Head, Neck, and Orofacial Infections: An Interdisciplinary Approach

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To Carmen, the love of my life, best friend, and wife; our unbelievably special children, Jamie, Justin, Joelle, and Jordan (Maime, Joost, Welly, and Jordo); our wonderfully talented daughters-in-law and son-in-law, Natacha, Jordan, and Joe; and our delightful granddaughters, Peyton and Morgan.

JRH

I dedicate this book to my wife Moniek, whose love, support, and devotion have made completion of this book possible. I would also like to express my love to my children Michael, Isabella, and Anthony, who inspire me and give me the strength and the will to strive to be the best I can be. Above all, I would like to thank my parents and brothers, especially Tony, who have encouraged me and taught me the value of education and hard work.

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Preface

Head, Neck, and Orofacial Infections: An Interdisciplinary Approach

Head, neck, and orofacial infections are commonly seen in medical and dental offices, and, in severe cases, in hospital emergency departments. Head and neck anatomy is complex, with many contiguous spaces, and thus infections in one anatomic region may easily spread to other regions, including the pharynx, eye, and brain. This can lead to devastating results, including airway obstruction, blindness, and altered mental status with cranial neuropathies. Early recognition and correct management of severe infections can be life-saving, especially in medically compromised patients and in those who present late in the infectious disease process. Knowledge of the surgical anatomy and the path of spread of infections in the head and neck is fundamental in correct diagnosis and treatment.

Head, Neck, and Orofacial Infections: An Interdisciplinary Approach is the first book of its kind to present this level of in-depth information for those diagnosing and managing pathology due to infections of the head and neck and orofacial complex. This comprehensive resource provides both time-tested and cutting-edge approaches to patient management.

This book is designed to be of value to a wide range of health professionals:

- Physicians including family physicians, infectious disease specialists, anesthesiologists, internists, hospitalists, critical care physicians, pediatricians, radiologists, and clinical pathologists
- Surgeons including general surgeons, head and neck surgeons, otolaryngologists, oral-maxillofacial surgeons, plastic surgeons, neurosurgeons, ophthalmologists, dermatologists, pediatric surgeons, and trauma surgeons
- Dental professionals including general dentists, periodontists, endodontists, pediatric dentists, oral-maxillofacial pathologists, and oral-maxillofacial radiologists
- Nursing professionals including midlevel providers, those working in hospital infection control, and nurses in emergency care facilities and operating rooms

Key Features

- Over 500 images (including photographs, radiographs, and illustrations) clearly demonstrate pathologies, procedures, and outcomes.
- Experts in the field provide authoritative, state-of-the art guidance.
- A variety of topics are covered such as the evolving principles of antibiotic therapy; odontogenic infections; the pathophysiology and management of nasal and paranasal sinus infections; acute ear infections; orbital infections; the infection issues surrounding dental implants; anesthetic management of the patient with a head and neck infection; and much more!

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JRH

I would like to acknowledge my mentors, colleagues, residents, students, and staff who have, over the years, directly or indirectly, contributed to this work. Their never-ending assistance and constant support have made this book possible. For that, I am eternally grateful. I also want to express my appreciation for the expertise of the entire publishing team at Elsevier.

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Immunobiology of Infectious Disease

JOSEPH V. CALIFANO AND PHILIP M. PRESHAW

Introduction

The human immune system is a biologic marvel designed to identify and destroy or alter pathogens, foreign material, and abnormal cells that threaten an individual. This chapter presents a detailed discussion of the immune system as it relates to infectious pathogens.

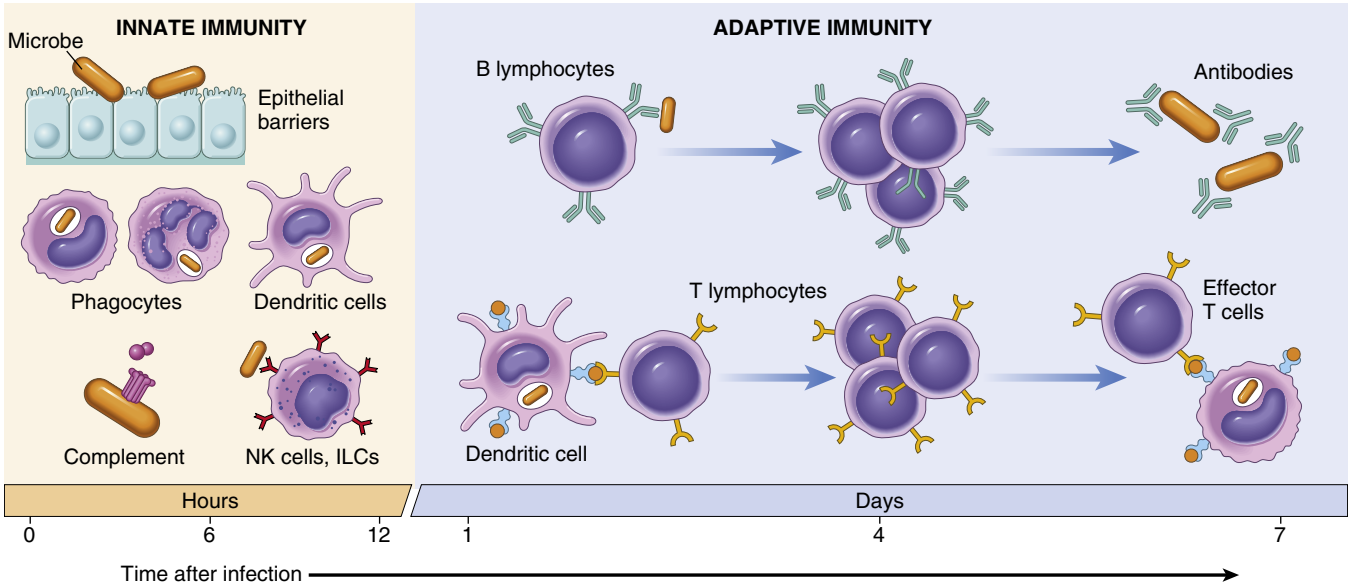
Throughout our lives we are constantly encountering microorganisms capable of causing infectious disease. We have, through the evolutionary processes of natural selection, developed a complex and highly organized immune system composed of molecules, cells, and tissues that protect us from agents of infection. Although infections clearly do occur, most interactions with potentially pathogenic bacteria, viruses, or fungi do not result in a productive infection. For infection to occur, the inoculum and virulence of the organism must be of a magnitude sufficient to overwhelm the immune system.

Our immune system has been divided, based on our understanding of function, into innate immunity and adaptive immunity (Figure 1-1). It is important to note that these aspects of immune functioning do not operate in isolation; indeed, there is close functional integration between the innate and adaptive arms of the immune response.[1] Innate immunity serves to provide the first line of defense in preventing infection. It includes physical barriers such as the skin and mucosa, phagocytic cells (e.g., neutrophils, macrophages), specialized receptors that bind and detect classes of macromolecules associated with pathogens not found in eukaryotic cells (e.g., lipopolysaccharide, lipoteichoic acid, single-stranded DNA, double-stranded RNA [dsRNA]), and molecules that promote inflammation, chemotaxis, and opsonization (e.g., cytokines, complement, acute phase proteins, arachidonic acid metabolites). In most cases, infectious agents are eliminated by innate immunity. When the innate immunity is not sufficient to prevent infection, the invading microorganism is successful in replicating within the host, and a productive infection ensues. Once this occurs, many of the elements of innate immunity are still active, but an adaptive immune response occurs over time.[2] This response may be dominated by a humoral response in which opsonizing antibody specific to the pathogenic organism facilitates phagocytosis and clearance of the microorganism and its toxins (typical for extracellular pathogens such as bacteria), or it may be dominated by a cellular response in which cytokines, phagocytes, or cytolytic T cells eliminate infected host cells to clear the pathogen (typical response for intracellular pathogens like viruses and some bacteria, such as *Mycobacterium tuberculosis*). Innate immunity is constitutive; it does not require prior exposure to the microorganism to respond to it. Adaptive immunity, on initial exposure to a pathogen, requires 3 to 7 days for a response to occur. With multiple exposures to the pathogen over time, either naturally or through immunization, there is a decrease in the lag time and an increase in the magnitude and efficacy of the adaptive immune response.

Cells of the Immune System

The cells of the immune system are derived from pluripotent stem cells in the bone marrow. The stem cells then differentiate into lymphoid and myeloid progenitors.

The lymphoid progenitor ultimately differentiates into B lymphocytes (produced in the bone marrow), T lymphocytes (produced in the thymus), and natural killer (NK) cells. NK cells are involved in early immune responses, and they recognize virally infected cells and neoplastic cells. B lymphocytes, when activated further, differentiate into memory B cells and antibody-secreting plasma cells. T lymphocytes further differentiate into helper T cells, cytotoxic T cells, and regulatory T cells. These different subpopulations of T cells are distinguished from each other by the types of cytokines they produce and by the surface molecules that they display (Figure 1-2).



• **Figure 1-1** Components and kinetics of innate and adaptive immunity. The mechanisms of innate immunity provide the initial defense against infections. Adaptive immune responses develop later and require the activation of lymphocytes. The kinetics of the innate and adaptive immune responses are approximations and vary in different infections. *ILC,* Innate lymphoid cell; *NK,* natural killer. (From Kumar V, Abbas AK, Aster JC: *Robbins and Cotran pathologic basis of disease,* ed 9, Philadelphia, 2015, Saunders.)

The myeloid precursor further differentiates into neutrophils (polymorphonuclear leukocytes), macrophage-monocytes, basophils, eosinophils, mast cells, and dendritic cells. It also can differentiate into an erythroblast from which red blood cells are derived, and a megakaryocyte from which platelets are derived (see Figure 1-2).

Polymorphonuclear leukocytes comprise the majority of the granulocytes circulating in the blood. Macrophages are also phagocytic cells, are derived from circulating monocytes, and are located in the tissues in readiness to encounter pathogens. Polymorphonuclear leukocytes and macrophages are the primary phagocytic cells of the body; they kill pathogens by ingesting them and exposing them to an array of enzymes, reactive oxygen species, and antimicrobial peptides contained within lysosomes.

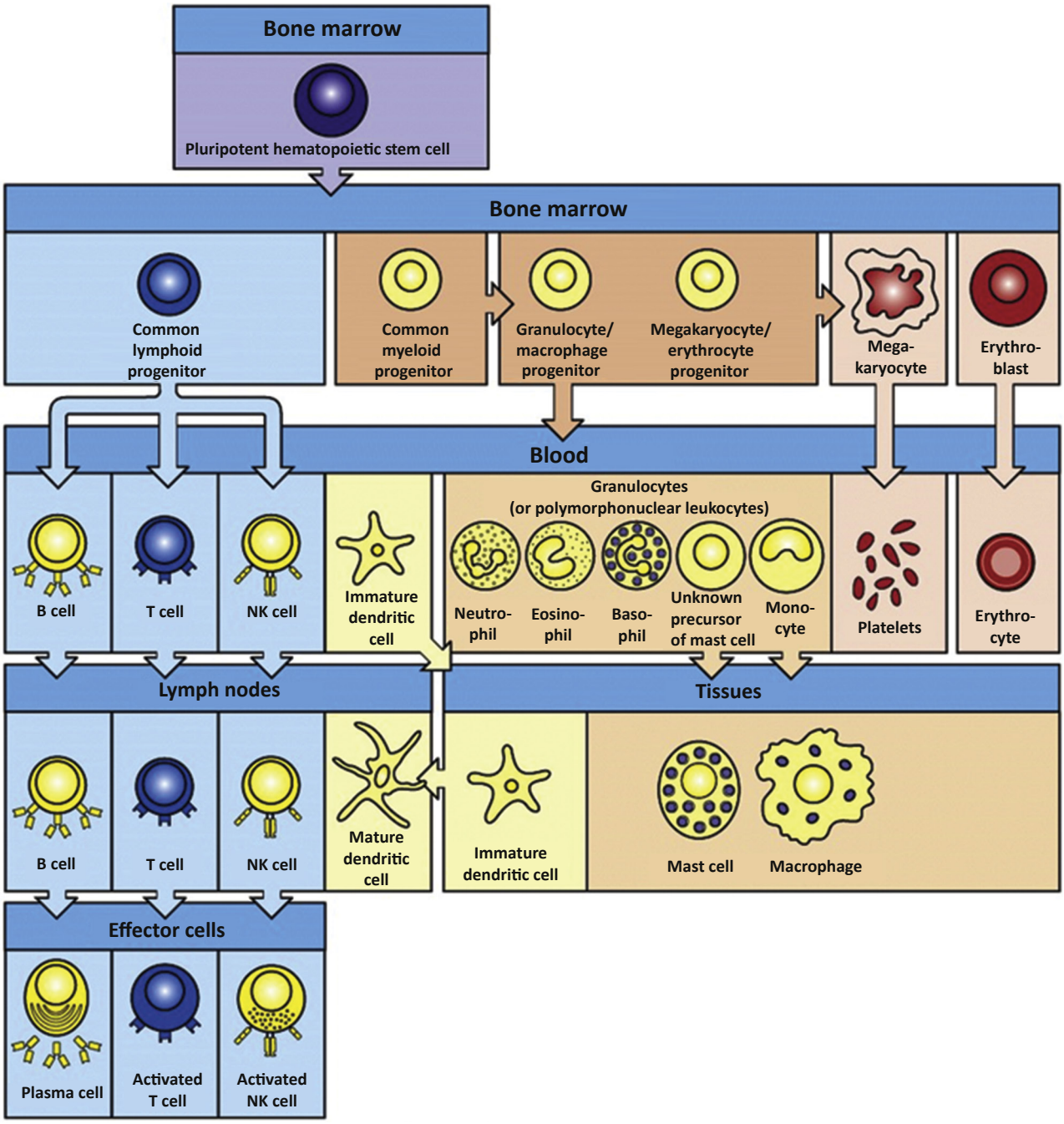
Mast cells are granulocytes that contain granules containing histamine and heparin. They are located throughout the body, being particularly present in tissues surrounding blood vessels and in close proximity to epithelial surfaces (skin and mucosa). They play a key role in allergic responses and anaphylaxis, and in immune responses to pathogens. Mast cells and basophils are functionally similar to each other, the difference being that mast cells are located in the tissues, whereas basophils are found in the circulation. Activation, and subsequent release of histamine by degranulation, results from binding with IgE and with complement proteins.

Dendritic cells are antigen-presenting cells that are located in the tissues. These phagocytic cells ingest microbes and then process (break up) antigen and present it to T cells as part of the immune response.

Primary and Secondary Lymphoid Tissues

The cells described in the previous section can be found in many locations throughout the body. Among these are the primary and secondary lymphoid tissues (Figure 1-3). The primary lymphoid tissues are the bone marrow and thymus. All cells involved in immunity are derived from the bone marrow. In addition, T lymphocyte development is completed in the thymus, where positive and negative selection allow T cells to be selected that can recognize antigen presented to them in association with self-major histocompatibility molecules with high affinity, but not recognize self-major histocompatibility molecules alone with high affinity. This allows them to be functional but not autoreactive, which could result in autoimmune disease. The secondary lymphoid tissues include lymph nodes in many locations throughout the body; spleen, tonsils, adenoids, and Peyer patches in the small intestine. All the secondary lymphoid tissues have T cell–rich zones where antigen presentation can occur and B cell–rich zones where B cells are part of lymphoid follicles. B cells in lymphoid follicles exhibit rapid proliferation and differentiation into antibody-secreting plasma cells in response to antigen and T cell-derived growth factors.

Sites in the body that are likely to encounter pathogenic organisms through ingestion or respiration have a higher concentration of secondary lymphoid tissue. Therefore, the head and neck have substantial lymph drainage of the oral cavity, pharynx, nose, nasal cavity, paranasal sinuses, nasal pharynx, and pharynx to help prevent infection as

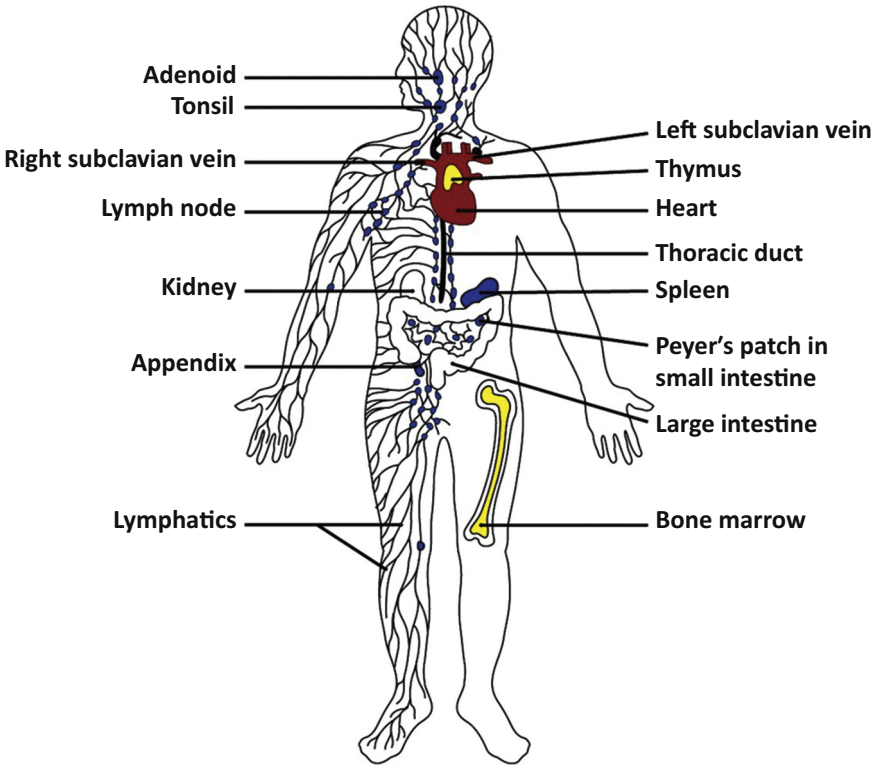


• **Figure 1-2** Cells of the immune system. NK, Natural killer. (From Murphy K, et al: *Janeway's immunobiology*, ed 7, 2008. Reproduced by permission of Garland Science/Taylor & Francis Group LLC.)

a result of exposure through ingestion of food, mastication, and respiration. Secondary lymphoid tissues in the head and neck include many lymph nodes, palatine tonsils, lingual tonsils, and adenoids. It is interesting to note that surgery in the oral cavity performed under aseptic but nonsterile conditions typically does not result in a postsurgical infection. Surgery under the same conditions in sterile tissue sites would likely result in such an infection. The relative resistance to postsurgical infection may result from the greater proportion of lymphoid tissue in this anatomic region.

