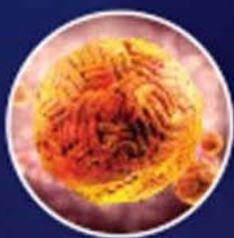
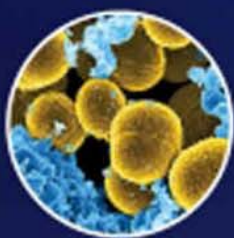
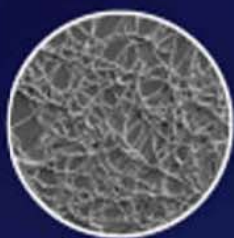


**Feigin** and **Cherry's**  
*Textbook of*  
**Pediatric**  
**Infectious Diseases**



EIGHTH EDITION | Volume 1

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*To my wife, Jeanne M. Cherry, who passed away on June 2, 2017  
To my children, James Cherry, Jeffrey Cherry and Kass Hogan,  
Susan Cherry, and Kenneth and Jennifer Cherry  
To my grandchildren, Ferguson, Dennis, and Siena Rose Cherry*

**James Cherry**

*To my husband, Neil Harrison  
To my children and sons-in-law, Emily Wolfe and Josh Wolfe,  
Kelly Green and Daniel Green, Matthew Demmler, Amy Demmler,  
Anna Rose Demmler, and Haley Harrison  
To my grandchildren, Jensen Wolfe and William Green  
To my brother, Loddie Naymola*

**Gail Harrison**

*To my wife, Marsha Kaplan  
To my children, Lauren Kaplan, Mindy Kaplan Langland,  
and son-in-law Lance Langland  
To my grandchildren, Reece and Macy Langland*

**Sheldon Kaplan**

*To my wife, Sandy Steinbach  
To my children, Amelia Steinbach, Aidan Steinbach, and Conner Franzen  
To my parents, Charles and Kathy Steinbach*

**William Steinbach**

*To my wife, Ann Hotez  
To my children, Matthew, Emily, Rachel, and Daniel Hotez  
To my mother, Jean Hotez, and mother-in-law, Marcia Frifield  
To the memories of my father, Ed Hotez, and father-in-law, Don Frifield*

**Peter Hotez**

# Ralph D. Feigin, MD

April 3, 1938–August 14, 2008

This eighth edition of the *Textbook of Pediatric Infectious Diseases* is dedicated to Ralph D. Feigin. As everyone in pediatrics and, in particular, pediatric infectious diseases, knows, Ralph was an extraordinary individual, and his untimely death in 2008 leaves a void that will never be filled.

Ralph Feigin was born in New York City on April 3, 1938. He graduated from Columbia College in New York City in 1958 and received his M.D. from Boston University School of Medicine in 1962. He married Judith S. Zobel, a childhood friend, in 1960 while in medical school. Ralph completed his first two years of pediatric residency at Boston City Hospital and his third year at the Massachusetts General Hospital. He then fulfilled his military service requirement at the United States Army Research Institute of Infectious Diseases, Ft. Detrick, Frederick, Maryland. While at the United States Army Research Institute, he participated in significant studies relating to circadian periodicity and susceptibility to infections, as well as other studies that resulted in eight publications for which he was the first author. After completing his service commitment, he was Chief Resident at Massachusetts General Hospital during the 1967-68 academic year.

Ralph was recruited to Washington University in St. Louis by Phil Dodge in 1968, and soon thereafter he and one of us (JDC), who was then at St. Louis University, got together and forged an academic and personal friendship that continued until the time of his death. Over 40 years ago, Ralph and Jim recognized the need for a comprehensive book on pediatric infectious diseases, but because of their busy schedules the plan was put on hold, and in 1973 Jim moved to California. In 1976, the pediatric research meetings were held in St. Louis, and at this time Jim and Ralph met with W. B. Saunders representatives, and the book was conceived. The first edition of the textbook was published 5 years later in the fall of 1981. In comparison with this 8th edition, it was a modest effort, with 44 chapters and 124 contributors.

At Washington University and St. Louis Children's Hospital, Ralph developed one of the finest infectious diseases divisions in the country. His "Feigin Rounds" were an unparalleled learning experience and were legendary among medical students and residents. In 1977, Ralph moved to Houston, Texas, to accept the challenge of being the Chair of Pediatrics for Baylor College of Medicine and the Physician-in-Chief at Texas Children's Hospital. During the ensuing 30 years, the Department grew from 43 faculty members to almost 500. One of us (SLK) came under Ralph's spell in St. Louis and moved to Houston with him. Another one of us (GJH), an intern in Houston in 1977, was waiting for Dr. Feigin when he arrived.

In Houston, Ralph served as the Chair of Pediatrics for Baylor College of Medicine and the Physician-in-Chief at Texas Children's Hospital for 31 years. For 7 years of his tenure, he also served as President and CEO of Baylor College of Medicine. In addition to his commitments in Houston, Ralph served in leadership roles on more than 100 local, regional, and national committees and professional societies. His efforts in persuading government officials of all ranks helped children in Texas, the United States, and in all parts of the world. Many consider him to have been the foremost pediatrician in the world.

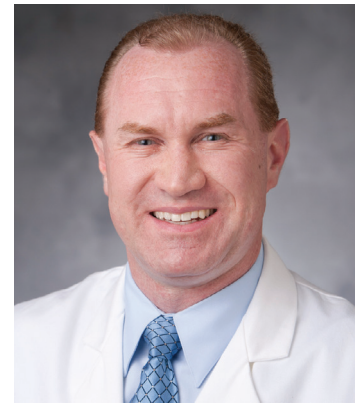
Not only was Dr. Feigin a powerhouse of energy, speed, and unsurpassed accomplishments, but he also was a gentleman, full of compassion, warmth, and kindness, and a man who kept people and patients first in his heart and mind. He was a loving husband to his wife, Judy, and a proud father to his three children, Susan, Debra, and Michael; doting grandfather to his six grandchildren, Rebecca, Matthew, Sarah, Rachel, Jacob, and Eli; and a mentor to so many in the field of pediatrics and pediatric infectious diseases. Ralph Feigin is missed by everyone who knew him, particularly by Judy Feigin and the family as well as by the present editors of this eighth edition of Feigin and Cherry.



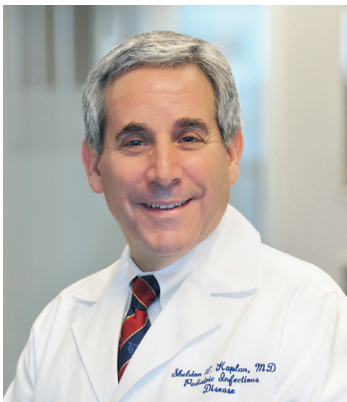
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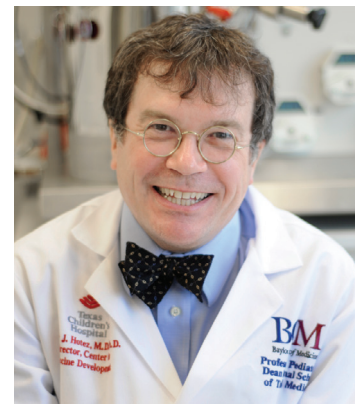
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Morbidity and mortality rates related to infectious diseases decreased dramatically during the first half of the 20th century in the developed world because of major improvements in public health (e.g., clean water, adequate sanitation, and vector control) and personal health. Further major reductions occurred in the second half of that century following the introduction of antimicrobial therapy, as well as active and passive immunization efforts. Despite these advances, infectious diseases in the developed world remain the leading cause of morbidity in infants and children in the 21st century. Children in the United States continue to experience three to nine respiratory infections and one to three gastrointestinal illnesses annually, requiring visits to physicians that outnumber the visits made for the purpose of well-child care. Infectious diseases are also the most common cause of school absenteeism.

Children in low and middle income countries also experience high rates of respiratory and gastrointestinal infections, which are often more severe and more frequent than those in children in the developed world. In addition, in the developing world there are great morbidity and mortality due to parasitic and vector-borne diseases. Also of importance are “spillover” infectious diseases such as Ebola, which because of increased urbanization resulted in an extensive epidemic in three African countries in 2014–15. In addition, mosquito-borne diseases such as Zika and Chikungunya have increased in prevalence in the Americas.

In more recent years, the emergence of resistance to multiple antibiotics by a large number of bacterial microorganisms (e.g., community-associated methicillin-resistant *Staphylococcus aureus*) has contributed to this infection-related morbidity and mortality, as have new infectious agents (e.g., SARS and MERS coronaviruses) and changes in the clinical manifestations and severity of established infectious agents (e.g., enterovirus 71, swine influenza).

The first edition of *Textbook of Pediatric Infectious Diseases* was written because Drs. Feigin and Cherry and many of their colleagues were concerned that no single reference text existed that comprehensively covered infectious diseases in children and adolescents. With each subsequent edition, including this one, the goal has been to provide comprehensive coverage of all subjects pertinent to the study of infectious diseases in children. Any attempt to summarize our present understanding of infectious diseases for serious students of the subject is a formidable task. In many areas, new information continues to accrue so rapidly that material becomes dated before it can appear in a text of this magnitude. Nevertheless, in this edition the editors and their author colleagues have endeavored to provide the most comprehensive and up-to-date discussion of pediatric infectious diseases ever compiled. This new edition is available online as well as in print. Purchasers can access the online version by registering their personal identification number (PIN) (found on the inside front cover of the book) at [expertconsult.inkling.com](http://expertconsult.inkling.com). Online access includes not only fully searchable text, photos, illustrations, and tables, but also references linked to PubMed.

To provide a text as comprehensive and authoritative as possible, we, the editors, have enlisted contributions from a large number of individuals whose collective expertise is responsible for whatever success we may have had in meeting our objective. We offer our most profound appreciation to the 307 fellow contributors from nearly 100 universities or institutions in 18 countries for their professional expertise and devoted scholarship. Their cooperation and willingness to work with us leave us deeply in their debt. Of note is the fact that 10 authors (Carol Baker, Ken Boyer, Jim Cherry, Morven Edwards, Chuck Grose, Scott Halstead, Maggie Hammerschlag, Shelly Kaplan, Ed Mason, and Barbara Stechenberg) have contributed to all eight editions of *Textbook of Pediatric Infectious Diseases*.

Once again, infectious diseases are discussed according to organ systems that may be affected, as well as individually by microorganisms. In all sections in which diseases related to specific agents are discussed,

emphasis has been placed, to the greatest extent possible, on the specificity of clinical manifestations that may be related to the organism causing the disease. Detailed information regarding the best means to establish a diagnosis and explicit recommendations for therapy are provided. In the present era of instant information, we have noted that historical perspectives relating to disease categories, as well as specific agents, are ignored. Because history is an important teacher, we have retained relevant historical details in this eighth edition.

Throughout the 37 years and eight-edition history of the *Textbook of Pediatric Infectious Diseases*, a number of classic chapters exist (e.g., measles, rubella, enteroviruses, and mycoplasma infections). The data in these chapters are unavailable in any other single-source publication.

The entire text of this eighth edition has been revised extensively. The seventh edition contained almost 4000 pages even though we included only new references in the print edition, which is close to the maximum that can be included in a two-volume book. Therefore, with this eighth edition, we were faced with a major dilemma: specifically, how to include new important material that had become available since the seventh edition but not to substantially increase the size of the book. We approached this dilemma in two ways. One problem in previous editions was redundancy, which we have addressed by combining information in some previous separate chapters into more concise single presentations and by shortening some chapters. The second way, which we introduced in the last edition, is to print only new references. The electronic version of the text contains all references.

This edition continues the format that was initiated in the fourth edition, in that infections with specific microorganisms have been organized to provide appropriate emphasis on the common features that may relate specific microorganisms to one another. Thus, all gram-positive coccal organisms are presented sequentially and are followed by gram-negative cocci, gram-positive bacilli, enterobacteria, gram-negative coccobacilli, Treponemataceae, anaerobic bacteria, and so forth. In addition, special sections of the text have been devoted to discussions of each of the following: molecular determinants of microbial pathogenesis; immunologic and phagocytic responses to infection; metabolic response of the host to infections; interaction of infection and nutrition; pathogenesis and treatment of fever; the human microbiome; epidemiology and biostatistics of infectious diseases; infections of the compromised host; Kawasaki disease; chronic fatigue syndrome; international travel issues for children; infectious disease problems of international adoptees and refugees; nosocomial infections; prevention and control of infections in hospitalized children; pharmacology and pharmacokinetics of antibacterial, antiviral, antifungal, and antiparasitic agents; immunomodulating agents; active and passive immunizing agents; public health considerations; infections in day care environments; and use of the bacteriology, mycology, parasitology, virology, and serology laboratories. The section on infections in the compromised host has again been expanded. This expansion has been necessitated by the large number of children, particularly transplant recipients, who have many infectious disease problems and constitute a large part of the consulting practice of many pediatric infectious disease physicians.

With some sadness, we have retained a section on bioterrorism, which is necessitated by the current state of world affairs. The section on immunomodulating agents and their potential use in the treatment of infectious diseases has been expanded because information on this subject has become more extensive since the publication of the last edition. We have also expanded the section on Ebola virus and included a new chapter on Zika virus.

This project could not have been brought to fruition without the help and assistance of many people whose names do not appear in the text. No words are sufficient to adequately convey our gratitude appropriately; we hope that they know they have our heartfelt thanks.



We would like to single out certain individuals for specific mention. First and foremost, we convey our appreciation to Laura Wennstrom Sheehan for the many hours she devoted to this edition. In her spare time between her position as Manager of Research Administration for the UCLA Department of Family Medicine and raising her young daughter, she coordinated the overall process of moving the book forward. She tended to numerous details relating to copyediting, transcribing, references, timing, and communication between the editors and Elsevier. Her expertise in EndNote was invaluable to the authors, editors, and publishing team for the organization of countless references throughout this edition. We are extremely grateful to have her as a part of our team. We would also like to acknowledge the hard work of Jordan Mann who assisted Laura throughout this process.

The following students at UCLA played a key role in processing chapters and in particular helping with references: Lauren M. Nguyen, David Dang, and Jewel Powe. We would also like to thank Nathaniel Wilder Wolf at Baylor who coordinated all the parasite chapters.

Of course this eighth edition of *Textbook of Pediatric Infectious Diseases* would not have been possible without Elsevier. We have been particularly

fortunate to have been able to work with Kate Dimock, Executive Content Strategist, Clinical Solutions at Elsevier throughout the whole process relating to this eighth edition. In addition, the initial planning contribution by Lauren Elise Boyle, Content Development Specialist, was invaluable. This was followed-up by the day-to-day contributions of Margaret Nelson, also a Content Development Specialist at Elsevier. Margaret kept everyone on track in meeting deadlines.

Finally, we thank the Baylor College of Medicine and Texas Children's Hospital in Houston, Texas, the David Geffen School of Medicine at UCLA and the Mattel Children's Hospital UCLA in Los Angeles, California, and Duke University School of Medicine in Durham, North Carolina for providing an environment that is supportive of intellectual pursuits.

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# Molecular Determinants of Microbial Pathogenesis

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Despite the availability of a wide variety of antimicrobial agents and expansion of vaccination programs, infectious diseases remain a leading cause of childhood morbidity and mortality worldwide. A number of factors contribute to the increasing importance of infectious agents: rates of antimicrobial resistance continue to rise, global travel has become routine, and the number of individuals with altered immunity has increased. Furthermore, in recent years, microorganisms have been implicated in diseases previously considered noninfectious, and a variety of new, emerging, and reemerging pathogens have been recognized.

*Pathogens* are defined as microorganisms that are capable of causing disease. However, not all pathogens are equal with respect to their pathogenic potential (i.e., their virulence). Many pathogens are, in fact, commensal organisms that live in harmony with their host under most conditions, causing disease only when normal immune mechanisms are disrupted or absent. Other pathogens produce disease even in the setting of intact host defenses and almost always cause symptoms.

For a given microbe, pathogenic potential is often determined by the genomic content and regulation of virulence-associated genes. Some bacterial species are capable of natural transformation and readily acquire fragments of DNA from other organisms, thus expanding or altering their genetic composition, occasionally with consequences related to virulence or antimicrobial resistance. A number of microorganisms carry virulence-associated genes on mobile genetic elements, including plasmids, transposons, and bacteriophages. These elements may equip the organism with genetic information that facilitates rapid adaptation to an unfavorable or changing environment. Comparison of genomes from pathogenic and nonpathogenic bacteria within a single genus or species has led to the identification of *pathogenicity islands*, which are large blocks of chromosomal DNA that are present in pathogens and absent from related nonpathogens. These blocks are flanked by insertion sequences or repeat elements and differ in nucleotide composition relative to the surrounding genome, suggesting acquisition by horizontal exchange. Pathogenicity islands in bacteria encode a variety of virulence factors, including protein secretion systems, secreted effector molecules, adhesins, and regulatory proteins. In an analogous way, some viral pathogens such as influenza virus are capable of exchanging nucleic acid segments with other viruses, leading to changes in pathogenicity, host tropism, and transmissibility.

To be successful, a pathogen must enter the host, occupy an appropriate niche, and then multiply. Sometimes the pathogen will induce damage to the host and then spread to other tissues, either near the initial site of infection or more distant. Often the pathogen will stop short of causing death to the host, maintaining latent infection or producing symptoms such as cough or diarrhea that facilitate spread to another host. This chapter addresses several key steps in the pathogenic process, each illustrated with examples of pathogens and paradigms of relevance to infectious diseases in children.

## COLONIZATION

Most bacterial infections begin with microbial colonization of a host surface, typically the skin, the respiratory tract, the gastrointestinal tract, or the genitourinary tract. Although colonization is not sufficient for an organism to produce disease, it is a necessary prerequisite. The process of bacterial colonization requires specialized microbial factors, called adhesins, that promote adherence to host structures and enable these organisms to overcome local mechanical defenses such as mucociliary function, peristalsis, and urinary flow. The cognate receptors for these

interactions are generally either carbohydrate or protein structures, in some cases expressed on host cells and in other instances present in mucosal secretions or in submucosal tissue.

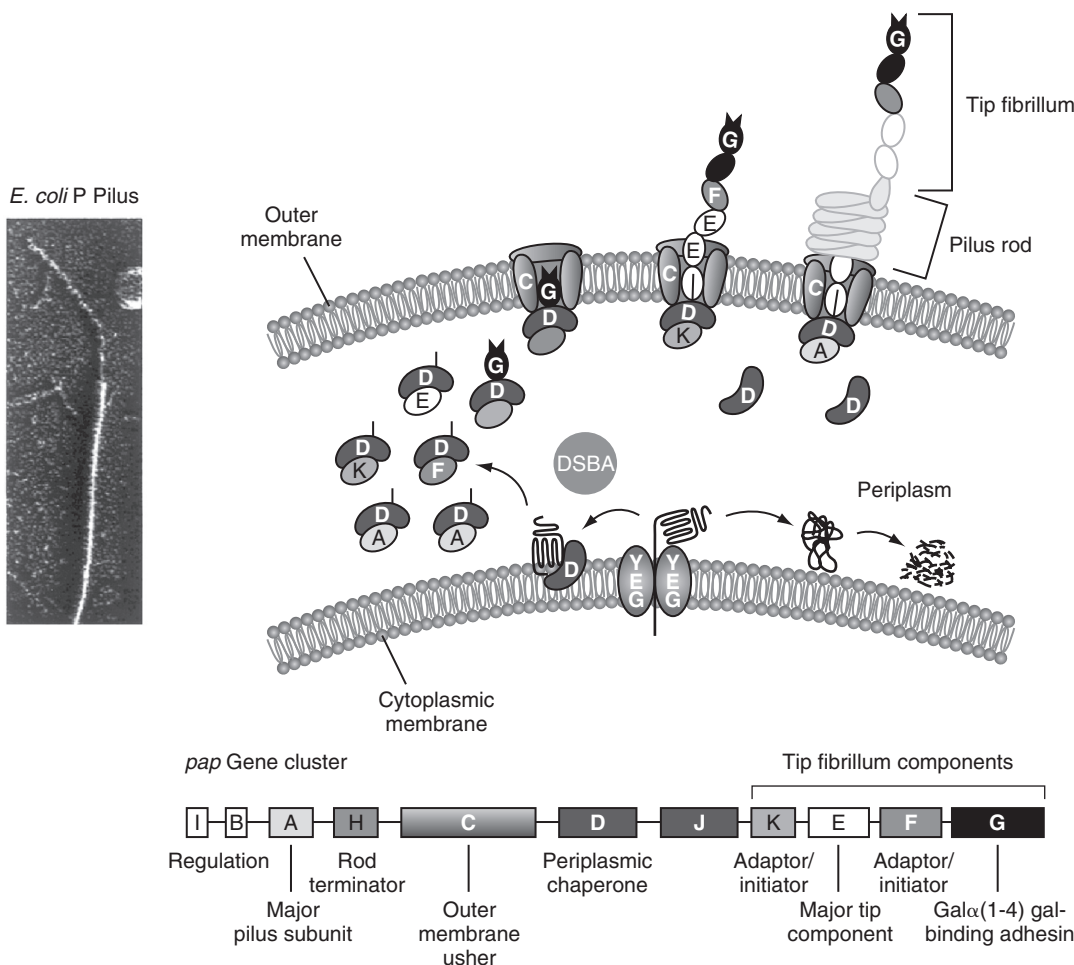
## Pilus Adhesins

Perhaps most common among bacterial adhesins are hairlike fibers called pili (also called fimbriae). Pili are heteropolymeric protein structures comprised largely of a major subunit usually ranging in size from 15 to 25 kDa. Because of their size and morphology, most pili can be visualized by negative-staining transmission electron microscopy.

The prototype example among adhesive pili is the P (or Pap) pilus, which is expressed by uropathogenic *Escherichia coli* (UPEC) and has been strongly associated with pyelonephritis. P pili recognize globoseries glycolipids, which are host molecules that are characterized by a core structure consisting of Gal- $\alpha$ 1,4-Gal. The globoseries glycolipids are especially abundant in renal epithelium,<sup>25</sup> thus accounting for the predilection of P-piliated *E. coli* to adhere to kidney tissue and cause pyelonephritis. Type 1 pili are analogous fibers expressed by UPEC and bind mannosylated uroplakin proteins in the mammalian bladder to initiate cystitis.<sup>153</sup> As shown in Fig. 1.1, P pili are composite structures and consist of two subassemblies, including a thick rod that emanates from the bacterial surface and a thin tip fibrillum that extends distally.<sup>173,270</sup> The pilus rod is a right-handed helical cylinder and is composed of repeating PapA subunits, whereas the tip fibrillum has an open helical configuration and contains mostly repeating PapE subunits. The two subassemblies are joined to each other by the PapK adaptor protein. PapG contains the adhesive moiety and is located at the distal end of the tip fibrillum, joined to PapE by the PapF adaptor.<sup>150</sup>

P pili are assembled through a canonical process termed the *chaperone-usher pathway* that involves a periplasmic chaperone (PapD) and an outer membrane usher (PapC) (see Fig. 1.1).<sup>65,174</sup> Subunit proteins (e.g., PapA) are translated in the bacterial cytoplasm, enter the periplasm through the inner membrane Sec machinery, and are stabilized by interaction with the chaperone, which ferries them to the usher. Extrusion of the nascent fiber is controlled by a “plug” domain in the usher pore; because the periplasm is devoid of adenosine triphosphate (ATP), the assembly process is energetically driven by the entropically favorable final conformation of the incorporated subunits.<sup>189,259</sup> More than 30 different bacterial adhesive structures are assembled via this chaperone-usher pathway, with distinct PapD-like chaperones and PapC-like ushers. The PapD-like chaperones can be divided into two distinct subfamilies based on conserved structural differences that occur near the subunit binding site.<sup>138</sup> One subfamily is involved in the assembly of rod-like pili similar to P pili, whereas the second subfamily participates in the biogenesis of more atypical filamentous structures, such as Caf1 of *Yersinia pestis* (the plague bacterium), which forms an amorphous “mat” on the bacterial surface. Thus the nature of the chaperone is directly correlated with the architecture of the adhesive appendage that it helps to assemble.<sup>285</sup>

Type 4 pili represent a second class of pili and are distinguished by a methylated first amino acid (usually phenylalanine); a short, positively charged leader sequence; a conserved hydrophobic N-terminal domain; and a tendency to form bundle-like structures. Type 4 pili have been identified in a number of gram-negative bacterial pathogens, including *Neisseria gonorrhoeae*, *N. meningitidis*, enteropathogenic *E. coli* (EPEC), *Vibrio cholerae*, *Pseudomonas aeruginosa*, *Kingella kingae*, *Eikenella corrodens*, *Haemophilus influenzae*, and *Moraxella* species.<sup>32,99,193,208,250,256,272,296,323</sup> Although the mechanism of



**FIG. 1.1** Biogenesis and structure of *Escherichia coli* P pili. The *pap* gene cluster and the function of each of the gene products are indicated in the lower portion of the figure. Nascent pilin subunits are complexed with the PapD chaperone and added to the base of the developing pilus via the PapC usher. The mature pilus rod is composed of repeating units of PapA; the tip fibrillum contains the adhesin PapG. The ultrastructure of the pilus is shown in the electron micrograph at the left side of the figure. (Courtesy S.J. Hultgren and F.J. Sauer.)

assembly of type 4 pili is still being elucidated, existing data suggest that the process is complex. For example, between 20 and 40 gene products are required for the assembly of *P. aeruginosa* type 4 pili, and at least 15 plasmid-encoded proteins are involved in the biogenesis of EPEC type 4 pili.<sup>127,294</sup> Based on studies of *P. aeruginosa*, EPEC, *Neisseria*, and *V. cholerae*, the presence of an inner membrane prepilin peptidase appears to be a general prerequisite for type 4 pilus biogenesis.<sup>161,176,226</sup> Type 4 pili are often glycosylated, with carbohydrate decoration affecting function in at least some cases and perhaps serving to obscure antigenic epitopes.<sup>33,190,278,319</sup> However, despite marked differences in the assembly pathways for type 4 pili and P pili, shared structural themes exist. For example, gonococcal type 4 pili are composed predominantly of PilE structural subunits polymerized into a helical rod.<sup>235</sup> A minor phase-variable adhesive protein called PilC is displayed at the tip of gonococcal pili and is essential for pilus-mediated binding to epithelial cells.<sup>154,267</sup> These observations suggest that *N. gonorrhoeae* pili may be composite structures with a tip-associated adhesin, analogous to P pili and other pili assembled by the chaperone-usher pathway.

Although adhesive pili are more prevalent in gram-negative bacteria, they are also found in some gram-positive species. One example is *Streptococcus parasanguinis*, an oral pathogen and a member of the *S. sanguinis* family. This organism binds to calcium phosphate (the primary mineral component of tooth enamel) and also to other oral bacteria, epithelial cells, platelets, and fibronectin. Several adhesins mediate these binding functions, including pili referred to as *long fimbriae*. Based on studies of *S. parasanguinis* strain FW213, long fimbriae are fashioned

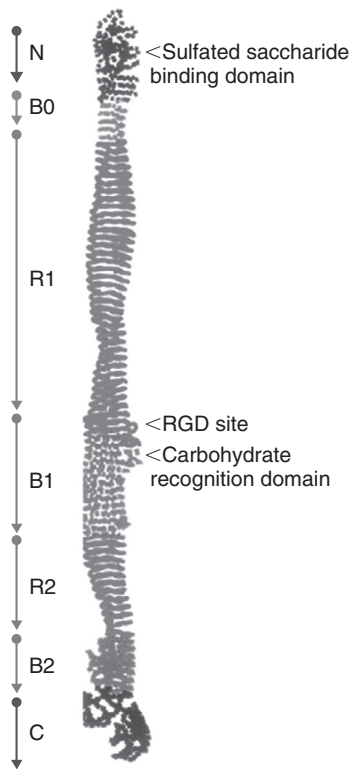
primarily from Fap1, a 200-kDa protein that includes an unusually long (50 amino acids) signal sequence and a cell-wall sorting signal typical of other gram-positive bacterial surface proteins.<sup>338,339</sup> Specific glycosylation of Fap1 appears critical to the adhesive function of this fimbrial protein.<sup>27,293,337</sup> Interestingly, similar to gram-negative bacterial pili, long fimbriae appear to have a composite structure with a pilus tip. The tip contains an additional adhesin called FimA, which in purified form is capable of blocking bacterial adherence to saliva-coated hydroxyapatite.<sup>81,231</sup> In work by Burnette-Curley and coworkers, disruption of the *fimA* gene resulted in a 7- to 20-fold reduction in the incidence of endocarditis after intravenous inoculation of rats.<sup>29</sup> Other gram-positive organisms capable of expressing pili include *Streptococcus pneumoniae* (a common cause of respiratory tract and invasive disease),<sup>11,216</sup> *Streptococcus agalactiae* (group B streptococcus; a common cause of neonatal pneumonia, sepsis, and meningitis),<sup>73,264</sup> and *Enterococcus faecalis* (a cause of endocarditis and urinary tract infections).<sup>215,277</sup>

### Nonpilus Adhesins

Beyond pili, a variety of nonpilus adhesins exist. In most cases, nonpilus adhesins are surface-expressed monomeric or oligomeric proteins, although isolated examples of carbohydrate- and lipid-containing adhesive structures have been identified. In general, these molecules are more difficult to visualize by electron microscopy, reflecting their smaller size. Similar to pili, for the most part nonpilus adhesins can be classified according to their mechanism of secretion and presentation on the bacterial surface.

Among the best-characterized bacterial nonpilus adhesins is filamentous hemagglutinin (FHA), a surface protein expressed by *Bordetella pertussis* and other *Bordetella* species. The export of FHA to the surface of the organism occurs via the so-called two-partner secretion (TPS) pathway, a conserved strategy in which a secreted protein (TpsA) interacts with a cognate outer membrane transporter (TpsB).<sup>128</sup> In *B. pertussis*, the TpsA-type protein FHA is transported by a TpsB-type outer membrane protein called FhaC, which has  $\beta$ -barrel pore-forming properties and facilitates translocation of FHA across the outer membrane.<sup>333</sup> Homologous TpsB proteins in other species export the hemolysins of *Serratia marcescens*, *Proteus mirabilis*, and *Haemophilus ducreyi*; the *H. influenzae* heme:hemopexin binding protein (HxuA); and the *H. influenzae* HMW1 and HMW2 adhesins, among others.<sup>9,46,234,249,287,316</sup> The crystal structure of FhaC reveals a 16-stranded  $\beta$ -barrel that is occluded by an N-terminal  $\alpha$ -helix and an extracellular loop and a periplasmic module composed of two polypeptide-transport-associated (POTRA) domains. Functional studies have demonstrated that the N terminus of FHA interacts with the FhaC POTRA 1 domain, illuminating what appears to be a general feature of interactions between TpsA and TpsB proteins.<sup>39,104</sup>

Examination of purified FHA by transmission electron microscopy and circular dichroism spectroscopy showed that the FHA molecule is 50 nm in length and adopts the shape of a horseshoe nail. It has a globular head, a 37-nm-long shaft that averages 4 nm in width but tapers slightly from the head end, and a small flexible tail (Fig. 1.2).<sup>157,188</sup> In the crystal structure of the N terminus of FHA (the so-called TPS domain that interacts with FhaC), a series of 19-residue repeat motifs form a  $\beta$ -helix that is central to the overall structure of full-length FHA.<sup>40</sup> Consistent with its large size, FHA contains at least five separate binding domains, four of which have been localized. The region involved



**FIG. 1.2** Ribbon representation model structure of filamentous hemagglutinin from *Bordetella pertussis*. There are five regions that are assigned  $\beta$ -helical coils, designated B0, R1, B1, R2, and B2. The N terminus of the protein is designated with "N," and the C terminus of the protein is designated with "C." The locations of the sulfated saccharide binding domain, the carbohydrate recognition domain, and the RGD tripeptide are noted. (From Kajava AV, Cheng N, Cleaver R, et al. Beta-helix model for the filamentous haemagglutinin adhesin of *Bordetella pertussis* and related bacterial secretory proteins. *Mol Microbiol.* 2001;42:279–92.)

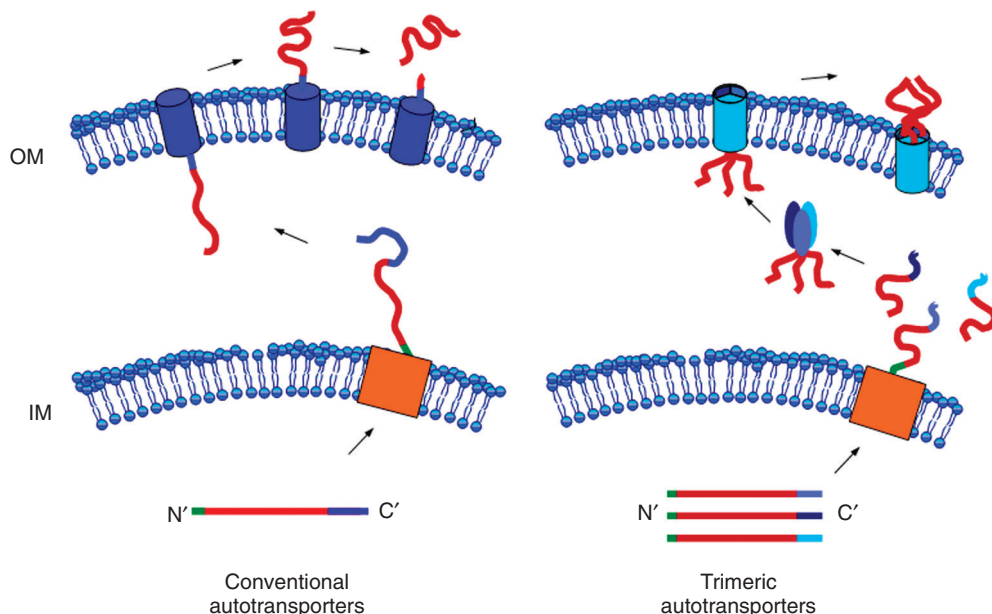
in adherence to sulfated saccharides has been mapped to the N terminus of the FHA molecule.<sup>206</sup> Sulfated saccharides such as heparin and heparan sulfate are a major component of mucus and extracellular matrix in the respiratory tract and are also found on the surface of epithelial cells.<sup>195,342</sup> The region that recognizes lactosylceramides and promotes adherence to ciliated respiratory epithelial cells and macrophages has been localized to amino acids 1141 to 1279 (the carbohydrate recognition domain).<sup>251</sup> An arginine-glycine-aspartic acid (RGD) tripeptide is located at amino acids 1097 to 1099 and interacts with leukocyte response integrin (LRI), a leukocyte integrin that stimulates upregulation of complement receptor type 3 (CR3).<sup>146</sup> The C terminus of mature FHA has been demonstrated to interact with epithelial cells and macrophage-like cells and appears to modulate the immune response to *Bordetella* infection.<sup>155</sup> Finally, FHA recognizes CR3 (CD11b/CD18), allowing organisms to be ingested by macrophages without stimulating an oxidative burst.<sup>258,336</sup> The location of the CR3-binding domain is currently unknown.

A growing number of nonpilus adhesins belong to the so-called autotransporter family. These proteins are synthesized as precursor proteins with three functional domains, including an N-terminal canonical signal sequence, an internal passenger domain, and a C-terminal outer membrane domain. The signal sequence directs the protein to the Sec machinery and is cleaved after it facilitates transport of the polypeptide from the cytoplasm to the periplasm. The C-terminal domain inserts into the outer membrane and forms a  $\beta$ -barrel with a central hydrophilic channel. Ultimately, the passenger domain is presented on the surface of the organism and influences interaction with host molecules.<sup>120</sup> Recent studies have established that autotransporter proteins can be separated into two distinct groups, designated *conventional autotransporters* and *trimeric autotransporters* (Fig. 1.3).<sup>52</sup> In conventional autotransporters, the C-terminal outer membrane domain contains roughly 300 amino acids and is a monomeric  $\beta$ -barrel with a single N-terminal  $\alpha$ -helix spanning the pore (Fig. 1.4A).<sup>232,299</sup> In trimeric autotransporters, the C-terminal outer membrane domain contains approximately 70 amino acids and forms heat- and detergent-resistant trimers in the outer membrane. Each trimer forms a  $\beta$ -barrel with four strands from each of the three subunits and with three N-terminal  $\alpha$ -helices spanning the pore (Fig. 1.4B).<sup>204</sup>

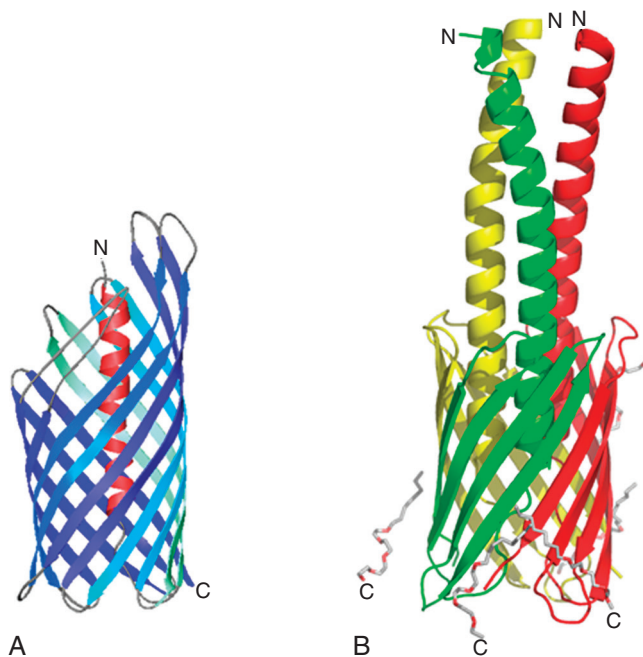
One example of a conventional autotransporter adhesin is the *H. influenzae* Hap protein, which was discovered based on its ability to promote adherence and low-level invasion in assays with cultured human epithelial cells.<sup>286</sup> Hap also promotes bacterial binding to extracellular matrix proteins and bacterial microcolony formation.<sup>83,121</sup> Examination of chimeric proteins and studies with purified protein have demonstrated that the adhesive activity responsible for Hap-mediated adherence, invasion, binding to extracellular matrix proteins, and microcolony formation localizes to the passenger domain, referred to as Hap<sub>s</sub>.<sup>83,121</sup> More detailed characterization of Hap<sub>s</sub> has established that the region responsible for interaction with host epithelial cells and microcolony formation resides in the C-terminal 311 amino acids and may have utility as a vaccine antigen.<sup>57,82,183</sup> This region folds into a triangular prism-like structure that can mediate Hap-Hap dimerization and higher degrees of multimerization, thus facilitating interbacterial interaction and microcolony formation.<sup>202</sup> A prototype member of the trimeric autotransporter subfamily is the *H. influenzae* Hia adhesin. This protein is expressed in a subset of nontypable *H. influenzae* strains and contains two homologous high-affinity trimeric binding domains, creating the potential for stable multivalent interaction with respiratory epithelial cells.<sup>175,203,343</sup>

Another group of nonpilus adhesins is typified by intimin, a protein expressed by enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), and the murine pathogen *Citrobacter rodentium*. Intimin contains a flexible N terminus, a central  $\beta$ -barrel domain that integrates into the outer membrane, and a C-terminal binding domain that interacts with the translocated intimin receptor (Tir).<sup>311</sup> Tir is an interesting example of a pathogen-derived receptor that is inserted into target host cells. After initial cell attachment mediated by type 4 pili, EPEC employs a type III secretion system (discussed in detail later in this chapter) to inject Tir into the host cell cytoplasm,<sup>164,335</sup> from where it is then inserted into the host cell membrane.<sup>254</sup> The subsequent interaction between





**FIG. 1.3** Autotransporter protein secretion pathway. Conventional autotransporter secretion is shown on the left, and trimeric autotransporter secretion is shown on the right. Autotransporter proteins are synthesized as preproteins with three functional domains, including an N-terminal signal sequence (shown in green), an internal passenger domain (shown in red), and a C-terminal outer membrane  $\beta$ -barrel domain (shown in blue). IM indicates inner membrane, and OM indicates outer membrane. Protein secretion begins with export of the protein from the cytoplasm via the inner membrane Sec machinery (Sec). Most conventional autotransporters are cleaved on the bacterial surface. (From Cotter SE, Surana NK, St Geme JW III. Trimeric autotransporters: a distinct subfamily of autotransporter proteins. *Trends Microbiol.* 2005;13:199–205.)



