Mechanisms and Targeted Therapies for *Pseudomonas aeruginosa* Lung Infection

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Abstract

Pseudomonas aeruginosa is a complex gram-negative facultative anaerobe replete with a variety of arsenals to activate, modify, and destroy host defense mechanisms. The microbe is a common cause of nosocomial infections and an antibiotic-resistant priority pathogen. In the lung, *P. aeruginosa* disrupts upper and lower airway homeostasis by damaging the epithelium and evading innate and adaptive immune responses. The biology of these interactions is essential to understand *P. aeruginosa* pathogenesis. *P. aeruginosa* interacts directly with host cells via flagella, pili, lipoproteins, lipopolysaccharides, and the type III secretion system localized in the outer membrane. *P. aeruginosa* quorum-sensing molecules regulate the release of soluble factors that enhance the spread of infection. These characteristics of *P. aeruginosa* differentially affect lung epithelial, innate, and adaptive immune cells involved in the production of mediators and the recruitment of additional immune cell subsets. Pathogen interactions with individual host cells and in the context of host acute lung infection are discussed to reveal pathways that may be targeted therapeutically.

Keywords: quorum sensing; type III secretion system; immunology; therapeutics; *Pseudomonas aeruginosa*

Contents

Pseudomonas aeruginosa is a predominant organism within the hospital environment, an increasingly multidrug-resistant microbe, and the most common gram-negative pathogen causing nosocomial pneumonia in the United States (1, 2). Nearly all *P. aeruginosa* infections are associated with compromised host defenses, which may include patients with severe burns, diabetes, cancer, organ transplants, or additional immunodeficiencies (3). In the lung, *P. aeruginosa* is known to opportunistically colonize patients with cystic fibrosis (4) and chronic obstructive pulmonary disease (5). The biology and impact of *P. aeruginosa* chronic lung infection in patients with cystic fibrosis and chronic obstructive pulmonary disease have been extensively reviewed elsewhere (6–9). The aim of this review is to assess the

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pathogenesis of acute *P. aeruginosa* lung infections and to provide insight into potential host- and pathogen-associated therapeutic targets.

P. aeruginosa is a leading cause of acute nosocomial infections and pneumonias in particular (10–12). Nosocomial pneumonia has a mortality rate ranging from 13% to 50%, lengthens hospital stays, and adds approximately US$40,000 in excess cost per patient (13, 14). Ventilator-associated pneumonia (VAP) is a significant cause of morbidity and mortality in critically ill patients, and the isolation of *P. aeruginosa* is associated with worse clinical outcomes (15). Prior exposure to quinolones and carbapenems, commonly used in ICUs, is linked to the development of multidrug-resistant *P. aeruginosa* (16), making this pathogen of great concern. *P. aeruginosa* expresses efflux pumps, β-lactamases, impermeable outer membrane proteins, and an adaptable genome that allows the microbe to acquire resistance to many classes of antibiotics (17). The number of multidrug-resistant *P. aeruginosa* strains (resistant to one or more drugs in three or more antibiotic categories) has steadily increased, and many clinical isolates possess carbapenem resistance or, rarely, colistin resistance (10, 18). Because of the increasing frequency of antimicrobial resistance in *P. aeruginosa*, the World Health Organization has listed it as a "Priority 1: Critical" pathogen in need of research and development of new therapeutic approaches to treating infections (19). The genetic diversity in antibiotic-resistant *P. aeruginosa* strains obtained from clinical isolates highlights the ability of the microbe to selectively adapt to environmental challenges (20). Therefore, the biology of the bacteria and the host microenvironment are essential to disease progression.

Clinical Presentation and Standard Therapy of Acute Nosocomial Pneumonia

P. aeruginosa accounts for up to 18% of nosocomial pneumonia cases, making it one of the most frequently isolated pathogens (10–13). The diagnosis of nosocomial pneumonia is associated with the appearance of new or progressive radiographic infiltrate plus fever, purulent sputum, leukocytosis, and a decline in oxygenation (21). Hospital-acquired pneumonia (HAP) is defined as pneumonia occurring 48 hours or more after admission to the hospital, whereas VAP is defined as pneumonia occurring 48 hours after endotracheal intubation (13). Some of the complications associated with pseudomonal HAP and VAP are empyema (in 4–8% of patients), bacteremia (in 4–17% of patients), and shock (in up to 46% of patients) (22, 23). Attributable mortality rates associated with VAP and caused by *P. aeruginosa* are estimated to range from 13% to 32% (14, 24). Delayed or inappropriate therapy is associated with increased mortality, extended hospital stays, and the development of resistant organisms (13). Therefore, microbial identification and selection of targeted therapies for this pathogen are essential to effective treatment. Initial empiric therapy for HAP and VAP includes a β-lactam (antipseudomonal penicillin or cephalosporin, or a carbapenem) plus a fluoroquinolone (ciprofloxacin, levofloxacin) or aminoglycoside (amikacin, gentamicin, tobramycin) (13). In some cases of multidrug-resistant *P. aeruginosa,* polymyxins (colistin) may also be considered (25). The prevalence of *P. aeruginosa* nosocomial infections, their associated morbidity and mortality, and the increased presence of multidrug-resistant *P. aeruginosa* strains (10, 18) highlight the need for new therapeutic approaches. In the sections that follow, we discuss the biology of *P. aeruginosa* and the host response to highlight potential molecules and pathways for targeted intervention.

The Bacterium

P. aeruginosa is a gram-negative, rod-shaped, facultative anaerobe that adheres to host airway epithelia via its flagellum, pili, and components of the outer plasma membrane (26). *P. aeruginosa* breach of the epithelium may require weakened tight junctions, as occurs during cell proliferation or death, or in response to a significant focal concentration of the bacterium, which circumvents the immune response and allows for *P. aeruginosa* exotoxin-S (ExoS)-dependent cytotoxic damage to the epithelium (27, 28). Individuals with a compromised immune system or a damaged epithelium, often present in mechanically ventilated patients, are highly susceptible to *P. aeruginosa* infections (3, 29). In addition, previous exposure to antibiotics has been identified as an additional risk factor (30), which may be linked to antibiotic-induced changes in the lung microbiome (31) and the innate immune subsets (e.g., macrophages) that reside in the microenvironment (32). The mechanisms that allow *P. aeruginosa* to home in on damaged tissue or colonize hospitalized patients with different underlying disorders (e.g., extrapulmonary infection, congestive heart failure, renal failure, or surgery) are not fully elucidated (27, 29, 30, 33, 34). Quorum sensing (QS), or the ability of the bacterium to "sense" and respond to its environment, in association with adhesion factors, membrane components, and secretion systems, is fundamental to the initiation, propagation, and maintenance of acute *P. aeruginosa* infection (35) (Figure 1).

Adhesion Factors

P. aeruginosa flagellum and pili play roles in the motility and adherence of the bacterium to host tissue and are therefore therapeutic targets of interest (36, 37). *P. aeruginosa* has a single, polar flagellum (38) that is essential for motility and establishing acute infections (39). The flagellar cap protein, FliD, is responsible for adhesion to mucins in the upper airways (40). On the apical surface of respiratory epithelial cells, both flagella and pili bind to glycosphingolipids called asialo ganglio-*N*-tetraosylceramides (41, 42), and on the basolateral surface, flagella and pili respectively bind heparan sulfate proteoglycans and N-glycans (26). Induced cell signals from interactions between glycosphingolipids and flagella or pili on the apical surface of respiratory epithelial cells initiate the transcription and mobilization of Toll-like receptor (TLR)-2 and TLR-5, where the latter is another well-characterized flagellar receptor (41). Damaged or immature epithelial cells express higher quantities of glycosphingolipids and heparan sulfate proteoglycans, which may explain *P. aeruginosa*'s predisposition for these cells (33). Pili can also operate as receptors for certain bacteriophages, and these bacterial viruses are currently being examined therapeutically in the treatment of *P. aeruginosa* infections (43).