Innate Immunity

Innate immunity includes barriers to infection (see Figure 1-1). Examples are skin, mucosa, saliva, mucus, tears, acid pH in the stomach, and ciliated respiratory epithelium. The importance of innate immunity becomes clear when one considers the risk of infection that follows severe burns in which the connective tissue is directly exposed to the environment. Infection is a significant cause of morbidity and mortality in patients with severe burns involving large areas of the body. Intact skin and mucosa thus are physical



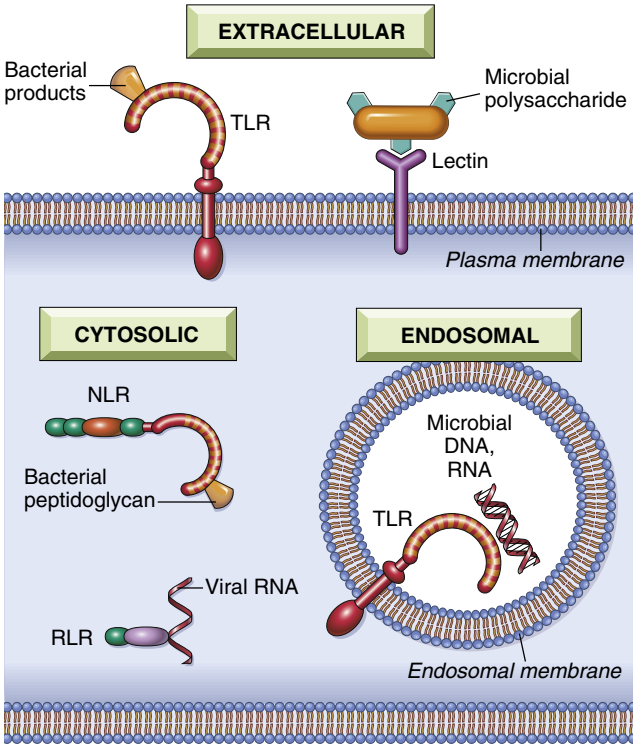
• **Figure 1-3** Primary and secondary lymphoid tissue. Primary lymphoid tissues are the bone marrow and thymus. Secondary lymphoid tissues include lymph nodes in many locations throughout the body; spleen, tonsils, adenoids, and Peyer's patches in the small intestine. (From Murphy K, et al: *Janeway's immunobiology*, ed 7, 2008. Reproduced by permission of Garland Science/Taylor & Francis Group LLC.)

barriers to infection. The epithelium of skin and mucosa is constantly sloughing or exfoliating. This impedes the attachment of pathogenic organisms as they are shed along with the dead cells to which they are attached. Secretions in the respiratory tract, gastrointestinal tract, and eyes serve to flush the epithelial surfaces and further cleanse them. In the respiratory tract, this action is enhanced as ciliated respiratory epithelial cells "sweep" mucus and any potential pathogens out of the lungs, nasal cavity, and paranasal sinuses, and from there into the esophagus to be swallowed and join the gastric contents in conditions of very low pH. Acidity of the stomach acts to inactivate and kill pathogens that enter the gastrointestinal tract.

Antimicrobial molecules are additional barriers to infection; among them are lysozyme, phospholipase A_2, antimicrobial peptides, and acute phase proteins. Lysozyme is secreted by neutrophils and macrophages; it is present in tissues and most secretions, including saliva, and catalyzes hydrolysis of peptidoglycan. Breakdown of bacterial peptidoglycan disrupts the cell wall, especially in gram-positive bacteria. Phospholipase A_2 is produced by neutrophils and epithelial cells. It digests fatty acids in the cell wall and leads to bacterial lysis. Phagocytes, epithelial cells, and salivary glands are all sources of antimicrobial peptides (e.g., defensins, cathelicidins). These molecules form pores or otherwise affect the permeability of the cell membrane, resulting in cellular lysis. Acute phase proteins (e.g., C-reactive protein [CRP], mannose-binding protein [MBP; also known as mannose-binding lectin]) are produced by the liver. They bind to surface carbohydrates of dead or dying host cells (i.e., CRP) or microbial carbohydrate (CRP and MBP) and in turn activate complement through the lectin pathway. Once complement is activated, the pathogen is opsonized by C3b.[3]

If the barriers and antimicrobial molecules fail to eliminate the pathogen, it will gain access to the subcutaneous tissues. As this occurs, cells of the immune system such as fibroblasts, epithelial cells, and endothelial cells begin to recognize broad classes of macromolecules associated with pathogenic organisms that are not found in humans (e.g., lipopolysaccharide, lipoteichoic acid, flagellin, hypomethylated CpG-rich DNA, dsRNA, *N*-formylmethionyl-leucyl-phenylalanine [FMLP]). These macromolecules are termed *pathogen-associated molecular patterns* (PAMPs). Similarly, the same cells recognize molecules from damaged host tissues that are elaborated as a result of infection. The receptors that recognize these pathogen-derived molecules are termed *pattern recognition receptors* (PRRs). Examples of PRRs include Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain–like receptors (NLRs), retinoic acid-inducible gene 1–like receptors (RLRs), and the FMLP receptor (Figure 1-4).[3]

There are as many as 13 different TLRs. Each recognizes particular macromolecules and molecular patterns. The patterns recognized by some of the key TLRs are summarized



• **Figure 1-4** Pattern recognition receptors. Engagement results in production of proinflammatory cytokines and promotes an antiviral state in cells. CDS, Cytosolic DNA sensor; NLR, nucleotide-binding domain–like receptor; RLR, retinoic acid–inducible gene 1–like receptor; TLR, Toll-like receptor. (From Kumar V, Abbas AK, Aster JC: *Robbins and Cotran pathologic basis of disease,* ed 9, Philadelphia, 2015, Saunders.)

in Figure 1-5. TLRs are found on macrophage–monocytes, neutrophils, fibroblasts, epithelial cells, endothelial cells, and dendritic cells. TLRs are transmembrane proteins, some of which are located on the surfaces of cells (e.g., TLR1, TLR2, TLR4, TLR5, TLR6, TLR11), whereas others are located within cells at the endosomal–lysosomal compartment (TLR3, TLR7, TLR8, TLR9). When a pathogen-derived molecule engages the TLR, intracellular second messengers in turn result in expression of genes for proinflammatory cytokines.[4,5]

The process by which interactions between PAMPs and TLRs lead to activation of expression of genes for proinflammatory cytokines is highly complex, and it is referred to as *signaling*. The process of signal transduction results from recruitment of adaptor proteins that are present within the cytoplasm as the means by which to transfer the signal from the surface-bound TLR to the nucleus, where gene expression will occur.[6,7] Adaptor proteins include myeloid differentiation primary response protein 88 (MYD88), TIR domain-containing adaptor protein inducing IFN-β (TRIF), TRIF-related adaptor molecule (TRAM), and TIR domain-containing adaptor protein (TIRAP). The adaptor proteins associate (by structural or conformational changes) with the cytoplasmic domains of TLRs via interactions between the Toll/IL-1 (TIR) domains present in each TLR and each adaptor. The MYD88 adaptor is used by all TLR family members except TLR3, which signals via the TRIF adaptor. TLR4 signals via both MYD88 and TRIF (Figure 1-6).[5]

The recruitment of adaptor proteins leads to a cascade of downstream signaling events that differ according to the adaptor molecule concerned. However, in broad terms, the outcome of each signaling pathway results in activation of transcription factors such as nuclear factor-κB (NF-κB) and interferon regulatory factors that control the transcription of DNA. TLR signaling leads to expression of proinflammatory cytokines including tumor necrosis factor-alpha (TNF-α), type I interferons, interleukin (IL) 1 (IL-1), IL-6, IL-8, and IL-12. These cytokines activate phagocytes, recruit phagocytes to sites of infection, increase the resistance of cells to viral infection, activate NK cells, and support the development of adaptive immunity for the pathogen (see Figure 1-6).

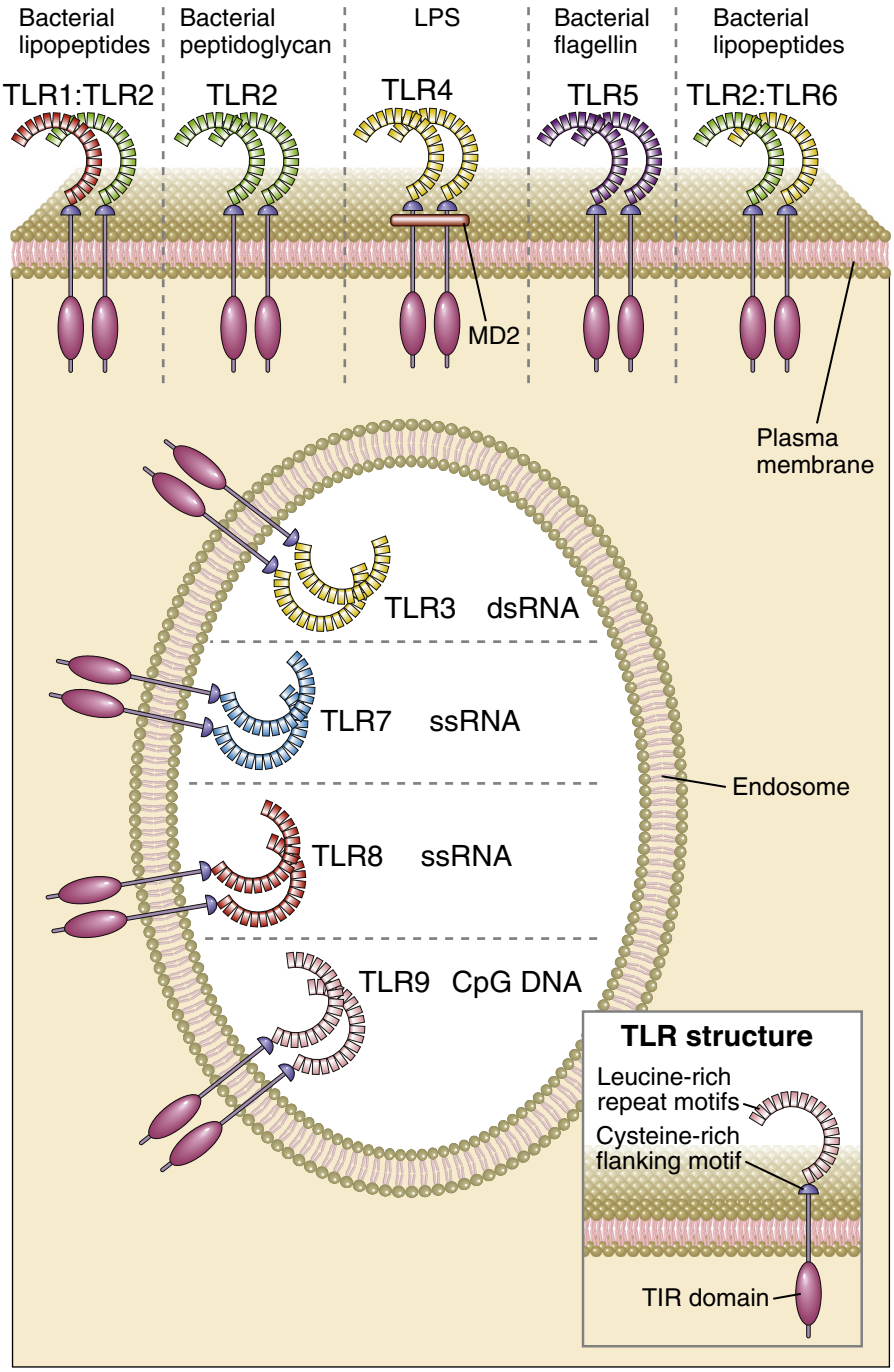
NLRs are another family of pattern recognition receptors. They are located intracellularly and, like TLRs, recognize the presence of molecular patterns uniquely associated with pathogens. Once engaged, they also result in the expression of genes for proinflammatory cytokines.

RLRs recognize dsRNA, which is unique to some RNA viruses and when present is indicative of a viral infection. Engagement of RLR by viral dsRNA also increases the expression of proinflammatory cytokines.

Neutrophils also have a receptor for FMLP. Every bacterial protein is synthesized beginning with these three amino acids, the first of which is unique to prokaryotes. Once protein synthesis is complete, this tripeptide is cleaved off. Where bacteria are present, so is FMLP; therefore, FMLP induces neutrophil chemotaxis toward increasing concentrations of this molecule.

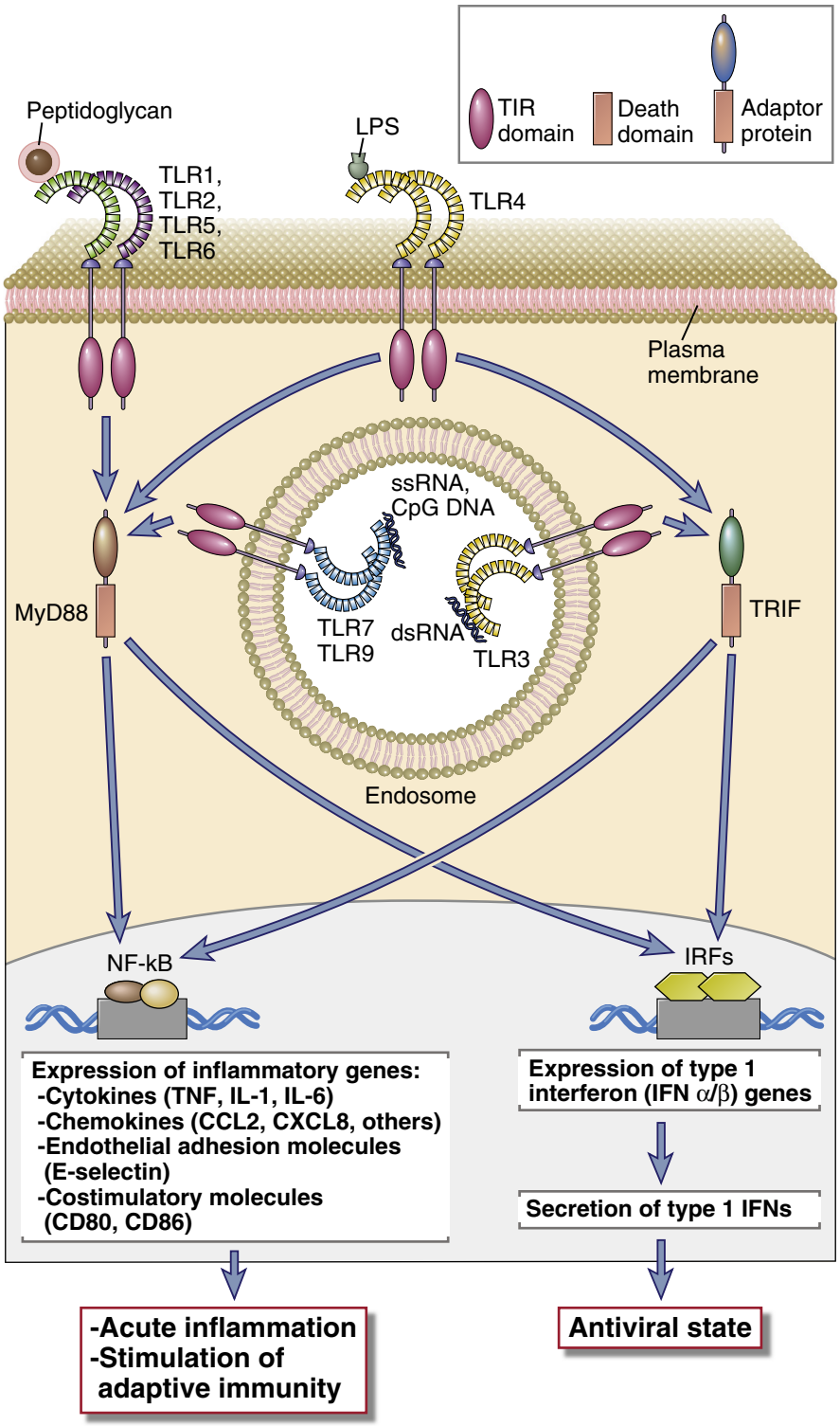
As cells begin to encounter a pathogen and their PRRs are engaged, they will secrete proinflammatory cytokines. These proinflammatory molecules play a significant role in innate immunity. They help in activating phagocytes (neutrophils and macrophages) and NK cells, and they help in recruitment of these cells to sites of infection. In addition, complement and cellular adhesion molecules participate in innate immunity and the inflammatory process.

As mentioned previously, some of the key proinflammatory cytokines that are released when PRRs (i.e., TLRs and NLRs) are engaged include TNF-α, type I interferons, IL-1, IL-6, IL-8, IL-12, and lipid mediators. The source of TNF-α in innate immunity is principally the macrophage. It stimulates endothelial cells to express intercellular adhesion molecule 1. Immune cells, especially neutrophils and monocytes, express leukocyte function–associated antigen 1 on their surface that binds to intercellular adhesion molecule 1 and allows these cells to exit the circulation at sites of inflammation, trauma, and infection. Neutrophils and macrophages are also activated by TNF-α. This cytokine also stimulates the liver to release acute phase proteins that activate complement, thereby promoting chemotaxis, opsonization, bacterial lysis, vasodilation, and increased vascular permeability.

