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TABLE 124-5 Initial Empirical Antibiotic Therapy for Common Infectious Disease Presentationsa CLINICAL SYNDROME COMMON ETIOLOGIES ANTIBIOTIC(S) COMMENTS SEE CHAPTER(S) Skin and soft tissue infection S. aureus, Streptococcus pyogenes Dicloxacillin, 250–500 mg PO qid or Cephalexin, 250–500 mg PO qid or Clindamycin, 300–450 mg PO tid or Nafcillin/oxacillin, 1–2 g q4h aThis table refers to immunocompetent adults with normal renal and hepatic function. All doses listed are for parenteral administration unless indicated otherwise. Local antimicrobial susceptibility profiles may influence the choice of antibiotic. Therapy should be tailored once a specific etiologic agent and its susceptibilities are identified. bTrough levels for vancomycin should be 15–20 µg/mL. cTrough levels for amikacin should be <4 µg/mL. dIn patients with late onset (i.e., after ≥5 days of hospitalization) or risk factors for multidrug-resistant organisms. eTrough levels for gentamicin and tobramycin should be <1 µg/mL. fIf P. aeruginosa is a concern, the dosage may be increased to 3.375 g IV q4h or 4.5 g IV q6h. gData on the efficacy of TMP-SMX in skin and soft tissue infections are limited. hComorbidities include chronic heart, lung, liver, or renal disease; diabetes mellitus; alcoholism; malignancy; or asplenia. Abbreviations: CNS, central nervous system; ICU, intensive care unit; MRSA, methicillin-resistant S. aureus; TMP-SMX, trimethoprim-sulfamethoxazole. ■ ■PERSPECTIVE The study of infectious diseases is really a study of host–microbial inter actions and represents evolution by both the host and the microbe—an endless struggle in which microbes have generally been more creative and adaptive. Given that nearly one-sixth of deaths worldwide are still related to infectious diseases, it is clear that the war against infectious diseases has not been won. For example, a cure for HIV infection is still lacking, there have been only marginal improvements in the methods for detection and treatment of tuberculosis after more than a half century of research, new infectious disease outbreaks (e.g., viral hemorrhagic fevers, SARS-CoV-2, mpox) continue to emerge, and the threat of microbial bioterrorism remains high. The subsequent chapters in Part 5 detail—on both a syndrome and a microbe-by-microbe basis—the current state of medical knowledge about infectious diseases. At their core, all these chapters carry a similar message: Despite numerous advances in the diagnosis, treatment, and prevention of infectious diseases, much work and research are required before anyone can confidently claim we have

achieved “the virtual elimination of infectious disease.” In reality, this goal will never be attained, given the rapid adaptability of microbes. PART 5 Infectious Diseases ■ ■FURTHER READING Baker RE et al: Infectious disease in an era of global change. Nat Rev Microbiol 20:193, 2022. Bartlett JG: Why infectious diseases. Clin Infect Dis 59:S85, 2014. Khabbaz RF et al: Challenges of infectious diseases in the USA. Lancet 384:53, 2014. Maillard A et al: Can chatbot artificial intelligence replace infectious diseases physicians in the management of bloodstream infections? A prospective cohort study. Clin Infect Dis 78:825, 2024. McQuillen DP, MacIntyre AT: The value that infectious disease physicians bring to the healthcare system. J Infect Dis 216:S588, 2017. Rubin EJ et al: Audio interview: Dr. Fauci on infectious disease chal lenges. N Engl J Med 388:e82, 2023. Verghese A et al: Inadequacies of physical examination as a cause of medical errors and adverse events: A collection of vignettes. Am J Med 128:1322, 2015. Jordan B. Jastrab, Marcia B. Goldberg

Molecular Mechanisms of Microbial Pathogenesis Infectious diseases involve intricate interactions among the infecting microbe, human tissue and immune system, and the host microbiome. The co-evolution of humans and microbes has led to the emergence of microbial factors that promote infection and corresponding human

(Continued) If MRSA is a consideration, clindamycin, vancomycin (15 mg/kg q12hb), linezolid (600 mg IV/PO q12h), or TMP-SMX

(1–2 double-strength tablets PO bidg) can be used. 134 and pathogenspecific chapters cellular responses to microbes. Among the microbial factors that promote disease are those that alter human cells, inhibit host immune responses, and respond to the microbiota. The process of infection can be divided into several stages: microbial entry into the human body (colonization), microbial attachment in its favored niche and avoid ance of host defenses (infection), deployment of microbial factors and host inflammatory responses that damage human tissue (disease), and microbial release into the environment, where the microbe can infect others (transmission). It is notable that for most pathogens, the host inflammatory response contributes substantially to symptoms and tissue damage. Moreover, the microbiota (the collection of microbes that reside in and on the human body) modulates, directly or indi rectly, every stage of infection (Chap. 484). This chapter describes the best-understood molecular and cellular mechanisms that contribute to human disease caused by bacterial pathogens. ENTRY INTO THE HUMAN HOST Infectious diseases occur when a live pathogen enters or a toxic patho gen product is ingested by the host. Entry of a live microbe into a host can occur by crossing a tissue barrier, through entry into or penetration of the skin or a mucous membrane of the respiratory, gastrointestinal, or genitourinary tract. If live bacteria spread to the connective tissue surrounding a barrier, an infectious collection may form (e.g., skin or intraabdominal abscess, empyema). Microbial penetration of barrier surfaces may also result in bloodstream infection, which can in turn lead to infection of other organ systems. Infection of the skin and underlying soft tissue (cellulitis) typically occurs when bacteria reach the dermis via a physical disruption of the epidermal barrier. This disruption can result from trauma, fungal infection, pressure ulcers, or venous stasis ulcers. Bacteria that are already associated with the skin are most likely to traverse this barrier defect; therefore, Staphylococcus aureus and group A streptococci are the most common causes of cellulitis. In patients with diabetic foot ulcers, infections with Pseudomonas aeruginosa and anaerobic bacteria are also common. Entry into the respiratory tract occurs via respiratory droplet nuclei (airborne particles 1–5 μm in diameter) or via fomites introduced on a contaminated hand. Infectious droplet nuclei are generated when an

individual with a communicable respiratory infection (e.g., tuberculo sis, influenza, COVID-19 infection) sneezes, coughs, or talks, or when environmental fluid contaminated with a noncommunicable infec tious agent is aerosolized (e.g., Legionnaires' disease, aspergillosis). A cough may generate 3000 particles, whereas a sneeze may generate up to 40,000 particles; large particles may evaporate down to 0.5 µm, and hygroscopic particles can increase in size as they pass through the moist respiratory tree. Entry into the gastrointestinal tract occurs via ingestion of con taminated food or water or via person-to-person contact. Patho gens for which the minimum infectious inoculum is large (e.g., 108 organisms for epidemic spread of Vibrio cholerae, 105 organisms for Salmonella enterica serovar Typhimurium) are generally acquired via

contaminated food or water, whereas pathogens for which the mini mum infectious inoculum is small (e.g., 101 to 102 organisms for Shigella spp.) are typically acquired by person-to-person spread. Entry into the genitourinary tract occurs via colonization of the urethral meatus or vaginal introitus with fecal organisms followed by ascension of the organisms into the bladder or kidneys or via instru mentation. Pyelonephritis can also result from hematogenous seeding of the kidney. ESTABLISHMENT OF INFECTION ■ ■NICHE Live Pathogens Many bacterial pathogens display tissue tropism; sites of infection are often pathogen-specific and restricted, such that adjacent tissues may be uninvolved. For example, group A streptococci cause pharyngitis and soft tissue infection, but rarely pneumonia. Cholera is an infection of the small intestine, whereas Shigella spp. cause disease only in the rectosigmoid colon. To establish infection, the pathogen must access and then survive within its niche (Table 125-1). In the respiratory tree, the site at which pathogens settle is determined by mode of spread: droplet nuclei reach the bronchial tree or alveoli, whereas fomites reach the pharynx or nasal passages. Pathogens move through the gastrointestinal tract via normal intestinal motility. Tissue association and invasion are dictated by bacterial interac tion with host factors, such as glycan-decorated receptors and the extracellular matrix. The environmental conditions of the niche trigger expression of virulence factors required for establishing infection; for example, bile salts in the gut stimulate the expression of V. cholerae adhesins and the germination of Clostridioides difficile spores. Bacteria commonly manipulate their niche environment in ways that facilitate TABLE 125-1 Bacterial Pathogens, Diseases, and Niches MOST COMMON TROPISM BACTERIUM DISEASE Skin, respiratory tract, small intestine Bacillus anthracis Anthrax Respiratory tract Bordetella pertussis Pertussis Systemic Borrelia burgdorferi Lyme disease Systemic Brucella abortus Brucellosis Systemic Burkholderia pseudomallei Melioidosis Eyes, venereal Chlamydia trachomatis Various chlamydioses, including trachoma Colon Clostridioides difficile Colitis Pharynx Corynebacterium diphtheriae Diphtheria Systemic Coxiella burnetii Q fever Colon Enterohemorrhagic Escherichia coli Stomach Helicobacter pylori Gastritis, gastric ulcers Respiratory tract Legionella pneumophila Legionnaires' disease Systemic, central nervous system Listeria monocytogenes Listeriosis Respiratory tract Mycobacterium tuberculosis Tuberculosis Urogenital tract Neisseria gonorrhoeae Gonorrhea Respiratory tract Pseudomonas aeruginosa Systemic Salmonella enterica serovar Typhi Typhoid fever Gastrointestinal tract Salmonella enterica serovar Typhimurium Colon, rectum Shigella spp. Dysentery, shigellosis Multiple sites Staphylococcus aureus Soft tissue Group A Streptococcus Small intestine Vibrio cholerae Cholera Systemic Yersinia pestis Plague

infection. For example, the gastroduodenal pathogen Helicobacter pylori converts urea into ammonia, thereby increasing the pH of the acidic stomach environment, which creates a more hospitable environment.

