Pathogen–Host Interactions in *Pseudomonas aeruginosa* Pneumonia

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***Pseudomonas aeruginosa* is an important pathogen causing a wide range of acute and chronic infections. *P. aeruginosa* rarely causes infection in the normal host, but is an efficient opportunistic pathogen causing serious infections in patients who are mechanically ventilated, individuals who are immunocompromised, and patients with malignancies or HIV infection. Among these risk groups, the most vulnerable hosts are neutropenic and patients who are mechanically ventilated. In addition, *P. aeruginosa* is the most prevalent chronic infection contributing to the pathogenesis of cystic fibrosis. Because of the ubiquitous nature of *P. aeruginosa* and its ability to develop resistance to antibiotics, it continues to be problematic from a treatment perspective. The pathogenicity of *P. aeruginosa* is largely caused by multiple bacterial virulence factors and genetic flexibility enabling it to survive in varied environments. Lung injury associated with *P. aeruginosa* infection results from both the direct destructive effects of the organism on the lung parenchyma and exuberant host immune responses. This article focuses on the major bacterial virulence factors and important aspects of the host immunity that are involved in the pathogenesis of serious *P. aeruginosa* infection. In addition to antibiotic therapy, strategies directed toward enhancing host defense and/or limiting excessive inflammation could be important to improve outcome in *P. aeruginosa* lung infections.**

Keywords: cystic fibrosis; cytokines; epithelium; host defense; nosocomial

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INTRODUCTION

Pseudomonas aeruginosa is a common pathogen associated with respiratory tract infections in diverse clinical settings. As a common cause of nosocomial infection in hospitalized and immunocompromised patients and the major pathogen associated with respiratory tract infection in cystic fibrosis (CF), there is a great deal of information available regarding the pathogenesis of *P. aeruginosa* pneumonia. The genomes of a prototypic laboratory strain of *P. aeruginosa* and several clinical isolates have been sequenced. Microarray studies have identified a variety of epithelial cell genes that are activated in response to contact with *P. aeruginosa*, and the contribution of numerous host receptors and signaling proteins has been established through the analysis of *P. aeruginosa* infection in transgenic mouse models. This article reviews recent data that elucidate host and pathogen factors that explain how this versatile pathogen causes pulmonary infection.

OVERVIEW OF *P. AERUGINOSA* RESPIRATORY INFECTIONS

P. aeruginosa is an important nosocomial pathogen in patients with significant underlying diseases, and colonization is frequently selected by broad-spectrum antimicrobial usage (1, 2). In patients with damaged airways from mechanical ventilation, trauma, or antecedent viral infection, *P. aeruginosa* colonization of the respiratory tract is often followed by acute pneumonia, sepsis, and death. As a cause of ventilator-associated pneumonia (VAP), *P. aeruginosa* has a high mortality compared with other pathogens (3). The epidemiology and mortality related to VAP has been recently reviewed (3). Some recent studies have reported an increased colonization of the oropharynx of patients with nasogastric tubes with *P. aeruginosa* and other gram-negative bacteria (4). The properties of the *P. aeruginosa* strains associated with VAP and acute respiratory failure are very different from those associated with the *P. aeruginosa* strains that chronically colonize the airways of patients with CF.

Patients who are immunosuppressed, particularly transplant recipients, neutropenic patients, and patients with HIV, are at increased risk for *P. aeruginosa* infection. Loss of mucosal barriers, mucositis from chemotherapy, and the selective pressure of broad-spectrum antimicrobial therapy are important risk factors for *P. aeruginosa* infection (5). Current guidelines for empiric therapy of fever and neutropenia insist on adequate anti–*P. aeruginosa* coverage (usually two drugs expected to have anti–*P. aeruginosa* activity). However, this ubiquitous organism remains a potentially serious threat to patients who are immunosuppressed. Patients with HIV whose viral load is not adequately controlled with antiretroviral drugs are also at an increased risk of *P. aeruginosa* infection (6, 7).

P. aeruginosa can cause community-acquired pneumonia (CAP). In the community, the incidence of *P. aeruginosa* pneumonia is increased in nursing home residents, patients with chronic

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obstructive pulmonary disease, and patients recently discharged from the hospital (8).

Molecular tools have aided in understanding the epidemiology of *P. aeruginosa* infections. Phenotypic typing methods using LPS, phage, pyocin, and antimicrobial serotyping are useful for understanding the epidemiology of acute infections; however, *P. aeruginosa* is phenotypically very unstable and therefore phenotypic typing is of limited value for chronic infections. Molecular typing techniques, such as restriction fragment length polymorphism, or RFLP, pulsed-field gel electrophoresis, and polymerase chain reaction–based methods, are more useful and discriminatory in chronic infections, permitting differentiation among many strains that may be considered identical by methods such as LPS serotyping. A recently published review on these methods for molecular diagnosis of *P. aeruginosa* infections provides further details (9).

EPIDEMIOLOGY OF *P. AERUGINOSA* RESPIRATORY INFECTIONS

Acute Infections

Nosocomial infections. According to the National Nosocomial Surveillance System of the Centers for Disease Control and Prevention, the overall incidence of *P. aeruginosa* infection in U.S. hospitals between 1985 and 1991 was 4.0 per 100 discharges, accounting for 10.1% of all hospital-acquired infections (10). In the SENTRY study performed in 1997 in Canada, the United States, and Latin America, among a total of 4,267 nosocomial and community-acquired blood stream infections, *P. aeruginosa* was the most common pathogen (10). Another recent survey indicated that, of the patients who develop nosocomial pneumonia, *P. aeruginosa* was isolated in at least 21% of cases (11). The most recent National Nosocomial Infection Surveillance indicates that *P. aeruginosa* is the second most common cause of nosocomial pneumonia after *Staphylococcus aureus* (12).

Ventilator-associated pneumonias. VAP caused by *P. aeruginosa* is a severe complication of intensive care, with mortality rates of 34 to 48% (13, 14). In a recent study, *P. aeruginosa* was the most frequently identified pathogen in patients who required a tracheotomy for continued mechanical ventilation (15). Septic shock and multiple organ dysfunction can complicate VAP caused by *P. aeruginosa* and significantly prolong hospital stays in these patients from 4 to 42 days (16–19). The excess morbidity and mortality in *P. aeruginosa* pneumonia appears to be related to dysregulated pathogen–host interactions with an exuberant host response to the pneumonia. The pathogenesis of VAP by *P. aeruginosa* is related to a breach of the epithelium, which results from mechanical injury associated with endotracheal intubation. These conditions favor attachment and growth of bacteria and allow for a greatly increased bacterial density. Intubation of the patient not only compromises the natural barrier between the oropharynx and trachea but also facilitates the entry of colonized pathogens through micro- and macroaspiration of infected oral and gastric contents (20). Bacterial colonization of the endotracheal tube and upper respiratory tract also facilitates the formation of biofilms (21).

Infections in the immunocompromised host. Patients who are immunosuppressed, particularly transplant recipients, burn patients, and patients with cancer and with neutropenia, are at increased risk of acquiring *P. aeruginosa* infection (22–24). In patients with cancer, the incidence of infections caused by *P. aeruginosa* was 1 to 2.5% among all the patients presenting with fever during neutropenia and 5 to 12% among patients with microbiologically documented infections (26). The mortality rate is increased by 40% among immunocompromised patients with *P. aeruginosa* pneumonia (24, 26).

With the advent of antiretroviral therapy, trends among persons dying of HIV infection in the United States show an increase in the percentage of deaths associated with bacterial pneumonias, including *P. aeruginosa* pneumonia. Vidal and coworkers (6) prospectively studied episodes of bacteremia in patients infected with HIV and found a substantially higher risk for *P. aeruginosa* bacteremia compared with the general population. Neutropenia, previous treatment with cephalosporins, and a low CD4 count were independent risk factors for developing *P. aeruginosa* bacteremia in patients infected with HIV. Afessa and Green (7) reported that *P. aeruginosa* pneumonia is a common pulmonary complication in patients with HIV with low CD4 counts. Compared with other pathogens, hospitalization was prolonged in patients with *P. aeruginosa* pneumonia by 14 days (7).