**FIG. 1.4** Crystal structures of the C-terminal outer membrane  $\beta$ -barrel of autotransporter proteins. (A) Crystal structure of NalP, a conventional autotransporter;  $\beta$  strands are shown in shades of blue, and the  $\alpha$ -helix that crosses the channel is shown in red. (B) Crystal structure of Hia, a trimeric autotransporter; individual subunits are shown in red, green, and yellow. (A, From Surana NK, Cotter SE, Yeo HJ, et al. Structural determinants of *Haemophilus influenzae* adherence to host epithelium: variations on type V secretion. In: Waksman G, Caparon MG, Hultgren SJ, editors. *Structural Basis of Bacterial Pathogenesis*. Washington, DC: American Society for Microbiology; 2005:129–148. B, From Meng G, Surana NK, St Geme JW III, et al. Structure of the outer membrane translocator domain of the *Haemophilus influenzae* Hia trimeric autotransporter. *EMBO J.* 2006;25:2297–304.)

intimin (on the bacterial surface) and Tir (now present on the host cell surface) triggers receptor clustering, dramatic rearrangement of the actin cytoskeleton, and formation of a distinctive pedestal referred to as an *attaching and effacing (A/E) lesion* (Fig. 1.5).<sup>164,263</sup> The bacterial genes essential for formation of A/E lesions reside within a 35-kb region of the EPEC chromosome called the locus of enterocyte effacement (LEE), an example of a pathogenicity island.<sup>70,196</sup> This locus is highly conserved in content and organization across all A/E pathogens and contains the genes encoding intimin, Tir, and the requisite type III secretion system. The interactions of Tir and other type III secreted effectors with host proteins influencing actin polymerization are beginning to be understood.<sup>265,344</sup> Tir contains domains analogous to host immunoreceptor tyrosine-based inhibition motifs (ITIM) important for regulation of eukaryotic signaling. On this basis, Tir recruits certain host proteins to regulate actin dynamics and inhibit proinflammatory signaling pathways.<sup>61,266,280,328</sup> Given its central role in EHEC/EPEC pathogenesis and its immunogenicity, intimin is also being examined as a target for the development of antivirulence therapeutics or vaccines in A/E diseases.

In recent years, investigators have identified a large family of nonpilus adhesins involved in adherence to host extracellular matrix proteins including fibronectin, laminin, vitronectin, collagen, fibrinogen, and a variety of proteoglycans. These adhesins have been classified as microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) and are especially prevalent among gram-positive bacteria.<sup>238</sup> In gram-positive organisms these proteins are covalently anchored to the cell wall peptidoglycan and have a characteristic primary amino acid sequence. In particular, the C terminus contains a segment rich in proline and glycine residues, an LPXTG motif (involved in sorting and covalently anchoring the protein to the cell wall), a hydrophobic membrane-spanning domain, and a short positively charged segment that resides in the cytoplasm and serves as a cell wall retention signal. Adhesive functions are typically located near the N terminus.<sup>85</sup>

*Staphylococcus aureus* is a common gram-positive pathogen in children and is capable of producing a variety of MSCRAMMs, including collagen-binding protein (CNA), fibronectin-binding proteins A and B, and clumping factors A and B. Recent work indicates that although





**FIG. 1.5** Enteropathogenic *E. coli* are perched on pedestals in the attaching and effacing lesion. (Courtesy B.B. Finlay; from Rosenshine I, Ruschkowski S, Stein M, et al. A pathogenic bacterium triggers epithelial signals to form a functional bacterial receptor that mediates actin pseudopod formation. *EMBO J.* 1996;15:2613–24.)

these proteins mediate typical binding interactions with host proteins, they are not monospecific, and a given MSCRAMM may bind multiple host connective tissue components or multiple motifs within a single host fiber type.<sup>284</sup> In addition, many are capable of provoking platelet activation. *S. aureus* strains recovered from patients with septic arthritis commonly express CNA, which mediates binding to cartilage in vitro and appears to play a key role in the pathogenesis of septic arthritis in experimental mice.<sup>257,239,300</sup> Fibronectin-binding protein A (FnBPA) shares homology with *S. pyogenes* protein F and mediates binding to fibronectin and the  $\gamma$  chain of fibrinogen, as well as to elastin and tropoelastin.<sup>162,262,322</sup> Accordingly, this protein is important in *S. aureus* endocarditis<sup>247</sup> and in infections of implanted biomaterials, which become coated with fibrinogen and fibrin soon after implantation. Clumping factor (ClfA) was named based on the observation that it mediates bacterial clumping in the presence of soluble fibrinogen.<sup>197</sup> Similar to FnBPA, ClfA mediates binding to fibrinogen-coated surfaces in vitro and probably contributes to infections of artificial surfaces.

### Other Mechanisms of Adherence

*Candida albicans* is a common inhabitant of mucosal surfaces and an important cause of systemic disease, especially in patients with compromised immunity. *Candida* blastospores are capable of efficient adhesion to epithelial cells, leading to budding and division. In addition, germ tube formation occurs, facilitating penetration through the epithelial barrier and then dissemination to distant sites.<sup>135</sup> In recent years, several candidate *C. albicans* adhesins have been identified.<sup>10,134,180,346</sup> Of particular interest is a protein called INT1, which shares functional homology with the vertebrate integrin family. Integrins are normally expressed by cells of the human immune system (neutrophils, monocytes, macrophages) and mediate cellular binding and shape-changing functions. Each integrin is a heterodimer of an  $\alpha$  chain and a  $\beta$  chain. There are a number of distinct  $\alpha$  and  $\beta$  chains, and each combination displays a unique binding specificity. INT1 is an  $\alpha$  integrin-like protein that recognizes the RGD sequence of the C3 fragment iC3b on epithelial cells. In in vitro assays, short peptides encompassing the RGD sequence are capable of inhibiting *C. albicans* adherence by 50%, confirming that INT1 plays a significant role as an adhesin and suggesting that other adhesins also exist.<sup>135</sup> Beyond promoting adherence to epithelium, INT1 disguises organisms as leukocytes, allowing evasion of phagocytosis. Of note, introduction of INT1 into *Saccharomyces cerevisiae* confers a

capacity for adherence and also results in germ tube formation, indicating a role for this protein in morphogenesis.<sup>95,94</sup>

The adhesive properties of *C. albicans* are closely tied to its morphologic state. For example, adherence to buccal epithelial cells is greater by organisms bearing germ tubes than by yeast forms.<sup>169</sup> With this information in mind, Staab and coworkers searched a germ tube cDNA library and identified a putative adhesin called hyphal wall protein 1 (Hwp1) encoded by the *hwp1* gene. Examination of the predicted amino acid sequence of Hwp1 revealed similarity to proteins that are substrates for mammalian transglutaminase enzymes.<sup>290</sup> These enzymes form a cornified envelope on squamous epithelial cells (including buccal epithelial cells) by cross-linking relevant substrates.<sup>302</sup> Interestingly, the interactions of germ tubes with buccal epithelial cells resist stresses (e.g., heating or treatment with sodium dodecylsulfate) capable of dissociating most typical microbe-host adhesive pairs, and elimination of expression of Hwp1 results in a marked reduction in adhesion to buccal epithelial cells.<sup>23,289</sup> Thus Hwp1 represents a unique adhesive strategy, employing host transglutaminase enzymes to cross-link Hwp1 (via a glycosylphosphatidylinositol remnant anchor) directly to surface proteins on buccal epithelial cells.<sup>288</sup> More recently, Hwp1 has been shown to be important for *Candida* biofilm formation,<sup>77,220</sup> indicating that a similar mechanism may also support interactions between candidal cells.

### TISSUE TROPISM

Most microorganisms demonstrate restriction in the range of hosts, tissues, and cell types that they colonize. This restriction is referred to as *tropism* and generally reflects the specificity of the interaction between a given microbial adhesin and its cognate receptor. Accordingly, tropism is determined by the distribution of the relevant host receptor.

Pili of uropathogenic *E. coli* serve as the platform for presentation of one of three different PapG variants, referred to as class I, class II, and class III PapG. All three variants recognize globoseries glycolipids, but each binds with a distinct specificity to the globoseries glycolipid isotypes. For example, class I PapG preferentially recognizes globotriosylceramide (GbO3, Gal- $\alpha$ 1,4-Gal- $\beta$ 1,3-Glc-ceramide), class II PapG preferentially recognizes globoside (GbO4, GalNAc- $\beta$ 1,3-Gal- $\alpha$ 1,4-Gal- $\beta$ 1,3-Glc-ceramide), and class III PapG preferentially interacts with Forssman antigen (GbO5, GalNAc- $\alpha$ 1,3-GalNAc- $\beta$ 1,3-Gal- $\alpha$ 1,4-Gal- $\beta$ 1,3-Glc-ceramide).<sup>297</sup> Globoside is the dominant globoseries glycolipid expressed in human kidney, and most human isolates of *E. coli* associated with pyelonephritis express class II PapG. In contrast, Forssman antigen is the most abundant globoseries glycolipid in dog kidney, and more than 50% of canine urinary isolates of *E. coli* express class III PapG.<sup>341</sup> *E. coli*-expressing P pili with class II PapG are not found as a cause of urinary tract infection in dogs. Thus the specificity of the PapG variant at the tip of the P pilus influences host range, favoring infection of either human or dog.

The crystal structure of class II PapG bound to Gal- $\alpha$ 1,4-Gal was solved by Dodson and coworkers, uncovering the structural basis of PapG binding specificity.<sup>66</sup> Of particular interest, the PapG receptor binding site is located on the side of the molecule and must be oriented with its N- to C-terminal axis parallel to the host cell membrane to allow docking to the receptor. This orientation may be facilitated by the flexibility inherent in the tip fibrillum. The PapG binding site consists of two regions. The first forms a  $\beta$ -barrel, and the second is composed of a central antiparallel  $\beta$ -sheet that is flanked on one side by two 2-stranded  $\beta$ -sheets and on the other side by an  $\alpha$ -helix. When class II PapG interacts with GbO4, the arginine residue at position 170 in PapG makes contact with the GbO4 side chain. Interestingly, in class I PapG, a histidine residue occupies position 170, interfering with potential contact with the GbO4 side chain. Similarly, class II PapG and class III PapG differ in amino acids required for interaction with the GbO5 side chain.<sup>66</sup>

Group A streptococcus (*S. pyogenes*) is a common cause of infections of skin and soft tissue, including impetigo, cellulitis, and necrotizing fasciitis. Adherence to host cells by *S. pyogenes* is influenced by nonpilus adhesins called M protein and protein F. M protein forms a fiber and consists of a C-terminal region that anchors the protein in the cell wall,

a coiled-coil rod region extending approximately 50 nm from the cell wall, and a short nonhelical domain extending more distally.<sup>84</sup> Protein F is a 120-kDa protein that is notable for a tandem repeat element consisting of up to six repeats of 32 to 44 amino acids adjacent to the C terminus.<sup>112,233</sup> M protein promotes adherence to human keratinocytes via interaction with the CD46 molecule (also called membrane cofactor protein, or MCP), whereas protein F mediates adherence to epidermal Langerhans cells, which are located in the basal layer of the epidermis.<sup>229,230</sup> Thus both M protein and protein F contribute to group A streptococcal adherence to the skin, but each protein directs interaction with a different population of epidermal cells.

Early studies demonstrated that human immunodeficiency virus type 1 (HIV-1) infects CD4<sup>+</sup> cells and interacts with the CD4 molecule but that CD4 alone is not sufficient to permit infection. More recent observations have established that a number of host cell chemokine receptors, especially CCR5 and CXCR4, serve as coreceptors for HIV-1 and are required for viral entry into CD4<sup>+</sup> target cells. These coreceptors appear to influence the cellular tropism displayed by different HIV-1 variants.<sup>62</sup> All HIV variants are able to replicate in primary T cells, but only some can also replicate in primary macrophages or in immortalized T-cell lines. Asymptomatic HIV-infected individuals carry strains that generally use CCR5 as a coreceptor (termed *M5 strains*) and are non-syncytium-inducing in vitro. Such strains have classically been described as macrophage tropic (M-tropic), but recent experiments have demonstrated that these M5 strains can also infect CD4<sup>+</sup> T cells and peripheral blood mononuclear cells.<sup>245</sup> Rapid viral mutation due to the error-prone HIV polymerase and HIV reverse transcriptase leads to the production within the host of syncytium-inducing, T-cell-tropic (T-tropic) HIV-1 strains, which predominate in the circulation of patients with acquired immunodeficiency syndrome.<sup>62</sup> These variants are generally restricted to CXCR4 (expressed on T cells) as a coreceptor, although some primary syncytium-inducing variants can use both CCR5 and CXCR4.<sup>71,79,276</sup> T-tropic, syncytium-inducing strains are characterized by positively charged residues at fixed positions of the V3 loop and changes in charge and length of the V2 region of the viral envelope glycoprotein gp120, which binds to CD4 and coreceptors before viral entry into host cells.<sup>86,87,106</sup> Thus cellular tropism is closely aligned, but not synonymous, with HIV coreceptor usage.

New HIV-1 infection is selectively established by M-tropic HIV-1 strains, even if the transmitting host harbors more pathogenic non-M-tropic strains as well.<sup>317,352</sup> CCR5 is also expressed on the surface of rectal and vaginal epithelial cells, which may be sites of initial encounter between HIV-1 and the human host.<sup>349</sup> The importance of CCR5 in HIV-1 binding to CD4<sup>+</sup> cells is underscored by the observation that individuals homozygous for a 32-bp deletion in CCR5 (the  $\Delta 32$  allele) are resistant to infection with HIV-1.<sup>137,184</sup> The  $\Delta 32$  heterozygous state does not necessarily protect against HIV-1 acquisition, although HIV disease in heterozygous patients may follow an attenuated course. This allele is surprisingly frequent (10%–14%) in white populations, leading to speculation that it provided a survival advantage during one or more historical epidemics of infectious diseases.<sup>225</sup> However, more recent data suggest that the  $\Delta 32$  allele may actually confer immune deficiency in the presence of challenge with certain viral pathogens, such as West Nile virus.<sup>100,101</sup> Of note, CCR5 may have a role in controlling the development of malignancy, including lymphoma, raising some concern about developing anti-HIV pharmacologic agents that target CCR5 function.<sup>178</sup> Finally, co-evolution of viral determinants and host cell receptors may determine the spectrum of tissue and organ involvement within the host. For example, the chemokine receptor CCR8 may facilitate the entry of neurotropic HIV-1 strains into brain cells,<sup>152</sup> and envelopes derived from brain isolates of HIV are adapted to infect cells with low-level CD4/CCR5 expression, such as neuroglia and brain macrophages.<sup>244</sup>

Other viruses also demonstrate tropism for specific cells or tissues within the host. Hepatitis C virus (HCV) has been demonstrated to use multiple cell surface molecules in sequence to locate and gain entry into target cells. HCV is bound to low-density lipoprotein (LDL) in serum and first binds to scavenger-receptor B1 (SR-B1), which is enriched on liver cells and serves to bind lipoprotein molecules.<sup>34</sup> After this initial binding event, HCV E2 protein interacts with the CD81 molecule, the

critical receptor for free virus.<sup>159,321,351</sup> Once virus has bound to CD81, this virus-receptor complex traffics to the gap junction, where the virus interacts with two key gap junction proteins, claudin-1 and occludin-1, to enter cells.<sup>78,114,172</sup> Human occludin-1 recently has been shown to be necessary for HCV entry into mouse cells, an advance that will facilitate disease modeling in the laboratory.<sup>248</sup> Although the liver does not exclusively express any of these four molecules, the combination of these four, the structural organization of liver cells, and other yet-undetermined intracellular factors account for the tropism of HCV for the liver.<sup>246</sup>

Similar observations have been made for coxsackievirus isolates, which bind to the coxsackie-adenovirus receptor (CAR) molecule located in the tight junction for entry into cells.<sup>12,42,53,158</sup> Brain, heart, and muscle cells are enriched for CAR in the fetal and neonatal period, whereas adult cells from these tissues express significantly lower levels of the receptor.<sup>131,147</sup> This developmental difference in CAR density is the likely explanation for severe coxsackievirus infections that are disproportionately seen in infants and young children compared with adults. CAR then allows for an interaction with the tight junction that is mediated by occludin-1, which allows for viral co-opting of host trafficking pathways.<sup>53</sup>

Among eukaryotic pathogens, tissue tropism can also be a major determinant of virulence. Cerebral malaria is a life-threatening consequence of infection with the protozoan parasite *Plasmodium falciparum* and results from adherence of parasite-infected erythrocytes to cerebral vascular endothelium.<sup>209</sup> During erythrocyte infection, the malaria parasite exports a variety of surface receptors to the host plasma membrane. The *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) multigene family represents a highly variable set of such receptors, only one variant of which is expressed at any given time. A substantial body of work has established that different forms of PfEMP1 possess distinct tissue adherence patterns.<sup>171</sup> Specific classes of PfEMP1 types (Group A DC8 and DC13) are associated with cerebral vascular adherence in vitro and are correlated with severe malaria in clinical populations.<sup>7,38,177</sup> More recent studies suggest that these PfEMP1 forms mediate adherence through interaction with the endothelial protein C receptor.<sup>314</sup>

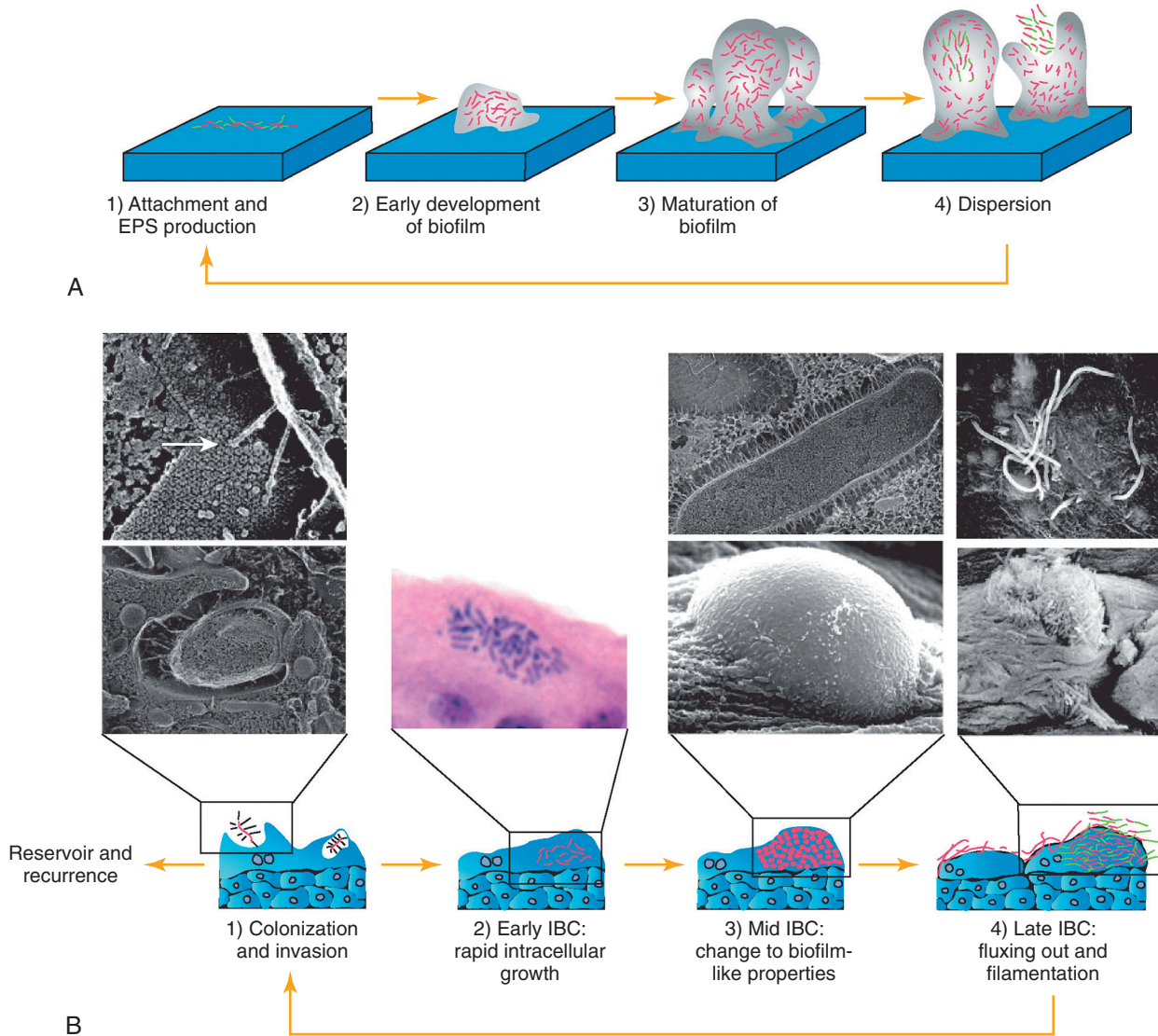
## BIOFILMS

After attachment to a particular surface, a number of pathogens are capable of forming *biofilms*, which can be defined as structured communities of microbial cells enclosed in a self-produced exopolysaccharide matrix. Although most studies of biofilms have involved a single species, it is likely that biofilms relevant to human infection often involve multiple species sharing the advantages of biofilm existence. Human infections associated with biofilms include dental caries, lower airway infection with *P. aeruginosa* and other organisms in patients with cystic fibrosis, and foreign body infections in patients with prostheses and implanted devices. In addition, biofilm formation likely occurs during osteomyelitis and endocarditis.<sup>49</sup>

*P. aeruginosa* is a model organism for the study of biofilms and forms pillars of stationary (sessile) bacteria held together by an extracellular polysaccharide called alginate. Interposed among these pillars are channels that facilitate the flow of nutrients and provide pathways for motile (planktonic) organisms to move about (Fig. 1.6A). In experiments directed at defining the early steps of *P. aeruginosa* biofilm formation, O'Toole and Kolter established that flagella are required for initial bacterial attachment, presumably because these appendages promote movement toward the relevant surface. After attachment, type 4 pili and pilus-mediated twitching motility promote formation of microcolonies<sup>227</sup> in which transcription of *algC*, *algD*, and *algU* is activated, resulting in synthesis of alginate.<sup>59</sup> Pulmonary isolates from patients with cystic fibrosis often form highly mucoid colonies (reflecting expression of alginate) or can form tiny colonies on agar plates, the so-called small-colony variant (SCV) phenotype associated with biofilm formation and increased antibiotic resistance.<sup>115,116,291</sup>

Development of the complex community present within a biofilm requires intercellular communication to coordinate the metabolic and other activities of members of the community. *P. aeruginosa* employs several identified *quorum-sensing* systems, which involve the production





**FIG. 1.6** In vitro *Pseudomonas* biofilm formation and parallel stages of formation of uropathogenic *Escherichia coli* intracellular bacterial communities (IBCs). (A) Dynamics of *P. aeruginosa* biofilm formation on an inert surface. Keys to the formation of the biofilm include flagella-mediated attachment, production of exopolysaccharide (EPS), type 4 pilus-based twitching motility, and a quorum sensing system. (B) Composite representation of the stages of IBC formation and maturation. (A, From Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. *Science*. 1999;284:1318–22, copyright 1999 American Association for the Advancement of Science. B, From Kau AL, Hunstad DA, Hultgren SJ. Interaction of uropathogenic *Escherichia coli* with host uroepithelium. *Curr Opin Microbiol*. 2005;8:54–9.)

of small molecules that are sensed by neighboring organisms and regulate gene expression in these neighbors. One well-studied system is based on the acyl-homoserine lactone called *N*-(3-oxododecanoyl)-L-homoserine lactone (3OC12-HSL).<sup>60,236</sup> 3OC12-HSL is synthesized in a reaction catalyzed by LasI and accumulates with increases in population density. Ultimately, 3OC12-HSL reaches a critical concentration and then interacts with LasR, serving to activate transcription of a number of genes. Host inflammatory pathways are also induced directly by accumulated 3OC12-HSL.<sup>279</sup> Organisms with a mutation in *lasI* are capable of attachment and microcolony formation, but the resulting microcolonies remain thin, undifferentiated, and sensitive to dispersion by detergents. Addition of the missing lactone signal to the *lasI* mutant restores development into structured, thick, biocide-resistant biofilms, as are observed with wild-type organisms.<sup>60</sup> In vivo, mutation in *lasI* impedes establishment of pulmonary infection in mice.<sup>242,340</sup>

Biofilms also play a prominent role in human infections with *Candida* species, with examples including oral thrush and catheter-associated infections. Although several pathogenic *Candida* species can form biofilms

in the host, the ultrastructure and the molecular strategies underlying biofilm formation vary from one species to another. *C. albicans* is the best studied *Candida* species and relies on the expression of certain cell wall proteins (including Hwp1),<sup>219</sup> a regulated yeast-to-hyphal switch described earlier, and quorum-sensing molecules such as *E,E*-farnesol, which represses filamentation and can suppress the growth of other nearby bacterial and fungal species.<sup>63,133,275</sup>

Biofilms constitute a protected mode of growth that allows survival in a hostile environment—for example, in the presence of host immune mechanisms or antimicrobial agents.<sup>49</sup> Based on studies of *P. aeruginosa*, sessile bacteria release antigens and stimulate production of antibodies, but these antibodies are ineffective in killing organisms within biofilms.<sup>41</sup> Similarly, sessile *P. aeruginosa* stimulate a diminished oxidative burst and are relatively refractory to phagocytic uptake. In addition, fungi and bacteria within biofilms are resistant to the effects of a number of antimicrobial agents, in part because these agents are unable to diffuse into the biofilm and in part because these organisms may exist in a slow-growing or otherwise protected phenotypic state.<sup>49</sup> Biofilm-like

microbial communities have also been described within host epithelial cells, as with uropathogenic *E. coli* in the mammalian bladder.<sup>6</sup> Recently, new approaches to antimicrobial therapy include novel natural products and other small molecules that inhibit quorum-sensing and biofilm formation.<sup>36,96</sup>

## CELL ENTRY AND INTRACELLULAR LIFE

After adherence to a host surface, many pathogenic bacteria are able to invade and survive inside epithelial cells and other nonprofessional phagocytes (i.e., M cells in intestinal Peyer's patches). In addition, some pathogens are able to survive inside professional phagocytes (macrophages and neutrophils). Invasion may represent a mechanism to breach host mucosal barriers and gain access to deeper or more distant tissues. Alternatively, invasion may provide the organism with a special niche (e.g., protecting it from host immune mechanisms). In the case of viruses, cell entry ensures access to the cell machinery required for viral replication.

Generally the process of bacterial invasion involves a class of molecules called invasins that mediate adherence and entry. For many bacteria, invasion is an active event that relies on underlying host cell functions and is associated with rearrangement of the host cell cytoskeleton. Once inside the host cell, the invading or internalized organism usually is localized within a membrane-bound vacuole that contains lysosomal enzymes. In some cases the pathogen escapes from this vacuole and enters the cytoplasm, a more permissive environment. In other cases, the pathogen remains in the vacuole and neutralizes lysosomal enzymatic activity. The processes of invasion into cells, survival within cells, cell-to-cell spread, and entry into the circulation define the extent of infection and dissemination.

### Invasion

In considering the molecular mechanism of bacterial invasion, perhaps best characterized are the enteropathogenic *Yersinia* species—namely, *Y. pseudotuberculosis* and *Y. enterocolitica*. These organisms are usually acquired by ingestion of contaminated food or water and typically cause self-limited enteritis or mesenteric adenitis. In infants and other individuals with compromised immunity, they sometimes produce systemic disease. The primary determinant of *Y. pseudotuberculosis* and *Y. enterocolitica* invasion is an adhesive outer membrane protein called invasin, which is encoded by a chromosomal locus called *inv* and binds tightly to a family of  $\beta 1$  integrins expressed on host cells, including  $\alpha 3 \beta 1$  integrin on the surface of intestinal M cells.<sup>145</sup> The interaction between invasin and  $\beta 1$  integrins initiates a cascade of signaling steps in the host cell, resulting in actin rearrangement and formation of large complexes of cytoskeletal elements (talin, vinculin,  $\alpha$ -actinin, and others) termed *focal adhesions*.<sup>144</sup> Bacterial entry into the host cell occurs via a “zipper-like” mechanism, with the plasma membrane zippering around the invading organism.

Beyond invasin, two additional proteins called YadA and Ail also influence invasion by enteropathogenic *Yersinia* species. YadA is a 45-kDa surface protein that is encoded by the 70-kb *Yersinia* virulence plasmid. It is highly expressed under environmental conditions (e.g., temperature of 37°C) in which invasin is repressed.<sup>75</sup> YadA reaches the bacterial surface via the autotransporter pathway and exists in a trimeric form that is essential for its adhesive activity.<sup>51</sup> Like invasin, YadA promotes invasion through binding to  $\beta 1$  integrins on the host cell surface, but its binding occurs indirectly via extracellular matrix molecules, including collagens, laminin, and fibronectin.<sup>76</sup> Based on studies using a mouse oral infection model, in *Y. enterocolitica* YadA is essential for survival and multiplication in Peyer's patches, whereas in *Y. pseudotuberculosis* YadA is dispensable for full virulence.<sup>22</sup> Ail is a 17-kDa outer membrane protein that also is encoded by a chromosomal locus (*ail*) and mediates high levels of adherence and low levels of invasion in assays with cultured epithelial cells. In addition, Ail mediates resistance to complement-mediated serum killing, independent of an effect on invasion.<sup>19</sup>

Similar to these pathogenic *Yersinia* species, *Listeria monocytogenes* invades epithelial cells via a zipper-like mechanism. Invasion is mediated by proteins called internalin A (InlA) and internalin B (InlB), which are required for virulence in animal models. InlA interacts with

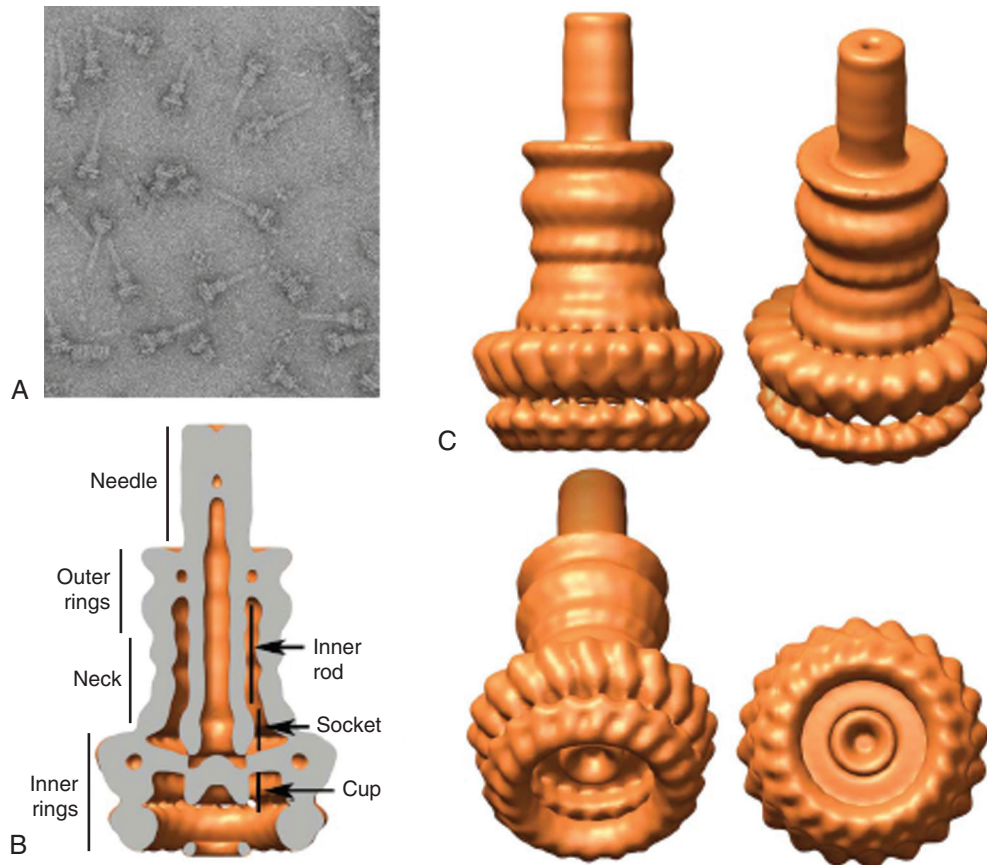
E-cadherin, a host cell transmembrane protein with an intracellular domain that interacts with the cytoskeleton.<sup>205</sup> InlB interacts with C1q on host cells and promotes invasion by activating the PI-3 kinase pathway.<sup>24</sup> Uropathogenic strains of *E. coli* also invade epithelial cells via a zipper-like mechanism mediated by the FimH adhesin expressed on the tip of type 1 pili. In experiments with cultured bladder epithelial cells, FimH is both necessary and sufficient for entry, as demonstrated by examination of a *fimH*<sup>-</sup> mutant and of latex beads coated with purified FimH.<sup>194</sup> In vitro experiments further suggest that FimH-mediated bacterial binding to a mannose-coated surface may be strengthened by shear forces, such as fluid flow over the surface.<sup>307,308</sup> After FimH-dependent invasion into superficial epithelial cells of the murine bladder, UPEC multiply rapidly to form intracellular bacterial communities, which display some features of biofilms, including community behavior, differential gene expression, and protection from antimicrobial agents (see Fig. 1.6B).<sup>6,156,160</sup> A subset of internalized bacteria ultimately form a quiescent bacterial reservoir within the uroepithelium that resists immune clearance and antibiotic therapy and may serve as a seed for recurrent infections.<sup>139,213,214</sup>

*Salmonella enterica* serovar *typhimurium* (*S. typhimurium*) is an example of a pathogen that invades cells by a mechanism distinct from zippering. On contact with the epithelial cell surface, *S. typhimurium* triggers a dramatic host cell response characterized by actin rearrangement, calcium and inositol phosphate fluxes, and a “splash” of membrane ruffling surrounding the point of entry. Bacterial internalization into the cell occurs rapidly, with organisms appearing in membrane-bound vacuoles within a few minutes of initial contact with the host cell. The determinants of *S. typhimurium* invasion are encoded by a pathogenicity island called SPI-1, located at centisome 63 on the bacterial chromosome.<sup>92</sup> Especially important in this region is a prototypical *type III secretion system*, which forms a needle-like complex on the bacterial surface that breaches the host cell membrane and serves to translocate bacterial proteins directly into the host cell, altering the host cell cytoskeleton<sup>43,186</sup> and influencing immune responses. The base of the needle complex spans both the inner and outer membranes and is about 40 nm in diameter, whereas the needle itself is 8 nm in width and approximately 80 nm in length (Fig. 1.7).<sup>45,93</sup>

The proteins secreted through the *S. typhimurium* needle complex (and other type III secretion systems) and into the host cell are referred to as *effector proteins*. SopE is an effector protein that mediates the initial rearrangement of actin and ruffling of the host cell membrane. It functions as a guanyl-nucleotide exchange factor (GEF) and activates two host cell Rho GTPase proteins called Rac and Cdc42.<sup>35,111,113</sup> SptP is an effector protein that functions as an antagonist of SopE, mediating reversal of actin rearrangement by converting Rac and Cdc42 to the inactive forms (GDP forms). Consistent with these functions, SopE and SptP directly antagonize each other when co-injected into cells.<sup>91</sup> Other effector proteins secreted by the *S. typhimurium* SPI-1 type III secretion system include the inositol phosphate phosphorylase SopB, which disrupts normal host cell signaling mechanisms,<sup>224</sup> and AvrA, which interferes with the nuclear factor  $\kappa B$  (NF- $\kappa B$ ) signaling pathway in host cells, thereby downregulating host inflammatory responses.<sup>44</sup>

Important accessory and regulatory genes are also present within SPI-1. As an example, the *sicA* gene is just upstream of the *sipB* and *sipC* genes and encodes an accessory protein with chaperone activity essential for stabilization and translocation of SipB, SipC, and SopE.<sup>313</sup> Other chaperones encoded by SPI-1 are involved in the stabilization and translocation of other effector proteins. The genetic and environmental factors that regulate the expression of type III secretion machinery and secreted proteins represent an area of ongoing study.<sup>5</sup>

In *Plasmodium falciparum*, the mechanisms of both host erythrocyte invasion and immune evasion are tightly linked. A mature parasite may release as many as 32 daughter parasites, called merozoites, into the bloodstream. In less than 30 seconds, merozoites attach to and invade new erythrocytes. High-titer antibodies to invasion proteins, as are present in the serum of semi-immune individuals living in endemic areas, can block these processes. For this reason, the molecular invasion machinery of *P. falciparum* is of considerable interest for vaccine development.<sup>240,303</sup> The initial contact between *Plasmodium* merozoites and host erythrocytes is weak and is thought to be mediated through



**FIG. 1.7** General structure of the gram-negative type III secretion system, according to electron micrographic and other data. Represented are (A) electron micrographs of isolated needle complexes, (B) cross-sectional schematic of the components of the needle complex, and (C) surface views of the assembly. (From Galan JE, Wolf-Watz H. Protein delivery into eukaryotic cells by type III secretion machines. *Nature*. 2006;444:567–73.)

merozoite surface proteins (MSPs) present along the entire merozoite surface. Stronger interactions are mediated by several additional receptors, notably the erythrocyte binding antigens EBA-175 and EBA-140, which engage the erythrocyte-specific receptors glycoporphin A and glycoporphin B, respectively. Recently, an additional parasite protein called PfRH5 was recognized as an indispensable mediator of parasite invasion via interaction with the human receptor basigin.<sup>54</sup> Anti-RH5 antisera have potent invasion-inhibiting activities,<sup>312</sup> and an RH5-based vaccine showed efficacy in an *Aotus* monkey infection model.<sup>72</sup> On the basis of these data, PfRH5 has emerged as a strong candidate vaccine antigen to prevent severe malaria.

