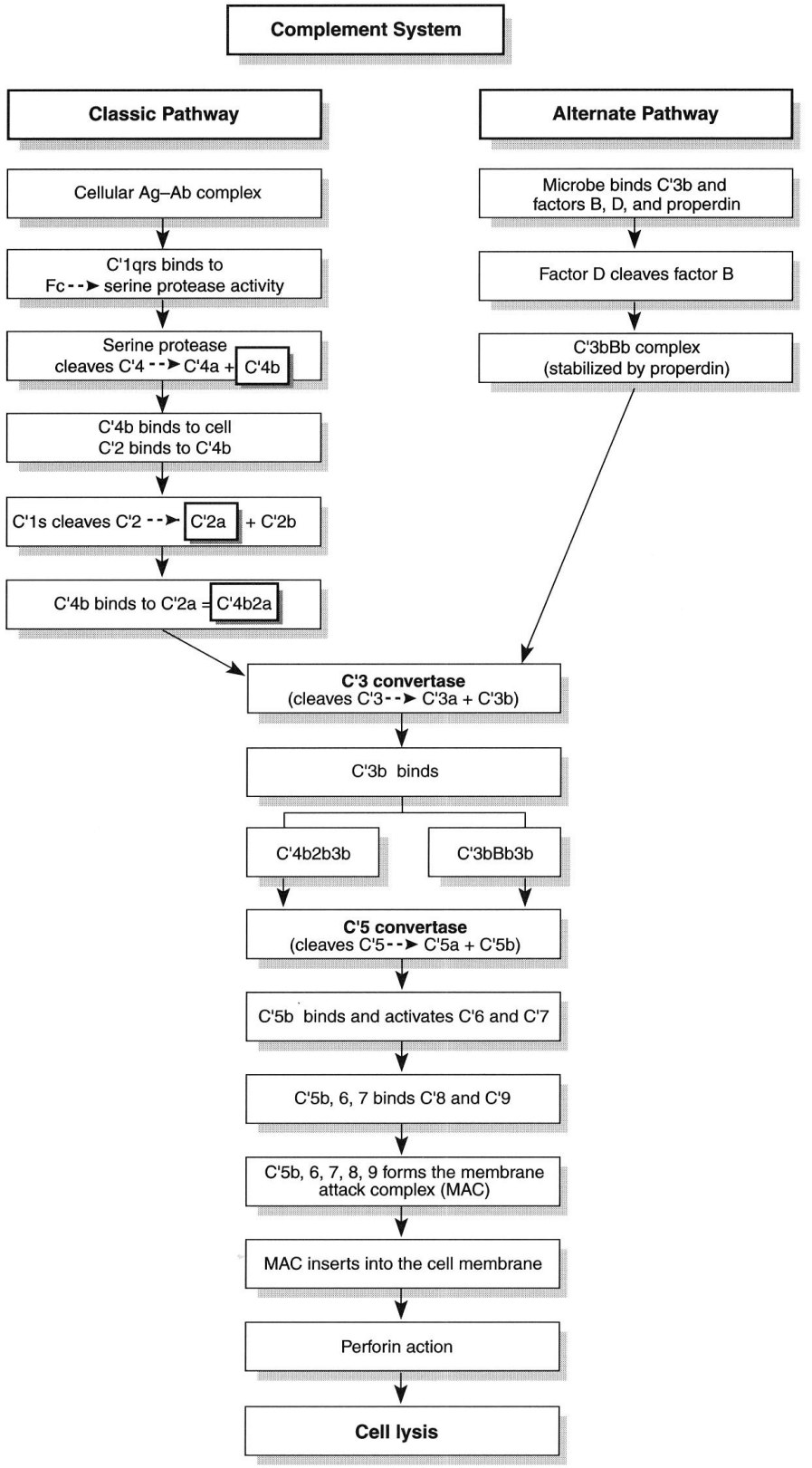


Figure 4-4. Complement is fixed via two pathways. Complement is lytic and promotes inflammation via its side products, C′3a and C′5a. A receptor for C′3 on phagocytic cells promotes the phagocytosis of Ag–Ab–C′ complexes. *Ab* = antibody; *Ag* = antigen; *C′* = complement.

2. **Alternate pathway.** This pathway is activated by cell walls of certain bacteria, yeasts, and aggregated IgA. It does not require antibody or C′1, C′4, or C′2.
   a. The cell walls bind to C′3b, which exists in normal serum. This complex binds with three other serum factors (B, D, and properdin), leading to a C′3 convertase. C′3bBb generates additional C′3b.
   b. A C′3bBbC′3b complex forms, which becomes a C′5 convertase leading to the reactions that result in the MAC.

B. Complement fixation test. When specific antibody combines with its antigen, complement is bound and its serum concentration diminished. The extent of diminution can be measured in a complement fixation test and reflects the extent of antigen–antibody union.

VI. FLUORESCENT ANTIBODY.

An antibody of concern is conjugated with **fluorescein isothiocyanate** or another dye that fluoresces under ultraviolet light. This can be used as a reagent to permit the visualization of either antigens or antibody in cells or tissues.

A. Direct technique. Fluorescinated antibody is added directly to the specimen (e.g., tissue) containing antigen and visualized under ultraviolet light.

B. Sandwich technique

1. If detection of the antigen in a specimen is desired, antibody is added, followed by fluorescinated anti-immunoglobulin antibody, and the specimen is visualized under ultraviolet light.
2. If detection of antibody in a specimen is desired, antigen is added, followed by fluorescinated antibody against the antigen, and the specimen is visualized under ultraviolet light.

VII. WESTERN BLOT

A. This technique is widely used as a **confirmatory test for AIDS.** The patient's serum is added to HIV antigens bound to the nitrocellulose matrix. A positive reaction is detected by the addition of a labeled, antihuman immunoglobulin antibody, as in the indirect ELISA test.

B. To **identify an antigen** in a mixture, the components of the mixture are separated by electrophoresis on a sodium dodecyl sulfate-polyacrylamide gel and "blotted" onto a nitrocellulose matrix. Labeled, known antibody is added to locate and identify the antigen of interest.

5

Immunogenetics

I. GENETIC CONTROL OF IMMUNOGLOBULIN CHAIN SYNTHESIS

A. Genetic diversity. Human antibodies exhibit an enormous range (~10^8) of specificities. The genetic basis for this diversity involves several factors:

1. Different genes code for the **variable** and **constant regions** of the heavy (H) and light (L) chains.

2. Rearrangement of variable region and constant region genes during differentiation within the genome. Any one of many different variable region genes can be linked to a single constant region gene, thus conserving DNA.

3. Joining segment. An additional gene sequence is required during the formation of the **L chain.** This sequence, the joining segment, joins the VL region gene to the CL region gene (Figure 5-1).

4. Diversity segment. An additional gene sequence is required during the formation of the **H chain.** This sequence, the **diversity segment,** links the VH gene to the J gene. These genes are then fused with the CH gene (Figure 5-2).

5. H-chain class switching from μ and δ to γ_3, γ_1, α_1, γ_2, γ_4, ε, and α_2 is dictated by a later rearrangement of the **class genes** in the CH region and is mediated by T cell cytokines (IL-4, IL-13, IFN-γ, TGF-β).

B. Random selection by each B cell from the variety of V, D, and J germ line genes available results in a large number of structural possibilities for the VL and VH epitope binding regions of the immunoglobulins. This random selection is primarily responsible for the vast diversity of antibodies.

C. Allelic exclusion. Only one of the two parental alleles is expressed by a single B cell, resulting in a single H-chain isotype and L-chain subtype receptor capable of reacting with only one antigenic epitope.

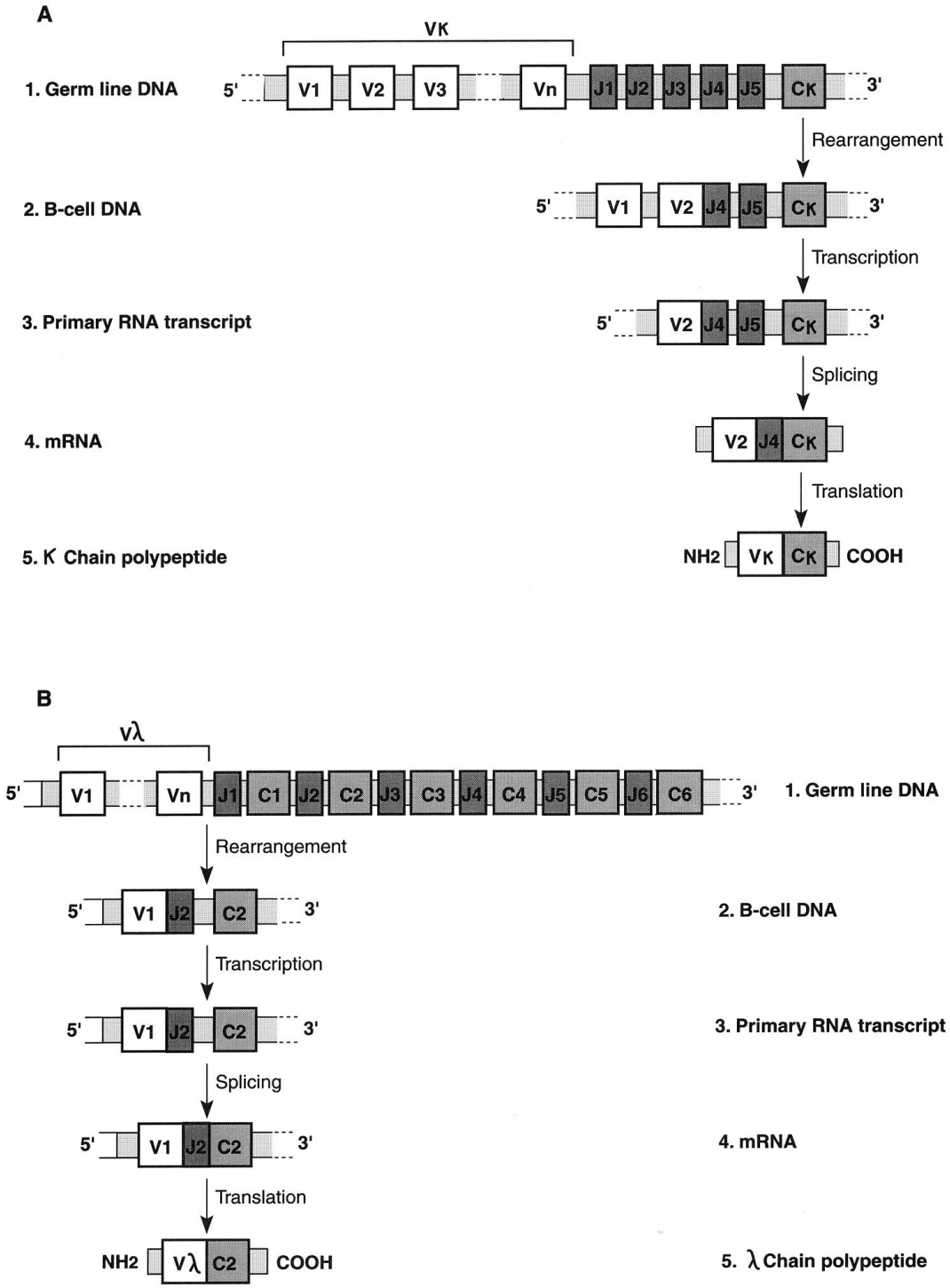
II. GENETIC CONTROL OF HUMAN LEUKOCYTE ANTIGENS (HLAs)

A. Functions of HLAs. HLAs control several elements, including:

1. Discrimination between self and nonself

2. Antigen presentation to T cells, but only of the same HLA type (self-MHC restriction)

3. Susceptibility to immunologic disorders and infectious agents



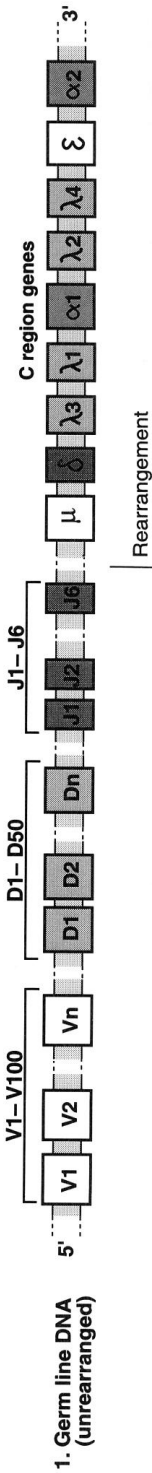


Figure 5-1. *(A)* Kappa (κ) light (L)-chain synthesis. From the pool of multiple variable *(V)* region genes on chromosome 2 in the germ line DNA *(1)*, one V region gene is joined to a joining *(J)* region gene, resulting in B-cell DNA *(2)*. Following removal of introns by recombinases, the primary RNA is transcribed *(3)*, resulting in mRNA *(4)* composed of one V region gene, one J gene, and the constant (Cκ) region gene. Translation of the mRNA results in the κ L-chain polypeptide *(5)*. (Redrawn with permission from Benjamini E: *Immunology: A Short Course*, 3rd ed. New York, Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc., 1996, p 98.) *(B)* Lambda (λ) L-chain synthesis. Rearrangement and synthesis of the λ L-chain genes occurs in an identical manner on chromosome 22, except for the availability of up to six Cλ exons for union to the VJ combined exon. This availability results in several subtypes.

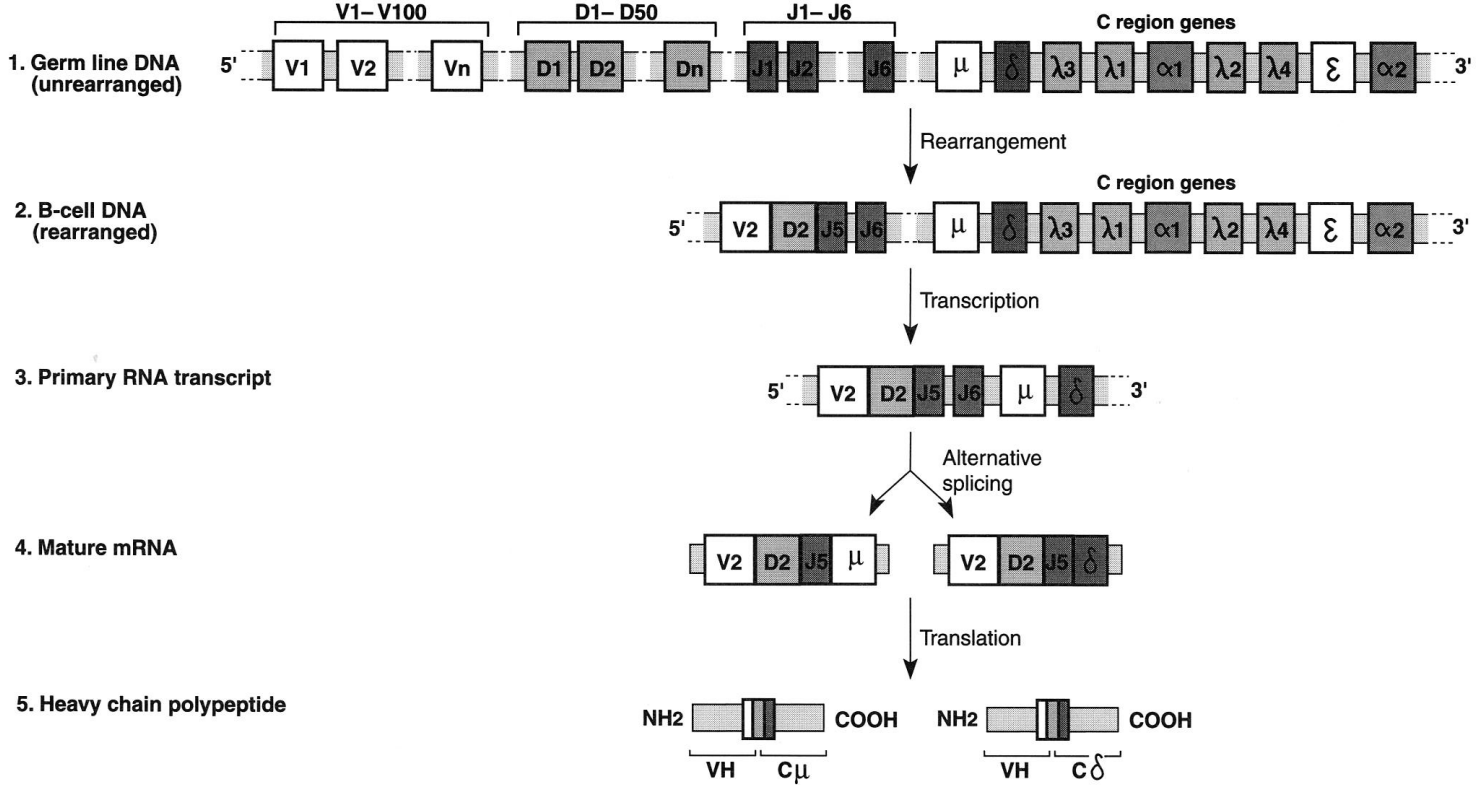


Figure 5-2. Heavy (H)-chain synthesis. *(1)* The variable region of the H chain is coded by three different gene complexes present on chromosome 14: variable *(V)* region genes, diversity *(D)* segment genes, and the joining *(J)* region genes. The constant *(C)* region gene complex harbors the genes controlling all of the immunoglobulin classes. *(2)* During rearrangement, a J region gene links to a D region gene, and then this complex links with a V region gene. The VDJ complex links to the μ or δ region genes. *(3)* A primary RNA transcript of the VDJμδ complex is made, and after splicing, mRNAs for a VDJμ and a VDJδ appear *(4)*. H chains of both IgM and IgD result after translation of the mRNAs *(5)*. These H chains combine with light (L) chains and deposit on the B cell membrane as the antigen receptors. Following antigen and cytokine stimulus, the IgM antibody is secreted (not illustrated). (Redrawn with permission from Benjamini E: *Immunology: A Short Course*, 3rd ed. New York, Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc., 1996, p 100.)

Table 5-1.
Human Leukocyte Antigen (HLA) Classes

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

From IMMUNOLOGY, 3/E by Janis Kuby. © 1992, 1994, and 1997 by W. H. Freeman and Company. Used with permission.
MHC = major histocompatibility complex; *TNF* = tumor necrosis factor.