Community acquired pneumonia. Several recent studies have highlighted *P. aeruginosa* as a cause of CAP, including two studies from Spain that found that 1.5 to 5% of cases of CAP were related to *P. aeruginosa* infection (7, 27). Also, in a large prospective study in patients hospitalized with CAP, 60 of 559 patients included in the study (11%) had CAP caused by gram-negative bacilli, including *P. aeruginosa* in 39 patients (28). In a multivariate analysis, previous hospital admission and pulmonary comorbidity were the strongest predictors of *P. aeruginosa* CAP. Elderly nursing home patients are another group at increased risk of *P. aeruginosa* pneumonia. A prospective study conducted by Ali and colleagues (29) showed that up to 4% of pneumonias in nursing home patients were caused by *P. aeruginosa*. In addition, Monso and coworkers (30) reported an incidence of *P. aeruginosa* infection of 6% in patients with severe chronic obstructive pulmonary disease who were frequently treated with antibiotics and continued to smoke (30). Recent outpatient procedures can also predispose to *P. aeruginosa* infection. In a recent study, *P. aeruginosa* infection was reported after bronchoscopy related to contamination of the bronchoscope (31).

P. aeruginosa rarely causes infection in previously healthy individuals. When *P. aeruginosa* infection occurs in these patients, it has been associated with heavy exposure to aerosols of contaminated water and can be rapidly progressive, with a reported mortality of 33% (32).

Chronic Infections

Infections in patients with cystic fibrosis. In CF, defective function of the CF transmembrane conductance regulator (CFTR) in airway epithelium and submucosal glands results in chronic disease of the respiratory tract, which manifests early in life by airway obstruction and recurrent infections of the lung and paranasal sinuses. The CF lung is particularly susceptible to *P. aeruginosa*, and this organism plays a critical role in the development and progression of pulmonary disease in these patients (33). Chronic airway inflammation with recurrent *P. aeruginosa* infections is the major cause of morbidity and mortality in patients with CF (34). In a longitudinal assessment of *P. aeruginosa* in young children with CF, Burns and coworkers (35) used bronchoalveolar lavage and showed that 97% of children with CF were colonized with *P. aeruginosa* by the age of 3 years. According to the data from the U.S. Cystic Fibrosis Patient Registry, the overall percentage of patients with *P. aeruginosa* infection diagnosed by a positive sputum culture, bronchoscopy, or oropharyngeal or nasal secretion was reported to be 58.7% (36).

An enormous amount of work has been done to explain why *P. aeruginosa* (and not other opportunistic pathogens) is the major cause of pulmonary disease in CF. Although there is still no simple explanation, there is a wealth of information to help understand the interactions of *P. aeruginosa* in both the normal and the CF lung. Because the clinical features and management of pulmonary infection in CF have recently been reviewed, we

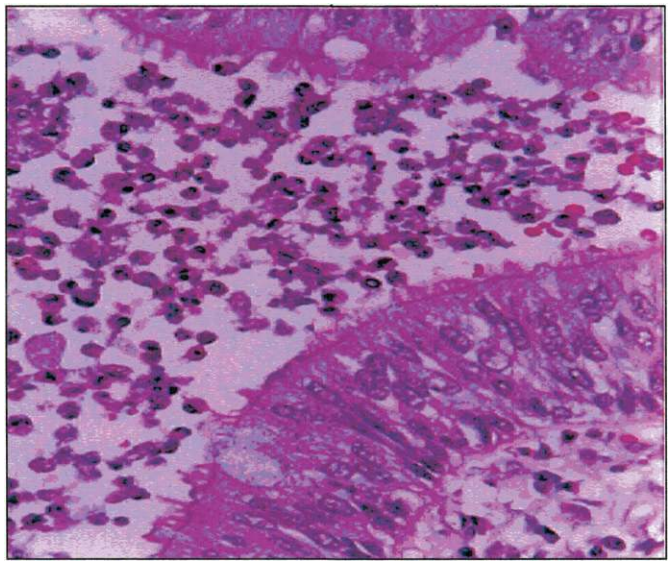

Figure 1. Histopathology of infected cystic fibrosis (CF) airways. A hematoxylin–eosin-stained section of CF lung tissue at the time of lung transplantation is shown. Note that, despite accumulation of polymorphonuclear neutrophils (PMNs), mucin, and bacteria in the airway, the epithelial tight junctions appear intact. Bacteria are enmeshed in mucin not juxtaposed to the airway surface or within cells. (Courtesy of Michael Welsh, University of Iowa.)

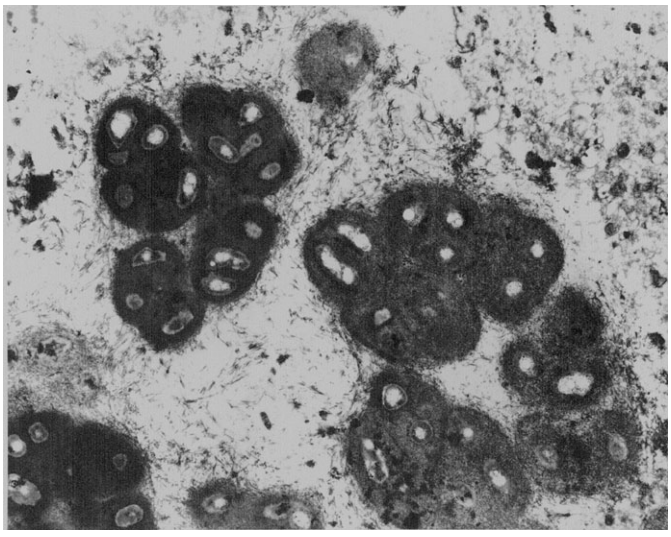

Figure 2. Electron photomicrograph of *Pseudomonas aeruginosa* microcolonies in CF sputum. (Courtesy of Pradeep Singh, University of Iowa.)

will summarize several current hypotheses that have been proposed to explain the predilection for *P. aeruginosa* lung infections in patients with CF and will refer readers to recently published reviews for more details (37–42).

The lack of normal CFTR chloride channel function in the airway epithelium and associated defects in sodium and water transport result in dehydrated airway secretions and mucus plugging (43). The contamination of these mucus plugs by ubiquitous *P. aeruginosa* and its rapid adaptation to the milieu of the airway result in initially intermittent, then chronic, infection. Despite the presence of large numbers of bacteria in the airway lumen, pathologic specimens obtained from patients with CF at the time of lung transplantation show intact epithelia (Figure 1). Numerous *in vivo* studies as well as animal models indicate that few *P. aeruginosa* are actually directly adherent to the airway epithelium. Instead, most of the bacteria are enmeshed in a biofilm composed of bacterial exopolysaccharides as well as host mucin (44). Thus, it is likely that much of the airway inflammation induced by these organisms is caused by the release of immunogenic bacterial components that can gain access to airway epithelium and immune cells in the lung and not have direct contact between bacteria and host cells.

CFTR has been proposed to function as a receptor that increases clearance of *P. aeruginosa*; therefore, lack of CFTR could directly impair host defense against this organism. Although there are substantial *in vitro* data supporting this hypothesis (45, 46), there are no human data that conclusively demonstrate a major role for CFTR in bacterial clearance mechanisms. Several reports have suggested that the activity of antimicrobial peptides is diminished in the purportedly high-salt environment of the CF airway surface liquid (47–50); however, these studies have not been substantiated (51). There is general agreement that the persistent inflammatory response to bacteria infecting CF airways eventually results in lung damage and fibrosis. However, it remains unclear whether excessive inflammation in CF is entirely caused by exogenous bacterial stimulation (52–55) or if CFTR dysfunction leads to endogenous "hyperinflammatory" responses in the CF airway cell.