Preformed Toxins A small number of diseases are caused by ingestion of preformed bacterial toxins. The most common among these are S. aureus enterotoxins and the Bacillus cereus emetic entero toxin. S. aureus enteroxins are strongly emetic and can be present in prepared foods such as dairy, meat, eggs, salads, and produce. B. cereus produces the peptide toxin cereulide, which acts as a potassium ionophore and induces emesis; this toxin is most often found in rice or other starchy food that has been improperly refrigerated. Although these pathogens may be killed when food is cooked, their toxins are heat stable. Less common but important is botulinum toxin, produced by Clostridium botulinum and the cause of botulism. Botulinum toxin blocks neurotransmitter release in motor neurons, inhibiting the cen tral nervous system and resulting in a flaccid paralysis that may be complicated by fatal respiratory failure. Although botulinum toxin is heat-labile, C. botulinum spores are heat-stable. ■ ■ATTACHMENT Attachment of bacteria to host tissue surfaces is a prerequisite for the pathogen's establishment of an infection and is mediated by specific receptor–ligand interactions. The tissue specificity of the host cell surface receptor repertoire is a critical factor in delimiting a patho gen's niche(s). These physical associations additionally facilitate the pathogen's avoidance of host clearance mechanisms (see "Avoidance of Innate Immune Responses," below) and may contribute to formation of biofilms by the pathogen (see "Biofilms," below). Because adhesion to cellular receptors often triggers cellular signal transduction and innate immune signaling, therapeutic blockade of this interaction may in some circumstances exacerbate infection. CHAPTER 125 Adhesins Bacterial pathogens have evolved a wide range of strate gies by which to attach to the diverse host cell structures they encoun ter. For many bacterial pathogens, ligands or adhesins for specific host receptors are known. Adhesins comprise a wide variety of surface structures, including single proteins, carbohydrates, glycoproteins, lip ids, lipoproteins, and multiprotein filamentous complexes that extend several micrometers from the bacterial surface, each anchored in the outer-surface cell envelope. Most bacteria produce multiple adhesins with varying specificity, enabling the pathogen to interact with mul tiple receptors, including those on several distinct cell and tissue types encountered during the process of infection. These interactions are often partially redundant, are serologically variable, and contribute additively or synergistically with other binding interactions. Molecular Mechanisms of Microbial Pathogenesis Common classes of adhesins are pili (also known as fimbriae), flagella, and autotransporter proteins (Table 125-2). Pili are hairlike extensions consisting of a polymer of the major pilin subunit capped with minor pilins that provide the adherence function of the structure. Pili are classified by type and are produced by many gram-negative bac teria and a smaller number of gram-positive bacteria. To date, efforts to prevent infection with pilus-based vaccines have been unsuccessful. Types of pili include type I, type P, and type IV. Type I pili frequently function at mucosal surfaces. For example, they mediate the close association of uropathogenic Escherichia coli (UPEC) with bladder epi thelial cells and the ability of this pathogen to persist, causing relapsing urinary tract infections. UPEC also produces type P pili and afimbrial adhesins. These adhesins bind sugar moieties on host surface glycopro teins, with varied specificity depending on the adhesin. The minor pilin lectins at the tip of the pili generally bind D-mannose glycans, albeit with strain specificity. For example, the type I pilus adhesin FimH of intes tinal E. coli strains often preferentially binds oligomannose, whereas the FimH of UPEC strains commonly binds monomannose. Thus, the same pilus structures in different bacterial strains can dictate adher ence to distinct tissues. Type IV pili (Tfp) are widespread among gramnegative bacteria, and similar structures exist among gram-positive bacteria. These are evolutionarily related to the type II secretion system (T2SS) and, in addition to mediating adherence, allow the uptake of

TABLE 125-2 Classes of Bacterial Adhesion Proteins and Their Host Receptors ADHESIN EXAMPLE RECEPTOR Type I pili Fim protein, uropathogenic Escherichia coli Terminal mannose of uroplakin N-glycan in urinary epithelial cells Type P pili Pap protein, uropathogenic E. coli Galactose disaccharides Type IV pili Tfp protein, Neisseria gonorrhoeae CD64, CR3, I domain– containing integrins MSCRAMM SdrC protein, Staphylococcus aureus β-Neurexin Opa Opa protein, Neisseria meningitidis CEACAMsa Flagellum FliC protein, Pseudomonas aeruginosa Asialo-GM1 ganglioside Autotransporter Invasin, Yersinia pseudotuberculosis β1-Integrins Autotransporter Ag85, Mycobacterium tuberculosis Fibronectin aCarcinoembryonic antigen–related cell adhesion molecules. DNA into bacteria and the motility of bacteria on surfaces. The Tfp of Neisseria spp. and V. cholerae mediate aggregation of individual bacte ria into microcolonies, which promotes colonization. Flagella are polymeric helical filaments that propel bacteria through liquid environments by rotating about their long axis. Because flagella confer the ability to swim toward a target surface, often following a chemotactic gradient where chemical sensing influences the direction of motility, they are vital virulence factors of many pathogenic bacteria. Flagella can be localized to one end of the bacterial cell (polar) or distributed around the bacterial surface (peritrichous). Flagella are evo lutionarily related to type III secretion systems (T3SSs) (see “Replicative Niche” and “Survival in the Vacuole,” below) and have been shown in some instances to be responsible for the secretion of bacterial toxins. Flagella may also act as adhesins, binding to mucins of mucosal sur faces in the case of the gastrointestinal pathogens enteropathogenic E. coli (EPEC) and enterohemorrhagic E. coli (EHEC). PART 5 Infectious Diseases Autotransporter proteins comprise a subdivision of type V secretion systems (T5SSs), which are prevalent among gram-negative bacteria Effector proteins Host cytosol Host Plasma membrane Outer membrane Peptidoglycan Bacterium Inner membrane Bacterial cytoplasm Type III secretion system Type IV secretion system Type VI secretion system Type V secretion system FIGURE 125-1 Major bacterial secretion systems involved in pathogenesis. Schematic of the types III, IV, V, and VI secretion systems (T3SS, T4SS, T5SS, and T6SS, respectively) of gram-negative bacteria. The T3SS, T4SS, and T6SS deliver bacterial effector proteins into host cells, whereas the T5SS participates in adhesion to the surface of cells. The architecture of the extracellular portion of the T4SS and how it translocates effector proteins remain poorly defined. Colored shapes with hooks in the host plasma membrane represent host membrane proteins that, it is thought, may participate in these processes.

(Fig. 125-1). Extended adhesive projections anchored in the bacte rial outer membrane, autotransporter proteins are pivotal virulence determinants for several human pathogens, including the filamentous hemagglutinin of Bordetella pertussis (the etiologic agent of whooping cough) and the IcsA adhesin and intracellular motility determinant of Shigella spp. (responsible for dysenteric diseases). The autotransporter proteins intimin and invasin are required for the intimate associa tion of adhering and effacing pathogens, such as EPEC, and invasion by Yersinia spp., respectively. Besides these main classes of adhesins, other bacterial surface proteins involved in adhesion include the Opa family of membrane proteins of Neisseria spp. and the gram-positive cell wall–anchored microbial surface component recognizing adhesive matrix molecules (MSCRAMMs) of S. aureus and various enterococci. Receptors Carbohydrates (glycans) on the surface of and secreted by human cells play major roles in the adherence of bacterial patho gens. The surfaces of human cells are coated with glycoproteins and glycolipids, whereas the extracellular matrix (ECM) scaffold of tissues is mainly composed of secreted proteoglycans. Most mucosal surfaces are covered with a layer of mucus, which consists primarily of mucins, a family of proteins that are

heavily glycosylated. Among the human enzymes involved in glycan decoration of secreted proteins is FUT2, for which ~20% of the human population harbors two nonfunctional alleles; the importance of human glycans in infection is highlighted by the observation that individuals who lack functional FUT2 dis play increased susceptibility to certain bacterial infections, decreased susceptibility to certain viral infections, and altered susceptibility to chronic noninfectious inflammatory diseases. These data are con founded by a relative increase in secretion of sialylated glycans in indi viduals lacking functional FUT2. Glycans participate in many adhesive interactions and serve as receptors for certain bacterial toxins. Certain bacteria enzymatically alter host glycans in a manner that enables improved access to the epithelial surface, as in the case of V. cholerae and the oral pathogen Tannerella forsythia. The GM1 ganglioside, a glycolipid, is the receptor for cholera toxin (CTX), Pseudomonas aeruginosa flagella, and the Clostridium per fringens α toxin, and contributes to tropism of the gastric pathogen H. pylori through interactions with the bacterial membrane proteins BabA and SabA. Heparan sulfate proteoglycans and other glycos aminoglycan-conjugated proteins are commonly associated with the basolateral membranes of epithelial layers and act as ligands for the