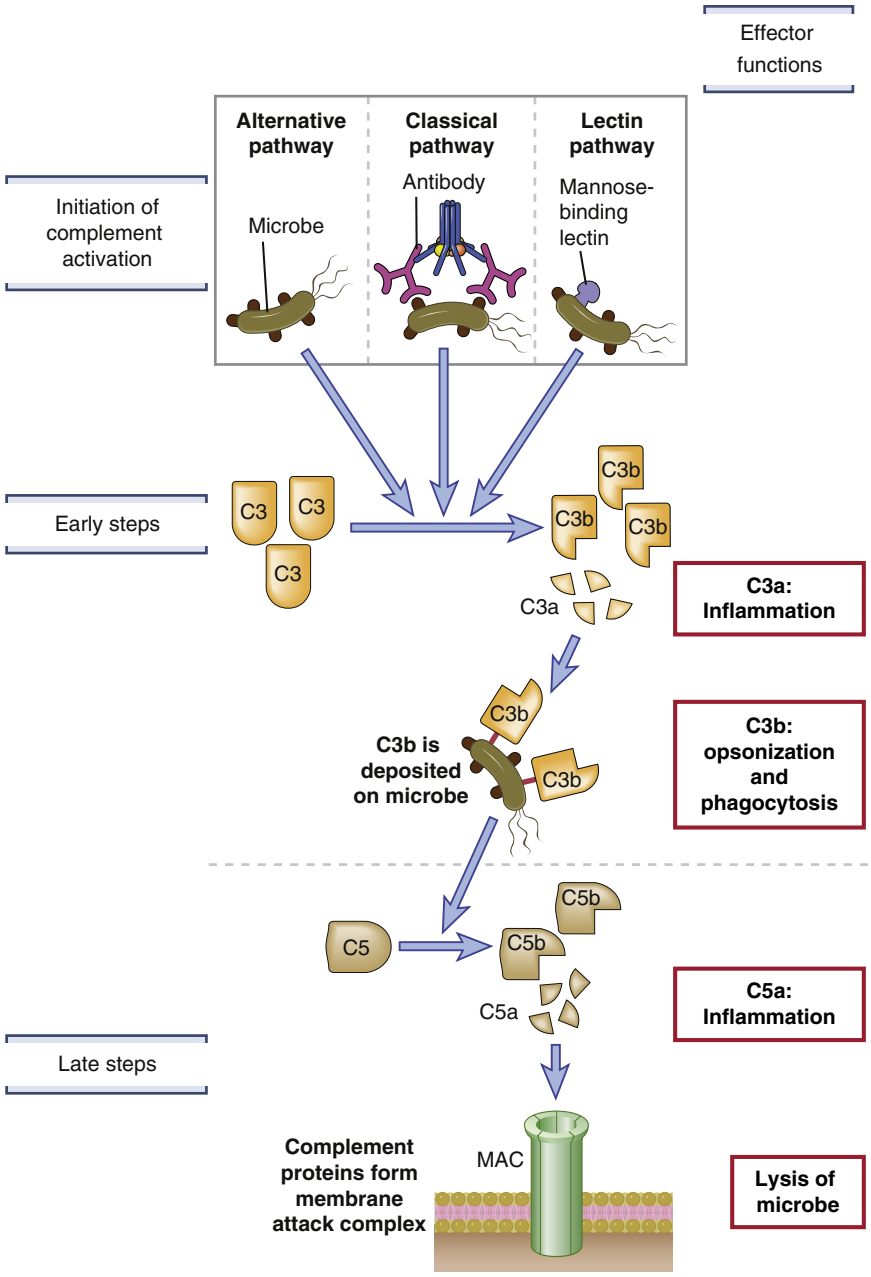


• **Figure 1-5** Toll-like receptors (TLR) 1 through 9 and their specificity. LPS, Lipopolysaccharide. (From Abbas AK, Lichtman AHH, Pillai S: *Cellular and molecular immunology,* ed 8, Philadelphia, 2015, Saunders.)

Macrophages and fibroblasts release type I interferons. Type I interferons activate NK cells and increase cellular resistance to viral infection. NK cells are important in the initial defense against viral infection. They recognize cells infected with a wide variety of viruses and then kill the infected cell. Macrophages and endothelial cells release IL-1, IL-6, and IL-8. The effects of IL-1 in innate immunity are similar to TNF-α. IL-6 stimulates the production of acute phase proteins in the liver and supports proliferation of B cells as an adaptive immunity is developing. Chemokines, including IL-8, are chemotactic for immune cells, especially neutrophils. They help to direct these cells to sites of infection. Macrophages and dendritic cells secrete IL-12. This cytokine stimulates NK cells to produce high levels of interferon (IFN)-γ, which in turn stimulates many cells, especially the macrophages. When activated, macrophages also release lipid mediators that have proinflammatory activity, including the metabolites of arachidonic acid, prostaglandins, and leukotrienes. Prostaglandins (e.g., prostaglandin E_2 [PGE_2]) increase vascular permeability and result in vasodilation, as well as have positive feedback effects on



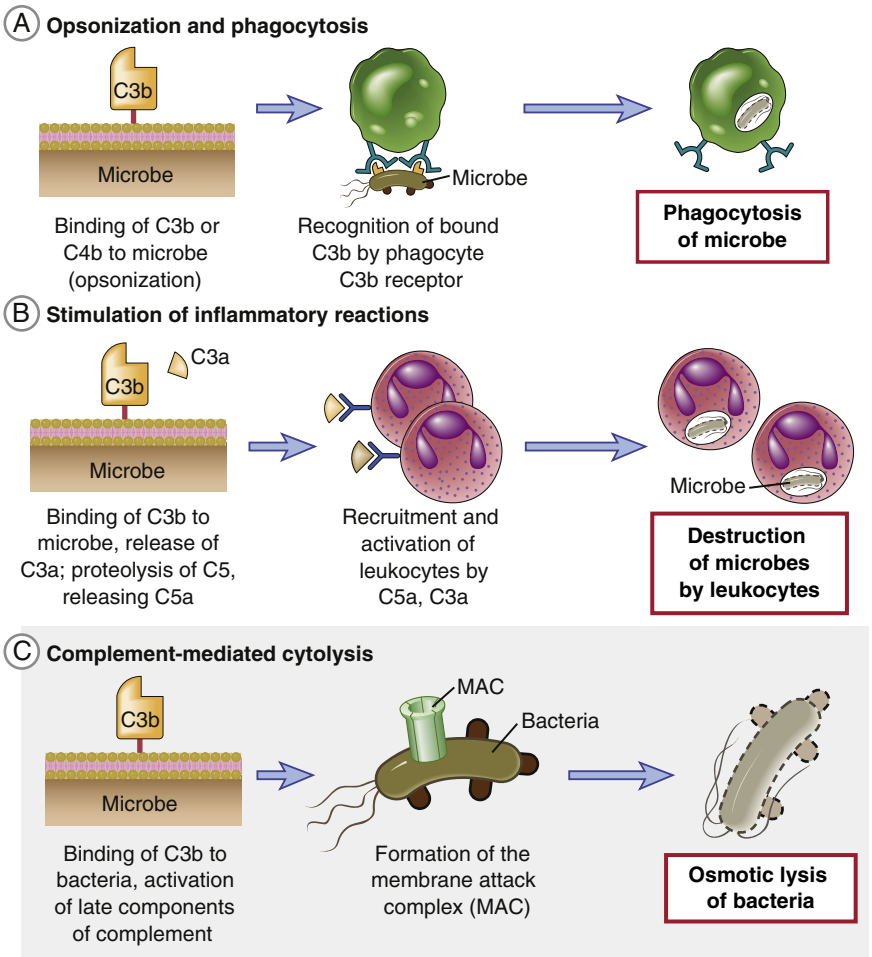
• **Figure 1-6** Toll-like receptors (TLR) signal transduction and inflammatory gene expression. TLRs 1, 2, 5, and 6 use adaptor protein MYD88 and activate transcription factors NK-κB and AP-1. TLR3 uses adaptor protein TRIF and activates the IRF3 and IRF7 transcription. TLR4 can activate both pathways. TLR 7 and 9 in the endosome use MYD88 and activate both NK-κB and IRF7. dsRNA, Double-stranded RNA; IFN, interferon; IL, interleukin; LPS, lipopolysaccharide; NF-κB, nuclear factor-κB; TNF, tumor necrosis factor; TRIF, TIR domain-containing adaptor protein inducing IFN-β. (From Abbas AK, Lichtman AHH, Pillai S: *Cellular and molecular immunology,* ed 8, Philadelphia, 2015, Saunders.)



• **Figure 1-7** Complement activation. The activation of the complement system may be initiated by three distinct pathways, all of which lead to the production of C3b (the early steps). C3b initiates the late steps of complement activation, culminating in the production of peptides that stimulate inflammation (C5a) and polymerized C9, which forms the membrane attack complex, so called because it creates holes in plasma membranes. The principal functions of major proteins produced at different steps are shown. (From Abbas AK, Lichtman AH, Pillai S: *Basic immunology: functions and disorders of the immune system,* ed 4, Philadelphia, 2014, Saunders.)

cytokine and prostaglandin secretion. Leukotrienes are chemotactic for neutrophils and support the vascular effects of prostaglandins.

In addition to the proinflammatory cytokines, complement proteins are also an important component of innate immunity (Figure 1-7). As indicated previously, acute phase proteins can activate complement through the lectin pathway. In addition, many bacterially derived molecules (especially lipopolysaccharide) can activate complement through the alternate pathway. Once activated, several important complement proteins are generated. Among them are C3a, C3b, and C5a. Phagocytes have receptors for C3b on their surface. As the complement cascade is activated, C3b is deposited on the surface of the pathogen, and it serves as an opsonin and facilitates phagocytosis. The complement protein C3a increases vascular permeability and results in vasodilation. A gradient of C5a emanating from sites



• **Figure 1-8** Complement-mediated opsonization, chemotaxis, and osmotic lysis. The major functions of the complement system in host defense are shown. Cell-bound C3b is an opsonin that promotes phagocytosis of coated cells **(A);** the proteolytic products C5a, C3a, and C4a stimulate leukocyte recruitment and inflammation **(B);** and the MAC lyses cells **(C).** (From Abbas AK, Lichtman AH, Pillai S: *Basic immunology: functions and disorders of the immune system,* ed 4, Philadelphia, 2014, Saunders.)

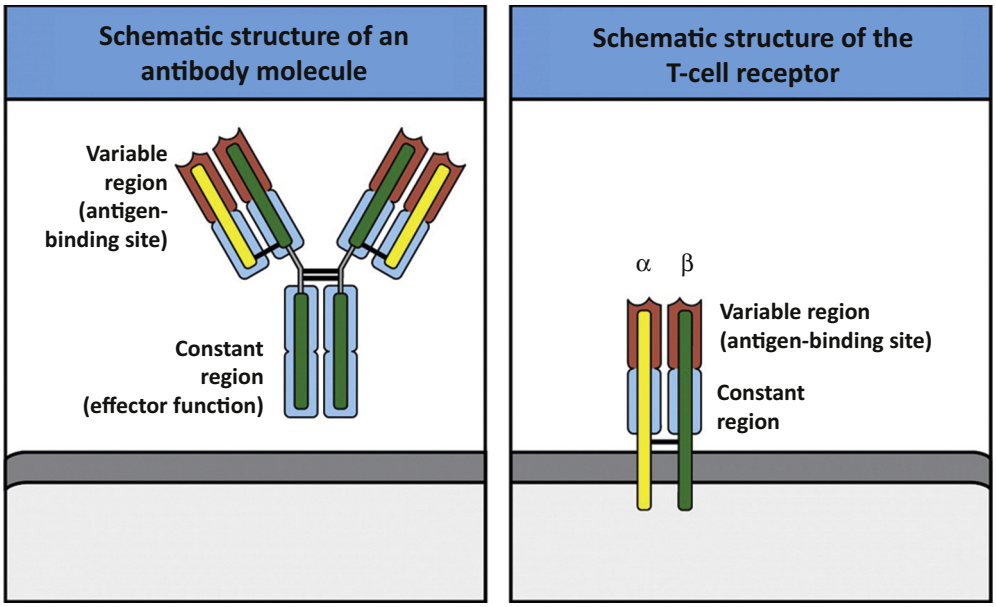
of infection serves as a potent chemotactic agent, particularly for neutrophils and monocytes. Finally, the terminal sequence of the complement cascade forms the membrane attack complex that creates a pore in the pathogen, disturbs osmotic balance, and thus results in cell lysis (Figure 1-8).[3]

Adaptive Immunity

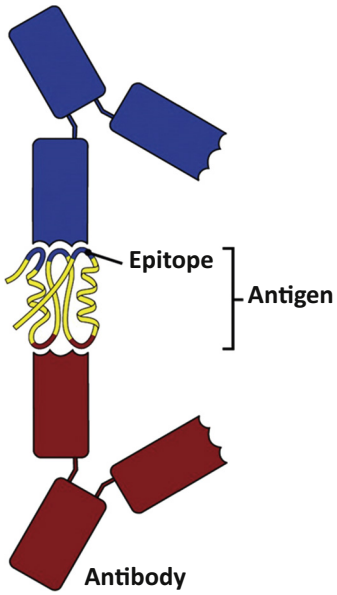
Once the barriers are breached, the pathogenic organism gains access to subcutaneous tissues. Immune cells release proinflammatory cytokines as part of innate immunity, and these molecules, as well as antigen from the pathogen, stimulate development of an adaptive immune response. If innate immunity quickly clears the pathogen locally, then an adaptive immune response will not occur. If instead the pathogen is successful in replicating within the host tissues and disseminates widely throughout the body, then an adaptive immune response occurs.

Antigens, Epitopes, and Antigen Receptors

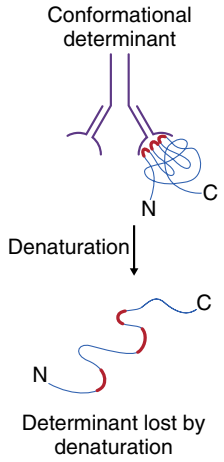
The adaptive immune response differs from innate immunity in that it recognizes specific antigens from a pathogen. Rather than responding through receptors with the ability to recognize broad classes of pathogen-associated macromolecules, the receptors in adaptive immunity recognize macromolecules (i.e., proteins, carbohydrates, lipids, nucleic acids) that derive from the particular invading organism at the species and strain level. In fact, if we examine a particular protein derived by the infecting pathogen, several receptors will recognize different sites or epitopes on the protein (an epitope is that part of an antigen that is recognized by the immune system, and typically constitutes five to seven amino acids of a protein). The receptors (antibody molecules and the T cell receptor) recognize epitopes in a specific "lock-and-key" manner (Figures 1-9 and 1-10). Therefore, if the secondary or tertiary conformation of the protein is affected by denaturing the protein (e.g., after heat



• **Figure 1-9** Antigen receptors: antibody and the T cell receptor. (From Murphy K, et al: *Janeway's immunobiology*, ed 7, 2008. Reproduced by permission of Garland Science/Taylor & Francis Group LLC.)



• **Figure 1-10** Antibody, antigen, and epitope. A protein antigen has a five- to seven-amino acid epitope that is recognized by the antibody's antigen-combining site. The amino acids are often not adjacent on the protein polypeptide chain, but are located near each other by virtue of the secondary structure. (From Murphy K, et al: *Janeway's immunobiology*, ed 7, 2008. Reproduced by permission of Garland Science/Taylor & Francis Group LLC.)

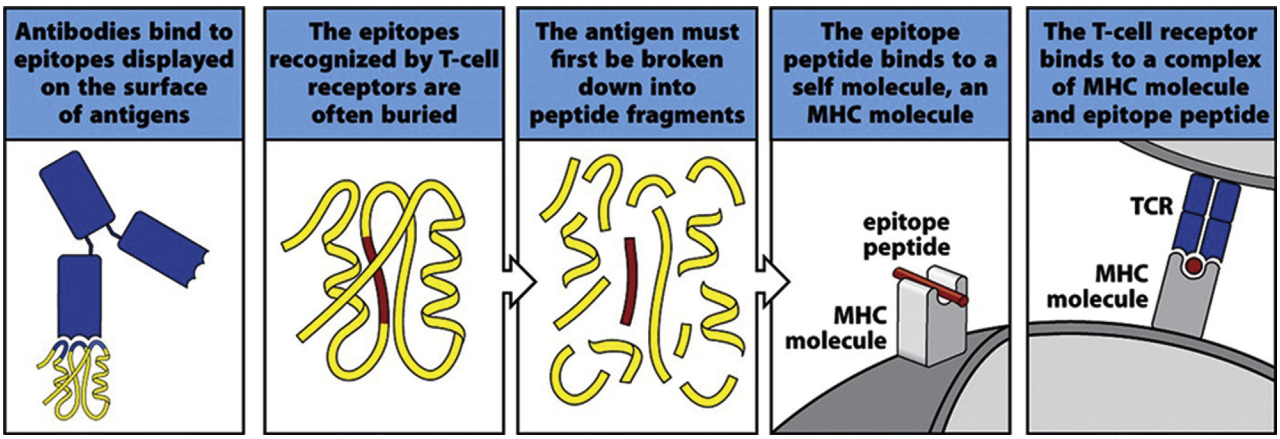


• **Figure 1-11** Denaturation loss of epitope. When a protein is denatured by heat and loses its secondary structure, the epitope/antigenic determinant recognized by the antibody's antigen-combining site is no longer present, and the antibody no longer recognizes the antigen. (From Rich RR, Fleisher TA, Shearer WT, et al: *Clinical immunology: principles and practice,* ed 4, London, 2013, Saunders.)

denaturation), then the epitope may no longer be present because the three-dimensional configuration of the epitope's amino acids may have been lost (Figure 1-11).

Adaptive immunity can be thought of as having two general types: (1) cellular, which is primarily focused on defense from intracellular pathogens (e.g., viruses, certain bacteria such as *M. tuberculosis*, malignant tumor cells), and (2) humoral, which is primarily focused on defense from extracellular pathogens (e.g., bacteria, fungi, parasites). The former involves cytokines from T helper (T_h) cells and effector cells, such as cytolytic T (T_{CTL}) cells, macrophage-monocytes, and NK cells. The latter involves B lymphocytes that differentiate into antibody-secreting plasma cells and B memory cells. They do so with the help of cytokines from helper T cells.