### Intracellular Survival

Once an organism invades a nonprofessional phagocyte or is ingested by a professional phagocyte, several potential outcomes exist. Often, the organism is killed. However, some pathogens have developed strategies to survive and replicate inside host cells, in some cases within a vacuole and in others by escaping from the vacuole.

There is general agreement that *S. typhimurium* resides within a membrane-bound vacuole in both professional and nonprofessional phagocytes. However, the vacuole lacks several lysosomal markers typical of the main endocytic pathway (the mannose-6-phosphate receptor pathway) and appears to be distinct from this pathway. Insight into the molecular determinants of intravacuolar survival came when two independent groups reported the discovery of a second *Salmonella* pathogenicity island, now called SPI-2.<sup>122,228</sup> This island maps to centisome 31 and encodes another type III secretion system, including structural proteins (*ssa* locus),<sup>122</sup> effector proteins (*sse* locus), and accessory proteins (*ssc* locus). In addition, this region encodes a two-component regulatory system consisting of a membrane-located sensor kinase (SsrA) and a

transcriptional regulator (SsrB).<sup>228</sup> Mutations in SPI-2 result in reduced survival inside macrophages, with no effect on adherence and invasion in assays with intestinal epithelial cells.<sup>228</sup> *Salmonella* SPI-2 mutants demonstrate reduced virulence in experimental mice (up to a 104-fold reduction in 50% lethal dose), suggesting that survival inside macrophages is a key factor in the pathogenesis of disease.<sup>273</sup> Expression of SPI-2 genes within the macrophage vacuole depends at least in part on the acidic intravacuolar environment. Inhibition of macrophage vacuolar acidification using bafilomycin A1 (an inhibitor of the vacuolar proton ATPase) results in a sharp attenuation in transcription of SPI-2 genes. This effect is not reproduced by low pH alone outside the vacuole, suggesting that other environmental effects within the vacuole influence SPI-2 expression.<sup>37</sup> Recent work indicates that *Salmonella* SPI-2 transcription is activated before invasion, apparently preparing the pathogen for the hostile intracellular environment.<sup>26</sup> As a group, the SPI-2 genes appear to modulate host endocytic and exocytic transport mechanisms and inflammatory signaling.<sup>1,142</sup>

A third *Salmonella* pathogenicity island called SPI-3 also promotes survival inside macrophages. This island is located at centisome 82 and was discovered by examining the *Salmonella selC* locus, a tRNA gene where pathogenicity islands reside in some strains of *E. coli*.<sup>15,17</sup> SPI-3 contains the *mgtBC* operon, which permits *S. typhimurium* growth in environments with low concentrations of Mg<sup>2+</sup>, including macrophages. In particular, mutation of the *mgtBC* operon abolishes the ability of *S. typhimurium* to replicate in low-Mg<sup>2+</sup> liquid media and in macrophages, and addition of Mg<sup>2+</sup> to the medium after phagocytosis restores the ability to survive intracellularly. Homologous *mgtBC* genes have been found in other organisms with intracellular lifestyles, such as *Brucella melitensis* and *Yersinia pestis*.<sup>16</sup> In *Salmonella*, the *mgtBC* genes are expressed after internalization into host cells under control of the



PhoP-PhoQ two-component regulatory system, a complex that directs expression of a number of virulence determinants.<sup>191</sup>

The ability to survive within phagocytic cells may provide *Salmonella* with a means to exploit an intrinsic host pathway and disseminate to distant sites. In particular, certain phagocytes express the  $\beta 2$  integrin CD18, which mediates leukocyte migration in response to various stimuli. During *S. typhimurium* infection, CD18-expressing phagocytes carry organisms from the intestine to the spleen. Indeed, bacterial loads in the liver and spleen are reduced after oral inoculation in CD18-deficient mice when compared with infection in wild-type mice.<sup>318</sup> On the one hand, this function of CD18 facilitates initiation of a systemic immune response and benefits the host. However, at the same time, it provides bacteria with a mechanism of transit from the gut to organs of the reticuloendothelial system and elsewhere.

*Mycobacterium tuberculosis* is another intracellular pathogen, and it uses an array of mechanisms to ensure intracellular survival. The *M. tuberculosis* vacuole lacks the usual amounts of the vesicular proton ATPase responsible for mediating acidification and fails to acidify to normal levels.<sup>298</sup> In addition, *M. tuberculosis* blocks fusion of the vacuole with acidic lysosomes, further preventing acidification.<sup>268</sup> Similar to intracellular gram-negative bacterial pathogens, *M. tuberculosis* contains an *mgTc* gene, and mutation of this gene results in impaired virulence in cultured human macrophages and in mouse spleen and lung. Low  $Mg^{2+}$  concentration and mildly acidic pH inhibit the growth of the *mgTc* mutant, suggesting that the gene is important for survival in the phagosome, where such conditions may exist.<sup>28</sup> Another factor that influences *M. tuberculosis* survival within macrophages is isocitrate lyase, an enzyme of the glycolytic shunt that is essential for metabolism of fatty acids. Expression of isocitrate lyase is upregulated during infection of activated macrophages and is required for full virulence in a murine model of infection, independent of an effect on bacterial growth.<sup>198</sup> The crystal structure of *M. tuberculosis* isocitrate lyase has been solved and may provide a target for new drug therapies against persistent infection because this enzyme is absent from vertebrates.<sup>271,282</sup>

During the course of interaction with macrophages, *M. tuberculosis* (at a low to moderate multiplicity of infection) is capable of stimulating caspase-1 and inducing macrophage apoptosis. Interestingly, less virulent strains of *M. tuberculosis* are more potent inducers of apoptosis, perhaps resulting in benefit to the host by preventing systemic spread of infection.<sup>163</sup> At the same time *M. tuberculosis* possesses at least two anti-apoptotic mechanisms that further influence the outcome of macrophage encounters. First, *M. tuberculosis* infection enhances host macrophage production of soluble TNFR2, a protein that binds to tumor necrosis factor alpha (TNF $\alpha$ ) and interferes with apoptosis.<sup>8</sup> Second, *M. tuberculosis* infection activates production of NF- $\kappa$ B, a transcriptional regulator that activates anti-apoptotic pathways within the host cell.<sup>97</sup> Of note, higher multiplicities of infection with virulent strains of *M. tuberculosis* can induce caspase-independent cell death in macrophages, a mechanism proposed to contribute to the formation of necrotic lesions during tuberculous disease.<sup>179</sup>

*L. monocytogenes* is an example of an organism that escapes from the phagocytic vacuole in macrophages and epithelial cells and moves into the cytoplasm. This organism causes meningitis and focal brain abscesses in humans and exhibits tropism for the fetoplacental unit. In pregnant women, listeriosis results in fetal loss in 30% of cases. Intravacuolar replication and escape from the vacuole are dependent on listeriolysin O, a hemolysin encoded by the *hly* gene.<sup>13</sup> Listeriolysin O interacts with cholesterol in host cell membranes and forms pores, leading to lysis of the phagosome.<sup>90</sup> Host enzymatic activities may also contribute to *Listeria* escape from the phagosome. In human epithelial cells the contributions of a broad-range phospholipase C (called PC-PLC) and a metalloproteinase called Mpl are most important for vacuolar escape in the absence of listeriolysin O.<sup>107,192,281</sup>

Intracellular survival of the protozoan parasite *Toxoplasma gondii* is thought to rely on parasite virulence factors that directly counter innate host defenses. In mice, interferon gamma (IFN $\gamma$ ) production is required to limit replication of *T. gondii*, in part through induction of immunity-related GTPases (IRGs). In mammalian cells, successful *T. gondii* strains replicate within a protected parasitophorous vacuole (PV) that does not fuse to the host lysosome. During infection with relatively

nonpathogenic parasites, recruitment of IRGs to the PV results in disruption of the PV and parasite death. In contrast, highly pathogenic *T. gondii* express an active serine-threonine kinase called ROP18, which is exported into the host cytoplasm and prevents the recruitment of IRGs.<sup>140,301</sup> The ROP kinase family is highly expanded in *T. gondii*, comprising 44 other proteins, many of which lack critical catalytic residues and are thought to function as regulatory “pseudokinases.” Interestingly, ROP kinases are absent from the related apicomplexan *Plasmodium* spp., presumably because prevention of IRG recruitment is unnecessary within the protected niche of the host erythrocyte.

### Viral Cell Entry

Viruses have developed a specialized family of proteins that specifically function to engage host cell proteins and fuse with cell membranes and that allow for transfer of viral genetic material. The details regarding structure and function of these proteins are reviewed elsewhere.<sup>168</sup> There are two known classes of viral fusion proteins: class I proteins form a hairpin structure with a known  $\alpha$ -helix domain (e.g., HIV gp41, influenza HA2), and class II proteins exist in  $\beta$ -sheets and transition from a moderately stable dimeric form to a very stable trimer (e.g., dengue E protein). These fusion proteins undergo conformational changes when transitioning from the prefusion to the postfusion state, resulting in a more stable form that favors the process of viral fusion and entry.

### Cell-to-Cell Spread

Movement from one cell to another may help an organism gain a stronger foothold in host tissues. *L. monocytogenes* is one example of a pathogen capable of cell-to-cell spread. Once this organism is free in the cytoplasm, actin begins to polymerize on the bacterial surface. Eventually the condensed actin forms a polar tail or comet, which propels the organism through the cytoplasm and into adjacent cells. The rate of bacterial movement within a cell correlates with actin tail length.<sup>305</sup> Actin accumulation and condensation is mediated by the *L. monocytogenes* ActA protein, which is tightly anchored to the bacterial surface and is expressed asymmetrically over the length of the organism.<sup>283,304</sup> ActA is the sole *Listeria* factor required for actin polymerization because actin tails form in *Xenopus* cytoplasmic extracts containing ActA-coated beads. However, in these experiments, motility occurs only when ActA is distributed asymmetrically on the beads.<sup>30</sup> ActA appears to interact directly with actin and also with a variety of other host cytoskeletal proteins.<sup>90,306</sup> Cytochalasin D is an inhibitor of actin polymerization and inhibits the cell-to-cell spread of *L. monocytogenes* in epithelial monolayers.<sup>58,210</sup>

On reaching the plasma membrane, bacteria protrude from the cell in filopodium-like structures (called listeriopods), which are then engulfed by neighboring cells. This engulfment may be part of a normal host process because MDCK cells demonstrate low-level endocytosis of adjacent cell membrane fragments even in the absence of bacteria.<sup>261</sup> The formation of listeriopods and the engulfment of these structures by neighboring cells are independent of listeriolysin O, PI-PLC, and PC-PLC.<sup>98</sup> Once inside a nascently infected cell, *Listeria* escapes from the double-membrane vacuole via the action of PI-PLC, PC-PLC, and Mpl.<sup>90</sup> On arrival in the cytosol, bacteria can enter another cycle of actin-based motility and cell-to-cell spread, although one or two bacterial generations may be necessary to regain motility.<sup>261</sup>

A second pathogen capable of actin-based motility and cell-to-cell spread is *Shigella flexneri*. In *Shigella*, a single protein called IcsA is sufficient to induce formation of an actin tail, similar to that observed in *L. monocytogenes*. IcsA is an autotransporter protein that is encoded on the *Shigella* virulence plasmid and is distributed on the bacterial surface in a polarized fashion, possibly as a result of specialized machinery for autotransporter protein secretion near the poles of some gram-negative bacteria.<sup>151</sup> Initially, IcsA is distributed over the whole bacterial surface, with a predominance at one pole. However, over time a secreted bacterial protease called IcsP cleaves roughly half of the surface IcsA, mostly at the opposite pole, further polarizing distribution.<sup>74,292</sup> Elimination of expression of IcsP leads to increased quantities of IcsA and increased actin-based motility, suggesting that IcsA (rather than host factors) is rate limiting in the motility process.<sup>274</sup> Like ActA, IcsA is necessary and sufficient to induce polymerization of the actin tail, and

the tail forms at the end where IcsA concentration is highest.<sup>102</sup> Despite the functional similarities between IcsA and ActA, there is no significant sequence homology between the two proteins. In contrast to ActA, no direct interaction between IcsA and actin has been demonstrated, and IcsA is found throughout the actin tail, not only at the bacterial pole-actin tail junction.

When considering cell-to-cell spread of viruses, it is helpful to return to the example of HCV. Claudin-1 and occludin-1 are the gap junction proteins that serve as the final entry point for this virus.<sup>78,248</sup> It has been demonstrated that HCV can infect neighboring liver cells directly via these gap junction proteins, likely bypassing the extracellular release of virus and subsequent SR-B1 and CD81 binding steps.<sup>309</sup> Experiments have demonstrated that antibodies blocking the E2-CD81 interaction inhibit infection from virus-infected media but do not affect infection of naïve cells when co-cultured with previously HCV-infected cells.<sup>309</sup> It has also been shown that after infecting a liver cell, HCV promotes breakdown of typical apical to basal organization of liver cells, exposing the gap junction complexes and presumably facilitating cell-to-cell spread.<sup>199,200</sup>

## DAMAGE TO THE HOST

Damage to host cells and host tissues represents a fundamental mechanism by which a pathogen is able to survive at a given site and then spread within a host. Generally, damage is induced by microbial toxins. Most toxins are released extracellularly and are capable of inducing damage at very low concentrations (*exotoxins*). Microbial attachment and invasion facilitate toxin delivery to target cells and target tissues and serve to enhance toxicity.

Historically, microbial toxins have been classified according to a variety of criteria, including cellular target of action (e.g., enterotoxins, leukotoxins, neurotoxins), mechanism of action (e.g., adenosine diphosphate [ADP]-ribosylating toxins, adenylate cyclase toxins, pore-forming toxins, proteolytic toxins), and major biologic effect (e.g., hemolytic toxins, edema-producing toxins). In recent years the term *toxin* has been applied more broadly to include enzymes that mediate damaging effects via phospholipase or hyaluronidase activity.

### *Bordetella pertussis* Toxins

Whooping cough (*B. pertussis* infection) is a classic example of a toxin-mediated disease and involves the interplay of multiple toxins.<sup>165</sup> The pathogenesis of whooping cough begins with *B. pertussis* colonization of the trachea, which is facilitated by a molecule called tracheal cytotoxin (TCT). TCT is a naturally occurring disaccharide-tetrapeptide fragment of peptidoglycan and belongs to the family of muramyl peptides.<sup>103</sup> Many gram-negative organisms produce an analogous fragment during normal turnover of cell wall components, but significant extracellular release appears to occur only in *Bordetella* species and gonococci. In most other species an inner membrane protein called AmpG recycles this fragment back into the bacterial cell.<sup>50</sup> TCT is toxic to tracheal epithelial cells in vitro, stimulating nitric oxide synthase and local production of interleukin-1 and causing inhibition of ciliary motility, inhibition of DNA synthesis, and cell death.<sup>117–119,124</sup> During natural infection, TCT is thought to paralyze the mucociliary escalator and thereby interfere with clearance of *B. pertussis* and respiratory mucus.

Pertussis toxin is believed to be a key determinant of the clinical manifestations of whooping cough. This toxin belongs to a family of bacterial ADP-ribosyltransferase enzymes. The target of pertussis toxin is host cell G proteins, resulting in disruption of normal signaling processes. A number of biologic effects have been ascribed to pertussis toxin, including induction of lymphocytosis, stimulation of insulin release, sensitization to histamine, and disruption of phagocytic cell function; however, the specific relationship between the effects of pertussis toxin and the symptoms of whooping cough remains unclear.<sup>125</sup> Of note, *B. parapertussis* is closely related to *B. pertussis* and produces a similar cough illness but fails to produce pertussis toxin because of mutations in the *ptx* promoter region.<sup>218</sup>

*B. pertussis* also elaborates a toxin called adenylate cyclase toxin (CyaA), a member of the RTX (repeat-in-toxin) family of bacterial cytolytins whose prototype is the *E. coli* hemolysin HlyA.<sup>50</sup> These toxins

cause target cell lysis by creating pores in the host cell plasma membrane, but at sublytic concentrations many of these toxins also manipulate host enzymatic and signaling pathways within the host cell. In the case of *B. pertussis*, CyaA inhibits host adenylate cyclase, resulting in accumulation of cyclic adenosine monophosphate (cAMP); elevated levels of cAMP within phagocytic cells inhibit oxidative activity and induce apoptosis, thus disabling this arm of the immune system.<sup>166,167,243</sup> In respiratory epithelial cells, elevated cAMP may result in increased fluid and mucus secretion, further impairing mucociliary function.

Among other examples of these dual-function RTX toxins, the prototypic HlyA of uropathogenic *E. coli* induces the degradation of host actin-associated proteins, resulting in exfoliation of the superficial epithelial layer in the bladder.<sup>64</sup> The  $\alpha$ -hemolysin of contemporary community-associated *S. aureus* strains activates a host epithelial cell surface molecule called ADAM10, which cleaves E-cadherin at cell-cell junctions to permit access of the pathogen across the epithelial layer.<sup>143,332</sup>

### Hemolytic-Uremic Syndrome and Shiga Toxins

A number of intestinal pathogens produce Shiga toxins, including *Shigella dysenteriae*, enterohemorrhagic *E. coli* (including *E. coli* O157:H7), and *Citrobacter freundii*, among others. Shiga toxins are classic A-B toxins, consisting of an A subunit that has toxic activity and five B subunits arranged in a pentameric ring-like structure that promotes binding to host cells and delivery of the A subunit. The B subunits interact with host cell globoseries glycolipids, especially the Pk trisaccharide moiety of globotriaosylceramide (GbO3). The A subunit is endocytosed by the host cell and traverses the cytoplasm in membrane-bound vesicles. Some of these vesicles travel in a retrograde fashion to the Golgi apparatus and then to the endoplasmic reticulum.<sup>269</sup> Shiga toxin then co-opts the function of the endoplasmic reticulum proteins HEDJ and BiP to enter the cytosol, where it enzymatically inhibits host 28S ribosomal RNA by cleaving a single adenine residue, resulting in inhibition of protein synthesis and cell death.<sup>269,347</sup>

In humans, *E. coli* O157:H7 is an important cause of hemorrhagic colitis and sometimes produces hemolytic-uremic syndrome. Infection begins with adherence to epithelial cells via intimin and other proteins encoded by the locus of enterocyte effacement (LEE), resulting in formation of attaching and effacing lesions analogous to those observed in EPEC infection.<sup>196</sup> After adherence, the organism releases Shiga toxin, which traverses the intestinal epithelial cell and enters the bloodstream.<sup>2</sup> Toxin circulates to distant organs and mediates damage via toxicity to endothelium. Diarrhea likely results from damage to endothelium in small mesenteric vessels, leading to ischemia and sloughing of the intestinal mucosa. The renal effects observed in human hemolytic-uremic syndrome arise from microvascular and glomerular damage with luminal occlusion by fibrin and platelets.<sup>353</sup> Hemolysis and thrombocytopenia likely develop as a consequence of microangiopathy.

### Tissue-Degrading Toxins

A number of toxins have enzymatic activity and are capable of degrading tissue components. One example is hyaluronidase, which degrades hyaluronic acid, a repeating disaccharide glycosaminoglycan involved in cell motility, adhesion, and proliferation in normal hosts. Hyaluronic acid contains alternating *N*-acetylglucosamine and glucuronic acid moieties, connected by  $\beta$  linkages. It is prominent in extracellular matrix when cell turnover and tissue repair are prominent—for example, in embryogenesis, wound healing, and carcinogenesis.<sup>55</sup> The primary host receptor for hyaluronic acid is CD44, which undergoes post-translational modification that varies according to host cell type. Interactions between hyaluronic acid and CD44 are critical to T- and B-cell stimulation, growth of certain lymphoid malignancies, and propagation of certain inflammatory responses.<sup>207</sup>

In *S. pyogenes*, hyaluronidase is a 96-kDa protein that is encoded by the *hylA* gene and is released extracellularly. It is proposed to promote invasion through cell layers and tissue planes and is considered one of several *S. pyogenes* spreading factors.<sup>141</sup> Interestingly, *S. pyogenes* also produces a thick “capsule” of hyaluronic acid that can interact with other host cellular and extracellular matrix proteins to contribute to tissue invasion by the organism. Other pathogens that produce a hyaluronidase include *S. agalactiae* (group B streptococcus),

*Treponema pallidum*, *Candida* spp., *Entamoeba histolytica*, and *Ancylostoma braziliense*.<sup>55</sup>

## EVASION OF IMMUNITY

To survive and replicate within the host, a pathogen must evade the host immune system. Initially the organism must circumvent innate immune mechanisms, including mechanical forces, resident phagocytes, and complement activity. Over time the organism must overcome adaptive immunity as well, including the presence of specific antibodies.

### Antiphagocytic Factors

As described earlier in this chapter, invasion-mediated entry into M cells plays an important role in the early stages of *Yersinia* infection. At the same time, evasion of phagocytosis is critical to the pathogenesis of *Yersinia* disease. The ability to avoid phagocytosis is dependent on the *Yersinia* virulence plasmid, which encodes a number of proteins called Yops.<sup>48,295</sup> Both YopE and YopH interfere with ingestion by macrophages and neutrophils via slightly different mechanisms. YopE shares sequence homology with the *Salmonella typhimurium* SptP protein and down-regulates all three of the Rho GTPases (Rho, Cdc42, and Rac), thus inhibiting actin rearrangement and blocking formation of membrane ruffles (lamellipodia) and spikes (filopodia).<sup>3,19</sup> YopH is a protein tyrosine phosphorylase that appears to act on a host cell cytosolic protein called Cas, interfering with recruitment of Rho, Cdc42, and Rac and preventing formation of actin stress fibers, focal complexes, and focal adhesions.<sup>14,18</sup> YopJ is an acetyltransferase that covalently modifies and inactivates intermediate kinases in the mitogen-activated protein kinase and NF- $\kappa$ B signaling pathways, leading to host cell apoptosis.<sup>212,345</sup> Importantly some Yop effectors also represent important immunogens; for example, the immunodominant epitope of YopE represents a major CD8<sup>+</sup> T-cell antigen in experimental plague and may facilitate a new direction in *Yersinia* vaccine development.<sup>182,350</sup>

*Shigella* employs another strategy to induce apoptosis in phagocytic cells. This pathogen produces hemorrhagic enterocolitis and is an important cause of bloody diarrhea in children. Infection begins with ingestion of organisms, which attach to intestinal M cells and then cross the intestinal epithelium.<sup>356</sup> On entry into the subepithelial space, organisms are engulfed by resident macrophages and contained in membrane-bound vacuoles. However, they quickly escape from macrophage vacuoles and move to the cytosol of the cell, where they induce apoptosis.<sup>355</sup> The mechanism of apoptosis involves a protein called IpaB, which is encoded by the *Shigella* virulence plasmid and is injected into host cell membranes via the *Shigella* type III secretion system.<sup>20</sup> Work by Zychlinsky and coworkers showed that IpaB binds to cytosolic interleukin-1 $\beta$  converting enzyme (caspase-1), a cysteine protease that cleaves IL-1 $\beta$  to its active form.<sup>126</sup> Of note, the *typhimurium* SipB protein shares homology with IpaB and also induces apoptosis by interacting with caspase-1.<sup>123</sup> Interestingly, recent work has shown that IpaB has contrasting effects in epithelial cells, binding to the cell-cycle regulator Mad2L2 to inhibit epithelial turnover and promoting epithelial colonization with *Shigella*.<sup>148</sup>

### Evasion of Complement Activity

*S. pyogenes* expresses at least three factors that interfere with host complement activity. Perhaps best known is M protein, which inhibits activation of the alternative complement pathway. This effect is mediated at least in part by the ability of M protein to bind complement factor H, a regulatory protein that inhibits assembly and accelerates decay of C3bBb. Recent studies indicate that serotype M1 and M57 strains express an extracellular protein called Sic (streptococcal inhibitor of complement-mediated lysis), which associates with human plasma proteins called clusterin and histidine-rich glycoprotein (HRG) and apparently blocks formation of the membrane attack complex (C5b-C9).<sup>4</sup> Studies of epidemic waves of M1 infection demonstrate that Sic undergoes significant variation over time, perhaps in response to the selective pressure associated with specific antibodies.<sup>129,130,201</sup> Of note, nonpolar inactivation of *sic* results in reduced mucosal colonization of mice.<sup>185</sup> In addition, *S. pyogenes* produces a serine protease called C5a peptidase,

which cleaves and inactivates C5a.<sup>330</sup> C5a is a cleavage product of C5 and serves as a powerful chemoattractant for neutrophils; thus, streptococcal C5a peptidase serves to attenuate the neutrophil response to infection.

*N. gonorrhoeae* is a common cause of cervicitis, urethritis, and pelvic inflammatory disease and is also capable of producing disseminated disease. Recent spread of antibiotic resistance in this pathogen highlights the need for understanding its pathogenic mechanisms to develop new mitigating strategies. Resistance to complement-mediated killing is important in gonococcal pathogenesis and is due in part to sialylation of lipo-oligosaccharide (LOS), which involves addition of host-derived cytidine monophospho-*N*-acetylneuraminic acid (CMP-NANA) by a bacterial sialyltransferase. Given the requirement for CMP-NANA, subcultivation in the absence of human serum or human neutrophils is associated with loss of sialylation and loss of resistance. Sialylated LOS binds factor H, resulting in downregulation of activity of the alternative pathway C3 convertase. In addition, sialylated LOS interferes with neutrophil phagocytosis and with the normal oxidative burst in neutrophils.<sup>260,329</sup> A second determinant of resistance to complement-mediated killing is Por1, an outer membrane porin protein that binds both factor H and C4b binding protein (C4b BP).<sup>255</sup> C4b BP binds C4b and serves to inhibit assembly and accelerate decay of C4b2a, the classical pathway C3 convertase. Gonococci also produce a third factor that influences resistance to complement—namely, an outer membrane-expressed nitrite reductase called AniA.<sup>21,31</sup>

### Evasion of Humoral Immunity

A number of pathogens have evolved mechanisms to vary surface-exposed immunogenic molecules, thus facilitating evasion of a specific antibody response. *Antigenic variation* represents one such mechanism and is characterized by the emergence of modified molecules with novel antigenic properties. *Phase variation* represents a second such mechanism and is typified by the reversible loss or gain of a given molecule or structure.

*N. gonorrhoeae* is capable of producing recurrent infection, reflecting the fact that the antibody response to infection fails to provide lasting immunity. In this context, it is noteworthy that *N. gonorrhoeae* pili are an important target of serum antibody and undergo frequent antigenic variation. Gonococcal pilin expression is controlled by the *pilE* locus (the expression locus), which contains an intact pilin gene along with promoter sequences. In addition to *pilE*, the gonococcal chromosome contains numerous copies of variant *pil* sequences, called *pilS* loci.<sup>110</sup> These loci are transcriptionally inactive because they lack a promoter and 5' coding sequence. However, they can be introduced into the expression locus by RecA-dependent recombination, resulting in an altered structural subunit and antigenically variant pili.<sup>136</sup> Because *N. gonorrhoeae* is naturally transformable, horizontal exchange of species-specific DNA may also give rise to new *pil* sequences.

The African trypanosomes (including *Trypanosoma brucei*) are parasites that cause sleeping sickness in sub-Saharan Africa and account for more than 50,000 deaths per year. These organisms are able to avoid humoral immunity by antigenic variation of a large family of proteins called variable surface glycoproteins (VSGs), which coat the entire surface of the trypanosome. VSGs are highly immunogenic and stimulate antibodies that lead to efficient and rapid clearing of parasites from the bloodstream. However, at any given point in time, the organism is able to express a new VSG, allowing some organisms to escape the antibody response against the previous VSG. Each parasite can express more than 100 different VSGs, with variation in expression occurring spontaneously at a rate of up to 10<sup>-2</sup> per cell per generation. Overall, the genome of *T. brucei* contains more than 1000 *vsg* genes, including so-called expression sites (ESs) located near telomeres on mini-chromosomes and silent loci in nontelomeric sites on large chromosomes.<sup>241,310</sup> In general, VSG antigenic variation occurs by two different mechanisms. The first is called *in situ* activation and involves the simultaneous activation of a new ES and inactivation of the old ES, occurring independently of DNA rearrangement. The second involves DNA recombination, either between the expressed *vsg* and another telomeric ES (reciprocal recombination) or between the expressed *vsg* and a silent *vsg* locus (gene conversion).<sup>310</sup>



In the case of *H. influenzae*, lipopolysaccharide (LPS) is likely a key factor in facilitating colonization and is also a major target of the antibody response to infection. Interestingly, *H. influenzae* LPS undergoes phase variation. LPS biosynthesis involves multiple enzymatic steps and a number of genes. Among these genes, *lic1A*, *lic2A*, *lic3A*, *lex-2*, *lgtC*, and an *oafA*-like gene contain long stretches of tandem four-base pair repeats within their 5' coding region. In studies of the *lic* loci, Weiser and coworkers observed that the number of repeats varies spontaneously, generating translational frameshifts with different ATG start codons falling in or out of frame.<sup>324</sup> Such frameshifts result in synthesis of a protein with a different N terminus or eliminate protein production altogether (when no in-frame start codon exists). The mechanism of variation in repeat number is presumed to be slipped-strand mispairing, which occurs during DNA replication and involves a single repeat looping out on either the template or the replicating strand. Changes in *lic2A* and *lic3A* influence glycotransferase activity and alter reactivity with monoclonal antibodies directed against specific LPS oligosaccharide epitopes.<sup>108</sup> The *lic2A* gene product is responsible for the addition of a Gal- $\alpha$ 1,4-Gal moiety, which resembles the globoseries glycolipids and protects *H. influenzae* from antibody-mediated killing, possibly by molecular mimicry.<sup>325</sup> *lgtC* may be involved in formation of a Gal- $\beta$ 1,4-Glu moiety.<sup>132</sup> Variation in the *lic1A* gene affects production of a choline kinase responsible for addition of phosphorylcholine to the LPS molecule, a physical change that enhances binding of C-reactive protein and results in susceptibility to serum bactericidal activity.<sup>187,326,327</sup> Expression of *lex2* results in addition of a tetrasaccharide (Gal- $\alpha$ 1,4-Gal- $\beta$ 1,4-Glc- $\beta$ 1,4-Glc) to the proximal heptose in LPS and increases resistance to complement-mediated serum killing.<sup>105</sup> Similarly, expression of the *oafA*-like gene results in LPS O-acetylation, which facilitates resistance to serum killing.<sup>88</sup>

Recent studies of hepatitis A virus (HAV) have identified a novel mechanism by which viruses can evade humoral responses. HAV was long considered to be nonenveloped, a characteristic that seemed well suited to promote fecal-oral transmission. However, elegant centrifugation studies demonstrated that HAV features an envelope as it exits an infected cell.<sup>80</sup> The enveloped HAV particle (eHAV) is fully infectious. Formation of eHAV requires proteins involved in host cell exosome formation (VPS4B and ALIX), suggesting that HAV has co-opted these pathways to facilitate spread. While temporary, this enveloped form of HAV is fully protected from neutralizing antibodies and likely facilitates cell-to-cell spread in the liver. Following these studies of HAV, additional viruses have been observed to generate a temporary envelope, indicating an established strategy for viruses to evade humoral responses.<sup>253</sup>

## Encapsulation

Expression of an extracellular capsule represents a common strategy to evade phagocytosis, complement activity, and humoral immunity among pathogenic bacteria, fungi, and parasites. One example is *H. influenzae*, a common cause of childhood bacteremia and meningitis in underdeveloped countries. Among isolates of *H. influenzae*, six structurally and antigenically distinct capsular types are recognized, designated serotypes a to f. Historically, serotype b isolates accounted for more than 95% of all *H. influenzae* invasive disease, reflecting the distinct virulence properties of the type b capsule, which is a polymer of ribose and ribitol-5-phosphate (PRP) and is encoded by the *capb* locus.<sup>211</sup> In animal studies comparing derivatives of *H. influenzae* strain Rd expressing type a, b, c, d, e, or f capsule, the strain expressing the type b capsule was associated with the highest incidence of bacteremia after intranasal inoculation of infant rats. Similarly, this strain was associated with the highest magnitude of bacteremia and incidence of meningitis after intraperitoneal inoculation of experimental rats.<sup>354</sup>

In considering the mechanism by which the type b capsule promotes intravascular survival and invasive disease, *in vitro* studies using mouse peritoneal macrophages and human peripheral blood monocytes provide some insights. The type b capsule inhibits bacterial binding to macrophages in the absence of complement and a source of C3.<sup>223</sup> In addition, the type b capsule interferes with ingestion by macrophages when anti-PRP antibody is lacking.<sup>223,222</sup> Furthermore the type b capsule blocks complement deposition on the bacterial surface and resultant

complement-mediated bacteriolysis. In almost all isolates of *H. influenzae* type b, the *capb* locus is a tandem repeat of 18-kb *capb* gene sequences.<sup>221</sup> As a consequence of this arrangement, the *capb* locus serves as a template for further amplification of capsule gene sequences *in vivo*, resulting in increased capsule production. In a study by Corn and colleagues, 23 of 66 minimally passaged invasive isolates had between three and five copies of the 18-kb repeat.<sup>47</sup> Further analysis demonstrated that amplification of the repeat results in augmented resistance to phagocytosis and complement-mediated bacterial killing.<sup>221</sup> The importance of the type b capsule in disease pathogenesis was recognized in vaccine development efforts, and the routine implementation of the Hib polysaccharide-conjugate vaccine in many countries has sharply curtailed the incidence of invasive Hib disease.

Capsule production is also a critical virulence determinant for a number of disease-causing fungi, including the opportunistic pathogen *Cryptococcus neoformans*, which causes infections primarily in HIV-infected and other immunocompromised hosts. *C. neoformans* elaborates a thick polysaccharide capsule that is the basis for the classic "halo" appearance of the organism upon India ink staining of cerebrospinal fluid in patients with cryptococcal meningitis. The capsule comprises a complex polymer with a galactose backbone modified by xylose, mannose, and glucuronic acid. The enzymes responsible for assembly have begun to be identified, suggesting new possible targets for new antifungal development.<sup>170,257</sup> However, several elements required for capsule biosynthesis remain to be elucidated, and major questions persist regarding the spatial organization of capsule components and the basis for interstrain variation in the chemical structure and antibody reactivity of the galactoxylomannan backbone.<sup>68</sup>

## Viral Immune Suppression and Latency

Infection with HCV is a potent inducer of interferon-stimulated gene expression (as with other viruses).<sup>331</sup> However, HCV has evolved several mechanisms to evade host innate immune responses. The viral protease NS3-NS4A interferes with nuclear localization of interferon regulatory factor-3 (IRF-3) in response to interferon in HCV-infected hepatocytes.<sup>89</sup> This disruption of IRF-3 signaling, which prevents cells from activating antiviral genes downstream of IRF-3, results from specific cleavage of the molecule IPS-1.<sup>181</sup> Similar observations regarding evasion of innate immunity have been made with the influenza virus NS1 protein and with West Nile virus and HIV.<sup>67,69,334</sup>

Among some other viral pathogens, *latency* represents an important mechanism for persistence in the presence of host immunity, especially in the case of viruses belonging to the herpesvirus family. Herpes simplex viruses (HSV types 1 and 2) commonly establish latency after either gingivostomatitis or genital tract infection. After infection of a host cell, HSV replication begins. Eventually cell death occurs, resulting in cell lysis and release of viral particles, which can then infect adjacent cells. This so-called lytic replication cycle is under control of a small number of immediate early (IE) genes, which must be transcribed in moderate amounts to allow expression of the remainder of the viral genome. IE gene expression is activated by VP16, a viral protein that binds to a sequence common to IE gene promoters.<sup>252</sup> After lysis of the host cell, new virions enter local nerve termini and travel up the long axon to sensory ganglia, where latency is established within days. In the latent state, viral DNA can be detected in the neuron, but infectious virions cannot be isolated. During latency,<sup>217</sup> IE genes are repressed and only one fragment of viral DNA is actively transcribed, yielding several latency-associated transcripts (LATs) via alternative splicing.<sup>348</sup> No protein product has been definitively attributed to the LAT; instead, recent work has demonstrated HSV-1 production of microRNAs, transcribed from LAT exons, that promote latency by inhibiting transforming growth factor- $\beta$  signaling, favoring survival of infected cells and regulating the expression of activation-associated viral genes.<sup>56,109,315</sup> LAT-deficient mutants are still able to establish initial latency, suggesting that IE gene expression may be under multiple controls.<sup>320</sup> The mechanism by which HSV is reactivated is an area of intense study and some controversy. Host cellular mechanisms may provide the inciting signals, and the actions of viral thymidine kinase and the protein ICP0 are required for a return to lytic replication.<sup>217</sup>

## CONCLUSION

With the proliferation of molecular techniques in recent years, our understanding of the specific microbial and host factors involved in the pathogenesis of a variety of infectious diseases continues to expand remarkably. As a consequence of this understanding, we have witnessed the development of new vaccines and potential targets for antivirulence therapeutics. In the coming years, it is likely that advances in immunology and microbial pathogenesis will inform novel approaches for treating and preventing human infections. Examples might include inhibitors of type III protein secretion systems,<sup>149</sup> antagonists of periplasmic chaperones, analogs of important host cell receptors, and vaccine adjuvants that direct polarization of T-cell responses. However, given the impressive adaptability of human pathogens, as new therapeutic agents become available, we must remain vigilant for new microbial strategies allowing evasion of our interventions.