B. Classes of HLAs. HLAs are organized into three classes of molecules (Table 5-1).

1. Class I HLAs are glycoproteins that are found on the **membranes of most nucleated cells.**

a. Gene regions. Class I molecules are encoded by three gene regions: **A, B,** and **C.**

b. Function. Class I molecules are linked to the cytotoxic T (Tc) cell through the CD8 molecule and present peptidic epitopes to **specific Tc receptors** (class I restriction). A single class I molecule can bind several different epitopes.

c. Structure (Figure 5-3)

(1) Two chains form the class I molecule.

(a) The α **chain** has three external domains, a transmembrane segment, and a cytoplasmic tail.

(b) The β_2**-microglobulin** is an invariant protein.

(2) The **peptide-binding site,** found between domains α1 and α2, binds peptides containing 8–10 amino acids.

2. Class II HLAs are glycoproteins that are found on the **membranes of dendritic cells, macrophages,** and **activated T cells** and **B cells.**

a. Gene regions. Class II molecules are encoded by three gene regions: **DP, DQ,** and **DR.**

b. Function. Class II molecules are linked to the T helper (Th) cell through the CD4 molecule and present peptidic epitopes to **specific Th cell receptors** (class II restriction). A single class II molecule can bind several different epitopes.

c. Structure (see Figure 5-3)

(1) Two chains, α and β, form the class II HLA molecule. Each chain has two domains plus a transmembrane segment and cytoplasmic tail.

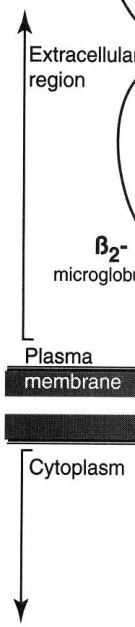
(2) The **peptide-binding site,** formed by juxtaposition of the α1 and β1 domains, binds peptides containing 13–18 amino acids.

3. Class III HLAs control certain **serum proteins,** including several complement components and tumor necrosis factors (TNFs). Class III molecules are encoded by three gene regions: **C4, C2,** and **BF.**

C. Polymorphism

1. Alleles. Many alleles of class I and II molecules are present at each locus on chromosome 6 and are the major obstacles to organ transplantation.

2. Haplotypes from both parents are inherited and expressed codominantly.



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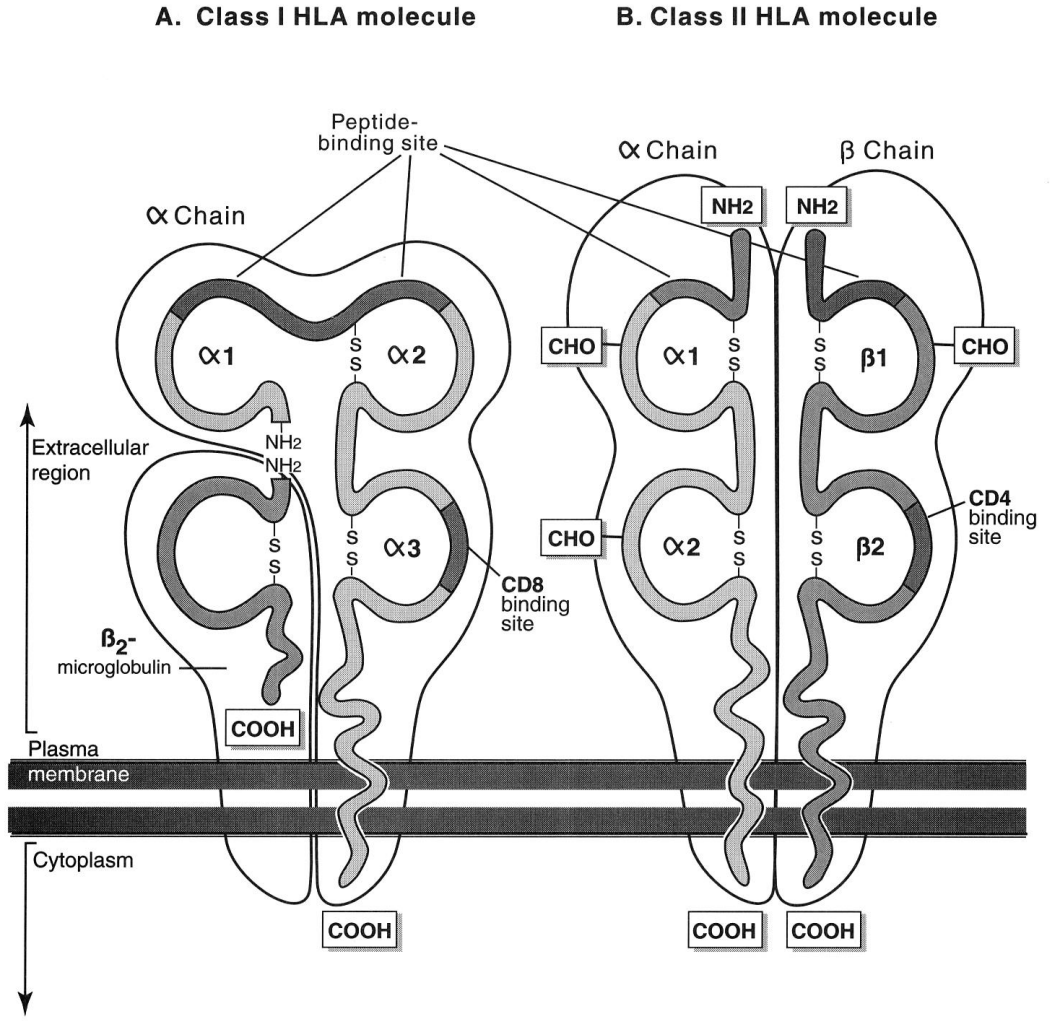

Figure 5-3. Structure of class I and class II human leukocyte antigen (HLA) molecules. —SS— = disulfide bond. (Redrawn with permission from Stites DP, Terr AI, Parslow TG: *Medical Immunology*, 9th ed. Stamford, CT, Appleton & Lange, 1997, p 86.)

III. GENETIC CONTROL OF THE T-CELL ANTIGENIC RECEPTOR (TCR)

A. Structure. The TCR is a **dimer** of either α and β chains (approximately 95%) or γ and δ chains (approximately 5%).

B. Function

1. In contrast to the monomeric IgM antigen receptor on the B-cell membrane, the **TCRs do not respond to soluble antigens.**
2. **TCRs recognize antigenic epitopes** only as peptidic fragments bound to either class I or class II HLA molecules on an antigen-presenting cell (APC) [e.g., dendritic cells, macrophages, B cells].

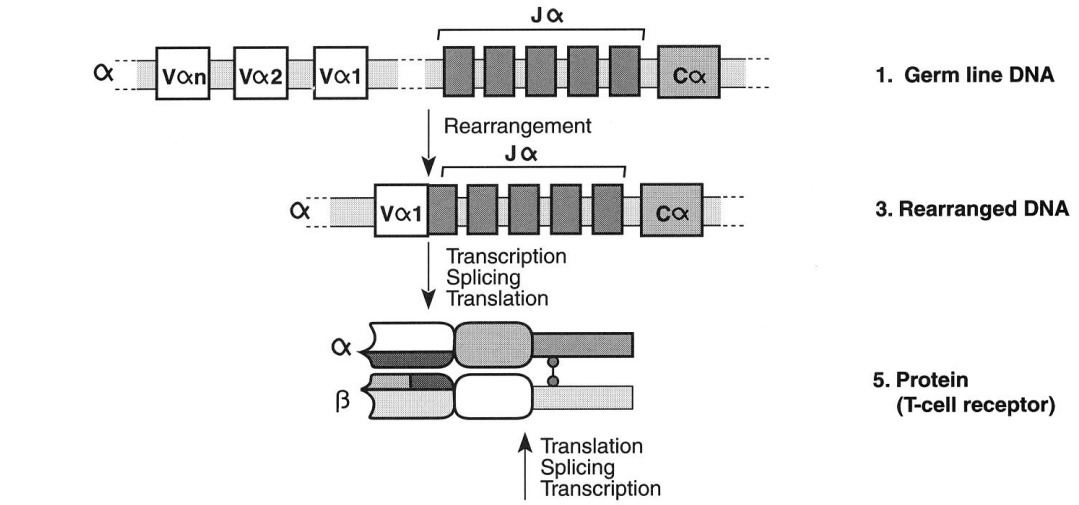

Figure 5-4. Synthesis of the human αβ T-cell receptor (TCR) genes. Synthesis of the γδ chains is thought to follow a similar pattern. *(1)* Multiple variable *(V)* region genes and joining *(J)* region genes occur at the TCRα locus on chromosome 14. *(2)* Similarly, multiple V region, diversity *(D)* segment, and J region genes occur at the TCRβ locus on chromosome 7. *(3)* During the rearrangement of α-chain genes, a randomly selected V gene is joined to a J gene and the exon is transcribed, combined with a constant (Cα) region gene, and translated. *(4)* Similarly, the β-chain exon is formed by the random linkage of a V region gene, first to a D region gene and a J region gene, and then to a Cβ gene. (Redrawn with permission from Janeway CA Jr, Travers P: *Immunobiology: The Immune System in Health and Disease*. New York, Garland Publishing, 1997, p 4:35.)

3. The **co-receptors, CD4 and CD8,** determine whether humoral immunity or cell-mediated immunity (CMI) occurs.
   a. Binding of the CD4 molecule to a class II HLA molecule on the APC results in **humoral immunity.**
   b. Binding of the CD8 molecule to a class I HLA molecule results in **CMI.**

4. Union of the specific TCR and co-receptor with the peptide–HLA membrane complex is associated with signal transduction into the cytoplasm by a complex of proteins. These proteins are collectively designated **CD3.**

C. Genetic makeup. Diversity among the TCRs is achieved through **gene rearrangements** similar to that of immunoglobulins (Figure 5-4). The phenomenon of **allelic exclusion** controls the genetic expression. Allelic exclusion occurs when only one of the parental alleles that code for the TCR is functional, rendering each T cell responsive to only a single epitope.

6

The Immune Response

I. HUMORAL IMMUNITY is mediated by antibodies that protect the body fluids (Figure 6-1).

A. Antigen entry

1. If antigen entry is **intravenous,** the antigen is phagocytized or pinocytosed in the **spleen.**

2. If antigen entry is **other than intravenous,** the antigen moves to the **lymph node** draining the site of entry.

B. **Antigen processing.** In the lymph nodes or spleen, the antigen encounters the **T-cell, B-cell, antigen-presenting cell (APC) triad** and is initially processed by the APC (Figure 6-2). Antigen processing results in the activation of T cells.

1. **Viruses and intracellular parasite antigens**

a. These antigens are **synthesized endogenously** within the APC cytoplasm and endoplasmic reticulum, then processed to peptides by proteasomes.

b. The **resulting peptides** bind to the heavy chains of **major histocompatibility complex (MHC) class I** molecules and migrate to the APC membrane, where they are presented to CD8+ T cells.

2. Exogenous protein antigens

a. These antigens enter the APC from the **extracellular environment** by pinocytosis and are processed in acidic endosomal vacuoles.

b. The **resulting peptides** bind to the cleft in **MHC class II** molecules and are transported to the cell membrane, where they are presented to **CD4+** T cells.

C. Activation of T and B cells

1. **Exogenous protein antigens.** After being transported to the APC cell membrane, the antigenic peptide–MHC class II complex is presented to CD4+ T helper (Th) cells (Figure 6-3A).

a. **Th1 cell response.** Following activation, the CD4+ Th1 cell clone differentiates, divides logarithmically, and secretes **IL-2, IFN-γ,** and **TNF-α.**

(1) IL-2 is necessary for T and B cell transformation.

(2) IFN-γ

(a) A **potent macrophage** and **natural killer (NK) cell activator,** IFN-γ enhances cell-mediated immunity (CMI).

(b) IFN-γ **triggers HLA antigen presentation** by endothelial cells.

(c) IFN-γ **downregulates IL-4 synthesis** by Th2 cells; thus, it can also suppress antibody formation.

(3) TNF-α

(a) Activates macrophages

(b) Stimulates the acute-phase response

(c) Synergizes with IL-1 in inducing the acute-phase response

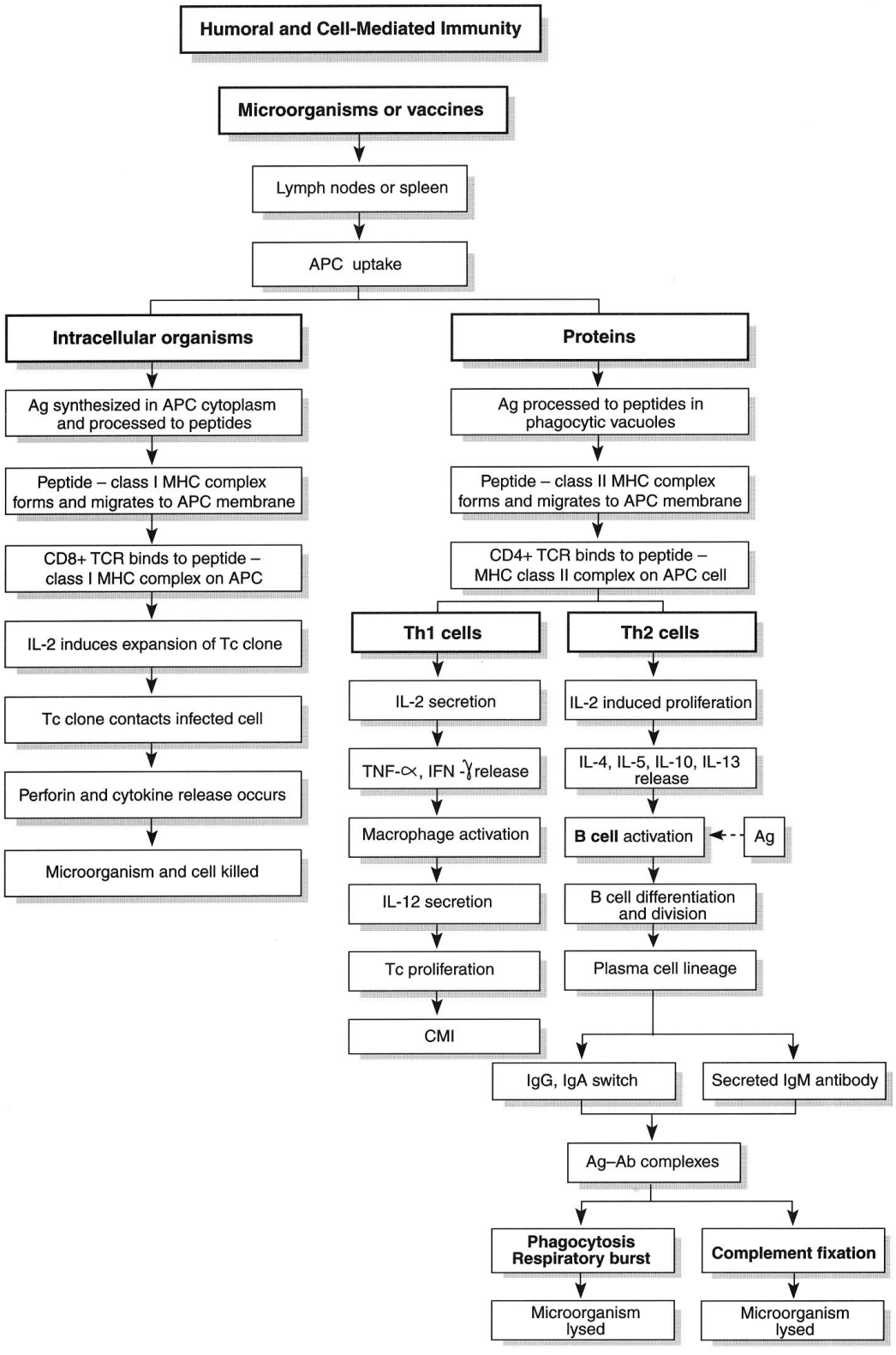

Figure 6-1. Humoral and cell-mediated immunity. *Ag–Ab* = antigen–antibody; *APC* = antigen-presenting cell; *CD* = cluster of differentiation; *IFN-γ* = interferon-γ; *Ig* = immunoglobulin; *IL* = interleukin; *MHC* = major histocompatibility complex; *Tc* = cytotoxic T cell; *TCR* = T-cell receptor; *TNF-α* = tumor necrosis factor-α.