chlamydial OmcB protein and the E. coli cytotoxic necrotizing factor toxin; these interactions promote initial bacterial adherence in prox imity to the plasma membrane and lead to engagement of additional surface molecules. In mammals, the ECM and integrin proteins are ubiquitous. The ECM consists of laminin, vimentin, and type IV collagen, which inter act via fibronectin with integrin receptors in the plasma membrane. Because of direct interaction with plasma membrane–embedded inte grins, ECM alterations can result in signal transduction that directly influences immune cell behavior. Many bacterial pathogens engage integrins and the functionally related carcinoembryonic antigen– related cell adhesion molecules (CEACAMs) as host cell receptors and as triggers for their internalization into human cells (see “Mechanisms of Microbial Entry into Cells,” below). The receptor for adherent-invasive E. coli, a pathogenic type of E. coli, is CEACAM6, whose levels are increased on epithelial cells in inflammatory bowel disease; adherentinvasive E. coli adhere at increased levels in Crohn disease, and sites of excessive bacterial adherence display increased inflammation. Among the many other host cell surface proteins that serve as receptors for bacterial adherence is the cystic fibrosis transmembrane conductance regulator (CFTR), a chloride channel involved in the maintenance of adequate hydration of mucosal surfaces. Mutation of the CFTR gene gives rise to the hereditary disease cystic fibrosis. These patients are hypersusceptible to respiratory infection because the cilia are unable to clear viscous mucus from the bronchial epithelial surface. The remarkably high frequency of cystic fibrosis (>2.5%) in Caucasian populations has been attributed to relative resistance to Salmonella enterica serovar Typhi infection, since S. Typhi adheres to CFTR via type IV pili. Epithelial cells utilize CFTR during internalization-mediated clearance of the extracellular pathogen P. aeruginosa, potentially con tributing to the decreased ability of cystic fibrosis patients to clear infections by this pathogen. ■ ■REPLICATIVE NICHE Once bacterial pathogens find their niche and associate with target host cells, they must replicate to persist. Some pathogens remain predominantly associated with the surface of epithelial cells (e.g., Bordetella, Pseudomonas, Vibrio, and Clostridium spp.); others pre dominantly enter into cells, where they may survive within the cytosol (e.g., Shigella, Francisella, and Listeria spp.) or within a membranebound compartment (vacuole) comprised of host-derived lipids (e.g., Salmonella, Legionella pneumophila). Phagocytic cells actively engulf pathogens for destruction (phagocytosis); to remain associated with the cell surface, extracellular bacteria must avoid or inhibit phagocytosis. Many pathogens deliver

cytoskeleton-disrupting proteins that perturb the phagocytic process. Others create structures, such as P. aeruginosa biofilms and streptococcal cell chains, that impede phagocytosis. A subpopulation of any infecting pathogen will nevertheless be phago cytosed by macrophages, neutrophils, and/or dendritic cells (phago cytes). Many pathogens have evolved mechanisms to survive within phagocytes; for example, during colonization of the nasopharynx, Streptococcus pneumoniae survives within vacuoles in dendritic cells and macrophages. Nonphagocytic cells, such as epithelial cells, may also internalize bacteria in processes triggered by either the host cell (as a clearance mechanism) or the pathogen (for tissue invasion). Extracellular Pathogens Extracellular pathogens replicate at the surface of host cells. Many secrete enzymes that liberate nutrients and other factors that fashion a hospitable niche. For example, V. cholerae secretes the mucinase vibriolysin that degrades the mucus barrier overlying intestinal epithelial cells, providing bacterial access to the cell surface. When phagocytosed, V. cholerae inhibits further phagocytosis of extracellular bacteria. Yersinia spp., which associates with the surface of leukocytes, delivers into these cells bacterial proteins that inhibit phagocytosis. EPEC and EHEC efface the microvilli of the brush border of intes tinal epithelial cells and adhere to the surface of these cells. These bac teria induce formation of actin-rich pedestals at the plasma membrane that inhibit bacterial internalization. Tight junctions mediate intimate

associations between neighboring epithelial cells, thereby maintaining tissue barrier function; the dissolution of tight junctions by pathogens facilitates bacterial penetration into the tissue.

BIOFILMS Some extracellular pathogens, including S. aureus and P. aeruginosa, establish chronic infections by producing extracellular polymeric matrices called biofilms, which encase the bacteria. Biofilms commonly develop where tissue integrity has been compromised, such as in burn wounds and on prosthetic material. The biofilm matrix is composed of extracellular polysaccharides and DNA, to which the bac teria adhere. Biofilms protect bacteria from phagocytosis while impair ing the diffusion of antibodies and antibiotics, enabling the bacteria to avoid elimination and persist. Mechanisms of Microbial Entry into Cells Bacterial patho gens that are predominantly intracellular during infection can induce internalization into host cells. Even pathogens that replicate within normally phagocytic cells such as macrophages (e.g., Salmonella spp., L. monocytogenes) possess mechanisms for inducing internalization into these cells. TRIGGER MECHANISM Bacterial systems that deliver proteins into human cells are critical to many aspects of pathogenesis (Fig. 125-1). An important example is the type III secretion system (T3SS), which delivers proteins from the bacterial cytoplasm directly into the cytosol of eukaryotic cells. Evolutionarily conserved T3SSs are found in many gram-negative bacterial pathogens. Delivery of bacterial proteins (effector proteins) into the host cell can have dramatic effects on host cell function; for many pathogens, these effects include induction of bacterial uptake into the host cell and alteration of cellular processes in ways that promote infection. CHAPTER 125 The T3SS forms an apparatus on the bacterial surface that resembles a needle and syringe, with a channel down its long axis (Fig. 125-1). The base of the organelle spans the two bacterial membranes. Anchored to the organelle base and protruding from the bacterial surface is a long needle-like structure. Upon contact of the needle tip with the eukary otic plasma membrane, two proteins secreted through the channel form a pore in the cell’s plasma membrane. The tip of the needle docks onto the extracellular face of the pore, forming a continuous conduit between the bacterial cytoplasm, where proteins are synthesized, and the host cell cytosol. Bacterial effector proteins are then delivered into the cell through this conduit. Molecular Mechanisms of Microbial Pathogenesis For those pathogens that use a T3SS to induce

entry into human cells (Salmonella, Shigella, and Chlamydia spp.), several of the first effector proteins to be translocated activate actin polymerization immediately beneath the point where the bacterium is docked on the plasma membrane. Polymerized actin pushes outwardly against the plasma membrane, generating large membrane ruffles that engulf the bacterium into a membrane-bound vacuole (Fig. 125-2). Shigella spp. escape the vacuole and reside in the host cell cytosol, whereas Salmonella and Chlamydia spp. predominantly reside within the vacuole. ZIPPER MECHANISM Several invasive pathogens enter host cells using a zipper-like mechanism that does not require a T3SS. In these instances, bacterial surface molecules engage with and stimulate the clustering of host cell receptors, which form close associations with the bacterium in a processive fashion until the plasma membrane is tightly opposed to and surrounding the bacterium (Fig. 125-2). For example, Y. pseudotuberculosis binds β1-integrins; this binding stimulates phos phoinositide 3-kinase (PI3K) and protein kinase B (Akt) signaling pathways, leading to zipper-mediated entry. L. monocytogenes engages E-cadherin and the Met receptor, activating β-catenin signaling and zipper-mediated entry. Subpopulations of bacteria classically consid ered extracellular, such as P. aeruginosa and S. aureus, may also pro mote bacterial uptake in a similar fashion. ENTRY OF CYTOSOLIC BACTERIA INTO NEIGHBORING CELLS Bacteria are initially taken up into a vacuole (nonphagocytic cells) or a phago some (phagocytic cells). Yet for some pathogens, residence in the vacu ole or phagosome is transient; the lifestyles of these pathogens include residence in the cytosol, actin-based motility, and cell-to-cell spread. L. monocytogenes, Shigella spp., spotted fever group Rickettsia spp. (the