In a recent study, Mall and colleagues (56) overexpressed individual epithelial sodium channel subunits using an airway cell–specific promoter and generated transgenic mice that absorb excess sodium in their airways. These mice show the key abnormalities of CF: airway obstruction with dehydrated mucus. The inflammation and airway remodeling (increased numbers of mucus-secreting goblet cells) were related to accumulation of cytokines and growth factors within the retained mucus, indicating that the excessive inflammation may be related to the structural changes and increased mucus viscosity. In cultured epithelial cells, mutant CFTR is associated with dysregulated inflammatory response and persistent activation of the transcription factor nuclear factor–κB, implying the chronic inflammation may be related to this persistent activation of NF-κB (57, 58). In addition to evidence that proinflammatory pathways may be upregulated in CF, a recent investigation suggests that a decrease in anti-inflammatory mediators could also influence an exaggerated inflammatory response in CF (59). This study showed that lipoxin, an antiinflammatory mediator, was markedly reduced in patients with CF compared with patients with other inflammatory lung conditions. Thus, there is evidence for a hyperinflammatory state in CF that may be multifactorial and related to structural changes with increased mucus viscosity, increased activation of inflammatory pathways, such as NF-κB, and/or a decrease in anti-inflammatory mediators.

Bacterial factors contribute to the development of chronic infections in CF, particularly the ability of *P. aeruginosa* to form biofilms (60, 61). *P. aeruginosa* isolates recovered from chronically colonized patients with CF are phenotypically different from those collected from other patients or from the environment (Figure 2). Patients with CF have a high frequency of *P. aeruginosa* isolates with a phenotypic variant capable of forming biofilms related to a regulatory protein (PvrR) (62). Alginate production or mucoidy is another prominent feature of *P. aeruginosa* encountered in patients with CF (63). Conversion to a mucoid strain, which is dependent on biofilm formation, has been associated with establishment of chronic infection. In a mouse model of acute lung infection, which compared a nonmucoid *P. aeruginosa* strain (PAO1) to its constitutive alginate-overproducing derivative, alginate production not only impeded

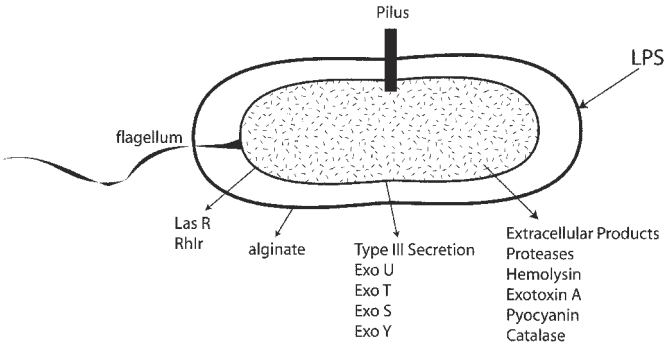

Figure 3. A representative image of *P. aeruginosa* with its virulence factors: surface factors include flagellum, pilus, LPS and secreted factors, including extracellular products, type III secretion proteins, quorum-sensing molecules, and alginate.

pulmonary clearance but also resulted in more severe lung damage (64). Other phenotypic changes in *P. aeruginosa* isolated from patients with CF include lack of flagellin or pilin expression (65–67).

PATHOGENESIS OF *P. AERUGINOSA* LUNG INFECTIONS

P. aeruginosa is a ubiquitous, gram-negative aerobic rod with polar, monotrichous flagella and protein structures on the surface (pili) that are responsible for adherence to respiratory epithelium. The single flagellum provides the bacteria with mobility and kinetic activity. *P. aeruginosa* produces several pigments, a fluorescent yellow-green as well as a blue-green pigment pyocyanin, which give *P. aeruginosa* colonies a characteristic appearance in culture media. The bacteria are widely distributed and can grow in almost any aqueous habitat, including soil, surface waters, sewage, plants, and various foods, such as leafy vegetables and fresh fruit juice (68). *P. aeruginosa* is also a common human intestinal bacterium. These organisms can be found in hand-washing sinks and humidifiers in the hospital environment and are often transmitted by medical personnel through direct health care worker–to-patient transfer (69).

Central to the success of *P. aeruginosa* as an opportunistic pathogen is the genetic flexibility provided by its large genome. Although the function of the majority of the predicted open reading frames in the *P. aeruginosa* PAO1 genome are unknown, many of the genes identified thus far are involved in the regulation, catabolism, and transport of organic compounds (70). Clinical isolates have been found to have pathogenicity islands, which contain discrete group of several genes that directly contribute to virulence (71, 72).

The pathogenesis of *P. aeruginosa* pneumonia is complex, and the outcome of an infection depends on the virulence factors displayed by the bacteria (Figure 3) as well as the host response. The large genome of *P. aeruginosa* (73) provides a tremendous amount of flexibility and the metabolic capabilities to thrive in environments that are inhospitable to most other organisms. A potent array of innate antimicrobial defense molecules expressed within the airway itself (e.g., lactoferrin, antimicrobial peptides, antibodies, neutrophil elastase, reactive oxygen intermediates) prevents colonization of the respiratory mucosa by most other bacteria. Once an organism does elude host mucociliary clearance, it must also adapt to the milieu, compete for iron, and avoid professional phagocytic cells and complement. Depending on the milieu imposed, *P. aeruginosa* mutants are selected that thrive in diverse circumstances. Surface appendages, such as flagella and pili, which are critical for the initial colonization phase of infection, function as ligands for phagocytic cells or stimulate the recruitment of neutrophils. Thus, mutants that fail to express pili or flagella and are less immunogenic are selected and persist (74–76).

Bacterial Factors

Surface components. *P. aeruginosa* expresses a limited number of polar pili, which are involved in attachment to eukaryotic cells. They bind to the GalNacβ1-4 gal moiety exposed on asialylated glycolipids and then activate NF-κB and proinflammatory gene expression through a receptor complex that includes asialoGM1, Toll-like receptor 2 (TLR2), and associated kinases in a lipid raft (77). Because the antigenically dominant epitope of *P. aeruginosa* pili is distinct from the cell-binding domain, strategies to prevent pilin-mediated bacterial adherence have been unsuccessful thus far.

P. aeruginosa also produces polar flagella, which are critical for motility. Flagella are involved in the initial stages of pulmonary infection and activate interleukin (IL-)-8 production by binding to TLR5 on the apical surface of airway epithelial cells (78). Shortly after colonization of the lung, flagella expression is turned off, coincident with the expression of genes involved in biofilm production.

Type III secretion system. *P. aeruginosa* encodes a type III secretion system that is a major determinant of virulence and allows the bacterium to inject toxins into the host cell. The type III secretion system is associated with acute invasive infections and requires pilin-mediated bacterial–epithelial contact (74, 79). This system is activated on contact with eukaryotic cell membranes and interferes with signal transduction, resulting in cell death or alterations in host immune responses. The type III secretion system consists of three components: the secretion apparatus, the translocation or targeting apparatus, and the secreted toxins (effector proteins) and cognate chaperones (80). *P. aeruginosa* secretes four known effector proteins via type III secretion system: ExoS, ExoT, ExoU, and ExoY. ExoT is a bifunctional protein possessing an N-terminal GTPase-activating domain and a C-terminal adenosine diphosphate (ADP-)–ribosyltransferase domain. ExoS, like ExoT, is a closely related ADP-ribosyltransferase and ExoY is an adenylate cyclase. In a recent study, ExoS was shown to induce tumor necrosis factor α (TNF-α) production via an MyD88-dependent pathway through activation of both TLR2 and TLR4. The ability to activate cells expressing TLR2 was attributed to the C terminus of ExoS, whereas the ability to activate TLR4/MD-2 complex was attributed to the N terminus of ExoS (81). ExoU is a potent cytotoxin whose host cellular targets and mechanism of action are not completely known (82–84). A recent study indicated that ExoU is a member of the phospholipase A family of enzymes, possessing phospholipase A2 activity. In mammalian cells, the direct injection of ExoU has been shown to cause irreversible damage to cellular membranes and rapid necrotic death (85). ExoY, the most recently discovered protein, has not been yet implicated directly in cellular toxic effects.

Several animal model systems have demonstrated the importance of type III secretion proteins in acute *P. aeruginosa* infections. The neutralization of the type III secretion proteins has been shown to prevent septic shock and improve survival (86). In a mouse model of *P. aeruginosa* pneumonia, intravenous administration of polyclonal antibodies against PcrV (a protein involved in translocation of type III–secreted toxin) of *P. aeruginosa* resulted in complete survival of the animals (86). Complementary studies in a rabbit model of *P. aeruginosa*–induced septic shock associated with lung injury showed that treatment with anti–PcrV IgG significantly reduced lung injury, bacteremia,

and plasma TNF-α levels compared with animals treated with control IgG, as well as improved hemodynamic parameters (86).