A

Figure 6-2. A
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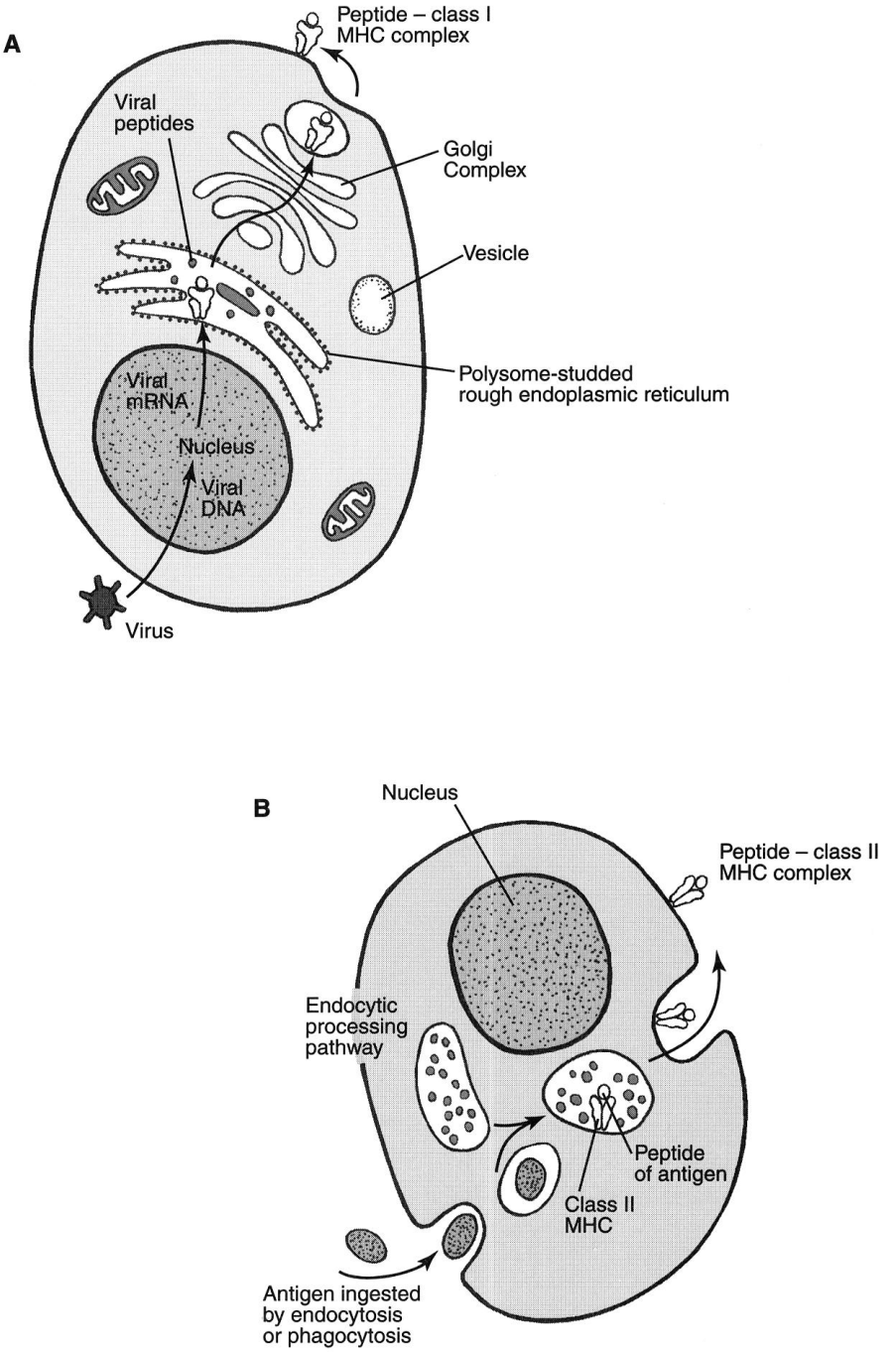


Figure 6-2. Antigen processing (*A*) of intracellular organisms and (*B*) of exogenous proteins. (From IMMUNOLOGY, 3/E by Janis Kuby. © 1992, 1994, and 1997 by W. H. Freeman and Company. Used with permission.)

b. Th2 cell response. Following antigen activation and stimulation by IL-2, the CD4+ Th2 cell responds by transforming, differentiating, and dividing logarithmically, while secreting **IL-4, IL-5, IL-10,** and **IL-13.**

(1) IL-4
- **(a)** Favors the development of antibody synthesis by stimulating B-cell differentiation
- **(b)** Downregulates IFN-γ by Th1 cells and, thus, can suppress CMI
- **(c)** Is necessary for the switch to immunoglobulin E (IgE) production

(2) IL-5
- **(a)** Functions synergistically with IL-4 and IL-2 to help B-cell differentiation
- **(b)** Facilitates IgA synthesis
- **(c)** Stimulates the growth and differentiation of eosinophils

(3) **IL-10,** like IL-4, inhibits Th1 cell release of IFN-γ and IL-2, thereby negating macrophage activation by IFN-γ.

(4) **IL-13** mimics IL-4 actions, inhibiting Th1 cytokine release.

c. B-cell response

(1) Antigen selects the **clone of B cells** with the membrane-bound IgM antigen receptor that is specific for the antigen epitope.

(2) **Binding of antigen** along with **stimuli from the T cell cytokines IL-2 and IL-4** triggers differentiation of that B-cell clone into a **large blast cell,** and **logarithmic division** occurs.

(3) IL-5 continues this process, during which the B cell acquires the **cytoplasmic "machinery"** necessary for **antibody synthesis.**
- **(a)** **H and L chains** are synthesized, assembled, and, under IL-6 influence, terminal differentiation into a plasma cell and secretion of IgM occurs.
- **(b)** Subsequent **gene rearrangements** result in a switch to IgG, IgA, and IgE synthesis and secretion.
  - **(i)** IL-4 and IFN-γ influence the switch to IgG; TGF-β influences the switch to IgA; and IL-4 influences the switch to IgE.
  - **(ii)** The binding of CD40 on the B cell to its ligand on the Th cell (CD40L), is necessary for switching to occur.

d. B memory cells. Memory cells of all classes are generated independently of the **plasma cell** lineage. **These memory cells migrate to various lymphoid tissues,** where they have an extended survival.

e. Secondary response. Further exposure to the same antigen can result in the following:

(1) A shorter induction period to antibody synthesis

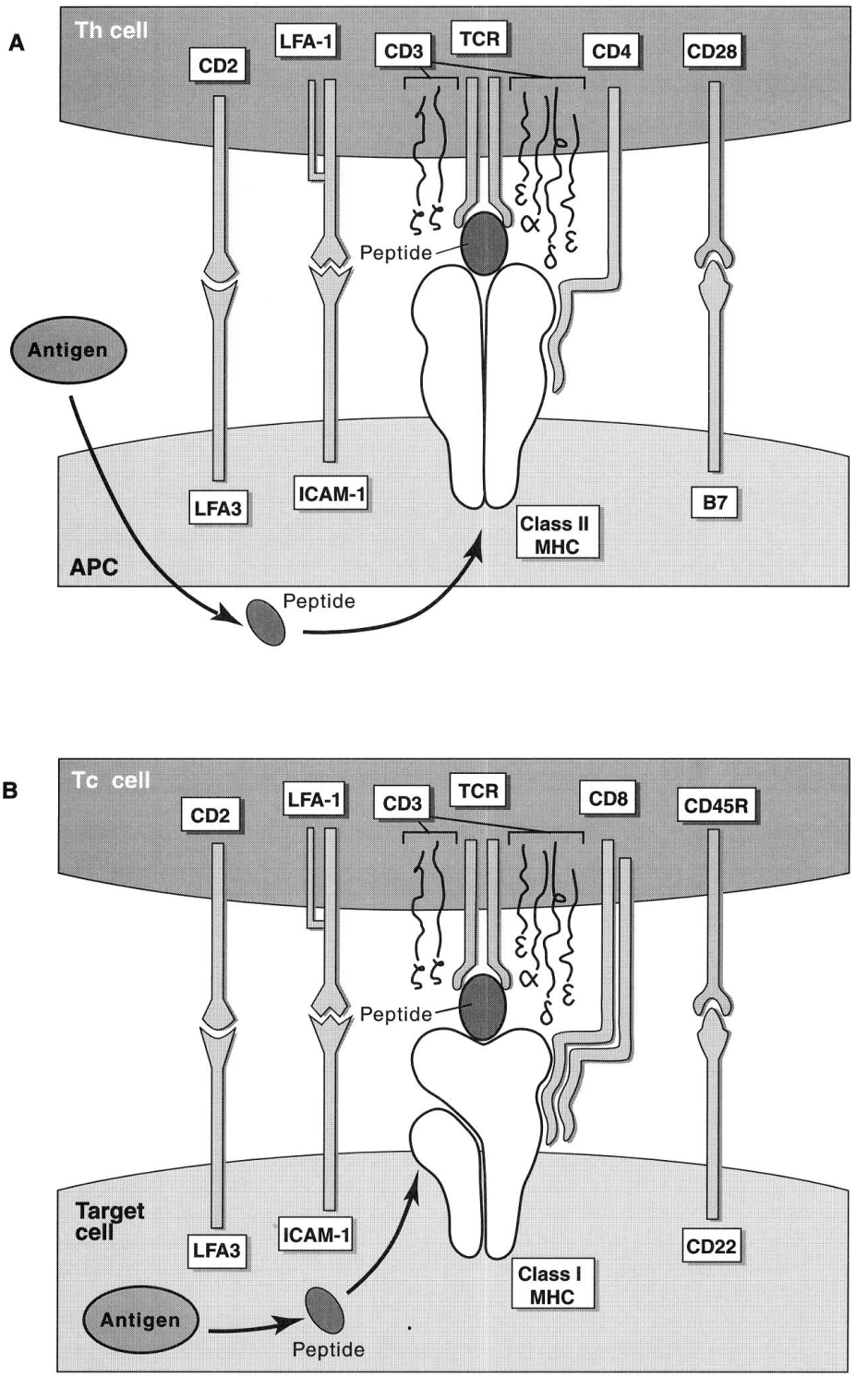
(2) More rapid class switching from IgM to IgG

(3) Increased IgG with antibodies of higher affinity

(4) Predominant IgA synthesis in mucosal tissues

2. Viruses and intracellular parasite antigens. Synthesized in the cytoplasm and transported to the APC membrane, the antigenic peptide–MHC class I complex is presented to CD8+ cytotoxic (Tc) cells (Figure 6-3B).

Figure 6-3. *(A)* Activation of CD4+ helper T (Th) cells. The specific T-cell antigenic receptor *(αβ:TCR* or *γδ:TCR)* binds to the peptide–class II major histocompatibility complex *(MHC)* complex by the antigen-presenting cell *(APC)*. The CD4 molecule links to the MHC complex. An activation signal is transduced by the TCR–CD3 complex, which is composed of three polypeptides (α, δ, ε) and two ζ chains. Accessory T-cell adhesion molecules [e.g., CD2, leukocyte function–associated antigen-1 *(LFA-1)*, and CD28] facilitate adherence of the Th cell to the APC and influence interleukin-2 (IL-2) synthesis. *(B)* Activation of CD8+ cytotoxic T *(Tc)* cells. (From IMMUNOLOGY, 3/E by Janis Kuby. © 1992, 1994, and 1997 by W. H. Freeman and Company. Used with permission.)


A
Th cell
CD2
LFA-1
CD3
TCR
CD4
CD28
Peptide
Antigen
LFA3
ICAM-1
Class II MHC
B7
APC
Peptide
B
Tc cell
CD2
LFA-1
CD3
TCR
CD8
CD45R
Peptide
Target cell
LFA3
ICAM-1
Class I MHC
CD22
Antigen
Peptide

II. CELL-MEDIATED IMMUNITY (CMI) is directed mainly against **intracellular-dwelling microorganisms** and **aberrant, endogenous cells** (e.g., cancers).

A. Mechanism. Immune reactivity is effected by sensitized **T cells, macrophages,** and **NK cells** on direct contact with the target cell.

1. Reactivity is transferrable to **normal, nonsensitized hosts** with sensitized effector cells.

2. Antibody is not involved, except in **antibody-dependent cellular cytotoxic reactions (ADCC).** In these cases, the effector cell is linked to the target cell by an antibody bridge, with the **Fab portion** binding to the specific membrane antigen on the target cell, and the **Fc portion** binding to the Fc receptor on an activated effector cell.

B. Types of CMI

1. Reaction to infectious agents (e.g., tuberculin test)

a. Function. The tuberculin test reveals immune reactions in internal organs (e.g., lungs).

(1) Domestic use. The tuberculin test is used in the United States to identify human beings exposed to, or actively infected with, *Mycobacterium tuberculosis*. The underlying principle can be extrapolated to apply to the detection of other intracellular microorganisms.

(2) Foreign use. Extensive use of bacille Calmette-Guérin (BCG) vaccine in other countries nullifies their use of the tuberculin test as a diagnostic test.

b. Procedure. A patient **previously exposed** to M. *tuberculosis* is injected intradermally with an extract of M. *tuberculosis* [called **purified protein derivative (PPD)**].

c. Reaction process [delayed-type hypersensitivity (DTH)]

(1) A contained lesion of **induration and erythema,** peaking in 1 to 2 days, results from the inflammatory response induced by sensitized T-cell action at the site of PPD deposition.

(2) The skin lesion is initiated by **Langerhans cell** presentation of antigen to previously sensitized delayed-type hypersensitivity T (TDTH) cells that have been recruited to the site of antigen deposition by chemokines.

(3) Subsequent APC– and T cell–secreted cytokines and chemokines attract **polymorphonuclear neutrophils (PMNs),** followed by CD4+ T cells and a dominant, nonspecific, perivascular accumulation of monocytic/macrophage cells.

(4) Destruction of the organisms, tissue, or both follows macrophage infiltration.

2. Granulomatous reactions occur if the antigen persists in the tissues and continues to stimulate host reactivity.

a. Chronic stimulation by intracellular organisms releases chemotactic agents (e.g., IL-1, IL-8), leading to an inflammatory cell influx.

b. IL-4 and **IFN-γ** promote the retention of macrophages and cause the fusion of monocytes at the site, leading to an **epithelioid cell granuloma** derived from macrophages, histiocytes, and epithelioid cells.

3. Contact dermatitis occurs when small-molecular-weight chemicals **(haptens)** or irritants are deposited into the skin, causing a CMI reaction. **Common eliciting agents** include nickel, dinitrochlorobenzene, rubber, poison ivy, and poison sumac.

a. The **haptenic agent** becomes antigenic by combining with intradermal proteins as carriers via NH_3 or S groupings. **Langerhans cells and endothelial cells** serve as APCs.

b. Subsequent reexposure to the agent results in chemokine and cytokine release, monocytic/macrophage infiltration, and a **vesiculating lesion** with **erythema and induration.**

C. Consequences of CMI

1. Although CMI is basically a defense mechanism against foreign substances, **cells** in the vicinity of antigen deposition, as well as those harboring microorganisms, are damaged if the inflammatory response induced is excessive.

2. The **magnified inflammatory response** induced by activated macrophages, cytotoxic T (Tc) lymphocytes, and NK cells causes this damage.

   a. Activated macrophages

   (1) Activation

   (a) Macrophages are activated **nonspecifically,** primarily by **IFN-γ** released by Th1 cells following antigenic stimulation.

   (b) Microbial products [e.g., bacterial lipopolysaccharides (endotoxins)] also are potent macrophage activators; these substances induce TNF-α and IFN-γ release.

   (2) Consequences. Activation results in **increased phagocytosis** and **microbicidal action.**

   (a) Microbial killing occurs mainly through **reactive oxygen species** (H_2O_2, O_2^- anion, and nitric oxide).

   (b) Activated macrophages generate many other microbicidal factors [e.g., IL-1, tissue factor, thrombin, platelet-derived growth factor (PDGF), TGF-β, TNF-α, and prostaglandins].

   b. Tc lymphocytes

   (1) Macrophage secretion of IL-12 acts synergistically with IL-2 to induce the differentiation of Th1 and NK cells into **Tc cells.**

   (2) Tc cells are mainly of the CD8+ phenotype. Binding to a class I MHC molecule on the target cell is facilitated by **multiple coreceptors (see Figure 6-3B).**

   (3) Unlike the IgM B cell antigenic receptor, the TCR is not secreted; immunity must be effected by **contact** with the target cells (e.g., virus- and bacteria-infected cells; foreign transplants; antigenic tumors; autoimmune susceptible, endogenous cells).

   (4) Following cell–cell contact, Tc lytic function emerges from **exocytosis of granzymes** (i.e., granules containing enzymes), **perforins, cytolysins, lymphotoxins,** and **serine esterases** (Figure 6-4).

   c. NK cells

   (1) Function. NK cells kill tumor cells and those infected by viruses, but they do not kill most normal cells. They are prominent in graft-versus-host reactions.

   (2) Morphology. NK cells are large granular lymphocytes that contain antagonists similar to Tc cells.

   (a) NK cells do not exhibit T-cell or B-cell phenotypes and lack CD3 and TCR markers.

   (b) NK cells do not require prior sensitization to exhibit cytolysis, but they can be activated by IL-2, IL-12, and IFN-γ.

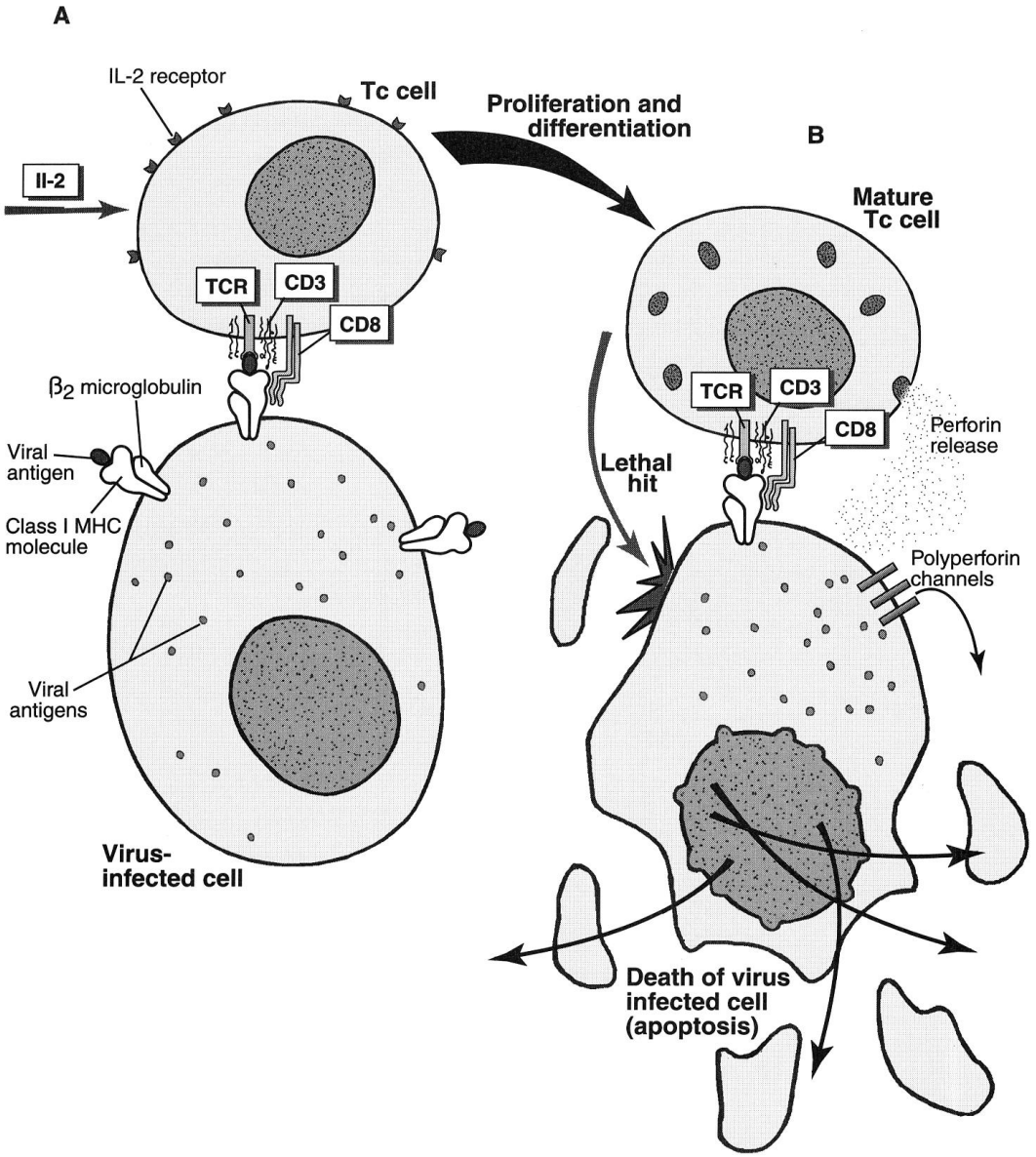
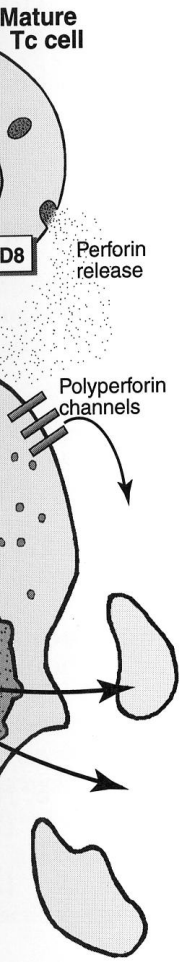