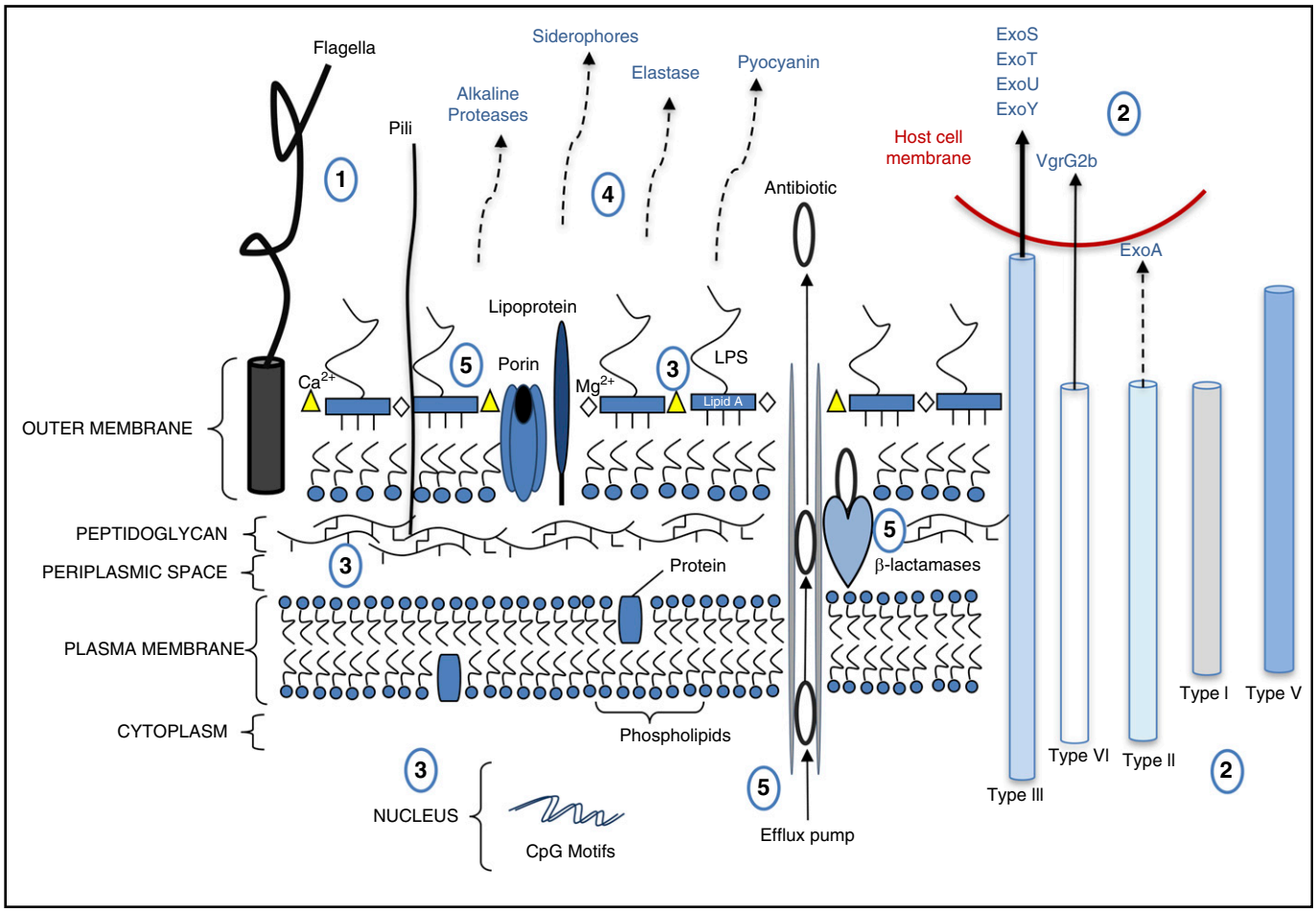

Figure 1. *Pseudomonas aeruginosa* structure and biology with respect to host interactions. (1) *P. aeruginosa* adheres to the host via flagella (e.g., binds to mucin, asialo ganglio-*N*-tetraosylceramide 1 [GM1], heparin sulfate proteoglycans, Toll-like receptor [TLR]-5, and TLR-2) and pili (e.g., binds to asialo GM1, asialo GM2, and N-glycans). (2) The microbe uses five secretion systems (type I, II, III, V, and VI). Type II secretion system releases exotoxin (Exo)A. Direct cell contact enables T3SS to inject toxins into host cells. T6SS-mediated injection of the effector molecule VgrG2b permits microtubule-dependent internalization of the pathogen. (3) Membrane proteins (lipoprotein:TLR-2, peptidoglycan:nucleotide-binding oligomerization domain [NOD]1 or NOD2) and molecules (LPS:TLR-4) as well as nucleic DNA motifs (CpG:TLR-9) also interact with host receptors. (4) Quorum-sensing (QS) molecules regulate the release of soluble factors (proteases, siderophores, elastase, and pyocyanin) in propagating infection. Pyocyanin also interacts with the host receptor, aryl hydrocarbon receptor. (5) *P. aeruginosa* efflux pumps, β-lactamases, and porins that regulate slow diffusion of solutes mediate antibiotic resistance.

Membrane Components

The *P. aeruginosa* outer membrane exhibits very low permeability because of the expression of certain porins and lipoproteins (44). These outer membrane proteins provide maintenance of cell structure, passive and active transport of extracellular molecules, adhesion to other cells, activation of TLR-2, and antibiotic resistance via various mechanisms (45–47). For example, the outer membrane porin F (OprF) allows nonspecific diffusion of solutes. However, in *P. aeruginosa*, OprF has much lower permeability than other gram-negative bacteria (48). This reduced permeability decreases the free diffusion of small hydrophilic antibiotics (e.g., β-lactams and tetracyclines) across the cell membrane and offers β-lactamases and efflux pumps greater efficiency in removing antibiotics that may gain access to the bacterium (44, 48). Outer membrane protein H (OprH) overexpression confers antibiotic resistance to cationic peptides and antibiotics such as aminoglycosides and polymyxin B by displacing Mg^{2+} and Ca^{2+} from the outer membrane and making tight cross-linking interactions with LPS, which consequently reduces outer membrane permeability (49). Increased production of additional proteins by the microbe, which may operate in either the diffusion or efflux of antibiotics (e.g., OprM, OprJ, and OprN), is also linked to multidrug resistance (45), whereas OprI is an identified susceptible target and potential receptor for the internalization of host cationic antimicrobial peptides/proteins (e.g., LL-37 and defensins) (50, 51). Therapeutic opportunities may therefore exist in targeting outer membrane proteins.

Also within the gram-negative outer membrane are molecules of LPS (Figure 1). This virulence factor contains a lipid A moiety that is recognized by the host cell TLR-4 complex consisting of TLR-4, the coreceptor MD-2, and either membrane or soluble CD14 (52). In addition to the lipid A moiety, LPS consists of inner and outer core oligosaccharides and the O antigen (53).

The O antigen can also serve as a receptor for bacteriophages (54). However, the heterogeneity of the lipid A and O antigen components in *P. aeruginosa* limits the opportunities to target LPS (52). Recent advances in understanding LPS biogenesis and the transport proteins involved in transporting the molecule from the inner membrane to the outer membrane have identified targets in modifying LPS virulence that warrant further study (53).

Peptidoglycan, another structural component of the membrane, is located just below the outer membrane (Figure 1). The muropeptides contained in peptidoglycan from either gram-negative or gram-positive bacteria are known to activate the intracellular innate immune receptor nucleotide-binding oligomerization domain (NOD)-2 (55). NOD-1, however, primarily responds to a unique muropeptide found in gram-negative bacteria (56). Because TLR-4 and NOD-1 are specific to gram-negative bacteria and induce synergistic cell signals, attempts to block or antagonize these pathways in severe infections have been investigated, albeit without success (57, 58). Routes of administration, animal models versus human clinical syndromes, patient comorbidities, and type, location, and duration of infection may contribute to the difficulties in developing effective interventions that target TLRs and NODs.

Secretion Systems

P. aeruginosa has secretion systems that release virulence factors directly into targeted cells or into the extracellular space. Gram-negative bacteria use at least seven secretion systems to transport bacterial proteins (59). *P. aeruginosa* secretes proteins via type I, type II, type III, type V, and type VI, but the major virulence factors of *P. aeruginosa* are secreted via either the type II secretion system (T2SS) or type III secretion system (T3SS) (60). General secretory pathway molecules have been identified in the transmembrane assembly of *P. aeruginosa* T2SS (61) that could be potential targets in antagonizing T2SS function. ExoA, elastases, lipases, and proteases are released into the extracellular space via the T2SS (62). Additional exotoxins, ExoS, ExoT, ExoU, and ExoY, are injected directly into host cells by the T3SS needle-like apparatus, which consists of a network of proteins (63). One product of the T3SS needle complex, PcrV, has been assessed as a therapeutic target (64, 65). Other molecules involved in the needle complex, translocation apparatus, or associated regulatory proteins and chaperones may also represent areas for therapeutic interventions.

QS

QS is a form of bacterial cell-to-cell communication that regulates gene expression. *P. aeruginosa* has three well-described QS pathways termed Las, Rhl, and Pqs that respectively generate the autoinducers 3-oxo-C12-homoserine lactone (3-oxo-C12-HSL), *N*-butyryl homoserine lactone (C4-HSL), and 2-heptyl-3-hydroxy-4-quinolone (PQS) (66). Each autoinducer freely diffuses into cells, and at high bacterial cell densities, these molecules reach a threshold concentration and bind to their respective receptor, which also serves as an inducible transcription factor for the induction of genes involved in the production of autoinducers, QS receptors, and virulence genes (67, 68). The Las pathway activates the transcription of the Las synthase, LasI, for the induction of 3-oxo-C12-HSL, the Rhl receptor (RhlR), and the RhlR cognate synthase, RhlI (68). In addition, the Las receptor (LasR) activates the transcription of the Pqs receptor (PqsR), but the transcription is negatively regulated by RhlR (69). The Pqs pathway is also dependent on anthranilate generated from chorismate (a nonmammal precursor of aromatic amino acids) by *P. aeruginosa* anthranilate synthases or via the kynurenine pathway, where tryptophan is catabolized (70, 71). PQS is produced by proteins that are encoded by the *pqsABCDH* genes, where *pqsE* is not involved but is linked to the increased production of pyocyanin and the expression of the efflux pump, MexGHI-OpmD (72). Additional virulence factors are generated by each pathway (35, 73, 74) and are highlighted in Figure 2.