Bacterial effector proteins Pathogencontaining vacuoles Trigger Zipper FIGURE 125-2 Common mechanisms of bacterial invasion. The mechanisms for internalization of bacteria into nonphagocytic cells are typically classified as trigger or zipper mechanisms. As examples of the trigger mechanism, Shigella and Salmonella spp. use their T3SSs to deliver into host cells effector proteins that manipulate the cytoskeleton in ways leading to the formation of cytoskeleton-supported membrane ruffles. These membrane ruffles extend and surround the pathogen, with consequent endocytosis of the bacterium. As examples of the zipper mechanism, bacterial membrane proteins of Yersinia and Listeria spp. induce clustering of host receptors. Clustering and subsequent intracellular signaling result in the uptake of the bacterium in a tightly opposed vacuole. etiologic agents of Rocky Mountain spotted fever, rickettsialpox, and other spotted fever diseases), and Burkholderia pseudomallei (the etio logic agent of melioidosis) rapidly lyse the vacuole. L. monocytogenes produces a pore-forming toxin, listeriolysin O, and phospholipases that mediate escape into the cytosol, whereas the mechanism of vacu olar escape of Shigella spp. is still uncertain except for a requirement of its T3SS. PART 5 Infectious Diseases Once they have escaped into the cytosol, L. monocytogenes, Shigella spp., B. pseudomallei, and spotted fever group Rickettsia spp. spread into adjacent cells via actin-based motility. These organisms recruit actin-polymerizing host factors that drive actin polymerization at one end of the bacterium, propelling the microbe through the cell cytosol. Actin-based motility pushes bacteria into host cell membranes to form protrusions that are taken up by the neighboring cell, resolving into a double-membrane vacuole containing the bacterium. This vacuole is lysed once more, releasing bacteria into the cytosol to begin another round of replication. Subversion of the host cytoskeleton aids bacterial cytosolic survival and promotes dissemination through epithelial tis sues. Burkholderia spp. instead trigger the infected host cell to fuse its membranes with adjacent cells in a T6SS-dependent manner, forming a multinucleated giant cell with contiguous cytoplasm. SURVIVAL IN THE VACUOLE After uptake into cells, the majority of invasive bacterial pathogens remain in a

vacuole. Uptake vacuoles normally enter the endosomal pathway, acquiring host proteins that promote vesicle maturation, acidification, and degradation of contents. Many bacterial pathogens contained in these endosomes subvert this intracellular trafficking in ways that block endosomal maturation and lysosomal degradation of the pathogen. A distinct subset of bacteria actively damage vacuolar membrane integrity, thereby escaping into the cytosol, where they replicate. For bacteria that remain in the vacuole, various bacterial effector proteins are delivered by secretion systems either into the vacuolar space or across the vacuolar membrane into the cytosol

(Fig. 125-1), where these proteins manipulate host processes to the benefit of the pathogen. ■ ■TYPE III SECRETION SYSTEMS T3SSs (Fig. 125-1) are versatile virulence systems, helping bacteria such as P. aeruginosa remain extracellular, but promoting both the uptake and the intracellular survival of the intracellular pathogens S. enterica serovar Typhimurium, Chlamydia spp., and Shigella spp.

S. enterica serovar Typhimurium uses distinct T3SSs for the invasion and subsequent maintenance of the Salmonella-containing vacuole (SCV), whereas Chlamydia and Shigella each encode only one such system. The effector proteins delivered are the major determinants of the lifestyles

of these pathogens; Shigella quickly escapes the vacuole in a T3SS-dependent manner, whereas Chlamydia remains in the vacu olar compartment (the inclusion) and uses effector proteins to hijack cellular traffick ing and perturb innate immune responses. The SCV and the Chlamydia inclusion exhibit distinct traits: the SCV is associated with thin membranous tubules that aid nutrient acquisition, whereas the inclusion is localized at the microtubule-organiz ing center, which is thought to facilitate recruitment of vesicles. ■ ■TYPE IV SECRETION SYSTEMS For other pathogens, type IV secretion systems (T4SSs; Fig. 125-1)—conceptually similar, evolutionarily distinct effector protein delivery systems—are key to the ability to survive in cellular phagocytic vacuoles. These T4SSs are similar to the T3SSs described above in that they form a multiprotein apparatus that contains a continuous channel between the bacterial cytoplasm and the eukaryotic cell cytosol. However, T4SSs are functionally more diverse than T3SSs, in that (1) they are present among both gram-negative and gram-positive bacteria as well as some archaea, (2) a large subset of T4SSs transport DNA in a process called conjugation, and (3) some T4SSs deliver proteins, typically toxins, into bacterial cells rather than eukaryotic cells. Not surprisingly, T4SSs also display great structural diversity. For the human pathogens L. pneumophila and Coxiella burnetii, bacterial effector proteins delivered across the vacuolar membrane into the cell cytosol by a T4SS alter maturation of the vacuole in a manner that makes it hospitable for bacterial survival; the resulting vacuole is known as a Legionella-containing or Coxiella-containing vacuole (LCV or CCV, respectively). The delivered effector proteins block fusion of lysosomes with the vacuole, thereby preventing bacterial degradation by lysosomal enzymes; manipulate host vesicular trafficking pathways; and remodel intracellular membranes to alter the lipid and protein content of the vacuolar membrane. For example, the L. pneumophila phospholipase VipD, a T4SS substrate, reduces the levels of phosphati dylinositol 3-phosphate on the LCV, thereby preventing recruitment of the host protein Rab5 GTPase, which is involved in endosomal matura tion. The mature LCV membrane contains many features that resemble cellular endoplasmic reticulum. In the case of C. burnetii, the vacuole displays more lysosome-like characteristics, including a relatively low pH, which induces the activation of T4SS effector delivery. The deliv ered bacterial effectors stimulate efficient vesicle recruitment from endosomal and autophagosomal (see “Autophagy,” below)

networks, massively increasing the membrane of the CCV until it occupies much of the cytosol. For both L. pneumophila and C. burnetii, the mature bacterium-containing vacuoles are hospitable to bacterial replication and long-term survival. ■ ■OTHER SECRETION SYSTEMS Francisella tularensis, the etiologic agent of the zoonotic infection tularemia, is a gram-negative facultative intracellular bacterium that displays a tropism for macrophages. F. tularensis resides in a phago some, the maturation of which it delays by remodeling the lipid content of the vacuolar membrane through the action of its type VI secretion system (T6SS; Fig. 125-1). F. tularensis then avoids destruction by lysosomal enzymes by escaping into the cytosol, where it replicates. Another pathogen that escapes into the cytosol of macrophages is the respiratory pathogen Mycobacterium tuberculosis. Here, the ESX-1 type VII secretion system (T7SS) is required for lysis of the phago somal membrane. For both of these pathogens, few effectors of their cognizant secretion system have been characterized, and the process of phagosomal membrane lysis remains poorly understood.

■ ■NUTRIENT ACQUISITION Regardless of the site of bacterial infection, microbes must acquire nutrients to replicate. Both extracellular and vacuolar pathogens must circumvent the nutrient-poor conditions of these sites. Also impacting extracellular bacteria is the microbiota, which contributes to nutritional immunity through competition for resources (Chap. 484). Bacterial pathogens possess an arsenal of nutrient acquisition mechanisms to overcome these challenges. For example, upon oxidative stress, to maximize nutrient uptake, several pathogens secrete metal ion-binding proteins through T4SSs (Fig. 125-1) and upregulate expression of cell surface receptors. Many bacteria manipulate host restriction of nutrients, with intracellular bacteria commonly delivering factors that inhibit host mRNA translation to increase the available pool of amino acids. Pathogens within vacuoles have evolved methods of redirecting host nutrient transport to these vacuoles (see “Cellular Trafficking,” below). ■ ■CELLULAR TRAFFICKING Because the vacuolar environment is poor in nutrients, bacteria that survive in vacuoles have evolved intricate strategies for the manipu lation of host processes to meet their metabolic needs. Vacuolar pathogens modulate endosomal trafficking to prevent degradation by lysosomal compartments. Whereas cytoskeletal rearrangements are regulated by small GTPases of the Rho family, the transport of vesicles through the cell cytosol is controlled by Rab and Arf GTPases. The coevolution of vacuole-residing bacteria with their eukaryotic hosts has promoted the acquisition of eukaryotic-like domains in many secreted bacterial effector proteins. Many of these effector proteins mimic host cellular trafficking proteins such as guanine nucleotide exchange fac tors (GEFs), which regulate GTPase activation, and soluble N-ethylma leimide-sensitive factor attachment protein receptors (SNAREs), which control vesicle fusion with vacuolar and other membranes. These bac terial effectors cause the accumulation of host protein and lipid mark ers on vacuolar membranes, thereby causing the vacuoles to resemble other host compartments. Consequently, host vesicles are redirected from the endoplasmic reticulum, Golgi apparatus, and secretory path ways to the pathogen-containing vacuole, thus delivering nutrients to the pathogen. Chlamydia spp., which are obligate intracellular bacteria, acquire host-derived lipids in the bacterial membranes. Dissection of the modes of action of bacterial effectors has revealed novel biochemi cal modifications of host trafficking regulators. For example, the L. pneumophila T4SS secretes two effector proteins that inactivate the cellular GTPase Rab1: AnkX inactivates Rab1 by transferring to it the phospholipid phosphatidylcholine, and SidM modifies Rab1 by adding an adenosine monophosphate molecule to it. This bacterium delivers

> 300 effector proteins through its T4SS; some of these proteins (metaeffectors) modulate the activity of other effectors rather than host factors. The coordinated activity of the numerous effector proteins provides precise spatiotemporal control of host processes. AVOIDANCE OF INNATE IMMUNE RESPONSES For the host to respond to and clear an infection, it must first be capa ble of sensing it. The route of infection and properties of the pathogen contribute to determining the nature of the immune response. The human immune system is generally considered to consist of two arms: the innate immune system, initiated by germline-encoded sensors, and the adaptive immune system, where lymphocytes are clonally selected in response to specific antigens. Initial interactions of pathogens are with specific host cell types, influenced by bacterial tropism; the result ing release of danger signals and cytokines from the innate immune response shapes the later adaptive immune response. Epithelia (e.g., in the intestinal and respiratory tracts) are commonly the first tissues that a pathogen encounters. The mucosal surfaces of these epithelia are covered in a gel-like mucus layer that contains secretory immunoglobulins, antimicrobial peptides, and commensal bacteria, which offer the first line of defense that infecting pathogens must overcome (Chap. 484). In the gastrointestinal tract, this barrier can be overcome through hijacking of microfold cells (M cells) in the

follicle-associated epithelia. M cells sample antigens, including intact pathogenic bacteria, from the gut lumen and deliver them to the underlying gut-associated lymphoid tissue in a process known as trans cytosis. Within the gut-associated lymphoid tissue, dendritic cells, mac rophages, and neutrophils engulf transcytosed material for destruction. Lysosomal degradation of pathogenic bacteria in macrophages and dendritic cells can then lead to antigen presentation, where the hydro lytic products are processed for display by the major histocompatibility complexes (MHCs). These antigen-presenting cells migrate through the lymphatic system to lymph nodes, where the adaptive immune response is stimulated through activation of B-cell and T-cell clones.