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# Normal and Impaired Immunologic Responses to Infection

# 2

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This chapter provides an overview of immunologic responses to infection and considers host interactions with different classes of pathogens, normal innate and adaptive immune mechanisms, the developing host responses of neonates, specific primary and secondary immunodeficiencies, and approaches to the evaluation of children suspected of having impaired immunity. Human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) are not considered here because they are addressed fully in Chapter 192B. This chapter is intended to supply a basic understanding of mechanisms involved in normal host responses to infection, an appreciation of the underlying basis and clinical presentation of important immunodeficiencies, and familiarity with general principles of evaluation and management of patients with suspected or documented disorders of immunity.

## HOST-PATHOGEN INTERACTIONS

### General Features of Host-Pathogen Interactions

Humans are constantly exposed to a daunting number and diversity of microorganisms that can cause infection. Many organisms that usually coexist harmoniously with the human host on the skin or on mucous membranes of the oral cavity, upper airways, or lower gastrointestinal tract may invade and become pathogens only if the balance of the commensal relationship is disrupted. Other organisms are more virulent, and they overtly challenge the host's normal surface barriers and internal defense mechanisms. The human host has evolved a complex array of

protective mechanisms designed to defend itself against these continuous microbial challenges.<sup>592</sup> To understand the pathogenesis, pathology, and natural history of infectious diseases, familiarity with the features of infectious agents that confer virulence is necessary; these topics are addressed elsewhere in this book. However, it is equally important to understand the elements of the host's response that contribute to containment, elimination, and protection against subsequent infection with these agents. Furthermore, it is important to recognize that host responses to infections also may contribute to the pathophysiology of infectious diseases and may injure the host in other ways.

The characteristic features of specific infectious diseases are determined by the interactions of structural components and released products of microbial pathogens with host tissue, cells, and their products. Virulence tactics commonly employed by organisms include adherence to host cell surfaces, internalization within or invasion of host cells, production of toxins, elaboration of surface barriers such as bacterial polysaccharide capsules, usurpation of host synthetic mechanisms, and direct inhibition of specific defense mechanisms within host cells. The successful evolution of host strategies to protect against microbial attack has resulted in defenses designed to interfere with or to counteract many of these modes of microbial virulence.<sup>592</sup> In recent decades, some of humanity's oldest microbial adversaries (e.g., smallpox, poliomyelitis, measles) systematically have been, or are being, eradicated with aggressive implementation of immunization programs. In the meantime, previously unrecognized human pathogens such as human immunodeficiency

virus-1 (HIV-1) and Ebola virus have emerged as new adversaries. Moreover, many of our oldest nemeses (e.g., tuberculosis, malaria) continue to elude our efforts to bring them under control, and they remain serious problems worldwide. Continued research at the interface between microbial pathogenesis and immunologic mechanisms is essential for the development of innovative approaches that can support and augment human immune responses to both old and new infectious diseases.

## Main Features of Host Responses to Specific Classes of Infectious Agents

### Viruses

Viruses are obligate intracellular parasites that consist of genetic material in the form of either DNA or RNA that usually is surrounded by a protein coat and may or may not be bound by a lipid envelope.<sup>372</sup> Diseases caused by viruses are remarkably diverse, ranging from mild and merely inconvenient to rapidly fatal, and from acute or brief to chronic or lifelong. However, certain features are common to the pathogenesis of most viral infections. First, viruses must enter host cells to replicate. Viral entry ordinarily is initiated by attachment of a viral surface protein to a specific receptor molecule on the host cell. The specific viral ligands or their corresponding host cell receptors have been identified for some viruses. For example, rhinovirus has evolved a capsid protein that binds to human intercellular adhesion molecule-1 (ICAM-1) on respiratory epithelium<sup>245</sup>; the envelope glycoproteins of HIV-1 interact with CD4 on T lymphocytes and distinct chemokine receptors on lymphocytes or macrophages<sup>154,308,589</sup>; and internalization of adenoviruses depends on interaction between a specific peptide sequence in the penton base complex of the viral capsid and  $\alpha_v$  integrins on host cell surfaces.<sup>584</sup> After the virus has entered the host cell, the cellular synthetic machinery is redirected to the synthesis of viral components. As with many native proteins synthesized by the host cell, a portion of newly synthesized viral protein is processed into peptides and presented on the infected cell surface by major histocompatibility complex (MHC) class I molecules (see later discussion). The host mechanisms most important in defense against the majority of viral pathogens include the production of specific neutralizing antibodies against viral surface proteins, the development of specific CD8<sup>+</sup> cytotoxic T-cell responses that eliminate infected cells, and the production by different immune cells of type 1 interferons (IFNs) that disrupt viral replication.<sup>38,345,347,476,536</sup> Natural killer (NK) cells appear to mediate the destruction of some virus-infected host cells,<sup>120,513</sup> and antibody-dependent cellular cytotoxicity (ADCC) may ensue after immunoglobulin (Ig)G antibodies bind to viral antigens on the infected cell, permitting subsequent attachment of either NK cells or cytotoxic T cells via IgG Fc receptors.<sup>200</sup> IFNs and other cytokines may enhance NK and ADCC activity, and cytokines such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) may exert cytotoxic actions on cells infected with certain viruses.<sup>347</sup> Additionally, opsonic complement components bound to viral surfaces can interfere with cell attachment, and the complement-derived membrane attack complex can lyse enveloped viruses.<sup>60</sup>

### Bacteria

The human host is colonized with a large variety of bacteria at skin and mucous membrane surfaces.<sup>108,404</sup> The integrity of these mechanical barriers ordinarily prevents systemic invasion of local commensal bacteria.<sup>89</sup> The epithelial cells that constitute these barriers, on recognition of an organism as a pathogen, also can release defensins and other microbicidal molecules.<sup>225</sup> In healthy hosts, circulating polymorphonuclear leukocytes (PMNs) help keep the resident flora in check by leaving the bloodstream at the mucosal sites containing the highest bacterial burdens, such as the lower intestine and the gingival crevices of the oral cavity.<sup>28</sup> This phenomenon helps account for the increased risk for local and systemic infection caused by oral and intestinal organisms in patients with severe neutropenia, including those who receive prolonged chemotherapy for malignancies, and in patients with phagocyte migration disorders such as leukocyte adhesion deficiency syndromes.<sup>28</sup> Important host defenses against most bacteria that invade the human host systemically include the complement system, specific

antibodies that promote both the opsonic and the bacteriolytic functions of complement, and phagocytes.<sup>1,28,60,293</sup>

### Fungi

Host mechanisms critical for defense against fungi are less well understood than those directed at bacteria and viruses, but phagocytes and cell-mediated immunity appear to be most important.<sup>187,215</sup> The relative importance of these factors appears to depend on the specific organisms involved, as is demonstrated by clinical observations in patients with isolated defects of one or the other. Severe mucosal infections caused by *Candida* spp. are common in patients with acquired or primary cell-mediated immune deficits, such as HIV infection, thymic aplasia (see later discussion), chronic mucocutaneous candidiasis, and some forms of severe combined immunodeficiency, as well as in patients with disorders of leukocyte migration.<sup>28,187</sup> In contrast to *Candida*, *Aspergillus* infections are not as great a problem for patients with cell-mediated immune defects as they are for patients with defects in phagocytic host defenses, such as neutropenia associated with cancer chemotherapy or stem cell transplantation, or genetic defects in phagocyte killing such as chronic granulomatous disease.<sup>58,588</sup> Fungi such as *Histoplasma* and *Cryptococcus*, like *Candida*, tend to cause severe infections in patients with defects in cell-mediated immunity, although phagocytes clearly are required for optimal clearance of these organisms.<sup>170,290,582</sup> The main role of antibodies and complement in protection from fungi probably is to provide opsonic activity to enhance phagocyte function.<sup>172</sup>

### Parasites

Parasites such as protozoa and helminths comprise such a widely varying group of pathogenic organisms that it is difficult to generalize about mechanisms of immunity to these organisms as a group. However, the importance of specific host mechanisms in defense against certain parasites may be appreciated by considering the characteristic host responses mobilized by parasitic infection or infestations. Some helminths induce production by host cells of chemokines that recruit eosinophils and stimulate their production. This suggests a likely role for these cells in antiparasitic defenses, and eosinophils have been shown to be important in protection against helminths such as *Strongyloides* and other parasites in this group that can invade tissues. IgE, among the immunoglobulins, appears to play a special role, often in concert with eosinophils, in anthelmintic defenses. IgG also may be important based on the susceptibility of individuals with hypogammaglobulinemia to hyperinfection with *Strongyloides*. Patients with hypogammaglobulinemia also are at risk for chronic or severe infestations with the flagellate intestinal parasite *Giardia lamblia*, suggesting a role for some degree of antibody-mediated protection in normal hosts. Patients with primary or acquired disorders of cell-mediated immunity are prone to development of serious central nervous system and ocular manifestations of infection with the protozoan *Toxoplasma gondii*, an obligate intracellular parasite, as well as hyperinfection with *Strongyloides*.<sup>299,429</sup>

## FEATURES OF NORMAL IMMUNE FUNCTION

The immune system can be viewed as consisting of two broad response categories: innate immunity and adaptive immunity. The former encompasses the more rapid and phylogenetically primitive, nonspecific responses to infection, such as surface defenses, cytokine elaboration, complement activation, and phagocytic responses. The latter involves more slowly developing, persistent, and highly evolved antigen-specific responses, such as cell-mediated immunity and antibody production that exhibit extraordinarily diverse ranges of specificities. The various arms of the immune system engage in a wide range of interactions that may enhance or regulate functions of other components of immunity, adding to the already remarkable complexity of the human immune response, and numerous examples of such interactions will be provided.

### Innate Immune Responses

#### Epithelia, Defensins, and Other Antimicrobial Peptides

The epithelium of skin and mucosal tissue functions as a mechanical barrier to the invasion of microbial pathogens. In recent decades, it has



become clear that epithelial cells also are a major source of antimicrobial peptides that play important roles in local host defense.<sup>48,224,223,421</sup> Studies of their structure, sources, expression, and actions also have revealed an unexpected range of immunologic activities for these molecules whose functions once were considered mainly antimicrobial in nature.<sup>2,33</sup>

Epithelial cells of mucous membranes of the airways and intestines, as well as keratinocytes, express the human  $\beta$ -defensins (HBD)-1, HBD-2, HBD-3, and HBD-4. These small cationic peptides are similar to the  $\alpha$ -defensins stored in the azurophilic granules of neutrophils, and they display antimicrobial activity against a broad range of bacteria, fungi, chlamydiae, and enveloped viruses.<sup>48,223,225,421</sup> Their production by epithelial cells may be constitutive, as for HBD-1, or inducible as for HBD-2, HBD-3, and HBD-4. For example, recent evidence indicates that epithelial cells of the airway or intestine can produce HBD-2 in response to activation by bacterial products via the Toll-like receptors TLR2 or TLR4 (see later discussion) on the epithelial cells.<sup>263,568,574</sup> Stimulation of epithelium by cytokines, including interleukin (IL)-1 or TNF- $\alpha$  also can induce defensin production.<sup>48,225</sup> Defensins have been reported to exert their antimicrobial action either by the creation of membrane pores or by membrane disruption resulting from electrostatic interaction with the polar head groups of membrane lipids, with more evidence now favoring the latter mechanism.<sup>48,275</sup> Some microorganisms have evolved mechanisms for evading the action of defensins. For example, bacterial polysaccharide capsules may limit access of microbial peptides to the cell membrane,<sup>112</sup> and an exoprotein of *Staphylococcus aureus*, staphylokinase, neutralizes the microbicidal action of neutrophil  $\alpha$ -defensins.<sup>288</sup>

Several immunoregulatory properties of defensins and related peptides, distinct from their antimicrobial actions, have been documented.<sup>223</sup> Several such peptides have been shown to facilitate post-translational processing of IL-1 $\beta$ .<sup>439</sup> Some of the  $\beta$  defensins have been shown to function as chemoattractants for neutrophils, memory T cells, and immature dendritic cells by binding to the chemokine receptor CCR-6.<sup>274,403,421</sup> Separately, HBD-2 has been shown to act, via a mechanism that requires TLR4, to activate immature dendritic cells and promote their maturation.<sup>69,591</sup> The  $\beta$ -defensins also act as chemoattractants for mast cells and can induce mast cell degranulation.<sup>402</sup> HBD-2 and several other antimicrobial peptides can interfere with binding between bacterial lipopolysaccharide (LPS) and LPS-binding protein (LBP), a process important in activating inflammatory cells via TLR4 (see later discussion).<sup>493</sup>

Additional antimicrobial peptides of epithelial cells include lysozyme and cathelicidin. Lysozyme, an antimicrobial peptide also found in neutrophil granules, attacks the peptidoglycan cell walls of bacteria and may be released from cells by mechanisms that involve TLR activation.<sup>431</sup> Cathelicidin, or LL37, like lysozyme, is released from both neutrophils and epithelial cells. It exhibits broad antimicrobial activity and can inhibit lentiviral replication.<sup>274,527</sup> Cathelicidin also exhibits chemotactic activity for neutrophils, monocytes, and T lymphocytes. This activity is mediated via a formyl peptide receptor-like molecule (FPRL-1), rather than the chemokine receptor (CCR)6 bound by  $\beta$ -defensins.<sup>590</sup>

The release of defensins in response to activation of TLRs and the various actions of these peptides, including their direct antimicrobial activities, their chemoattractant actions for a wide range of immune cells, and their activation of dendritic cell maturation, already suggest a highly complex and regulatory role in the development of host defense and immunity. Genomic evidence for the possible existence of many additional human defensins that have not yet been characterized suggests that current knowledge describes but a small sample of the overall contribution of these peptides to immune responses.<sup>48,490</sup>

### Toll-Like Receptors

Mononuclear phagocytes, including circulating monocytes and tissue macrophages, other phagocytic cells, and many epithelial cells, express a family of receptors that is highly homologous to the *Drosophila* receptor called Toll.<sup>95,263,370,568,574</sup> These receptors mediate a phylogenetically primitive, nonclonal mechanism of pathogen recognition based on binding, not to specific antigens, but to structurally conserved pathogen-associated molecular patterns.<sup>8,412,413,595</sup> At least 10 human TLRs with a range of microbial ligands have been identified, such as gram-negative bacterial LPS, bacterial lipoproteins, lipoteichoic acids of gram-positive bacteria, bacterial cell wall peptidoglycans, cell wall components of yeast and mycobacteria, unmethylated CpG dinucleotide motifs in bacterial DNA, some viral particles, and viral RNA.<sup>8,412,413,595</sup> Gram-positive cell wall components bind mainly to TLR2, and TLR2 also can bind components of herpes simplex virus.<sup>323,538</sup> TLR2 forms dimers with either TLR1 or TLR6 when bound jointly by their ligands.<sup>288,342</sup> Gram-negative LPS activates TLR4 indirectly by first binding to LBP, which transfers the LPS to the host accessory protein CD14 at the cell surface. The bound CD14 has no transmembrane domain but associates directly with an extracellular domain of TLR4.<sup>413,538</sup> MD-2, an additional accessory protein associated with TLR4, also plays a role in binding LPS.<sup>434</sup> TLR5 has been identified as the receptor for bacterial flagellin, TLR9 recognizes CpG motifs of bacterial and viral DNA, and TLR3 has been shown to bind synthetic and viral double-stranded RNA.<sup>56,255,319,323</sup> A listing of known human TLRs with their major ligands and cellular distribution is summarized in Table 2.1.

Signaling by TLRs occurs via a well-described pathway in which receptor binding generates a signal via an adaptor molecule, myeloid differentiation factor 88 (MyD88), that leads to intracellular association with IL-1 receptor-associated kinase (IRAK). In turn, this leads to activation of TNF receptor-associated factor-6 (TRAF-6), which results in nuclear translocation of nuclear factor- $\kappa$ B (NF- $\kappa$ B).<sup>133</sup> NF- $\kappa$ B is an important transcription factor that activates the promoters of the genes for a broad range of cytokines and other proinflammatory products, such as TNF- $\alpha$ , IL-1, IL-6, and IL-8. This signaling pathway, based on studies with TLR4, is similar but not identical to the signaling pathways activated by other TLRs.<sup>133</sup> The activation of cytokine production by TLRs plays an important role in recruiting other components of innate host defense against bacterial pathogens. However, with large-scale cytokine release, the deleterious effects of sepsis or other forms of the systemic inflammatory response syndrome demonstrate that these

TABLE 2.1 Human Toll-Like Receptors: Their Ligands and Cellular Distribution

TLR	Ligands	Cellular Distribution
TLR1 (+TLR2) TLR2 (+TLR6)	Mycobacterial lipoarabinomannans, bacterial lipoproteins, bacterial lipoteichoic acids, bacterial and fungal $\beta$ -glucans	Mo, DC, MC, Eos, Bas, AEC
TLR3	Viral double-stranded RNA	NK cell
TLR4 (+CD14, MD-2)	Bacterial lipopolysaccharide	M $\Phi$ , DC, MC, Eos, AEC
TLR5	Bacterial flagellin	AEC, IEC
TLR7	Viral single-stranded RNA	PDC, NK, Eos, BL
TLR8	Viral single-stranded RNA	NK cell
TLR9	Unmethylated CpG dinucleotides	PDC, Eos, BL, Bas (bacteria, herpesvirus)
TLR10	Unknown ligands	PDC, Eos, BL, Bas

AEC, Airway epithelial cell; Bas, basophil; BL, B lymphocyte; DC, dendritic cell; Eos, eosinophil; IEC, intestinal epithelial cell; M $\Phi$ , macrophage; MC, mast cell; Mo, monocyte; NK, natural killer; PDC, plasmacytoid dendritic cell; TLR, Toll-like receptor.

pathways have both beneficial and potentially harmful effects for the host.<sup>133</sup> Genetic polymorphisms in TLRs may play a role in determining the balance of these effects in certain individuals responding to the challenge of systemic infection.<sup>133,352,353</sup>

In addition to their “first responder” roles in generating an inflammatory response to invading pathogens, TLRs may network with other components of innate and adaptive immunity. TLR4 function is suppressed by activation of cells via the chemokine receptor CXCR4.<sup>307</sup> Activation of some TLRs also can induce expression of the costimulatory molecule B7 on antigen-presenting cells, which is required for activation of naïve T cells.<sup>370</sup>

### Cytokines

A heterogeneous group of soluble small polypeptide or glycoprotein mediators, often collectively called cytokines, forms part of a complex network that helps regulate immune and inflammatory responses. Included in this group of mediators, whose molecular weights range from about 8 to about 45 kDa, are the ILs, IFNs, growth factors, and chemokines (see separate discussions later). Most cells of the immune system and many other host cell types release cytokines, respond to cytokines via specific cytokine receptors, or both. A list of cytokines and related molecules that play a role in immune function, with selected characteristics, is provided in Table 2.2.<sup>322,422,443</sup> Excellent general reviews are available,<sup>321,322,347,422,443</sup> and the use of cytokines as immunomodulating

agents is discussed in Chapter 242. However, two cytokines, IL-1 and TNF- $\alpha$ , are of such fundamental importance in acute host responses to infection that they warrant specific attention here.

IL-1 and TNF- $\alpha$  are small polypeptides, each with a molecular weight of approximately 17 kDa, that exhibit a broad range of effects on immunologic responses, inflammation, metabolism, and hematopoiesis.<sup>66,422</sup> IL-1 originally was described as “endogenous pyrogen,” referring to its ability to produce fever in experimental animals, and TNF- $\alpha$ , which produces some of the same effects produced by IL-1, was originally named “cachectin” after the wasting syndrome it produced when injected chronically in mice.<sup>66,422</sup> Many of the physiologic changes associated with gram-negative sepsis can be reproduced by injecting experimental animals with these cytokines, including fever, hypotension, and either neutrophilia or leukopenia.<sup>66,422</sup> In the development of endotoxic shock resulting from gram-negative sepsis, IL-1 and TNF- $\alpha$  are produced by mononuclear phagocytes in response to activation of TLRs by bacterial LPS. They in turn activate the production of other cytokines and chemokines, lipid mediators such as platelet-activating factor and prostaglandins, and reactive oxygen species. They also induce expression of adhesion molecules of both endothelial cells and leukocytes, stimulating recruitment of leukocytes by inducing release of the chemokine IL-8 and activating neutrophils for phagocytosis, degranulation, and oxidative burst activity.<sup>66,135</sup> These are all important, usually beneficial host responses to infection. However, at very high levels of activation,

**TABLE 2.2 Features of Selected Human Cytokines and Growth Factors**

Cytokines and Growth Factors	Main Cellular Sources	Biologic Effects
IL-1	Mo, TL, BL, NK, PMN, others	Broad range of cellular activation in inflammatory and immune responses
IL-2	TL, BL, NK	TL, BL proliferation and activation; enhances TL and NK cytotoxicity
IL-3	TL	General stimulation of hematopoiesis
IL-4	TL, BL, Mast, Mo	TL, BL proliferation; BL isotype switching; stimulates IgE synthesis; enhances MHC class II expression
IL-5	TL	Stimulation of Eos production
IL-6	TL, BL, Mo	Broad inflammatory activity; stimulates BL differentiation and megakaryocyte production
IL-7	Marrow and thymus stromal cells	TL, BL growth and differentiation
IL-8	Mac, Mo, Endo, Epi, PMN, Eos	Activation and chemotaxis of PMN, Eos
IL-9	TL	Mast growth and differentiation; growth of activated TL
IL-10	TL, BL, Mast, Mac	Broad antiinflammatory actions; inhibits synthesis of several other cytokines (TNF, IL-2, IL-3, IFN- $\gamma$ )
IL-11	Marrow stromal cells	General stimulation of hematopoiesis; BL growth and differentiation
IL-12	BL, Mo	Stimulation of TL growth; induction of IFN- $\gamma$ production; enhancement of TL and NK cytotoxicity
IL-13	TL	BL proliferation and isotype switching; enhances MHC class II expression; inhibits production of cytokines by Mac
IL-14	TL, malignant BL	Induces BL growth
IL-15	Epi, Endo, Mo, Mac, marrow stromal cells	Enhances NK growth, development, function; enhances TL growth and migration
IL-17	TL	Enhances TL growth; induces Mac cytokine release
IL-18	Kupffer cells, Epi, spleen, Mac	Promotes TL, BL, NK cytokine release; promotes TL, BL cytotoxicity
IL-21	TL	Promotes BL, TL proliferation; NK cytotoxicity
IL-23	Dendritic cells, Mac	Similar to IL-12
IL-25	TL (T <sub>H</sub> 2), Mast	TL, Mac T <sub>H</sub> 2 cytokine secretion
IL-27	Dendritic cells, Mac	TL responsiveness to IL-12
IFN- $\alpha$	Mo, TL	Interference with viral replication; increases MHC class I expression
IFN- $\beta$	Epi, Fibro	Similar to IFN- $\alpha$
IFN- $\gamma$	TL, NK	Similar to IFN- $\alpha$ , IFN- $\beta$ ; stimulates Mac inflammatory functions
TNF- $\alpha$	Mo, Mac, TL, NK	Broad inflammatory effects; fever; cachexia; stimulates catabolism; activation of leukocytes and Endo
GM-CSF	TL, BL, Mo, PMN, Eos, Fibro, Mast, Endo	Growth of PMN, Eos, Mo, and Mac precursors; enhances leukocyte function
G-CSF	Mo, Epi, Fibro	Enhances production and function of granulocytes
M-CSF	Mo, TL, BL, Endo, Fibro	Promotes Mo production; stimulates Mo and Mac function

BL, B lymphocyte; Endo, endothelial cell; Eos, eosinophil; Epi, epithelial cell; Fibro, fibroblast; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; Mac, macrophage; Mast, mast cell; M-CSF, macrophage colony-stimulating factor; MHC, major histocompatibility complex; Mo, monocyte; NK, natural killer cell; PMN, polymorphonuclear leukocyte; TL, T lymphocyte; TNF, tumor necrosis factor.

pathologic effects of this proinflammatory cascade may occur, including vascular instability, decreased myocardial contractility, capillary leak, tissue hypoperfusion, coagulopathy, and multiple organ failure.<sup>133,569</sup> For some systemic actions, notably the production of hemodynamic shock, IL-1 and TNF- $\alpha$  are synergistic. Both IL-1 and TNF- $\alpha$  also induce production of IL-6, a somewhat less potent cytokine that exhibits some of the actions of IL-1 and TNF- $\alpha$ .<sup>422</sup> The human host produces several soluble antagonists of IL-1 and TNF- $\alpha$  that can modulate their effects, including IL-1 receptor antagonist (IL-1ra), soluble TNF- $\alpha$  receptor (sTNF- $\alpha$ R), and antiinflammatory cytokines, especially IL-10.<sup>133</sup>

The importance of effects mediated by IL-1 and TNF- $\alpha$  in the pathophysiology of septic shock has prompted much active research aimed at blocking their direct and downstream effects to reduce sepsis morbidity and mortality. To date, despite promise and progress, clinical strategies to interfere with the cytokine-induced cascade that leads to endotoxin shock have continued, overall, to meet with limited success.<sup>29,43,96,133,212,422,456,562,572</sup>

### Chemokines

A specialized group of small cytokine-like polypeptides, chemokines, which all share the feature of being ligands for G-protein-coupled, seven-transmembrane-segment receptors, play a complex role in the immune response as cellular activators that induce directed cell migration mainly of immune and inflammatory cells.<sup>44,285,304,363,393,472</sup> The chemokines and their receptors have been classified into four families based on the motif displayed by the first two cysteine residues of the respective chemokine peptide sequence. Each of at least 16 CXC chemokines binds to one or more of the CXCRs, CXCR1 to -6. Examples of CXC chemokines include IL-8 and Gro- $\alpha$ . Similarly, at least 28 CC chemokines, such as macrophage inflammatory protein (MIP)-1 $\alpha$ ; regulated and normal T cell expressed and secreted (RANTES); and eotaxin-1, -2, and -3 bind to one or more of the CCRs, CCR1 to -10. The sole CX3C chemokine, fractalkine (neurotaxis), binds to CX3CR1, currently the only receptor in its family. The two XC chemokines, including lymphotaxin, bind to the sole receptor in this family, XCR1. A chemokine nomenclature currently designates each of the chemokines as a numbered ligand for its respective receptor family. In this system, Gro- $\alpha$  is CXC ligand (L)-1 (or CXCL-1), and IL-8 now becomes CXCL-8. Similarly, RANTES becomes CCL-5, fractalkine is CX3CL-1, and lymphotactin is XCL-1.<sup>285,472</sup> A review of this nomenclature system tabulates the members of each family with their respective ligands and receptors, as well as with the traditional names in both human and murine systems.<sup>285</sup>

Virtually every cell type of the immune system expresses receptors for one or more of the chemokines. The cells of virtually any inflamed tissue can release a range of chemokines, and tissues infected with different bacteria or viruses release chemokines that recruit characteristic sets of immune cells.<sup>235,304</sup> For example, whereas rhinoviruses induce the release of chemokines that result mainly in recruitment of neutrophils (early in the course of infection), Epstein-Barr virus induces a set of chemokines that result in recruitment of B cells, NK cells, and both CD4<sup>+</sup> and CD8<sup>+</sup> T cells.<sup>235</sup> It is of interest that almost mutually exclusive sets of chemokines are induced by cytokines associated with T<sub>H</sub>1 (IFN- $\gamma$ ) versus T<sub>H</sub>2 (IL-4, IL-13) versus T<sub>H</sub>17 (IL-17) immune responses (see later discussion), indicating a tight interplay between cytokines and chemokines in determining the type of immune response to specific infectious challenges generated under differing conditions.<sup>71</sup> The specificity of such responses is strongly influenced by the type of chemokines released by specific tissues, the vascular adhesion molecules expressed in those tissues, the chemokine receptors expressed by different leukocyte populations, and the specific adhesion molecules expressed by leukocytes.<sup>71,235,304</sup>

Modulation of chemokine functions may occur by several mechanisms. Chemokines themselves may be potentiated or inactivated by tissue proteases including tissue peptidases and matrix metalloproteases.<sup>369</sup> Heparin sulfate-related proteoglycans on endothelial cell surfaces tether chemokines locally, where they can most efficiently activate circulating leukocytes for adhesion (see later discussion). However, similar proteoglycans free in the extracellular environment may act to bind and sequester chemokines, keeping them from interacting with their cellular receptors.<sup>136,324</sup> Finally, in addition to the well-described use of chemokine

receptors as coreceptors for viral entry by HIV-1, other viruses, especially members of the herpesvirus family, encode soluble decoy receptors that compete with native host receptors for chemokine binding, thereby disrupting normal host responses.<sup>136,469</sup>

### Natural Killer Cells

NK cells are an important cellular component of innate immunity. They are lymphoid cells found in the peripheral circulation, spleen, and bone marrow that do not express clonally distributed receptors, such as T-cell receptors or surface immunoglobulin, for specific antigens.<sup>387,388,513</sup> They respond in an antigen-independent manner to aid in the control of malignant tumors and to help contain viral infections, especially those caused by members of the herpesvirus family, before the development of adaptive immune responses.<sup>513,514</sup> Activated NK cells are an important source of IFN- $\gamma$ , which limits tumor angiogenesis and promotes the development of specific protective immune responses.<sup>387,388,513,514</sup>

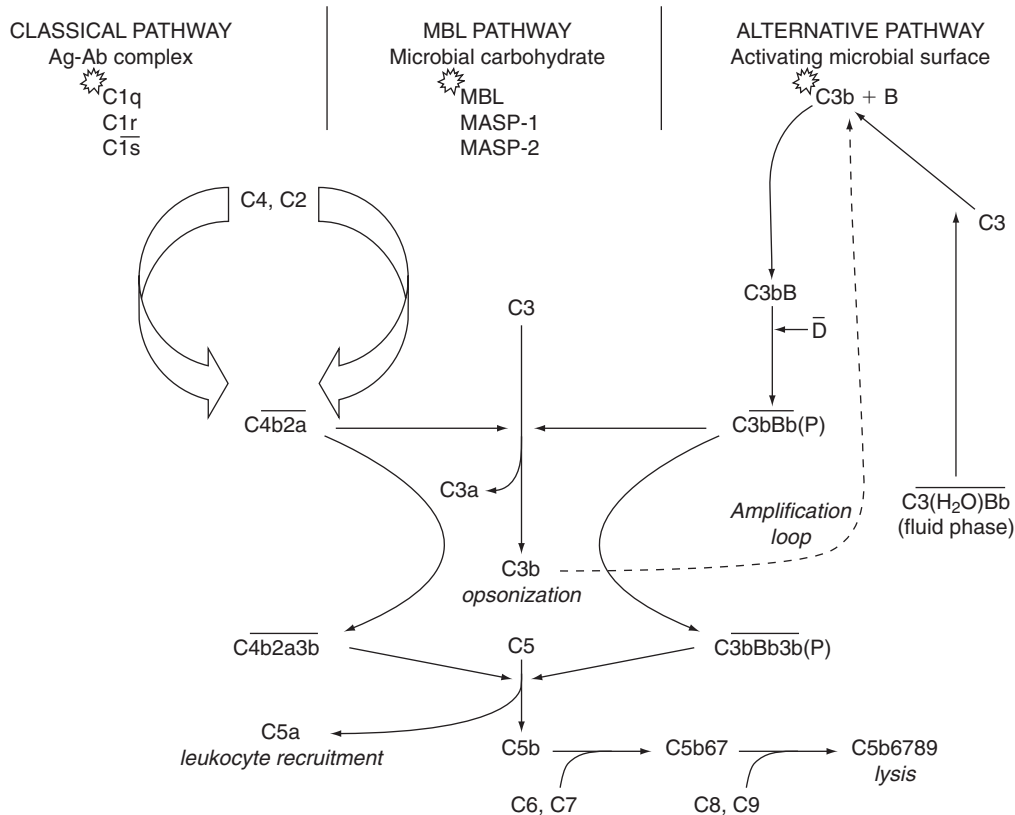
Regulation of NK cell activity involves a complex balance between activating and inhibitory signals. Several cytokines can activate NK cell proliferation, cytotoxicity, or IFN- $\gamma$  production, including IL-12, IL-15, IL-18, IL-21, and IFN- $\alpha\beta$ .<sup>514</sup> Activating signals via other receptors on NK cells, such as NKG2D, may lead either to cytotoxicity or cytokine production or both, depending on the receptor's association with distinct intracellular adaptor proteins that signal via different kinases.<sup>514,566</sup> Other molecules on NK cells may act as either costimulatory or adhesion receptors, including CD27, CD28, CD154 (CD40 ligand), and lymphocyte function-associated (LFA)-1 (CD11a/CD18).<sup>50,514</sup> Additionally, Fc $\gamma$ RIII (CD16) can contribute to NK cell-mediated antibody-dependent cell cytotoxicity.<sup>200,387</sup> NK cells are able to distinguish normal cells of self-origin via receptors that recognize specific MHC class I molecules. Activation of such receptors provides an inhibitory signal that protects healthy host cells from NK cell-mediated lysis. Virus-infected cells and malignant cells may express MHC class I molecules at reduced levels, rendering them more susceptible to attack by NK cells.<sup>120,513</sup> NK cell inhibitory receptors, some of which have been characterized, appear to contain intracytoplasmic tyrosine-based inhibition motifs and antagonize NK cell activation pathways via protein tyrosine phosphatases.<sup>454,514</sup>

NK cells kill virus-infected or malignant cells by the release of perforin and granzymes from granular storage compartments and by binding of the death receptors Fas and TRAIL-R on target cells via their respective NK cell ligands.<sup>485,513,514</sup> The mechanisms by which perforin and granzymes mediate target cell death are not fully understood. One or more of the granzymes appear to activate intracellular pathways leading to target cell apoptosis via pathways that involve the mitochondria or caspases or both.<sup>300,553</sup> Separately, binding of the death receptors also activates caspases, causing target cell apoptosis.<sup>494,514</sup> NK cells engage in several kinds of interactions with other cells of the immune system, including dendritic cells and other antigen-presenting cells. Dendritic cells can influence the proliferation and activation of NK cells both by release of cytokines, including IL-12, and by cell surface interactions, including CD40/CD40L, LFA-1/ICAM-1, and CD27/CD70.<sup>164</sup> In return, NK cells can provide signals that result in either dendritic cell maturation or apoptosis.<sup>120,513</sup>

### Complement System

The complement system consists of more than 30 different free and membrane-bound activation and regulatory proteins. It has multiple key roles in the clearance of invading microbes, including opsonization, recruitment of phagocytic cells, and lytic destruction of pathogens.<sup>59,168,169,188,189,208,290,289,292,392</sup>

Approximately 90% of complement proteins are synthesized in the liver, but some components can be produced locally at sites of infection by tissue mononuclear phagocytes and fibroblasts.<sup>134,438</sup> In healthy persons the majority of complement is found in the circulation. Circulating complement levels vary over time, particularly in the presence of inflammation. The inflammatory response may lead to increases in levels of those complement components such as C3 that are acute-phase reactants or to decreases in individual components and total complement activity as a result of consumption.



**FIG. 2.1** The complement cascade. The initial binding events of the classical, mannan-binding lectin (MBL) and alternative pathways are indicated by a starburst. These pathways intersect at the conversion of C3 to C3b. This is followed by activation of the terminal components, beginning with the binding and cleavage of C5, releasing C5a and leaving bound C5b to initiate assembly of the remaining components to form the membrane attack complex (C5b6789). Enzymatically active proteases of the classical and alternative pathways that cleave and activate subsequent components are, by convention, shown with an overbar. The alternative pathway C3 and C5 convertases are shown associated with properdin (P), which increases their stability. B, Complement factor B; D, complement factor D; MASP, MBL-associated serine proteases.

The importance of normal complement component levels and activity in host defense has been well established and is based primarily on the increased susceptibility of patients with specific complement component deficiencies to recurrent or severe bacterial infections.<sup>166,168,188,289,290</sup> Although the complement response to infection usually is beneficial to the host, it also may be associated with adverse clinical manifestations such as septic shock and acute respiratory distress syndrome.<sup>158,203,576</sup>

**Complement activation.** Complement proteins are activated in a specific sequence or “cascade” via one or more of three pathways: the classical pathway, the alternative pathway, and the more recently described MBL pathway, as shown in Fig. 2.1. These pathways converge at C3, and the complement cascade downstream from C3 proceeds identically, irrespective of the pathway by which activation occurs. The C3 convertases, C4b2a for the classical and MBL pathways and C3bBb for the alternative pathway, cleave the C3 molecule at exactly the same location, producing C3b, which binds to the target surface, and C3a, which is released into the fluid phase. Cleavage and activation of C3 lead to a conformational change in C3b that transiently renders its reactive thioester group capable of forming covalent ester or amide bonds with acceptor molecules on the target surface.<sup>276,331</sup> Surface-bound C3b can act as an opsonin to promote phagocytosis, or it can bind with the classical and alternative pathway C3 convertases to form the C5 convertases C4b2a3b and C3bBb3b, respectively.<sup>452</sup> C5 convertases bind and then cleave C5, with release of the chemoattractant C5a fragment into the fluid phase. The bound C5b fragment then can initiate formation of the membrane attack complex by the sequential incorporation of the remaining terminal components, C6, C7, C8, and multiple molecules of C9. The membrane attack complex can insert into the outer membrane of target cells, such as erythrocytes or gram-negative bacteria, and cause cell lysis and death.<sup>292</sup>

**Classical pathway.** Ordinarily the classical pathway is activated by IgM or IgG bound to microbial antigenic targets or by other kinds of antigen-antibody complexes.<sup>169</sup> IgM activates complement more efficiently than IgG because only one molecule of polymeric IgM is required in contrast to at least two molecules of IgG.<sup>141</sup> Activation typically is initiated when C1q binds directly to an immunoglobulin molecule on the surface of an organism. C1r and C1s are activated and bound to C1q sequentially, forming C1qr. The enzymatic activity of this complex, which resides in the C1s molecule, can cleave multiple molecules of C4 and C2 into two fragments each. The C4a and C2a fragments are released into the environment, whereas C4b and C2a remain bound to each other on the target surface to form the classical pathway C3 convertase, C4b2a. C4b2a can cleave and activate C3 and localize C3b binding to nearby sites on the target surface. As noted earlier, some C3b binds with C4b2a to form the classical pathway C5 convertase, C4b2a3b.