In addition, activation of QS results in the production of virulence factors that not only damage host cells but also exhibit crosstalk with host cells through direct and indirect mechanisms (60) (Figure 2). For example, the Las pathway generates the siderophore pyoverdine, which sequesters iron away from host cells (35), and this pathway also generates the autoinducer 3-oxo-C12-HSL, which induces myeloid cell production of IL-10, the expression of the tolerogenic nonclassical class I HLA-G, and an inhibition of tumor necrosis factor (TNF)-α release (75, 76). Both 3-oxo-C12-HSL and the PQS autoinducer inhibit *in vitro* mitogen-induced human T-cell proliferation and IL-2 release (76). The Rhl and Pqs pathways produce hydrogen cyanide and pyocyanin that generate reactive oxygen species (ROS) (35, 73, 77). Pyocyanin also induces the activation of a characterized host receptor called the aryl hydrocarbon receptor (AHR) (78). This receptor is also activated by kynurenine generated in the Pqs system (79). Adding to the complexity, AHR is an identified receptor for various xenobiotic chemicals, a transcriptional activator of cytochrome P450 enzymes, and an essential factor in the development and function of hematopoietic cells (80).

Moreover, the QS pathways are activated by host factors (Figure 2). Although natriuretic peptides are predominantly characterized as cardiac hormones known to regulate blood pressure (through control of sodium and water balance, for example) (81), endothelial cell-produced C-type natriuretic peptide also induces LasI transcription and the subsequent production of soluble 3-oxo-C12-HSL (81, 82). The human cathelicidin, LL-37, is a host defense peptide secreted by a variety of cells (e.g., macrophages, natural killer cells, and epithelial cells) but is best characterized in neutrophils (83). Treatment of *P. aeruginosa* with LL-37 induces the production of pyocyanin, which may occur through the activation of *pqsE* (84). Dynorphin, a κ-opioid peptide produced by pulmonary neuroendocrine cells and alveolar macrophages, has been implicated in the activation of *pqsABCDE* (85, 86). Last, IFN-γ has been shown to bind to the outer membrane protein OprF, subsequently activating the Rhl system and the production of C4-HSL and pyocyanin (87). The above QS studies reflect a constant interplay between the microbe and the host and highlight the need to understand the biology of both the microbe as well as the host response in designing novel therapies.

The Innate Immune Response

In the development of a pulmonary bacterial infection, the microbe must first overcome innate host defense responses. Goblet, ciliated, basal, club, and neuroendocrine

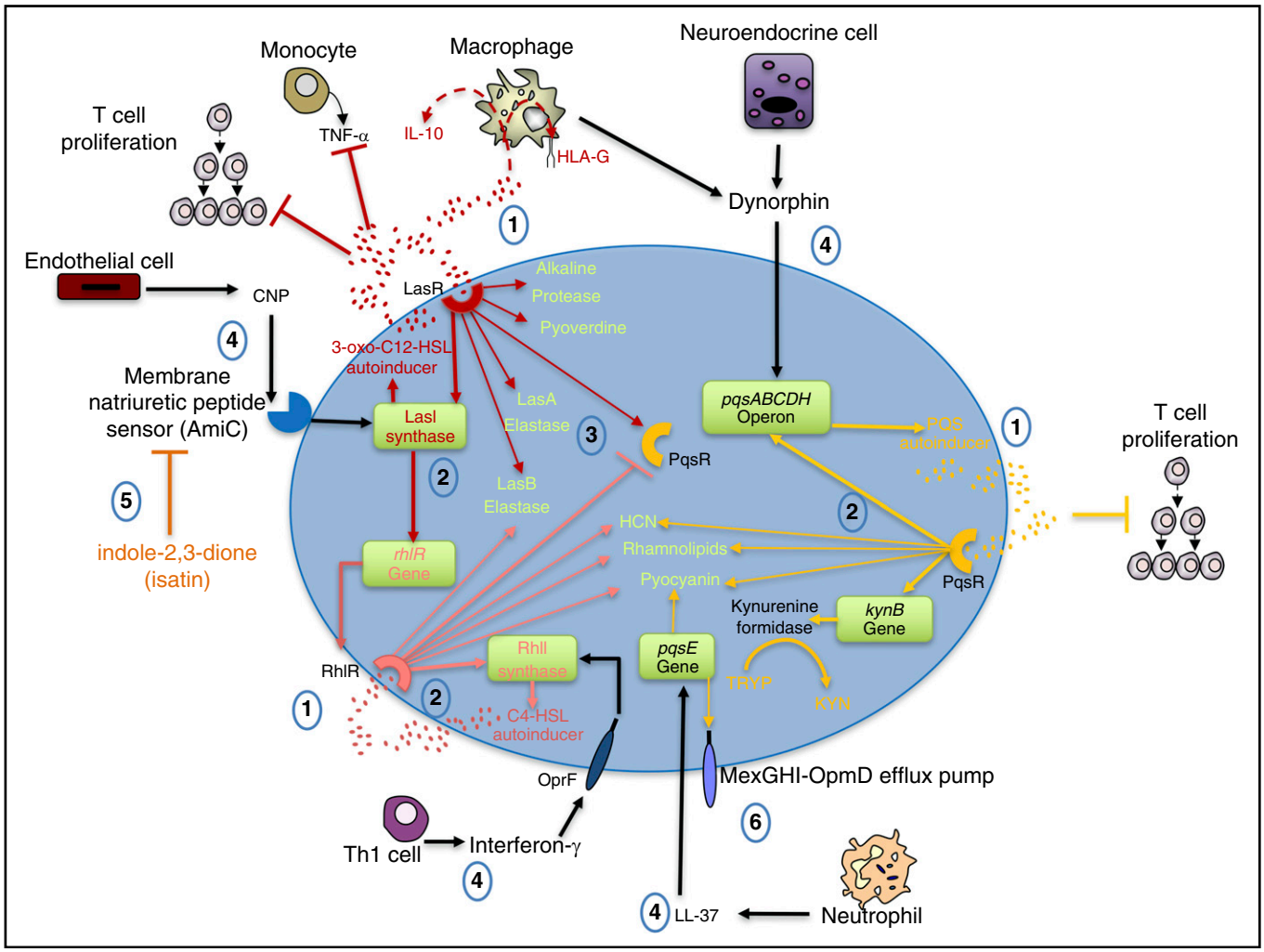

Figure 2. Quorum sensing (QS). *Pseudomonas aeruginosa* QS is a bacterial intercellular communication system that also interacts with the host. (1) The autoinducers 3-oxo-C12-homoserine lactone (3-oxo-C12-HSL), *N*-butyryl homoserine lactone (C4-HSL), and 2-heptyl-3-hydroxy-4-quinolone (PQS) activate their receptors (LasR, RhlR, and PqsR, respectively) and modulate the immune response. 3-oxo-C12-HSL inhibits tumor necrosis factor (TNF)-α release and induces IL-10 and HLA-G expression in myeloid cells. Both 3-oxo-C12-HSL and PQS can inhibit T-cell proliferation. (2) The receptors' binding their ligands stimulates the transcription of autoinducer enzymes and genes (e.g., *LasI* synthase, *RhlI* synthase, and *PqsABCDH* operon) and the transcription of virulence factors: elastases, proteases, siderophores (pyoverdine), reactive oxygen species (hydrogen cyanide [HCN]), and pyocyanin. PqsR activation also generates kynurenine formidase involved in the catabolism of tryptophan (TRYP) into kynurenine (KYN). (3) LasR activates while RhlR suppresses PqsR transcription. (4) Environmental stimuli such as IFN-γ, C natriuretic peptide (CNP), dynorphin, and the antimicrobial peptide LL-37 can induce the activation of the QS system at various points. CNPs secreted from endothelial cells are detected by the membrane natriuretic peptide sensor AmiC, which activates the transcription of *lasI*. Likewise, when IFN-γ binds to the outer membrane porin F (OprF), *rhlI* transcription is stimulated. Dynorphin activates the *pqsABCDH* operon. (5) The indole isatin antagonizes AmiC activation. (6) LL-37 activates *pqsE*, which generates the multidrug efflux pump MexGHI-OpmD. Th1 = T-helper cell type 1.

cells line the epithelium in the upper airway mucosa and contribute to an extensive mucociliary escalator to defend against particulate matter and organisms (88). Mucins protect the epithelium in association with plasma cell–secreted immunoglobulins, which opsonize foreign matter (89, 90). P450 enzymes detoxify xenobiotics throughout the lung (91). In the alveolar lumen, macrophages are charged with regularly clearing debris, surfactant, and microorganisms in maintaining lung compliance and protecting the epithelium composed of type I and II pneumocytes (92, 93) (Figures 3 and 4).