In a human study, the relative risk of mortality was sixfold higher in acute infections associated with expression of the type III secretory proteins ExoS, ExoT, ExoU, or PcrV. Similarly, Hauser and coworkers (76) showed that, in patients with VAP, type III–secreting isolates were associated with worse clinical outcomes. Furthermore, the prevalence of the type III secretion phenotype was found to be significantly higher in acutely infected patients rather than in chronically infected patients with CF, suggesting that the type III protein secretion system is integral to increased *P. aeruginosa* virulence, particularly in the acute setting (87).

Type III secretion protein phenotype analysis may help distinguish respiratory tract colonization from potentially lethal infection. Because antibodies against type III secretory proteins have been protective in animal models, antibodies targeted against these proteins (e.g., anti-PcrV immunoglobulin) may prove to be useful as adjunctive therapy in patients with *P. aeruginosa* infection who demonstrate the type III secretory phenotype.

Quorum sensing (cell-to-cell signaling). In contrast to the invasive properties mediated by type III–secreted gene products, *P. aeruginosa* has also developed a mechanism to coordinate expression of genes important for adaptation to the environment. This response is controlled by quorum-sensing systems, a complex regulatory circuit involving cell-to-cell signaling (88, 89). This signaling mechanism enables *P. aeruginosa* to regulate genes in a density-dependent manner through the production of small diffusible molecules called autoinducers (90).

In many bacteria, quorum-sensing signaling molecules are acyl homoserine lactones (AHL), which are freely diffusible. When a threshold AHL concentration is reached, AHL binds LasR/RhlR transcriptional activators to induce expression of certain genes. *P. aeruginosa* predominately makes two autoinducers: *N*-3-oxododecanoyl homoserine lactone (3-O-C12-HSL, also called PAI-1) and *N*-butyryl-L-homoserine lactone (C4-HSL, also called PAI-2) (90, 91). The activation of the quorum-sensing cascade promotes the formation of biofilms, structured communities that coat mucosal surfaces and invasive devices. The formation of biofilms makes conditions more favorable for bacterial persistence in the lungs. Bacteria in biofilms are inherently more difficult to eradicate than those in the planktonic form (92, 93). It is important to note that production of alginate, the mucoexopolysaccharide characteristic of *P. aeruginosa* isolated from patients with CF, is a separate genetic event, even though these organisms also are enmeshed in a protective carbohydrate polymer.

Quorum-sensing molecules have the potential to directly modulate the host immune system. Smith and coworkers (94) showed that AHL induced cyclooxygenase-2 (COX-2) production in host cells through activation of NF-κB. COX-2 expression was linked to production of Prostaglandin E_2 (PGE2), which has been shown to induce mucus secretion and promote vasodilation and edema. Tateda and colleagues (95) showed that AHL were able to induce apoptosis of neutrophils and macrophages but not epithelial cells. Quorum-sensing molecules have also shown to increase the production of inflammatory cytokines from airway cells (96, 97) and macrophages (98). These studies suggest that AHL not only regulate bacterial adaptation to the lungs but also modulate functions of eukaryotic cells important for inflammation and immune defenses.

Recent studies suggest that macrolide antibiotics inhibit quorum sensing and biofilm formation by *P. aeruginosa* (99). Although macrolides lack intrinsic antibacterial activity against *P. aeruginosa,* after prolonged exposure they inhibit synthesis of gene products that are expressed during late logarithmic and stationary phase of growth, such as the gene products involved in quorum sensing (100). Although bacterial density is not altered by drugs, such as azithromycin, immunostimulatory exoproduct expression may be inhibited. Clinical trials in patients with CF have shown modest improvement in lung function, and there is considerable enthusiasm for the use of these well-tolerated antibiotics in CF.

Iron scavenging. A successful human pathogen needs to be able to acquire iron from host tissues, where it is tightly bound to transferrin or, in the airways, lactoferrin (101). *P. aeruginosa* and other human pathogens must be able to compete with transferrin and lactoferrrin for iron, and they have developed a complex regulatory system to accomplish this. *P. aeruginosa* produces two major siderophores: pyochelin and pyoverdin (102–104). These siderophores bind iron efficiently and are then taken up by the bacteria through specific cell-surface receptors. The siderophores are major virulence factors important not only for providing iron to support bacterial metabolic processes but also for controlling the expression of other *P. aeruginosa* virulence factors, such as exotoxin A, endoprotease, and pyoverdine itself (105).

Other virulence factors. In addition to the type III–secreted proteins and the quorum-sensing systems, *P. aeruginosa* expresses many other virulence factors that contribute to its pathogenicity. Some of these factors help colonization, whereas others facilitate bacterial invasion. Bacterial colonization involves multiple factors, including fimbriae or pili, flagella, and surface polysaccharides. Tissue invasion by *P. aeruginosa* is promoted by the production of elastase, alkaline proteases, hemolysins (phospholipase and lecithinase), cytotoxin (leukocidin), siderophores with their uptake systems, and diffusible pyocyanin pigment. *P. aeruginosa* elastases cleave collagen, IgG, IgA, and complement. The elastases disrupt the integrity of the epithelial barrier by disrupting epithelial cell tight junctions and interfering with mucociliary clearance. *P. aeruginosa* elastase degrades surfactant proteins A and D (SP-A and SP-D), which have an important role in innate immunity (106). Alkaline proteases lyse fibrin, interfere with fibrin formation, and inactivate important host defense proteins, such as antibodies, complement, IFN-γ, and cytokines. Leukocidin is a pore-forming protein that has cytotoxic effects on host cells. Phospholipase and lecithinase are hemolysins that act synergistically to break down lipids and lecithin. These proteins promote invasion by causing cytotoxic effects on host cells (107).

Most *P. aeruginosa* strains secrete pyocyanin (N-methyl-1-hydroxyphenazine), the pigment that gives blue-green color to the bacterial colonies (108). High concentrations of pyocyanin are detected in pulmonary secretions of patients with CF, where it exerts a proinflammatory effect, disrupts the bronchial epithelium, and impairs ciliary function. Pyocyanin also interferes with the antioxidant defenses in the lung and facilitates oxidative damage to the lung epithelium through inhibition of catalase activity (109, 110).

P. aeruginosa can cause direct tissue damage and necrosis, often a consequence of exotoxin A production. Exotoxin A is an exceptionally potent ADP-ribosylating enzyme that enters eukaryotic cells by receptor-mediated endocytosis and catalyzes the ADP-ribosylation of eukaryotic elongation factor-2. This inhibits protein synthesis, ultimately leading to cellular death (111, 112). Exotoxin A facilitates the dissemination of infection and is highly lethal in animal models of infection. Increased titers of anti–exotoxin A antibodies in serum from patients with *P. aeruginosa* sepsis has shown to be associated with better survival in some human studies (113, 114)

LPS is another important component of *P. aeruginosa* and other gram-negative bacteria. The antigenic O-side chains of LPS were the basis of serologic typing schemes historically used

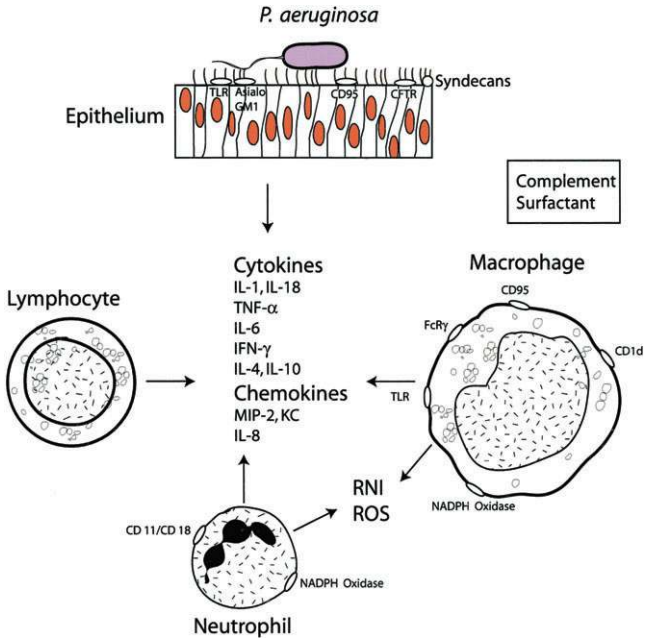

Figure 4. Host responses to *P. aeruginosa*. Airway epithelial cells, macrophages, neutrophils, and lymphocytes release mediators that enable the host to mount a response to the invading bacteria. IL = interleukin; MIP = macrophage inflammatory protein; NADPH = nicotinamide adenine dinucleotide phosphate-reduced; RNI = reactive nitrogen intermediates; ROS = reactive oxygen species; TLR = Toll-like receptor; TNF-α = tumor necrosis factor α.