The antigen receptor for the B cell is the surface antibody molecule. It recognizes soluble or free native antigen (i.e., not denatured or processed). The antigen-combining sites on the antibody molecule recognize the epitope in a specific lock-and-key fashion. The antigen receptor for the T cell is the T cell receptor (Figure 1-12). The T cell receptor recognizes only processed antigen that must be



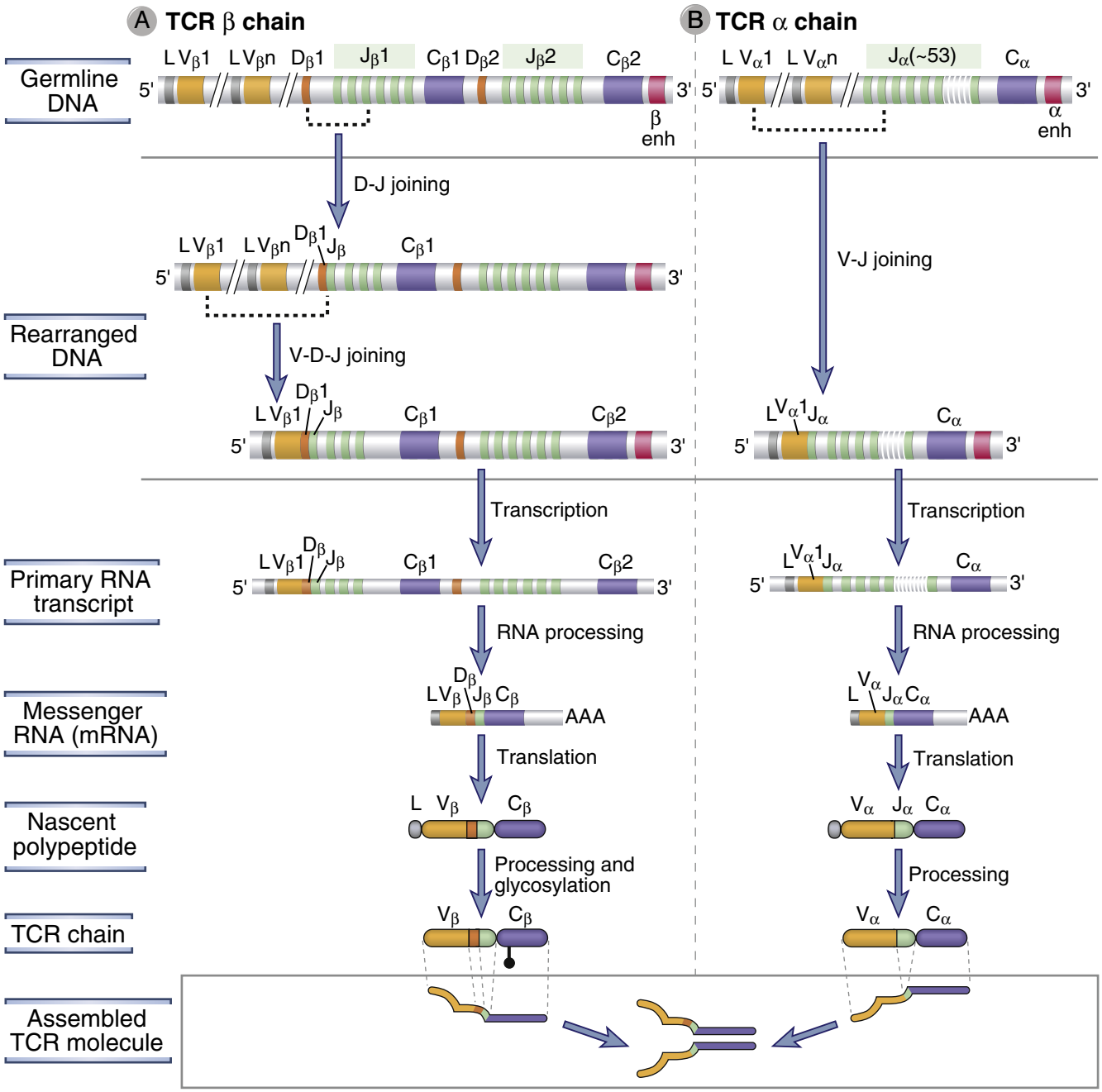
• **Figure 1-12** Antibody recognizes native antigen, whereas the T cell receptor (TCR) recognizes processed and presented antigen. MHC, Major histocompatibility complex. (From Murphy K, et al: *Janeway's immunobiology*, ed 7, 2008. Reproduced by permission of Garland Science/Taylor & Francis Group LLC.)

presented to the T cell (i.e., it is not a native antigen and is not free or soluble antigen). Antigen presentation involves internalizing of the native molecule by an antigen-presenting cell (e.g., a dendritic cell), digestion of the molecule into small pieces, association of the fragments with a special cleft on the major histocompatibility complex (MHC) molecules, and expression of the same on the cell surface. The T cell and its T cell receptor then recognize the processed antigen in association with self-MHC molecules in a specific lock-and-key fashion (see Figure 1-12).

The T cell and B cell antigen receptors and, in particular, their antigen-combining sites are unique to the particular cell clone and relate to its antigen specificity. Immature lymphocytes have germ line DNA sequences in the genes responsible for their antigen receptors. Once the cell is mature, these genes eventually encode for two glycoprotein transmembrane proteins that compose the α and β chains of the T cell receptor or the two heavy-chain transmembrane glycoproteins and two associated light chains that compose the surface antibody molecule that is the B cell receptor. These genes are composed of a large number of DNA segments separated by noncoding DNA (Figures 1-13 and 1-14). There are constant region gene segments that do not vary among different cell clones and are present for each chain (four for antibody and two for the T cell receptor). There are also variable region (V), diversity (D), and joining (J) gene segments. There are many different V, D, and J segments. For antibody molecules, the variable portions of the heavy chains are composed of V, D, and J gene segments and the light chains only V and J. For the T cell receptor, the variable region of the α chain is composed of V and J gene segments, and the β chain is composed of V, D, and J segments. As the lymphocyte develops into a mature B or T cell, the germ line DNA is subjected to recombination and mutation among the gene segments and deletion of many of the V, D, and J genes, so that a particular set of V and J or V, D, and J genes are directly joined to one another. This process occurs on each of the heavy and light chains of the antibody genes and the α and β chains of the T cell receptor. There are also some inaccuracies in the splicing of these gene segments and insertions of a small number of nucleotides at the joints, resulting in additional diversity. This genetic recombination and rearrangement in the germ line DNA allows for an extremely high level of variation in the final DNA sequence in the antigen-combining sites of the antigen receptors; this is referred to as *generation of diversity*. It allows, through this random process, the production of unique antigen-combining sites in the B cell surface antibody or T cell receptor that allows an immune response to essentially any possible antigen in the environment.

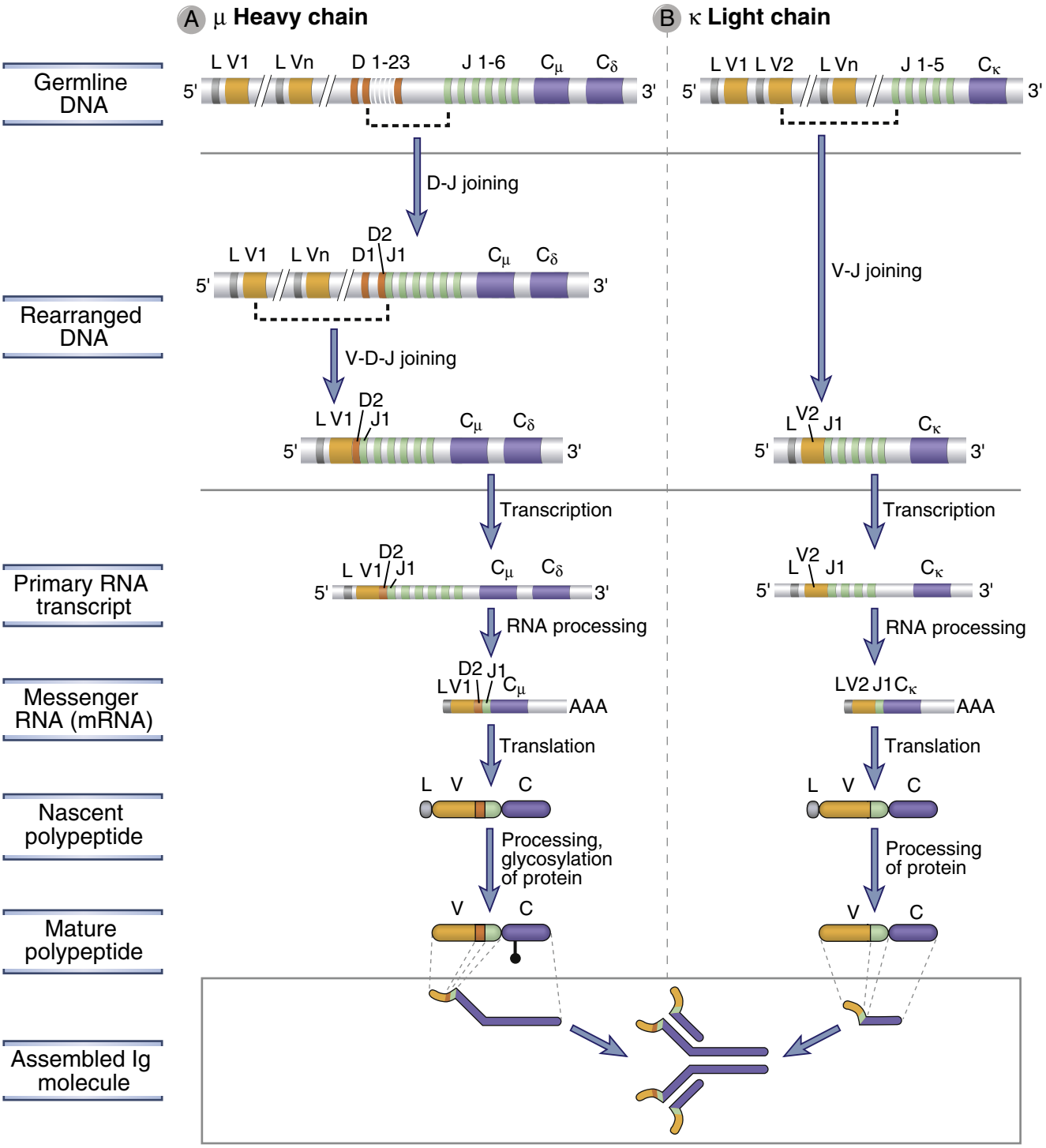
Antigen Presentation and the Major Histocompatibility Complex

T lymphocytes have several subsets: T_h cells, which can be further subdivided into T_{h1}, T_{h2}, T_{h17}, T_{fH} cells; T_{CTL} cells, which are the primary effector cells in cellular immunity; and regulatory T (T_{reg}) cells, which help in regulation of immunity. They encounter and recognize antigen that is processed and presented to them. T cells require processing and presentation of antigens rather than responding directly to antigen, as this permits them to be active against intracellular pathogens, rather than responding to soluble antigen that might be present in the circulation. Thus, T_h and T_{reg} cells are $CD4^+$, which indicates that they recognize antigen presented in association with MHC class II molecules (i.e., they recognize antigen together with components of self that are involved in the presentation of pieces of the antigen). Not all cells possess class II MHC; therefore, only certain cells can present antigen to $CD4^+$ cells. Antigen-presenting cells with high levels of MHC class II molecules on their surface include dendritic cells, macrophage-monocytes, Langerhans

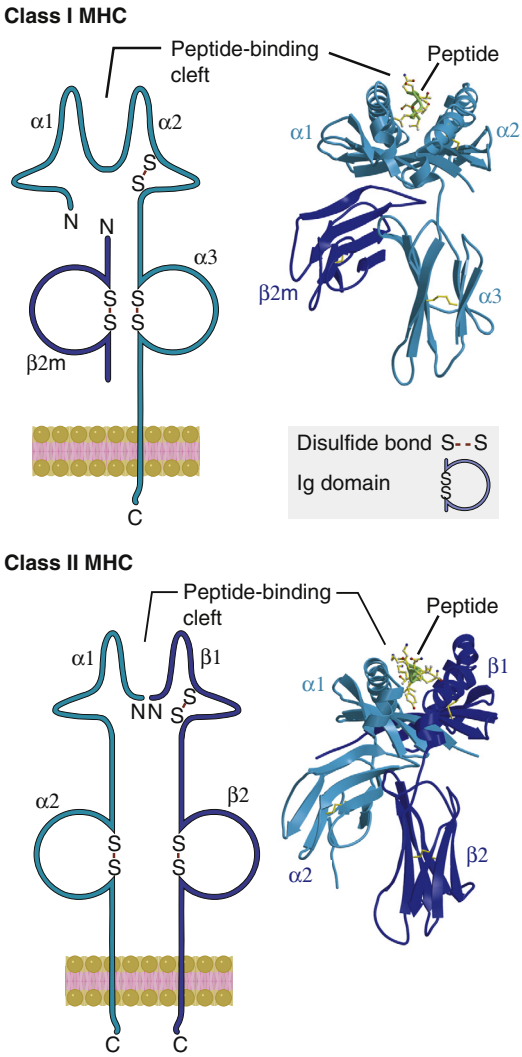


• **Figure 1-13** **A** and **B** Generation of diversity T cell receptor (TCR). As the lymphocyte develops into a mature T cell, the germ line DNA is subjected to recombination and mutation among the gene segments and deletion of many of the V, D, and J genes, so that a particular set of V and J or V, D, and J genes are directly joined to one another. This process occurs on each of the α and β chains of the T cell receptor. This genetic recombination and rearrangement in the germ line DNA allows for an extremely high level of variation in the final DNA sequence in the antigen-combining sites of the antigen receptors. (From Abbas AK, Lichtman AHH, Pillai S: *Cellular and molecular immunology,* ed 8, Philadelphia, 2015, Saunders.)

cells, and B cells. They all have the ability to present antigen to $CD4^+$ T_h and T_{reg} cells. T_{CTL} cells are $CD8^+$, which indicates that they recognize antigen in the context of MHC class I. MHC class I molecules are expressed on all nucleated cells. When a cell is infected with a virus, the T_{CTL} cells can then recognize viral antigens that are presented by the infected cell in association with class I MHC molecules (Figures 1-15 and 1-16). The T cell receptors in each case recognize the combination of the fragment of the foreign antigen and the self-MHC molecule. It is important to note that T cells can only recognize antigen that is processed and presented to them in association with self-MHC molecules. Therefore, antigen-presenting cells from one person cannot present antigen to another person's T cells, unless they are monozygotic twins, because they will not share the same MHC molecules. As an aside, MHC molecules are also the



• **Figure 1-14** **A** and **B** Generation of diversity B cell receptor/antibody. As the lymphocyte develops into a mature B cell, the germ line DNA is subjected to recombination and mutation among the gene segments and deletion of many of the V, D, and J genes, so that a particular set of V and J or V, D, and J genes are directly joined to one another. This process occurs on each of the heavy and light chains of the antibody genes. There are also some inaccuracies in the splicing of these gene segments and insertions of a small number of nucleotides at the joints, resulting in additional diversity. This genetic recombination and rearrangement in the germ line DNA allows for an extremely high level of variation in the final DNA sequence in the antigen-combining sites of the antigen receptors. (From Abbas AK, Lichtman AHH, Pillai S: *Cellular and molecular immunology,* ed 8, Philadelphia, 2015, Saunders.)

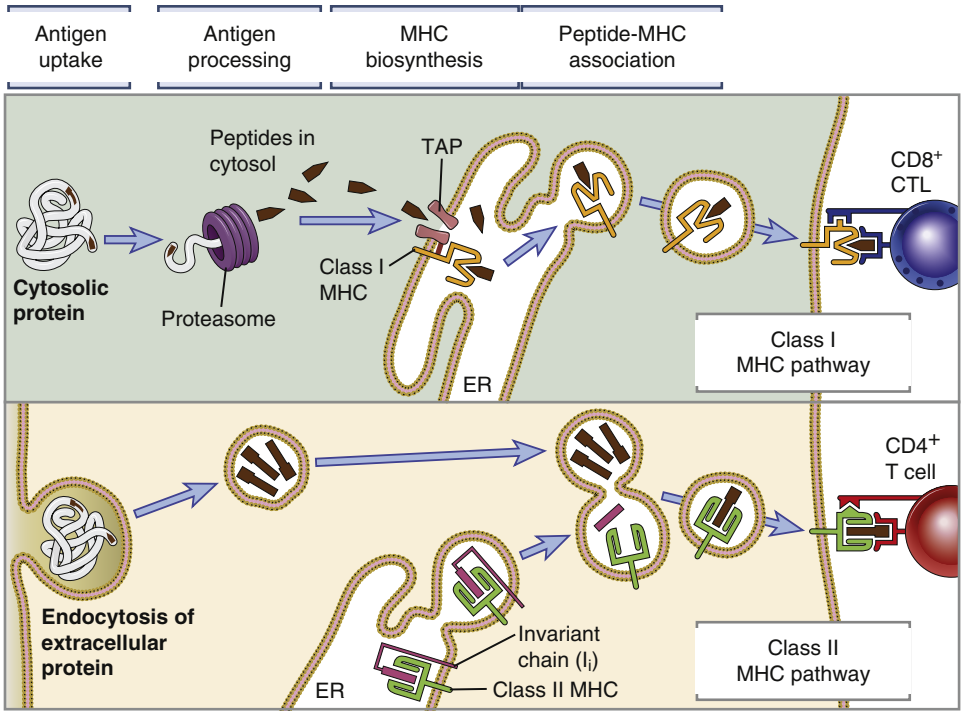


• **Figure 1-15** The schematic diagrams illustrate the different regions of the MHC class I *(left)* and class II *(right)* molecules (not drawn to scale). (From Abbas AK, Lichtman AH, Pillai S: *Basic immunology: functions and disorders of the immune system,* ed 4, Philadelphia, 2014, Saunders. Crystal structures courtesy Dr. P. Bjorkman, California Institute of Technology, Pasadena, CA.)

transmembrane glycoproteins involved with tissue typing and responsible for graft rejection in tissue transplantation (see Figures 1-15 and 1-16).

Clonal Selection

At any given time, the large numbers of T and B lymphocytes we have are composed of many clones, with each clone comprising only a small number of cells, all of which share the same receptor and specificity (Figures 1-17 and 1-18). When we have an infection (or receive a vaccine immunization), almost all the lymphocytes lack reactivity with the antigens from the pathogen. Lymphocytes that have receptors with affinity for specific antigen of the invading organism will be activated by engagement of their receptor by the antigen along with costimulatory signals. When this occurs, these cells will undergo rapid cell division and, through the resulting proliferation, temporarily represent a large proportion of the circulating T and B cells in the body. This will remain so until the pathogen is cleared by the resulting immune response. It usually takes 3 to 7 days of lag time for this response to occur on initial exposure. Once that pathogen is cleared, the number of lymphocytes will return to normal. Some of the cells that are reactive with the pathogen will persist as B and T memory cells in secondary lymphoid tissues. If the same pathogen is encountered again in the future, the lag time will be shorter (Figure 1-19). In addition, each time there is an exposure to antigens of a particular pathogen, there is competition for antigen among the memory cells. As a result, the cells with higher affinity (i.e., antigen receptors that bind the antigen more tightly and effectively) will be more likely to be stimulated. This process of "natural selection" of cells with the most avid and effective receptors results in the phenomenon of affinity maturation. Thus, multiple exposures not only decrease the lag time, but also increase the efficacy and efficiency of the adaptive immune response.



• **Figure 1-16** Antigen processing and presentation. Antigen presentation to CD8+ T cells *(upper panel):* Protein antigens for viral or tumor-specific antigens present in the cytosol of any nucleated cell are broken up into peptides by a proteasome. They are then associated with class I major histocompatibility complex (MHC) molecules on the surface of the cell and recognized by the T cell receptor on the CD8+ cell. Antigen presentation to CD4+ T cells *(lower panel):* Protein antigens are taken up by endocytosis or phagocytosis and are then within a lysosome of an antigen-presenting cell. Once in the lysosome, they are broken up into peptides by hydrolytic enzymes, associated with class II MHC molecules on the surface of the cell, and recognized by the T cell receptor on the CD4+ cell. (From Abbas AK, Lichtman AH, Pillai S: *Basic immunology: functions and disorders of the immune system,* ed 4, Philadelphia, 2014, Saunders.)