Phagocytes

During infection, macrophages and neutrophils preferentially phagocytose motile bacteria irrespective of opsonization (94). Nonopsonic phagocytosis mechanisms are not well defined, might be strain specific, and may require *P. aeruginosa* flagella, macrophage complement receptor 3 (CR3), or CD14 (95, 96). Antibodies (IgA, IgG) (97), complement (C2, C3, C3b, C4) (98–100), and surfactant protein-A (101) opsonize *P. aeruginosa* and enhance phagocytic uptake via binding interactions with Fc or complement receptors. To evade these mechanisms, *P. aeruginosa* uses 3-oxo-C12-HSL to modulate the activation state of

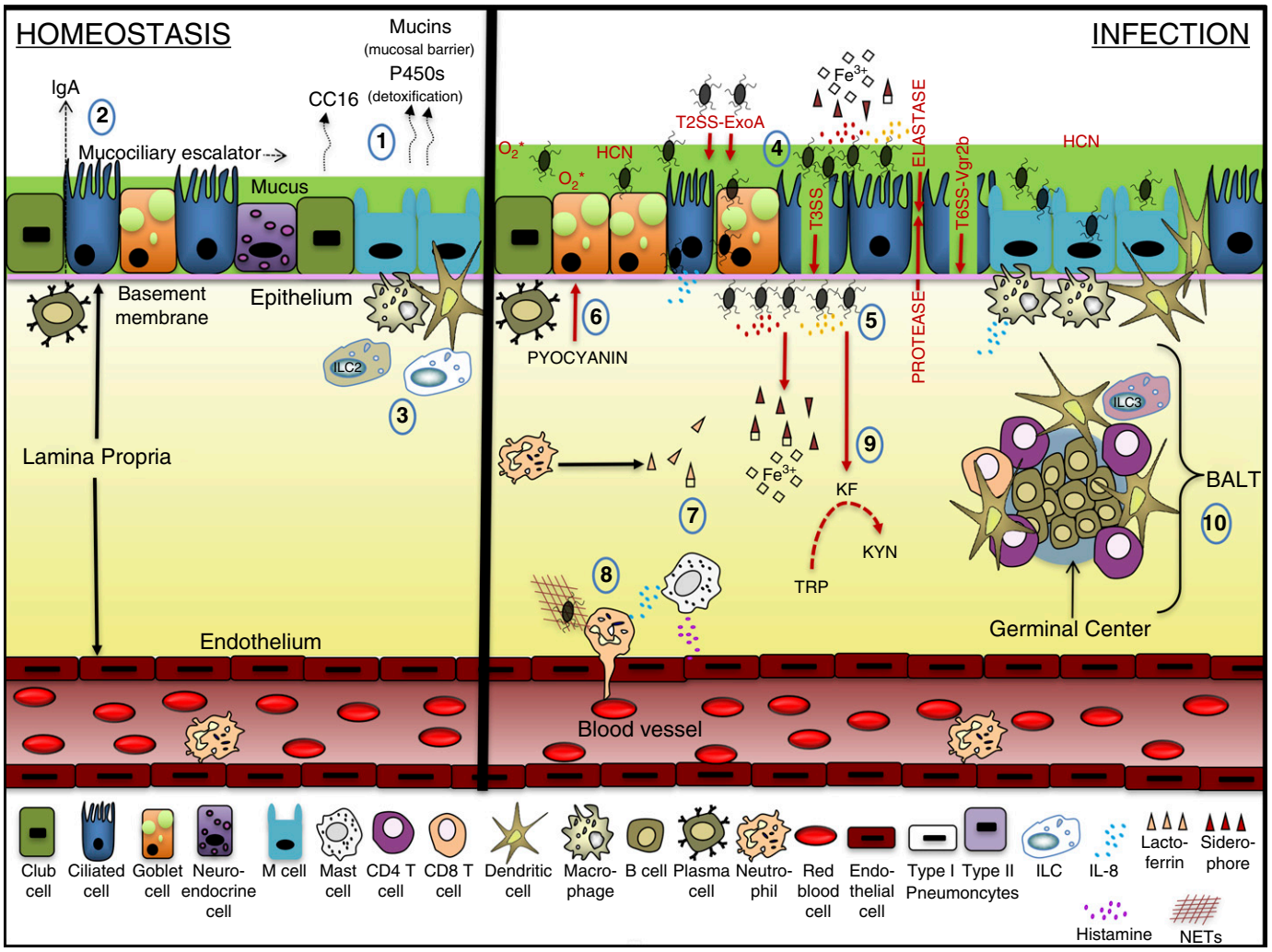

Figure 3. Upper airway homeostasis and infection. (1) During homeostasis, the respiratory bronchial epithelium (club cell, ciliary, goblet, neuroendocrine, microfold [M] cells) protects against environmental insult by maintaining tight junctions between cells, producing mucin and p450 detoxifying enzymes and generating the antiinflammatory molecule club secretory protein (CC16). (2) Plasma cells generate antibody to opsonize foreign pathogens, and the mucociliary escalator acts as an additional host defense mechanism. (3) The vascular endothelium is separated from the epithelium by the lamina propria, where dendritic cells, macrophages, and innate lymphoid cells (ILCs) that are mostly ILC2 localize. (4) During infection, *Pseudomonas aeruginosa* adheres to the epithelium and injects toxins through the type III secretion system (T3SS) and the effector molecule involved in T6SS-induced internalization, VgrG2b. (5) Activation of quorum sensing (QS) encourages the accumulation of *P. aeruginosa* and the production of QS molecules that in turn generate exotoxin (Exo)A, hydrogen cyanide (HCN), reactive oxygen species, proteases, and elastase in the destruction of the epithelium. (6). QS also induces the production of pyocyanin, which is characterized in goblet cell hyperplasia and enhanced mucin production. (7) Released siderophores compete with cells in the microenvironment for available iron. (8) Mast cell production of histamine and IL-8 production by various cells induce the recruitment of neutrophils. (9) The production of kynurenine formidase (KF) induces tryptophan (TRP) catabolism into kynurenine (KYN). (10) Infection triggers cell signals for the formation of the inducible bronchus-associated lymphoid tissue (BALT) that coordinates with the lymphatic system in mediating secondary responses. NET = neutrophil extracellular trap.

myeloid cells (75, 76) (Figure 2). The virulence factors elastase and alkaline protease degrade opsonins (100, 102–104). ExoA and pyocyanin induce apoptosis (62, 105), and pyocyanin also impairs macrophage ingestion of apoptotic cells (106). In a murine model of *P. aeruginosa* pneumonia, macrophages and neutrophils are also susceptible to T3SS ExoS cytotoxicity (28). *P. aeruginosa*–induced release of IL-8 by macrophages (107), mast cells (108), and epithelial cells (109) elicits the recruitment of neutrophils from the peripheral blood (110). To replace a depleted pool of macrophages in the lung is more complex, given that the vast majority of tissue-resident macrophages originate from the yolk sac and not hematopoietic stem cell precursors (111). In a murine *Escherichia coli* primary pulmonary infection model followed by secondary *P. aeruginosa* pneumonia, populations of dendritic cells (DCs) and macrophages were reconstituted in an altered phenotype involving diminished antigen presentation and reduced production of proinflammatory cytokines (IL-6, TNF-α,

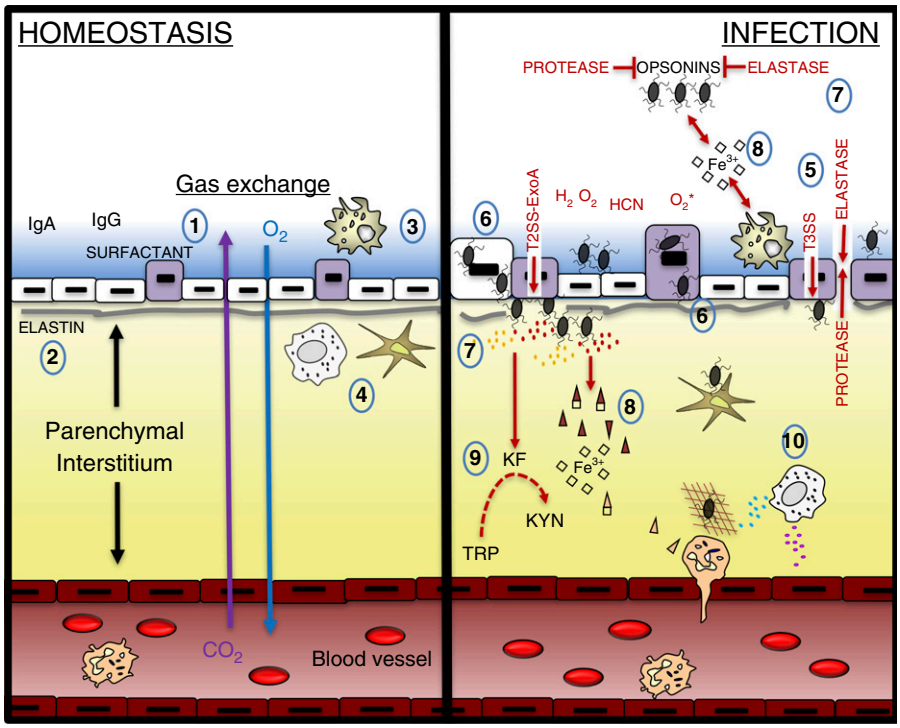

Figure 4. Lower airway homeostasis and infection. (1) The alveolar epithelium consists of mainly type I pneumocytes that mediate gas exchange and type II pneumocytes that secrete surfactant. (2) Intact elastin fibers provide structural support and aid in expansion of alveoli during respiration. (3) Macrophages persistently survey the lumen for debris and antigen that may or may not be coated with opsonins. (4) Additional innate immune cells (dendritic cells and mast cells) stand vigil over their microenvironment. (5) During infection, *Pseudomonas aeruginosa* adheres to the epithelium and injects toxins through the type III secretion system (T3SS). (6) In some instances, *P. aeruginosa* will proliferate within type I or II pneumocytes. (7) Activation of quorum sensing (QS) encourages the accumulation of *P. aeruginosa* and the production of QS molecules that in turn generate exotoxin (Exo)A, hydrogen cyanide (HCN), reactive oxygen species, proteases, and elastase in the destruction of the epithelium, surfactant, opsonins, and elastin. (8) Released siderophores compete with cells in the microenvironment for available iron. (9) The production of kynurenine formidase (KF) induces tryptophan (TRP) catabolism into kynurenine (KYN). (10) Innate immune cells interact with the pathogen and begin to recruit additional immune subsets.