**Alternative pathway.** Alternative pathway activation of C3 is the principal means by which a nonimmune host can activate the effector functions of complement until a specific antibody response can be mounted.<sup>167,205</sup>

A spontaneous low level of hydrolysis of the thioester of C3 in the fluid phase results in an activated form of C3, C3(H<sub>2</sub>O). This activated form of C3 can bind factor B, and the latter is then cleaved by factor D to form the fluid phase C3 convertase C3(H<sub>2</sub>O)Bb. The constitutive presence of small amounts of this convertase in the fluid phase ensures that a small amount of C3b always is available to bind to microbial surfaces and initiate the alternative pathway.<sup>432</sup> The alternative pathway protein factor B can bind to surface-bound C3b, after which factor B undergoes proteolytic cleavage by factor D to release a small soluble fragment, Ba, while the larger fragment, Bb, remains associated with



C3b. C3bBb, the alternative pathway C3 convertase, is analogous to the classical pathway C3 convertase, C4b2a. Properdin stabilizes the C3 convertase C3bBb, permitting more efficient activation of C3 to form more C3b, creating the C3 amplification loop (see Fig. 2.1).<sup>202,205</sup>

The most important factor in determining whether a specific microbial pathogen will activate the alternative pathway is the biochemical nature of its surface. On surfaces rich in sialic acid, bound C3b is less able to bind factor B because another molecule, factor H, has a strong competitive advantage over factor B under these conditions. When bound by factor H, C3b becomes highly susceptible to further cleavage by factor I (C3b inactivator), resulting in C3bi (or iC3b). Although C3bi is an effective opsonin, it cannot bind factor B. Thus no alternative pathway convertases can be formed, and no amplification loop is established.<sup>39,325,392</sup> Organisms whose surfaces do not support activation of the alternative pathway, such as K1 *Escherichia coli*, groups A and B streptococci, *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Haemophilus influenzae* type b, and some salmonellae are some of the most successful pathogens in infants and young children who lack specific protective antibodies.<sup>115,292</sup>

**Mannan-binding lectin pathway.** The most recently described complement activation pathway is the MBL pathway. It is similar to the classical pathway but does not involve antibodies. MBL is a serum protein of the collectin family that has structural and functional similarities to those of C1q. However, it does not require antigen-antibody complexes to initiate its complement-activating function. MBL binds to mannose-containing carbohydrates on microbial surfaces, leading to its association at the microbial surface with activated MBL-associated serine proteases (MASP-1 and -2). These latter proteases have structural and functional similarities to C1r and C1s, respectively, and result in activation of C4, with sequential binding of C4b and C2a and formation of C4b2a, the C3 convertase of the classical pathway. C3 is activated, and the cascade proceeds as described. A more detailed characterization of the MBL pathway and its role in immune responses to infection may be found in an excellent review.<sup>440</sup>

**Effector functions of complement in host defense.** The principal complement effector functions in host defense include opsonization via bound fragments of C3; phagocyte recruitment, especially via release of C5a; lysis of microorganisms, especially gram-negative bacteria, via the membrane attack complex (C5b–C9); and immune regulation via interactions with host cells involved in adaptive immunity. Complement sometimes may be activated and bound to microbial surfaces but unable to carry out these functions if it is bound to a disadvantageous location; for example, C3b bound to a pneumococcal cell wall beneath a thick polysaccharide capsule or C5b–C9 bound to long lipopolysaccharide molecules distant from the gram-negative bacterial outer membrane.<sup>93,240,277,292</sup>

**Opsonic activity.** Complement opsonic activity is essential for effective phagocytic removal of organisms from the circulation by macrophages in the liver and spleen and from other sites by local macrophages and neutrophils.<sup>78</sup> Opsonins facilitate recognition, binding, ingestion, and killing of microorganisms by phagocytes. Opsonization particularly is important for protection against gram-positive bacteria and fungi because their thick cell walls prevent them from being killed by the membrane attack complex.

The major complement-derived opsonins are the C3 fragments C3b and iC3b. Surface-bound C3b and iC3b permit microbes to be recognized by circulating and tissue phagocytes by interacting with the phagocyte surface complement receptors CR1 (CD35) and CR3 (CD11b/CD18), respectively. These interactions lead to binding, ingestion, and intracellular killing of the organisms.<sup>117,240,277,276,331</sup>

Antibodies, especially of the IgG class, are important opsonins in their own right, but they also facilitate more rapid complement activation via the classical pathway and more effective localization of C3b binding to the surface of encapsulated organisms, where it is accessible to phagocyte receptors.<sup>93,277,294</sup>

**Inflammation.** The cleavage products of several complement proteins contribute to the development of inflammatory responses. C3a stimulates an increase in the number of circulating granulocytes, and C5a serves as a potent stimulus for monocyte, neutrophil, and eosinophil migration toward the source of C5a gradients being produced at infected tissue sites. C5a also upregulates phagocyte expression of CR1 and CR3 and

stimulates these cells to release granule contents that also are important mediators of inflammation and microbicidal activity. C5a-induced neutrophil aggregation and stasis in the pulmonary circulation can be an important feature of the respiratory distress syndrome associated with sepsis.<sup>576</sup>

The anaphylatoxins, C4a, C3a, and especially C5a, induce release of histamine from mast cells and basophils, causing increased vascular dilation and permeability, which, in turn, permit local diffusion of other inflammatory mediators.<sup>279,576</sup> When large quantities of anaphylatoxins are produced rapidly, they can contribute to septic shock.<sup>203</sup>

**Microbicidal activity.** As noted earlier, C5b and the terminal complement proteins C6, C7, C8, and C9 form the membrane attack complex, which can lyse gram-negative bacteria by penetrating their outer membranes.<sup>292</sup> The C5b–C8 complex serves as a polymerization site for several molecules of C9, which increases the efficiency of lysis.<sup>68,539</sup> As has been noted, the membrane attack complex cannot penetrate the thick cell walls of gram-positive bacteria and fungi and therefore cannot kill these organisms directly. The membrane attack complex can lyse some virus-infected host cells and some enveloped viruses themselves.<sup>143</sup>

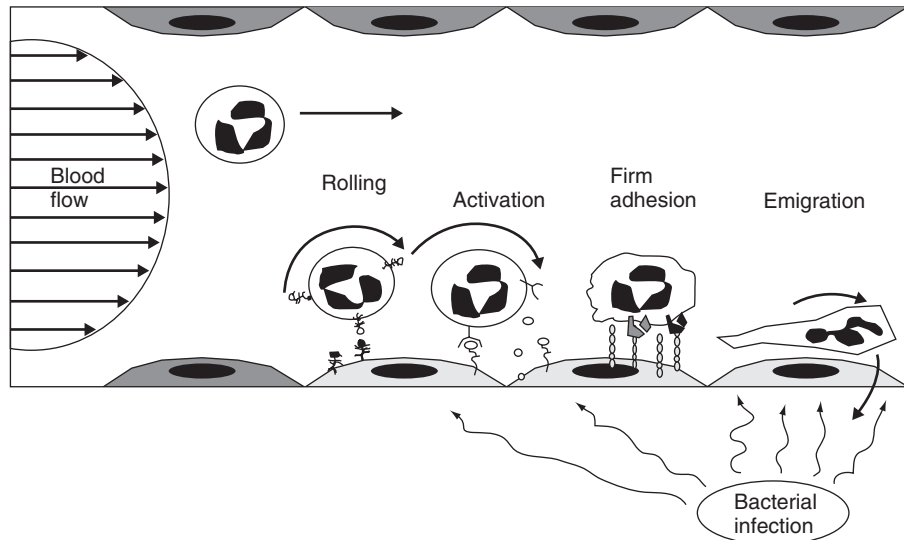
**Immune regulation.** Complement components and fragments can modulate immune responses, both directly by binding to CR1, CR2, and CR3 on the surfaces of T cells, B cells, and other cells involved in antigen recognition and indirectly by stimulating the synthesis and release of cytokines.<sup>195</sup> For example, the C3b cleavage product, C3dg, when covalently bound to antigen, brings the antigen close to B cells by binding to B-cell CR2 (CD21).<sup>70,84,113</sup> C3 influences antigenic localization within germinal centers, and it is involved in anamnestic responses and isotype switching. Additionally, C1-, C2-, C4-, and C3-deficient animals have decreased antibody responses that can be restored by providing the missing protein,<sup>70,84,113</sup> and C2 deficiency in humans also has been associated with antibody deficiencies.<sup>15,113</sup>

## Phagocytes

PMNs, the most abundant circulating phagocytes in the human host, will serve as a model for discussing phagocyte functions. These cells constitute a major line of defense against invading bacteria and fungi. The proliferation of myeloid marrow progenitors and their differentiation into mature progeny are regulated by specific growth factors and cytokines.<sup>45,345,346,547</sup> The normal half-life of circulating PMNs is approximately 8 to 12 hours.<sup>365,570</sup> In the absence of active infection, most PMNs leave the circulation via the gingival crevices and the lower gastrointestinal tract, where the resident flora stimulate ongoing local extravasation of PMNs, a process that helps maintain the integrity of these tissues.<sup>29</sup> In response to invasive bacterial infection, circulating PMNs engage in three major functions: (1) migration to the site of infection, (2) recognition and ingestion of invading microorganisms, and (3) killing and digestion of these organisms.

**Phagocyte recruitment to infected sites.** Activation of endothelial cells that line the microvessels of acutely infected tissue occurs via locally produced cytokines, eicosanoid compounds, and microbial products.<sup>110,505</sup>

As a result, the endothelial cells rapidly upregulate their surface expression of P-selectin from preformed intracellular storage pools and, subsequently, of E-selectin by new synthesis.<sup>344,509</sup> These selectins interact with the fucosylated tetrasaccharide moiety sialyl Lewis X, which is presented on constitutively expressed glycoproteins on PMNs including L-selectin and P-selectin glycoprotein ligand-1 (PSGL-1).<sup>328,344,598</sup> These early interactions slow the PMNs in this first adhesive phase of leukocyte recruitment, sometimes described as “slow rolling.”<sup>67,110,505</sup> Within several hours, newly synthesized ICAM-1 is expressed at the endothelial surface.<sup>110,505,508</sup> The slowly rolling PMNs are activated by transient selectin-mediated interactions and locally produced mediators, especially endothelium-derived chemokines such as IL-8.<sup>343</sup> These chemokines are most effective in PMN activation when they are bound by complex proteoglycans at the endothelial cell surface.<sup>324,573</sup> The activated PMNs then signal the conformational activation of binding function of their surface  $\beta_2$  integrins LFA-1 and Mac-1,<sup>171,563</sup> as well as translocating an additional large quantity of Mac-1 from intracellular storage pools to the cell surface.<sup>60,80,82</sup> This newly translocated Mac-1 also may undergo conformational activation as the PMN is exposed to increasing



**FIG. 2.2** Events during leukocyte (polymorphonuclear leukocytes [PMNs]) recruitment to infected sites. Interactions between microorganisms in infected tissue and host cells and proteins result in elaboration of mediators that diffuse to the local microcirculation and stimulate the endothelial cells. This induces new surface expression of P-selectin and E-selectin, release of interleukin-8 and other chemokines, and new surface expression of intercellular adhesion molecule 1 (ICAM-1). The endothelial selectins bind to constitutively expressed carbohydrate ligands on circulating PMNs and slow the passage of the PMNs through the microvessels. As the PMNs slow further, they become activated by interaction with chemokines bound to complex glycopeptides on the endothelial surface. This activation of PMNs increases their expression and binding activity of the  $\beta_2$  (CD11/CD18) integrins, Mac-1 and lymphocyte function-associated antigen-1 (LFA-1). Interactions between these integrins and ICAM-1 (and ICAM-2 in the case of LFA-1) lead to tight adhesion and spreading on the endothelial surface. These latter adhesive interactions also are used for migration between endothelial cells and through the subjacent extracellular matrix in response to the gradient of chemoattractants, such as C5a, chemokines, and bacterial peptides, released at the infected site. Homophilic interactions between PECAM-1 on the PMNs and endothelial cells (not diagrammed) also appear to contribute to transendothelial migration. (Courtesy Scott Seo, MD.)

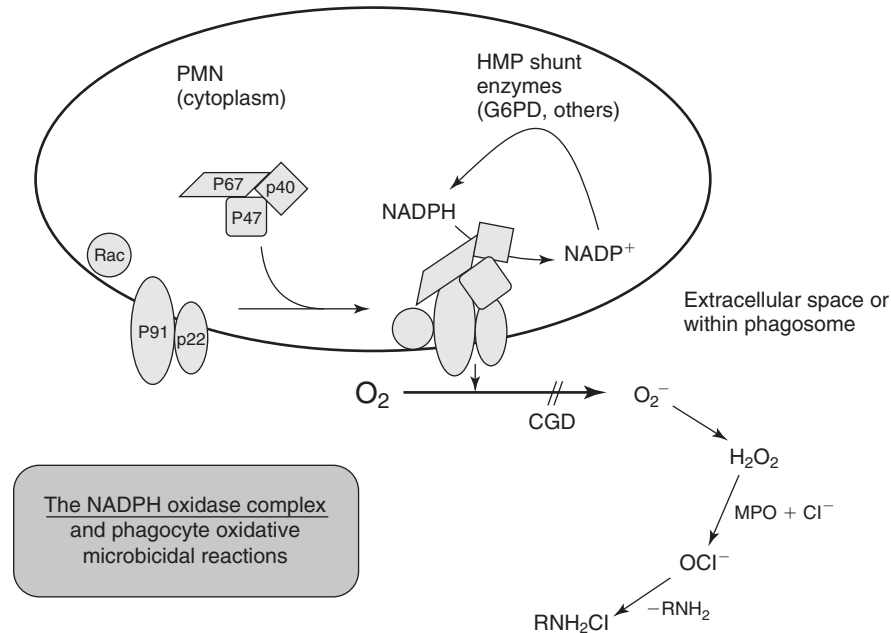
concentrations of mediators.<sup>171,269</sup> These activated  $\beta_2$  integrins interact with the endothelial cell ICAM-1 in this second, firm adhesion phase, which is necessary for transendothelial migration of the PMNs.<sup>60,110,170,343,496,505,508</sup> Other chemoattractants, such as C5a, N-formyl bacterial oligopeptides, and leukotrienes (e.g., LTB<sub>4</sub>) that diffuse from the site of infection further activate PMNs and provide a chemotactic gradient for PMN migration into tissue.<sup>177,232,393</sup> The receptors for these chemoattractants, like the chemokine receptors, are G-protein coupled and have a seven-transmembrane-domain structure.<sup>232,393</sup> They constitute important sensory mechanisms of the PMNs for activating adhesion, directional orientation, and the contractile protein-dependent lateral movement of adhesion sites in the PMN membrane necessary for cell locomotion.<sup>24,232,393,531</sup> A scheme for PMN recruitment from the microcirculation into infected tissue is presented in Fig. 2.2. Although the specific stimuli and adhesion molecules may vary, this general scheme applies to the local recruitment of virtually all circulating cells of the immune system.<sup>71,235,304</sup>

**Phagocytosis.** After PMNs reach the site of infection, they must recognize and ingest, or phagocytose, the invading bacteria. Opsonization, especially with IgG and fragments of C3, greatly enhances phagocytosis.<sup>277,293</sup> Although nonopsonic phagocytosis may occur, only opsonin-mediated phagocytosis is considered here.<sup>483,546</sup> CR1 and CR3 are the main phagocytic receptors for opsonic C3b and iC3b, respectively.<sup>60–62,204</sup> When PMNs are activated by chemoattractants or other stimuli, CR1 and CR3 are rapidly translocated to the cell surface from intracellular storage compartments, thus increasing surface expression up to 10-fold.<sup>60,204</sup> Note that CR3 is identical to the adhesion-mediating integrin Mac-1.<sup>35,60</sup> CR1 and CR3 act synergistically with receptors for the Fc portion of antibodies, especially IgG.<sup>34,293</sup> Phagocytic cells may express up to three different types of IgG Fc receptors, or Fc $\gamma$ R, all of which can mediate phagocytosis.<sup>200,556</sup> Fc $\gamma$ RI (CD64) is a high-affinity receptor that is expressed mainly on mononuclear phagocytes.<sup>556</sup> The two Fc $\gamma$ R

ordinarily expressed on circulating PMN are Fc $\gamma$ RII (CD32) and Fc $\gamma$ RIII (CD16).<sup>547,556</sup> Fc $\gamma$ RII is conventionally anchored in the cell membrane, exhibits polymorphisms that determine preferences for binding of certain IgG subclasses, and can directly activate PMN oxidative burst activity.<sup>547,556,557</sup> Fc $\gamma$ RIII is expressed on PMNs as a glycolipid-anchored protein, although it is anchored conventionally on NK cells and macrophages.<sup>482,549,556</sup> Many phagocytes also express IgA FcRs, which promote phagocytosis and killing of IgA-opsonized bacteria.<sup>278,385</sup>

The engagement of phagocyte receptors with microbial opsonins on microbes locally activates cytoskeletal contractile elements, leading to engulfment of the microbe within a sealed phagosome.<sup>530</sup> This is followed by fusion of the phagosome with lysosomal compartments containing the phagocyte's array of microbicidal products.

**Phagocyte microbicidal mechanisms.** Intracellular killing by phagocytes, usually within the fused phagolysosome, involves microbicidal weapons that can be categorized as either oxygen-dependent or oxygen-independent.<sup>466</sup> The oxygen-dependent microbicidal mechanisms of phagocytes depend on a complex enzyme, reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, which catalyzes the conversion of molecular oxygen (O<sub>2</sub>) to superoxide anion (O<sub>2</sub><sup>-</sup>), the reaction that is deficient in chronic granulomatous disease (see later discussion).<sup>39,40,129</sup> As the name suggests, the reaction catalyzed by this enzyme requires a supply of NADPH, which is supplied in turn by reactions of enzymes of the hexose monophosphate shunt. The NADPH oxidase is assembled at the plasma or phagolysosomal membrane of activated cells from six known components that include a cytochrome ( $\alpha$ - and  $\beta$ -subunits, designated gp91<sup>phox</sup> and p22<sup>phox</sup>, respectively) and at least three cytosolic proteins, p40<sup>phox</sup>, p47<sup>phox</sup>, and p67<sup>phox</sup> (“phox” refers to phagocyte oxidase), along with a Rac-1 GTPase, which assemble with the membrane-associated components to form the active enzyme complex (Fig. 2.3).<sup>40,80,129</sup> Each of the main oxidant products derived from this enzyme's activity exhibits microbicidal activity, including the earliest



**FIG. 2.3** The phagocyte reduced nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase enzyme complex and the major reactions in the evolution of oxygen-dependent PMN microbicidal activity. The diagram depicts the six main components of the NADPH oxidase complex: the 91-kDa and 22-kDa subunits of the membrane-bound cytochrome; the 40-kDa, 47-kDa, and 67-kDa cytosolic components; and a Rac-1 signaling molecule. After assembly at the plasma or phagolysosome membrane, the enzyme catalyzes the conversion of molecular oxygen ( $O_2$ ) to superoxide anion ( $O_2^-$ ), the initial step in the sequence of production of oxidant antimicrobial products. This reaction requires a supply of NADPH, most of which is derived from activity of enzymes of the hexose monophosphate shunt (not shown). Shown in sequence are subsequent reactions for the spontaneous formation of hydrogen peroxide ( $H_2O_2$ ), the myeloperoxidase-catalyzed formation of hypochlorite ( $OCl^-$ ), and formation of chloramines ( $RNH_2Cl$ ).

products,  $O_2^-$  and  $H_2O_2$ , and the more potent downstream products hypochlorite ( $OCl^-$ ) and chloramines ( $NH_3Cl$ ,  $RNH_2Cl$ ), with chloramines being the most stable.<sup>248,466</sup>

The oxygen-independent microbicidal activity of PMNs resides mainly in a group of proteins and peptides stored within their primary (azurophilic) granules and, to a lesser extent, in their secondary (specific) granules.<sup>80,81,83,225</sup> Lysozyme is contained in both the primary and the secondary (specific) granules of PMN.<sup>522</sup> It cleaves important linkages in the peptidoglycan of bacterial cell walls and is most effective when it can act in concert with the complement MAC.<sup>293</sup> The primary granules contain several cationic proteins with important microbicidal activity. A 59-kDa protein, bactericidal/permeability-increasing protein, is active against only gram-negative bacteria.<sup>578</sup> Smaller arginine- and cysteine-rich peptides, the  $\alpha$ -defensins, similar to the  $\beta$ -defensins of epithelial cells, are active against a range of bacteria, fungi, chlamydiae, and enveloped viruses; other related molecules include cathelicidin and a group of peptides called p15s.<sup>221,224,225,337,341</sup> Some of these PMN proteins and peptides interact with each other synergistically to enhance overall antimicrobial activity.<sup>340</sup>

### Important Interactions Among Innate Immune Mechanisms

A schematic overview of many of the main features of innate immunity discussed earlier, along with some of their important interactions, is diagrammed in Fig. 2.4. Several levels of interactions are depicted, from initial host-pathogen contact, through a variety of activating signals, to the attack by host effector mechanisms on their respective pathogenic targets.<sup>545</sup>

### Adaptive Immune Responses

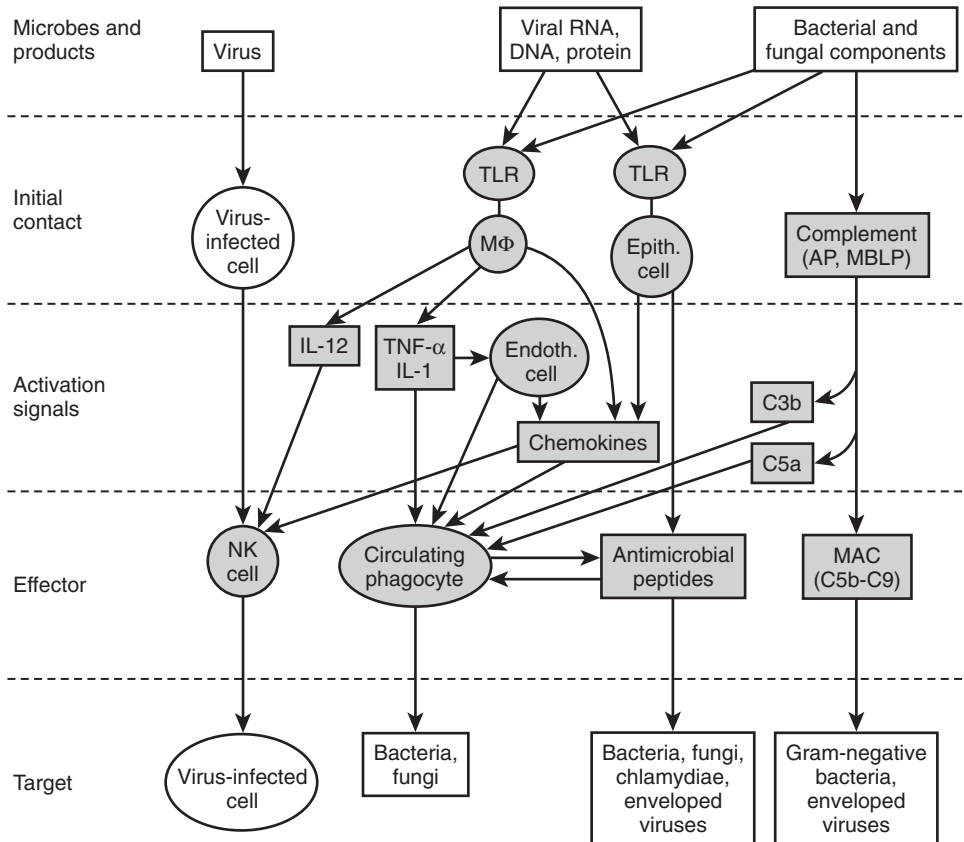
Adaptive immunity involves the host's antigen-specific responses to infectious challenges that can provide specific protection against subsequent challenges by the same infectious agent. The major steps in the development of adaptive immunity include the processing and presentation of specific antigens to T lymphocytes (T cells) by antigen-presenting cells (APCs); the activation and differentiation of T cells for specific cytotoxic T-cell activity, T-cell cytokine production, and T-cell help in

activating antigen-specific B cells; and the differentiation of activated B cells into plasma cells for the production of specific antibodies. Whereas the innate immune responses described earlier often occur in a matter of minutes to hours and may activate early cellular responses that are essential for the development of adaptive immunity, the full development of most adaptive immune responses requires days to weeks. Once developed, however, the latter often can provide durable protection. A summary of the major events in the adaptive immune response to infection is diagrammed in Fig. 2.5.

### Antigen Presentation and Specific Cell-Mediated Immunity

Specific cell-mediated immunity provides T-cell help for antibody production by B cells, cytokine production for the stimulation and regulation of a range of immune responses, and cytotoxic T-cell activity against host cells infected with viruses.<sup>175,391,436</sup> The development of cell-mediated immunity requires complex interactions between T cells and APCs via several types of surface molecules on the respective cell surfaces. These include binding of an antigen-specific T-cell receptor on the T lymphocyte to a peptide antigen presented on the class I or II MHC by the APCs, with concurrent binding of the class I or class II MHC by CD8 or CD4, respectively,<sup>144,543</sup> as represented in Fig. 2.6. Other respective pairs of cell-surface molecules that enhance interactions between T cells and APCs include CD40 ligand/CD40, LFA-1/ICAM-1, and CD28/B7. An additional molecule, cytotoxic T lymphocyte antigen-4 (CTLA-4), expressed on activated T cells, also can bind to B7 molecules on APCs to generate a suppressive signal that may terminate T-cell activation.<sup>543</sup> The sustained physical interface between T cells and APCs at which these molecular interactions take place has been characterized as the "immunologic synapse."<sup>32,92,242</sup>

**Class I major histocompatibility complex.** Virtually all human cells except neurons express class I MHC.<sup>152,153</sup> The class I MHC molecule presents antigenic peptides to CD8<sup>+</sup> cytotoxic T lymphocytes.<sup>67,407</sup> It consists of a heavy chain that contains both the peptide-binding domain and a transmembrane domain and a smaller extracellular subunit,



**FIG. 2.4** Innate immunity: first contact, intermediate signals, and effector mechanisms. Diagrammed are important host responses to infection that are independent of specific cell-mediated immunity or antibodies. Initial contact between the host and microbes or their products may result in viral infection of cells, activation of Toll-like receptors (TLR) on macrophages (MΦ) and epithelial cells, and activation of the alternative pathway (AP) or mannose-binding lectin pathway (MBLP) of complement. The resulting activation signals, including cytokines (e.g., interleukin [IL]-12, tumor necrosis factor  $\alpha$  [TNF- $\alpha$ ], IL-1), chemokines, and products of the complement cascade mobilize both cellular (natural killer [NK] cells, phagocytes) and humoral (antimicrobial peptides, membrane attack complex [MAC]) effectors that attack their respective microbial targets. (From Tosi MF. Innate immune responses to infection. *J Allergy Clin Immunol* 2005;116:241–9.)

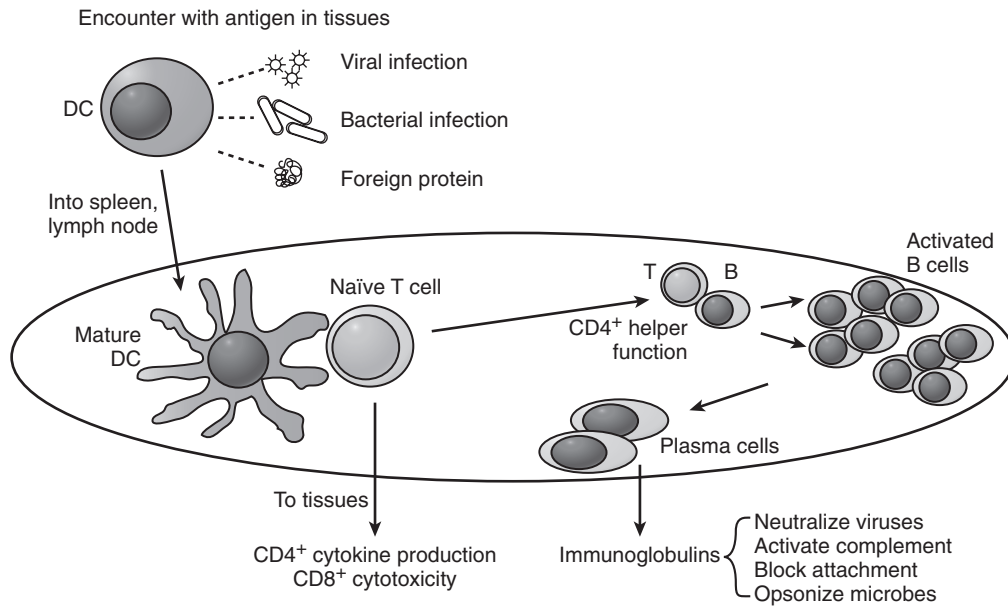
$\beta_2$ -microglobulin.<sup>73,462</sup> The three major types of class I MHC heavy chains in humans, human leukocyte antigen (HLA)-A, HLA-B, and HLA-C, have at least 22, 31, and 12 different alleles, respectively.<sup>597</sup> This polymorphism permits a great diversity in the peptide-binding repertoire in individuals and within populations. A restricted degree of MHC genetic polymorphism has been invoked as a possible explanation for the predisposition of certain populations to develop infections.<sup>74</sup> Class I MHC molecules within the cell ordinarily bind peptides derived from recently synthesized proteins, either of self-origin or of infecting viruses.<sup>198,199</sup> A portion of newly synthesized proteins is processed into peptides at a cytoplasmic site, the proteasome.<sup>236</sup> These peptides are actively transported into the endoplasmic reticulum, where they are bound in the peptide-binding cleft of MHC class I. Suitable peptides usually are restricted to 8 to 10 amino acids in length, and they must contain certain amino acids at specific "anchor" positions on the peptide to bind.<sup>280</sup> Allelic variants of MHC class I may require different amino acids at these anchor positions.<sup>218</sup> The other amino acids of the peptide constitute the specific antigenic determinant. After trafficking of the MHC-peptide complex to the cell surface, the peptide antigen is recognized and bound by a specific T-cell receptor on CD8<sup>+</sup> cytotoxic T cells, which concurrently bind the heavy chain of MHC class I via CD8.<sup>67,218,280,551</sup>

**Class II major histocompatibility complex.** Mononuclear phagocytes, B lymphocytes, and dendritic cells, including specialized tissue-specific dendritic cells, such as the Langerhans cells of the skin, serve the immune system as "professional" APCs.<sup>365</sup> Dendritic cells, the most efficient APCs

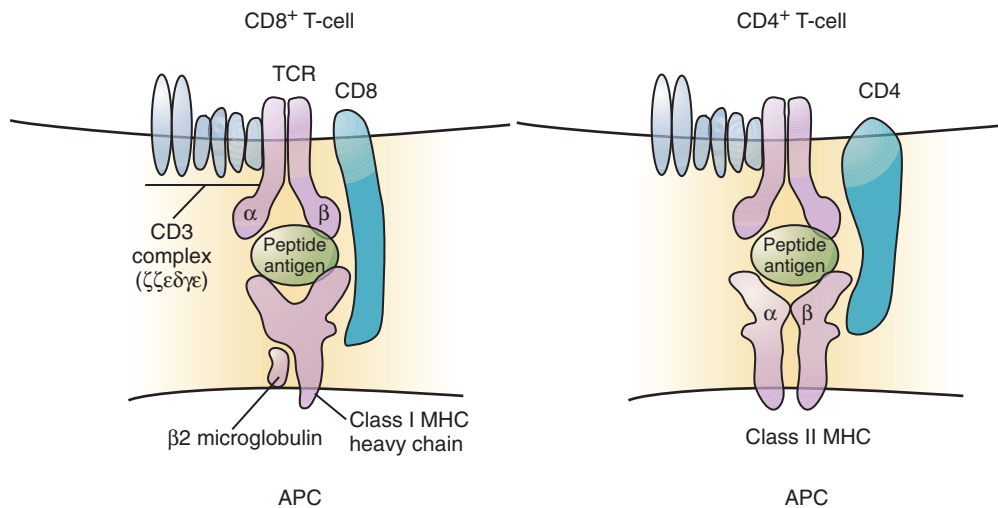
for primary activation of naïve T cells, are macrophage-like cells of a distinct lineage that take up and process antigens in tissues and migrate to local lymph nodes or to the spleen, where they are likely to encounter T cells specific for the presented antigens.<sup>182,183,253,463</sup> A defining feature of these professional APCs is their expression of class II MHC molecules in addition to class I MHC.<sup>153</sup> Class II MHC molecules consist of an  $\alpha$  and a  $\beta$  chain, which together form a peptide-binding cleft.<sup>94,475</sup> Class II MHC molecules present peptides, 13 to 17 amino acids in length, derived from proteins that are internalized by endocytosis or during phagocytosis of microorganisms.<sup>251,281,475</sup> The three major types of class II MHC  $\alpha$  and  $\beta$  chains are HLA-DR, HLA-DP, and HLA-DQ, each exhibiting a high degree of polymorphism.<sup>460</sup> MHC class II, bound to a separate smaller molecule known as the "invariant chain," traffics via the Golgi to endosomal/lysosomal compartments, where it must dissociate from the invariant chain to bind antigenic peptides derived from internalized proteins.<sup>461,541,542</sup> The class II MHC-peptide complexes then move to the cell surface, where the peptide antigens are bound by specific T-cell receptors of CD4<sup>+</sup> T cells, which concurrently bind class II MHC via CD4.<sup>183,351</sup>

Fig. 2.7 depicts the essential features of the conventional antigen presentation pathways that involve class I and class II MHC molecules, as described earlier. Alternative mechanisms have been documented by which class I MHC can present peptides derived from internalized exogenous proteins, and class II MHC may present peptides derived from newly synthesized proteins. The importance of these unconventional pathways of antigen presentation in the immune response is not fully

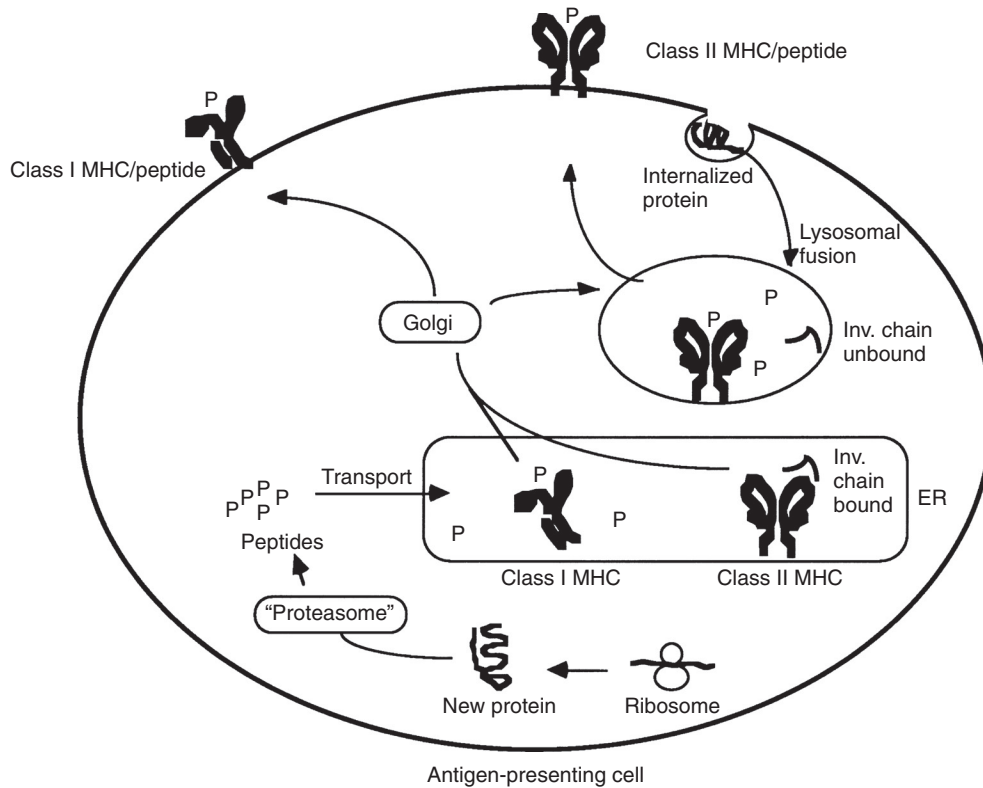




**FIG. 2.5** A simplified scheme of major events in the development of adaptive immune responses. When antigen-presenting cells (APCs) such as dendritic cells (DCs) encounter and internalize microbes or their protein antigens in peripheral tissues, they process the microbial proteins and present the resulting antigenic peptides on either class I or class II MHC molecules. The activated APCs migrate to lymphoid tissue, where they undergo maturation. When mature dendritic cells encounter  $CD4^+$  or  $CD8^+$  T cells expressing T-cell receptors specific for the peptides presented in the appropriate major histocompatibility complex (MHC) context ( $CD4/MHC-II$ ;  $CD8/MHC-I$ ), binding between the cells occurs via TCR-peptide, MHC- $CD4/8$ , and other pairs of accessory molecules, all necessary for stimulating the T cells to become effector cells. Cytotoxic effector  $CD8^+$  T cells migrate into the periphery and kill virus-infected cells that present viral peptides via MHC class I. Effector  $CD4^+$  cells either migrate to the periphery where they produce cytokines and otherwise regulate immune responses or remain in the lymphoid tissue to provide help to antigen-specific B cells, promoting their proliferation, differentiation, and eventual production by their progeny plasma cells of specific antibodies that can neutralize viruses, prevent microbial attachment, opsonize microorganisms, or activate complement.



**FIG. 2.6** Principal cell surface interactions between  $CD8$  and  $CD4$  T lymphocytes and peptide antigens complexed with major histocompatibility complex (MHC) class I and class II molecules, respectively.  $CD3$  (composed of six subunits,  $\zeta$ ,  $\epsilon$ ,  $\delta$ ,  $\gamma$ ,  $\eta$ ) is associated closely with the T-cell receptor (TCR), which recognizes a specific peptide presented on MHC molecules. Class I and class II MHC determinants are recognized by  $CD8$  and  $CD4$ , respectively. Additional or accessory interactions are discussed in the text. APC, Antigen-presenting cell. (Modified from Lewis DB, Wilson CB. Developmental immunology and role of host defenses in neonatal susceptibility to infection. In: Remington JS, Klein JO, editors. *Infectious Diseases of the Fetus and Newborn Infant*. 6th ed. Philadelphia: WB Saunders; 2006:92.)