Figure 6-4. *(A)* Activation of a CD8+ cytotoxic T *(Tc)* cell leads to *(B)* killing of a virus-infected cell. The virus-infected cell synthesizes viral antigens, which, when processed, bind to major histocompatibility complex *(MHC)* class I. The viral peptide–MHC class I complex binds to the T cell antigenic receptor *(TCR)*, triggering the Tc cells to proliferate and differentiate into mature Tc cells. *(B)* The mature Tc cells release perforin, which binds to the virus-infected cell via calcium ions. The perforin causes channels to form in the membrane of the virus-infected cell, causing the cell contents to leak out. The resultant osmotic imbalance causes the death of the virus-infected cell. (Modified with permission from Benjamini E: *Immunology: A Short Course*, 3rd ed. New York, Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc., 1996, p 212.)



rus-infected cell. The
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cells release perforin,
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imbalance causes the
: A *Short Course*, 3rd

7

Inflammation

I. INTRODUCTION

A. Definition and cause

1. Inflammation occurs in response to injury resulting from **infection, foreign substances** or **other causes,** including antigen–antibody (Ag–Ab) complexes. Inflammation is necessary for **alleviating and repairing injury;** however, excessive inflammation can be **damaging to host tissues.**
2. Inflammation is characterized by the **controlled passage of cells and plasma** from the blood into the traumatized area.

B. Phases. There are two phases of inflammation:

1. **Acute**—mediated primarily by neutrophils
2. **Chronic**—mediated primarily by lymphocytes and macrophages

C. Clinical signs include:

1. **Redness,** caused by increased blood flow, dilation of arterioles, and vascular perfusion of the area
2. **Swelling,** caused by diapedesis of blood cells and plasma from the postcapillary venules into the damaged tissue
3. **Heat,** resulting from swelling and the release of endogenous pyrogens [e.g., interleukin-1 (IL-1), IL-6]
4. **Pain,** caused by the stimulation of neuronal pathways

II. INFLAMMATION PROCESS

A. Initiation

1. Inflammation is initiated by the injury-induced release of **pro-inflammatory mediators** (see IV B), including the cytokines IL-1 and tumor necrosis factor-α (TNF-α), as well as complement activated by the alternate pathway.
2. The release of these mediators induces **adhesion molecules** on leukocytes, endothelial cells, and epithelial cells.

B. Recruitment of inflammatory cells into the site by **chemokines** [mainly IL-8 and monocyte chemotactic protein (MCP); see IV A] follows.

1. Initially, **neutrophils** are recruited, followed by **monocytes, macrophages,** and, in immune-mediated inflammation, **lymphocytes.**

2. Binding of neutrophil **integrins** to **selectins** and **intracellular adhesion molecules (ICAM)** on the vascular endothelium precedes **diapedesis** into the injury site (Figure 7-1).

C. Cell destruction

1. Removal of the inciting condition or agent occurs via phagocytic cells that are activated by **IL-8, macrophage inflammatory protein (MIP),** and **interferon-γ (IFN-γ).**

2. The phagocytized, membrane-enclosed organisms are destroyed in the phagocytic vacuole by **lysosomal enzymes and hydrogen peroxide (H_2O_2), nitric oxide (NO), and O_2^- anion,** resulting from oxygen-dependent killing.

D. Repair of the damage caused by excessive inflammation requires two phases.

1. IL-4, IL-10, and **transforming growth factor-β (TGF-β)** must **downregulate** IL-8 (a chemokine) and cytokines IL-1 and TNF-α, which initially induced the inflammatory response.

2. Platelet-derived growth factor (PDGF), TGF-β, and other growth factors produce an **extracellular matrix** following increased proliferation and activation of fibroblasts.

III. KINETICS (see Figure 7-1). Diapedesis is initiated by the slowing and stoppage of the circulating neutrophil.

A. Initiating events

1. Injury-induced, pro-inflammatory molecules activate endothelial cells and trigger the appearance on their membrane of molecules called **selectins.**
a. Thrombin and **histamine** elicit **P-selectin.**
b. IL-1 and **TNF** elicit **E-selectin.**

2. Selectins bind loosely to **counterreceptor molecules (L-selectins),** which are present on circulating neutrophils.

B. Rolling adhesion. Binding of the selectin to the counterreceptor slows the neutrophil to a **"rolling adhesion."**

C. Stationary adhesion. Rolling adhesion triggers the substitution of integrins [called **leukocyte function–associated antigen-1 (LFA-1)** or CD11a/CD18] on the neutrophil surface. The integrins bind firmly to **intracellular and vascular adhesion molecules (ICAM, VCAM)** after the ICAM and VCAM have been elevated on the endothelium by IL-1, IL-4, and TNF.

D. Diapedesis occurs at intracellular junctions on the endothelium. Migration to the site of injury is facilitated by **chemotactic gradients,** and the cell destruction and later repair begin.

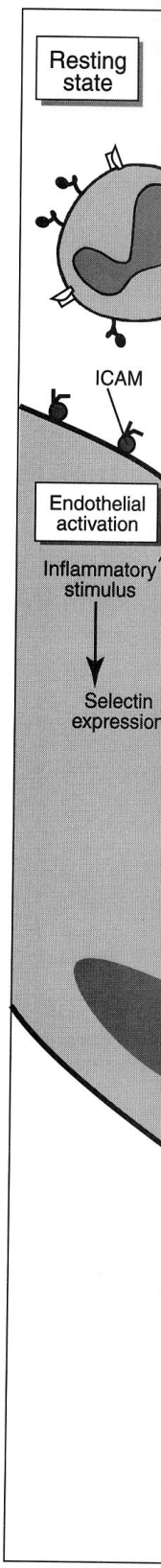

Figure 7-1. Following injury and the release of interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α), the resting state of the endothelium is changed by the appearance of selectins on the cell surface. These selectins bind to the counterreceptor on circulating neutrophils, slowing the polymorphonuclear neutrophils (PMNs) to a "rolling adhesion." The release of IL-8, macrophage inflammatory protein (MIP), and monocyte chemotactic protein (MCP) results in the activation of integrins on the neutrophil surface. The integrins bind tightly to intracellular adhesion molecules *(ICAMs)* on the endothelial cell surface. Diapedesis (transendothelial migration) follows, facilitated by platelet–endothelial cell adhesion molecules *(PECAM-1)*. Antagonism at any point can reduce the inflammatory process. (Modified with permission from Zimmerman GY, McIntyre TM, Prescott SM: Cell adhesion molecules. In *Manual of Vascular Mediators*. Edited by Ward PA. Kalamazoo, MI, The Upjohn Company, 1993.)

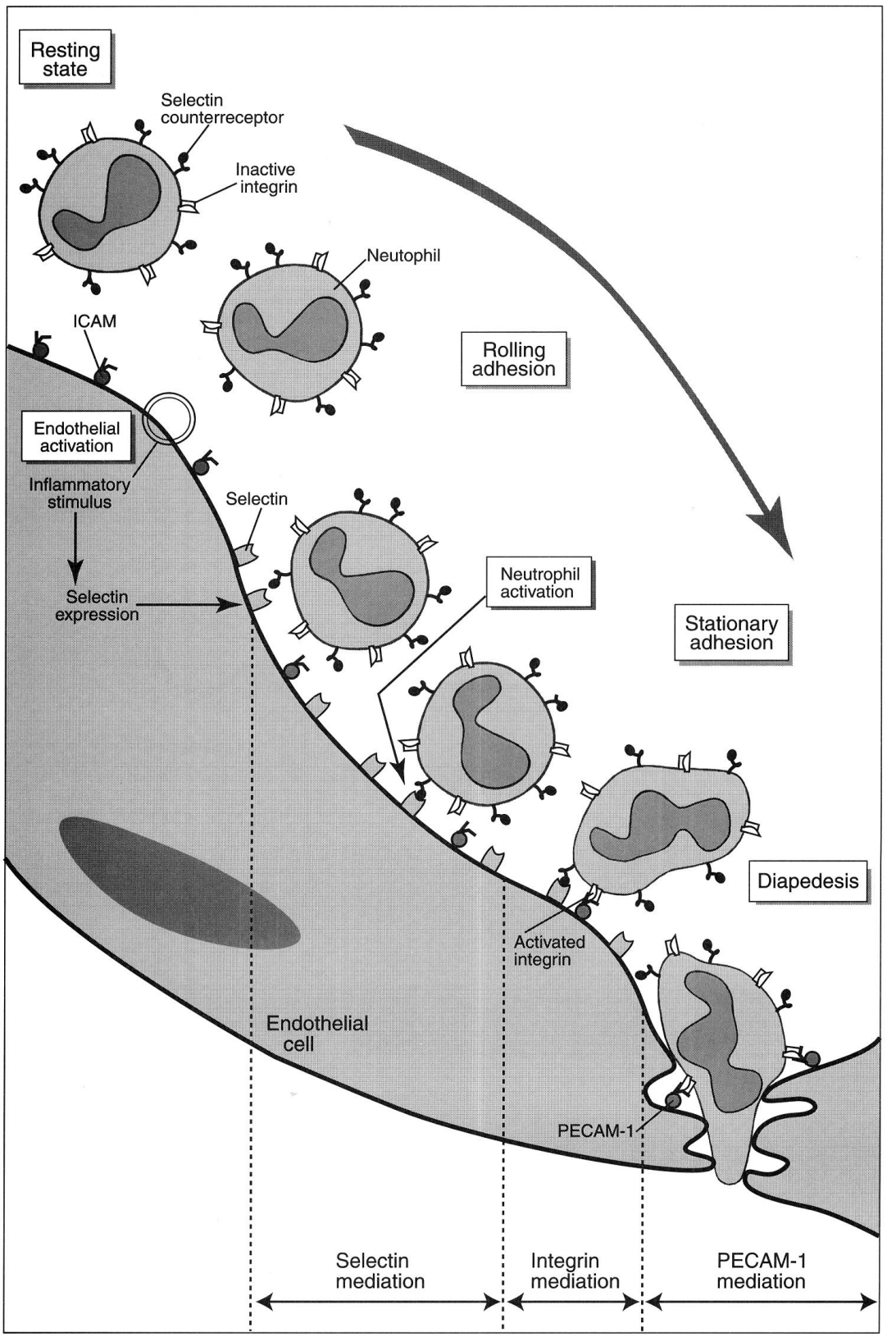
Resting state
Selectin counterreceptor
Inactive integrin
Neutophil
ICAM
Rolling adhesion
Endothelial activation
Inflammatory stimulus
Selectin
Selectin expression
Neutrophil activation
Stationary adhesion
Diapedesis
Activated integrin
Endothelial cell
PECAM-1
Selectin mediation
Integrin mediation
PECAM-1 mediation

Table 7-1.
Major Inflammatory Mediators

Cytokine or Chemokine	Cell Sources	Principal Activities
IL-1	Macrophages Fibroblasts Endothelial cells Others	Upregulates adhesion molecules Activates T cells Induces acute phase reactants Synergizes with TNF-α Endogenous pyrogen
IL-4	Th2 cells	Inhibits IL-8, IL-1, TNF-α Stimulates growth of B cells Induces IgE synthesis
IL-5	Th2 cells	Eosinophil chemotaxis and growth
IL-8	Monocytes Endothelial cells Fibroblasts	Neutrophil chemotaxis, adhesion, and angiogenesis
IL-10	Th2 cells	Inhibits IL-8, IL-1, TNF-α, and IFN-γ
TNF-α	Macrophages, Th1 cells	Activates macrophages, PMNs, and Tc cells Induces PMN–endothelial cell adhesion Causes sepsis, cachexia, pyrexia, acute phase proteins Tumor cell lysis
Monocyte chemotactic protein	Endothelial cells Fibroblasts Smooth muscle cells	Induces monocyte, T cell, and NK cell chemotaxis Activates macrophages
Macrophage inflammatory protein (MIP)	Macrophages	Activates neutrophil integrins and adhesion to ICAM
Adhesion molecules ICAM-1 ICAM-2 E-selectin (ELAM-1) VCAM-1 L-selectin (LECAM-1) P-selectin	 Endothelial cells Endothelial cells Endothelial cells Endothelial cells Neutrophils Platelets, endothelial cells	 Binds leukocyte integrins to vascular endothelium (same for all adhesion molecules)
LFA-1 (CD11/CD18 integrins)	Leukocytes	Binds neutrophils, monocytes, and lymphocytes to vascular endothelium via ICAMs
IFN-γ	Th1 cells NK cells	Induces class I and II MHC Stimulates differentiation of monocytes into macrophages Activates macrophages Inhibits Th2 cytokines
Interleukin receptor antagonist protein	Monocytes	Blocks binding of IL-1 to its receptor
Transforming growth factor-β (TGF-β)	Monocytes, T cells	Induces synthesis of extracellular matrix proteins Assists in wound healing Immunosuppressant
RANTES	Activated T cells	Monocyte chemotaxis
C′5a	Complement source	Neutrophil chemotaxis and activation Increases capillary permeability

ICAM = intracellular adhesion molecule; *IFN-γ* = interferon-γ; *IgE* = immunoglobulin E; *IL* = interleukin; *LFA-1* = leukocyte function–associated antigen-1; *MHC* = major histocompatibility complex; *NK* = natural killer; *PMN* = polymorphonuclear neutrophil; *RANTES* = Regulated on Activation, Normal T Expression and Secreted; *Tc* = cytotoxic T cells; *Th* = T helper cell; *TNF-α* = tumor necrosis factor-α; *VCAM* = vascular adhesion molecules

IV. MEDIATORS OF INFLAMMATION (Table 7-1)

A. Chemokines

1. **Definition.** Chemokines are small-molecular-weight peptides (8,000 daltons–16,000 daltons) that are released by injury. These peptides are active at very low concentrations (10^{8}–10^{11} molar) and exhibit approximately 30%–50% amino acid sequence homology.

2. **Function**
   - **a.** Chemokines activate and attract leukocytes to sites with tissue damage.
   - **b.** Chemokines transmit signals through seven transmembrane, rhodopsin-like receptors.

3. **Classification.** Chemokines are classified into two subcategories based on the sequence of two pairs of the amino acid **cysteine.**
   - **a. C-X-C chemokines (α)** have their first two cysteines separated by one amino acid.
     - **(1)** Most attract **neutrophils.**
     - **(2)** The most potent include IL-8, platelet factor 4, and IFN-γ-induced proteins, macrophage activation factors, and IFN-γ inducible protein-10.
   - **b. C-C chemokines** (β) have two adjacent cysteine residues.
     - **(1)** Most attract **monocytes and T lymphocytes,** while a few attract **eosinophils, basophils,** and **natural killer (NK) cells.**
     - **(2)** C-C chemokines include the **monocyte chemotactic proteins (MCP), MIP,** and **RANTES (Regulated on Activation, Normal T Expression and Secreted).**

B. Cytokines

1. **Definition.** Cytokines are **intracellular signaling proteins** acting in a **paracrine** or **autocrine** manner. They usually **act locally** by binding to high affinity receptors.

2. **Function.** Cytokines have frequently overlapping functions.
   - **a.** A single activity can be caused by multiple cytokines.
   - **b.** Multiple activities (pleiotropism) can be caused by a single cytokine.

3. **Classification**
   - **a. Lymphokines** are cytokines that are produced by lymphocytes.
   - **b. Monokines** are cytokines that are produced by monocytes or macrophages.
   - **c.** The cytokine receptors can have **circulating forms,** consisting of only the extracytoplasmic portion of the receptor that blocks the cytokine before it reaches its cellular target.

4. **Examples** of cytokines include:
   - **a. IL-1, IL-6,** and **TNF-α.** These cytokines:
     - **(1)** Induce the acute phase response
     - **(2)** Are endogenous pyrogens
     - **(3)** Induce MCP and IL-8
   - **b. TGF-β.** This cytokine:
     - **(1)** Acts as a potent wound-healing agent
     - **(2)** Acts as a potent immunosuppressive agent, inhibiting IL-2 effects and proliferation of many cell types
     - **(3)** Promotes the switching of B cells to immunoglobulin A (IgA) synthesis

8

Hypersensitivities

I. OVERVIEW. There are four types of hypersensitivity reactions (Table 8-1).

II. ANAPHYLAXIS (TYPE I) REACTIONS are termed **immediate hypersensitivity** because symptoms usually begin within minutes.

A. General characteristics

1. Classification. There are two categories of type I reactions:
- **a. Atopy** (local)
- **b. Anaphylactic shock** (systemic)

2. Definition. Anaphylaxis is a hypersensitive response by **genetically susceptible individuals** to extremely small amounts of antigen (i.e., **allergen)** to which they already have been sensitized.