■ ■COMPLEMENT Once bacteria have crossed epithelial barriers into the lamina propria, they encounter components of humoral immunity. The complement system is a complex of plasma and tissue-resident proteins that can undergo activation from their pre-protein or zymogen forms to either label pathogens for phagocytosis (opsonization) or lyse them directly (Fig. 125-3). Complement activation commences with detection of the pathogen through the binding of circulating antibodies, which are recognized by the C1 complex (classical pathway), or of lectins (lectin pathway) to surface carbohydrates. These pathways converge at the deposition of the C3 convertase complex on the bacterium. The alter native pathway is the third route of complement activation, whereby an alternative C3 convertase complex is formed either spontaneously or by the action of plasma factors, promoting amplification of the cascade. Activation of further complement components results in chemokine production, bacterial opsonization, or assembly of the membrane attack complex pore on the bacterial surface, which lyses the pathogen. Bacterial virulence factors have been described that inhibit each step of the complement pathway, highlighting the importance of this anti microbial system to host immunity. The various strategies of bacterial interference include prevention of initial recognition by assembly of a polysaccharide capsule,

modification of bacterial lipopolysaccha ride (LPS), degradation of complement components, and masking of surfaces with host proteins. Streptococci employ numerous mecha nisms for complement evasion. The streptococcal proteases ScpA and SpeB degrade many complement proteins, whereas the bacterial surface-exposed M proteins bind the complement inhibitor C4BP, thereby preventing C3b deposition. The thick peptidoglycan layer of gram-positive bacteria provides modest resistance to membrane attack complex–mediated lysis. Furthermore, streptococci produce a hyaluronic capsule that shields the bacterial surface from complement recognition. In comparison, Borrelia burgdorferi, the etiologic agent of Lyme disease, encodes a CD59-like protein that inhibits the comple tion of membrane attack complex assembly. Whereas many pathogens inhibit opsonization, thereby avoiding phagocytosis, other bacteria, including Francisella and Yersinia spp., promote this process, leading to uptake into the phagosomal compartment, wherein they replicate and prevent lysosomal fusion. CHAPTER 125 Molecular Mechanisms of Microbial Pathogenesis ■ ■LYSOSOMES Lysosomes are vesicular organelles of the endosomal system that con tain hydrolytic and antimicrobial enzymes within an acidic environ ment. These enzymes are crucial for recycling of cellular material but also participate in direct killing of microbes and initiation of antibac terial immunity. Lysozyme is a lysosomal enzyme that hydrolyzes the polymeric glycan chains in peptidoglycan, resulting in bacterial lysis. Liberated peptidoglycan fragments may then stimulate innate immune receptors to drive antibacterial immunity. Lysozyme is highly effec tive against most gram-positive bacteria; however, the peptidoglycan layer of gram-negative bacteria lies between the bacterial outer and inner membranes, making it inaccessible to lysozyme. Gram-positive bacteria can decrease their susceptibility to lysozyme with peptidogly can modifications that block access of lysozyme to the glycan chain, such as acetylation of sugars in the glycan strand. Metabolites present within the lysosome may also contribute to immunity; for example, the mitochondria-derived metabolite itaconate accumulates within lysosomes during infection of macrophages with S. enterica serovar Tymphimurium, where it limits bacterial growth.

C1 complex Classical pathway Lectin pathway C3 convertase Streptococcus pyogenes C5 convertase C3 cleavage Neisseria gonorrhoeae Membrane attack complex PART 5 Infectious Diseases Borrelia burgdorferi FIGURE 125-3 Overview of the complement system in bacterial infection. The classical and lectin pathways of complement activation are initiated by the binding of the C1 complex to antibodies bound to bacteria or to lectins binding carbohydrate moieties on the bacterial surface, respectively. A cascade of proteolytic cleavage generates the C3 convertase complex on the bacterial surface, which, upon activation, leads to C5 convertase formation. In the alternative pathway, spontaneous C3 cleavage forms alternative C3 products that lead to bacterial opsonization and to amplification of C3 convertase activation. The three complement pathways converge at the point of C3 convertase activation. In a manner similar to that seen for bacteria opsonized with circulating immunoglobulin, opsonized bacteria bind to surface receptors of phagocytes, triggering their engulfment for destruction and stimulation of the adaptive immune system. The C5 convertase, generated by conventional C3 convertase activation, recruits further complement proteins to form the membrane attack complex that directly lyses bacteria. Many bacterial pathogens inhibit specific points in the complement cascade, a selection of which are shown with blunt-ended arrows; in contrast, others, such as Francisella spp., promote their own opsonization and subsequent phagocytosis, enabling them to reach their intracellular replicative niche. ■ ■ANTIMICROBIAL PEPTIDES Antimicrobial peptides, such as the positively charged defensins and cathelicidins, are concentrated in degradative compartments. These molecules bind

the anionic surface of bacteria and perturb the integ rity of LPS of the gram-negative outer membrane and wall teichoic acids in the gram-positive cell wall. Many bacterial pathogens modify their surfaces to reduce the net negative charge, thereby decreasing the binding of positively charged antimicrobial peptides. S. aureus and the respiratory pathogen Burkholderia cenocepacia secrete proteases that cleave antimicrobial peptides, facilitating survival at mucosal surfaces and within phagosomes. ■ ■OXIDATIVE BURST Pathogens are exposed to reactive oxygen species and reactive nitrogen species produced by cells responding to infection. A well-characterized example is the neutrophil oxidative burst, in which the membrane com plex nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase) produces superoxide radicals through the transfer of electrons to molecular oxygen. NADPH oxidase activation at the site of infec tion generates reactive oxygen species in the phagosome of neutrophils and/or in the extracellular space. Bacterial detoxification enzymes,

Pseudomonas aeruginosa Alternative pathway Immunoglobulins Opsonization Francisella tularensis Phagocytosis Lysis including superoxide dismutases, peroxiredoxins, and catalases, enable bacteria to survive this oxidative burst. Many bacteria can also block the activity of NADPH oxidase; several pathogens prevent assembly of the NADPH oxidase complex by T3SS effector protein–mediated posttranslational modification of small GTPases. The importance of NADPH oxidase in immune defense is highlighted clinically by the marked susceptibility to certain bacterial and fungal infections in patients with chronic granulomatous disease, which results from non functional alleles for essential subunits of this complex. ■ ■NEUTROPHIL EXTRACELLULAR TRAPS The oxidative burst also stimulates the release of the proinflammatory tumor necrosis factor α, azurophilic granules, and neutrophil extracel lular traps (NETs). NETs are an extruded mesh of decondensed chro matin that are thought to ensnare pathogens, thereby restricting their spread and destroying them. Streptococci encode deoxyribonucleases (DNases) and S. aureus nuclease degrades NETs. S. aureus then con verts the nucleotide products of this degradation into deoxyadenosine, which functions as a potent macrophage toxin. This process showcases how bacterial pathogens may utilize antimicrobial responses to their own benefit.

■ ■PATTERN RECOGNITION RECEPTORS, GUARD PROTEINS, AND THEIR EVASION The first line of detection of pathogens by the innate immune system is via recognition of molecular patterns that are indicative of the pres ence of pathogens, termed pathogen-associated molecular patterns (PAMPs). Host proteins referred to as pattern-recognition receptors (PRRs) recognize PAMPs, enabling cells to establish that an infection is occurring. Membrane-associated PRRs surveil the extracellular or endosomal milieu, whereas cytosolic PRRs monitor for threats within the cytosol. Cytosolic ligands tend to stimulate stronger proinflamma tory responses than extracellular ligands, and detection of bacteriaderived PAMPs tends to elicit a more powerful inflammatory response than detection of virus-derived PAMPs. Thus, both the subcellular localization and ligand specificity of PRRs play important roles in dic tating the character and amplitude of an immune response. Another class of innate immune proteins, termed guards, can also initiate innate immune signaling in a process referred to as effector-triggered immunity. In contrast to the direct binding of PRRs to PAMPs, guards become activated upon sensing activities or cellular changes associated with infection. TOLL-LIKE RECEPTOR DETECTION OF EXTRACELLULAR AND PHAGOSOMAL LIGANDS The most extensively studied PRRs for bacterial PAMPs are Toll-like receptors (TLRs), which are membrane-associated. On phagocytic cells, TLRs may employ scavenger receptors as co-stimulatory surface recep tors. Different TLRs recognize distinct conserved molecules that include