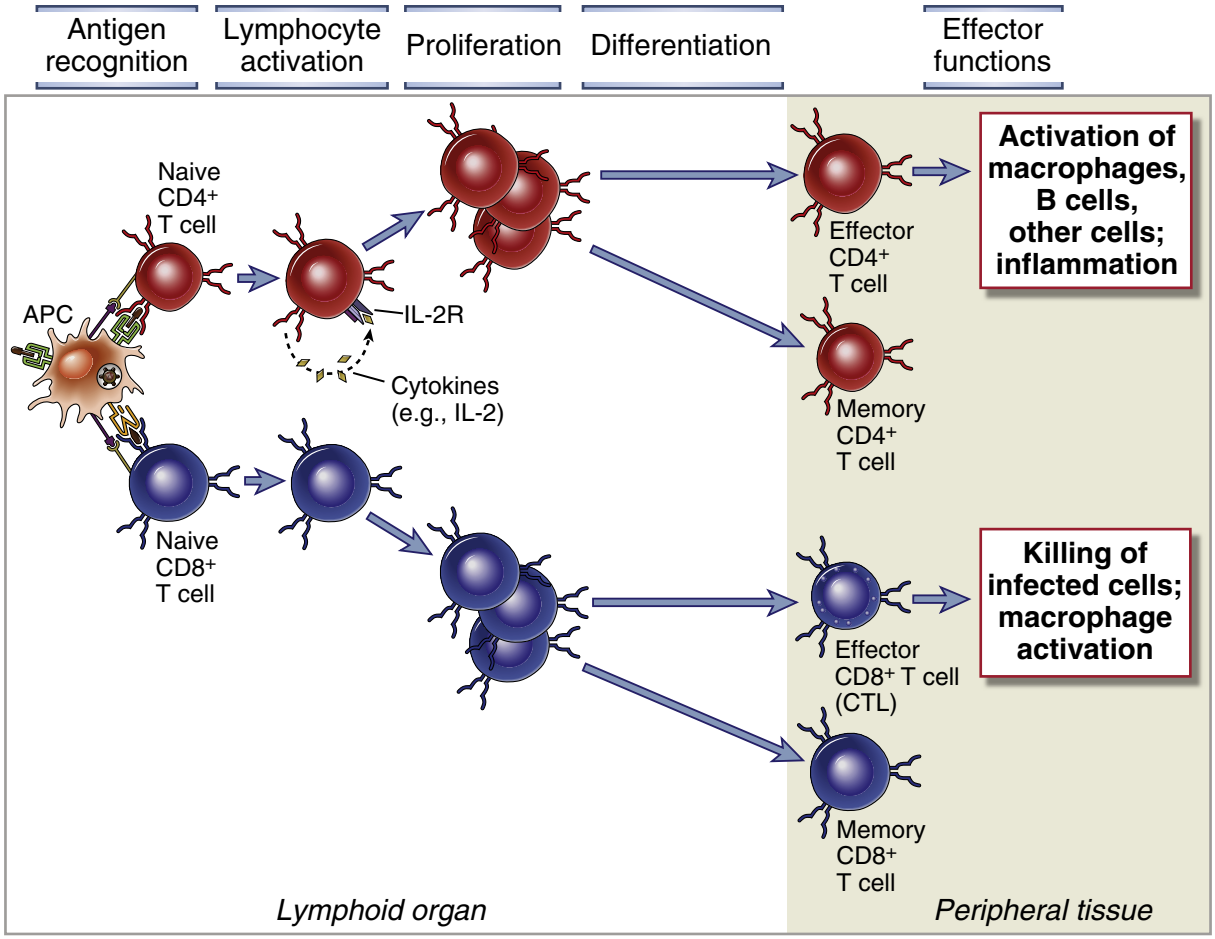
T Cell Maturation in the Thymus

B cells mature in the bone marrow, and essentially all B cells that effectively rearrange their germ line DNA to a functional surface antibody receptor become part of the B cell repertoire. This can result in B cells that generate antibody molecules that recognize self antigens (i.e., autoantibody), and this sets up the potential for autoimmune disease. For most people, autoimmunity is prevented by the T cell maturation process that occurs in the thymus. Immature T cells generated in the bone marrow mature and rearrange their germ line DNA and express T cell receptors in the thymus. As the T cells mature, thymic stromal cells assess the level of affinity of the T cell receptor for the self-MHC molecules on their surface. T cells with receptors that have low affinity for self-MHC are positively selected because they have good potential to recognize processed foreign antigen in association with self-MHC with high affinity. Those without affinity for self-MHC are not selected. T cells that have high affinity for self-MHC are deleted because they would likely be autoreactive and promote autoimmune disease. This process prevents cell-mediated autoimmune disease and prevents autoimmune disease mediated by autoantibody, because autoreactive B cells will not have T cells reactive with autoantigens to provide the required T cell help in the form of cytokine growth factors.

Cellular Immunity

Once CD4+ T_h cells leave the thymus, they circulate throughout the body. As they move through T cell–rich zones in the secondary lymphoid tissue, they sample foreign antigen presented by dendritic cells. If they encounter antigen that their T cell receptor recognizes, they are stimulated. They exchange stimulatory signals in the form of cytokines and reciprocal receptor engagement. The T cell then proliferates and expands so that many clones of the pathogen- or antigen-specific T cell are produced and circulate throughout all the secondary lymphoid tissues to help eliminate the pathogenic organism.

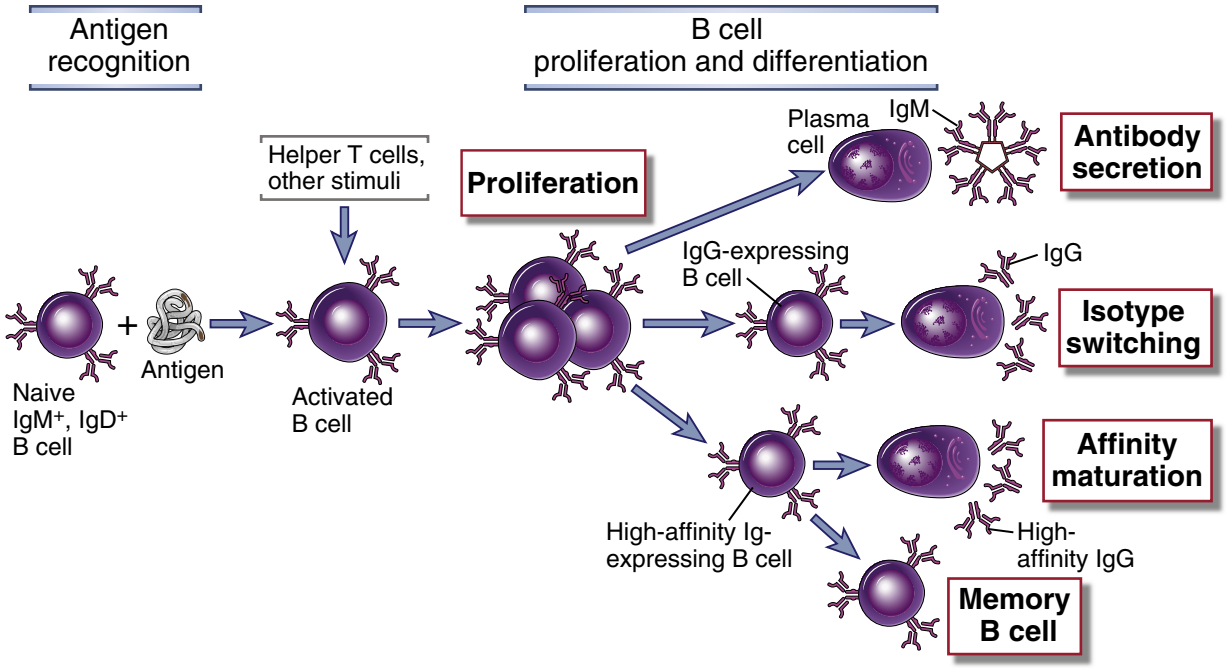
Depending on the type of interaction and signals received from the antigen-presenting cell, the T_h cell is further differentiated into one of several T_h subsets.[8] Each produces a different set of growth factors that support an immune response that is tailored to the particular pathogen (e.g., bacteria/extracellular versus virus/intracellular). The T cell subsets are T_{h1}, T_{h2}, Th_{17}, and T_{fH} cells. T_{h1} cells secrete IL-2, IFN-γ, and TNF-α and especially support cellular immune responses to viral infections.



• **Figure 1-17** Clonal selection T cells. Processed antigen is presented to CD4+ and CD8+ T cells that have receptors specific for the antigen (i.e., there is clonal selection of these particular T cells). There is then activation and clonal expansion followed by effector helper and cytolytic function. (From Abbas AK, Lichtman AHH, Pillai S: *Cellular and molecular immunology,* ed 8, Philadelphia, 2015, Saunders.)

These cytokines are important in stimulating macrophage–monocytes, T_{CTL} cells, and NK cells. T_{h2} cells secrete IL-4, IL-5, IL-10, and transforming growth factor (TGF)-β, which support B cell growth and differentiation and, therefore, antibody responses important for extracellular bacterial infections. Th_{17} cells secrete IL-6, IL-17, IL-22, and TNF-α, which support innate immunity and antibody responses (Figure 1-20).[9] T_{fH} cells are unique helper cells that reside in the B cell–rich zone of secondary lymphoid tissue where lymphoid follicles are located. They help support and regulate B cell growth and maturation within the lymphoid follicle (Figure 1-21).[10,11] T_{reg} cells secrete IL-10 and TGF-β, which regulate and dampen the adaptive immune response. These cytokines especially inhibit T_{h1} cytokines. It is important to note that during an adaptive immune response to an infection, no one particular T helper subset is exclusively active. Rather, all or many are active, and some predominate as appropriate to the particular type of infection. For example, one may have both T_{h1} and T_{h2} helper cells supporting antibody production in response to a bacterial infection. The T helper cell functions to provide growth factors to stimulate and support the proliferation and differentiation of the appropriate effector cells to control the infection (see Figures 1-17, 1-20, 1-22, and 1-23).

When the infection is intracellular and demands a cellular immune response, T_{h1} cytokines (IL-2, IFN-γ, TNF-α) predominate. Phagocytes, T_{CTL} cells, and NK cells are activated and kill virally infected cells to clear the pathogen. In the case of T_{CTL}, viral antigens expressed on the surface of infected cells are presented in the context of MHC class I molecules. The cytolytic T cell then kills the infected cell by releasing perforin (cytolytic proteins located in the granules of T_{CTL} that, upon degranulation, insert into the plasma membrane of the target cell, forming a pore) to assist in delivering granzymes that are also released to the cytoplasm, where they induce apoptosis or programmed cell death. NK cells also kill virally infected cells. Rather than the specific way in which T_{CTL} cells recognize viral antigen, NK cells can recognize a wide variety of virally infected cells and kill them by inducing apoptosis similarly to T_{CTL} cells. In addition, NK cells have Fc receptors that can bind the constant region of opsonizing antibody. If antibody is produced that binds to viral antigens on the surface of a virally infected cell, the NK cell can use



• **Figure 1-18** Clonal selection B cells. B cells recognize native antigen and receive T cell help in the form of cytokine growth factors. They are then activated, and clonal expansion occurs. During this process the B cells further differentiate into antibody-secreting plasma cells and B memory cells. (From Abbas AK, Lichtman AHH, Pillai S: *Cellular and molecular immunology,* ed 8, Philadelphia, 2015, Saunders.)

antibody-dependent cellular cytotoxicity to identify and then kill the cell (see Figures 1-22 to 1-26).

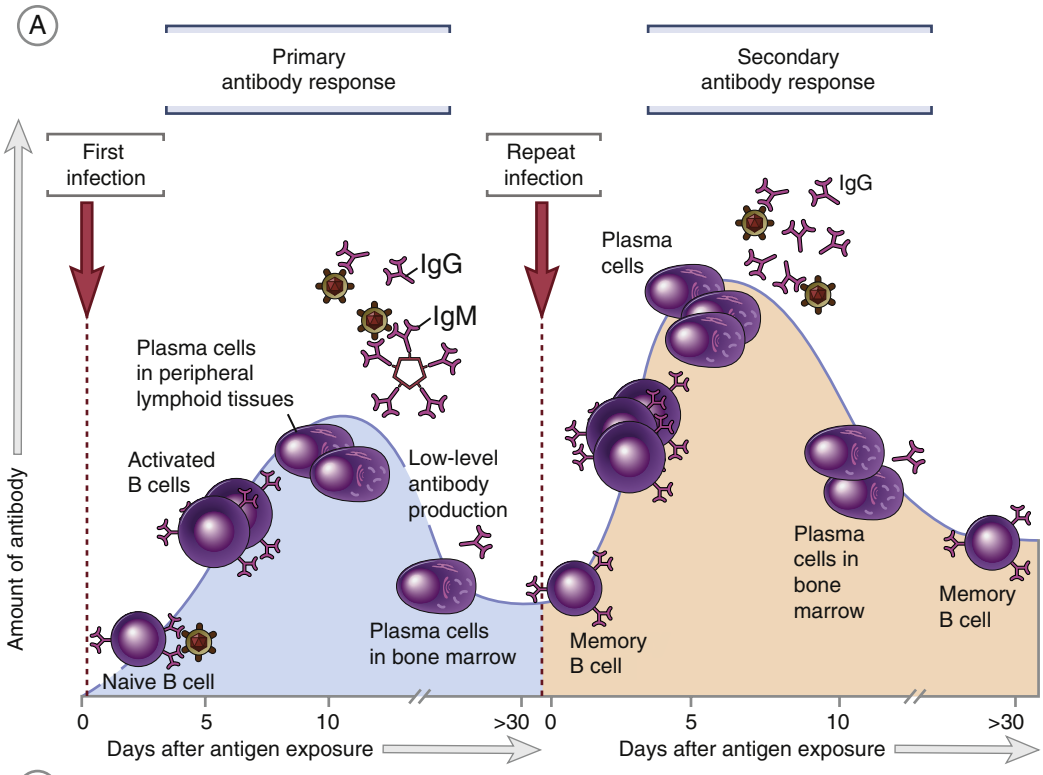
Humoral Immunity

When an infection involves an extracellular pathogen such as a bacterial species, antibody production becomes important for antigen-specific opsonization and toxin neutralization and clearance (see Figures 1-18, 1-19, 1-23, and 1-27). T_{h2} (and sometimes T_{h1} and Th_{17}) cells provide the cytokine growth factors that support proliferation and differentiation of antigen-specific B cells. B cells express antibodies, all with the same specificity (i.e., the same hypervariable region) on a particular B cell, on their cell surface to function as their antigen receptors. Upon activation, these B cells then further differentiate into B memory cells and antibody-secreting plasma cells that produce the same antibody that bound to the antigen.[12] Antibody then binds to the surface of the pathogen, opsonizing it. The antibody may also fix complement through the classical pathway that can further opsonize the bacterium by depositing C3b on its surface. Phagocytes have Fc receptors that bind the constant region of antibody molecules that have bound to the surface of a pathogen or toxin. They also have C3 receptors that allow them to utilize the opsonin C3b. Furthermore, the bacteria can be lysed by the complement membrane attack complex (see Figure 1-27).

Antibody molecules have a characteristic Y-shaped structure comprising two heavy and two light polypeptide chains, each containing hypervariable regions (fraction antibody binding, $F(ab)_2$), which account for the specificity of the molecule in binding to antigen, and constant regions (fraction crystallizable [Fc]), which result in biological activity such as binding to Fc receptors on phagocytic cells. Antibody can be of different functional classes (immunoglobulin [Ig] M, IgG, IgA, IgE, and IgD) and subclasses (IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2). Functions of antibodies include activation of complement, prevention of attachment of microbes, opsonization, and agglutination and immobilization of pathogens.

In the primary antibody response, IgM is usually produced. It forms a structure of five basic IgM units (a pentamer) bound together by small polypeptide chains, resulting in a large molecule in the circulation that is highly effective in activating complement and in immobilizing bacteria by binding to flagella. Monomeric IgM (single unit) is found on the surface of B cells, where it functions as an antigen receptor. With continued exposure to antigen or infection, class switching can occur and is under the control of T_h cells based on the cytokines being produced. Antibodies of the IgM and IgG classes are typically found circulating in serum. Similar to IgM, IgG also activates complement, and being a smaller molecule existing as a single unit (a monomer), it can leave the circulation and enter the tissues of the body. IgG performs a unique role in pregnancy, crossing the placenta to enter the fetal circulation and thus provide immune protection to the fetus while its immune system is still developing.

Antibody of the IgA class is typically found in secretions, including saliva, tears, and mucus. It is found as a monomer and a dimer, and the dimeric form is transported across mucosal surfaces to enter the lumen of the gastrointestinal, genitourinary, and respiratory tracts. This secreted form of IgA (S-IgA) provides the main form of antibody



B

	Primary response	Secondary response
Induced by	All immunogens	Mainly protein antigens
Peak response	Smaller	Larger
Antibody isotype	Usually IgM>IgG	Relative increase in IgG and, under certain situations, in IgA or IgE (heavy-chain isotype switching)
Antibody affinity	Lower average affinity, more variable	Higher average affinity (affinity maturation)

• **Figure 1-19** Primary and secondary response. When B cells encounter antigen in the primary response, there is a long lag time before the B cell differentiates into a plasma cell and secretes antibody. This antibody is mostly IgM and of lower affinity. With subsequent exposure in the secondary response, B memory cells compete for antigen. Cells with surface antibody receptors with the highest affinity will more readily bind antigen and become stimulated. This natural selection process increases the affinity of the antibody with each exposure to antigen. During the secondary response, class switch usually occurs and the lag time is shorter. (From Abbas AK, Lichtman AH, Pillai S: *Basic immunology: functions and disorders of the immune system,* ed 4, Philadelphia, 2014, Saunders.)

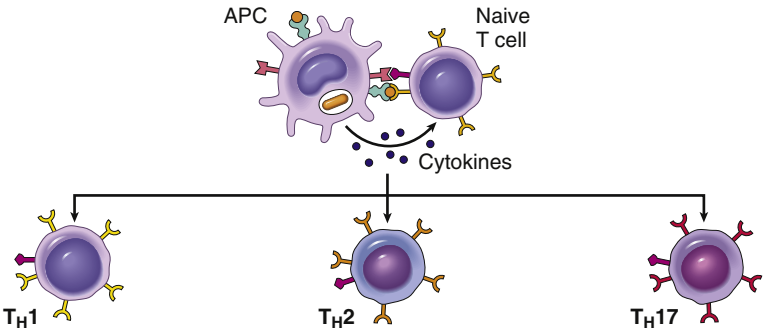
protection for mucosal surfaces. S-IgA therefore plays a key role in defense against infection by blocking the attachment of microorganisms to the mucosa.