3. Pathogenesis
- **a. Sensitization** occurs during an initial or repeated exposure to antigens by inhalation, ingestion, injection, or insect sting.
  - **(1) Excess IgE antibody** is produced and binds avidly via its Fc domain to its receptor **(FcεR)** on the surface of **mast cells** and **basophils.** Although a hereditary predisposition to sensitization exists, the IgE antibody does not cross the placenta.
  - **(2)** The **F(ab′)2** containing the antigen-binding sites remains free to bind the allergen.
- **b. Reaction**
  - **(1)** When antigen is **reintroduced** into the **sensitized host,** it binds to and aggregates several cell-bound IgE antibody molecules.
  - **(2)** The resulting membrane perturbation causes **degranulation of the mast cells** and release of pharmacologically active agents (e.g., histamine, leukotrienes, serotonin, bradykinin).
  - **(3)** These agents **rapidly contract smooth muscle, increase vascular permeability and secretions, change coagulability,** and **induce hypotension.**
- **c. Signs and symptoms.** The location of target cells (i.e., mast cells or basophils) determines the resulting signs and symptoms.
  - **(1) Mast cells** are abundant in the **skin, lungs,** and **mucosae** and do not circulate.
  - **(2) Basophils** occur in the **circulation** but **can be recruited into the tissues.**

4. Common allergens include:
- **a.** Penicillin (active metabolite is penicilloyl)
- **b.** Procaine
- **c.** Insect venom

Table 8-1.
Hypersensitivity Classifications

Type	Conditions	Distinguishing Characteristics
I: Anaphylaxis	Atopy Urticaria Asthma Allergic rhinitis Anaphylactic shock	IgE Fc adherence to mast cells and basophils Degranulation and histamine release Smooth muscle contraction
II: Cell surface Ag–Ab cytotoxicity	Hemolytic disease of the newborn Transfusion reactions	Exogenous cell antigens Complement-induced target cell lysis Phagocytosis ABO, Rh blood cell destruction
	Goodpasture's syndrome Glomerulonephritis	Endogenous cell antigens Autoimmunity Complement-mediated neutrophil influx and damage
III: Ag–Ab complex disease	Arthus reaction Serum sickness Polyarteritis nodosa Glomerulonephritis Systemic lupus erythematosus Rheumatoid arthritis	Ag–Ab complex disease Vasculitis Complement-mediated neutrophil influx and damage dsDNA–anti-dsDNA complexes Rheumatoid factor
IV: Delayed-type hypersensitivity	Tuberculosis Granulomatous reactions Contact dermatitis	Cell-mediated immunity Activated macrophages Epithelioid cells TDTH, Th1, CD8+ cells Haptens

Ag–Ab = antigen–antibody; CD = cluster of differentiation; dsDNA = double-stranded DNA; TDTH = delayed-type hypersensitivity effector cells; Th1 = T helper cell type 1.

d. Pollens
e. Molds
f. Foreign serum

B. Atopic disease

1. Urticaria (hives), a **cutaneous form** of immediate hypersensitivity, is characterized by vasodilatation and increased vascular permeability of the skin.
a. Incidence. Urticaria affects approximately 15%–20% of the United States population.
b. Pathogenesis. Histamine release is mainly responsible for the wheal and flare lesion and pruritus.

2. Asthma is characterized by **airway obstruction** resulting in acute respiratory distress.
a. Types
(1) Extrinsic asthma results from **chronic exposure** to occupational, environmental, and food allergens.
(2) Intrinsic asthma can be induced by nonimmunologic means.
b. Pathogenesis
(1) Inflammation results from the influx of **mast cells, CD4+ cells, Th2 cells, basophils,** and **eosinophils.**

(2) **Obstruction** is caused by **mucus secretion** and mediator-induced **constriction of the smooth muscle surrounding the bronchioles** following allergen–IgE union.

(a) **Important mediators** are the leukotrienes, platelet-activating factor (PAF), eosinophil chemotactic factor (ECF), and histamine.

(b) **Important triggers** are respiratory infections, environmental pollutants, aspirin, nonsteroidal anti-inflammatory drugs (NSAIDs), and isocyanate inhalants.

c. **Signs and symptoms** include wheezing, dyspnea, chest tightness, and cough.

d. **Treatment** includes:

(1) Avoidance of allergen

(2) Drugs that promote bronchial smooth muscle cell relaxation by elevating cyclic adenosine monophosphate (cAMP) [e.g., β-adrenergic bronchodilators, theophylline, aminophylline]

(3) Corticosteroids

(4) Cromolyn sodium

3. **Allergic rhinitis** is the most common clinical expression of atopy.

a. **Clinical features** involve inflammation of the mucous membranes of the nose, leading to profuse rhinorrhea, paroxysmal sneezing, nasal obstruction, itching, and conjunctivitis.

b. **Pathogenesis**

(1) **Common allergens** resulting in allergic rhinitis include pollens, fungal spores, house dust, and animal danders.

(2) **Mediators.** The binding of allergen to IgE releases mediators, including histamine, leukotrienes, prostaglandin D_2, and ECF, which attracts eosinophils.

c. **Treatment** is mainly symptomatic. **Desensitization** can be attempted in unresponsive patients.

(1) With certain allergens, a **hyposensitive state** can be achieved by repeated injection of the agent in subliminal doses.

(2) Parenteral exposure to repeated subliminal doses favors the synthesis of circulating **IgG** antibody, which **combines avidly with the allergen in the circulation,** thus **blocking** union with cell-associated IgE and mediator release.

C. **Anaphylactic shock** is a severe, generalized reaction that occurs when the allergen-induced mediators are released **systemically. Anaphylactoid shock** refers to anaphylactic shock induced by nonimmunologic means.

1. **Pathogenesis**

a. **Common triggers** are *Hymenoptera* venom, foods, drugs, and antibiotics (particularly penicillin).

b. **Major mediators** include tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), IL-6, PAF, leukotrienes, prostaglandins, and histamine.

c. **Death** results from hypotension and shock caused by generalized vasodilation of arterioles, increased vascular permeability, and upper airway edema, leading to organ failure.

2. **Signs and symptoms.** Multiple organs can be affected, leading to symptoms such as hypotension, dyspnea, vomiting, abdominal cramping, angioedema, and urticaria.

3. **Treatment** includes **epinephrine** (which acts as a potent vasopressor), **diphydramine, aminophylline,** and **corticosteroids.**

III. CELL
CYTO

A. Path
on

1.

2.

3.

B. Exa

1.

2.

RBC Genotype
OO
AA or AO
BB or BO
AB

III. CELL SURFACE ANTIGEN–ANTIBODY (Ag–Ab) CYTOTOXICITY (TYPE II) REACTIONS

A. Pathogenesis. Cytotoxicity occurs when antibody is directed against epitopes that occur on the **surface membrane** of host cells. **Damage** results from:

1. **The osmotic, lytic action of complement.** Complement is activated when the antibody binds to the membrane antigen.
2. **Opsonization by phagocytic cells or killing by antibody-dependent cell-mediated cytolysis (ADCC).** Linkage of the Fc receptor and/or the C′3b receptor on the cytotoxic cell to the Fc domain on the antibody bound to the target cell is necessary for ADCC.
3. **Killing of the target cell by cytotoxic T (Tc) lymphocytes, natural killer (NK) cells, or both.** The destruction of the target cell mainly results from the release of perforins and serine proteases (granzymes), which cause pore formation and osmotic lysis.

B. Examples of cytotoxic reactions

1. **Transfusion reactions** occur following the transfusion of blood containing red blood cell (RBC) antigens foreign to the recipient (Table 8-2). **ABO incompatibility** reactions are the most common. **Rh reactions** are the most severe.
   - **a. Pathogenesis. Preformed antibodies** in the recipient's blood cause the donor's RBCs to agglutinate, resulting in **complement-mediated RBC lysis** or rapid **phagocytosis.**
   - **b. Signs and symptoms**
     - **(1) Fever** is the most common reaction.
     - **(2) Chest pain, hypotension,** and **disseminated intravascular coagulation (DIC)** may occur in severe reactions.
2. **Hemolytic disease of the newborn (erythroblastosis fetalis)**
   - **a. Pathogenesis**
     - **(1)** The **major cause** of this disorder is the placental transfer of a non–saline agglutinating, maternal, anti-Rh IgG antibody (usually **anti-RhD**), which binds to RhD+ fetal erythrocytes.
     - **(2) Loss of fetal erythrocytes** occurs through complement-mediated lysis or rapid phagocytosis.
       - **(a)** Hemolysis results, causing **hemoglobinuria** and conversion to **indirect bilirubin.**
       - **(b)** The accumulation of indirect bilirubin can result in **respiratory and brain damage.**

Table 8-2.
Blood Grouping

ABO System				Rh Genotype	
RBC Genotype	**Phenotype**	**Serum Antibody**	**Terminal Epitope**	**Rh+**	**Rh–**
OO	O	Anti-A and Anti-B	Fucose, galactose	DCe	dce
AA or AO	A	Anti-B	N-Acetylgalactosamine	DcE	dCe
BB or BO	B	Anti-A	Galactose	DCE	dcE
AB	AB	Neither	. . .	Dce	dCE

b. Signs and symptoms include **hemoglobinuria** and **kernicterus (jaundice).**

c. Prevention. Sensitization of the mother can be prevented by injecting the Rh-negative mother with **anti-Rh antibody** (i.e., **RhoGAM)** within 1–2 days of delivery. This antibody **neutralizes the fetal Rh-positive antigens** entering the mother's circulation during the removal of the placenta, thereby preventing stimulation of the maternal immune system and injury to future Rh-positive newborns.

3. Autoimmune reactions occur in **genetically susceptible individuals** who produce antibodies against their own cellular membrane antigens by unknown mechanisms.

a. Goodpasture's syndrome is characterized by **glomerulonephritis** and **pulmonary hemorrhage.**

(1) The **antigen** is a **glycoprotein** dispersed uniformly on the **glomerular basement membrane (GBM).**

(2) Susceptible hosts produce an **IgG antibody,** which binds to the membrane antigen and activates complement, releasing the potent chemotactic factor C′5a.

(3) Neutrophils are attracted to the antibody–GBM complex, where they release lysosomal enzymes. These lysosomal enzymes cause **severe necrosis of the glomeruli** and a **loss of filtration capacity.**

b. Other autoimmune reactions. Autoantibodies against many other tissue antigens can occur in the genetically susceptible host and are discussed in Chapter 10.

IV. ANTIGEN–ANTIBODY (Ag–Ab) COMPLEX (TYPE III) REACTIONS

A. Pathogenesis. Circulating Ag–Ab complexes of small size, with antigen in slight excess escape phagocytosis and deposit in tissues or on the surface of blood vessels (Figure 8-1). These complexes can cause damage by:

1. Activating complement and **releasing the chemotactic factor C′5a, anaphylatoxins,** and **clotting factors**

2. Attracting neutrophils to the area of deposition, where they release lysosomal enzymes that attack the tissue

B. Examples of Ag–Ab complex reactions

1. Arthus reaction. This reaction is a rare, severe inflammatory response to gross, intravascular Ag–Ab precipitates of intermediate size.

a. The response occurs when **highly sensitized** humans or animals are injected with antigen.

b. Complement-activated chemotactic factors (C′3a, C′5a), polymorphonuclear neutrophil (PMN) infiltration, and **platelets** result in thrombi and hemorrhagic, necrotic lesions.

2. Serum sickness

a. Etiology

(1) Injection of foreign serum or its products results in a complement-dependent, systemic immune complex reaction.

(2) A milder allergic vasculitis can be elicited by **drugs** (e.g., sulfonamides, penicillin, cephalosporins, phenytoin, thiourea).

b. Signs and symptoms include fever, a pruritic rash, lymphadenopathy, and joint pain.

c. Incidence. This condition is rare because the use of serum is restricted to the treatment of a few diseases (e.g., hepatitis, tetanus) and immunosuppression.

3. Polyarteritis nodosa is characterized by continuous insult of arteriolar walls by the deposition of circulating Ag–Ab complexes, causing **thrombosis** and **obliteration of blood flow.** Frequently, **hepatitis B-antibody complexes** are involved.

Figure 8-1. Th
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(B) Normal serum c
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a.

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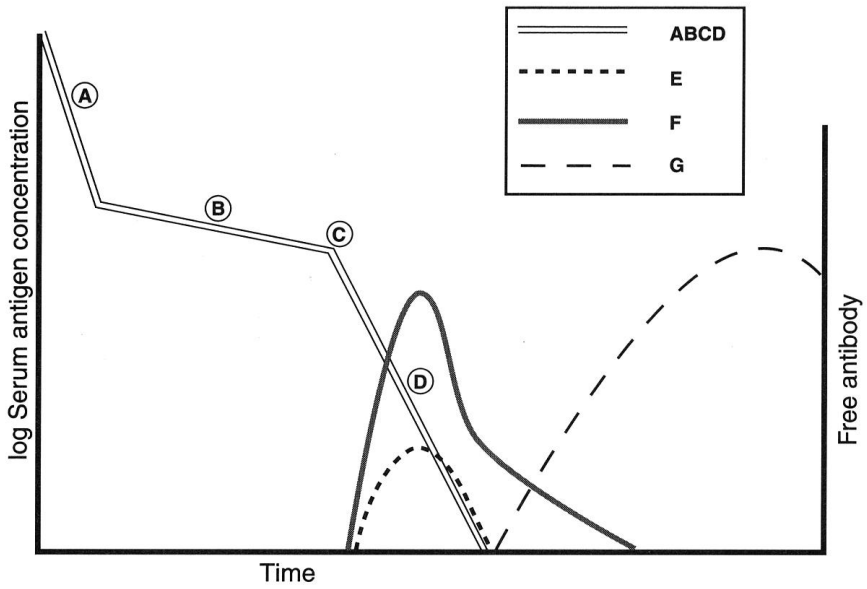


Figure 8-1. This graph illustrates the association of pathology (i.e., damage) with the formation of antigen–antibody (Ag–Ab) complexes in an antigen-excess environment. Ag–Ab complex reactions (type III hypersensitivity) result when Ag–Ab complexes, with antigen in slight excess, escape phagocytosis and deposit in the tissues or on the surface of blood vessels. *(A)* Equilibration of antigen between intra- and extravascular spaces. *(B)* Normal serum catabolic phase of antigen. *(C)* Antibody synthesis is initiated; antigen is in considerable excess with a molecular complex ratio of approximately 5:1 (Ag:Ab). *(D)* Rapid disappearance of circulating antigen owing to complex formation with antibody; as antibody synthesis increases, the molecular complex ratio reverses. Curves *E* and *F* describe the appearance of immune complexes (curve *E*) and the interval over which the complexes elicit pathology (curve *F*). Curve G describes the later appearance of free antibody in the circulation.

4. Glomerulonephritis
   - a. Pathogenesis
     - (1) **Soluble Ag–Ab complexes** (with antigen in excess) deposit on and behind the renal GBM, causing an **inflammatory response.** The release of enzymes by neutrophils attracted to the GBM results in destruction of the glomeruli and loss of filtration capacity.
     - (2) The **most commonly implicated antigens** are DNA, insulin, thyroglobulin, group A nephritogenic streptococci, and foreign serum.
   - b. **Diagnosis.** Complexes can be detected using **fluorescent antibody** against either the antigen, the antibody, or complement. A **lumpy-bumpy pattern** of fluorescence results from the random deposition of the complexes.

5. **Systemic lupus erythematosus (SLE)** is a chronic, exacerbating inflammatory disease that usually affects young women between the ages of 20 and 45 years. The cause of SLE is unknown, but the disorder may be initiated by an antibody response against bacterial or viral DNA, followed by loss of regulatory control of self tolerance.
   - a. **Pathogenesis.** SLE is characterized by the **formation of autoantibodies** to many endogenous antigens, such as RBCs, white blood cells (WBCs), platelets, double-stranded RNA (dsRNA), and nuclear antigens [e.g., antinuclear antibodies (ANA)], with **anti-dsDNA** predominating.

(1) **dsDNA–anti-dsDNA** and other complexes in slight antigen excess randomly lodge in the kidney, giving rise to the **cardinal lesion of glomerulonephritis.**

(2) **Inflammation** is mediated by a C′5a-induced influx of neutrophils, which results in lysosomal enzyme damage.

b. Signs and symptoms. Clinical manifestations of SLE include mainly **polyarthralgia** or **arthritis.** An **ultraviolet light–induced skin rash, facial "butterfly rash," pleurisy, pericarditis,** or **vasculitis** may also be present.

c. Diagnosis

(1) A **lumpy-bumpy pattern** of fluorescence distinguishes SLE glomerulonephritis from the Goodpasture type of glomerulonephritis, which is characterized by a smooth pattern.

(2) The **LE cell** (i.e., a neutrophil or macrophage with a phagocytized nucleus), although pathognomonic for SLE, is rarely sought because the technique is cumbersome.

(3) **Rheumatoid factor** may be present.

6. Rheumatoid arthritis is a chronic, recurrent inflammatory disease thought to be initiated by an unknown antigen that stimulates local antibody formation in the synovium. Approximately 70% of patients with rheumatoid arthritis possess the **HLA-DR4 haplotype.**

a. Pathogenesis

(1) The **union of antigen and antibody** alters the tertiary structure of the antibody, revealing previously buried amino acid sequences "foreign" to the immune system.

(2) These **newly available epitopes** stimulate the local production of an antibody (usually IgM), called **rheumatoid factor.** This antibody can react with the Fc domain of IgG molecules (i.e., an antibody against an antibody). Consequently, IgM–IgG complexes form in synovial fluid and activate complement and chemokines.

(a) **Neutrophils** are attracted to the site. While attempting to phagocytize the complexes, the neutrophils release lysosomal enzymes, destroying articular cartilage.

(b) **Delayed-type hypersensitivity effector (TDTH) cells** predominate and contribute to the damage. Also causing damage are **macrophages,** which release IL-1, IL-6, and TNF, and **osteoclasts,** which injure bone.

(3) **Inflammation** of the pannus and **loss of cartilage** characterize the joint lesions.

b. Diagnosis. Rheumatoid factor can be detected by **latex agglutination tests,** which involve the addition of IgG-coated latex particles to the patient's serum.