LPS, peptidoglycan, flagellin, and nucleic acids (Table 125-3). Upon binding their cognate ligands, TLRs trigger assembly of supramolecular organizing centers, large protein complexes that are signaling platforms for signal transduction. Ultimately, TLR signaling results in nuclear translocation of transcription factors, including nuclear factor-κB (NF-κB), AP-1, and interferon-regulatory factors (IRFs), which induce expression of cytokines, chemokines, and other immunity-related genes (Fig. 125-4). ALTERATION OF PAMPS TO PREVENT IMMUNE RECOGNITION Patho gens have evolved a plethora of strategies to avoid immunity; one com mon strategy is to mask PAMPs. For example, TLR4 binds efficiently to LPS that contains hexa-acylated lipid A. Y. pestis, the etiologic agent of plague, produces hexa-acylated LPS in the flea vector, but upon transmission into a human host switches to production of tetraacylated LPS, which does not activate TLR4. Tetra-acylated LPS is also synthesized by F. tularensis, H. pylori, and the periodontal pathogen Porphyromonas gingivalis. Bacteria can also mask immune recognition of flagella. The flagel lum of H. pylori facilitates bacterial access to the gastric epithelium and is essential for virulence. Flagellin, the monomeric protein subunit of the flagellum, is a ligand for TLR5, but H. pylori bypasses immune recognition by producing a flagellin epitope that does not bind TLR5. CpG motifs in mammalian DNA are usually methylated, but CpG TABLE 125-3 Pattern Recognition Receptors of the Innate Immune System and Their Ligands PATTERN RECOGNITION RECEPTOR LIGAND OR MODE OF ACTIVATION TLR2 (with TLR1 or TLR6) Lipoproteins TLR4 LPS TLR5 Flagellin TLR9 CpG DNA NLRP1 Enzymatic cleavage NLRP3 Ionic flux, mitochondrial damage NLRP6 Lipoteichoic acid, RNA NAIPs/NLRC4 Flagellin/T3SS STING Cyclic dinucleotides NOD1 and NOD2 Peptidoglycan

motifs in bacterial DNA are not; thus, methylation state provides a method of discriminating self from nonself. TLR9 recognizes CpGcontaining unmethylated DNA in intracellular membrane compart ments of the endosomal system. Group A streptococci secrete the Sda1 DNase that degrades CpG DNA, thereby reducing the immunogenicity of lysed bacteria.

INHIBITION OF NF-κB AND MAPK SIGNALING NF-κB signaling plays a central role in controlling both antibacterial and steady-state immu nity. A plethora of bacterial effector proteins target components of the NF-κB pathway in ways that maintain repression of NF-κB-dependent proinflammatory genes, thereby counteracting activation of innate immune responses triggered by their conserved PAMPs. Many bacteria produce virulence factors with eukaryotic-like domains that enable pathogens to hijack host signal transduction, including the NF-κB pathway. Ubiquitination, a posttranslational modification in which a chain of the small protein ubiquitin is cova lently linked to a target protein, is a common bacterial mechanism of host signaling inhibition. Ubiquitin is added to a target protein by an E3 ubiquitin ligase, which transfers ubiquitin from an E2 ubiquitinconjugating enzyme to the target protein. Depending on the precise structure of the ubiquitin chain, the modified protein is subsequently degraded by the proteasome, targeted to particular vesicular compart ments, or recruits adaptor proteins that initiate signal transduction. The translocation of NF-κB into the nucleus requires the succes sive phosphorylation, ubiquitination, and degradation of IκBα by the IκB kinase (IKK) complex, which in resting cells inhibits NF-κB; the dissociation of the IKK complex from NF-κB enables the latter to translocate into the nucleus, where it functions as a transcription factor (Fig. 125-4C). The T3SS of Shigella spp. delivers into cells sev eral E3 ubiquitin ligases that target components of the NF-κB pathway for degradation. The intracellular pathogens C. trachomatis and B. pseudomallei secrete bacterial deubiquitinases through T3SS and T2SS, respectively, which cleave the ubiquitin moiety from IκBα, thereby pre venting its degradation, maintaining inhibited NF-κB in the cytosol, and repressing

proinflammatory gene expression. CHAPTER 125 Molecular Mechanisms of Microbial Pathogenesis Other enzymatic activities of bacterial effectors can also inhibit NF-κB activation by ubiquitin-dependent or -independent processes. Shigella spp. and L. pneumophila deliver effectors via their T3SS and T4SS, respectively, that inactivate E2 enzymes to prevent modification and degradation of the IKK complex. EPEC and Shigella spp. release cysteine methyltransferases that inhibit IKK modification, whereas S. enterica serovar Typhimurium targets NF-κB directly for degrada tion via T3SS effectors with metalloprotease activity. Another cellular pathway of pro-inflammatory signaling stimu lated by PRRs is the mitogen-activated protein kinase (MAPK) phos phorylation cascade, which activates transcription factor AP-1. The B. anthracis metalloprotease exotoxin lethal factor ablates this kinase cascade by cleaving the N-terminal region of MAPKs. Homologous T3SS effectors of Yersinia spp., S. enterica serovar Typhimurium, and V. parahaemolyticus acetylate MAPKs, preventing their phosphorylation. Finally, Shigella spp. and S. enterica serovar Typhimurium produce phosphothreonine lyases that cleave phosphorylated threonine resi dues from MAPKs, preventing their activities. The broad range of effector protein mechanisms that bacteria employ to subvert TLR and MAPK signaling underscores the criti cal roles of these pathways in preventing pathogens from establishing infection (Fig. 125-4). PRR SENSING OF CYTOSOLIC INFECTION Whereas the extracellular environment is surveilled for signs of infection by TLRs, the cytosol is monitored by other PRRs. Among the signaling pathways activated upon recognition of intracellular bacterial pathogens are the cytosolic PRRs NOD1 and NOD2, which activate NF-κB signaling upon binding modified peptidoglycan fragments. These PRRs are critical for defense against numerous bacterial pathogens, yet polymorphisms in the NOD1 and NOD2 genes confer susceptibility to inflammatory bowel disease. Another important cytosolic (and occasionally nuclear) innate immune surveillance system is the cyclic guanosine monophosphate–adenosine monophosphate (cGAMP) synthase-stimulator of interferon genes

Flagellin GBPs Innate immunity mechanisms Degradation Ubiquitination SMOC assembly A Immune GTPases K+ Nutrient depletion Vacuole rupture Inflammasome activation Cytosolic PAMPs / DAMPs Phagophore formation Autophagosome formation Degradation Lysosome PART 5 Infectious Diseases Autophagy Inflammasomes B C FIGURE 125-4 Overview of innate immune recognition and response to bacterial pathogens. A. Immune GTPases. Proteins of the GBP family associate with the bacterial surface, LPS shed from the bacterial surface, or pathogen-containing vacuoles, disrupting the integrity of the pathogen and leading to bacterial lysis. Alternatively, labeling of intracellular pathogens with immune GTPases may stimulate ubiquitination of the bacterium and its destruction by cell-autonomous immune mechanisms such as autophagy. B. Autophagy. Recognition of bacterial invasion leads to host-mediated sequestration of the pathogen in an autophagosome and destruction of the pathogen upon fusion of the autophagosome with lysosomes. Bacteria may be directly labeled by ubiquitination, damaged vacuoles are recognized by the binding of galectins to exposed glycans, and/or the cellular autophagy may be induced in response to sensing starvation. C. Pattern recognition receptors and innate immune signaling. Conserved PAMPs, DAMPs, and danger signals are recognized by all cells, triggering innate immune signaling pathways and proinflammatory gene expression. TLRs surveil the cell surface and endosomal interior for PAMPs that include LPS, nucleic acids, and flagellin. Cytosolic PRRs and guards, including NLRs and ALRs, recognize similar ligands as well as distinct stimuli, including dysregulation of ion homeostasis. TLR activation promotes supramolecular organizing center assembly and NF-κB signaling. Under resting conditions, NF-κB is sequestered in the cytosol by IκBα, but TLR activation leads to IκBα phosphorylation, ubiquitination, and degradation, with the consequent release of NF-κB. Resultant

translocation of NF-κB into the nucleus leads to pro-inflammatory gene expression and the production and release of cytokines. Stimulation of cytosolic PRRs and guards may lead to assembly of supramolecular organizing centers known as inflammasomes, resulting in activation of inflammatory caspases. A well-characterized example is the activation of caspase-1 by canonical and noncanonical inflammasomes, subsequent activation of the pore-forming protein GSDMD, maturation and release of proinflammatory cytokines IL-1β and IL-18, and pyroptotic death of the cell. (cGAS-STING) pathway. In response to recognition of cytosolic DNA, cGAS synthesizes the second messenger cGAMP. Upon bind ing cGAMP, STING activates interferon regulatory factor 3 (IRF3) and NF-κB, which subsequently promote transcription of interferonregulated proinflammatory genes. The products of these genes include cytokines that alert bystander cells and recruit immune cells from the circulation, creating a heightened immune state in the local area. INFLAMMASOMES Some cytosolic PRRs, including members of the NOD- and AIM2-like receptor (NLR and ALR, respectively) families, can seed formation of supramolecular organizing centers known as inflammasomes. Upon binding to a PAMP, these PRRs oligomerize and recruit the zymogen procaspase-1; some PRRs recruit procaspase-1 directly, whereas others bind the adaptor protein ASC, which then recruits procaspase-1. Upon its recruitment, procaspase-1 undergoes autocatalytic cleavage, thereby converting into active caspase-1 and completing formation of an active inflammasome. PRRs capable of triggering inflammasome assembly include NAIP-NLRC4, AIM2, and NLRP6. Select guard proteins can also trigger inflammasome assem bly, including NLRP3, Pyrin, and NLRP1. In addition, caspase-4 and -5 (and their murine homolog caspase-11) serve as PRRs that drive assembly of the “non-canonical” NLRP3 inflammasome (see below). The importance of inflammasome signaling is underscored by a pleth ora of polymorphisms within inflammasome-associated genes that predispose to autoinflammatory disorders or susceptibility to infection.