IL-12) (112). CD11b+ DC populations characterized in this study exhibited increased B-lymphocyte–induced maturation protein (Blimp)-1, which is an identified transcription factor regulated by AHR (112, 113). In neutrophils, the *P. aeruginosa*–produced AHR ligand, pyocyanin, induces nicotinamide adenine dinucleotide phosphate(H) (NADPH) oxidase and the uncontrolled release of neutrophil extracellular traps (NETs) (114). Neutrophil bacterial uptake also involves the activation of NADPH oxidase, the release of antibacterial molecules, and ROS involved in the killing of the pathogen (115). These activities may be countered by an additional *P. aeruginosa*–derived AHR ligand, kynurenine, which scavenges neutrophil ROS (116). In addition, T3SS blocks neutrophil ROS production via ADP-ribosyltransferase activities of ExoS and ExoT (117). These competing processes may allow the microbe to evade host phagocytes and interact with additional immune subsets and the epithelium.

Innate Lymphoid Cells

Lung innate lymphoid cells (ILCs) are composed of three subsets (ILC1, ILC2, ILC3), which are derived from common lymphoid progenitors. The ILC2s have been characterized in murine and human lung tissue and may be recruited to the airway (118). This subset is maintained by various cytokines (e.g., IL-33 and IL-25) and is implicated in the maintenance of lung homeostasis (118, 119). In a murine model, *P. aeruginosa* infection altered mediator production and induced the formation of the ILC3 variant (120). This subgroup is formed not only in response to IL-1β and IL-23 but also in response to AHR cell signals (119), suggesting that the AHR ligands, particularly pyocyanin and kynurenine from QS (Figure 2), or the production of kynurenine via indoleamine-2,3-dioxygenase (IDO) released from CpG DNA-activated dendritic cells (121), may influence the proliferation or recruitment of ILC3s (122). These cells are also characterized by the production of IL-17 and IL-22 (119), which are essential cytokines in the formation of bronchus-associated lymphoid tissues (BALT) during the adaptive immune response (123).

Bronchial Epithelium

P. aeruginosa flagella and pili interact with mucin and cell surface receptors on the apical surface of the respiratory bronchial epithelium (41, 42) (Figures 1 and 3). Epithelial cell invasion by the microbe may be induced by T6SS-mediated injection of the effector molecule VgrG2b, which targets the host cell microtubule network and permits microtubule-dependent internalization of the pathogen (124, 125). This mechanism may be linked to the activation of epithelial cell signals, specifically phosphatidylinositol 3-kinase (PI3K) and protein kinase B/Akt (Akt), which are required for internalization of the bacterium (126). Release of ExoA via T2SS (62) and injection of additional toxins via T3SS (63) into epithelial cells damage the epithelium and provide pathways to the basolateral surfaces. There the bacteria preferentially adhere and localize via binding interactions between the epithelium and bacterial flagella (26, 127) (Figure 3). QS activates cell signals involved in the production of virulence factors (35) (Figure 2). Proteases and elastases degrade mucin (128), IgA (102), and the epithelial tight junctions (3). Pyocyanin slows ciliary beating (129) and induces goblet cell hyperplasia and mucin hypersecretion (130), possibly as a result of increased club cell differentiation into goblet cells (130, 131). Furthermore, the differentiation of club cells may also involve pyocyanin or kynurenine (79) binding to AHR in club cells, because AHR is highly expressed in these cells and known to bind additional AHR ligands in human club cells *in vitro* (132). Distinct xenobiotic AHR

ligands (curcumin, indole-3-carbinol, 2,3,7,8-tetrachlorodibenzo-p-dioxin) are also characterized in modulating lung inflammation and fibrosis (133), suggesting that microbe AHR ligands are integral to the host response.

Alveolar Epithelium

In the alveolar epithelium, type I pneumocytes cover approximately 95% of the gas exchange surface area, and the type II pneumocytes cover the remaining area and secrete surfactant in maintaining lung compliance (134). *P. aeruginosa* type IV pilus accounts for about 90% of the adherence capability of the microbe to the adenocarcinomic human alveolar basal epithelial cell line, A549 (135). Invasion of rat and murine type I–like pneumocytes *in vitro* and *in vivo* is mediated by *P. aeruginosa* coopting host cell caveolin-2–dependent lipid raft endocytosis (136, 137), suggesting that this host–microbe interaction could be a therapeutic target. In cell lines and murine models, type I and type II pneumocytes endocytose *P. aeruginosa*, protect the bacteria from host defenses by shielding them from phagocytes, and allow bacterial proliferation (138, 139). In a murine pneumonia model, type I pneumocytes accounted for nearly 40% of all cells injected with ExoS 18 to 24 hours after *P. aeruginosa* infection (28). Damaged type I pneumocytes are replaced by type II pneumocytes, and this increases the production of surfactant and decreases gas exchange in the alveoli (134). In A549 cells, pyocyanin accepts electrons from nicotinamide adenine dinucleotide(H) (NADH), NADPH, or reduced glutathione and transfers the electrons to O_2, generating superoxide (O_2*) and hydrogen peroxide (H_2O_2) (77). These ROSs also induce the release of histamine from murine primary mast cells (140), leading to increased vascular permeability and therefore encouraging immune cell influx from circulation (Figure 4).

The Adaptive Immune Response

The adaptive immune response is a delayed response that requires directives from the innate immune system for the generation of pathogen-specific lymphocytes. *P. aeruginosa* antigens in the lung are taken up, processed, and presented to T-cell subsets mainly by DCs (141, 142). Additional professional antigen-presenting cells, such as B cells and macrophages, can also perform this function (143). Atypical epithelial cells called microfold (M) cells and ILC3s may also present antigen and interact with T-cell subsets (143, 144). In mice, M cells are localized above nasopharynx-associated lymphoid tissue and BALT (145) and may translocate pathogens to macrophages and dendritic cells directly beneath the epithelium (145, 146). The activation of T cells occurs mostly in lymph nodes but can also occur in the BALT, where distinct subsets (T-helper cell type 22 [Th22], Th17, T regulatory) are generated (123). Animal studies examining the effect of Th1 cytokines, such as IFN-γ and IL-18, in response to *P. aeruginosa* pulmonary infection, demonstrate that a Th1 response impairs bacterial clearance in the lung (147, 148). This contrasts with a murine model involving *P. aeruginosa* subcutaneous footpad injections, where IFN-γ is critical to the local containment of bacteria and the prevention of systemic dissemination (149). Possibly, this is due to the ability of *P. aeruginosa* to "sense" IFN-γ through the QS system in the lung microenvironment, thus leading to increased virulence (Figure 2). This may explain the proliferation of additional T-cell subsets and B cells that can both be influenced by AHR ligands.

T Cells and BALT

The most robust adaptive immune response occurs within the regional mediastinal lymph nodes, where migratory dendritic cells interact with T cells to generate and induce the proliferation of antigen-specific T cells (142, 150). Dendritic cells pulsed with the heat-killed bacterium *in vitro* are able to protect mice against an *in vivo* lethal challenge in $CD8^{-/-}$ but not $CD4^{-/-}$ mice, indicating a requirement for $CD4^{+}$ cells (151). Subsets of CD4 cells that are generated after *P. aeruginosa* challenge include Th17 and Th22 cells (152) that provide cytokines (IL-17, IL-22) in promoting the organization of B cells and T cells into BALT (123). IL-17 also contributes to lymphangiogenesis within BALT (153), forming around major bronchi and pulmonary blood vessels, thus allowing for enhanced recruitment of innate and adaptive immune cells into the lung (154). IL-17 and IL-22 are also important to host defense, as deletion or blockade of IL-17 or IL-22 in murine models of *P. aeruginosa* and other gram-negative pulmonary infections results in increased lung injury and mortality (155–158). Cell signals that yield IL-17 and IL-22 in T-cell subsets are similar, particularly in that some murine Th17 cells can produce both IL-17 and IL-22 (159). Similar to the ILC3s (119), both Th17 and Th22 cells are regulated by the cytokines IL-1β and IL-23 (160, 161) and are influenced by the transcription factor AHR.

Moreover, the activation of T-cell subsets by AHR is critical to the development and function of immune responses. The microenvironment and type of AHR ligand determine the formation of Th17, Th22, the immunosuppressive T regulatory lymphocytes (80), and ILC3s (120, 162). *P. aeruginosa* pigment molecules, pyocyanin and 1-hydroxyphenazine, in association with the tryptophan catabolic product kynurenine, produced by both the bacterium and host, appear sufficient to activate AHR during infection (78, 79, 163). In a rat catheter-related lung infection model examining *P. aeruginosa* QS mutants, the production of suppressive cytokines (IL-10, TGF-β) and the presence of T regulatory lymphocytes were higher in the animals infected with the wild-type bacteria than in animals challenged with the mutants (164), suggesting that QS molecules also affect the induction of certain T-cell subsets.

B Cells

In a murine model of *P. aeruginosa* lung infection, IL-17–producing γδ T cells were found to be essential for B-cell production of antibodies detected in serum and bronchial lavage fluid (165). An additional model demonstrated that B cells also produced IL-17, but in mice lacking B cells (μMT mice), pathogenesis was not affected (166), which highlights the lack of target specificity of the antibodies generated. Of interest, B-cell receptor cross-linking significantly induces the expression of AHR, and AHR activation affects the processes of class-switch recombination and plasma cell differentiation, which induce the formation of distinct effector B cells and antibody-secreting cells, respectively (113). The production of AHR ligands by the host and microbe may therefore affect the humoral response to

P. aeruginosa infection. These adaptive immune responses involving lymphocyte subsets, coupled with innate host defense mechanisms, provide insight into the pathophysiology of clinically evident *P. aeruginosa* pneumonia.