IgD is mainly located on the surface of B cells and, along with monomeric IgM, functions as an antigen receptor.

Antibody of the IgE class is typically found free in only minute amounts, and it has an important role in anaphylaxis. Almost all the IgE produced by plasma cells is rapidly bound by mast cells, basophils, and eosinophils that have very high affinity Fc_ε receptors on their surface that bind IgE. When multiple IgE molecules bound by Fc_ε receptors on the cell surface bind antigen and are therefore cross-linked, mast cell, basophil, or eosinophil degranulation occurs. This can result in immediate type I hypersensitivity. These cells appear to be important in the immune response to parasitic infections (see Figures 1-24 and 1-28).

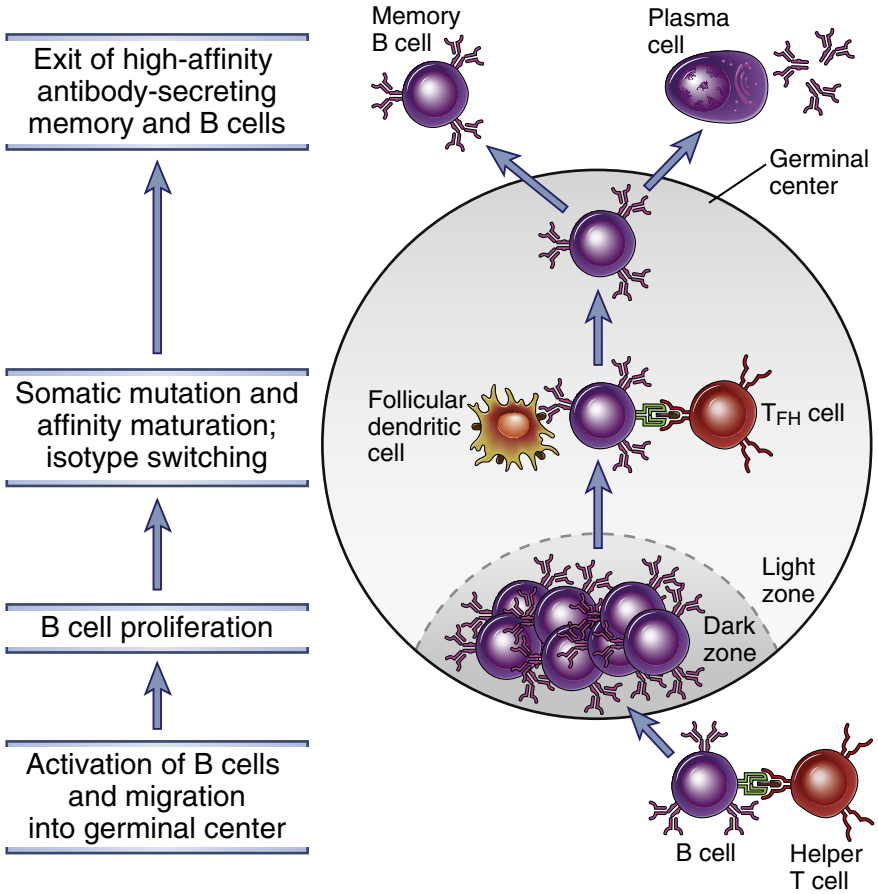
Regulation of the Immune Response

The immune response is heavily regulated at every level. Every cell and cytokine feeds back in some fashion to prevent an excessive response to the invading pathogen.

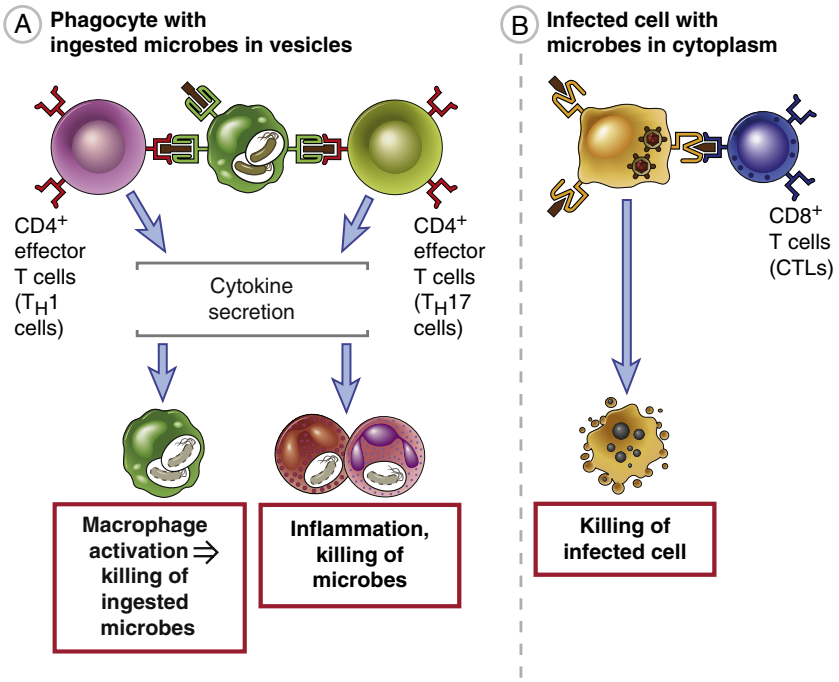


	T_H1	T_H2	T_H17
Major cytokines produced	IFN-γ	IL-4, IL-5, IL-13	IL-17, IL-22
Cytokines that induce this subset	IFN-γ, IL-12	IL-4	TGF-β, IL-6, IL-1, IL-23
Immunological reactions triggered	Macrophage activation, stimulation of IgG antibody production	Stimulation of IgE production, activation of mast cells and eosinophils	Recruitment of neutrophils, monocytes
Host defense against	Intracellular microbes	Helminthic parasites	Extracellular bacteria, fungi
Role in disease	Autoimmune and other chronic inflammatory diseases (such as IBD, psoriasis, granulomatous inflammation)	Allergies	Autoimmune and other chronic inflammatory diseases (such as IBD, psoriasis, MS)

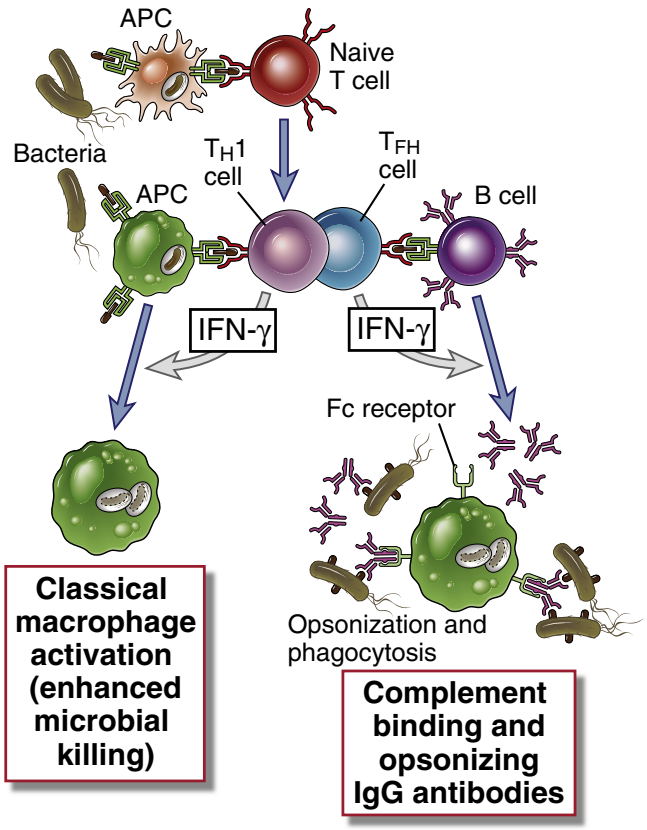
• **Figure 1-20** T helper (T_h) cell subtypes and their function. Naive $CD4^+$ T cells may differentiate into distinct subsets of effector cells in response to antigen, co-stimulators, and cytokines. The columns to the right list the major differences between the best-defined subsets. (From Kumar V, Abbas AK, Aster JC: *Robbins and Cotran pathologic basis of disease,* ed 9, Philadelphia, 2015, Saunders.)



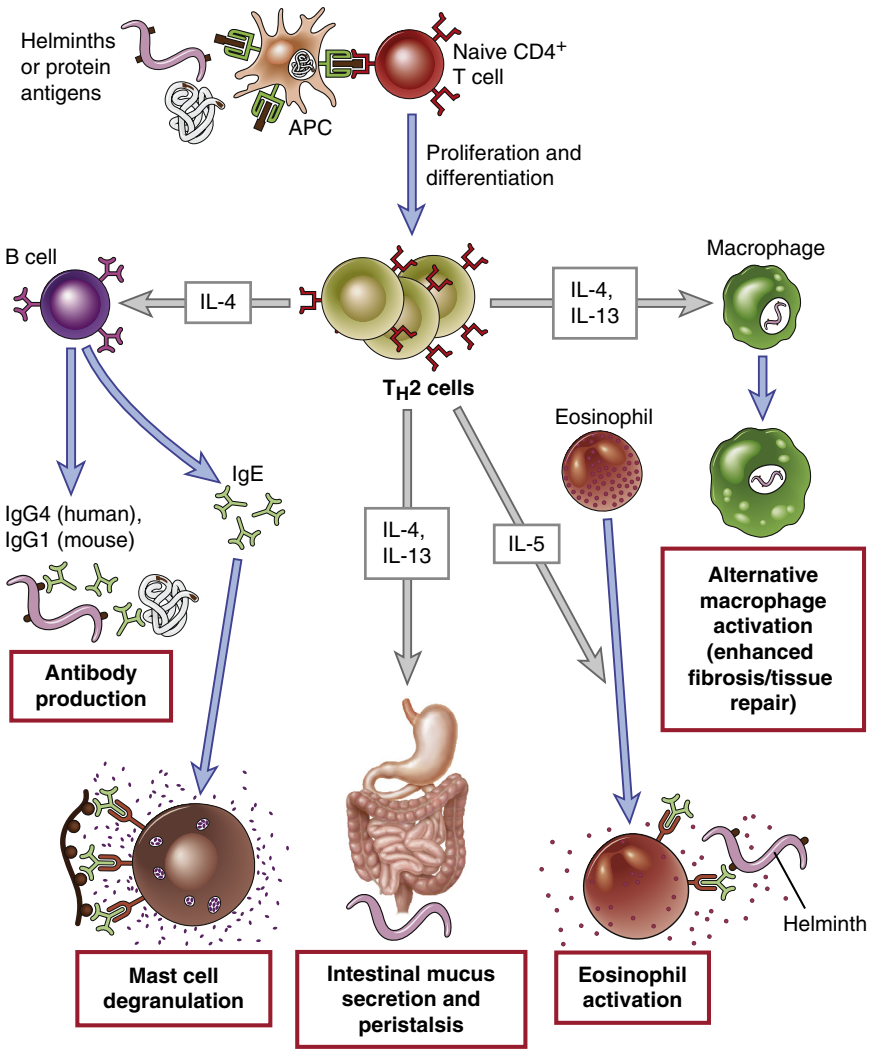
• **Figure 1-21** T_{fH} helper cells and B cell growth and differentiation. T_{fH} cells are unique helper cells that reside in the B cell–rich zone of secondary lymphoid tissue where lymphoid follicles are located. They help support and regulate B cell growth and maturation within the lymphoid follicle. (From Abbas AK, Lichtman AHH, Pillai S: *Cellular and molecular immunology,* ed 8, Philadelphia, 2015, Saunders.)



• **Figure 1-22** T cells and killing of extracellular and intracellular pathogens.T_{h1} and T_{h17} cells secrete cytokines that support clearance of extracellular pathogens **(A);** T_{CTL} cells that are stimulated by cytokines identify host cells with intracellular infection and kill those cells **(B).** (From Abbas AK, Lichtman AH, Pillai S: *Basic immunology: functions and disorders of the immune system,* ed 4, Philadelphia, 2014, Saunders.)



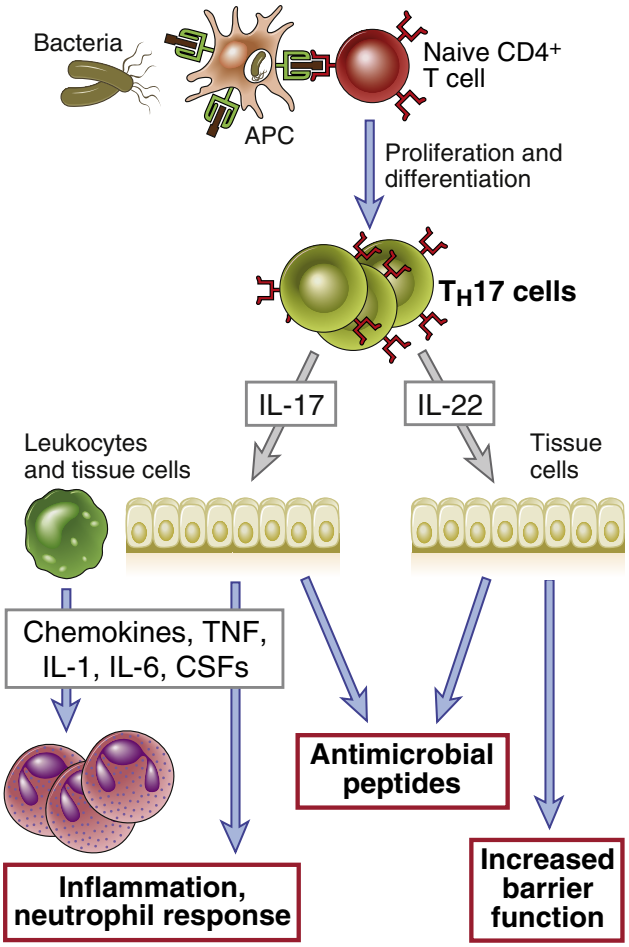
• **Figure 1-23** T_{h1} cells and response to bacterial pathogens.T_{h1} cells secrete cytokines (e.g., interferon γ [IFN-γ]) that support activation of phagocytic cells and stimulate IgG antibody production by B cells/plasma cells and therefore opsonization of bacteria directly and also through complement fixation. APC, Antigen-presenting cell. (From Abbas AK, Lichtman AHH, Pillai S: *Cellular and molecular immunology,* ed 8, Philadelphia, 2015, Saunders.)



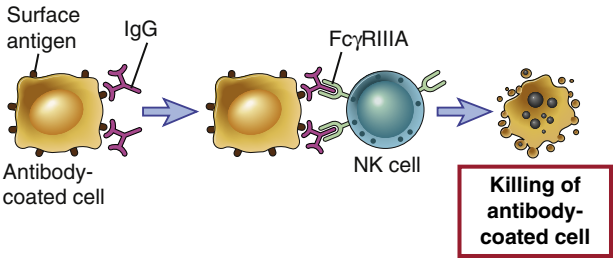
• **Figure 1-24** T_{h2} cells and the response to parasitic infection.T_{h2} cytokines induce inflammation, support production of IgG_4 and IgE, and activate mast cells and eosinophils in response to parasitic infection. APC, Antigen-presenting cell. (From Abbas AK, Lichtman AH, Pillai S: *Basic immunology: functions and disorders of the immune system,* ed 4, Philadelphia, 2014, Saunders.)

An inflammatory response that is excessive, especially if it is not directed specifically toward the pathogen, has the potential to cause tissue damage and harm the host (which can be recognized as the signs and symptoms of specific chronic diseases). This text does not allow for a comprehensive and complete review of every regulatory process in the immune response. What follows are some examples of immunoregulation.

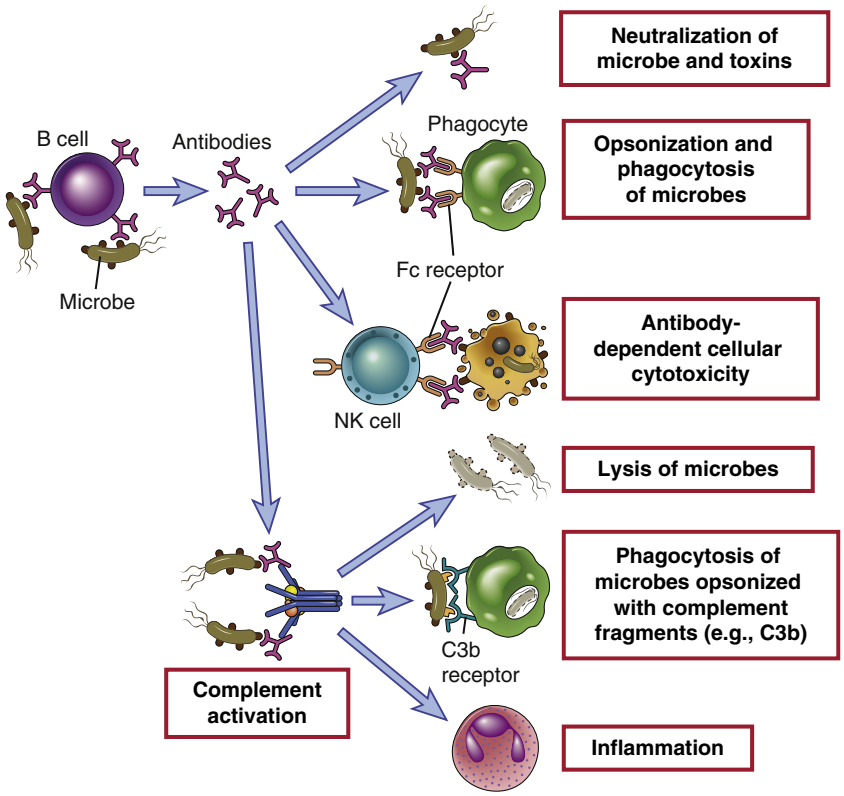
One such example involves the pathogenic condition *Papillon-Lefèvre syndrome*. In this condition, a defective enzyme prevents normal immunoregulation and results in a systemic disease that includes severe periodontitis. Affected individuals have a defective cathepsin C gene. As bacterial dental plaque (biofilm) accumulates on the teeth, bacterial macromolecules induce cells of the periodontal tissues to release the chemokine macrophage inflammatory protein-1α (MIP-1α). This chemokine (also known as chemokine [C-C motif] ligand 3 [CCL3]) is then chemotactic for phagocytes, especially neutrophils. As neutrophils accumulate at the site, they release inactive serine proteases. In systemically healthy individuals, the serine proteases are activated when they are cleaved by the protease cathepsin C. When activated, the serine proteases digest the MIP-1α and thus prevent excessive accumulation of neutrophils. In patients with Papillon-Lefèvre syndrome, the cathepsin C gene has a mutation rendering the enzyme inactive. The serine proteases from the neutrophils are therefore also inactive and unable to digest and limit the local concentration of MIP-1α. As a result, neutrophils accumulate at high levels in the periodontal tissues and are activated by bacteria-specific macromolecules and inflammatory cytokines present in the local environment. The resulting high numbers of activated neutrophils in the connective tissues release hydrolytic enzymes that destroy the connective tissue and bone that normally support the teeth. This typically results in early-onset and rapidly progressive periodontitis, typically leading



• **Figure 1-25** T_{h17} cells and the response to bacterial infection.T_{h17} cytokines induce inflammation and support innate immunity in addition to antibody production. APC, Antigen-presenting cell; CSF, colony-stimulating factor; IL, interleukin; TNF, tumor necrosis factor. (From Abbas AK, Lichtman AH, Pillai S: *Basic immunology: functions and disorders of the immune system*, ed 7, Philadelphia, 2014, Saunders.)