LPS Nucleic acids Peptidoglycan Extracellular PAMPs TLRs IκBα degradation NF-κB Nucleus Pro-inflammatory gene expression Cytokine release Gasdermin D pore formation IL-1β IL-1β Caspase-1 activation Pyroptosis Upon their activation within an inflammasome, caspases drive immunity by cleaving inflammasome effector proteins. The bestdescribed inflammasome effectors are gasdermin D (GSDMD) and IL-1 family cytokines, including interleukin-1β (IL-1β) and IL-18. Cleavage of GSDMD liberates its N-terminal domain, which forms pores in the plasma membrane; cleavage of the zymogen forms of IL-1 family cytokines converts them into mature, active cytokines, which are released through GSDMD pores and bind the IL-1 receptor, in turn driving robust inflammation (Fig. 125-4). The formation of GSDMD pores in the plasma membrane is often associated with cell death, termed pyroptosis (inflammatory cell death). Inflammasome-seeding PRRs are activated by binding to PAMPs. NAIPs sense the needle protein of the T3SS or the related flagel lin protein and subsequently recruit NLRC4 to seed inflammasome formation. Many pathogens have evolved mechanisms to overcome activation of the NAIP-NLRC4 inflammasome. In L. pneumophila, L. monocytogenes, P. aeruginosa, and S. enterica serovar Typhimurium, flagellin expression is repressed during infection; similarly, in S. enterica serovar Typhimurium, expression of an immunogenic T3SS is repressed and that of a less immunogenic T3SS is upregulated.

S. enterica serovar Typhimurium also produces a T3SS effector that blunts NLRC4 expression. AIM2 is activated upon binding cytosolic double-stranded DNA, which is released during lysis of cytosolic bac teria. Bacterial cell wall and membrane modifications that reduce bac teriolysis dampen AIM2 activation during cytosolic infection. Overall,

the inhibition of inflammasomes by bacterial pathogens enables patho gens to avoid elimination by the innate immune system. The NLRP3 inflammasome appears to be activated upon sens ing cell stress and homeostatic dysregulation, including via ion flux, mitochondrial damage, and release of oxidized mitochondrial DNA. Because these processes are triggered by membrane damage, NLRP3 can be activated by many bacterial pore-forming toxins; bacteria may avoid stimulating NLRP3 by downregulating the production of toxins. The pyrin inflammasome becomes activated upon the inactivation of Rho GTPases, thereby serving as a sensor of cytoskeletal perturbations. C. difficile, C. botulinum, and B. cenocepacia all produce effectors that inactivate RhoA via distinct covalent modifications, which induces cell death by alteration of the actin cytoskeleton yet ultimately drives pyrin activation. Meanwhile, NLRP1 acts as bait for cleavage by microbial proteases or E3 ubiquitin ligases; partial degradation of NLRP1 stimu lates its activity and subsequent pyroptosis. Caspases-4 and -5 trigger formation of the noncanonical NLRP3 inflammasome in response to LPS. In human cells, LPS is bound by the PRR NLRP11, which facilitates activation of caspase-4; alterna tively, caspases-4 and -5 can themselves serve as PRRs for LPS. Upon activation, these caspases cleave GSDMD but not IL-1β; the forma tion of GSDMD membrane pores indirectly drives activation of the NLRP3 inflammasome, which subsequently cleaves IL-1β. S. flexneri produces an effector that inactivates caspase-4 via ADP-riboxanation, which blocks host cell mechanisms of cytosolic bacteriolysis, thereby preventing release of LPS into the cytosol. Although caspases-4 and -5 play important roles in host defense, they also contribute to pathologic inflammation during gram-negative sepsis, as the murine homolog caspase-11 drives mortality in murine models of sepsis. Immune GTPases During many infections, the initial pathogen interaction triggers expression of host immunity-related genes that prime host cells for a more robust response. For example, detection of bacteria can drive interferon signaling, which induces production of the guanylate binding protein (GBP) family of immune GTPases. GBPs are recruited to pathogen-containing vacuoles or bacterial membranes and promote the release of PAMPs, making them available as ligands for PRRs. Some GBPs extract LPS from the surface of cytosolic gramnegative bacterial pathogens, facilitating noncanonical inflammasome activation. GBPs contribute to lysis of F. novicida (a subspecies of F. tularensis) and Neisseria meningitidis, causing release of bacterial DNA into the cytosol. Recruitment of GBPs to Shigella spp. prevents bacte rial actin-based motility and promotes bacterial ubiquitination, which targets the bacteria for proteasomal degradation; simultaneously, Shigella spp. secrete an effector that ubiquitinates GBPs, targeting them for degradation. Inhibition of Nonpyroptotic Cell Death Several types of regu lated (programmed) cell death besides pyroptosis can occur, of which inflammatory necroptosis and noninflammatory apoptosis are best characterized. In necroptosis, activation of any of a variety of innate immune signaling pathways (interferon-γ, TLRs) leads to kinasedependent assembly of a plasma membrane pore complex that induces cell lysis and release of DAMPs into the extracellular space, events that induce an intense inflammatory response. Necroptosis can drive both pathologic inflammation and antibacterial immunity. In apoptosis, cells condense and form membranous blebs without releasing cytosolic contents and thus without inducing inflammation in adjacent tissues. Apoptotic blebs and dead cells are disposed of by macrophages in a process known as efferocytosis. In addition to its role during infection, apoptosis plays a key role in development and is required for normal cell turnover in many epithelial tissues, includ ing the intestine. Apoptosis can be initiated by intrinsic or extrinsic cellular stimuli, resulting in activation of apoptosis-specific caspases that demolish cellular contents. The intrinsic pathway occurs upon dis solution of the mitochondrial membrane potential, which commonly occurs during infection due to cellular stress or the action of bacterial toxins. The extrinsic pathway can be stimulated by interactions of cell-surface death receptors with

proinflammatory ligands like TNF-α.

The gastrointestinal pathogens S. enterica serovar Typhimurium, Yersinia enterocolitica, and pathogenic E. coli inhibit signal transduc tion from cell-surface death receptors by modifying or cleaving the components of death receptor–associated supramolecular organizing centers (Fig. 125-4), thereby blocking cell death. Inhibition of extrinsic apoptotic signaling can stimulate alternative inflammatory cell death pathways, including necroptosis, highlighting the functional redun dancy of cell death pathways in some contexts.

Vacuolar Chlamydia spp. activate survival pathways through phos phoinositide 3-kinase (PI3K) and Wnt/β-catenin signaling, while simultaneously inhibiting apoptosis. T4SS effectors of vacuolar L. pneumophila and C. burnetii inhibit apoptosis by sequestering proapoptotic host factors. Cyclomodulins, a group of bacterial effector proteins that arrest the cell cycle, are delivered into cells by EPEC and EHEC. The cyclomodulin Cif deamidates ubiquitin and related proteins, inactivating host E3 ligases involved in cell cycle progression and thereby reducing epithelial turnover; this disruption can be sensed by host cells and triggers other cell death pathways. The diversity of mechanisms that bacteria have accumulated to modulate host cell death pathways highlights the importance of these pathways during infection. Autophagy Host cells deploy mechanisms of cell-autonomous immunity to neutralize microbial threats. One such mechanism is autophagy, which recycles organelles and protein complexes by gener ating double-membrane compartments (autophagosomes) around the cargo and delivering their contents to the lysosome; the cargo is subse quently degraded to amino acids and other molecules. Autophagy can isolate and dispose of cytosolic pathogens; host polysaccharide-binding molecules termed galectins can bind microbes and induce ubiquitina tion of the bacterial surface, which drives recruitment of LC3, a host protein that triggers engulfment into autophagosomes. CHAPTER 125 Intracellular bacterial pathogens must inhibit autophagy to survive. Common mechanisms used by bacteria to interfere with autophagy include masking bacterial ubiquitination sites, inhibiting autophago some maturation, and altering host pathways that regulate autophagy. L. monocytogenes recruits the host factor Arp2/3 to mask the bacterial surface, F. tularensis produces an LPS O-antigen that prevents surface access of E3 ligases, and S. flexneri produces an effector protein that blocks ubiquitination of the bacterial surface protein IcsA. To inhibit autophagosomal flux, L. pneumophila secretes an effector protein that cleaves LC3, S. enterica serovar Typhimurium secretes effector proteins that block activation of a kinase required for autophagosome genera tion, and M. tuberculosis and C. trachomatis prevent autophagosomelysosome fusion. An important metabolic trigger of autophagy is intracellular depletion of amino acids, which occurs upon replication of metabolically active intracellular pathogens and triggers inactivation of a master regulator of cell metabolism, mammalian target of rapamy cin (mTOR). mTOR inactivation derepresses autophagy; invasive pathogens including M. tuberculosis, S. enterica serovar Typhimurium, and Shigella spp. can reactivate mTOR signaling during infection, thereby blunting autophagy. Molecular Mechanisms of Microbial Pathogenesis Epigenetic Control of Innate Immune Responses Dysregu lated immune responses (e.g., sepsis, severe COVID-19 infection) can cause morbidity or death, whereas weak immune responses may lead to pathogen-induced morbidity and mortality. A cellular mecha nism that promotes appropriate amplitude of the immune response is innate immune training, in which cells are epigenetically primed for immunity via alterations in chromatin structure. Posttranslational modification of histones influences chromatin structure and access of transcription factors to regulatory elements in the DNA. Mounting evidence indicates that the