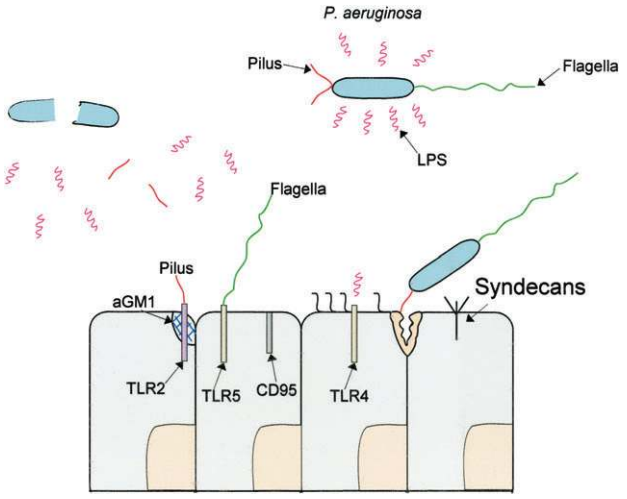

Figure 5. Interaction of *P. aeruginosa* and individual bacterial products with important receptors on airway epithelial cells.

for *P. aeruginosa* epidemiologic studies before genomic typing methods were available (115). Environmental isolates of *P. aeruginosa* typically express smooth (typable) LPS with long O-side chains, as opposed to the strains that have adapted to CF lung, which are often nontypable and have lost these O-side chains. Ernst and coworkers (67) have demonstrated that LPS from CF isolates has a characteristic penta- or hexa-acylated lipid A structure. This is associated with increased immunogenicity, although it should be noted that *P. aeruginosa* LPS is much less immunogenic and evokes a more modest cytokine response from macrophages than *Escherichia coli* or *Salmonella* LPS (67).

Another extracellular polysaccharide expressed by CF isolates of *P. aeruginosa* is alginate, usually the consequence of a bacterial *muc* mutation (116). This polymer of D-mannuronic acid and L-glucuronic acid is generally pathognomonic for CF isolates of *P. aeruginosa* (117). Although these organisms *in vivo* produce copious amounts of slimy exopolysaccharide, the phenotype is relatively unstable, often reverting to the wild-type phenotype on subculture.

Host Factors

The clearance of *P. aeruginosa* from the airways involves the coordinated effort of multiple cell types, including respiratory tract epithelium and both resident and recruited phagocytic cells (Figure 4). In addition to innate immunity, adaptive immune responses are also required for effective host defense, especially in cases of chronic bacterial infections. We will discuss the roles of different host cell types in *P. aeruginosa* infection, followed by a discussion of individual mediators that have been shown to be important in host defense against this pathogen.

Role of individual cell types. RESPIRATORY EPITHELIUM. The airway epithelium is remarkably resistant to bacterial invasion. Epithelia provide a formidable mucosal barrier and contribute mucociliary clearance functions. The tight junctions between cells help to prevent microbial invasion (118–120). In addition, there are numerous signaling cascades that result in epithelial expression of mucins (121), antimicrobial peptides (122, 123), and chemokines (124–126) to recruit and activate neutrophils. Normal epithelial cells secrete antimicrobial peptides (127, 128), such as β-defensins and lactoferrins, which directly contribute to host defense (129). There are few bacterial receptors displayed on the apical surface of airway epithelial cells, which may serve to prevent inadvertent activation by transient contamination of the lower airways. However, in response to a significant bacterial challenge or exposure to bacterial products, airway cells actively mobilize signaling components to the apical surface of the airway cell to initiate inflammatory responses (77). As shown in Figure 5, *P. aeruginosa* interacts with epithelial cells via cell-surface receptors, such as asialoGM1 and TLRs, to induce signal transduction cascades leading to production of inflammatory mediators. Contact between *P. aeruginosa* and airway epithelial cells results in clustering of asialoGM1 receptors on the epithelium at sites of bacterial contact (Figure 6).

Several bacterial components, including both *P. aeruginosa* flagella and pili, activate an intracellular calcium-dependent signaling cascade that includes Ras, Src, and Erk 1/2MAP (mitogen-activated protein) kinases (130, 131). *P. aeruginosa* flagellin has also been shown to stimulate matrilysin expression in lung epithelial cells, and the overexpression of this proteinase in CF lungs may contribute to the lung pathology (132).

Cytotoxic bacteria that express the type III secretion system attach to the epithelium and produce specific toxins that interfere with cytoskeletal function cause the loss of tight junctions, and expose the basolateral surface of the epithelium. Damaged airway epithelium is much more readily colonized by bacteria, as increased amounts of the receptor asialoGM1 are available on the basolateral aspects of the epithelial cell. Thus, once the epithelial barrier is breached, the organisms can invade and may disseminate.

P. aeruginosa can induce apoptosis of a variety of cell types, including bronchial epithelial cells. The role of epithelial cell apoptosis in the pathogenesis of *P. aeruginosa* lung infection is currently not well defined. In one model system, apoptosis induced by the interaction of CD95 and CD95 ligand was found to be critical for the clearance of *P. aeruginosa* from murine

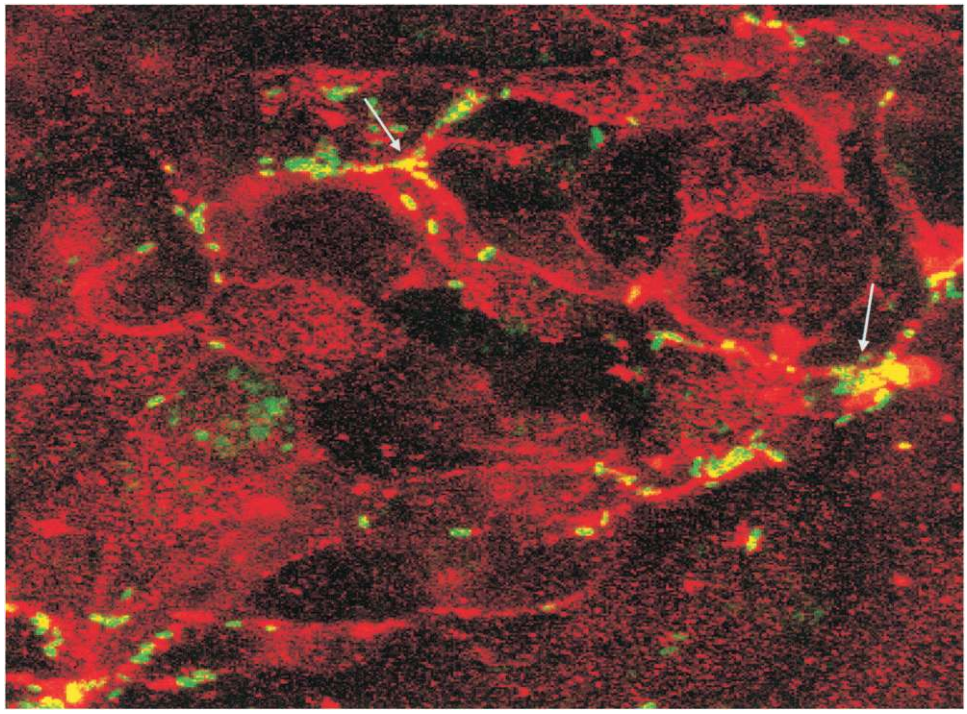

Figure 6. Distribution of bacterial receptors. AsialoGM1 (*red*) and green fluorescent protein–labeled *P. aeruginosa* (*green*) colocalize (*yellow; see arrows*) at regions of cell–cell contact in polarized airway epithelial cells.

lungs through the internalization of apoptotic cells containing ingested bacteria (133). Apoptosis induced by the CD95–CD95 ligand interaction requires clustering of the CD95 receptors, which involves the generation of ceramide by the action of acid sphingomyelinase on sphingolipids (134). *P. aeruginosa* infection can trigger activation of acid sphingomyelinase and induce the formation of lipid rafts containing CD95, which appear to regulate apoptosis and cytokine responses by infected cells (135). In contrast, infections caused by more indolent organisms, such as those that form biofilms and fail to invade, have not been found to be associated with apoptosis, even when high inocula are used to infect murine airways (136–138).