• **Figure 1-26** Natural killer (NK) cell killing of intracellular pathogen through antibody-dependent cellular cytotoxicity. NK cells utilize their Fc receptor to recognize host cells that are infected with an intracellular pathogen (e.g., virus, *Mycobacterium tuberculosis*) and also bound by pathogen-specific antibody. NK cells kill infected cells once they are recognized. (From Abbas AK, Lichtman AH, Pillai S: *Basic immunology: functions and disorders of the immune system,* ed 4, Philadelphia, 2014, Saunders.)



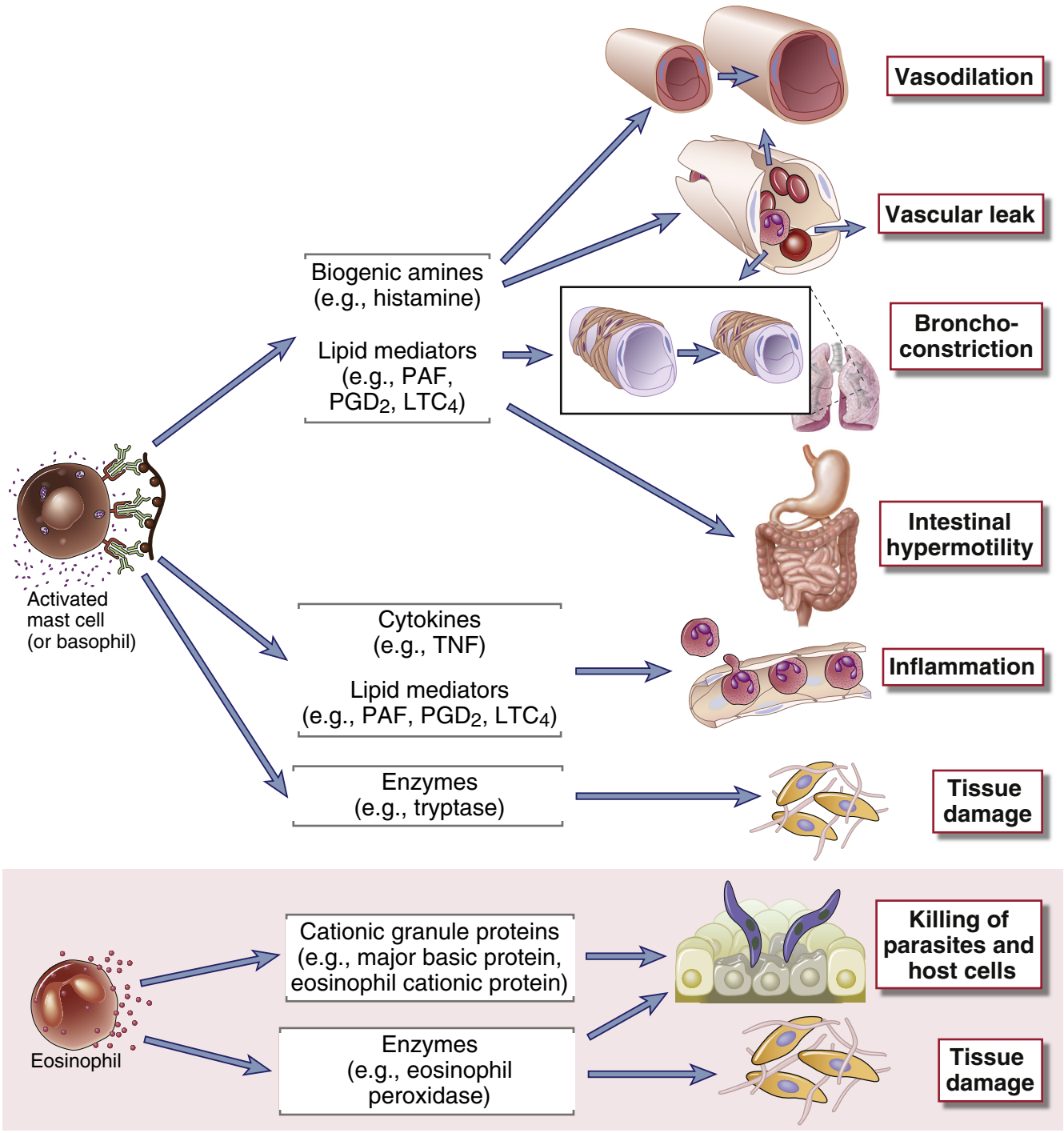
• **Figure 1-27** Antibody and the response to infection. Antibody molecules function in the defense against infection in several ways. They neutralize toxins, opsonize microbes to facilitate phagocytosis (either directly or indirectly through complement fixation), and can potentiate inflammation and cell lysis through complement activation. NK, Natural killer. (From Abbas AK, Lichtman AH, Pillai S: *Basic immunology: functions and disorders of the immune system,* ed 4, Philadelphia, 2014, Saunders.)

eventually to loss of all teeth. An additional symptom of this disease is palmar-plantar keratosis.

T_h cells, especially $CD4^+$ cells, are central to regulation of immunity. In a T_{h1}-dominated response to an intracellular pathogen, high levels of IL-2 and IFN-γ are released. This promotes the activation of macrophage–monocytes and the release of additional cytokines that support cellular immunity including TNF-α, IL-1, IL-12, and IL-18. In addition to supporting cellular immunity, IFN-γ downregulates the activity of T_{h2} cells and their cytokines, which would dampen B cell activation and the production of antibody. Conversely, when there is an infection with an extracellular pathogen, T_{h2} cells and their cytokines predominate; these include IL-4, IL-5, IL-6, IL-10, and IL-13, which support the development of B cells that eventually differentiate into antibody-secreting plasma cells. In addition, IL-10 limits the release of IFN-γ by T_{h1} cells. The T_{h2} cytokines IL-4, IL-5, IL-6, and IL-13 also limit the release of cytokines by the macrophage–monocytes.

T_{reg} cells (also $CD4^+$) are important cells in immunoregulation. They recognize antigen presented to them and, as they are activated, release IL-4, IL-10, and TGF-β. These cytokines reduce the activity of dendritic cells so that antigen presentation is dampened. These cytokines also directly inhibit the release of IL-2, IL-5, and TNF-α, thereby reducing the activity of T_h and T_{CTL} cells.

The activity of the immune system is also subject to the effects of the emotional state of the individual. Psychological stress, especially if chronic, induces the release of cortisol and catecholamines. Cells involved with immunity have receptors for circulating cortisol and catecholamines, both of which are immunosuppressive. In fact, chronic stress can lead to an increased susceptibility to infection.



• **Figure 1-28** Mast cells and eosinophils and the response to parasitic infection. Mast cells and basophil mediators include biogenic amines and lipid mediators stored preformed in granules as well as lipid mediators, which are largely newly synthesized on cell activation. The biogenic amines and lipid mediators induce vascular leakage, bronchoconstriction, and intestinal hypermobility, all components of the immediate response. Cytokines and lipid mediators contribute to inflammation, which is part of the late-phase reaction. Enzymes probably contribute to tissue damage. Activated eosinophils release preformed cationic proteins as well as enzymes that are toxic to parasites and host cells. Some eosinophil granule enzymes probably contribute to tissue damage in chronic allergic diseases. LTC_4, Leukotriene C_4; PAF, platelet activating factor; PGD_2, prostaglandin D_2; TNF, tumor necrosis factor. (From Abbas AK, Lichtman AHH, Pillai S: *Cellular and molecular immunology,* ed 8, Philadelphia, 2015, Saunders.)

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2

Bacteriology of the Head and Neck Regions

THOMAS S. MURRAY AND TODD CASSESE

The head and neck regions of the human body are home to a wide variety of bacteria that inhabit both the skin and mucosal surfaces. Traditionally, the clinical microbiology laboratory has used various nutrient-rich culture media to grow and identify bacteria from patient specimens. Phenotypic and biochemical properties of the isolated bacteria distinguish pathogens from commensal organisms. Although these methods remain the cornerstone of work in the microbiology laboratory, the introduction of molecular techniques to identify bacteria that both colonize and infect the human body is changing our understanding of the important role bacteria play in both causing disease and maintaining health.

The human microbiome is the microbial population, numbering in the trillions, that inhabits the human body. The vast majority of these bacteria are not recovered with standard growth techniques in the clinical microbiology laboratory. A recent revolution has occurred in our understanding of the interaction between humans and bacteria, a result of deep sequencing DNA technology that has expanded our knowledge of the microbes that constitute this diverse, complex population.[1] This technology allows for the sequencing of hundreds of thousands of bacterial DNA sequences from a single specimen to identify all bacteria present in the sample. Generally, samples from a specific location or niche of the human body (e.g., oral cavity, sinuses, skin, adenoids) are collected and the microbial DNA from the sample is extracted. Next, a variable region of the bacterial gene encoding the 16s ribosomal RNA is amplified and sequenced, and compared with a reference database for bacterial identification. Major advantages of this technology are the ability to identify organisms that are not readily recovered by bacterial culture and the ability to gather information on large numbers of different bacteria from a single specimen.[2] The final result is a detailed population profile of bacteria for specific regions of the body, including different anatomic sites of the head and neck (Table 2-1).[3-8]

The National Institutes of Health recently established the Human Microbiome Project to document the different populations of bacteria that colonize the human body, including areas of the head and neck, to explore how differences in colonizing bacterial flora impact human health. Data produced from this project have led to a paradigm shift with renewed thinking about how infectious processes are likely due to the complex interactions between the host immune system, pathogenic bacteria, and the resident commensal microbes.[1] Literature is emerging specifically examining these relationships as they relate to infections of the head and neck, confirming the important role of commensal organisms and the host immune response in the clinical presentation of these infections.

Commensal Flora of the Head, Neck, and Oral Cavity

The application of deep sequencing to the human microbiome suggests that we are possibly colonized *in utero* and are certainly colonized with bacteria immediately at birth. A number of factors influence the evolution and development of the bacterial populations that colonize the skin, oral cavity, and gut. These factors include, but are not limited to, the type of delivery (vaginal versus cesarean section), breast feeding or formula, length of time in the hospital, exposure to antibiotics, transition to solid food, and the presence or absence of teeth.[9] The local environment also plays an important role in the kinds of bacteria present in a given location of the body. Environmental factors that will determine the bacterial population include, but are not limited to, the available nutrients, pH, moisture levels, other competitive bacteria occupying the same niche, and exposure to the host immune system. The microbiome is unstable early in life. A more mature, stable bacterial population that resembles those of adults emerges by 2 to 3 years of age.[9]

TABLE 2-1 Bacteria Flora Identified in Selected Niches of the Head and Neck from Children and Adults

Location (Reference)	Bacteria*
Outer ear (3)	*Alloiococcus otitis* *Corynebacterium otitidis* *Staphylococcus auricularis*
Inner ear† (4)	*Moraxella* Viridans streptococcus *Pasteurella* *Staphylococcus* *Corynebacteria* *Flavobacterium* *Carnobacterium* Comamonada
Sinuses, middle meatus (12, 13)	*Cyanobacteria* *Propionibacterium* *Staphylococcus* *Corynebacteria*
Esophagus (5)	Viridans streptococcus *Fusobacterium* *Neisseria* *Haemophilus* *Prevotella*
Larynx (11)	*Streptococcus* *Fusobacterium* *Prevotella* *Neisseria* *Gemella* *Parvimonas*
Tonsils, hyperplasia (7)	*Streptococcus* *Neisseria* *Prevotella* *Haemophilus* *Porphyromonas* *Gemella* *Fusobacterium*
Adenoids (14)	*Streptococcus* *Staphylococcus* *Haemophilus* *Fusobacterium* *Moraxella* *Prevotella* *Gemella* *Neisseria*
Nose (6)	*Staphylococcus* *Haemophilus* *Neisseria* *Moraxella* *Streptococcus*
Oral cavity, saliva, and plaque (8)	*Streptococcus* *Veillonella* *Corynebacterium* *Actinomyces* *Fusobacterium* *Rothia* *Prevotella* *Neisseria* *Haemophilus* *Porphyromonas*

*More common genera of bacteria have been selected. This is not a comprehensive list.
†These bacteria were recovered from healthy infants.

However, for a given individual, the microbiome can change over time with changes in overall health. In addition, there is significant variation across different individuals examining bacterial populations at the same anatomic site. Different physiologic states and behaviors have profound effects on the commensal flora of the individual presumably by altering the microenvironment for bacterial growth in favor of certain organisms at the expense of others. For example, smoking alters the microflora of the oral cavity, with increased numbers of potentially pathogenic bacteria present compared with the oral flora of nonsmokers.[10] In one study, patients with squamous cell carcinoma of the larynx had vastly different microbial populations compared with control patients with vocal cord polyps.[11] Changes to the mucosal surfaces because of chemotherapy can alter the microbial populations present. Through these studies and others, bacterial populations of different regions of the head and neck are currently being characterized for both healthy and diseased populations (see Table 2-1).

Given that laboratory cultivation techniques recover a limited number of organisms, it is not surprising that culture-independent techniques have identified previously unrecognized commensal flora, especially among the anaerobic bacteria. The increased sensitivity of these molecular techniques compared with culture-based approaches has also revealed large numbers of bacterial populations in healthy subjects at sites not previously thought to be colonized. For example, DNA-based studies of healthy adults have identified bacterial populations in the middle meatus of the sinus that include *Staphylococcus aureus, Staphylococcus epidermidis, Propionibacterium acnes,* and anaerobic *Cyanobacterium* sp.[12,13] While the diversity and populations of bacteria vary between individuals and studies, the common conclusion is that the sinuses of healthy asymptomatic individuals are colonized with bacterial populations, including potential pathogens such as *S. aureus*. It also raises the possibility that there are as yet unrecognized organisms contributing to clinical disease that do not grow under routine laboratory growth conditions.

In many cases bacterial infections of the head and neck are caused by bacterial flora that often colonize the skin, oral cavity, or respiratory tract and contiguous spaces without causing disease. When there is disruption of this homeostasis between host and microbes because of a change in the host or bacterial population, certain bacteria (e.g., *S. aureus, Streptococcus pneumoniae*) behave as pathogens that cause invasive infection and clinical symptoms. Surgically removed adenoids and tonsils contain common pathogens that cause acute otitis media, and these organisms can migrate from the posterior airway to the inner ear to cause infection when homeostasis is disrupted.[14,15]

Pathogenesis of Acute and Chronic Bacterial Infections

Acute Infection

Acute infections occur when a change in the host environment or the bacterial population in a region permits

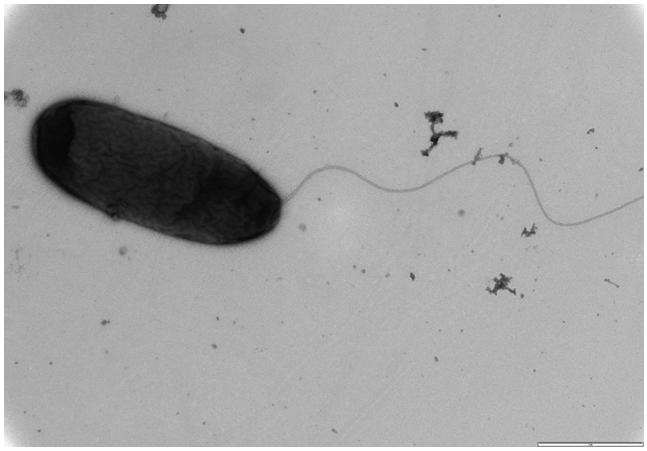
invasion of a pathogenic bacteria, resulting in clinical signs and symptoms consistent with infection. Acute infection initially requires that bacteria express surface molecules that facilitate adhesion to an epithelial, mucosal, or artificial surface. Attached bacteria then secrete a variety of virulence factors that interact with host tissue and the immune response to produce inflammation consistent with symptomatic infection. If the bacteria are able to penetrate the host surface into a previously sterile site and replicate, effectively dealing with host defenses, then invasive disease occurs. These general principles of acute infection are applicable to multiple pathogens discussed below.

Surface Adhesion

The first step in acute infection is adhesion to a surface, typically a mucosal surface for head and neck infections. Bacteria may possess one or more multiple surface structures or surface-exposed proteins that facilitate adhesion to the epithelial cell layer. For example, both *S. pneumoniae* and *Haemophilus influenzae*, pathogens of the upper respiratory tract and contiguous spaces, express the surface phosphorylcholine ChoP, which in pneumococcus binds the platelet-activating factor receptor found on epithelial cells of the nasopharynx. Multiple pathogens—including *Pseudomonas aeruginosa*, a common cause of otitis externa—display fimbriae on the cell surface, thin structures that extend and retract, attaching the bacteria to surfaces such as epithelial cells. *P. aeruginosa* also expresses a single flagellum used for motility to get the bacterium to the host surface and attachment once it makes contact with the surface (Figure 2-1).

Virulence Factor Production

In many cases, bacteria successfully colonize the mucosal cell layer without causing clinical symptoms and disease. However, when bacteria invade a sterile space or secrete virulence factors, the ensuing tissue damage and inflammatory response results in clinical signs and symptoms.



• **Figure 2-1** Bacterial flagellum. Electron micrograph showing *Pseudomonas aeruginosa* with a single, unipolar flagellum important for interaction with the host innate immune system (via Toll-like receptor 5) and bacterial adhesion to host surfaces.