Pathophysiology of *P. aeruginosa* Pneumonia

Barriers exist in the respiratory tract to prevent the establishment of infection, such as the presence of mucus, opsonins, innate immune cells, and additional factors (Figures 3 and 4). These barriers may be altered by physical injury (29), a compromised immune system (3), or previous exposure to antibiotics (30), which render the host susceptible to *P. aeruginosa* lung infections. Activation of *P. aeruginosa* QS alters innate and adaptive responses (Figure 2) and, along with the associated cytotoxic effects of the virulence factors, allows for the establishment of a severe lower respiratory tract infection, as discussed in detail above (Figures 5 and 6). The corresponding inflammatory response includes *P. aeruginosa*–mediated release of IL-8 by various cell types and the recruitment of neutrophils to the lung (110). NET proteins can induce endothelial and epithelial cell death, alter the extracellular matrix, and expose autoantigens, which may contribute to mucus viscosity, autoantibody production, and impaired lung function in certain *P. aeruginosa* infections (167, 168). Red hepatization, tissue necrosis, thrombosis of the blood vessels, and obstruction of small airways by lymphocytic infiltrates are linked to the oxidative effects of QS-mediated release of pyocyanin in the murine lung (169). These various interactions combined with sequestration of iron by the pathogen converge to create a hypoxic environment where host lung function declines (170, 171).

Moreover, ineffective clearance of the pathogen occurs in response to *P. aeruginosa*–mediated phagocyte apoptosis (62, 105). This decreased clearance allows the bacteria to further degrade tight junctions (172), which induces the expression of adhesion molecules (173) that permit fluid and immune cell influx into the lung parenchyma and subsequently impairs gas exchange. Preferential damage to club cells in the bronchioles and type I pneumocytes in the alveoli increases the proliferation of mucin-producing goblet cells and surfactant-generating type II pneumocytes, respectively, thus altering the mix of cytochrome P450 enzymes that are produced by these cells to mediate the clearance and detoxification of AHR ligands (91). The AHR ligand, pyocyanin, also enhances the production of free radicals (77) that are also produced by neutrophil respiratory burst activity (115). Increased production of ROS, damage-associated molecular patterns, and pathogen-associated molecular patterns sustain cellular inflammasome activation and induce the production of mediators that enhance the alveolar filling process, dramatically decreasing gas exchange within the alveoli (174).

Last, bacterial virulence factors also have a role in promoting bacteremia. *P. aeruginosa* elastase degrades surfactant proteins A and D, which are known to opsonize bacteria and affect the phenotype and function of macrophages (93, 175). *P. aeruginosa* proteases that degrade tight junctions also degrade IL-22 (176), which protects the integrity of the epithelium (156). The combined effect is increased destruction of the epithelial barrier, which may allow the bacteria to interact with the endothelium, where proteases and toxins released from T2SS and T3SS further disrupt endothelial tight junctions and destroy endothelial cells (172). Migration of *P. aeruginosa* into the bloodstream produces bacteremia (29). In a murine *in vivo* model of severe infection, TLR-4–activated platelet-bound neutrophils within the vasculature significantly and predominantly induced NET activity, mediating intravascular bacterial trapping while at the same time causing tissue damage (177). In summary, the delicate balance between bacterial factors and host responses determines the severity of infection and thus host outcomes. Therefore, modulating bacterial factors, the host response to infection, or both offer opportunities for novel therapeutic interventions.

Novel Therapeutic Interventions

The acquisition of resistance genes (e.g., genes for β-lactamases and enzymes inactivating aminoglycosides), overexpression of efflux pumps, or decreased expression of porins may affect treatment success of *P. aeruginosa* infections (178). Ceftolozane/tazobactam and ceftazidime/avibactam are new combinations of β-lactam/β-lactamase inhibitors that have been approved for use against certain infections caused by multidrug-resistant strains of *P. aeruginosa* (179). Vaccines using live-attenuated or irradiated *P. aeruginosa* (180–182), the LPS O-antigen (52), a 3-oxo-C12-HSL-carrier protein conjugate (183), and various recombinant proteins (PcrV [184, 185], flagellin B [186], OprL [187], OprF [186], OprI [184, 186], OprF/I fusion [188], pili [189, 190], T6SS hemolysin coregulated protein 1 [184]) are being investigated to prevent *P. aeruginosa* infections. Because *P. aeruginosa* is a World Health Organization "Priority 1: Critical" pathogen in need of new approaches to treatment (19), preclinical models of biologics are being assessed (Table 1). Expanding on these factors that are unique to the pathogen as well as those linked to the host response may aid in devising new therapeutic interventions to combat *P. aeruginosa* infections.

Interventions Targeting the Pathogen

Protein Epitope Mimetics

Small synthetic molecules called protein epitope mimetics resemble functional epitopes of physiologically important proteins. A protein epitope mimetic of LptD, a porin protein critical to LPS transport to the outer membrane, has demonstrated antimicrobial activity *in vitro* (191). Intratracheal administration of this mimetic significantly reduced lung bacterial counts and pulmonary inflammation in an *in vivo P. aeruginosa* pneumonia mouse model (192) (Table 1). LptE forms a complex with LptD and is being similarly assessed as a target for peptidomimetic antibiotics (191, 193). In addition to targeting LPS transport, examination of antibiotic resistance OM proteins (e.g., OprF, -H, -J, -L, -M, and -N) or structural proteins of secretion systems (T2SS, T3SS, and T6SS) as potential targets of protein mimetics or binding molecules such as aptamers may provide a needed mechanism in treating resistant *P. aeruginosa* infections.