V. DELAYED-TYPE HYPERSENSITIVITY (TYPE IV) REACTIONS. The pathologic consequences of cell-mediated immunity (CMI) are referred to as delayed-type hypersensitivity (DTH) and are described in detail in Chapter 6. Tissue damage results from excessive activation of macrophages by cytokines from CD4+ cells, Th1 cells, and CD8+ cells.

9

Immunodeficiency Diseases

I. OVERVIEW

A. Disease course

1. **Developmental immunodeficiency disorders** manifest during the **prenatal period** or **early childhood.**

2. A **depressed immune response** is associated with the **aging process.**

B. **Clinical signs** include:

1. A **history of recurrent infections**

2. **Below-normal enzyme-linked immunosorbent assay (ELISA)** values for **IgG, IgM,** and/or **IgA** [normal levels, in mg%, are IgG = 800–1400; IgM = 60–200; IgA = 100–300]

3. **Abnormal T cell:B cell ratios, CD4:CD8 ratios,** or **both**

a. **T cells.** Because all T cells possess membrane-bound CD3, they can be counted by adding **fluorescent-labeled, monoclonal anti-CD3 antibody** to a blood or tissue sample.

b. **B cells** can be quantified by their reactivity with **fluorescent-labeled, monoclonal antibody** against membrane-bound IgM, CD19, or CD20.

c. **CD4 and CD8. Monoclonal antibodies** against CD4 and CD8 differentiate **T helper (Th)** and **suppressor** subtypes, respectively.

4. **Diminished *in vivo* humoral immunity, cell-mediated immunity (CMI),** or **both** against standard vaccines [e.g., diphtheria-pertussis-tetanus (DPT)]

II. DEVELOPMENTAL IMMUNODEFICIENCY DISORDERS

A. **Transient physiologic hypogammaglobulinemia** occurs in infants between the ages of approximately 3 and 6 months. Although infants are born with **adult levels of placentally transferred IgG,** a low level of IgG results from:

1. The disappearance of maternal antibody, which has a half-life of 22–28 days

2. The infant's low early rate of synthesis of secretable immunoglobulins

B. **Congenital agammaglobulinemia (Bruton's disease)** is a sex-linked (male) disorder that affects infants between the ages of 5 and 6 months. These patients have an apparently normal thymus and CMI.

1. **Clinical features** include:

a. Recurrent pyogenic infections

b. Digestive tract disorders

2. **Cause.** The defect that causes Bruton's disease may occur in the transition from pre-B to B cells and involves the loss of a tyrosine kinase gene. The pre-B cells are normal.

3. **Diagnosis** is made by noting the **absence of tonsils** (on physical examination), **germinal centers** (on lymph node biopsy), and **B cells** (on a peripheral blood smear). **Serum immunoglobulin levels** of less than 10% also suggest the disease.

4. **Treatment.** Passive transfer of **adult serum immunoglobulin** can be administered prophylactically to diminish infections.

C. Dysgammaglobulinemia. Patients of varying age present with a **selective immunoglobulin class deficiency** (i.e., one or more immunoglobulins, but not all).

1. **Diagnosis.** Most patients have decreased IgA levels, with 1 in 600–800 of these patients having IgA levels of less than 5 mg/dl.

2. **Immunologic features** include:
   - **a.** Loss of mucosal surface protection
   - **b.** Failure of IgA-bearing cells to differentiate into secreting plasma cells, although their numbers are normal
   - **c.** Increased susceptibility to autoimmune diseases

D. Congenital thymic aplasia (DiGeorge syndrome) is characterized by a **hypocalcemia, tetany,** and an **absence of T cells.**

1. **Cause.** DiGeorge syndrome is **not hereditary.** It is caused by an **unknown intrauterine injury** to the third and fourth pharyngeal pouches that occurs between the **fifth and sixth weeks of gestation.**

2. **Clinical features**
   - **a.** The **thymus** and **parathyroid glands** are **not developed.**
   - **b.** **Depressed CMI** permits infections caused by opportunistic organisms (e.g., *Candida*, *Pneumocystis*, viruses).
   - **c.** Patients have apparently **normal germinal centers, plasma cells,** and **serum immunoglobulin.**

3. **Treatment.** These patients usually die early.
   - **a.** **Vaccination** with live vaccines (e.g., measles) is contraindicated.
   - **b.** The **transplantation of fetal thymic tissue** is experimental and may be complicated by a graft-versus-host reaction.

E. Chronic mucocutaneous candidiasis is a highly **specific T cell disorder** that is characterized by an **absence of immunity to *Candida*.** Patients have apparently normal T cell absolute numbers and functions. Approximately 50% of patients with this disorder also have endocrine dysfunctions (e.g., hypothyroidism).

F. Wiskott-Aldrich syndrome is a sex-linked (male) disorder occurring mainly in children. The syndrome has three main features: **thrombocytopenia** (manifested by bleeding), **eczema,** and **recurrent infections.** An increased incidence of lymphoreticular malignancies or lymphomas may occur.

1. **Immunologic features** include:
   - **a.** Depressed CMI and a low serum IgM level, but normal IgG and IgA levels
   - **b.** Poor response to bacterial capsular polysaccharide antigens

2. **Cause.** The primary defect may be an absence of specific glycoprotein receptors on T cells and platelets.

3. **Treatment. Bone marrow transplantation** may be effective.

G. Severe combined immunodeficiency disease (SCID) is a rare disorder characterized by a **genetic defect in stem cells** that results in the **absence of the thymus gland** and **T and B cells.** Affected children are extremely susceptible to infections and have a very **short life span.**

1. **Immunologic features.** A deficiency in the enzyme **adenosine deaminase (ADA)** occurs in 50% of patients. This deficiency results in the accumulation of **toxic deoxyadenosine triphosphate (DATP),** which inhibits ribonucleotide reductase and prevents DNA synthesis. A mutation in the γ chain of the interleukin-2 (IL-2) receptor gene is found in other patients with SCID.
2. **Treatment. Gene therapy** with the ADA gene is experimental.

H. Chronic granulomatous disease (CGD) results from a genetic defect in the **nicotinamide adenine dinucleotide phosphate (NADPH) oxidase system** in neutrophils. Patients are **susceptible to infections** by age 2 years, especially by organisms of low virulence.

1. **Immunologic features. Neutrophil bactericidal activity** (i.e., respiratory burst) is **defective** because of **depressed NADPH oxidase, superoxide dismutase activity** and **decreased hydrogen peroxide levels.**
2. **Diagnosis** is based on failure of neutrophils and macrophages to reduce a **nitroblue tetrazolium dye.**
3. **Treatment** with **interferon-γ (IFN-γ)** has been successful.

III. ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS)

A. Cause. AIDS is caused by **human immunodeficiency virus (HIV),** an immunosuppressive RNA retrovirus that is a member of the Lentivirus (slow virus) family.

1. **Two variants** of HIV exist. Both variants closely resemble the simian immunodeficiency virus harbored by African green monkeys.
   a. **HIV-1,** the predominant variant, currently causes disease only in humans.
   b. **HIV-2,** found mainly in Africa, is more readily transmitted heterosexually than HIV-1.
2. **Genetic structure** (Figure 9-1)
   a. The HIV genome consists of **three major genes:**
      (1) ***env*** codes for the **envelope protein,** gp160, which is cleaved into gp120 and gp41.
      (2) ***gag*** codes for the **core proteins** p24, p17, p9, and p7.
      (3) ***pol*** codes for **enzymes** (i.e., **reverse transcriptase, integrase,** and a **protease).**
   b. **Other regulatory genes** include:
      (1) ***tat,*** which activates transcription of viral DNA
      (2) ***nef,*** which helps with virus replication
      (3) ***rev,*** which regulates mRNA activity

B. Pathogenesis. The **major target cell** is the **CD4+ Th cell,** which is eventually lysed by the virus. Several other cells (e.g., macrophages, astrocytes, dendritic cells) with much lower membrane levels of CD4 can be infected by low numbers of HIV. Because these cells are not readily lysed, they may serve as reservoirs of latent virus.

1. **Virus entry**
   a. HIV enters cells by binding both to the CD4 receptor and an obligate chemokine coreceptor (CCR5) via gp120.
   b. After the virus binds to the CD4+ Th cell, fusion and entry of the virus through the cell membrane is mediated by gp41.

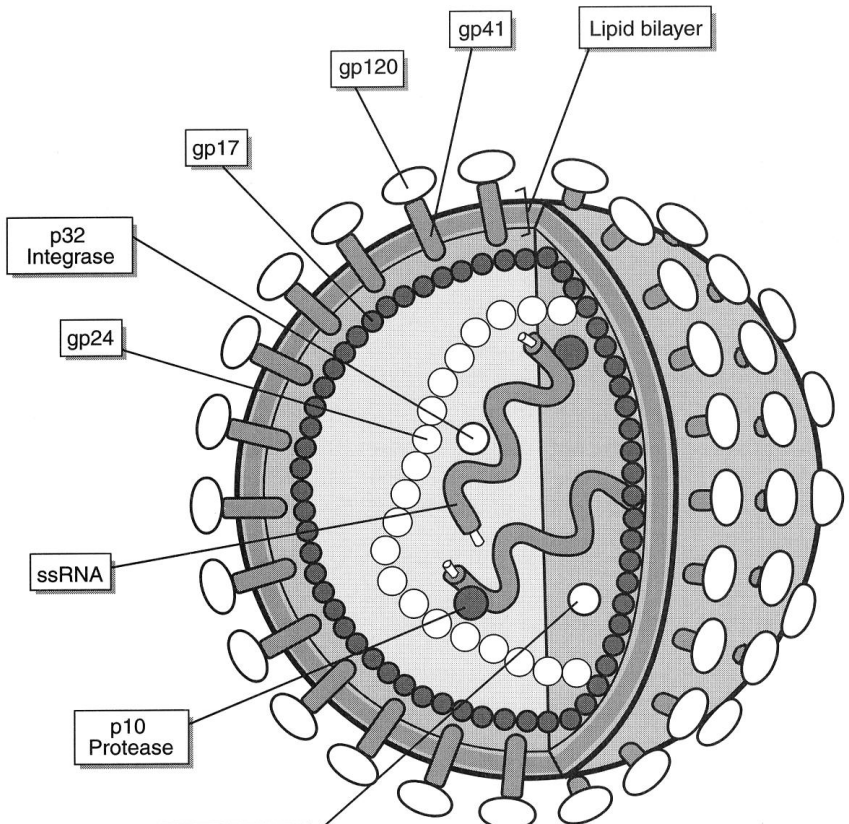


Figure 9-1. Components of the human immunodeficiency virus (HIV). The virus consists of an envelope formed from glycoproteins (i.e., gp120 and gp41) that houses several core proteins (e.g., p17, p24). The virus has several genes that code for enzymes (e.g., integrase, reverse transcriptase, protease) that play a role in integrating viral DNA into the host genome and degrading polyprotein precursors into smaller proteins and peptides.

2. **Transcription of the viral RNA into DNA** is accomplished enzymatically through a viral **reverse transcriptase.**
3. **Integration of viral DNA into the target cell** genome is facilitated by an **integrase,** leading to the formation of a **provirus** that may lie latent for years.
4. **Host cell lysis.** Following activation of the infected T cell (by other viruses or antigens), the provirus is transcribed, translated into viral proteins, assembled, and replicated, leading to lysis of the host cell.
5. **Infections and cancer**
   a. **Infections.** Depletion of the Th cell population results in a loss of cytokines (which activate other immunocompetent cells) and a diminished capacity to offset normally noninvasive, infectious agents. Infections in patients with AIDS are caused primarily by **endogenous** and **nosocomial agents.** Common organisms include ***Pneumocystis,*** **cytomegalovirus (CMV),** ***Toxoplasma, Candida, Mycobacterium,*** **herpesvirus,** and ***Cryptococcus.***

(1) At CD4 T cell levels of 200–400 cells/μl, ***Candida albicans, Mycobacterium avium-intracellulare*** and **varicella-zoster** infections dominate.
(2) At **CD4 T cell levels of less than 200 cells/μl, *Pneumocystis carinii,* CMV,** and ***Cryptococcus neoformans*** are common causes of infection.

b. Cancer
(1) HIV-positive patients are predisposed to **Kaposi's sarcoma** (mediated by herpes simplex virus-8).
(2) There is a markedly increased susceptibility to highly aggressive **B cell lymphomas,** notably **Burkitt's lymphoma** and **immunoblastic sarcoma,** among HIV-positive patients.

C. Diagnosis

1. Signs and symptoms. Patients with HIV can present with any number of **vague symptoms** (e.g., fever, weight loss), making clinical diagnosis difficult. One notable clinical sign is **increased frequency of infections by opportunistic organisms.** These infections often occur at relatively predictable CD4+ counts.

2. Laboratory tests
a. ELISA. Positive results on two separate tests are suggestive. Indirect ELISA tests detect serum antibody to HIV.
b. Western blot. This test confirms the ELISA tests.
c. Polymerase chain reaction (PCR). If necessary, small amounts of HIV DNA can be amplified using PCR.

D. Treatment is based primarily on interference with some stage of the HIV life cycle.

1. Reverse transcriptase inhibitors. Inhibition of reverse transcriptase by the dideoxynucleotides has slowed but not ceased the progression of the disease. Agents include:
a. Zidovudine (ZDV)
b. Azidodideoxythymidine (AZT)
c. Dideoxyinosine (ddI)
d. Dideoxycytidine (ddC)

2. Protease inhibitors have recently become available. These agents prevent the cleavage of protein precursors, which are essential for HIV maturation.
a. Agents. Saquinavir, ritonavir, indinavir, and **nelfinavir** reduce viral loads when used in various combinations.
b. Effectiveness. None of these protease inhibitors has completely eradicated viral reservoirs, and HIV resistance is beginning to develop.

IV. CYTOKINE AND CHEMOKINE DEFICIENCIES.

A functional deficiency in any of the 18 cytokines or 40 chemokines described to date, or their receptors, would contribute to an immunocompromised state.

A. Cytokines regulate cell–cell signaling.

B. Chemokines have chemoattractant properties.

C. Specific receptors on target cells control cytokine and chemokine action.

V. SENESCENCE (AGING) OF THE IMMUNE RESPONSE

A. T cells. Although T cell levels remain in the normal range, there is a loss in some T cell functions in the elderly. This loss results in **depressed humoral and cellular immune responses,** which are highly variable in the aged.

B. Autoimmune diseases are more prevalent, suggesting defects in immune regulation.

10

Autoimmune Disorders

I. OVERVIEW

A. Definition. Autoimmune disorders result in antibody or cell-mediated immunity (CMI) against the host's own tissues.

B. Immunologic mechanisms

1. Self-tolerance. Normally, humans are **immunologically unresponsive to endogenous molecules** because of self-tolerance. However, in normal humans, B-cell clones do exist, with receptors reacting with endogenous molecules (self-antigens).

2. Tolerance to self-antigens can be achieved by **clonal deletion, clonal anergy,** or **peripheral suppression.** A breakdown in any of these three mechanisms results in aberrant immunologic regulation and autoimmunity.

a. Clonal deletion. This hypothesis postulates a loss in self-reactive T and B cells, which appear during maturation in the fetal thymus and bone marrow.

(1) Immature CD4+ T cells, which bear receptors for endogenous molecules, are eliminated after contact with self-antigens in the **thymus gland.**

(2) B cells with self-reactive receptors are destroyed after contact with potential self-antigens in the **bone marrow.**

b. Clonal anergy describes the loss of T- and B-cell functions after exposure to antigens in the absence of mandatory costimulatory signals (e.g., B7–CD28 binding), or following exposure to cells lacking major histocompatibility complex (MHC) class II molecules.

c. Peripheral suppression can occur if **CD8+ T cells or macrophages secrete cytokines** [e.g., transforming growth factor-β (TGF-β)], which downregulate the immune response, or if **tolerogenic doses of antigen are administered.**

(1) High-dose tolerance is an anergic condition that occurs after systemic exposure to large amounts of unaggregated proteins.

(2) Low-dose tolerance occurs after repeated administration of small amounts of protein antigens.

(3) A **tolerant state** also can be induced by certain antigens when administered orally.

(4) Tolerance is specific, more readily induced, and lasts longer in T cells than in B cells.

II. IMMUNOREGULATION BREAKDOWN.

The theories of why immunoregulation breakdown results in autoimmunity vary with the disorder. These theories include:

A. Cross-reaction of microbial antigens with human tissue antigens

1. Although this reaction induces an immune response against the host, this is **not true autoimmunity** because the antigenic stimulus is of **exogenous origin.**

2. **Examples** of this theory include:
   a. **Streptococcal antigens,** which cross-react with sarcolemmal heart muscle and the kidney
   b. **Anti-DNA antibodies** (which may be induced by microbial DNA) that react with cells in patients with **systemic lupus erythematosus (SLE)**
   c. Deposition of **viral antigens** on host-cell membranes

B. Sequestration of potential self-antigens from fetal clonal deletion-inducing mechanisms. Antibodies to self-antigens in thyroid and heart tissue emerge after the tissue is **damaged by microbes or surgery.** These antibodies **rarely cause injury.**

C. Formation of new antigenic determinants. The alteration of host molecules exposes the host to new antigenic determinants that were **unavailable when fetal tolerance was induced.** For example, patients with rheumatoid arthritis exhibit **rheumatoid factors,** which are mainly IgM antibodies against the Fc fragment of IgG. The Fc is altered and becomes antigenic when IgG formed in the synovium complexes with its antigen.

D. Formation of a hapten-carrier complex. The adsorption of a foreign hapten (e.g., **quinidine, sulfathiazole**) onto an endogenous molecule (e.g., **platelets**) leads to the formation of a hapten-carrier complex. Antibody to the drug is formed and reacts with the drug on the platelet membrane. Complement is activated, resulting in **platelet lysis.**

E. Depletion of suppressor cells

1. If the normally occurring suppression (by T-suppressor cells) of B-cell clones that arise with idiotype specificity for self-antigens is lost or diminished (as in elderly patients), autoantibodies result.

2. An inordinate **switch from Th1 to Th2 cell activation** during antigenic stimulus may favor **autoantibody synthesis.**

III. SYSTEMIC AUTOIMMUNE DISORDERS

A. SLE is an episodic multisystemic disease that usually occurs in young women.

1. **Clinical signs.** An **erythematous rash, vasculitis,** and **arthritis** are the major lesions. In addition, **nephritis** may be seen.