Immune cells. *Macrophages*. Alveolar macrophages, the resident mononuclear phagocytes of the lung, provide the first line of defense against organisms that reach the lower airways. They have roles in both the innate and adaptive immune responses to infection. In addition to their phagocytic function, alveolar macrophages can synthesize and release various protein and lipid mediators on contact with pathogens or pathogenic substances. Macrophages become activated when microbial products bind to cell-surface receptors, including TLRs (139, 140).

In response to *P. aeruginosa* infection of the distal airways, macrophages have the capacity to ingest bacteria and produce inflammatory mediators that are important for host defense. The interaction of *P. aeruginosa* with macrophages occurs via multiple cell surface receptors and is accompanied by the formation of pseudopods. Several phagocytosis-promoting receptors, including a variety of Fc receptors, complement receptor 1 and 3, and macrophage mannose receptor, have been implicated in phagocytosis of *P. aeruginosa* (141). The phagocytic response also requires the small GTPases Rac1 and Cdc42 (142). *P. aeruginosa* is ingested by macrophages in a unique two-step glucose-dependent process. Binding occurs in the absence of sugars, but ingestion requires D-glucose or D-mannose and is delivered via facilitative glucose transporter GLUT1 to the macrophage (143).

Although lung macrophages have the capacity to participate in the host response to *P. aeruginosa*, the role of alveolar macrophages in acute *P. aeruginosa* infection has not been clearly defined. In studies using strategies to eliminate macrophages or investigate the importance of macrophage phagocytosis in lung host defense, contradictory findings have been reported concerning the requirement for macrophages in the protective response to acute *P. aeruginosa* pneumonia. Kooguchi and coworkers (144) reported that depletion of lung macrophages by aerosolized clodronate-containing liposomes resulted in decreased early neutrophil recruitment and chemokine production. This treatment resulted in persistent neutrophilic alveolitis and decreased bacterial clearance at 48 hours, suggesting that macrophages were important for the coordinated innate immune response to *P. aeruginosa* infection. A separate study, however, found that depletion of alveolar macrophages by 78 to 88% using airway administration of liposomal clodronate did not affect survival or bacterial clearance in mice infected with *P. aeruginosa* (145). Also, tissue macrophage depletion using intravenous liposomal clodronate treatment 48 hours before infection did not impact the severity of bacteremia or the survival of infected mice, but decreased plasma TNF-α, macrophage inflammatory protein (MIP)-2, and IL-10 levels (146).

In a syngenic bone marrow transplantation model in mice, increased susceptibility to *P. aeruginosa* pneumonia after engraftment was not found to be related to altered neutrophil recruitment, but rather, defective phagocytosis of the bacteria by alveolar macrophages (147). In contrast, other studies using strains of mice resistant or susceptible to *P. aeruginosa* infection concluded that the ability of macrophages to ingest and kill bacteria was not a critical determinant of the outcome of the infection (148).

Neutrophils. The recruitment of neutrophils is a major component of the protective host response to *P. aeruginosa*, and appears to outweigh the contributions of other immune cells, at least in the acute setting (149). The depletion of neutrophils results in excessive mortality after *P. aeruginosa* infection (126) and resistant (BALB/c) mice have a substantially greater neutrophil influx into the lungs than susceptible (DBA/2) mice in re-

sponse to *P. aeruginosa* (148). Neutrophil recruitment in *P. aeruginosa* is dependent on production of neutrophil chemotactic chemokines. The potential cellular sources of neutrophil chemokines (CXC chemokines) include macrophages, lung epithelium, endothelial cells, and lymphocytes. In a mouse model of intratracheal administration of *P. aeruginosa*, antibody-mediated depletion of a single CXC chemokine (MIP-2 or KC) resulted in modest changes in neutrophil influx but no change in survival or bacterial clearance. However, neutralization of the receptor CXCR2 resulted in a striking increase in mortality, which was associated with a marked decrease in neutrophil recruitment and bacterial clearance (126).

In addition to chemokines and their receptors, Kumasaka and coworkers (150) showed that the emigration of neutrophils into the lungs was dependent on CD11/18 in a rabbit model of acute *P. aeruginosa* pneumonia. Similar studies in murine models of *P. aeruginosa* pneumonia confirmed that the presence of CD11a(b)/18 is required for neutrophil emigration to the lungs in response to *P. aeruginosa* infection (151, 152). Urokinase-type plasminogen activator receptor (uPAR), a modulator of β_2 integrins, may also have a role in host response to *P. aeruginosa* infection. Mice deficient in uPAR were found to have diminished neutrophil recruitment in response to *P. aeruginosa* lung infection compared with the wild-type mice (153). *P. aeruginosa* pyocyanin has been shown to induce apoptosis of neutrophils and may be a mechanism by which the bacteria resist host defenses, leading to persistent infection (154, 155)

T lymphocytes. The role of lymphocytes in the immune response against *P. aeruginosa* is not well characterized, but some studies have shown an immunomodulatory role for T cells, particularly during chronic *P. aeruginosa* infections (156). Mice strains show variability in T-cell responses that correlates with susceptibility to *P. aeruginosa* infection. A Th2-dominated pulmonary response is seen in susceptible strains, whereas mice that are resistant to *P. aeruginosa* infection show a Th1-dominated pulmonary response (157, 158). There is also evidence that a Th1-dominated immune response improves the prognosis of patients with CF with chronic *P. aeruginosa* lung infections. In a small human study of 14 patients with CF and *P. aeruginosa* infection, a Th1-type immune response was associated with better lung function compared with those patients that had a predominant Th2 response (159, 160). Thus, the type of T-cell response in the lung may contribute to the level of resistance to *P. aeruginosa*. Future studies will help delineate the mechanisms by which these effects are mediated and may help develop therapies to modulate the host response using specific T-cell products or cytokines.

A recent study suggests that CD1d-restricted T cells appear to play a key role in host defense against *P. aeruginosa* in acute infections. CD1 is a major histocompatibility complex (MHC) class I–like protein that is expressed on the macrophage and presents glycolipid antigens to CD1-restricted T cells. These CD1d-restricted natural killer T cells play an important role in defending against bacteria. In a murine *P. aeruginosa* pneumonia model, CD1d knockout mice showed markedly reduced pulmonary eradication of *P. aeruginosa* compared with wild-type mice. The treatment of wild-type mice with α-galatosyl-ceramide, a lipid that activates CD1d-restricted T cells, was associated with rapid pulmonary clearance through enhanced phagocytosis by alveolar macrophages (161).

Molecules important for host defense. TOLL-LIKE RECEPTORS. TLRs are a family of pattern-recognition molecules that initiate intracellular signaling cascades on exposure to specific microbial components. Eleven TLRs have been identified in humans (12 in mice), and these receptors are predominantly expressed on cell types that are likely to encounter microbes (162). Phagocytic cells, such as macrophages, neutrophils, and dendritic cells, exhibit the broadest repertoire and express the highest levels of TLRs. However, it is increasingly recognized that other cell types, such as epithelial cells, express TLRs. TLRs initiate a signal transduction cascade that results in the activation of several intracellular pathways, leading to activation of MAP kinases, NF-κB, and activated protein (AP)-1 (163).

Several members of the TLR family interact with surface components of *P. aeruginosa*. TLR2 is engaged by pili and appears to play an important role in epithelial activation by *P. aeruginosa*. Furthermore, a recent study by Lorenz and coworkers (164) indicates that TLR2 may also interact with nonpilus adhesins. *P. aeruginosa* flagella have been shown to initiate signaling through TLR5 and TLR2 (76, 165). Recent data also indicate that ExoS of *P. aeruginosa* may activate monocytes by binding to both TLR2 and TLR4 (81). In addition, *P. aeruginosa* has been shown to signal through TLR4 with its LPS moiety (166–168). Although TLR4 is expressed in airway epithelial cells, it does not appear to be prominently involved in signaling of *P. aeruginosa* presented at the apical surface of polarized epithelial cells (169, 170).