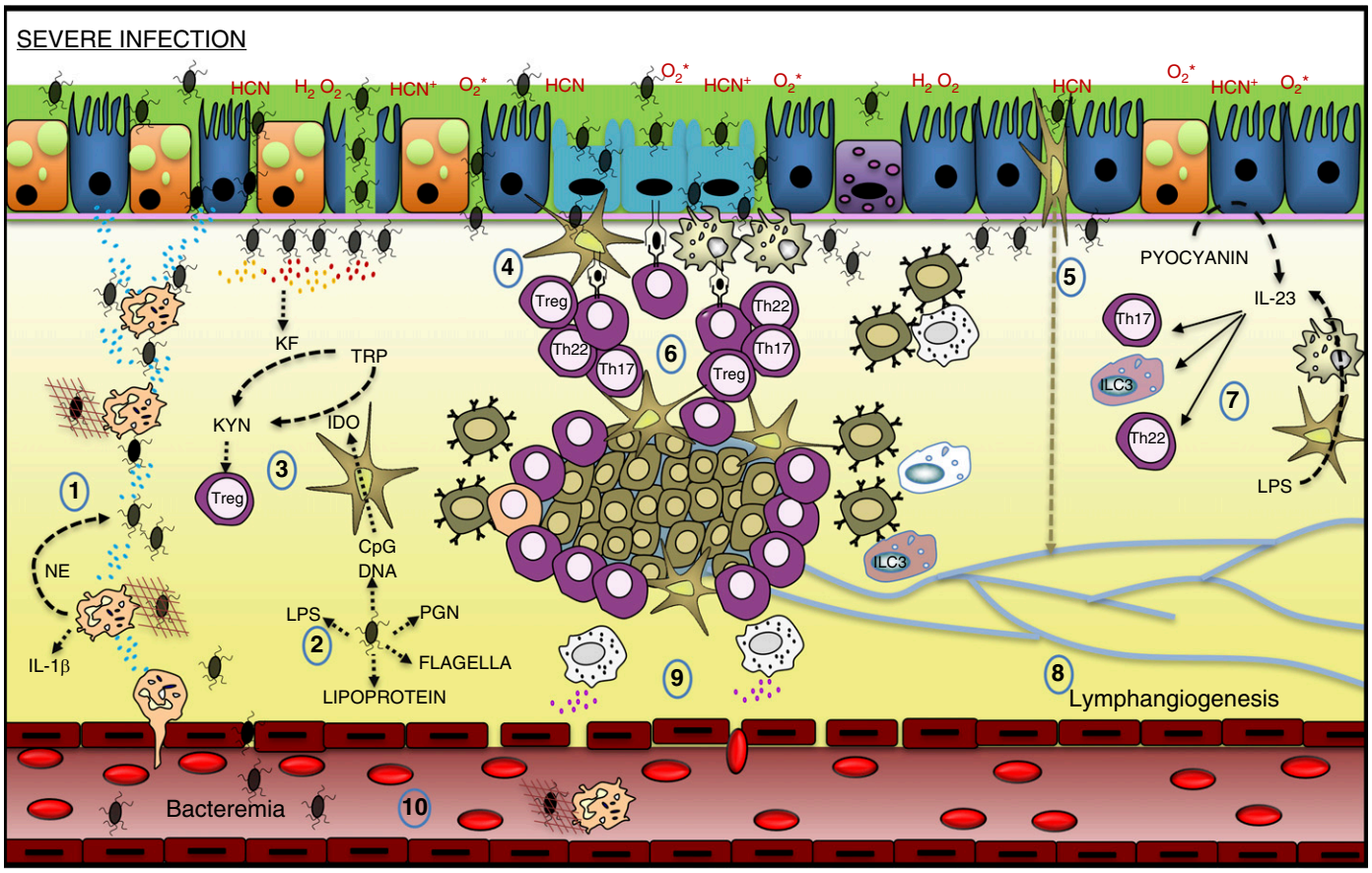

Figure 5. Upper airway severe infection. (1) IL-8 produced by epithelial cells and additional cell types encourages the recruitment of neutrophils that trap *Pseudomonas aeruginosa* with neutrophil extracellular traps and degrade *P. aeruginosa* cell walls with neutrophil elastase (NE). Active IL-1β can also be generated by NE. (2) Release of components of the bacterial wall induces the activation of immune and nonimmune cells. (3) CpG DNA induces the production of indoleamine-2,3-dioxygenase (IDO) that synergistically with *P. aeruginosa* kynurenine formidase (KF) induces the catabolism of tryptophan (TRP) to kynurenine (KYN), which encourages the production of T regulatory lymphocytes (Tregs). (4) Antigen may be directly presented by microfold (M) cells or transported through M cells to dendritic cells and macrophages under the epithelium. (5) Dendritic cells transport antigen retrieved from the epithelium directly to draining lymph nodes. (6) *P. aeruginosa* infections generally induce the production of T-cell subsets (T-helper cell type 22 [Th22], Th17, and Tregs). Th22 and Th17 provide cytokines (IL-22 and IL-17, respectively) in promoting the organization of B cells and T cells into bronchus-associated lymphoid tissue. (7) Th22, Th17, and innate lymphoid cell (ILC) 3 are known to be induced by IL-23, which can be generated by *P. aeruginosa*–activated epithelial cells, dendritic cells, and macrophages. (8) Excessive inflammation induces the formation of new lymphatic vessels. (9) Mast cells regulate vascular dilation via histamine production. (10) Breaks in the vascular endothelium may, in some cases, lead to infection spread or bacteremia. HCN = hydrogen cyanide; PGN = peptidoglycan.

Iron

P. aeruginosa siderophores, pyochelin and pyoverdine, sequester iron from the surrounding environment and are respectively internalized by their outer membrane receptors, FpvA and FptA (194). Tagging pyochelin or pyoverdine with an antibiotic or a redox-inactive metal ion such as gallium, which interferes with *P. aeruginosa* iron metabolism, has been proposed as a potential therapeutic intervention (194). In the absence of siderophores, some *P. aeruginosa* strains may use another iron chelating system involving nicotianamine (171), which could be similarly explored.

Pyocyanin

Strategies to neutralize pyocyanin activity or block pyocyanin production in the host with antioxidants, antibodies, or acyl-homoserine lactone analogs may prevent severe *P. aeruginosa* infections (195, 196) (Table 1). Selective estrogen receptor modulators have unique effects on pyocyanin production. One member of this class of drugs, raloxifene, binds and inhibits the activity of a *P. aeruginosa* biosynthesis protein, PhzB2, involved in the conversion of chorismate to pyocyanin (197). In additional *in vitro* analyses, raloxifene and similar compounds (toremifene, tamoxifen) in combination with polymyxin B were shown to exhibit synergistic activity against polymyxin-resistant *P. aeruginosa* proliferation and survival (198), suggesting that modifying cell signals leading to pyocyanin production may be beneficial in severe infections.

Adhesion Factors

Because flagella and pili are the primary means by which *P. aeruginosa* adheres to the host (36, 37), these molecules are being explored as therapeutic targets. Human antiflagellar antibodies administered intravenously have shown efficacy in a rat *P. aeruginosa* pneumonia model (36) (Table 1). Studies with chicken antiflagellar

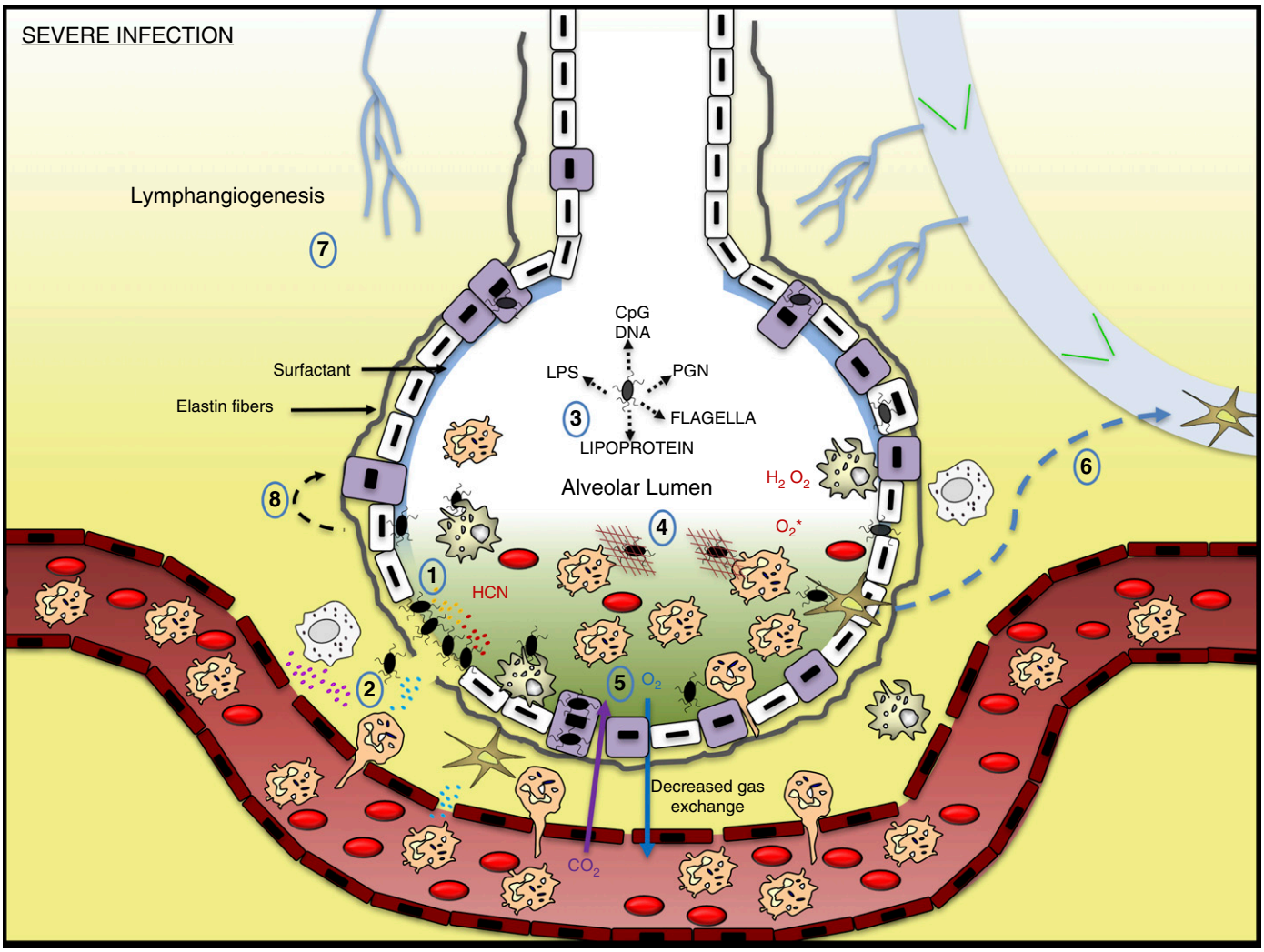

Figure 6. Lower airway severe infection. (1) *Pseudomonas aeruginosa* production of quorum-sensing molecules degrades the epithelium and the supporting elastin fibers. (2) Production of histamine by mast cells and IL-8 by various cells in the microenvironment encourages the recruitment of immune cells into the alveolus. (3) Components of *P. aeruginosa* activate immune cells and induce the production of mediators. (4) Leakage of fluid from the interstitium and mediators produced from immune and nonimmune cells fill the lumen space. This edema, in association with reactive oxygen species and various *P. aeruginosa* toxins, induces hypoxia. (5) Gas exchange is reduced. (6) Dendritic cells migrate to draining lymph nodes. (7) New lymphatic vessels are formed. (8) Damaged type I pneumocytes are replaced by type II pneumocytes. HCN = hydrogen cyanide; PGN = peptidoglycan.

antibodies have shown a potential prophylactic effect against *P. aeruginosa* colonization in patients with cystic fibrosis who gargled the antibodies (199, 200). In a murine burn wound sepsis model involving *P. aeruginosa*, mouse antiflagellar antibodies given intraperitoneally significantly improved morbidity and mortality (201), highlighting the therapeutic potential of antiflagellar antibodies. With respect to pili, extensive study of *P. aeruginosa* type IV pili biogenesis is anticipated to identify small molecule inhibitors that may block the dynamic assembly and disassembly of pili or the adhesion of pili (202, 203). However, *P. aeruginosa* pili and flagella are also susceptible to genetic changes in expression in certain microenvironments and species, and this may create difficulties in targeting these adhesive factors (204–206).