**FIG. 2.7** Conventional pathways for peptide antigen presentation by class I and class II major histocompatibility complex (MHC) molecules. In the antigen-presenting cell, a proportion of newly synthesized proteins, whether of viral or host origin, undergo proteolysis into peptides by enzymes that constitute the “proteasome.” The peptides are transported actively into the endoplasmic reticulum (ER), where those with the appropriate length and sequence bind to MHC class I molecules. MHC class II cannot bind peptides in the ER because of interference by the associated “invariant (inv.) chain.” The class I MHC/peptide complex is transported via the Golgi to the cell surface, where it may be recognized by CD8<sup>+</sup> lymphocytes. Class II MHC molecules pass via the Golgi to a lysosomal compartment, where conditions favor the release of the invariant chain. This permits class II MHC to bind peptides derived from internalized proteins that have entered the lysosomal compartment via fusion of endosomes or phagosomes with the lysosome. The lysosome translocates to the cell surface, where the class II MHC/peptide complex may be recognized by CD4<sup>+</sup> lymphocytes.

understood, but evidence indicates that such “cross-presentation” may be important for generation of CD8<sup>+</sup> cytotoxic T-cell response against some viruses or fungi taken up via endocytosis by antigen-presenting cells.<sup>36,348</sup>

**CD1 family of antigen-presenting molecules.** The CD1 family includes proteins with significant homology and structural similarity to that of the MHC class I heavy chain but that present lipid and glycolipid antigens. All mammalian species express one or more members of the CD1 family, principally on professional antigen-presenting cells. Four human CD1 proteins, CD1a, CD1b, CD1c, and CD1d, have been identified, each tightly associated with a  $\beta_2$ -microglobulin subunit. Mycolic acid, lipoarabinomannans, and other related components of mycobacteria are the best-documented foreign antigens presented by CD1 molecules, and both internalized antigens and antigens synthesized within the antigen-presenting cells by ingested mycobacteria may be presented via distinct trafficking patterns of the CD1-antigen complexes. Antigens presented on antigen-presenting cells by CD1 molecules are recognized by a specialized subset of CD1-restricted T cells that usually lack CD4 and CD8; these are known as NK T cells. These cells share characteristics of both NK cells and T cells and exhibit a limited range of T-cell receptor specificity. Greater detail regarding the structure, function, phylogeny, trafficking, expression, and T-cell interactions for members of the CD1 family may be found in a recent review.<sup>489</sup>

**Plasmacytoid dendritic cells.** A specialized class of dendritic cells known as “plasmacytoid” dendritic cells plays a multifactorial role in both innate and adaptive immune responses. These cells are early responders to viral infections by virtue of their expression of TLR7/9 and their

ability to produce large amounts of type I interferons that disrupt viral replication.<sup>38,536</sup> Additionally they can play an auxiliary role in the adaptive immune response by providing help to conventional dendritic cells during antigen presentation, apparently by helping to sustain IL-12 production by the latter in response to IFN- $\gamma$  released by interacting T cells.<sup>38,536</sup>

### T Lymphocytes

The development of T lymphocytes, or T cells, begins when prothymocytes leave the marrow and enter the subcapsular region of the thymus.<sup>181</sup> By mechanisms that are poorly understood, the thymic environment induces the rearrangement of T-cell receptor V (variable), D (diversity), and J (joining) gene segments with the eventual expression of mature  $\alpha$ - $\beta$  T-cell receptors complexed with CD3. The T cells, now coexpressing CD4 and CD8, migrate to the thymic cortex, where they undergo screening for T-cell receptor specificity both to optimize the repertoire for distinguishing self from nonself and to eliminate T-cell receptor rearrangements that result in undesirably high self-reactivity. Thymocytes that do not pass this dual screening procedure receive signals that induce programmed cell death (apoptosis).<sup>373,394,567</sup> Only about 5% of the original thymocytes pass this screening, after which they express either CD4 or CD8 but not both.<sup>373,394,458,567</sup> Mature thymocytes are released into the periphery, where the CD4<sup>+</sup> cells serve as the main source of IL-2 and provide help for B-cell antibody production, and the CD8<sup>+</sup> cells engage in specific cytotoxic activity.<sup>231,391</sup> This discussion of T cells and T-cell receptors specifically relates to T cells that express T-cell receptors composed of  $\alpha$  and  $\beta$  chains, or  $\alpha$ - $\beta$  T cells. T

cells of a distinct type,  $\gamma\delta$  T cells, are far less numerous in most tissues (intestinal epithelium is a notable exception), exhibit much less T-cell receptor diversity than do  $\alpha\beta$  T cells, may not require an intact thymus for development, and play a role in host responses to certain intracellular bacterial pathogens, including *Listeria* and mycobacteria.<sup>106,254</sup>

Antigen specificity of  $\alpha\beta$  T cells resides in their T-cell receptors, which are integral membrane proteins that exhibit structural homology with immunoglobulins. T-cell receptor diversity results from a rearrangement of V, (D), and J segments.<sup>226</sup> There are up to 100 different V segments, one (D) segment, and as many as 100 different J segments in the complete germline configuration of the T-cell receptor genes. Rearrangement of these gene segments into a mature VDJ sequence occurs by the action of a recombinase enzyme complex formed by two proteins, RAG-1 and RAG-2.<sup>417,488</sup> T-cell receptor diversity is generated by several factors, including the range of possible combinations of V, (D), and J segments; the imprecise action of the recombinase complex; the variability in the number of nucleotides deleted during rearrangement; and the action of another enzyme, terminal deoxynucleotidyl transferase, which appears to add nucleotides at random to extend segments during rearrangement.<sup>206,504</sup> The actions of Artemis and DNA ligase IV, two enzymes critical for the processing and joining of DNA ends, introduce additional sources of variability.<sup>99,191,355,585</sup> It has been estimated that as many as  $10^{15}$  different T-cell receptor specificities theoretically could result from the preceding mechanisms.<sup>159</sup>

Stimulation of naïve CD4<sup>+</sup> or CD8<sup>+</sup> T cells occurs as they circulate through peripheral lymphoid tissue and encounter dendritic cells and other professional APCs. Localized T-cell migration is highly regulated by specific chemokines and adhesive interactions with local endothelium and involves mechanisms similar to those discussed earlier for circulating phagocytes.<sup>185,320</sup> When T cells engage APCs presenting specific peptide antigens on the appropriate MHC molecules, they are activated via their T-cell receptor and several costimulatory molecules, especially CD28, to produce IL-2 and proliferate and differentiate into effector T cells.<sup>128,284</sup>

Effector CD4<sup>+</sup> T cells may be of the T<sub>H</sub>1 or T<sub>H</sub>2 type, and this type is influenced by several factors, including the specific cytokines elicited by a particular microbial pathogen.<sup>395</sup> Naïve T cells activated in the presence of IL-12 and IFN- $\gamma$  are likely to develop into T<sub>H</sub>1 cells, whereas IL-4 and IL-6 tend to drive development in the direction of T<sub>H</sub>2 cells.<sup>390,395,397</sup> Preferential development of T<sub>H</sub>1 effector cells leads mainly to macrophage activation and cell-mediated immunity, whereas T<sub>H</sub>2 effector cells help drive certain aspects of humoral immunity, including immunoglobulin class switching to IgE in allergic responses.<sup>395</sup> Until recently, before the identification of the T<sub>FH</sub> subset (see later discussion), T<sub>H</sub>2 cells were thought to be the principal cell in providing T-cell help for B-cell antibody production.

A third major subset of effector CD4 T cells are T<sub>H</sub>17 cells, whose main function appears to involve protection against extracellular bacteria and fungi by stimulating phagocytic cell responses to these pathogens.<sup>272,349,382</sup> Their development is favored by the presence of IL-6 and TGF- $\beta$  and by the absence of IL-4 and IL-12. They are distinguished by their ability to produce IL-17 cytokines, which in turn stimulate local tissues to produce chemokines, such as IL-8, that recruit neutrophils and other phagocytic cells to tissue sites.<sup>272,349,382</sup> Development of T<sub>H</sub>17 cells involves production of IL-21, which acts in an autocrine fashion to activate signal transducer and activator of transcription 3 (STAT3), a transcription factor that drives T<sub>H</sub>17 cell development.<sup>272,382</sup>

In contrast to T<sub>H</sub>1, T<sub>H</sub>2, and T<sub>H</sub>17 CD4 T cells, which exert their main effector functions in the periphery, a fourth T-cell subset, T follicular helper cells, or T<sub>FH</sub> cells, appears to account for most of the CD4 T cells that provide help to B cells in the lymphoid follicles for antibody production.<sup>306,411</sup> T<sub>FH</sub> cells are characterized by their location in lymphoid follicles, expression of the CXCR5 chemokine receptor, and their ability to secrete cytokines typical of both T<sub>H</sub>1 and T<sub>H</sub>2 cells.<sup>306,411</sup> The developmental origins of these cells in humans and their relationships and interactions with the other T-cell subsets are subjects of current research.

Activation of naïve CD8<sup>+</sup> T cells by antigen binding, costimulation by accessory binding molecules on antigen-presenting cells, and exposure to cytokines, including IL-2, all lead to clonal proliferation of specific CD8<sup>+</sup> cells and their differentiation into cytotoxic effector cells. Effector

CD4<sup>+</sup> T cells bound in common to an APC may play a role in activating naïve CD8<sup>+</sup> T cells, either by releasing IL-2 or by activating the antigen-presenting cell to provide greater costimulation to the CD8<sup>+</sup> T cell to make its own IL-2.<sup>31</sup> Antigenically experienced effector CD8<sup>+</sup> T cells respond to specific antigenic peptides and costimulatory molecules on infected host cells by activating cytotoxic mechanisms similar to those described earlier for NK cells, including the release of both perforin and granzymes and the generation of receptor-mediated signals for target cell apoptosis.<sup>345,476,552</sup>

**Regulatory T cells.** The existence of T suppressor cells was long a subject of debate among immunologists. Within the past decade solid evidence has been developed to support the existence of suppressor T cells, now referred to as regulatory T cells, or T-regs. These cells were discovered when thymectomized mice were noted to develop autoimmune disease. Transfer of T cells that expressed CD25, the  $\alpha$  chain of the IL-2 receptor, from normal adult mice to thymectomized mice prevented autoimmune disease. This population of CD4<sup>+</sup>CD25<sup>+</sup> T-regs can suppress the activity of other immune cells and has been shown to prevent graft-versus-host disease and allograft rejection.<sup>449</sup> The mechanism of suppression by T-regs is uncertain but may involve direct contact with other cells or secretion of inhibitory cytokines, including IL-10.<sup>349,484</sup> These inhibitory cytokines can interfere with T-cell proliferation and inhibit the ability of antigen-presenting dendritic cells to promote T-cell activation.<sup>349,484</sup> The role of T-regs in immunity to infection is only beginning to be studied, but some current evidence suggests that the action of T-regs with specificity for microbial antigens may suppress protective immune responses to some infections but may also suppress excessive or injurious host responses.<sup>449</sup>

**T-cell memory.** Some proportion of activated CD4<sup>+</sup> and CD8<sup>+</sup> T cells become endowed with the capacity for long-term antigenic memory and can rapidly become effectors on re-exposure to specific antigen. Whether these cells develop directly from naïve T cells or previously have been effector cells, or both, is uncertain, and the mechanisms by which they become memory T cells are poorly understood. Among the features of memory T cells are high-level expression of CD45RO, the ability to suppress activation of naïve T cells of the same specificity, and a homeostatic level of ongoing proliferation in bone marrow and peripheral lymphoid organs.<sup>296,357,481,599</sup>

**T-cell activation by superantigens.** The term *superantigen* describes a class of proteins, mainly microbial exotoxins, including most staphylococcal enterotoxins, staphylococcal toxic shock syndrome toxin-1 (TSST-1), and related streptococcal TSST-1-like toxins. These bacterial toxins are potent pyrogens, can induce a potentially lethal toxic shock syndrome, and contain binding domains for both T-cell receptor V regions and MHC class II molecules. Superantigens bypass normal antigen-processing and presentation pathways by binding directly to class II MHC molecules on antigen-presenting cells and to specific variable regions on the  $\beta$ -chain of the T-cell antigen receptor. Through these interactions, superantigens induce a polyclonal activation of T cells at orders of magnitude above levels induced by antigen-specific activation, resulting in massive release of cytokines from T cells and antigen-presenting cells, including TNF- $\alpha$  and TNF- $\beta$ , IL-1, IL-2, and IFN- $\gamma$ , that are believed to be responsible for the most severe features of toxic shock syndromes.<sup>14,450</sup>

## B Lymphocytes and Immunoglobulins

**B lymphocytes.** B lymphocytes (B cells) are the source of humoral immunity in the form of specific immunoglobulin. The earliest recognizable marrow precursors of B cells are pro-B cells whose surfaces bear the pan-B marker CD19. Further differentiation produces pre-B cells and then mature B cells, the latter expressing cell-surface immunoglobulin by which they recognize and bind antigen. B lymphocytes constitute approximately 20% of the lymphocytes in the circulation and peripheral lymphoid tissues, including the lymph nodes, spleen, bone marrow, tonsils, and intestines, and they are identified by the presence of surface immunoglobulin and the pan-B differentiation markers CD19 and CD20.<sup>109,371</sup>

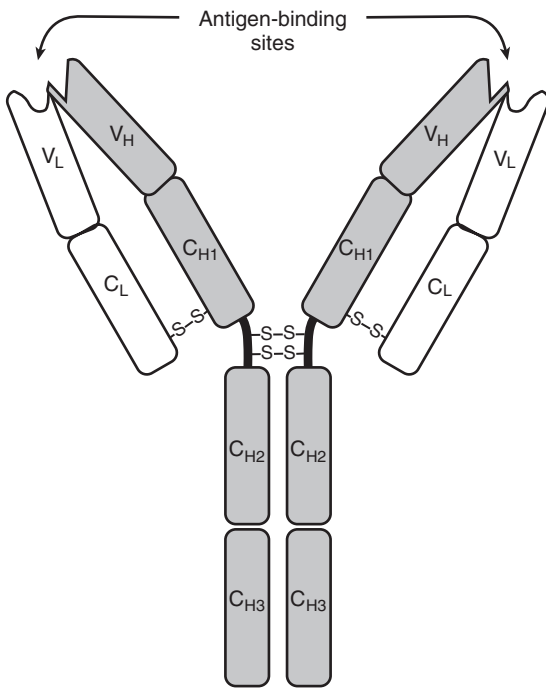
B-cell activation is initiated by recognition and binding of specific antigens to B-cell surface immunoglobulins. Early activation leads to increased expression of receptors that either bind cytokines (e.g., IL-2, IL-4, and IL-6) or interact with T cells,<sup>327</sup> leading in turn to clonal

proliferation and differentiation into memory B cells and plasma cells in the germinal centers of peripheral lymphoid tissue.<sup>302</sup> Some data suggest that B-cell differentiation into memory B cells is favored by exposure to the CD40 ligand on dendritic cells in lymphoid organs, whereas differentiation into plasma cells is favored by exposure to CD23, IL-1 $\alpha$ , IL-6, and IL-10.<sup>302,540</sup> The plasma cells, later found in bone marrow and liver as well as peripheral lymphoid tissue, are responsible for most free immunoglobulin production.<sup>540</sup>

The B-cell response to protein antigens depends on T-cell help. B cells can process and present antigen to CD4<sup>+</sup> T<sub>H</sub> cells they encounter in the lymph nodes and spleen.<sup>301,302,540</sup> In the typical sequence of events, B-cell surface immunoglobulin binds to a protein antigen, which is internalized, processed, and presented to the T cell via class II MHC molecules. B cell–mediated activation of T cells during antigen presentation is much more effective for memory T cells, whereas naïve T cells are more likely to be turned off or rendered tolerant.<sup>197,220</sup> T-cell help is provided for B-cell proliferation and production of antibody against the specific protein antigen. This is mediated by signaling via CD40–ligand interactions with CD40 on the B cell and by the release of cytokines, which also can induce isotype switching.<sup>163,405,526</sup> Most B-lymphocyte responses to polysaccharide antigens proceed largely without formal T-cell help, although antibody responses to some such antigens may be enhanced in the presence of T cells.<sup>384</sup>

**Immunoglobulin.** Immunoglobulin molecules may be bound at the surface of B cells or free in the circulation, mucosal secretions, or tissues. Free immunoglobulins function in host defense against infection by binding to microbial surfaces to prevent microbial attachment, activating complement via the classical pathway, neutralizing viruses and toxins, and participating in the formation of immune complexes.<sup>128</sup>

Ig molecules are composed of two identical heavy and two identical light chains, as diagrammed in Fig. 2.8.<sup>186,430</sup> The carboxyl terminus of the immunoglobulin molecule is the heavy chain constant, or C<sub>H</sub>, region. The amino acid sequence of this region determines the immunoglobulin isotype. The heavy chain is encoded by V, (D), J, and constant (C) regions on chromosome 14.<sup>63,571</sup> Each immunoglobulin molecule has a pair of either  $\kappa$  or  $\lambda$  light chains, defined by distinct constant regions.



**FIG. 2.8** Structure of an immunoglobulin molecule. The schematic structure of immunoglobulin G (IgG) is shown, depicting the variable (V) and constant (C) regions of both the heavy (H) and light (L) chains, the disulfide bonds that link the two heavy chains at the hinge region and the CL region with CH<sub>1</sub>, and the antigen-binding sites formed by the complementarity-determining regions of VH and VL.

The variable region of the immunoglobulin molecule contains the antigen-binding site. Like the T-cell receptor, the Fab region consists of two identical heavy and light chain pairs; similarly, broadly diverse antigen specificity results from the variable nature of recombinase-mediated DNA rearrangements of the three hypervariable, or complementarity-determining, regions (CDR1, CDR2, and CDR3) and the four framework regions during B-cell development.<sup>201,334,359</sup> The imprecision inherent in this rearrangement, involving mechanisms similar to those described for the T-cell receptor, leads to the generation of more than 10<sup>12</sup> potential antigenic specificities. Somatic hypermutation of variable regions after gene rearrangement adds to the repertoire, and further diversity results from differences in approximation of the three CDRs in relation to each other, affecting the three-dimensional structure of the antigen recognition site.<sup>408,433</sup> Thus unlike most T-cell receptors, which recognize specific peptide sequences, the antigen-binding domain of an immunoglobulin molecule recognizes the three-dimensional structure of its respective antigen.<sup>43,156</sup>

All immunoglobulin is derived from B cells expressing surface IgM. B cells may change immunoglobulin isotype when they differentiate into plasma cells, which produce only one class or subclass of immunoglobulin each. Isotypes other than IgM are the result of isotype switching by replacing a part of the constant region of the immunoglobulin heavy chain with another isotype-specific segment.<sup>447,526</sup> As already noted, isotype switching primarily depends on specific B-cell interactions with cytokines and T cells. The variable region remains unchanged during isotype switching; thus there is no change in antigen specificity. However, important features of immunoglobulins, including half-life, localization in tissues, ability to activate complement, and interactions with cellular IgG receptors, are directly determined by isotype.<sup>91,287,556</sup>

In addition to isotype switching, immunoglobulins undergo the process of “affinity maturation.” As B cells proliferate in lymphoid tissue in response to persistent or repeated antigen exposure and T-cell help, they undergo V-region somatic hypermutation. When this mutation results in a reduced or absent affinity for antigen, B cells are less able to become activated and elicit T-cell help. Such B cells die by apoptosis, removing lower affinity immunoglobulin from the repertoire. Alternatively, B cells that undergo a mutation resulting in increased affinity for antigen are better able to bind antigen, present antigenic peptides to T cells, receive T-cell help, and survive to give rise to plasma cells, which in turn will produce immunoglobulin with higher affinity. This is a process that occurs as a result of booster doses of vaccines or during persistent infections, as with cytomegalovirus, for example.<sup>26,444</sup>

**Immunoglobulin isotypes.** IgG accounts for about 80% of circulating immunoglobulin and includes the subclasses IgG1, IgG2, IgG3, and IgG4.<sup>64</sup> The half-life of IgG ordinarily is about 21 days (7 days for IgG3).<sup>287</sup> Initial exposure to most microbial protein antigens first induces IgM and then an IgG response consisting of IgG1 and IgG3. IgG2 and IgG4 usually are produced during the secondary immune response. IgG1 usually is made in response to protein antigens.<sup>287</sup> In adults, the main antibody response to polysaccharides is IgG2, whereas in infants IgG1 predominates.<sup>6,17</sup> The functions of IgG in host defense include blocking microbial attachment, opsonization, complement activation, toxin and virus neutralization, and promoting antibody-dependent cell cytotoxicity. IgG1, IgG2, and IgG3, but not IgG4, can trigger complement activation via the classical pathway by binding to C1q.<sup>91</sup>

Free IgM usually exists as an immunoglobulin pentamer that has a molecular weight of approximately 950,000 and is stabilized by a single J chain.<sup>186,314,374,433</sup> Present mainly in the circulation, its half-life is approximately 8 to 10 days. The IgM response is the earliest of the isotype responses, appearing within the first few days of infection, but it is transient. The formation of an IgM response in the absence of an IgG response to infection is not associated with the formation of memory B cells. The main direct action of IgM in host defense is the activation of complement via the classical pathway.<sup>374</sup>

IgA exists in monomeric circulating and polymeric secretory forms and has a half-life of about 7 days.<sup>287</sup> Both forms are produced mainly by plasma cells that have migrated to mucosal sites. Secretory IgA is made up of two or three IgA molecules joined by a stabilizing J segment



that is secreted by plasma cells and a secretory component produced by mucosal epithelial cells.<sup>303,314</sup> The secretory component permits delivery of IgA to mucosal surfaces.<sup>406</sup> There are two subclasses, IgA1 and IgA2, that differ in the composition of their heavy chains. Most IgA in the circulation is IgA1, whereas most IgA in secretions is IgA2. IgA1 may be cleaved at mucosal sites by bacterial proteases.<sup>313</sup> IgA neutralizes viruses at mucosal sites, may block bacterial adhesion, and can act directly as an opsonin to promote phagocytosis and via Fc $\alpha$  receptors.<sup>278,303</sup>

The IgE molecule has a molecular weight of 200,000 and a half-life of only 2.3 days.<sup>287</sup> Most IgE is produced by plasma cells in lymphoid tissue near gastrointestinal and respiratory mucosal surfaces and released into the circulation.<sup>286,534</sup> IgE acts via Fc $\epsilon$  receptors to trigger activation and degranulation of mast cells and basophils, leading to immediate hypersensitivity reactions.<sup>534</sup> Persons with intestinal metazoan parasites often have elevated serum levels of IgE, and IgE may have a role in protecting against parasitic disease by stimulating mediator release from mast cells that can recruit eosinophils and cause intestinal smooth muscle contraction and expulsion of parasites.<sup>534</sup>

IgD has a molecular weight of approximately 180,000 and a half-life of 3 days.<sup>287</sup> It is expressed along with IgM on surfaces of naïve B cells but is present in normal adult serum and secretions in very low concentrations. Some antigenic specificity for IgD has been demonstrated, and although its function in host defense is unclear, it may serve as a secondary antigen receptor on B cells, where it may regulate the development of B-cell antibody responses.<sup>76</sup>

## CLINICAL CONDITIONS ASSOCIATED WITH DEFICIENT HOST RESPONSES TO INFECTION

### Immature Host Responses of the Newborn Infant

It is well recognized that newborn infants are much more susceptible to serious infections from many types of organisms than are older children and adults. This predisposition to infection is even more profound in infants born prematurely. The basis for this special vulnerability of the neonate is complex and encompasses all arms of the immune system.<sup>332,342,583</sup>

#### Cell-Mediated Immunity

Antigen presentation per se, via the mechanisms discussed earlier, appears to be relatively intact in the newborn infant. Expression of class I and II MHC molecules has been documented in a broad range of fetal tissues by 12 weeks' gestation,<sup>271,419</sup> and levels of expression are sufficient to mediate normal MHC class II–restricted antigen presentation by neonatal monocytes to maternal or paternal CD4<sup>+</sup> T cells, as well as to induce vigorous rejection of allogeneic fetal tissue by CD8<sup>+</sup> cytotoxic T cells.<sup>258,270</sup>

By about 20 weeks' gestation, the fetal repertoire of diversity of T-cell receptors has developed fully.<sup>561</sup> At the time of birth, although most basic functions of cell-mediated immunity are present, a high proportion of immature T cells are in the peripheral circulation, which can be identified by their coexpression of CD4 and CD8.<sup>342</sup> This phenotype typifies type II thymocytes, which usually are not found in the periphery in older persons.

Neonatal T cells appear to be relatively deficient in most of their major functions, including CD8<sup>+</sup> T cell–mediated cytotoxicity, delayed hypersensitivity, T-cell help for B-cell differentiation, and diminished cytokine production. Lack of prior antigenic exposure largely explains these defects because memory T cells are much more efficient in all of these functions.<sup>256,342</sup>

#### B Cells and Antibody

**B cells.** Pre-B cells are found in the fetal liver and omentum by 8 weeks' gestation and in the fetal bone marrow by 13 weeks' gestation.<sup>228,342,516</sup> Pre-B cells with surface IgM have been detected as early as 10 weeks' gestation. After 30 weeks' gestation and delivery, pre-B cells are seen only in the bone marrow. Mature B cells are present in the circulation by the eleventh week and have reached adult levels in the bone marrow, blood, and spleen by the twenty-second week of gestation.<sup>161,228,516</sup>

Fetal B cells express only IgM, whereas most adult B cells express both IgM and IgD. Neonatal B cells may express three immunoglobulin isotypes (e.g., different combinations of IgG, IgA, IgM, and IgD) on their surfaces.<sup>228,250</sup>

Although germinal centers are not present in lymphoid tissue at birth, they begin to develop in the first few months of life concomitant with the infant's exposure to antigens.<sup>544</sup> Despite conflicting *in vitro* data, neonatal T-cell help for B cells probably is comparable to that of adult T cells, as is reflected by the excellent T-dependent antibody response of the newborn to immunization with protein antigens.<sup>165</sup> In contrast to B cells of older individuals, B cells of neonates and young infants cannot respond to pure polysaccharide antigens. The recruitment of T-cell help to enhance this immature response to polysaccharides has been achieved with the advent of protein-polysaccharide conjugate vaccines. Such vaccines elicit help from T cells specific for peptides derived from the protein component as presented by polysaccharide-specific B cells that have internalized the protein-polysaccharide complex, allowing peptide-specific T cells to activate polysaccharide-specific B cells.<sup>75</sup>

**Antibody.** Maternal IgG accounts for the great majority of the newborn's circulating immunoglobulin because almost none is made by the healthy fetus and IgG is the only isotype of maternal immunoglobulin that crosses the placenta.<sup>311,362</sup> Maternal transport of IgG can be detected as early as 8 weeks' gestation, and the newborn's IgG level is directly proportional to gestational age, reaching 100 mg/dL by 17 to 20 weeks' gestation and 50% of the maternal level by 30 weeks' gestation (Fig. 2.9).<sup>47,90,111</sup> Maternal IgG is transported both passively and actively via trophoblast Fc receptors. Trophoblast Fc receptors have higher affinity for IgG1 and IgG3 than for IgG2 and IgG4, and thus more of those subclasses are transported from the mother.<sup>336</sup>

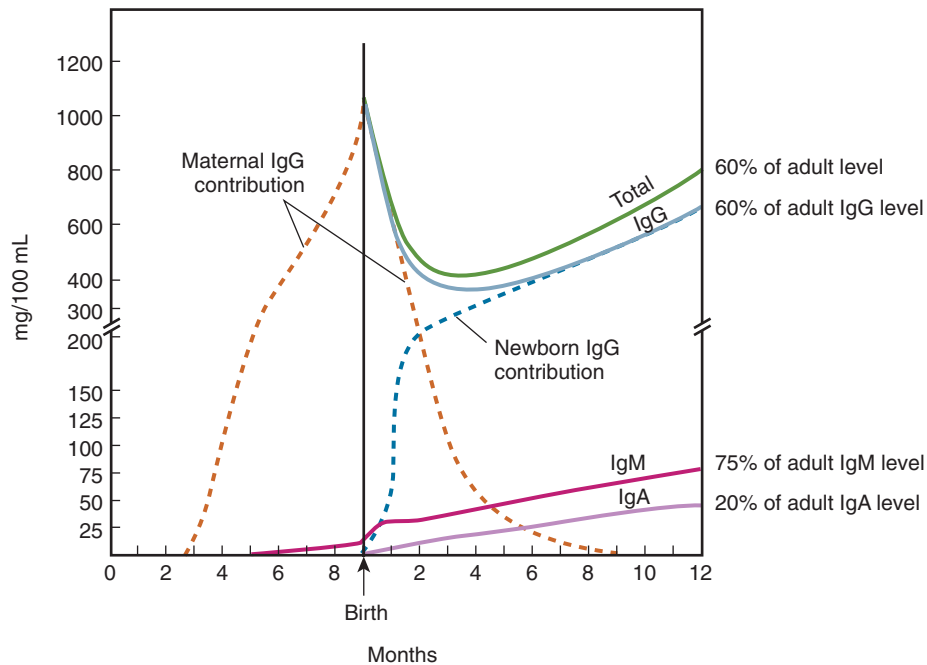
The concept of passive transfer of protective IgG is the basis for development of vaccines for maternal immunization before or during pregnancy so that passive transfer of vaccine-induced antibody will result in protection during the neonatal period. Examples of organisms for which such strategies have been investigated include group B streptococcus, *H. influenzae* type b, meningococcus, pneumococcus, rotavirus, and respiratory syncytial virus.<sup>46,194,282</sup>

By about 2 months of chronologic age, approximately half of the term infant's quantitative IgG is of maternal and half is of infant origin. The physiologic nadir of IgG in all infants is about 3 to 4 months of age and ranges from less than 100 mg/dL in preterm infants with very-low-birth weight to about 400 mg/dL in term infants (see Fig. 2.9).<sup>47,593</sup> Maternal IgG usually has waned completely by about 12 months of age, at which time infant levels are approximately 60% of adult levels. Production of IgG1 and IgG3 matures more rapidly than that of IgG2 and IgG4, reaching adult levels by approximately 8 years of age, versus 10 and 12 years of age, respectively.<sup>416</sup>

Little IgM, IgA, IgE, or IgD normally is produced by the fetus, and none is transported from the mother.<sup>311,362</sup> The presence of total IgM levels greater than 20 mg/dL at birth suggests an intrauterine infection, and documentation at birth of specific serum IgM or IgA against relevant organisms, such as *T. gondii* and others, would be diagnostic.<sup>192,398,418</sup> Serum IgA levels at birth in both preterm and term infants usually are less than 5 mg/dL and consist of both IgA1 and IgA2. Secretory IgA is not detectable until after birth but usually is present within the first few weeks of life. IgM and IgA reach approximately 60% and 20% of adult levels by 1 year of age, respectively (see Fig. 2.9). Secretory IgA reaches adult levels by 6 to 8 years of age.<sup>342</sup>

It has been documented that the fetus can respond to antigenic stimulation in the form of maternal immunization with tetanus toxoid vaccine and be primed for a secondary antibody response to repeat immunization after birth.<sup>233,234</sup> The amount of fetal antibody produced in response to intrauterine antigenic stimulation is proportional to gestational age.<sup>162,528</sup>

Maternal antibody inhibits the infants' ability to respond to live-virus vaccines against certain organisms, such as measles, but it does not prevent them from mounting protective immune responses to most childhood vaccine antigens, such as tetanus, diphtheria, polio, hepatitis B, and protein-conjugated polysaccharide vaccines.<sup>12</sup> In general, neonates have protective responses to T-dependent antigens even though they



**FIG. 2.9** Immunoglobulin (IgG, IgM, and IgA) levels in the fetus and infant in the first year of life. The IgG of the fetus and newborn infant solely is of maternal origin. The maternal IgG disappears by 9 months of age, by which time endogenous synthesis of IgG by the infant is well established. The IgM and IgA of the neonate are synthesized entirely endogenously because maternal IgM and IgA do not cross the placenta. (From Braun J, Stiehm ER. The B-lymphocyte system. In: Stiehm ER, editor. *Immunologic Disorders in Infants and Children*. 4th ed. Philadelphia: WB Saunders; 1996:67.)

may produce less antibody to some antigens than do older infants and adults.<sup>11,155,165,209,506,512</sup>

The newborn infant's response to T-independent antigens, such as polysaccharides, is poor.<sup>237</sup> The antibody response to most such antigens, including the polysaccharide capsules of group B streptococci, pneumococci, and *H. influenzae* type b, is not mature until 18 to 24 months of age.<sup>510</sup> In contrast, in the first few weeks of life, infants mount excellent antibody responses to T-independent polysaccharide antigens that have been rendered T-dependent by covalent conjugation of the polysaccharide to a protein carrier, as noted earlier.<sup>75</sup>

The response of premature infants to most routine childhood vaccines by 2 months of age, including diphtheria, tetanus, pertussis, and oral and inactivated polio, is comparable to that of 2-month-old term infants.<sup>7,65,116,512</sup> However, premature infants may not respond as well to hepatitis B vaccine for reasons that are unclear.<sup>137,330</sup>

### Complement

Complement proteins do not cross the placenta, but there is evidence for fetal synthesis of complement beginning as early as 5.5 weeks' gestation, and most complement proteins are present by 10 weeks' gestation.<sup>135,311</sup> Levels of complement activity and of individual complement components vary significantly among infants, but, in general, classical pathway hemolytic activity of term neonates ranges from 60% to 90% of normal adult values.<sup>187,594</sup> Alternative pathway hemolytic activity is decreased to approximately 50% to 70% of normal adult values at term.<sup>5,184,409,502</sup> Complement activity usually is lower in premature than term infants.<sup>409,502</sup>

Hemolytic activity of both the classical and alternative pathways rises rapidly and reaches adult levels by 3 to 6 months of age and by approximately 6 to 18 months of age, respectively. In addition to hemolytic activity, complement-mediated opsonic and bactericidal activity is decreased in newborn sera and generally correlates with C3 and factor B levels.<sup>187</sup> Studies of opsonic and bactericidal activity of newborn sera have been reviewed in detail elsewhere.<sup>187,289</sup> Levels of individual complement proteins do not always correlate with their functional activity.<sup>229,277</sup> Zach and Hostetter<sup>594</sup> reported not only that

total C3 levels in neonates were decreased but also that C3 thioester reactivity was decreased and that it correlated with gestational age.

### Phagocytes

The newborn infant exhibits both quantitative and qualitative deficits in phagocytic defenses. Although the number of circulating PMNs usually does not differ greatly from that in older children and adults, under conditions of stress, including systemic infection, the availability of marrow reserves of PMNs is impaired markedly.<sup>125</sup> Whereas the ratio of marrow neutrophil reserves to circulating cells in older persons is nearly 15:1, in the newborn infant this ratio is more often between 2:1 and 3:1.<sup>125</sup> Thus, neutropenia is more likely during severe systemic infections in the newborn than in older children and adults.<sup>125</sup> Distinct from this quantitative deficiency in marrow reserves of PMNs, functional impairments of PMNs also are important in understanding neonatal phagocytic defenses.

The most important and best-documented functional impairments of neonatal PMNs are related to defective adhesion and migration.<sup>3,4,23–25,27,317,367,375,376</sup> Specific structural, functional, and biochemical abnormalities have been documented, any or all of which may contribute to the overall impairment in adhesion and migration of these cells.<sup>367</sup> Impaired adhesion of neonatal PMNs to endothelial cells and other biologic substrates has been linked with deficiencies in the expression or function of the  $\beta_2$  integrins Mac-1 (CD11b/CD18) and LFA-1 (CD11a/CD18).<sup>3,26,95,295,367</sup> Perhaps the best documented of these is the diminished level of surface expression of Mac-1 on activated neonatal PMNs, although expression on resting PMNs is similar to that of adults.<sup>95,295</sup> The total cell content of Mac-1 in PMN at the time of birth is related directly to gestational age, and cell lysates of PMNs from very early premature infants (less than 30 weeks' gestation) have been found to contain less than 20% of the Mac-1 content of an equal number of adult PMNs, increasing to about 60% by term.<sup>367</sup> The PMN content of LFA-1, which is normal at term, appears to be reduced in infants born before 35 weeks' gestation.<sup>367</sup> In addition to reduced integrin expression, reduced adhesive function of the  $\beta_2$  integrin molecules themselves at the surface of activated PMN has been documented.<sup>26</sup> Several other

defects of neonatal relative to adult PMNs that might influence chemotaxis have been documented. These include defective redistribution of surface adhesion sites,<sup>24</sup> impaired uropod formation during stimulated shape change,<sup>25</sup> reduced cell deformability,<sup>293</sup> impaired microtubule assembly,<sup>25</sup> deficient F-actin polymerization,<sup>252,479</sup> reduced lactoferrin content and release,<sup>23</sup> reduced ability to effect membrane depolarization and intracellular calcium ion flux,<sup>478</sup> and impaired uptake of glucose during stimulation by chemoattractants.<sup>4</sup>

Evidence suggests that the number and binding efficiencies of neonatal PMN receptors for chemoattractants are normal.<sup>24,478,532</sup> In some studies in which assay conditions are designed to expose a potential defect (e.g., limiting concentrations of opsonins and high bacterial inocula), defects in phagocytosis and killing have been demonstrated.<sup>376,377</sup>

### Primary and Heritable Immunologic Deficiencies

The infant or toddler who experiences even six to eight presumed viral upper respiratory tract infections during the course of a winter season, without other complications, ordinarily would not be considered likely to have an immunodeficiency. In contrast, a child who had experienced several episodes of acute otitis media in the previous 4 months, perhaps some accompanied by sinusitis or pneumonia, has displayed reasonable cause to suspect a humoral immunodeficiency.<sup>140</sup> For certain organisms, infection in the healthy host is so decidedly uncommon that even a single episode should prompt a high suspicion of impaired host defenses. *Pneumocystis jiroveci* pneumonia strongly suggests a severe defect of T-cell number or function.<sup>140</sup> Similarly, lymphadenitis or osteomyelitis caused by gram-negative enteric bacilli suggests a defect of phagocytic killing, such as chronic granulomatous disease.<sup>291,448</sup>

The International Union of Immunological Societies, through an expert committee on primary immunodeficiency diseases, periodically publishes an updated classification of all known primary immunodeficiency disorders based on phenotypic features.<sup>85</sup> The following discussion of specific immunologic defects, their genetic basis (if known), and their infectious consequences focuses on well-characterized prototypic disorders within most major classes of defects but also will address other related disorders.