The TLRs induce signal transduction via adaptor proteins, five of which have been described. It has been shown that different TLRs interact with distinct adaptor proteins. Differential use of these adaptor molecules may provide the specificity in the TLR signaling (171). The role of myeloid differentiation factor 88 (MyD88), which is one of the key adaptor proteins used by TLRs, has been investigated in the host response to *P. aeruginosa* lung infections. Skerrett and colleagues (172) compared the responses of MyD88-deficient and wild-type mice to pulmonary infection with *P. aeruginosa* and *S. aureus*. They showed that MyD88-dependent signaling is integral to the initiation of cytokine and inflammatory responses to both pathogens after infection of the lower respiratory tract; however, MyD88 is essential for innate immunity to *P. aeruginosa* but not *S. aureus*. Another recent study confirmed that MyD88-deficient mice have impaired bacterial clearance of *P. aeruginosa* from the lungs compared with the wild-type controls. This defect in host defense was found to be related to impaired activation of NF-κB and reduced production of mediators, such as MIP-2, TNF-α, and IL-1β (173). An understanding of the specific pathways downstream of individual TLRs will provide insights into mechanisms involved in the pathogenesis of bacterial infection and may even help develop new therapies for immunomodulation.

Cytokines and chemokines. Pulmonary infection caused by *P. aeruginosa* is associated with increased production of various cytokines that regulate lung host defense and inflammation. The role of proinflammatory cytokines is intriguing, as most data suggest that TNF-α is critical for activating phagocytic cells to clear the bacteria. However, IL-1 and IL-18 seem to have deleterious effects in host defense against *P. aeruginosa*. Neutrophil chemotactic chemokines are clearly important for neutrophil recruitment after *P. aeruginosa* infection. Experimental work has also demonstrated a beneficial effect of IL-4 and IL-10 in host defense against *P. aeruginosa*. Although the effect of many of these cytokines has been investigated in animal models, their role in human disease has not been established.

The role of TNF-α has been studied by a number of investigators. Most studies suggest that TNF-α plays an important role in innate immunity and confers a protective effect in animal models of *P. aeruginosa* lung infection. The differences in the host response to *P. aeruginosa* among inbred mice have shown to be related to altered TNF-α production. Morissette and colleagues (174) showed that a defect in TNF-α production renders DBA/2 mice susceptible to *P. aeruginosa* infection. Similarly Gosselin and colleagues (175) have shown that BALB/c mice,

which are known to be resistant to *P. aeruginosa* infection, succumb to *P. aeruginosa* lung infection if treated with anti-TNF antibodies. These studies suggest that inherent differences in the TNF-α production may contribute to the differences in the host response in these mice. In another study, Chen and coworkers (176) administered replication-deficient adenoviruses expressing TNF-α to mice that received a bacterial challenge with *P. aeruginosa* after cecal ligation and puncture. They showed that TNF-α expression enhanced bacterial clearance and improved survival by 25% compared with control animals. Perhaps the more convincing evidence of the role of TNF-α in host defense against *P. aeruginosa* is provided by studies of TNF-α knockout mice, which exhibit a major defect in bacterial clearance of *P. aeruginosa* (177). Although several studies point to a beneficial effect of TNF-α in host response to *P. aeruginosa,* mice deficient in TNF receptors have not shown a diminished resistance to infection with *P. aeruginosa* in some animal models (178, 179). The differences in the results may be related to the study design or the model of infection used. The exact mechanism by which TNF-α confers host resistance is not known.

Although TNF-α appears to be necessary for effective defense against *P. aeruginosa*, the IL-1 family of cytokines appears to have deleterious effects on the host. IL-1 receptor type 1 (IL-1R–)–deficient mice were found to be protected from *P. aeruginosa* infection as reflected by increased *P. aeruginosa* replication in wild-type mice as compared with *IL-1R*−/− mice. Treatment of wild-type mice with IL-1R antagonist also improved clearance of *P. aeruginosa* (180).

IL-18 is a member of the IL-1 family of ligands and is produced mainly as a precursor protein (24 kD) that requires proteolytic activation by an IL-1β–converting enzyme to liberate the 18-kD mature active protein (181). IL-18 is secreted mainly by macrophages and stimulates IFN-γ production by natural killer cells and T cells (182). The IL-18 receptor system and its signal transduction pathway are analogous to those of IL-1. In addition to IFN-γ, IL-18 can induce the synthesis of IL-1β, TNF-α, and various chemokines, probably through the activation of NF-κB (183–185). The role of IL-18 in *P. aeruginosa* lung infection was recently investigated using IL-18 null mice (184). Compared with wild-type control mice, mice deficient in IL-18 had increased bacterial clearance and did not show dissemination of the bacteria. Similarly, the neutralization of IL-18 in wild-type mice resulted in improved bacterial clearance and host survival (184). Thus, it is apparent that both IL-1 and IL-18 activity impairs bacterial clearance in *P. aeruginosa* lung infections; however, the molecular mechanisms by which these cytokines impair host defense in this setting have not been elucidated.

Studies have demonstrated that several *P. aeruginosa* virulence factors are potent inducers of neutrophil chemotactic chemokines (CXC), particularly human IL-8. Bacterial products that induce chemokine production include pili, flagella, peptidoglycan, and the homoserine lactone autoinducer (186). These products may initially interact with discrete receptors, but downstream activation of NF-κB and/or MAP kinases is necessary to regulate CXC chemokine expression (187). Animal models of *P. aeruginosa* lung infection have demonstrated an increased level of CXC chemokines in bronchoalveolar lavage of mice infected with *P. aeruginosa,* and blockade of CXC chemokine receptors impairs neutrophil recruitment and host defense (126).

IFN-γ plays an essential role in the host response to many pathogens, but its role in *P. aeruginosa* pneumonia is not clearly defined. The administration of exogenous IFN-γ via adenoviral vectors before bacterial challenge with *P. aeruginosa* has been shown to enhance bacterial clearance (188). In this model, there was an increase in production of TNF-α and an increased expression of alveolar macrophage MHC-II molecules related to overexpression of IFN-γ. In addition, using a rat model of chronic *P. aeruginosa* lung infection, Johansen and coworkers (189) administered recombinant IFN-γ by intraperitoneal injection and found a significant reduction in the severity of lung inflammation in IFN-γ–treated rats compared with control animals. In contrast, IFN-γ–receptor null mice demonstrated enhanced clearance of *P. aeruginosa* from their lungs when compared with wild-type mice (190). In this study, IFN-γ–receptor null mice had similar levels of cytokines, but higher nitric oxide (NO) levels in bronchoalveolar lavage as compared with wild-type mice. These studies suggest that endogenous IFN-γ expression does not benefit host defense against *P. aeruginosa*, but increased levels of IFN-γ may improve bacterial clearance. The reasons for these disparate findings are not apparent and further studies are needed before any definitive conclusion can be drawn about the beneficial or detrimental effect of this cytokine.

IL-10 is an antiinflammatory cytokine that has been shown to be important in sepsis and in the evolution of bacterial pneumonia (191–193). Antiinflammatory cytokines are involved in regulating the potentially damaging effects of neutrophilic inflammation. IL-10 null mice show prolonged and excessive proinflammatory cytokine production and neutrophil infiltration in the airways after *P. aeruginosa* infection (194). Similarly, Sawa and colleagues (195) reported improved survival from *P. aeruginosa* pneumonia after administration of IL-10 to mice infected with cytotoxic strains of *P. aeruginosa*. These studies demonstrate that antiinflammatory signaling pathways (in addition to proinflammatory signaling pathways) are important in *P. aeruginosa* pneumonia.