2. **Immunologic features**
   a. SLE is characterized by **multiple autoreactive antibodies** against diverse cellular constituents.
      (1) **Antinuclear antibody (ANA).** The most dominant antibody is ANA, which is nonspecific and may be induced by microbial infection.
      (2) Antibodies to **double-stranded DNA (dsDNA)** are **specific for SLE.**
   b. **Immunologic mechanisms**
      (1) Small **antigen–antibody (Ag–Ab) complexes** with host antigen in excess continuously deposit on and behind the glomerular basement membrane (GBM).
      (2) The accompanying **complement fixation releases C′5a,** which attracts inflammatory cells into the area of complex deposition.
      (3) **Damage.** Subsequent release of leukocytic lysosomal enzymes **damages the GBM** and **impairs renal filtration.**

3. **Differential diagnosis**
   a. SLE can be confused with **rheumatoid arthritis** because 30% of SLE patients exhibit serum rheumatoid factor.
   b. **Certain drugs** (e.g., procainamide, hydralazine, quinidine, chlorpromazine) can induce a **lupus-like syndrome.**

B. **Rheumatoid arthritis** is a chronic, systemic inflammatory disease that is characterized by **granulation tissue (pannus) formation** and **subcutaneous nodules** in the joints.

1. A **genetic predisposition** (HLA-Dw4 and HLA-DR4) for this condition exists.
2. **Immunologic features.** Antibodies against immunoglobulins, called **rheumatoid factors,** appear in serum and synovial fluids. Rheumatoid factor formation may be an **immune response** by synovial lymphocytes **against unknown Ag–Ab complexes.**
   a. **Complement** is activated, and the resulting chemotactic factors attract inflammatory cells into the joints.
   b. **Damage.** These inflammatory cells damage tissues by releasing cytokines and pharmacologically active mediators.
3. **Diagnosis.** Rheumatoid factors can be detected by the agglutination of latex particles coated with altered IgG.

C. **Sjögren's syndrome** is a chronic inflammatory disease of **unknown etiology** that primarily affects **postmenopausal women.** This syndrome may occur **secondary to rheumatoid arthritis** and **SLE.**

1. **Clinical features** include **dryness of the mouth, trachea, bronchi, eyes, nose, vagina,** and **skin.**
2. **Immunologic features.** Sjögren's syndrome is characterized by autoantibodies against salivary duct antigens, lymphocytic infiltration, and immune complex formation in the salivary gland.

D. **Polyarteritis nodosa** is one of several **human vasculitides** of varying cause. The condition often involves hepatitis B Ag–Ab complexes, which are found in the vessel walls of 30%–40% of patients. Similar lesions can be reproduced in animals using other Ag–Ab complexes.

IV. ORGAN-SPECIFIC AUTOIMMUNE DISORDERS

A. Blood disorders

1. **Anemia, leukopenia, and thrombocytopenia.** Autoantibodies that react with **red blood cells (RBCs), white blood cells (WBCs),** and **platelets** result in anemia, leukopenia, and thrombocytopenia, respectively.
2. **Multiple myeloma,** the malignant transformation of a single plasma cell clone, results in an excess of IgG or another immunoglobulin class **(paraproteins).** Patients may secrete **Bence Jones proteins** (monoclonal light chains) in their urine.

B. Central nervous system (CNS) disorders

1. **Allergic encephalomyelitis** is a demyelinating disease that can occur after infection or immunization.
   a. The condition can be mimicked experimentally by immunizing animals with **homologous extracts of brain** or a **nonapeptide** from the basic protein of myelin.
   b. The experimental disease can be transferred to normal animals with **lymphocytes sensitized to the nonapeptide,** implicating CMI in the demyelination process.

2. **Multiple sclerosis** is a chronic, relapsing disease of unknown etiology that is characterized immunologically by mononuclear cell infiltrates and demyelinating lesions (plaques) in the white matter of the CNS.
   a. **Clinical features.** Patients usually have **increased levels of IgG** in the cerebrospinal fluid. Elevated titers to measles and other viruses appear in the cerebrospinal fluid, suggesting a viral etiology.
   b. **Immunologic features.** Patients with MS generally exhibit a **decrease in suppressor T-cell function,** which indicates an immunoregulatory disorder.

3. **Myasthenia gravis** results from a defect in neuromuscular transmission.
   a. **Clinical features** include **muscle weakness** and **fatigue.** Patients often exhibit **thymic hyperplasia** or a **thymoma.**
   b. **Immunologic features**
      (1) Myasthenia gravis is associated with the presence of an **anti-acetylcholine receptor antibody.** Binding of this antibody with the receptor at the postsynaptic membrane of the neuromuscular junction results in the loss **(endocytosis)** of the receptor.
      (2) An **inability to transmit the acetylcholine-induced signal** to muscle fibers causes the clinical signs.

C. Endocrine disorders

1. **Chronic thyroiditis (Hashimoto's disease, hypothyroidism)** is a self-limiting disease with a probable genetic basis that affects mainly women.
   a. Chronic thyroiditis is characterized by autoantibodies and CMI to thyroglobulin or thyroid peroxidase. This reactivity causes progressive **destruction of the thyroid gland.**
   b. **Antibody-dependent cell-mediated cytotoxicity (ADCC)** may be responsible for the tissue damage.

2. **Graves' disease (hyperthyroidism)** is characterized by T cell and B cell infiltration of the thyroid gland, leading to the formation of **autoantibodies** to the **thyroid-stimulating hormone (TSH) receptor.** The autoantibodies may compete with TSH, bind to the TSH receptor site, and induce uncontrolled TSH-like activity. Clinical features include a diffuse goiter and thyrotoxicosis.

3. **Diabetes mellitus (insulin-dependent diabetes, juvenile onset, type I diabetes)** is characterized by the destruction of insulin-producing cells in the pancreas. Either humoral or cell-mediated anti-islet cell activity can be operative. There is no evidence of an autoimmune pathogenesis for non–insulin-dependent (maturity onset, type II) diabetes.

D. Gastrointestinal tract disorders

1. **Pernicious anemia** is caused by impaired gastrointestinal absorption of vitamin B_{12}, resulting in weakness and chronic fatigue.
   a. **Immunologic features**
      (1) Pernicious anemia occurs secondary to **T cell damage** to the **gastric parietal cell.** The gastric parietal cell normally synthesizes **intrinsic factor,** the agent responsible for the transport of vitamin B_{12} into the blood.
      (2) **Anti-parietal cell** and **anti-intrinsic factor antibodies** are found in most patients. The latter block the transport function of intrinsic factor, and contribute to the disease process.
   b. **Treatment.** Injection of vitamin B_{12} bypasses the need for gastric absorption and corrects the deficiency.

2. **Ulcerative colitis** is characterized by chronic **inflammatory lesions** that are confined to the **rectum** and **colon.** These lesions are accompanied by the **infiltration of monocytes, lymphocytes,** and **plasma cells.**
   - **a.** Patients' lymphocytes exert cytotoxicity against colonic epithelial cells in **culture.**
   - **b.** Patients may also have antibodies that are cross-reactive with ***Escherichia coli,*** but the disease is of **unknown etiology.**
3. **Crohn's disease**is an inflammatory, granulomatous disease that involves **T and B cells, macrophages,** and **neutrophils.** The disease usually occurs in the **submucosal area** of the **terminal ileum.** This chronic progressive disease is often suspected but has not been established as being of **microbial etiology.**
4. **Chronic active hepatitis** is characterized by an **infiltration of the liver by T cells, B cells,** and **monocytes.** The condition may result from **faulty immunoregulation** because of decreased suppressor cell numbers.

11

Immunologic Aspects of Transplantation

I. HISTOCOMPATIBILITY. Rejection of grafts is antigenically specific and is determined primarily by allogeneic differences in the **histocompatibility antigens** [i.e., **human leukocyte antigens (HLAs)**]. The genes for the HLAs are located in the major histocompatibility complex (MHC) on chromosome 6.

A. HLA function. HLAs have two functions:

1. Their major **physiologic function** is to **bind and present processed, foreign antigenic peptides** to T cells, thus initiating the immune response (see Chapter 5).
2. They also **artificially distinguish the membrane antigens** on the transplanted donor organ from those of the recipient, provoking an attack by the recipient's sensitized T cells.

B. HLA classification. Class I and class II HLA genes, which encode antigens, exhibit enormous polymorphism, inasmuch as multiple different alleles exist at each locus.

1. **Class I antigens** are found on all **nucleated cells.**
   - **a. Structure.** Class I antigens have three gene loci: **HLA-A, HLA-B,** and **HLA-C.**
   - **b. Identification.** These antigens are defined serologically with anti-HLA antibodies.
   - **c. Function.** Class I antigens present foreign antigenic peptides to **CD8+ cells.**
2. **Class II antigens** are found on **immunologic effector cells** (e.g., macrophages, B cells, activated epithelial cells).
   - **a. Structure.** Class II antigens have three gene loci within the D region: **HLA-DP, HLA-DQ,** and **HLA-DR.**
   - **b. Identification.** These antigens are defined by cellular reactions.
   - **c. Function.** Class II antigens present foreign antigenic peptides to **CD4+ cells.**

C. Tests for histocompatibility. Matching the donor and recipient at the HLA locus improves graft acceptance (Table 11-1).

1. Both donor and recipient are typed for **HLA profiles** using DNA sequence analysis of the HLA genes or more than 200 specific anti-HLA antisera.
2. Both donor and recipient are **typed for ABO and Rh antigens** with specific antisera.
3. The donor must be tested for **preexisting anti-HLA antibodies** and **cell-mediated immunity (CMI)** because sensitization to HLA antigens can occur as a result of **prior blood transfusions, pregnancy,** or **other organ grafts.** One way to test for preexisting anti-HLA antibodies and CMI is with a **mixed lymphocyte culture.**
   - **a. Procedure**
     - **(1)** **Blood lymphocytes** from the donor and the recipient are cultured together, and **tritiated thymidine** is added.
     - **(2)** The donor cells are treated with an **antimitotic agent;** therefore, any response can be attributed to the recipient's reaction against the donor's cells.

Table 11-1.
Types of Grafts

Type	Condition	Fate
Autograft	Within the confines of one's own self	Accepted
Isograft	Between members of an inbred species	Accepted
Allograft	Between members of a species (humans)	Rejected
Xenograft	Between members of different species	Rejected
Homovital	Viable, functional graft required	. . .
Homeostatic	Nonviable graft, used for support	. . .

b. Results
- **(1)** If the lymphocytes are antigenically incompatible, DNA is synthesized, and the cells divide.
- **(2)** The extent of the genetic disparity can be determined by scintillation counting.

II. COMPLICATIONS OF ORGAN TRANSPLANTATION

A. Graft rejection. The vigor and speed of rejection is related to the genetic disparity between the donor and the recipient (i.e., the degree of foreignness). CD8+ cytotoxic T (Tc) cells and macrophages (activated by CD4+ T cells) mediate most rejection.

1. Acute rejection

a. Definition. Acute rejection, characterized by swelling and tenderness over the allograft, occurs within weeks. The exact time of onset varies with the host, the organ, and the immunosuppressive regimen.

b. Mechanism of rejection. HLA antigens on allografts stimulate recipient CD4+ T cells, which respond by secreting cytokines and by inducing adhesion molecules.
- **(1)** **Interleukin-2 (IL-2)** activates **CD8+ T cells** to a state of cytotoxicity.
  - **(a)** These Tc CD8+ cells bind to the graft antigens via specific receptors and release **effector molecules,** such as perforins.
  - **(b)** **Perforins** create pores in the target cell membrane and enable serine proteases (i.e., granzymes) to enter the cell and initiate cell death by **apoptosis.**
- **(2)** **Interferon-γ (IFN-γ)** activates monocytes/macrophages to express delayed-type hypersensitivity (DTH). This reaction results in increased lysosomal activity, phagocytosis, respiratory burst, and the release of tumor necrosis factor-α (TNF-α).
- **(3)** Activated **selectins, integrins, intercellular adhesion molecules (ICAM),** and **vascular cell adhesion molecules (VCAM)** promote **leukocyte extravasation** into the graft bed.

c. Acute renal allograft rejection results from injury to the renal vasculature by the Tc cells and their products, with resulting ischemia of the renal parenchyma.

d. Second-set phenomenon describes the rejection of a second allograft from the same donor as the initial allograft. The second allograft is rejected more quickly than the initial allograft (exhibiting the memory response).

2. Chronic rejection

a. Definition. Chronic rejection is characterized by episodic bouts of rejection, occurring months to years after transplantation.

b. Mechanism of rejection. Both cellular and humoral mechanisms are functional, eventually resulting in interstitial fibrosis, vascular occlusion, and loss of function.

3. **Hyperacute rejection**
   a. **Definition.** Hyperacute rejection occurs when a graft never takes because of preexisting sensitivity (white graft). **Rejection occurs within minutes.**
   b. **Mechanism of rejection.** The presence of **alloantibodies** against the donor's HLA or ABO antigens at the time of transplantation does not permit the graft to take. These antibodies react with the vascular endothelium of the graft and promote clotting, causing rapid death of the organ.
   c. **Prevention. Cross-matching** donor and recipient cells and serum identifies this potential problem.

B. Graft-versus-host reaction

1. **Definition.** When **immunocompetent tissues** (e.g., bone marrow, thymus, spleen, organs harboring passenger leukocytes) are allografted, they may recognize the recipient (i.e., host) as foreign, resulting in CMI damage to the recipient. If the recipient is immunoincompetent, a host-versus-graft reaction does not take place.
2. **Signs and symptoms.** The reaction is characterized by a skin rash, diarrhea, and jaundice.
3. **Treatment.** Immunosuppressive therapy has proven useful in reducing lethality.

III. IMMUNOSUPPRESSION is used to prolong graft acceptance; however, it predisposes the individual to infection.

A. Prevention of infection. Appropriate **killed vaccines** should be administered before transplantation. **Major organisms that cause infection** include the following:

1. **Cytomegalovirus (CMV),** present in more than 50% of donors
2. ***Candida,*** present in more than 90% of donors
3. **Epstein-Barr virus,** present in more than 90% of donors
4. ***Aspergillus***
5. **Respiratory syncytial virus**

B. Immunosuppressive agents

1. **Cyclosporine A** is a metabolite of the fungus *Tolypocladium inflatum Gams*.
   a. **Mechanism of action.** Cyclosporine A **inhibits resting T cell activation** by blocking IL-2 mRNA synthesis. The drug **binds to** a **cyclophilin,** and then the cyclosporine–cyclophilin complex binds to **phosphatase calcineurin,** interfering with the transmission of intracellular signals necessary for IL-2 formation.
   b. Cyclosporine A has little effect on already activated cells; thus, **Rapamycin,** a macrolide isolated from *Streptomyces hygroscopicus* that binds to cyclophilins and inhibits the G1 phase of activated T cells, may be added.
2. **Tacrolimus (FK 506)** is a macrolide compound derived from *Streptomyces tsukabaensis* that also inhibits resting Th cell activation by blocking IL-2 synthesis. Tacrolimus binds to a different cyclophilin, FK-binding protein, and then the tacrolimus–cyclophilin complex inhibits calcineurin and IL-2 formation.
3. **Mycophenolate mofetil** inhibits inosine monophosphate dehydrogenase, an enzyme that converts inosine monophosphate to guanosine monophosphate. In this way, mycophenolate mofetil **inhibits T and B cells,** because guanosine monophosphate is required for nucleic acid synthesis in these cells.

4. Other drugs

a. **Azathioprine,** a derivative of 6-mercaptopurine, is used early following transplantation. Azathioprine inhibits the synthesis of inosinic acid, thus blocking DNA synthesis in actively replicating cells.

b. **Corticosteroids** are anti-inflammatory agents that synergize with cyclosporine A.

(1) Corticosteroids dissociate nuclear factor kB (NF-kB) from its inhibitor (I-kB), thereby inhibiting transcription of cytokine genes.

(2) Corticosteroids also suppress IL-2 synthesis indirectly by blocking macrophage release of IL-1.

c. **Antilymphocyte globulin (ALG)** is an antibody that causes lysis of recipient T lymphocytes reactive against the graft. ALG is most effective during acute rejection episodes.

12

Cancer Immunology

I. DEFINITION. A **cancer** is a **malignant tumor growth** that expands locally by invasion and systemically by metastasis.

II. ONCOGENES. Cancers arise from cells in which growth-regulating and repair genes **(protooncogenes)** have become ineffective as a result of random mutation or following viral infection or physical or chemical damage. When protooncogenes become altered or damaged, they are termed **oncogenes** and are capable of causing neoplastic growth. Examples of oncogenes and their actions include the following.

A. p53 gene. As a protooncogene, p53 encodes a nuclear phosphoprotein that inhibits cell division, thus suppressing tumor growth. Mutations in p53 result in uncontrolled growth.

B. *ras*. As a protooncogene, *ras* controls a guanosine triphosphate (GTP) binding–protein involved in signal transduction. Mutation results in failure of guanosine triphosphatase (GTPase) inactivation of *ras* and continuous *ras* activity.

C. *c-myc*. When this protooncogene is translocated onto a different chromosome (e.g., as in Burkitt's lymphoma), it becomes oncogenic, resulting in loss of regulation of B cell growth and a B cell lymphoma.

D. Bcl-2 gene. In normal concentrations, the cellular protein produced by this gene (Bcl-2) inhibits apoptosis; high concentrations of Bcl-2 in B cells promote cell expansion and follicular lymphoma.

E. *bcr/abl* gene fusion results in a protein with increased tyrosine kinase activity, and is involved in chronic myeloid leukemia.

III. CANCER AND THE IMMUNE SYSTEM

A. Cancer antigens. Cancer cells arise from normal cells. In order for the immune system to attack cancer cells, the **cancer cells** need to be distinguished from self (i.e., they **need to possess antigens).**

1. Types. Two types of antigenic molecules have been found on cancer cells.

a. Tumor-specific antigens (TSA) are unique to cancer cells. They are induced by viruses (e.g., papovaviruses, herpesviruses, adenoviruses) or chemical or physical carcinogens.

(1) Virus-induced TSA are **cross-reactive** (i.e., the genome of a particular virus synthesizes the same viral antigens in whatever cell that virus infects). Consequently, immunotherapy should be applicable to all individuals infected by the same virus.