#### Antibody Deficiencies

Humoral immunity is provided by specific antibody and plays an important role in host defense against most pathogens, as is illustrated by the finding that patients with significant antibody deficiencies develop recurrent and sometimes life-threatening infections.<sup>139,147</sup> They characteristically are prone to recurrent otitis media, sinusitis, pneumonia, and, less often, sepsis and meningitis.

**X-linked agammaglobulinemia.** X-linked agammaglobulinemia (XLA), first described by Bruton, is a primary immunodeficiency disorder of the B-cell lineage and is the most serious disorder of humoral immunity.<sup>98,335,468</sup> It is characterized by absent or severely decreased numbers of circulating B lymphocytes and absent or extremely low levels of all classes of circulating immunoglobulins. It is caused by several different mutations in the gene encoding for a B-cell-specific tyrosine kinase, *Btk*, which maps to the long arm of the X chromosome at Xq22.<sup>554,564</sup> This abnormality in kinase activity results in an arrest in the development of B cells, usually at the pre-B stage, and thus few B cells or their progeny (e.g., plasma cells) are in the circulation or lymphoid tissues.<sup>239</sup>

Most persons with XLA develop chronic or recurrent pyogenic bacterial respiratory or gastrointestinal tract infections, and some may have recurrent skin infections.<sup>98,335</sup> Sepsis and serious focal infections resulting from bacteremia do not occur as frequently but are more common and more severe than in normal hosts. The causative agents of most of these infections are *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis*, but *Staphylococcus aureus* and *Pseudomonas aeruginosa*, as well as other gram-negative organisms, may be implicated. The most troublesome gastrointestinal tract infections in XLA are caused by *Salmonella*, *Campylobacter*, and chronic infestation with *G. lamblia*. These patients have been found to have unusually severe or chronic enterovirus infections that can be manifested by chronic arthritis, meningoencephalitis, dermatomyositis, hepatitis, or a combination thereof, and several patients with XLA have developed vaccine-related paralytic poliomyelitis after receiving the live oral polio vaccine.<sup>335</sup>

The only typical abnormality on physical examination in XLA that is not related directly to infections is the absence or a paucity of normal B-cell-containing lymphoid tissues, such as tonsils, adenoids, and peripheral lymph nodes.

The diagnosis of XLA can be confirmed by studying lymphocyte markers and demonstrating a lack of circulating cells that stain for surface immunoglobulin or with B-cell-specific monoclonal antibodies against CD19, CD20, or both. The number and function of T lymphocytes are normal in XLA. It may be difficult to establish the diagnosis based on immunoglobulin levels in the newborn period because of the presence of maternally derived IgG. However, if suspected, the diagnosis can be made in the newborn period by documenting a paucity of circulating B cells by flow cytometry.

Although individuals with XLA ordinarily are thought of as having a “pure” B-cell disorder, recent evidence reveals that the absence of B cells in XLA is associated with a contracted T-cell receptor repertoire and that mice that lack B cells are unable to prime CD4<sup>+</sup> T cells for their effector function in clearing *Pneumocystis* infection, findings that are consistent with the important role of B cells in presenting antigen to CD4<sup>+</sup> T cells.<sup>420,451</sup>

In contrast to carriers of some other X-linked diseases examined later (see discussion of chronic granulomatous disease), circulating B lymphocytes of XLA carriers express only one population of B cells, those with the normal allele on the X chromosome, presumably because B cells with the mutant allele are at a selective disadvantage and do not develop. Advances in genetic techniques have enabled detection of maternal carriers of XLA.<sup>139</sup> Prenatal diagnosis can be made by genetic studies of amniotic fluid cells or quantitation of fetal circulating B cells.<sup>139</sup>

The prognosis for patients with XLA has improved markedly with earlier diagnosis, high-dose intravenous immunoglobulin (IVIG) therapy, and aggressive use of antibiotics. Before the availability of IVIG, most patients who survived to the third decade of life had chronic lung disease from recurrent pulmonary infections and hearing loss from recurrent otitis media.<sup>335</sup>

**IgG subclass deficiency.** Persons with IgG subclass deficiencies have levels of one or more IgG subclass that are more than two standard deviations below normal for age, normal to slightly decreased total IgG, normal levels of other immunoglobulin isotypes, and, often, a poor antibody response to certain antigens.\*

Patients with IgG subclass deficiency who also have IgM and IgA deficiency may have another immunodeficiency disorder such as common variable immunodeficiency (CVID).

The most common kinds of infections in patients with any clinically significant IgG subclass deficiency include otitis media, sinusitis, and pneumonia. Ordinarily, these patients do not have life-threatening systemic infections.

Deficiency of IgG1 is likely to be associated with subnormal levels of total IgG because this subclass accounts for about 60% of total IgG, and it often is associated with other subclass deficiencies.<sup>16,17,492,498–500</sup>

IgG2 deficiency usually is associated with normal total serum IgG levels and is more likely to be clinically significant if accompanied by IgG4 or IgA deficiency. Patients with IgG2 deficiency typically have poor antibody responses to polysaccharide antigens but normal responses to protein antigens. Like most patients with deficiencies of humoral immunity, their infections primarily are due to encapsulated bacteria and are localized to the respiratory tract.<sup>498,500</sup>

IgG3 deficiency has been associated with low total levels of serum IgG and recurrent respiratory infections, which also may lead to chronic pulmonary disease.<sup>415</sup>

IgG4 deficiency is difficult to diagnose because many normal persons have low serum levels of IgG4, and most normal infants have no detectable IgG4.<sup>416</sup> IgG4 deficiency appears to be of clinical significance, however, if it is associated with IgG2 and IgA deficiency.

The treatment for children with IgG subclass deficiency typically is individualized according to the frequency and severity of symptoms. Noninvasive infections usually can be treated successfully with appropriate antibiotics. Patients with more severe presentations may benefit from

\*References 260–262, 386, 416, 425, 426, 491, 492, 497–501.



regular IVIG therapy, but those who also are completely IgA-deficient should be treated only with IgA-depleted IVIG preparations.

**IgA deficiency.** IgA deficiency is the most common immunodeficiency, occurring as frequently as 1/400. This disorder appears to occur sporadically, but familial cases have been described.<sup>22,147</sup> Most of the functions of serum IgA can be performed by IgG and IgM.<sup>147,416</sup> Thus, although deficiencies of secretory IgA may lead to recurrent respiratory or gastrointestinal tract infections, deficiency of serum IgA alone usually is not associated with increased susceptibility to systemic infections.<sup>22</sup> IgA deficiency has been associated with many other conditions, including recurrent infections, IgG2 deficiency, autoimmune disorders, and malignancy.<sup>21</sup> Recurrent infections are most likely to occur in the subset of IgA-deficient patients who also have IgG2 deficiency.<sup>147,425</sup> The infections usually are relatively mild and involve the upper respiratory and gastrointestinal tracts. Chronic gastrointestinal tract disease in these patients can be caused by *G. lamblia* infestations, nodular lymphoid hyperplasia, lactose intolerance, malabsorption, or inflammatory bowel disease. Other autoimmune diseases associated with IgA deficiency include rheumatoid arthritis, systemic lupus erythematosus, thyroiditis, myasthenia gravis, and vitiligo.<sup>147</sup> About 20% of IgA-deficient patients have allergy, and many have elevated levels of IgE.<sup>147</sup> Food allergy is common and may be the result of abnormal processing of antigen at mucosal surfaces.

Rare patients with serum IgA levels less than 5 mg/dL who receive transfusions may make antibody against donor IgA and have severe reactions when transfused again. IVIG reactions also may occur because IVIG preparations contain varying amounts of IgA. IgA-depleted preparations are available and usually are well tolerated.<sup>150</sup>

**Transient hypogammaglobulinemia of infancy.** The syndrome of transient hypogammaglobulinemia of infancy can be differentiated from the physiologic hypogammaglobulinemia in infants because immunoglobulin levels of normal infants begin to rise by about 6 months of age, whereas those of infants with transient hypogammaglobulinemia of infancy do not begin to increase until between 18 and 36 months of age.<sup>416</sup> Infants suspected of having this syndrome should be evaluated for XLA and CVID (see later discussion) and followed closely until their immunoglobulin levels normalize for age.

**Antibody deficiency with normal or elevated levels of immunoglobulins.** Some persons with normal levels of all circulating immunoglobulin isotypes are at increased risk for infections similar to those seen in specific deficiencies of immunoglobulin levels described earlier.<sup>16,18,416</sup> As in other forms of humoral immunodeficiency, the most common infections in these patients are recurrent bacterial respiratory tract infections, although a few patients have developed pneumococcal sepsis.<sup>18</sup> Such persons can be identified by their inability to make antibody in response to stimulation with specific antigens. A good way to test for this disorder is to immunize with protein antigens, such as tetanus and diphtheria toxoids, and with polysaccharide antigens, such as pneumococcal and *H. influenzae* type b capsular polysaccharide vaccines. Patients who can respond to protein but not to polysaccharide antigens usually will respond to protein-polysaccharide conjugates. Treatment with IVIG may help prevent recurrent infections in these patients, although their normal overall levels of immunoglobulin can pose difficulties in determining the appropriate doses of IVIG and intervals between infusions.

### Defects of Cell-Mediated Immunity: DiGeorge Syndrome

The prototypic pure T-cell defect, DiGeorge syndrome, is characterized clinically by congenital heart disease (usually involving the aortic arch), hypocalcemic tetany, unusual facial features, and recurrent infections.<sup>173</sup> The classical, or complete, form of this disorder has absence or hypoplasia of the thymus and parathyroid glands, cardiac or aortic arch deformities, and a stereotypical constellation of abnormal facial features, most notably micrognathia and hypertelorism, all associated with malformation of the 4th and 5th branchial arches during embryogenesis.<sup>173,318,350,537</sup> Although the condition usually is considered to be associated with immunodeficiency because of the thymic hypoplasia, only about 25% of patients actually exhibit an immunologic defect.<sup>55</sup> The term *partial DiGeorge syndrome* sometimes has been used to describe patients with the typical constellation of anatomic findings but without

immunodeficiency or similar patients with mild immunologic impairment.<sup>273</sup> Some sources designate this disorder as an “anomaly” or “sequence” rather than a syndrome because of confusion about its relationship to 22q11 deletion syndrome (del22q11) or the more recently defined microdeletion, del22p11.2, a deletion also associated with velocardiofacial syndrome.<sup>97</sup> Robin and Shprintzen<sup>459</sup> hold that the findings in DiGeorge sequence, although often associated with del22q11.2, are etiologically heterogeneous and have been associated with other chromosomal deletions such as del10p and del17p or del10q13. Moreover, some individuals with del22p11.2 exhibit abnormalities quite distinct from those of the DiGeorge sequence.<sup>243,244,273,326,437,459</sup> One candidate gene, *TBX1*, encoding a T-box transcription factor and located in 22q.11.2, has been a recent focus of research into the underlying genetic defect in DiGeorge syndrome. Although mice with mutations in *TBX1* exhibit some features consistent with DiGeorge syndrome, data remain insufficient to confirm the precise role of *TBX1* in human patients with this disorder.<sup>249</sup>

Because of the serious nature of the cardiovascular defect, many patients with DiGeorge syndrome in earlier decades have not survived long enough for the immune defect to become a clinical problem.<sup>273</sup> However, with improvements in surgical treatment of the heart defects, more of these infants now survive long enough to display manifestations of the immunodeficiency that results in an increased frequency or severity of viral and fungal infections, as well as *Pneumocystis* pneumonia. In such patients, management often has included prophylaxis against *Pneumocystis*, avoidance of live virus vaccines, and, because antibody production is poor as a result of lack of T-cell help, periodic IVIG infusions.<sup>273</sup> HLA-matched bone marrow transplantation has been successful in some cases.<sup>83,238,273</sup> Earlier work with transplantation of fetal or postnatal thymic tissue provided some long-term success in correcting the immunologic defect.<sup>238,273</sup> Recently, a highly promising large series of cases of transplantation with postnatal cultured thymic tissue in patients with complete DiGeorge syndrome yielded immune reconstitution with 73% survival at 2 years.<sup>361</sup>

### Combined Defects of Cellular and Humoral Immunity

**Severe combined immunodeficiency disease.** Severe combined immunodeficiency (SCID) describes a heterogeneous group of heritable immunodeficiencies that involve serious impairments of both cellular and humoral immunity, thus leading to recurrent severe infections by a wide range of viral, bacterial, and fungal organisms. SCID has multiple forms, which have been reviewed in greater detail elsewhere.<sup>102,103,123</sup> At this writing, at least 10 genes have been identified with abnormalities known to result in SCID. X-linked SCID, the most common form, is due to a mutation in the common  $\gamma$  chain of the receptor for IL-2 and several other cytokines ( $\gamma_c$ ).<sup>103,339</sup> The other known forms of SCID are either known or presumed to be autosomal recessive. These include a deficiency of adenosine deaminase, a purine salvage pathway enzyme; a deficiency in Janus kinase 3 (Jak3), a cytokine receptor signaling molecule; and a defect in the  $\alpha$  chain of the IL-7 receptor, IL-7R $\alpha$ .<sup>103,297,555</sup> Mutation of one of at least six different genes whose products play a role in T-cell receptor or immunoglobulin gene recombination or T-cell receptor signaling, including RAG1, RAG2, Artemis, DNA ligase IV, CD3- $\delta$ , and CD3- $\epsilon$ , also results in SCID.<sup>103,123,191,210,297,356,457</sup> Additionally, SCID is caused by a mutation in CD45, a phosphatase that regulates signaling thresholds in immune cells.<sup>297</sup> Flow cytometry analysis of lymphocyte markers reveals very low to absent B- and T-cell numbers in patients with most forms of SCID.

Long-term management of patients with SCID involves modalities employed in both B- and T-cell disorders, including prophylaxis against *P. jiroveci* pneumonia, avoidance of live viral vaccines, and immunoglobulin replacement therapy.<sup>103</sup> Bone marrow transplantation from HLA-matched siblings has corrected the defect successfully in some cases and is considered the current treatment of choice.<sup>101</sup> Adenosine deaminase deficiency is of historical interest in that it is the first heritable disorder for which gene therapy was attempted, although early success was limited.<sup>101</sup> Approaches using retroviral-based gene therapy for X-linked SCID initially appeared to be successful. However, at least three patients developed lymphoproliferative disorders similar to lymphocytic leukemia, with malignant cells demonstrating insertion



of the vector into the promoter or first intron of a proto-oncogene, *LMO2*.<sup>79,102,118,211,446</sup>

Because the earliest possible treatment with stem cell transplantation often is the key to meaningful survival in patients with SCID, newborn screening programs for SCID based on assays of bloodspots have been introduced in several states and in some countries. These assays are based on quantitation of T-cell receptor excision circles—circular fragments of DNA that are a by-product of T-cell receptor rearrangement during fetal development.<sup>46,121,445</sup>

**Common variable immunodeficiency.** CVID is a heterogeneous group of combined immunodeficiencies that differ from most other primary immunodeficiencies in that they often present in the second or third decade of life, although they may present at any age.<sup>146,148</sup> Patients with CVID characteristically have normal or only modestly decreased numbers of circulating B cells; low, but not absent, levels of IgG, IgM, and IgA; poor responsiveness to antigens; and abnormal T-lymphocyte function.<sup>149</sup>

Although both T- and B-cell abnormalities often can be demonstrated, the clinical presentation usually is most like that in patients with humoral or B-cell defects (i.e., recurrent bacterial otitis media and sinopulmonary infections).<sup>146,259,468</sup> Occasionally, these patients also have infections with organisms more common in persons with T-lymphocyte abnormalities, such as *Pneumocystis*, recurrent herpes simplex virus, and herpes zoster virus infections. Chronic gastrointestinal problems may be due to *G. lamblia* or other intestinal pathogens. CVID patients are prone to nodular lymphoid hyperplasia, autoimmune diseases, and malignancies.

Several gene mutations have been found in patients with CVID. These include the genes encoding for CD19, transmembrane activator and CAML interactor (TACI), receptor for B-cell activating factor of the TNF receptor family (BAFF-R), and inducible costimulatory molecule (ICOS).<sup>114,487,596</sup>

Patients with CVID usually can benefit from therapy with IVIG, which reduces the incidence of acute infections. However, most patients with CVID, even some of those who undergo long-term treatment with IVIG, develop chronic sinopulmonary disease.<sup>148,424</sup>

**Hyper-immunoglobulin M syndrome.** Immunoglobulin deficiency with increased IgM is characterized by low levels of IgG, IgA, and IgE but normal to increased levels of IgM in the circulation and normal numbers of circulating B cells.<sup>12,37,157</sup> The disorder is caused by an intrinsic T-cell abnormality that impairs class switching from IgM to other isotypes. The basis for the various genetic forms of this defect is in one of several possible defects that involve interactions between CD40 on B cells and CD40 ligand (CD40L) on T cells. B cells from patients with the hyper-IgM syndrome only make IgM antibody, and their B cells only express surface IgM and IgD. The originally described and most common form of the defect is X-linked recessive and results from a mutation in the gene encoding CD40L, a protein expressed transiently on activated T cells.<sup>12,37,157</sup> More recently described autosomal recessive forms of this disorder involve mutations either in CD40 itself<sup>57,207</sup> or in a CD40-activated RNA editing enzyme, activation-induced cytidine deaminase.<sup>455</sup> Other cells express surface CD40, thus neutropenia and the increased incidence of infections caused by *Pneumocystis* and of malignancies in patients with hyper-IgM syndrome also may be the result of impaired cell interactions via CD40.

Clinically, hyper-IgM syndrome is manifested by recurrent bacterial infections, especially of the respiratory tract.<sup>157</sup> Such persons are susceptible to the same kinds of recurrent pyogenic infections associated with other immunoglobulin deficiencies, as well as to infections with organisms more commonly encountered in patients with T-cell defects (e.g., *P. jiroveci*).<sup>49</sup> Some patients with this syndrome have recurrent diarrhea as a result of *G. lamblia* and *Cryptosporidium* infection that is severe enough to require parenteral nutrition. About half of patients have persistent or recurrent neutropenia. Those with autoantibodies may have thrombocytopenia, hemolytic anemia, nephritis, hypothyroidism, or arthritis.

The diagnosis of X-linked hyper-IgM syndrome may be established by using immunofluorescence to document absent expression of CD40L on activated T cells, absent CD40 expression on B cells, or demonstrating a mutation in one of the genes encoding CD40, CD40L, or the enzyme activation-induced cytidine deaminase.

Treatment of patients with the hyper-IgM syndrome with IVIG usually results in significant clinical benefit.<sup>49</sup>

**Wiskott-Aldrich syndrome.** Wiskott-Aldrich syndrome is a rare X-linked disorder characterized by thrombocytopenia, small platelets, eczema, recurrent infections, autoimmune disease, and hematologic malignancy. Although both T- and B-cell compartments are affected, it presents phenotypically more like a B-cell or humoral deficiency, with recurrent otitis media, sinusitis, and pneumonia. The defect involves a mutation in the gene encoding a signaling molecule, Wiskott-Aldrich syndrome protein, which regulates “immune synapse” formation and IL-2 production.<sup>414,415</sup>

**Ataxia-telangiectasia.** Ataxia-telangiectasia is a rare autosomal recessive disorder characterized by progressive cerebellar dysfunction with ataxia, oculocutaneous telangiectasias, recurrent bacterial respiratory infections such as those seen in humoral deficiencies, and a predilection to hematologic malignancy and breast cancer. Serum immunoglobulins often are low, as are lymphocyte counts. The disorder is due to a mutation in the *ATM* gene, located on chromosome 11. This gene encodes a serine-threonine kinase that is important in cell cycle regulation and double-stranded DNA repair, and it influences expression of *BRCA1* genes associated with breast cancer. The risk for breast cancer in patients with ataxia-telangiectasia is increased 15- to 20-fold over that in the general population. Heterozygotes for missense mutations of the *ATM* gene can be affected because copies of the abnormal protein can interfere with function of the wild-type protein.<sup>368,410</sup>

### Defects of the Interferon-Gamma (IFN- $\gamma$ ) and Interleukin-12 (IL-12) Pathways

Macrophages infected by intracellular pathogens, especially *Mycobacterium* or *Salmonella* spp., are stimulated via a TLR4-dependent mechanism to release IL-12, along with IL-18, IL-23, and IL-27. These cytokines stimulate T and NK cells to produce IFN- $\gamma$  via interactions with cellular receptors for the aforementioned cytokines. The released IFN- $\gamma$ , in turn, further stimulates the macrophage to release more IL-12 and to activate killing. The mechanism by which this intracellular killing occurs is unknown. This cycle of mutual activation is essential for normal defense against mycobacterial pathogens, and some genetic defects of these cytokines, their cellular receptors, or related molecules critical for receptor-mediated signaling have been associated with increased susceptibility to mycobacterial infections. Deficiencies in this system have resulted from mutations in either of the two receptors for IFN- $\gamma$ , IFN- $\gamma$ R1 and IFN- $\gamma$ R2, as well as mutations in STAT1, a molecule critical for transducing signals from both IFN- $\gamma$  receptors. Mutations also have been described in the 40-kDa subunit of IL-12, IL-12p40, and the IL-12 receptor, IL-12R $\beta$ 1. These disorders are rather uncommon, all involving fewer than 100 known patients.<sup>423,470</sup>

Mutation of either of the IFN- $\gamma$ Rs is associated with increased risk for mycobacterial infections. Deficiencies of IFN- $\gamma$ R1 may be either autosomal recessive or dominant and either complete or partial. Most recessive defects are complete and result in absent IFN- $\gamma$  responsiveness. Dominant IFN- $\gamma$ R1 deficiency results from heterozygous truncations of the cytoplasmic domain of the receptor with excessive accumulation of nonfunctional receptors at the cell surface. Patients with the recessive complete form of this deficiency have a much more severe clinical phenotype than those with the dominant partial form, although the latter have a fivefold greater frequency of nontuberculous mycobacterial osteomyelitis. Defects in IFN- $\gamma$ R2, much less common, also may be recessive or dominant in inheritance and either complete or partial. Rare deficiencies in the receptor signaling molecule STAT1 have led to increased mycobacterial infections in a partial deficiency or, in two patients with a recessive complete form, to postvaccination disseminated bacille Calmette-Guérin (BCG) disease followed later by death from severe viral infections. The latter probably relates to the additional role of STAT1 in development of IFN- $\alpha/\beta$ -mediated antiviral activity.<sup>470</sup>

Deficiencies of IL-12p40 or its receptor IL-12R $\beta$ 1 are associated with disseminated nontuberculous mycobacterial infections, tuberculosis, and *Salmonella* infections. The receptor deficiency results in unresponsiveness to IL-12. This defect is apparently autosomal recessive with variable clinical penetrance. Deficiency of IL-12p40 also is variable in its clinical phenotype and has resulted in deaths from severe mycobacterial infections.<sup>427,470,525</sup>

The approach to specific diagnosis of defects of the IFN- $\gamma$  pathway has been well systematized, but such studies should be undertaken only in a highly specialized reference laboratory.<sup>470</sup>

### Complement Deficiencies

Approximately 0.03% of the general population have complement deficiencies resulting from acquired or congenital abnormalities of single or multiple complement components or regulatory proteins. Excellent reviews of complement deficiencies are available elsewhere.<sup>166,167,169,176,265,289,471,580</sup>

Congenital or hereditary deficiencies of complement more often are manifested by abnormality or complete absence of a single complement protein, and most of these have been well documented to predispose to potentially life-threatening infections. Most primary complement abnormalities (C1q dysfunction and C1rs, C4, C2, C3, C5, C6, C7, C8, and C9 deficiencies) are inherited as autosomal codominant traits.<sup>166</sup>

Patients with homozygous or heterozygous deficiency of the early classical pathway proteins C1, C2, and C4 are more prone to develop autoimmune disease than difficulty with infections. However, approximately 20% of patients with homozygous deficiency of early components have problems with recurrent or severe infections that are similar to those seen in C3 deficiency.<sup>166,208,471</sup> Their predilection for autoimmune disease probably is due, at least in part, to abnormal solubilization and removal of immune complexes. C2 deficiency has been associated with antibody deficiencies in individuals with recurrent infections.<sup>15,113</sup>

Deficiencies of alternative pathway proteins predispose to serious, often fatal, infections because of the lack of ability to respond promptly to organisms not previously encountered.<sup>167</sup> Properdin deficiency, the only X-linked complement deficiency, has been associated with fulminant, usually fatal, meningococcal infection.<sup>167</sup> Factor D deficiency is rare and appears to predispose to recurrent neisserial infection.<sup>309</sup>

Mutations or variants in the gene for MBL, the initiator of the MBL pathway (see earlier discussion), have been associated with an increased risk for recurrent infections.<sup>176,265</sup> In particular, homozygosity for such mutations or variants was found to be associated with an increased risk for systemic meningococcal disease.<sup>214</sup>

Because all three complement activation pathways converge at the activation of C3, patients who are deficient in C3 are unable to mobilize any of the three main effector functions of complement in host defense—opsonization, phagocyte recruitment, or bacteriolysis. Thus it is not surprising that the most serious complement deficiency state is the rare total absence of C3.<sup>166,208,471</sup> Patients with deficiencies or mutations of factors H and I have low but detectable levels of C3 because absence of either of these regulatory factors allows continuous activation of the alternative pathway and uncontrolled C3 consumption. Patients with C3 deficiency caused by any of these mechanisms have increased susceptibility to infections caused by encapsulated bacteria such as *S. pneumoniae*, *N. meningitidis*, and *H. influenzae* type b. Most of these infections involve the respiratory tract (otitis, sinusitis, bronchitis, and pneumonia), but C3-deficient patients also are predisposed to sepsis and meningitis.<sup>166,208,471</sup> In addition, some C3-deficient persons develop autoimmune diseases.<sup>166,208,471</sup>

Deficiencies of terminal complement proteins C5, C6, C7, and C8 greatly increase the risk for developing systemic infections with *N. meningitidis* or *Neisseria gonorrhoeae*.<sup>72</sup> C9 deficiency increases the risk for infection to a lesser degree than do deficiencies of other terminal components. In one study, the risk for meningococcal disease was increased 5000-fold in C7-deficient persons and about 700-fold in C9-deficient persons.<sup>396</sup>

The risk for infection is higher in patients with C5 deficiency than in those with deficiencies of other terminal proteins because, in addition to the role of C5 in initiating assembly of the membrane attack complex, the free C5 fragment, C5a, is important for leukocyte recruitment to sites of microbial invasion.<sup>15,53</sup>

At least one episode of meningococcal disease occurs in approximately 60% of persons who have been identified as having C5, C6, C7, C8, or properdin deficiency, and 75% to 85% of documented bacterial infections in complement-deficient persons are meningococcal.<sup>166,208,471</sup> Conversely, approximately 14% of patients presenting with sporadic meningococcal disease have a defect in one of the late complement components, and

this percentage rises to about one third among individuals with two or more meningococcal infections. The mortality due to meningococcal infection in such patients is lower than in normal persons, probably because patients with these deficiencies often have antibodies to meningococci that can activate the classical pathway leading to normal opsonization and phagocyte activation, which are effector functions upstream in the cascade from the membrane attack complex. In contrast, individuals with normal complement levels who develop meningococcal infection usually do so because they do not have specific antibodies with which to mobilize any effector functions via the classical pathway, and, as noted earlier, meningococci are poor activators of the alternative pathway.

Currently no specific treatment exists for patients with hereditary complement deficiencies. Replacement of missing complement proteins is not practical because of the short half-life of most of the components.<sup>52,329,474</sup> Immunization of complement-deficient patients and their close household contacts against encapsulated organisms, especially *N. meningitidis*, is important.

### Disorders of Phagocyte Function

**General features of phagocyte disorders.** The most frequently encountered reminder of the importance of an adequate supply of well-functioning phagocytes comes from patients who develop chemotherapy-associated neutropenia and are thus at high risk for bacterial and fungal infections.<sup>442</sup> The qualitative disorders of phagocyte function discussed in this section result in similar susceptibilities to these infections, either because the circulating cells are unable to migrate to an infected site or because, once having migrated to the infected tissue, they are unable to effect normal microbicidal activity. There is some overlap among the types of infectious complications associated with disorders of migration versus killing. However, as a rule, defects of neutrophil migration tend to be associated with infections at skin, subcutaneous tissue, and mucous membrane sites. In contrast, killing defects are more likely to result in infections of deeper soft tissues and internal organs, although skin infections are not uncommon.

#### Intrinsic disorders of cell migration

**Type 1 leukocyte adhesion deficiency.** In the late 1970s and the first half of the ensuing decade, several reports described patients with recurrent bacterial infections, diminished neutrophil motility, and delayed separation of the umbilical cord.<sup>1,28–30,34,145,213,257</sup> The neutrophils of these patients were discovered to be markedly deficient in adherence to both natural and artificial surfaces, response to complement-opsonized particles, and expression of members of a family of heterodimeric glycoproteins: LFA-1, Mac-1, and p150,95, each defined by its own unique  $\alpha$  subunit, CD11a, CD11b, and CD11c, respectively, but sharing a common 95-kDa  $\beta$  subunit designated CD18.<sup>29,30,34,35,523</sup> A fourth  $\alpha$  subunit, CD11d, whose importance remains poorly understood, has been described more recently. The defective expression of these proteins, also called the  $\beta_2$  leukocyte integrins, appeared to be directly responsible for the striking adherence-dependent defects that characterized the function of leukocytes from patients with this disorder.<sup>28–30,34,35,523</sup> Various called Mac-1 deficiency, MO1 deficiency, LFA-1 deficiency, CD11/CD18 deficiency, or CR3 deficiency, this disorder, now usually called type 1 leukocyte adhesion deficiency (LAD-1), is an autosomal recessive disorder with one of numerous mutations in the  $\beta_2$  integrin subunit, CD18, localized to chromosome 21.<sup>30,35,523,524</sup> It has been identified in more than 150 persons worldwide and encompasses a broad ethnic diversity.<sup>30,35</sup> Patients may exhibit a moderate or severe phenotype, depending on the extent of the defect in protein expression.<sup>29,30</sup> The documented mutations of the  $\beta_2$  subunit (CD18) range from complete absence of the protein to extensions of the molecule, truncations of the extracellular portion or of the cytoplasmic domain of the molecule, small deletions, and point mutations.<sup>31,35,269</sup>

Patients with LAD-1 develop recurrent necrotic skin and soft tissue infections with poor or absent pus formation, and they exhibit poor wound healing.<sup>20,29</sup> They develop severe periodontitis, often losing their primary and secondary dentition along with alveolar bone.<sup>29,30,558</sup> They may develop enterocolitis much like that seen in neutropenic patients.<sup>26,29</sup> Delayed separation of the umbilical cord, presumably resulting from an impaired inflammatory response, is a common feature of the more

severe phenotype of this disorder,<sup>29,30</sup> but this finding alone in infants without infectious complications or other characteristic features is of doubtful significance.<sup>586</sup> Pronounced leukocytosis is a common feature of LAD-1, even in the absence of active infection.<sup>29,30</sup> Recent studies in CD18-null LAD-1 mice reveal abnormally high circulating granulocyte colony-stimulating factor (G-CSF) levels and suggest the absence of a negative feedback mechanism on G-CSF production that occurs during normal transendothelial migration of leukocytes and involves IL-17. Absent ongoing transendothelial migration results in failure of this putative feedback mechanism, resulting in elevated G-CSF levels and higher circulating granulocyte counts.<sup>216</sup>

Functional studies of neutrophils from patients with LAD-1 reveal a marked impairment of all adherence-dependent functions that require the  $\beta_2$  integrins.<sup>28–30,107,227,269</sup> PMNs and NK cells from patients with LAD-1 exhibit impaired ADCC for virus-infected target cells, suggesting that CD11/CD18-mediated cell-cell adhesion is essential for normal killing of virus-infected cells by this mechanism<sup>310</sup> and that the increased severity of viral infections in a few of the most severely affected patients could be related to defective ADCC. Currently the diagnosis of LAD usually is made by demonstrating absent or markedly deficient expression of the CD11/CD18 family of glycoproteins on circulating leukocytes by immunofluorescence flow cytometry.<sup>28–30,35</sup>

Careful attention to skin and oral hygiene, aggressive management of infections, and meticulous local care of wound sites are important in the care of patients with LAD-1 or any serious disorder of neutrophil migration. The efficacy of prophylactic antibiotics has not been well established. Bone marrow transplantation with HLA-matched allogeneic marrow has had mixed results, from complete correction of the phagocytic defect to death 9 months after transplantation from graft-versus-host disease.<sup>30,213</sup> The human CD18 gene has been cloned and sequenced, and human LAD-1 cells have been corrected successfully in vitro with the normal CD18 complementary DNA carried by retrovirus vectors, hinting at the future promise of gene therapy for patients with LAD-1.<sup>26,30,316</sup>

**Type 2 leukocyte adhesion deficiency.** In 1992, two unrelated patients were reported, both products of consanguineous matings, who exhibited clinical characteristics virtually identical to those described for LAD-1.<sup>219</sup> However, expression of the  $\beta_2$  (CD18) integrins on leukocytes was normal. In addition to defects in neutrophil motility, these children exhibited short stature, psychomotor retardation, and the Bombay (hh) erythrocyte phenotype (homozygous for absence of the H antigen). Phagocytosis by PMNs was normal. Recently it has been documented that this defect is due to one or more mutations of a specific guanosine diphosphate (GDP)-fucose transporter,<sup>354</sup> resulting in the absence of fucosyl residues on sialyl Lewis X, the tetrasaccharide moiety that serves as the principal ligand for members of the selectin family of adhesion molecules.<sup>219,328,567</sup> In vivo and in vitro studies comparing the adhesive functions of PMNs from LAD-1 and this new disorder, now called LAD-2, provided elegant validation of the distinct roles of selectins and integrins in the recruitment of leukocytes in vivo, with the initial selectin-mediated “rolling” stage (deficient in LAD-2) required first for the second integrin-mediated “firm adhesion and extravasation” stage (deficient in LAD-1) to occur (see Fig. 2.2).<sup>567</sup> The other somatic and neurologic features of LAD-2 may be related to more widespread consequences of the generalized defect in fucosylation of glycoproteins.<sup>354</sup>

**Type 3 leukocyte adhesion deficiency (integrin activation defect).** During the past decade, several patients have been reported who have a clinical phenotype that includes features of both type 1 LAD and Glanzmann thrombasthenia, a bleeding disorder associated with mutations in the  $\alpha_{IIb}\beta_3$  integrin on platelets. Laboratory studies of these patients revealed markedly deficient integrin-mediated adhesive functions of both leukocytes and platelets despite normal surface expression of both leukocyte and platelet integrins. Further studies led to the conclusion that this defect in integrin function was the result of defective “inside-out” signaling pathways that normally lead to integrin activation.<sup>196,305,366</sup> Recent data on several kindreds with this disorder, studied in different laboratories, confirmed the presence of mutations in the gene encoding Kindlin-3, a molecule that, during cell activation, forms a critical bridge between the actin cytoskeleton and the cytoplasmic domain of the  $\beta$

subunit of multiple classes of integrins expressed by cells of hematopoietic lineages.<sup>266,358,389,535</sup> This bridging by Kindlin-3 is essential for normal integrin activation in both leukocytes and platelets.

**Specific granule deficiency.** Rare patients with hereditary specific granule deficiency have been reported, beginning with Spitznagel’s original description in 1972.<sup>86,222,521</sup> These persons exhibited recurrent and severe infections, primarily of the skin and mucous membranes, sometimes involving the lung and, in one patient, the mastoid. Neutrophils from patients with this disorder exhibit absent specific granules on Wright-stained blood smears. Lactoferrin released from specific granules reduces the negative surface charge of the plasma membrane, contributing to nonspecific adhesiveness of the cell.<sup>222</sup> The specific granule membrane also contains some of the intracellular store of the important adhesion molecule Mac-1 (CD11b/CD18) that is mobilized to the plasma membrane upon cell activation.<sup>60,81</sup> Recurrent skin and mucous membrane infections resulting from *S. aureus*, gram-negative bacilli, and *Candida* spp. characterize the natural history of patients with this disorder.<sup>86,222,521</sup> Neutrophils in this disorder also exhibit diminished microbicidal activity, presumably because of diminished amounts of the cytochrome component of NADPH oxidase that normally reside in the membrane of specific granules. In this rare disorder, males and females are represented equally. A few patients with specific granule deficiency have a deletion in the gene encoding the myeloid cell transcription factor known as CCAAT/enhancer binding protein epsilon (*C/EBP $\epsilon$* ) with absent expression of this transcription factor, although not all patients with this disorder have a mutation of this gene.<sup>174,338</sup>

**Chédiak-Higashi syndrome.** Chédiak-Higashi syndrome is a complex, rare autosomal recessive disorder characterized by partial oculocutaneous albinism, recurrent pyogenic infections, peripheral neuropathy, and neutropenia.<sup>77</sup> The illness also may involve an accelerated lymphoproliferative phase.<sup>77</sup> Granular cells, including neutrophils, contain giant lysosomal granules that are the apparent result of spontaneous intracellular fusion of azurophilic granules and, to a lesser extent, specific granules.<sup>77</sup> Corresponding disorders of intracellular pigment granules and vesicle trafficking in axons account for the albinism and other manifestations of this disease.<sup>77</sup> The genetic basis of the defect is now known to involve either a mutation in the gene encoding a large protein called the *lysosomal trafficking regulator* (*LYST*), homologous to the “beige” gene in mice, with all mutations studied so far resulting in a truncated protein.<sup>51,119</sup> Patients with Chédiak-Higashi syndrome develop recurrent skin and mucosal infections, most often caused by *S. aureus*.<sup>30,77</sup> A cell migration defect appears to be related to abnormal regulation of microtubule polymerization upon cell activation.<sup>77</sup> The possible role of intracellular levels of cyclic adenosine monophosphate and guanylic acid in this microtubule abnormality has been suggested.<sup>89</sup> Studies of two brothers with Chédiak-Higashi syndrome demonstrated abnormally increased tyrosinylation of the  $\alpha$  subunit of tubulin.<sup>77,399</sup> The diagnosis of Chédiak-Higashi syndrome usually is suspected clinically on the basis of partial oculocutaneous albinism and recurrent pyogenic infections. A Wright stain demonstrating giant lysosomal granules and laboratory studies showing defective cell migration are confirmatory, and genetic confirmation is now possible.