IL-4 is produced primarily by Th2 lymphocytes and by mast cells. IL-4 induces the differentiation of uncommitted precursor CD4+ T cells toward the Th2 subset and inhibits the differentiation of Th1 cells, in addition to enhancing differentiation and activation of various inflammatory cells. Transgenic mice overexpressing IL-4 in respiratory epithelial cells are able to clear *P. aeruginosa* much more rapidly than are wild-type mice (196). Intranasal administration of IL-4 enhances bacterial clearance from the lungs of wild-type mice and decreases mortality after infection (196). The protective effects of IL-4 may be related to its ability to modulate leukocyte function. IL-4 enhances expression of complement receptors CR1, CR3, and CR4 and increases complement-dependent phagocytosis. IL-4 is also a potent stimulator of mannose receptor expression on macrophages (197).

Complement system. The complement system has been shown to be important in host defense against *P. aeruginosa*. C5a receptor–deficient mice were unable to clear *P. aeruginosa* despite a marked increase in neutrophil influx and succumbed to pneumonia (198). Complement-deficient mice were also found to have a higher mortality after *P. aeruginosa* inoculation into the airway (199, 200). The lack of complement or its receptor did not affect the nature of the inflammatory response; however, complement-dependent killing appears to have an important role in *P. aeruginosa* clearance and host survival.

Reactive oxygen and nitrogen intermediates. Microbicidal effects of phagocytes are mediated by the generation of reactive oxygen species and reactive nitrogen species (201). A principal mechanism for generation of reactive oxygen species is the nicotinamide adenine dinucleotide phosphate-reduced oxidase system present in macrophages and neutrophils. Reactive oxygen species are capable of killing *P. aeruginosa in vitro* and *in vivo*. Mice deficient in the p47*phox* component of nicotinamide adenine dinucleotide phosphate-reduced oxidase have impaired activation of NF-κB and production of NF-κB–dependent cytokines despite increased *P. aeruginosa* proliferation (202). Therefore, nicotinamide adenine dinucleotide phosphate-reduced oxidase appears to contribute to host defense against *P. aeruginosa* not

only through microbicidal action but also through modulation of redox-sensitive signaling pathways (e.g., NF-κB) in phagocytes.

NO is another key mediator produced in many inflammatory and infectious conditions by inducible NO synthase. The antimicrobial effects of NO have been demonstrated against a variety of pathogens (203). In a rat model of *P. aeruginosa* pneumonia, the administration of inhaled NO markedly reduced pulmonary bacterial load and lung myeloperoxidase activity (204). However, defining the role of inhaled NO as an antimicrobial agent against *P. aeruginosa* requires further investigation.

Syndecans. Syndecans are a family of cell-surface heparan sulfate proteoglycans expressed on epithelial cells. Syndecans bind and modulate the activity of a diverse group of soluble and insoluble ligands, such as extracellular matrix components, growth factors, chemokines, cytokines, and proteases, through the action of their heparan sulfate chains (205). LasA production by *P. aeruginosa* was found to markedly stimulate the shedding of syndecan-1 ectodomains, whereas other gram-negative bacteria had only low levels of activity to induce shedding (206). Syndecan shedding is a protective response activated during tissue injury. Shed syndecan-1, via the heparan sulfate chains, binds to and neutralizes proinflammatory cytokines. Thus, by enhancing the shedding of syndecan, *P. aeruginosa* takes advantage of a host-protective mechanism to promote its survival. The shed ectodomains also inhibit the activity of antimicrobial peptides released by the host (207, 208). Syndecan-1 null mice are resistant to *P. aeruginosa* lung infection, suggesting the importance of this mechanism in pathogenesis of *P. aeruginosa* pneumonia (209).

Surfactant proteins. SP-A and SP-D are members of the collectin family of mammalian lectins that contribute to distinct aspects of pulmonary host defense. The immunomodulatory role of the surfactant proteins has been reviewed (210–212). Surfactant proteins enhance the phagocytosis and killing of microbes. Both *in vitro* and *in vivo* studies provide evidence that SP-A and SP-D have important roles in the innate immune response to *P. aeruginosa* (212). Levels of SP-A and SP-D have been shown to be decreased in the lungs of patients with CF (213, 214). The binding of SP-A or SP-D is associated with agglutination of *P. aeruginosa* (215); however, SP-D is the more potent agglutinating agent (216, 217).

SP-A–deficient mice have an increased inflammatory cell infiltrate and proinflammatory cytokines in response to *P. aeruginosa* infection (218). However, because SP-A does not bind to *P. aeruginosa in vitro*, it is likely that SP-A enhances phagocytosis of *P. aeruginosa in vivo* by stimulating macrophage activity by a process independent of opsonization (218). *In vitro* studies support a role for SP-D in phagocytosis of mucoid and nonmucoid strains of *P. aeruginosa* (219, 220) SP-D can enhance phagocytosis of *P. aeruginosa* expressing either smooth or rough LPS (215). Surfactant proteins may also have a direct microbicidal function (215). A recent study showed that *P. aeruginosa* elastase causes degradation of SP-A and SP-D, suggesting that inactivation of these important host defense molecules is a mechanism of virulence used by *P. aeruginosa* (106).

SUMMARY AND CONCLUSIONS

P. aeruginosa is an exceptionally diverse, opportunistic pathogen capable of many different types of interactions in the lung. Depending on the environmental conditions and the immune status of the host, *P. aeruginosa* can be a quiescent colonizer, enmeshed in a carbohydrate biofilm, only occasionally shedding a few gene products capable of immunostimulation. *P. aeruginosa* can also be highly virulent invader, attaching to damaged epithelial cells, injecting toxins that interfere with eukaryotic cytoskeletal integrity, and rapidly triggering apoptosis and breaches in epithelial integrity. The recognition that different clinical entities may be the consequence of infection by the same organism is important in devising strategies to prevent or treat *P. aeruginosa* pulmonary infection.

A great deal has been learned about the epidemiology and behavior of *P. aeruginosa* in biofilms, such as those present in infected lungs of patients with CF. Strategies to impair biofilm formation through drugs that block quorum-sensing systems are being actively sought. The understanding that these biofilms are dynamic bacterial communities provides targets for therapy. The recent success of azithromycin in CF may reflect its activity in blocking quorum-sensing systems *in vivo*.

Analysis of the complete genome sequence of *P. aeruginosa* reveals many clues to the versatility of this pathogen. The large genome and the genetic complexity allow *P. aeruginosa* to thrive in diverse ecologic conditions. Multiple bacterial virulence factors impact the pathogenesis of *P. aeruginosa* infections.

Host factors have been extensively explored in the pathogenesis of *P. aeruginosa* infection. The respiratory epithelium plays a critical role in defending against *P. aeruginosa*. The role of lipid rafts and clustering of cell-surface receptors in host defense against *P. aeruginosa* is being investigated and these studies will provide further insights into mechanisms that modulate host response. Neutrophils are the key phagocytic cells involved in bacterial clearance, with some contribution by the alveolar macrophage. Alveolar macrophages participate in generating the host inflammatory response through activation of cell-surface receptors and production of mediators, such as cytokines and chemokines. The role of T cells is being investigated and suggests that a Th1-type response is beneficial to the host. Studies with the CD1d-restricted T cells suggest that activation of CD1d cells by lipids may also help to enhance bacterial clearance in *P. aeruginosa* pneumonia.

A variety of cytokines play important roles in the host response to *P. aeruginosa* infection. TNF-α production appears to be critical in bacterial clearance, but IL-1 and IL-18 are counterproductive. Precise mechanisms by which these effects are mediated require further investigations, and it is uncertain whether neutralization of these cytokines would be beneficial in human lung infections. The modulation of other mediators, including CXC chemokines and the cytokines IL-4 and IL-10, has promise for future human studies.

The recognition that shed bacterial components efficiently activate proinflammatory signaling in epithelial cells may provide novel targets for antiinflammatory therapy. The control of airway inflammation through modulation of inflammatory signaling may prove to be an effective mode of therapy to prevent lung injury. The development of vaccines directed at flagella or pili may be useful in preventing the initial acquisition of environmental isolates, either in CF or in an intensive care unit setting, but is unlikely to be useful in established infection caused by organisms that no longer express flagella or pili. The prevention of invasive infection associated with type III–secreted toxins of *P. aeruginosa*, which are rarely expressed in CF isolates, is a realistic goal in an intensive care unit or nosocomial setting. It is hoped that better understanding of host immune mechanisms will lead to the development of successful adjuvant therapies targeted toward immunomodulation.

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