Bacteria have evolved an incredible variety of surface-associated and secreted virulence factors to cause cellular damage and inactivate host defenses. For example, gram-negative pathogens have lipopolysaccharide (LPS) on the cell surface that interacts with Toll-like receptor (TLR) 4 of the innate immune system to generate a robust proinflammatory response. Although this response can lead to bacterial death and resolution of infection, it can also cause tissue damage that facilitates bacterial invasion across surfaces into sterile sites. Several different pathogens of the head and neck secrete proteins that form pores in host cell membranes, disrupting the host cell and resulting in lysis. Common examples discussed later in more detail include streptolysin produced by *S. pneumoniae* and the *S. aureus* Panton-Valentine leukocidin (PVL) toxin. In addition to secreting virulence factors directly into the environment, several bacteria possess needlelike structures that insert into host cell membranes. These structures allow bacteria to pump virulence factors directly into the cytosol of host cells, resulting in cell death and facilitating bacterial invasion. *P. aeruginosa* has a type 3 secretion system that injects molecules into host cells including ExoU, a phospholipase expressed by some strains that causes rapid cell necrosis.

Surviving the Host Response to Infection

Given the vast array of defenses the body has to protect against infection, bacteria are armed with multiple mechanisms to survive within a dangerous host environment. Pathogenic bacteria avoid phagocytosis by neutrophils and macrophages through a variety of means. Many gram-negative and gram-positive bacteria possess a polysaccharide capsule that is antiphagocytic and offers a competitive advantage compared with unencapsulated strains. In addition to the extrapolysaccharide (EPS) capsule, surface proteins such as the M-protein of *Streptococcus pyogenes* (group A streptococcus) also prevent engulfment by host cells. A variety of secreted proteins disrupt opsonization by averting deposition of complement on the bacterial surface. Another important immune defense mechanism that bacteria must deal with is the neutrophil extracellular trap (NET), extracellular fibrils that engulf and destroy bacteria. Respiratory pathogens have developed numerous ways to protect themselves from NETs. Some secrete enzymes that degrade the DNA component of NETs (e.g., the DNAse of *S. pyogenes*), whereas others exhibit surface molecules that allow for survival within the NET after engulfment. In addition, many of the cytolytic pore-forming toxins described previously target immune cells. For example, PVL and pneumolysin can both lyse white blood cells recruited to the site of infection. Additional secreted proteins cleave antibody or complement or inactivate host defensin molecules to survive the host response.

Chronic Infection and Biofilm Formation

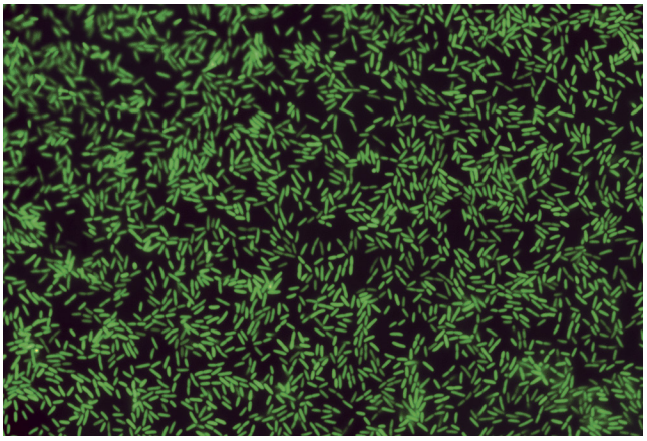
Under certain circumstances, an acute infection is not completely resolved despite an aggressive immune response with or without antimicrobial therapy, and chronic infection

results. A number of chronic infections of the head and neck, such as chronic otitis media and chronic rhinosinusitis, are challenging to treat with antibiotic therapy alone. The bacterial strategy for long-term survival in the host now known to be an important component of most chronic infections is the formation of surface-associated bacterial biofilms. Virtually all the pathogens of the head and neck described in this chapter form biofilms, which are organized communities of bacteria that colonize a surface and are resistant to antibiotic therapy.

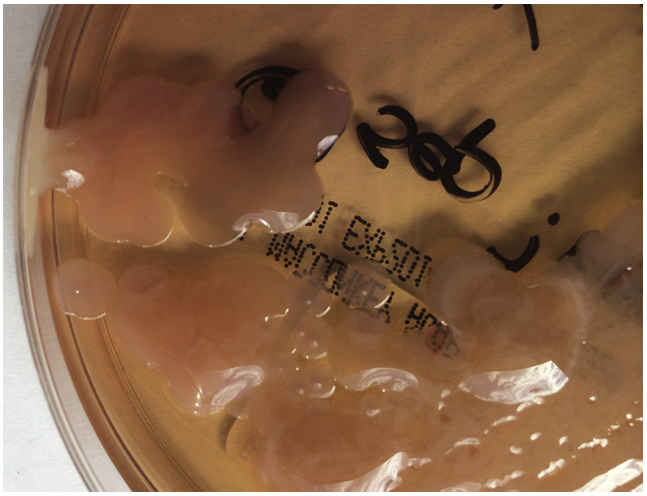
There is an inverse relationship comparing the bacterial lifestyle of tissue destruction, invasion, and acute infection with that of surface colonization, biofilm formation, and chronic disease. When the process of biofilm formation is initiated, genes encoding proteins required for biofilm formation are upregulated, whereas genes encoding proteins that are virulence factors during acute infection are downregulated. Alternatively, during acute infection when virulence factors for invasion are upregulated, genes encoding proteins required for biofilm formation are turned off. This paradigm argues that the same bacterial strain may display an acute or chronic phenotype, depending on the environment and host response, and may serve to explain how initial acute infections become chronic and more difficult to treat over time with the same strain of bacteria.

Biofilm formation follows a series of organized steps and is initiated with surface contact and adhesion (Figure 2-2). The presence of a foreign body provides an ideal surface for bacterial colonization, chronic infection, and biofilm formation. Biofilms form on teeth and contribute to dental caries, and they allow for bacterial colonization of mucosal and epithelial surfaces. Attachment to a foreign or host surface requires many of the same factors mentioned for acute infection, such as flagella, pili, or surface adhesins. Next, the bacteria aggregate into microcolonies followed by cell division and the formation of a mature biofilm with channels that allow for gas exchange and nutrient acquisition. Bacteria at the base of the biofilm exhibit decreased metabolism compared with bacteria closer to the surface; therefore, antibiotics that require metabolic activity, such as protein or cell wall synthesis, are less active if the drugs do penetrate the biofilm. The biofilm is protected by an extracellular matrix consisting of both bacterial and host material that inhibits both phagocytosis and antibiotic penetration. Some bacterial strains produce a mucoid phenotype because of an increase in the production of EPS (Figure 2-3). These mucoid strains are particularly difficult to eradicate and commonly cause chronic infection. Importantly, biofilms are not static, and bacteria released from the biofilm are capable of colonizing nearby surfaces, spreading infection within the host.

While the list of potential pathogens of the head and neck region is extensive (Table 2-2), several pathogens that more commonly cause invasive disease in multiple locations of the head and neck and those that cause disease in the oral cavity are discussed here in more detail.



• **Figure 2-2** Bacterial surface colonization. The initial step in both colonization and biofilm formation is attachment to a surface. In this example, *Pseudomonas aeruginosa* expressing green fluorescent protein adhere to the surface, colonizing a glass slide.



• **Figure 2-3** Exopolysaccharide, a bacterial virulence factor. Mucoid *Klebsiella pneumoniae* grown on MacConkey agar plates secrete an abundance of exopolysaccharide that in vivo prevents phagocytosis and antibiotic penetration.

Common Bacterial Pathogens of the Ear, Sinuses, and Contiguous Regions

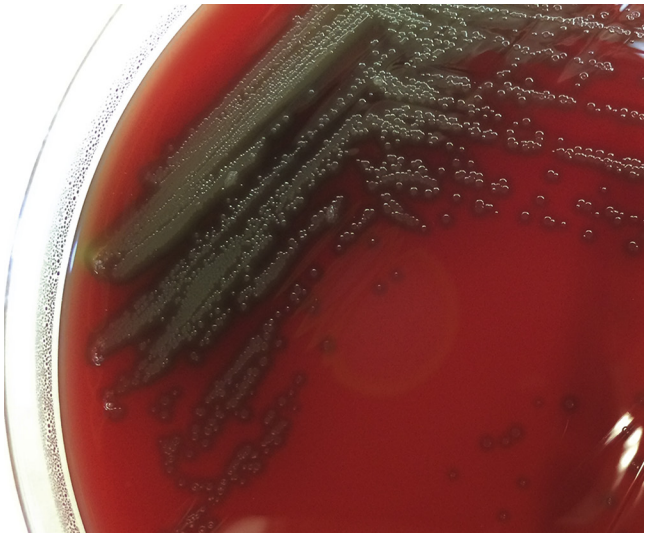
Streptococcus pneumoniae

Pneumococcus is a gram-positive, catalase-negative, facultative anaerobic bacterium that typically produces α-hemolysis (partial hemolysis) on blood agar plates (Figure 2-4). Gram stain classically reveals lancet-shaped diplococci. Pneumococci are susceptible to optochin, which is used for presumptive identification and distinguishes it from other pathogenic streptococci. The introduction of routine pneumococcal vaccination for all children in the United States to reduce invasive pneumococcal disease has also reduced the rates of acute otitis media in children caused by vaccine containing *S. pneumoniae* serotypes; however, it has not eliminated pneumococcus as a pathogen of the inner ear and upper respiratory tract, as 90 serotypes exist and the current protein conjugate vaccine formulation in routine use covers only 13 serotypes.

TABLE 2-2 Infections Caused by Bacteria Commonly Found in the Head and Neck

Bacteria	Common Infections of Head and Neck	Systemic Infectious Complications
Actinomyces species	Dental plaque, caries	Actinomycosis
Aggregatibacter actinomycetemcomitans	Periodontitis	Association with increased risk for CAD
Capnocytophaga species	Periodontitis (particularly juvenile periodontitis)	Endocarditis, fulminant sepsis in asplenic patients
Fusobacterium species	Periodontitis, pharyngitis, tonsillitis, peritonsillar abscess	Lemierre syndrome (septic thrombophlebitis of the internal jugular vein), sepsis
Haemophilus influenzae	Epiglottitis, rhinosinusitis, otitis media	Meningitis, septic arthritis, osteomyelitis, cellulitis, pneumonia, bronchitis
Moraxella catarrhalis	Otitis media, rhinosinusitis	COPD exacerbation, pneumonia
Prevotella intermedia	Dental plaque, caries, periodontitis	Brain or lung abscess
Staphylococcus aureus	Peritonsillar abscess, cervical lymphadenitis, rhinosinusitis	Pneumonia, endocarditis, skin and soft tissue infection, sepsis, toxic shock syndrome, osteomyelitis, septic arthritis, pneumonia
Streptococcus pneumoniae	Otitis media, rhinosinusitis	Pneumonia, sepsis, meningitis
Streptococcus pyogenes	Pharyngitis, peritonsillar abscess, cervical lymphadenitis	Rheumatic fever, post-streptococcal glomerulonephritis, skin and soft tissue infection, toxic shock syndrome
Viridans streptococci	Dental plaque, caries	Endocarditis, sepsis

CAD, Coronary artery disease; COPD, chronic obstructive pulmonary disease.



• **Figure 2-4** α-Hemolysis of *Streptococcus pneumoniae*. The green around each pneumococcal colony is due to the conversion of hemoglobin to methemoglobin in the blood agar plate. Viridans streptococci colonies are similar in appearance and distinguished from pneumococcus by optochin susceptibility.

S. pneumoniae has a complex array of surface-associated and secreted factors that facilitate mucosal surface colonization, mitigate the host immune response, and promote invasion of the host.[16] The negatively charged polysaccharide capsule is required for virulence and protects against upper respiratory secretions and phagocytosis. However, the capsule does not promote binding to epithelial cells, and its size is decreased when the bacteria interacts with host epithelial cells. Additional surface adhesions bind components of the extracellular matrix, such as fibronectin, as well as epithelial cell receptors. *S. pneumoniae* strains secrete antimicrobial peptides, including those that target other pneumococcal serotypes to colonize a mucosal surface already crowded with other commensal flora.[16]

The immune system is also the target of several secreted virulence factors. Pneumolysin is a cytotoxic protein that targets the cholesterol-containing membranes of host membranes for pore formation and cell lysis. Pneumolysin is important for pneumococcal respiratory infections and invasion into the bloodstream. In addition to lysing cells, it also activates the immune response with proinflammatory cytokines leading to the recruitment of $CD4^+$ T cells.[16] Examples of pneumococcal mitigation of the host immune response include a secreted metalloprotease that cleaves immunoglobulin A1 (IgA1) to degrade antibody in the respiratory tract and surface expression of PsPA, a protein that prevents C3 complement fixation on the bacterial surface.[16]

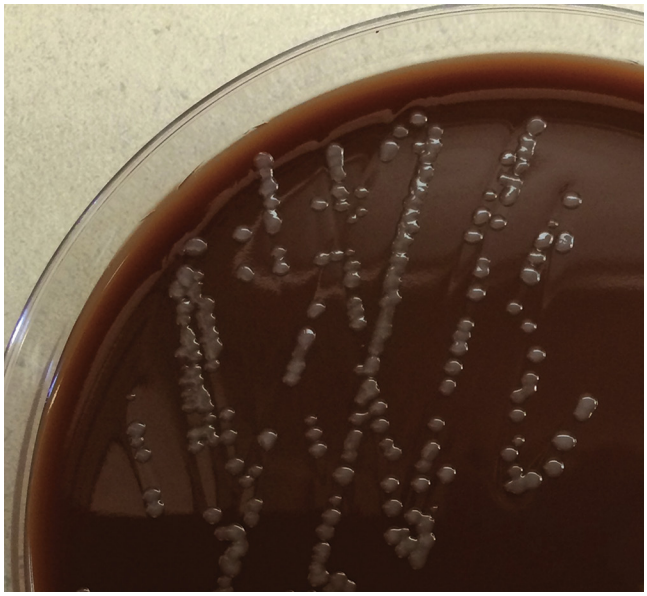
In recent years, *S. pneumoniae* has developed resistance mechanisms to many commonly used antipneumococcal antimicrobial agents. For example, resistance to penicillin resulting from mutations in penicillin-binding proteins required for cell wall synthesis has been described. Resistance to other drugs such as the macrolides and quinolones

is also increasing. Thus, antibiotic susceptibility testing is recommended to guide therapy when pneumococcus is isolated from an invasive head and neck infection.

Moraxella catarrhalis

Moraxella catarrhalis is an oxidase-positive, catalase-positive, gram-negative diplococci that exhibits γ-hemolysis (no hemolysis) when grown on blood agar plates (Figure 2-5). *M. catarrhalis* specifically binds the respiratory epithelium and the extracellular matrix of the human upper respiratory tract, and it has been recovered from the biofilms of children with chronic otitis media.[17,18] Although *M. catarrhalis* does not possess a polysaccharide capsule, it does have a number of surface adhesins. Examples of important adhesins include the outer membrane proteins, lipooligosaccharide, and ubiquitous surface protein A (UspA) proteins. UspA1 binds both epithelial cells and extracellular matrix to facilitate colonization. UspA2 functions to bind and inactivate complement and lipooligosaccharide and outer membrane proteins function in reducing serum-dependent bacterial killing.[17] Similar to *P. aeruginosa, M. catarrhalis* also possesses surface fimbriae called *type IV pili*, which are important for epithelial cell binding and biofilm formation. After mucosal colonization, *M. catarrhalis* is capable of invading epithelial cells, although the exact mechanisms remain to be elucidated.[18] This has also been observed in vivo, as *M. catarrhalis* has been identified in adenoid and tonsillar specimens from patients.[14]

The vast majority of *M. catarrhalis* secrete a β-lactamase that confers resistance to therapy with ampicillin alone. Successful treatment requires either the addition of a β-lactamase inhibitor to a penicillin, or a cephalosporin if a β-lactam antibiotic is preferred, or an antibiotic from an alternative class.

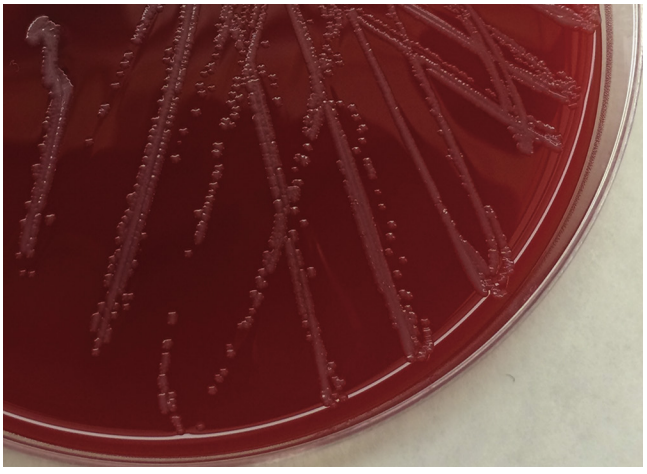


• **Figure 2-5** *Moraxella catarrhalis* growth on blood agar plate. Note the complete absence of hemolysis (γ-hemolysis) around the colonies.

Haemophilus influenzae

Haemophilus influenzae is a fastidious, thin, gram-negative rod that is oxidase positive and that requires both factors V (nicotinamide adenine dinucleotide) and X (hemin) for growth. It does not typically grow well on sheep blood agar plates, but it does grow well on chocolate agar that contains lysed red blood cells (Figure 2-6). Six encapsulated serotypes (a-f) exist, and historically *H. influenzae* type B (Hib) was the cause of most invasive disease. The introduction of routine childhood vaccination against Hib has virtually eliminated invasive disease with this strain in the United States. The majority of infections caused by *H. influenzae,* such as acute otitis media, are due to unencapsulated, nontypeable strains.[19] Nontypeable *H. influenzae* has a number of virulence factors that are similar to *M. catarrhalis*, a competitor for mucosal colonization of the upper airway. Surface pili bind both host cells and extracellular matrix components. The outer membrane protein P2 displays antigenic variation to avoid recognition by antibody, allowing for the persistence of chronic infection despite the host immune response.[20] Another well-studied group of adhesins are the autotransporters.[20,21] An example is HapA, a protein that promotes bacterial aggregation and adherence to respiratory epithelial cells.[21]

H. influenzae invasion of epithelial cells is postulated to occur by multiple endocytic pathways and requires actin. *H. influenzae* can also penetrate epithelial cell layers between cells, disrupting tight junctions, without causing cell death. In addition to causing invasive disease, strong evidence from animal models and human patients demonstrate that certain nontypeable strains of *H. influenzae* exist in biofilms, often coexisting with other bacteria.[22] The aggregation of bacteria promoted by HapA on the epithelial cell surface contributes to the initial stages of biofilm formation. These biofilms are stabilized by extracellular DNA and can even survive within NETs produced by activated neutrophils.[22] Nontypeable *H. influenzae* has been recovered from surgically removed



• **Figure 2-6** *Haemophilus influenzae* growth on chocolate agar plate. *Haemophilus influenzae* is fastidious and requires factor V and factor X for growth. These factors are available to the bacteria in chocolate agar composed of lysed erythrocytes.