**Neutrophil actin dysfunction.** Filamentous actin constitutes the main contractile mechanism of neutrophils for migration and phagocytosis.<sup>530</sup> An extremely rare and apparently heterogeneous disorder, neutrophil actin dysfunction has been characterized by recurrent skin infections caused by *S. aureus* and *Candida albicans*. In vivo and in vitro studies revealed severely impaired neutrophil chemotaxis and phagocytosis.<sup>87</sup> The capacity for polymerization of actin from cell extracts also was diminished markedly. It is of interest that PMNs from family members of this patient also were found to be variably deficient in the CD11/CD18 family of glycoproteins that are the basis of LAD-1.<sup>518</sup> One similarly affected infant was found to have abnormally high levels of a 47-kDa protein, now identified as *lymphocyte-specific protein-1* (*LSP-1*), which exhibits actin-binding activity.<sup>132</sup>

**Glycogen storage disease type 1B.** Beaudet and colleagues<sup>57</sup> first reported the association of recurrent infection, neutropenia, and impaired neutrophil migration with glycogen storage disease type 1B, a metabolic disorder characterized by defective microsomal transport of glucose-6-phosphate. In 1985, Ambruso and coworkers<sup>19</sup> reviewed



the features of 21 patients with glycogen storage disease type 1B, 15 of whom suffered from frequent infections, especially of the skin and subcutaneous tissues. Impaired neutrophil motility was found in 8 of 11 patients in whom this was evaluated. A specific relationship between the underlying metabolic defect in glycogen storage disease type 1B and the mechanism of impaired cell motility has not been established. However, exogenous glucose is an important energy source for chemotaxis,<sup>577</sup> and it is interesting to note that the uptake of glucose by PMNs in response to chemoattractant stimulation is impaired in glycogen storage disease type 1B, as well as in neonates, both examples of patients with impaired PMN migration.<sup>4,53</sup>

#### **Extrinsic or secondary defects of polymorphonuclear leukocyte migration**

**Defective neutrophil chemotaxis associated with serum inhibitors of cell function.** Investigators have reported the presence of inhibitors of PMN chemotaxis in the serum of patients with recurrent infection.<sup>315,507,517,559,573</sup> In each case, the patient's neutrophils exhibited diminished chemotaxis in the presence of autologous serum or plasma, whereas identical assays in the presence of control serum or plasma resulted in a normal chemotactic response. Most such inhibitors appear to be immunoglobulins or immunoglobulin-like molecules.

**Hyper-immunoglobulin E syndrome.** In 1966, Davis and colleagues<sup>160</sup> described two young girls with coarse facial features, reddish hair, fair skin, severe eczema, dystrophic nails, "cold" staphylococcal skin abscesses, and recurrent sinopulmonary infections. The term *Job syndrome* was suggested, referring to the similar biblical affliction. Additional patients were described with a similar disorder, first associated by Buckley and associates<sup>105</sup> with very high serum IgE levels, including a patient who exhibited a defect in neutrophil chemotaxis reported in 1973 by Clark and associates.<sup>131</sup> Features common to all of the patients with the disease now termed *hyper-IgE syndrome* include a history of staphylococcal infections of the skin and sinopulmonary tract beginning in infancy or early childhood and serum levels of IgE that are greater than 2000 IU/mL.<sup>100,105,179</sup> Based on extensive reviews, other characteristic but variable features of this disorder include coarse facies, cold abscesses of the skin and subcutaneous tissues, a chronic eczematoid rash, eosinophilia, and mucocutaneous candidiasis.<sup>100,179,217</sup> Consistent abnormalities of cell-mediated immune functions in patients with hyper-IgE syndrome suggest that the pathogenic basis involves a defect of T-cell regulation.<sup>104,126,179,230</sup> An extensive study of 19 kindreds revealed autosomal dominant inheritance with a genetic locus for hyper-IgE syndrome on chromosome 4, in the proximal 4q region.<sup>246,247</sup> In 2007, it ultimately was determined that this disorder can be attributed to dominant negative mutations in STAT3, a factor critical for signal transduction by at least 10 different cytokines, some with proinflammatory and others with antiinflammatory functions.<sup>272,381,519</sup> The immunologic defect is characterized further by a paucity of T<sub>H</sub>17 lymphocytes, which normally promote protective leukocyte responses to bacteria and fungi.<sup>382</sup> Recently a rare and clinically distinct autosomal recessive form of hyper-IgE syndrome has been described, resulting from a mutation in *DOCK8*, a member of a family of atypical guanine nucleoside exchange factors highly expressed in lymphocytes.<sup>533</sup> Patients with this form of hyper-IgE syndrome have developed chronic viral infections, severe allergies, and early-onset malignancies.<sup>79,246,519,533</sup>

Although hyper-IgE syndrome might more properly belong in discussions of defective T-cell regulation, some patients with hyper-IgE syndrome may have a defect in neutrophil chemotaxis.<sup>179</sup> The defect sometimes has been intermittent, and, in several cases, the presence of a serum inhibitor of chemotaxis has been recognized.<sup>178,179</sup> Recent data suggest that keratinocytes and other epithelial cells from patients with hyper-IgE syndrome may produce reduced amounts of neutrophil-attracting chemokines, and neutrophils of these patients exhibit reduced expression of chemoattractant receptors.<sup>382,383</sup>

**Other secondary or poorly defined disorders of polymorphonuclear leukocyte migration.** Patients with protein-calorie malnutrition have defective PMN chemotaxis that appears to be based on systemic preactivation of circulating cells resulting from chronic low-level endotoxemia from impaired intestinal mucosal integrity.<sup>122,484</sup> Shwachman-Diamond syndrome, in addition to pancreatic insufficiency, neutropenia, and growth retardation, also is associated with defective PMN migration.<sup>10</sup>

Two kindreds with congenital ichthyosis and an associated defect of PMN migration have been described.<sup>380</sup> Patients with severe thermal injuries develop an acquired form of specific granule deficiency with impaired PMN migration beginning about 14 days after injury.<sup>222</sup> Several reports have been published of a poorly defined disorder of neutrophil migration referred to as *lazy leukocyte syndrome*,<sup>9,378,379</sup> marked by recurrent staphylococcal skin infections, rhinitis, gingivitis, stomatitis, neutropenia despite adequate marrow precursors, and diminished in vivo and in vitro migration of neutrophils.

**Defects in phagocyte microbicidal activity.** As described earlier, the broad array of available phagocyte microbicidal mechanisms may be divided into oxygen-dependent and oxygen-independent mechanisms. To date, no specific deficiency of any oxygen-independent microbicidal mechanism has been described. Thus this section is concerned mainly with the known deficiencies of oxygen-dependent microbicidal mechanisms of phagocytes, especially chronic granulomatous disease, the prototypical defect in this group. PMNs, monocytes, and the fixed phagocytes of the reticuloendothelial system generally share in the deficient microbicidal activity observed.

**Chronic granulomatous disease.** Chronic granulomatous disease (CGD) was one of the earliest syndromes of phagocyte dysfunction to be characterized.<sup>448</sup> It is recognized now to be a family of biochemically and genetically heterogeneous disorders of distinct components of the phagocyte NADPH oxidase complex (see Fig. 2.3 and related text)<sup>122,174</sup> that result in the inability of phagocytes to generate superoxide anion and other reactive oxygen species.<sup>174</sup> Organisms that produce catalase pose a special problem for patients with this disease.<sup>174,180,291,333</sup> This encompasses a broad range of pathogens, including staphylococci, gram-negative enteric bacteria, *Pseudomonas* spp., yeast, fungi, *Nocardia* spp., and numerous other pathogenic species.<sup>180,289,333,588</sup> Most microorganisms produce H<sub>2</sub>O<sub>2</sub>, which might be used, even by the CGD phagocyte, as an effective microbicidal weapon because it feeds into the sequence of oxidant reactions downstream from the defective oxidase enzyme (see Fig. 2.3).<sup>465</sup> Organisms that produce catalase are able to survive within these deficient cells because catalase is an enzyme that degrades H<sub>2</sub>O<sub>2</sub> to oxygen and water.<sup>291,466</sup> Infections with catalase-negative bacteria, such as *Streptococcus*, *Haemophilus*, and *Neisseria* spp., do not occur with increased frequency in CGD patients,<sup>588</sup> and these organisms are killed normally in vitro by CGD phagocytes. Phagocyte functions not directly related to oxidative mechanisms of intracellular killing, including adherence, chemotaxis, phagocytosis, and degranulation, usually are normal.<sup>42,381,448,515</sup>

The genetic defect in CGD may be inherited by either X-linked recessive or autosomal recessive mechanisms.<sup>83,130,151</sup> In the report of a registry of 368 patients with CGD in the United States,<sup>588</sup> more than two-thirds of the patients had the X-linked recessive form with absent gp91<sup>phox</sup>, the larger subunit of the cytochrome b<sub>558</sub>; about 12% had an autosomal recessive form with absent cytosolic p47<sup>phox</sup>; and fewer than 5% each had autosomal recessive disease with absent cytosolic p67<sup>phox</sup> or absence of p22<sup>phox</sup>, the smaller subunit of the cytochrome b<sub>558</sub>. Approximately 12% had an unknown genetic form of the disease. A single individual with an autosomal recessive form of CGD due to mutations in the cytosolic p40<sup>phox</sup> has been reported.<sup>364</sup> About 5% of patients with CGD have normal levels of an abnormal protein that is inactive, and at least 410 different mutations have been reported to result in CGD.<sup>264</sup> These genetically diverse defects all result in defective function of the oxidase and the characteristic CGD phenotype. In the female obligate carriers of X-linked CGD, the proportion of cells that express the defect usually is between 35% and 65%, depending on the proportion of cells in which random inactivation of the normal versus the affected X chromosome occurs.<sup>355,588</sup> Overall, patients with X-linked disease have more severe courses and experience higher yearly death rates than patients with autosomal recessive forms of the disease.<sup>588</sup>

Patients with CGD experience recurrent serious bacterial and fungal infections, usually beginning in the first few months of life. *S. aureus* and gram-negative bacilli, especially *Serratia marcescens* and *Burkholderia cepacia*, are the most common causes of infection in patients with CGD. Fungi, especially *Aspergillus* spp., also are prominent etiologic agents,<sup>333,453,588</sup> and infections caused by *Aspergillus* are the most common cause of death in these patients.<sup>588</sup> Granuloma formation at infected



sites is one of the histologic hallmarks of this disorder.<sup>290,453</sup> Pulmonary infections and their complications have been the reported cause of death in up to 50% of these patients in some series, and *Aspergillus* predominates.<sup>588</sup> These infections often are protracted and respond slowly to appropriate antibiotic therapy.<sup>291,453</sup> Progression to lung abscess, empyema, or both occurs in about 20% of patients with CGD with pneumonia.<sup>291</sup> Liver abscesses occur in about half of patients and may be recurrent.<sup>59,127</sup> The hepatosplenomegaly common in CGD may result from these infections but more likely results from chronic infections at various sites with systemic lymphoid hyperplasia.<sup>45,291</sup> Osteomyelitis occurs in about one third of patients.<sup>291,453,588</sup> In contrast to normal children, in whom this infection usually involves the metaphyseal area of long bones, patients with CGD more often develop infections of the small bones of the hands and feet. In normal children, *S. aureus* is the most common etiologic agent and causes a significant proportion of cases in CGD. However, gram-negative bacilli and *Aspergillus* appear to be the predominant etiologies, and other agents, including *Nocardia*, also may be important etiologic agents of bone infection in CGD.<sup>437,588</sup> Skin infections in this disorder may include pyoderma, purulent dermatitis, and cutaneous or subcutaneous abscesses and often are preceded by a chronic eczematoid skin rash.<sup>453</sup>

Although localized infections are the rule in patients with CGD, these patients also may develop septicemia.<sup>291,453,588</sup> The most common cause of septicemia in most series has been *Salmonella*, but other gram-negative enteric bacilli also have been prominent.<sup>290,333</sup> Of note, *S. aureus* is a proportionally less common cause of septicemia in these patients.<sup>453,588</sup>

Granuloma formation adjacent to hollow viscera in patients with CGD can produce clinically significant obstruction, including obstruction of the gastric outlet, esophagus, small intestine, and ureters. This complication usually responds to treatment with corticosteroids.<sup>20,180,588</sup>

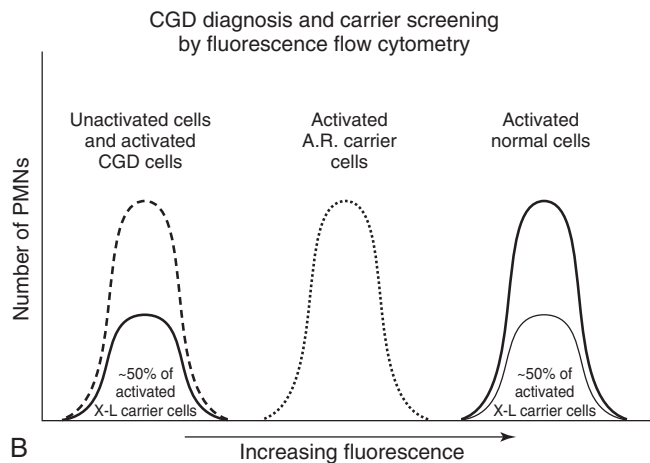
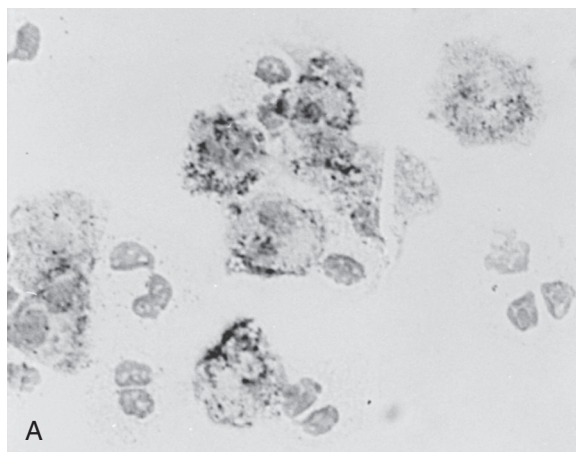
CGD should be suspected in patients with a history of recurrent indolent infections caused by catalase-positive organisms such as those

described earlier, especially if granulomas are found in biopsy specimens of lymph nodes or other tissues. Confirmation of the diagnosis usually rests on the demonstration of an absent or nearly absent oxidative metabolic burst in the patient's phagocytes. This can be detected classically by the slide NBT test or by various other measurements of oxidative burst activity, most recently by flow cytometry of PMNs loaded with oxidant-sensitive fluorescent dyes.<sup>13,40,54,465,548</sup> Fig. 2.10A is an example of the slide NBT test in an X-linked carrier; Fig. 2.10B is an idealized set of flow cytometry histograms with typical patterns for a normal control, a patient with CGD, and both X-linked and autosomal recessive carriers. Prenatal diagnosis has been achieved by the use of the slide NBT test with blood from placental vessels obtained at fetoscopy.<sup>401</sup>

The management of patients with CGD includes antibiotic prophylaxis, usually with trimethoprim-sulfamethoxazole, and an aggressive approach to the specific diagnosis and treatment of acute infections.<sup>453</sup> Antifungal prophylaxis with itraconazole or voriconazole that has activity against *Aspergillus* also has become standard. Bone marrow transplantation met with early limited success,<sup>241,298,581</sup> but allogeneic peripheral blood stem cell transplantation from HLA-identical sibling donors, with prior myeloablative conditioning, has been considerably more promising, as suggested by the European experience from 1985 to 2000, with 22 of 23 patients "cured" at median follow-up of 12 years.<sup>495</sup>

A multicenter study reported that daily subcutaneous injections of IFN- $\gamma$  reduced the requirement for hospitalization of CGD patients for serious infections by about two-thirds.<sup>283</sup> The mechanism by which IFN- $\gamma$  exerts its beneficial effect in CGD has not been determined. Despite some systemic side effects, such as fever, fatigue, and myalgia,<sup>283</sup> it generally has been well tolerated,<sup>360</sup> and it continues to be used in the management of many patients with this disorder.<sup>588</sup>

**Deficiencies of glucose-6-phosphate dehydrogenase, glutathione peroxidase, and glutathione synthetase.** The normal activity of the NADPH oxidase enzyme complex depends on the continued availability of NADPH to reduce molecular oxygen to form superoxide anion.<sup>129,465</sup> The primary source of NADPH for this enzyme is the hexose



**FIG. 2.10** (A) Photomicrograph of a slide nitroblue tetrazolium (NBT) test of polymorphonuclear leukocytes (PMNs) isolated from the blood of a maternal carrier of X-linked recessive chronic granulomatous disease (CGD). Because of random inactivation of either the normal or the affected X chromosome in maternal carriers of this disorder, approximately half of the PMNs exhibit the granular blue-black staining characteristic of the oxidative reduction of NBT by normal PMNs. In contrast, the remaining PMNs, which express the reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase defect of CGD, are visible only by their nuclear counterstain. (B) Flow cytometry in CGD diagnosis and carrier testing. These idealized flow cytometry histograms demonstrate the oxidative burst activity of populations of PMNs that have been loaded with an agent such as dihydrorhodamine that becomes fluorescent when it reacts with products formed during the oxidative burst. The taller curve on the far left represents the fluorescence typical of both inactivated normal PMNs and activated PMNs from a patient with CGD with an absent oxidative burst. The taller histogram on the far right demonstrates the increased level of fluorescence exhibited by activated normal PMNs. The two smaller curves on the left and the right represent the two populations of activated PMNs from a typical maternal carrier of X-linked CGD, indicating about 50% normal PMNs and 50% expressing the defect. The taller histogram in the middle represents typical results for the cells of a carrier of autosomal recessive CGD, with all cells exhibiting an intermediate level of fluorescence.

monophosphate shunt, and this pathway is provided with the hexose substrate, 6-phosphoglucose, by the enzyme glucose-6-phosphate dehydrogenase (G6PD), which also generates NADPH in a coupled reaction.<sup>466</sup> The reactions of the hexose monophosphate shunt itself are coupled to two other enzymes, glutathione reductase and glutathione peroxidase, which recycle oxidized and reduced glutathione.<sup>465</sup> A deficiency in any of these three enzymes results in a lack of available NADPH to drive the NADPH oxidase and may result in a phagocyte killing defect similar to CGD.<sup>41,477</sup> G6PD deficiency usually involves erythrocytes and is associated with hemolytic anemia, especially in conjunction with the administration of sulfonamides.<sup>142</sup> Only when the defect also involves myeloid cells and is severe or complete (<5% of normal enzyme levels) is the CGD-like disorder manifested.<sup>41,477</sup> A partial deficiency of glutathione reductase has been reported, presenting with hemolytic anemia and early cataracts, but no increased incidence of infection was noted.<sup>464</sup>

The synthesis of an adequate supply of glutathione also is critical to these cells. Two brothers with glutathione synthetase deficiency who presented with neutropenia, hemolytic anemia, acidosis, 5-oxyprolinuria, and recurrent infection were reported by Spielberg and colleagues<sup>520</sup> and Boxer and associates.<sup>88</sup> The PMNs from these patients exhibited elevated cytosolic hydrogen peroxide levels, diminished oxidative microbicidal activity, and impaired microtubule assembly. Antioxidant therapy with vitamin E normalized the *in vitro* abnormalities of the patients' PMNs, with no further difficulty with recurrent infections.<sup>88</sup>

**Myeloperoxidase deficiency.** Congenital deficiency of neutrophil myeloperoxidase has come to be recognized as the single most common heritable disorder of neutrophil function, occurring in about 1/2000 persons.<sup>400,435</sup> The precise mode of inheritance of this defect has not been established, but myeloperoxidase is known to be a product of a single gene on chromosome 17.<sup>560</sup> Although PMNs from some patients with myeloperoxidase deficiency have been found to exhibit delayed killing of *C. albicans* and *S. aureus*, myeloperoxidase deficiency rarely has been associated with unusual infectious complications.

## Important Examples of Secondary Immunodeficiency (Excluding Human Immunodeficiency Virus Infection)

### Asplenia

Fulminant infections can occur in patients who have anatomic or functional asplenia.<sup>78</sup> The mortality rate from these infections in asplenic persons ranges from 40% to 80%.<sup>78,587</sup> The most common pathogens are encapsulated bacteria, including *S. pneumoniae* (50–70%), *H. influenzae*, and *N. meningitidis*.<sup>587</sup> Other streptococci, *S. aureus*, *Salmonella*, and other gram-negative bacilli are important but less common pathogens. Malaria and babesiosis also are more severe in asplenic persons. Infections can occur at any time but are most common within the first 2 years after splenectomy.

Both the liver and the spleen are important in phagocytic clearance of bacteria from the circulation, and the spleen is an important site for antibody production. Young children who become asplenic are much more susceptible to fulminant infection than are adults because adults are more likely to have encountered antigens before splenectomy than are children. Persons whose indication for splenectomy is thalassemia or Hodgkin disease are at higher risk for dying of overwhelming infection than are those who develop functional asplenia from sickle-cell disease. Patients who undergo spleen removal for spherocytosis or idiopathic thrombocytopenia have a lower risk for infection. The lowest risk group consists of individuals whose spleens are removed surgically after trauma.<sup>78,138</sup>

Congenital asplenia usually is associated with complex congenital cardiac disease and occasionally with structural abnormalities of the gastrointestinal or genitourinary tracts. Thus asplenia should be suspected in any patient with congenital heart disease and sepsis resulting from encapsulated organisms. Anatomic or functional asplenia also should be suspected in patients whose erythrocytes are found to contain Howell-Jolly bodies because these typically are removed from the circulation by the normally functioning spleen. Ultrasound or radionuclide liver and spleen scan usually can confirm asplenia or significant hyposplenia.<sup>78</sup>

Asplenic persons are managed using prophylactic antibiotics, usually penicillin V or amoxicillin, until at least 5 years of age.<sup>138</sup> They also should be immunized against encapsulated organisms at the appropriate ages (e.g., *H. influenzae* type b and pneumococcal conjugate vaccines and meningococcal conjugate vaccine beginning at 2 months), and heightened attention to clinical evaluation and treatment of febrile episodes is recommended.<sup>138,473</sup> Patients should be warned about the increased risk for serious infections from malaria and babesiosis.

### Sickle-Cell Disease

Immunodeficiency in sickle-cell disease patients is due in large part to their functional asplenia.<sup>484</sup> Part of the risk for infection stems from local infarction and tissue necrosis resulting from sickling, which causes sludging and resultant tissue hypoxia. The reticuloendothelial system also may be obstructed by having to deal with chronic hemolysis. Patients with sickle-cell disease experience a high incidence of sepsis and meningitis caused by encapsulated organisms (e.g., *S. pneumoniae*, *H. influenzae* type b, *N. meningitidis*) and *Salmonella*.<sup>215</sup> *Salmonella* infections often are associated with osteomyelitis.<sup>193</sup>

Patients with sickle-cell disease appear to have normal antibody responses to most antigens, including age-appropriate responses to vaccines, and they usually have normal complement function.<sup>289</sup>

Patients with sickle-cell disease should be managed with prophylactic antibiotics until at least 5 years of age and should be immunized against *H. influenzae* type b, pneumococci, and meningococci at the appropriate ages.<sup>138</sup>

### Cystic Fibrosis

Cystic fibrosis (CF) is an autosomal recessive disorder caused by mutations in both alleles of the gene encoding the protein called cystic fibrosis transmembrane conductance regulator (CFTR), a cyclic AMP-regulated chloride channel expressed at the luminal surface of airway epithelial cells.<sup>579</sup> Most patients with CF develop chronic endobronchial infection with *P. aeruginosa* of the mucoid phenotype. This infection is accompanied by an intense chronic airway inflammation with an exuberant influx of neutrophils that leads to bronchiectasis and destruction and fibrosis of lung and airway tissue and early death.<sup>124,312</sup> The airway inflammatory milieu, including neutrophil-derived proteases such as elastase, contributes to secondary impairments in opsonic and phagocytic host defenses by cleaving opsonic antibody and complement fragments, as well as important phagocytic receptors for these opsonins.<sup>550</sup> Mucociliary clearance of bacteria from the CF airway is impaired because of viscous, dehydrated epithelial lining fluid, and large quantities of DNA from dying neutrophils impair clearance of secretions by further enhancing their viscosity. The unresolved question of the relationship between the cystic fibrosis genetic defect and the pathogenesis of *Pseudomonas* chronic lung infection continues to stimulate active research.<sup>124,441,480,511</sup> Diagnosis of CF is classically made by documenting elevated levels of sweat chloride, but genetic diagnosis is now undertaken more frequently. Management of patients typically includes nutritional support, pancreatic enzyme replacement, frequent aggressive chest physiotherapy to mobilize secretions, intermittent courses of aerosolized tobramycin, aggressive management of pulmonary exacerbations, and recombinant aerosolized DNase to help liquefy secretions. Gene therapy has been explored but has not resulted in correction of the defect at the clinical level. Recently a novel and promising trial of pharmacotherapy targeted the basic defect in CF. The drug ivacaftor, an orally administered potentiator of CFTR chloride channel function in CF patients with at least one G551D-CFTR mutation, resulted in sustained improvement of lung function through 48 weeks of treatment; ivacaftor is now licensed for CF patients with this mutation.<sup>190</sup>

### Ciliary Dyskinesia

Primary ciliary dyskinesia is a rare disorder of deranged ciliary motility that predisposes patients to recurrent respiratory infections such as otitis media, sinusitis, bronchitis, and pneumonia as a result of poor clearance of secretions.<sup>529</sup> It is due to a mutation in one of several genes encoding for a component of either the dynein arms or radial spokes of the cilia that provide the energy, movement, and structural integrity of the specific tubulin structures that make up the normal cilia of

respiratory epithelium.<sup>529</sup> Approximately 50% of these patients may have dextrocardia and situs inversus. In the absence of these features, this disorder should be suspected in patients who have recurrent infections, as noted earlier, but who have undergone a comprehensive evaluation of humoral immunity that has not detected an abnormality. Diagnosis ordinarily requires a brush biopsy of nasal epithelial cells, best evaluated by transmission electron microscopy, which usually will reveal an abnormality of the inner or outer dynein arm or of the radial spokes of the cilia.<sup>503,529</sup> Patients in whom this diagnosis is suspected should be referred to clinicians with expertise at centers with the capability to perform the necessary diagnostic studies. Long-term management can be challenging and often involves antimicrobial prophylaxis and attempts to assist in mobilization of secretions. Many of these patients develop bronchiectasis as a result of recurrent and chronic airway and lung infection. There is no specific treatment for the ciliary dysfunction.<sup>529</sup>

## EVALUATION FOR IMMUNODEFICIENCY IN THE CHILD WITH RECURRENT OR SEVERE INFECTIONS

Most immunodeficiency disorders can be diagnosed readily by employing a methodical process that begins with careful analysis of the child's presenting history and physical examination.<sup>140,147,167,484,486</sup> This information serves as the foundation, when indicated, for a rational laboratory evaluation. It should be borne in mind that it is normal for children to have several infections every year. It is not unusual for normal children who are exposed to other children, particularly older school-aged siblings or classmates, to develop approximately one infection per month, especially from late fall to early spring, when respiratory viruses are most active. The overwhelming majority of infections in immunocompetent children are characterized by being mild and localized to the gastrointestinal or upper respiratory tracts, and they either are self-limited or respond rapidly to conventional therapy. Immunocompromised hosts tend to have more frequent, severe, and unusual infections that may not respond readily to appropriate therapy.

### History

A detailed history alone often is sufficient to determine whether an immunologic evaluation should be pursued in many children who have recurrent or severe infections. If tests for immunity are indicated, the history also serves as a guide to the types of studies that should be performed initially.

The age of onset of suspicious infections usually helps in defining the underlying problem. For example, children with isolated immunoglobulin deficiencies tend to do well during the first few months of life because they are protected by maternal antibody. They usually start developing serious infections later in the first year of life. Those with cell-mediated or phagocytic disorders may begin developing infections in the newborn period (see earlier discussion). In contrast, healthy children who have been cared for at home by their mothers and who have no siblings often have relatively few infections in the first few years of life but may be brought to the physician for immunologic evaluation when they develop recurrent respiratory tract infections beginning the first few weeks after entering daycare, nursery school, or kindergarten because of new exposure to various respiratory viral pathogens.

The number, nature, etiology, and severity of infections help in determining how aggressively to pursue an immunologic evaluation. Certain clinical presentations of disease and causative organisms are associated with a high likelihood of an immunodeficiency. Antibody or complement deficiencies or functional asplenia should be suspected in children with recurrent or life-threatening infections such as sepsis and meningitis caused by encapsulated organisms (e.g., *S. pneumoniae*, *H. influenzae* type b).<sup>98,167,289,335,467</sup> Complement deficiencies should be considered in persons with recurrent or severe neisserial disease.<sup>167</sup> CGD should be suspected in the presence of recurrent or unusual deep tissue infections, such as liver abscess, lymphadenopathy, pneumonia, or osteomyelitis, especially when caused by unusual gram-negative bacteria, such as *Serratia marcescens*, or by *Aspergillus* species.<sup>588</sup> Bacterial skin infections in patients who have never formed pus at infected sites may

suggest a leukocyte adhesion deficiency. Pneumonia caused by *Pneumocystis* suggests a T-cell deficiency, either hereditary or as a result of HIV infection. In contrast, recurrent or even severe infections with group A streptococcus have not been associated with immunodeficiency, and recurrent urinary tract infections usually are associated with anatomic abnormalities of the urinary tract rather than immunodeficiency.

Documentation of the child's growth pattern is essential because children who are thriving are much less likely to have serious immune disorders than are those with failure to thrive.

The immunization history should be documented carefully because it may prove useful in evaluating the child's ability to mount an antibody response to specific vaccine antigens. The history of recent live viral immunization should be obtained in children who have clinical presentations compatible with polio or measles because infection caused by vaccine strains of these viruses may be the first indication of immunodeficiency in some children.

Children who attend daycare facilities or preschools are constantly being exposed to common infections. Familiarity with community patterns of disease such as prevalent clinical manifestations of enterovirus infection or the beginning of croup, respiratory syncytial virus, influenza, or rotavirus seasons can be used to reassure families of normal children with frequent mild infections.

A detailed family history should be obtained that includes questions about the presence of immunodeficiency, recurrent or severe infections, contributing factors to any early deaths, the gender of affected persons, and consanguinity. A history of recurrent or severe infections in maternal male relatives should be given special attention because of the prominence of X-linked disorders among the primary immunodeficiencies. Auto-immune diseases might suggest a familial disorder of complement or cell-mediated immunity.<sup>167</sup>

### Physical Examination

Physical examination may provide valuable clues to the nature of an immune disorder. In certain cases, such as some patients with the hyper-IgE syndrome<sup>100,268</sup> and the DiGeorge syndrome,<sup>173</sup> the characteristic facies may suggest the diagnosis.

As noted earlier, one of the most obvious signs that a child may have a serious underlying medical problem is failure to thrive. Every immunologic evaluation must include documentation of current growth parameters and comparison with past growth.

Many immunodeficiency disorders have dermatologic manifestations. Eczematoid rashes are seen in patients with the hyper-IgE syndrome and CGD.<sup>100,268</sup> CGD and leukocyte adhesion deficiencies also are characterized by slow wound healing and abnormal scarring patterns.<sup>588</sup> Patients with Chédiak-Higashi syndrome have partial albinism.<sup>77</sup> Severe gingival disease and early loss of teeth are prominent clinical features in disorders of neutrophil migration, such as LAD.<sup>27,33</sup>

The chest should be evaluated for physical signs of active disease, such as rales and rhonchi, as well as evidence of chronic infection, such as an increased anterior-posterior diameter. Pneumonia, bronchitis, bronchiectasis, and scarring can occur with many immunodeficiencies but are associated most frequently with immunoglobulin deficiencies.<sup>98,259</sup>

Cardiac abnormalities may be associated with primary asplenia or immunodeficiency disorders such as DiGeorge syndrome,<sup>173</sup> and dextrocardia with situs inversus should suggest ciliary dyskinesia as a cause of recurrent infections.<sup>140</sup>

Although hepatosplenomegaly may be found in many types of primary immunodeficiency diseases, it is more common in patients with disorders of phagocyte function, especially CGD.<sup>588</sup>

### Laboratory Studies

The laboratory evaluation should be guided by the history and physical findings. Relatively simple and inexpensive screening tests often can help narrow the differential diagnosis and streamline the evaluation. One of the first tests that should be performed is a complete blood count with differential and evaluation of the blood smear. This simple test can detect several immunologic abnormalities, including neutropenia, lymphopenia associated with HIV-1 or forms of SCID, the abnormal neutrophil granules associated with the Chédiak-Higashi syndrome,



Howell-Jolly bodies found with asplenia, and some malignancies. Chest radiographs should be examined for thymic tissue, mediastinal lymphadenopathy, pneumonia, bronchiectasis, and other evidence of chronic pulmonary infections. Consideration also should be given to evaluating patients with chronic pulmonary disease for CF with a sweat chloride test.

In most patients, the specific clinical features of infections and their etiologies provide an optimal guide to choosing the most relevant and specific laboratory tests for evaluating for immunodeficiency when this is indicated. All too frequently, a comprehensive and expensive evaluation of every major aspect of immunity is undertaken for a patient whose clinical presentation suggests a more carefully considered and targeted approach.

Children with a clear history of recurrent bacterial otitis media, sinusitis, or pneumonia are candidates for evaluation of humoral immunity by measuring levels of the major classes of immunoglobulin and IgG subclasses. Enumeration of B cells and marker studies by immunofluorescence flow cytometry should be considered if more severe forms of humoral deficiency, such as XLA or some forms of CVID, are suspected. Very high IgE levels may be helpful in establishing the diagnosis of hyper-IgE syndrome; it should be noted that many laboratories do not include IgE levels in the standard Ig quantitation, and this must be ordered separately. Children who have normal quantitative Ig and IgG subclass levels but who continue to have frequent sinopulmonary infections that do not respond well to appropriate medical and surgical management (e.g., ventilation tubes) also can be evaluated by measuring antibody responses to specific antigens such as tetanus and diphtheria toxoids, *H. influenzae* type b, and meningococcal and pneumococcal capsular polysaccharides. Antibody levels can be measured before immunization and approximately 1 to 2 months after immunization to evaluate the child's ability to respond to different kinds of antigens, including T-cell-dependent antigens, such as diphtheria and tetanus toxoid, or T-cell-independent antigens such as unconjugated pneumococcal capsular polysaccharide vaccine. In a patient with recurrent bacterial respiratory infections in whom no antibody-related abnormality can be identified after a comprehensive evaluation, the possibility of ciliary dyskinesia should be considered and a brush biopsy of nasal epithelial cells performed for electron microscopic studies (see earlier discussion).

The complement system should be evaluated in persons with recurrent or life-threatening neisserial disease, including systemic gonococcal infections and sporadic meningococcal disease. The best screening test for hemolytic complement is the CH<sub>50</sub>. A normal CH<sub>50</sub> reflects a normal quantity and function of classical pathway proteins (C1, C4, C2), C3, and terminal components through C8, as noted earlier.<sup>134</sup> An abnormal CH<sub>50</sub> should be confirmed, being careful that the specimen is handled correctly. If a very low CH<sub>50</sub> is confirmed, it is important to determine both the serum levels of individual complement proteins and their functional activity in consultation with a reference laboratory.

In patients with infections suggesting a T-cell defect, such as *Pneumocystis*, *Cryptococcus*, or recurrent candidiasis, delayed hypersensitivity skin testing with antigens such as *Candida* or mumps may be useful to screen cell-mediated immunity, but results are not always reliable. Lymphocyte subset quantitation may be helpful in diagnosing such conditions as SCID, but more sophisticated testing of lymphocyte function such as mitogen and antigen stimulation should be performed in patients with recurrent or severe fungal infection or in whom CVID is suspected. Such studies ideally should be directed by a clinical immunologist.

Similarly suspected phagocyte function disorders should be evaluated in consultation with experts in phagocyte function because lack of proper standardization and expertise often produces misleading results from commercial laboratories. Phagocyte function studies should be directed toward defects of adhesion and migration in patients with recurrent skin and mucosal infections, poor or absent pus formation, and persistent leukocytosis suggestive of a leukocyte adhesion defect. Tests of oxidative metabolic activity and killing should be performed in patients with recurrent staphylococcal or unusual gram-negative or fungal tissue infections suggestive of CGD.

When clinical suspicion of an inherited immunodeficiency is confirmed by appropriate functional laboratory studies, it may be desirable to arrange for specific genetic testing to determine the precise nature of a patient's genetic mutation or variation when such testing is available. This may assist greatly in subsequent genetic counseling and will add important information to the clinical database on patients with the specific disorder.

### Prevention of Infection

Patients and household members should be immunized with appropriate vaccines as soon and as fully as possible after a diagnosis of a family member with an immunodeficiency.<sup>138</sup> Although many immunodeficient patients, such as those with XLA or SCID, cannot respond normally to vaccines, immunization of household members and other close contacts may reduce the patients' risk for infection. Patients with complement deficiencies, asplenia, and sickle-cell disease should be immunized with vaccines directed against encapsulated organisms, such as meningococci, pneumococci, and *H. influenzae* type b. However, it should be remembered that these persons may not have normal responses to immunization and, therefore, if possible, their antibody responses to these vaccines should be measured; if responses are poor, they may benefit from additional doses of the vaccines. Live viral vaccines should not be administered to most patients with primary defects of cell-mediated immunity.

Patients with disorders characterized by recurrent or severe bacterial or fungal infections may require prophylactic antibacterial or antifungal therapy. Although this is an essential component of management for many of the more severe immunodeficiencies, such as DiGeorge syndrome, SCID, and CGD, the benefit of this approach has not been documented for all immune disorders. For many other disorders the uncertainty of the benefits of long-term antimicrobial prophylaxis must be weighed carefully along with the risk for emergence of antimicrobial resistance.

### Prospects for Correction of Serious Primary Immunodeficiencies

Bone marrow or stem cell transplants have been successful in a few patients with specific immunologic disorders, including SCID,<sup>103,118</sup> Wiskott-Aldrich syndrome,<sup>428</sup> LAD, and CGD.<sup>30,213,581</sup> Current protocols for thymus transplantation are showing great promise in treatment of patients with DiGeorge syndrome.<sup>361</sup> Gene therapy has raised new possibilities for correcting certain immunologic defects, having demonstrated early promise for some forms of SCID and early success in animal models of CGD.<sup>174,446</sup> The development of malignancies associated with retroviral gene therapy in some cases has raised serious concerns about the ultimate feasibility of this approach,<sup>102,446</sup> but the means of overcoming these and other potential obstacles to gene therapy continue to be sought.

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