microbiota modifies the methylation state of promoters of genes involved in immunity, facilitating appropriately tuned responses to infection. Pathogens also regulate host responses at the epigenetic level. B. anthracis and L. pneumophila deliver into cells histone methyltransferases that control inflammation and ribosome activity. L. monocytogenes listeriolysin O, the P. aeruginosa T3SS pore, and other pore-forming toxins exert epigenetic modulation through

alterations in ion homeostasis, thereby manipulating the gene expres sion profiles of infected host cells.

■ ■INHIBITION OF ADAPTIVE IMMUNE RESPONSES Early interactions of pathogens with host cells trigger the innate immune response, releasing cytokines that recruit additional antigenpresenting immune cells to the site of infection. To establish chronic infection, bacterial pathogens need not only to suppress the innate immune system but also to avoid elimination by the adaptive immune response. The adaptive immune system comprises clonally expanded lineages of B and T lymphocytes that have been activated by antigenpresenting cells, the best characterized of which are dendritic cells and macrophages. B cells are required for humoral immunity; genetic dis eases that affect B-cell function often manifest as the inability to pro duce adequate antibody titers to clear extracellular bacterial infections. T cells generally mediate cell-mediated immunity, helping the host clear infected cells. The activation of T cells occurs by the presentation of processed antigen on MHC molecules of antigen-presenting cells. The activation of T cells is controlled by the specificity of MHC mol ecules and of their receptors. The large variety of receptors possessed by T cells enables collective recognition of a wide variety of antigens. Mutations that ablate the development of the adaptive immune system result in severe combined immunodeficiency, rendering the individual extremely vulnerable to infection. M. tuberculosis is notorious for its ability to establish latent infec tions, avoiding elimination by the immune system for decades. Once phagocytosed, the pathogen induces secretion of immunosuppressive cytokines, including IL-6, IL-10, and transforming growth factor β. The result is inhibition of interferon-γ-dependent gene expression, with downregulation of MHC class II and other immune-stimulatory molecules, which inhibits induction of helper CD4+ T lymphocytes. Similarly, B. pertussis induces dendritic cells to produce IL-10, which skews T-cell maturation into regulatory T cells, dampening the immune response. The S. enterica serovar Typhimurium T3SS effec tor protein SteD depletes MHC class II molecules from the surface of dendritic cells by the activation of the E3 ubiquitin ligase MARCH8. MARCH8 ubiquitinates MHC class II, interfering with its trafficking to the cell surface and thereby decreasing interaction with and subsequent activation of T lymphocytes. PART 5 Infectious Diseases In addition to preventing antigen-presenting cells from stimulating lymphocytes, pathogens may also directly alter B-cell and T-cell activ ity. The Yersinia T3SS effector YopH, a protein tyrosine phosphatase, dephosphorylates B-cell and T-cell receptors and thus prevents both signal transduction upon stimulation by antigen-presenting cells and lymphocyte activation. Staphylococcal toxic shock syndrome is induced by the production of superantigens by S. aureus. These extremely inflammatory exotoxins are potent activators of T cells, stimulating exuberant and at times fatal cytokine production. Rather than binding the specificity-determining variable regions of MHC molecules and the T-cell receptor, superantigens bind invariable regions and are therefore able to nonspecifically activate vast numbers of T cells, with a consequent cytokine storm. ■ ■BACTERIAL CYTOTOXINS Many bacteria produce cytotoxins—toxins that trigger host cell death. The best-characterized group of bacterial cytotoxins are the AB tox ins, which are composed of an enzymatically active (A) subunit and a binding (B) subunit, which interacts with the cellular

receptor. This family of toxins includes Shiga toxins of Shigella spp. and some strains of pathogenic E. coli, diphtheria toxin of Corynebacterium diphtheriae, pertussis toxin of B. pertussis, and CTX of V. cholerae. In general, upon binding to cell surface receptors, the toxin is endocytosed, whereupon the A-subunit translocates across the endosome membrane into the cytosol where its toxic enzymatic activity is stimulated. CTX and per tussis toxin ADP-ribosylate G-protein regulators of adenylate cyclases, thereby increasing cellular cyclic AMP (cAMP) concentrations. Within the cell, increased cAMP perturbs ion homeostasis and apoptosis; on a tissue level, increased intracellular cAMP induces chloride secretion via CFTR and inhibits sodium chloride absorption, driving massive

fluid secretion into the lumen of the small intestine and the diarrheal symptoms of cholera. C. perfringens and P. aeruginosa produce non-AB family toxins that ADP-ribosylate elongation factor-2, thereby inhibit ing host translation. S. enterica serovar Typhimurium and C. difficile produce toxins that target actin, inhibiting normal cellular cytoskeletal rearrangements. As dictated by the host targets modified, the effects of bacterial toxins on the host cell generally fall into the few broad categories of cytoskeletal manipulation, inhibition of innate immune signaling, and hijacking of cellular trafficking. Pore-forming toxins are secreted virulence factors produced by many extracellular pathogens, including S. pneumoniae, C. perfringens, B. anthracis, and L. monocytogenes, among many others. S. aureus produces several types of pore-forming toxins, including leukocidins, hemolysins, and phenol-soluble modulins. Leukocidins and α- and γ-hemolysins oligomerize within host plasma membranes, leading to host cell lysis. The cell-type specificities of leukocidins and hemolysins are driven by toxin binding to specific cell surface receptors. Conversely, phenolsoluble modulins are small amphipathic peptides that directly insert into cell membranes. Lytic toxins protect S. aureus from phagocytosis and prevent an infected host from developing a protective adaptive immune response. Host cells can counter cytolysins by releasing exosomes, vesicles that act as decoys by removing cytolysins from the local environment. ■ ■TISSUE DAMAGE AND PATHOGEN DISSEMINATION Much of the pathology associated with bacterial infection results from proinflammatory immune responses. Infected cells may continually signal in ways that alert the immune system, even though, as described above, many bacteria avoid elimination by cell-autonomous and immune cell–mediated mechanisms. In the intestine, tight junctions mediate inti mate associations between epithelial cells that maintain tissue barrier function, linking the cytoskeletal networks of adjacent cells through intimate association of protein complexes across the cell membranes. Many intestinal pathogens perturb the integrity of the gut epithelium either by manipulating cell polarity or by disrupting intercellular junc tions. C. perfringens, V. cholerae, pathogenic E. coli, Shigella spp., and S. enterica serovar Typhi all produce toxins in the gastrointestinal tract that disrupt tight junctions, consequently disrupting the barrier function of the tissue and facilitating access of the pathogen to deeper tissue. The RtxA multifunctional repeats-in-toxin (MARTX) toxin of V. cholerae causes cell rounding and barrier failure through actin-crosslinking activ ity, yet avoids eliciting substantial immune responses by simultaneously inactivating phospholipases and Rho GTPases. The serine protease autotransporters (SPATEs) of Shigella spp. and some pathogenic E. coli are typically secreted into the gut lumen or mucus layer, whereupon they cleave components of epithelial junctions and mucins in ways that facili tate tissue penetration. Tissue damage permits access to the underlying mucosal layers, the lymphatics, and the bloodstream; for some patho gens, this access enables seeding of other organs. Transmission to New Hosts The host represents a replicative niche for bacterial pathogens, in which they multiply and are transmitted to new hosts. The mode of transmission is typically aligned with the mode of entry. For example, for respiratory pathogens, coughing induced by tissue damage in the lung aerosolizes the pathogen, enabling inhalation by

and colonization of a new host. Similarly, gastrointestinal pathogens elicit diarrhea and are transmitted via the direct fecal–oral route or via contamination of crops or food with waste from an infected individual. The understanding of the spread of infectious diseases permits the institution of basic hygiene procedures that greatly diminish transmis sion rates—for example, hand washing, decontamination of communal surfaces, and adoption of social distancing measures. Bacteriophages and Pathogen Lifestyle The reservoir of V. cholerae is aquatic environments. Disease is acquired through the inges tion of contaminated seawater or seafood. In regions where cholera is endemic, the disease displays seasonal peaks. This seasonality is associ ated with blooms of bacteria-targeting viruses (bacteriophages), which infect V. cholerae organisms, replicate, and lyse and kill the bacterial host. Bacterial lysis releases viral particles into the aquatic environment,

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