(2) **Carcinogen-induced TSA.** Carcinogens induce **random mutations** in the genome of affected cells. Consequently, each mutated gene product (antigen) differs (depending on which gene has been affected by the carcinogen) and immunologic cross-protection is not feasible.

b. Tumor-associated antigens (TAA) are not found exclusively on cancer cells; however, they are generally present in higher quantities in cancer patients, and aid in diagnosis.

(1) **Carcinoembryonic antigen (CEA)** reappears in the serum of most patients with colorectal cancer. (High concentrations of CEA on fetal gastrointestinal and liver cells disappear at birth.)

(2) **β-Fetoprotein (AFP)** attains high levels in patients with hepatomas and testicular teratocarcinomas. Levels are normally very low in adults (although high AFP levels are normal in fetal and maternal serum).

2. Immune response to cancer antigens

a. Antigen-responsive T cells. Immunocompromised hosts with diminished T cell function have a higher incidence of lymphoproliferative cancers.

(1) **CD4+ T cells** secrete cytokines [e.g., interleukin-2 (IL-2), interferon-γ (IFN-γ)] that activate **CD8+ cytotoxic T cells (Tc)** and **macrophages.**

(2) **CD8+ T cells** lyse cancer cells through cytotoxic factors and perforins.

b. Macrophages are found frequently in the bed of regressing tumors. They must be activated by macrophage-activating factors (MAF), such as IFN-γ, in order to eradicate tumor cells. Mechanisms of destruction may include the respiratory burst, nitric oxide release, neutral proteinases, tumor necrosis factor-α (TNF-α), and antibody-dependent cell-mediated cytotoxicity (ADCC).

c. Natural killer (NK) cells kill cancer cells through ADCC and lysis following contact. NK cell cytolytic activity is increased by IL-2, IL-12, and IFN-γ and is not major histocompatibility complex (MHC)-restricted.

3. Cancer cell evasion of the immune system. Cancer cells can evade the immune system in multiple ways. Examples include the following.

a. The relatively weak immune response may be overwhelmed by rapid tumor growth.

b. Certain cancers may possess subliminal numbers of human leukocyte antigen (HLA) or costimultory signal molecules, rendering them unable to trigger a TSA T cell response.

c. Non–complement fixing antibodies that arise as a result of the cancer may actually enhance cancer growth by blocking the TSA from attack by cell-mediated immunity (CMI).

4. Certain cancers may elicit a dominant T cell suppressor (Ts) response or secrete immunosuppressive molecules [e.g., prostaglandins, transforming growth factor-β (TGF-β)].

5. Antigenic modulation can occur, causing the cancer cells to change or lose their TSA.

6. The cancer may arise in an immunologically privileged site [e.g., eye, central nervous system (CNS)].

IV. CANCER IMMUNOTHERAPY

A. Lymphokine-activated killer (LAK) cells are tumor-reactive lymphocytes that are isolated from the blood of the cancer patient, expanded *in vitro* with IL-2, and reinfused into the patient. Although heterogenous, they are thought to constitute a population with some specificity. Their lineage is unknown.

B. Tumor-infiltrating lymphocytes (TIL) are T cells isolated from the tumor bed (therefore, they should have higher specificity for the tumor antigens). TIL cells are expanded *in vitro* with IL-2 and reinfused into the patient.

C. Monoclonal antibodies specific for TSA can be infused into the patient, either directly or with a toxin, drug, or radioisotope conjugated to the antibody. The antibody directs the conjugate exclusively to the cancer cell.

D. Cytokine therapy—using various cytokines capable of elevating humoral immunity, CMI, or both—is the goal of current clinical trials. The multiple variables (e.g., dose, concentration, bolus or multiple injections, route, patient population, cytokine specificity, toxicity) have impeded research progress.

13

Immunization

I. OVERVIEW

A. Benefits

1. Immunization is the most cost-effective weapon available against infectious diseases.
2. Immunization has permitted eradication of smallpox around the world and eradication of polio from the Western hemisphere. These successes are largely attributable to the Childhood Immunization Initiative passed by Congress in 1977.

B. Types of vaccines

1. **Live attenuated vaccines** permit replication of the organism (mainly viruses) in the host, increasing antigenic stimulation.
   a. Attenuation occurs mainly by passages in cell culture, growth in embryonic tissue, or at low temperatures, or selective deletion of genes involved in pathogenesis.
   b. A single inoculation frequently stimulates life-long immunity.
2. **Killed vaccines** contain organisms that have been inactivated by chemical or physical means. Multiple doses must be given, and adjuvants might be required for a protective response.
3. **Recombinant vaccines** (e.g., hepatitis B vaccine). Formulation requires identification of an epitope involved in the organism's pathogenicity. Synthesis of the vaccine antigen follows isolation and expression of the gene coding for the epitope in an appropriate host cell.
4. **Plasmid DNA vaccines,** based on the isolation of microbial DNA containing the genes coding for an antigen involved in pathogenicity, are under development. Results to date indicate that DNA vaccines elicit both potent humoral immunity and cell-mediated immunity (CMI) to multiple viruses and bacteria in animals; human clinical trials will follow.
   a. **Advantages.** The potential advantages of DNA vaccines include stability, low cost, ease of production, and long-lasting protection.
   b. **Technique**
      (1) The DNA (gene) is inserted into an expression plasmid and transfected into bacteria, where the plasmid is replicated in large amounts. DNA from multiple pathogens can be inserted into a single large plasmid.
      (2) The isolated DNA can be either injected in saline solution or adsorbed to microscopic gold beads and fired into muscle cells with a "gene" gun.
      (3) The DNA is translated in the cells and the **antigen** of concern is released *in vivo*, stimulating humoral immunity and CMI over an extended period of time.

C. Requirements for an effective vaccine

1. **Protective effect.** The vaccine must induce a humoral or CMI response directed against an antigen involved in pathogenesis.
2. **Safety.** Potential safety problems must be recognized.
   - **a. Live attenuated vaccines.** Potential safety problems include:
     - **(1)** Insufficient attenuation
     - **(2)** Reversion to wild type
     - **(3)** Contamination by live organisms or toxins
     - **(4)** Unsuspected immunodeficient patients
   - **b. Killed vaccines.** Potential safety problems include:
     - **(1)** Contamination by live organisms or toxins
     - **(2)** Autoimmune or allergic reactions
     - **(3)** Incomplete killing
   - **c. Recombinant vaccines** are not associated with any safety concerns.
   - **d. Plasmid DNA vaccines.** Continuous antigenic stimulus may lead to tolerance or autoimmunity.
3. **Stability.** Vaccines are stable for 1 year when maintained at a temperature of 4°C. They can deteriorate in 2–3 days at a temperature of 37°C.

II. SPECIFIC VACCINES. Current vaccine recommendations are given in Table 13-1.

A. Hepatitis B vaccine. Hepatitis B virus, a hepadnavirus, is a major cause of hepatitis and cirrhosis and is a known human carcinogen.

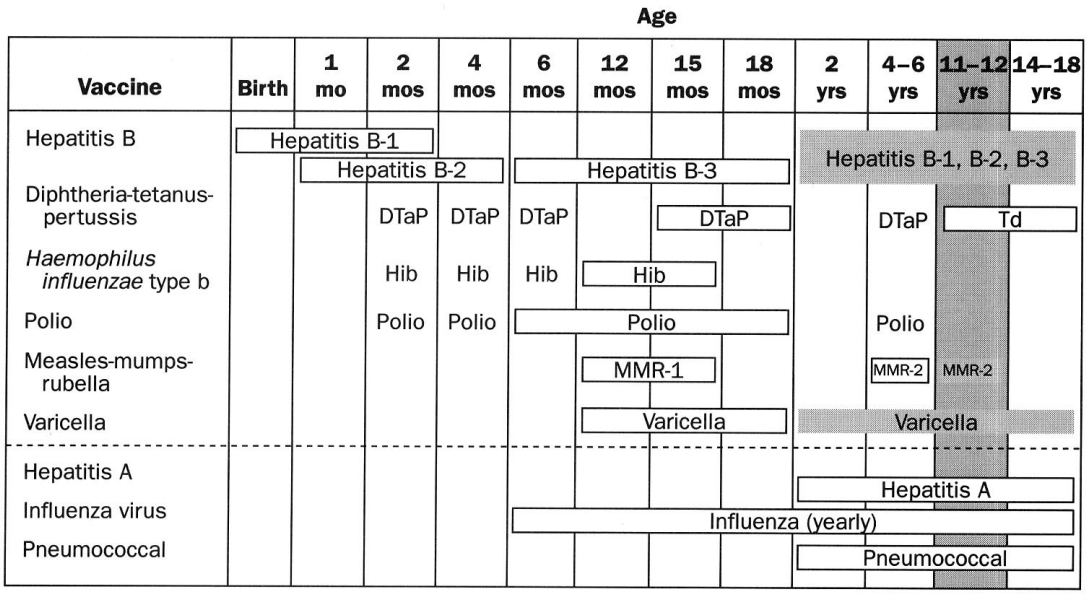
1. **Vaccine production.** The vaccine antigen, **hepatitis B surface antigen (HBsAg)** appears on the surface membrane of the virus and in the blood of infected individuals. The noninfectious vaccine is produced by recombinant DNA technology.
   - **a.** HBsAg is synthesized in *Saccharomyces cerevisiae* after the yeast is transfected with a plasmid containing the gene for HBsAg.
   - **b.** The isolated and purified HBsAg protein is adsorbed onto aluminum hydroxide gel.
2. **Vaccine administration.** The hepatitis B vaccine is administered as a one-time vaccine during routine childhood vaccination.
3. **Postexposure prophylaxis. Hepatitis B immune globulin (HBIg)** is recommended for postexposure prophylaxis. Candidates include personnel in blood banks or transfusion units, pregnant women positive for circulating HBsAg, and newborns born to HBsAg-positive mothers.

B. Diphtheria and tetanus toxoids and acellular pertussis (DTaP) vaccine

1. **Vaccine production**
   - **a. Diphtheria** and **tetanus** toxins are administered in the **toxoid form.**
   - **b.** Pertussis is administered in the **acellular form,** which is associated with fewer adverse reactions than the whole-cell form.
2. **Vaccine administration.** The DTaP vaccine can be given at the same time as the trivalent oral polio vaccine (TOPV) and the measles-mumps-rubella (MMR) vaccine, but must be administered at a different site.

C. *Haemophilus influenzae* type b (Hib) vaccine. *H. influenzae* type b was the major cause of bacterial meningitis in the United States prior to licensure of the Hib conjugate vaccines.

Table 13-1.
Recommended Childhood Immunization Schedule, Minnesota, 1998



A routine early adolescent immunization visit is now recommended at age 11–12 years. *Bars* indicate the range of acceptable ages for vaccination. *Shaded bars* indicate catch-up vaccination. Vaccines below the *dotted line* are appropriate for selected populations. (Adapted from the *Minnesota Department of Health Disease Control Newsletter* 26(4):30, 1998.)
DTaP = diphtheria and tetanus toxoids and acellular pertussis; *Hib* = *Haemophilus influenzae* (type b);
MMR = measles-mumps-rubella; *Td* = tetanus and diphtheria (toxoids, absorbed, for adult use).

1. Vaccine production. The Hib vaccine incorporates the polysaccharide antigen, a polymer of polyribosyl ribitolphosphate (PRP) found in the type b capsule (the major virulence component in 90% of the invasive strains). PRP, a thymus-independent antigen, can be conjugated to protein carriers [e.g., diphtheria toxoid, *Neisseria meningitidis* outer membrane protein (OMP), tetanus toxoid], rendering it highly protective in children older than 2 months.

2. Vaccine administration. PRP-OMP and PRP-tetanus toxoid are given in 3–4 doses, beginning at the age of 2 months; PRP-diphtheria toxoid is recommended only for children older than 1 year.

D. Poliomyelitis vaccines. Poliomyelitis is caused by three serotypes of poliovirus; type 1 causes paralytic disease most often.

1. Available vaccines. Two vaccines are available.

a. Trivalent oral polio vaccine (TOPV) contains **live attenuated** strains of all three serotypes of poliovirus, which are propagated in monkey kidney cell cultures.

(1) TOPV is administered **orally.**

(2) TOPV induces **local** and **systemic immunity**—local immunity is accomplished by proliferation of the virus in the gastrointestinal tract, and systemic immunity is accomplished by spread of the attenuated virus to the circulation.

b. Inactivated polio vaccine (IPV) contains **killed virus.**

(1) IPV is administered **subcutaneously.**

(2) IPV induces a **systemic** immune response.

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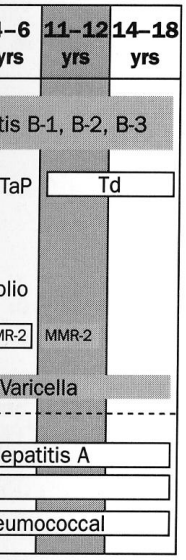

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2. **Vaccination schedule**
   a. The recommended schedule of two doses of IPV followed by two doses of TOPV decreases the risk of vaccine-associated poliomyelitis. The initial antibody response induced by the IPV protects the patient against the remote possibility that vaccine-associated disease might be caused by the subsequent TOPV.
   b. TOPV-only and IPV-only regimens are also acceptable for human use.

E. Measles-mumps-rubella (MMR) vaccine. This vaccine has decreased the incidence of measles, mumps, and rubella by 99%. In addition, the MMR vaccine has decreased the incidence of major birth defects (associated with rubella), encephalitis (associated with measles), and aseptic meningitis and parotitis (associated with mumps).

1. **Vaccine production**
   a. **Measles** and **mumps,** paramyxoviruses, are grown in chick embryo cell cultures.
   b. **Rubella,** a togavirus, is grown in human diploid fibroblast cell cultures.
2. **Vaccine administration**
   a. Two doses are required prior to school enrollment.
   b. MMR, a live vaccine, should not be administered to pregnant women.
   c. The vaccine is heat- and light-sensitive.

F. Varicella vaccine. Varicella-zoster virus causes chickenpox.

1. **Vaccine production.** The vaccine is formulated from a **live attenuated virus.**
2. **Vaccine administration.** Vaccination is recommended for children between the ages of 1 and 12 years and for adults who have not contracted chickenpox.

G. Hepatitis A vaccine

1. **Vaccine production.** Hepatitis A, a picornavirus (enterovirus), is grown in cultures of human fibroblasts. The purified virus is inactivated by formalin and adsorbed onto an aluminum hydroxide gel to make the vaccine.
2. **Vaccine indications.** People in close contact with patients with hepatitis A, people with high occupational risk, homosexuals, and travelers should be vaccinated.
3. **Postexposure prophylaxis** with **anti-hepatitis A immunoglobulin** is warranted.

H. Influenza virus vaccine

1. **Classification of influenza virus.** The classification of influenza virus into types A, B, and C is based on differences in the nucleoproteins.
2. **Vaccine production**
   a. Influenza type A virus has two important surface antigens, a **hemagglutinin (H)** and a **neuraminidase (N);** variations in each determine the subtypes of the type A influenza virus.
      (1) Three subtypes of hemagglutinins are recognized (H1, H2, H3). **Antibody to the hemagglutinins reduces the likelihood of infection and lessens the severity of disease.**
      (2) Two subtypes of neuraminidase (N1 and N2) exist.
   b. The vaccine is an **attenuated virus vaccine** that **contains three virus strains,** usually **two type A strains** and **one type B strain.** (Type C is not an important human pathogen.)
      (1) The strains chosen each year represent the influenza viruses that are most likely to circulate in the United States during the upcoming influenza season.
      (2) **Antigenic drift** results from mutations in the RNA segment coding for either of the major membrane antigens. Type A strains exhibit much greater

antigenic variation than type B strains. No cross protection between strains occurs.

c. The vaccine is made from highly purified, egg-grown viruses that have been inactivated. Whole virus, subvirion, and purified surface antigen preparations are available.

3. Vaccine indications. The vaccine is indicated for most patients, especially those older than 65 years and residents of nursing homes or other chronic care facilities.

4. Vaccine administration

a. Subvirion or **purified surface antigen vaccines** cause fewer febrile reactions, and are used in **patients younger than 12 years.**

b. The vaccine should be **withheld from patients with known anaphylactic hypersensitivity to eggs.**

5. Vaccine nomenclature. Influenza vaccines are named according to the following sequence: virus type, geographic origin, strain number, year of isolation, and the type A virus subtype. Thus, the vaccine for the 1998–99 season includes A/Beijing/262/95-like (H1N1), A/Sydney/05/97-like (H3N2), and B/Harbin/07/94.

I. *Streptococcus pneumoniae* vaccine. The capsule is the primary pathogenic element of the pneumococcus. Anticapsular antibody, which appears after a 2- to 3-week induction period, is completely protective, increasing opsonization, phagocytosis, and killing of the bacteria.

1. Vaccine production. The *S. pneumoniae* vaccine is composed of **23 polysaccharides** purified from the capsules of the most important serotypes.

a. The polysaccharides are **thymus-independent antigens,** which are not effective in children younger than 2 years. Complexing the antigens with carrier proteins or absorbing them into liposomes can convert them to a thymus-dependent state.

b. Little or no memory is produced; however, the polysaccharides persist in tissues and continue to stimulate antibody synthesis.

2. Vaccine administration. The antibody response persists for approximately 5 years, at which time revaccination is recommended for asplenic patients, those with chronic illnesses, and those older than 65 years. Revaccination is not recommended if the individual was vaccinated initially after the age of 65.

III. ADJUVANTS are substances that can be added to vaccines to increase their immunogenicity.

A. Aluminum hydroxide gel is the only adjuvant approved currently for human use.

1. The antigen is adsorbed onto the gel when aluminum chloride is treated with sodium hydroxide.

2. The antigen is released slowly and large numbers of antigen-presenting cells (APCs) are attracted to the injection site, increasing and prolonging antibody formation.

3. The adsorbed antigen exhibits minimal toxicity; occasionally, granulomas form at the site of injection.

B. Other compounds with diverse capabilities for enhancing any of the many steps leading to humoral immunity and CMI are under study for inclusion in human vaccine preparations, including **cytokines, chemokines,** and **synthetic bacteria-derived lipids.**

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