Bacteriocins and QS Inhibition

Bacteriocins are bacterial toxins that an individual bacterium uses to kill neighboring bacteria (207). Bacteria have also devised mechanisms to inhibit QS of neighboring bacteria. This phenomenon is termed quorum quenching and occurs by bacterial production of bioactive molecules that affect a neighboring cell's autoinducer gene activation, structure, or receptor binding interactions (208). Attempts to identify and engineer *P. aeruginosa*–specific bacteriocins called pyocins, native quorum quenchers, and synthetic or plant-derived QS inhibitors as antimicrobial agents against *P. aeruginosa* are in process. For example, the pyocin SD2 binds the common polysaccharide antigen of *P. aeruginosa* LPS and interacts with an outer membrane receptor, which facilitates the killing of the microbe (209). In diabetic foot ulcer isolates, a quorum quencher lactonase (SsoPox-W263I), which cleaves

Table 1. Biologics Targeting *Pseudomonas aeruginosa* Pneumonia in Animal Models

Target	Biologic	Model	Outcomes
LPS transport protein LptD	Intratracheal POL7001 protein epitope mimetic molecule (192)	Mouse Acute pneumonia	↓ cfu/lung ↓ Leukocyte recruitment ↓ Cytokine/chemokine profiles
Bacteriophage-induced *Pseudomonas aeruginosa* lysis	Intranasal PAK-P1 bacteriophage (245)	Mouse Acute pneumonia	↓ IL-6 and TNF-α ↑ Survival rate ↓ Bacterial load
Bacteriophage-induced *P. aeruginosa* lysis	Intranasal YH6 or YH30-phage (246, 247)	Mouse or mink Hemorrhagic pneumonia	↓ cfu/lung, spleen, blood ↑ Survival rate
PcrV, nonspecific *P. aeruginosa* neutralization, FcγRs on target cells	Intravenous anti-PcrV antibodies and immunoglobulins (248)	Mouse Lethal *P. aeruginosa* infection	↓ cfu/lung ↑ Survival rate ↓ Cytokines
Nonspecific *P. aeruginosa* neutralization, FcγRs on target cells	Intravenous immunoglobulins (249)	Immunocompromised mice Acute pneumonia	↑ Serum TGF-β ↑ T regulatory cells ↓ Lung injury
Lymphocytes	Recombinant human IL-7 immunotherapy via subcutaneous injection (250)	Murine secondary pneumonia (intratracheal *P. aeruginosa* infection subsequent to cecal ligation puncture)	↑ Lung ILCs and CD8 T cells expressing IL-17, IFN-γ, TNF-α ↑ Survival rate ↑ Spleen T cells expressing IFN-γ, TNF-α, IL-17, IL-22
Pyocin S2, pyocin AP41 (DNase activity), pyocin S5 (pore-forming toxin), pyocin L1 (cytotoxic mechanism is unknown)	Intranasal or peritoneal recombinant protein toxins (molecules produced by *P. aeruginosa* to inhibit the growth of closely related strains) "pyocin cocktail" (251)	Murine acute lung injury	↓ Bacterial counts ↑ Survival rate
Pyocin SD2 (tRNase activity)	Intranasal recombinant *P. aeruginosa* SD2 1 h postinfection (209)	Murine Acute lung injury	↓ Bacterial counts ↑ Survival rate
3-oxo-C12-HSL	Intratracheal lactonase (252)	Rat Acute lung injury	↑ Survival rate ↓ Lung injury
All TLR-4/MD2–expressing cells, primarily immune cells	Intraperitoneal TLR-4/MD2 agonistic monoclonal antibody UT12 (253)	Murine chronic lung infection (bronchi-implanted sterile plastic *P. aeruginosa*–coated tubes)	↓ cfu counts/lung ↑ Neutrophil recruitment and bactericidal activity
P. aeruginosa outer membrane	Aerosolized recombinant human lysozyme (254)	Hamster Acute pneumonia	↓ cfu/lung ↓ BAL neutrophils ↓ Lung injury
P. aeruginosa flagella	Intravenous anti–type a and type b flagellar protein antibodies (36)	Rat Acute pneumonia	↑ Pa_{O_2} ↓ Respiratory rate ↓ Lung injury
P. aeruginosa quorum sensing	Subcutaneous injections of a halogenated furanone (C-30) (255)	Murine Acute lung injury	↓ cfu counts/lung ↓ QS as identified by *LasB-GFP* reporter in infected lungs

Definition of abbreviations: cfu = colony-forming unit; HSL = homoserine lactone; ILC = innate lymphoid cell; *LasB-GFP* = *las* gene that encodes elastase labeled with green fluorescent protein; QS = quorum sensing; TGF = transforming growth factor; TNF = tumor necrosis factor; TLR = Toll-like receptor.

acyl side chains from 3-oxo-C12-HSL (210), inhibits the virulence of *P. aeruginosa* by decreasing the secretion of proteases and pyocyanin (211). The *PA2385* gene of *P. aeruginosa* encodes a quorum quencher acylase that removes the fatty acid side chain from 3-oxo-C12-HSL and effectively reduces the production of PQS, elastase, and pyocyanin *in vitro* (212). A synthetic C4-HSL inhibitor, *N*-(2-pyrimidyl) butanamide, called C11, decreases expression levels of both *las* and *rhl* virulence genes (213). Targeting *pqsA* via substrates that bind the gene reduces autoinducer production for this pathway (214). The compounds brominated furanone C-30 and 5-fluorouracil as well as plant-derived zingerone from ginger root and garlic extract also inhibit *P. aeruginosa* QS systems *in vitro* (215–218). Additional *in vivo* studies are highlighted in Table 1.

Bacteriophages

Bacteriophages are ubiquitously expressed in the biosphere and provide a reservoir of genetic diversity to the bacterial hosts they infect (219). These bacterial viruses, consisting of a single-stranded or double-stranded DNA or RNA, either integrate their genome into the host chromosome or inject and replicate their DNA in the host, resulting in lytic death and the release of progeny phages (219, 220). Lytic phages (Myoviridae, Podoviridae, Siphoviridae) are being assessed for the treatment of *P. aeruginosa* infections (220). Some of these bacteriophages also bind to additional intervention targets. For example, the Myoviridae phage ϕCTX uses LPS as a receptor, and the Podoviridae 116 phage as well as Siphoviridae D3112 and B3 phages bind type IV pili (219). A recently isolated Myoviridae, OMKO1, depends on the porin OprM for entry, and, in the absence of OprM, *P. aeruginosa* becomes resistant to the phage but susceptible to drugs such as tetracycline and erythromycin, because these antibiotics are commonly removed from the bacterium by OprM-specific multidrug efflux systems (MexAb-OprM, MexXY-OprM) (221). This research suggests that microbial defenses involved in the generation of phage resistance may also increase the microbe's susceptibility to antibiotics, which may suggest that these approaches could be combined therapeutically (221). Bacteriophages, used individually or as a mixture of various phages, have also shown improved survival in some animal models of *P. aeruginosa* infection (220) (Table 1). Despite regulatory hurdles involved in the manufacturing and handling of bacteriophages for personalized patient use as well as safety concerns regarding the host immune response and changes to mucosal microbiota (222–224), bacteriophages continue to be evaluated as noted in a clinical trial involving patients with severe burns or diabetic foot ulcers (225).

Interventions Targeting the Host

IL-22

In animal models of ventilator-induced lung injury or allergic inflammation, inhalation or intranasal doses of IL-22 ameliorated disease progression (226). Because IL-22 supports epithelial integrity (156, 227, 228), and the activity of the cytokine may be weakened by *P. aeruginosa* protease degradation (176), inhalation therapy with IL-22 may be an approach to protect host airways against *P. aeruginosa* damage.

Desulfated Heparin

During a bacterial infection, heparin may inhibit the activation of platelets and disrupts neutrophil NET activity (229, 230). Heparin also inhibits mast cell degranulation, which may be important in mast cell protection of the host epithelium (231). However, the clinical use of heparin in the treatment of patients with severe infections has not shown significant benefits (229). Partially desulfated heparin (2-O, 3-O-desulfated heparin: ODSH), a heparin derivative with significant antiinflammatory properties but minimal anticoagulative effects, reduced *P. aeruginosa*–induced neutrophilic lung injury and also increased *P. aeruginosa* clearance when delivered subcutaneously to mice. This may be due to the ability of ODSH to inhibit neutrophil elastase-induced macrophage release of the proinflammatory molecule high-mobility group box 1, which is known to bind to TLR-2, TLR-4, and the receptor for advanced glycation end products (RAGE) (232, 233). Understanding the functions of ODSH with respect to mast cells, platelets, or additional cells in the lung may increase insight into the use of ODSH as a therapeutic for *P. aeruginosa* infections.

ACE2 Activation

Mast cells produce heparin and renin (234). The latter cleaves angiotensinogen into angiotensin I, which is also cleaved by angiotensin-converting enzyme (ACE) into angiotensin II, which then binds type 1 and type 2 receptors involved in vasoconstriction and blood pressure regulation (235). The ACE homolog, ACE2, further cleaves angiotensin II into angiotensin 1 to 7, inactivating angiotensin II, thus functioning as an endogenous inhibitor of the ACE pathway (235). In a murine ACE2 knockout model of lung injury, knockout mice exhibited increased vascular permeability, lung edema, and neutrophil influx in association with decreased lung function, which is a phenotype that was rescued with the administration of recombinant ACE2 or with a pharmacological inhibitor of the angiotensin II type 1 receptor. Additional research in this study also revealed that ACE knockout mice showed reduced disease severity, whereas ACE2 knockout mice increased disease severity in an endotoxin-induced model of lung injury (236), highlighting the divergent function of these two enzymes. These responses may be explained by ACE2 catabolism of angiotensin II into seven peptides (angiotensin 1–7) that exhibit additional mechanisms that are not yet elucidated (235). These studies emphasize the potential of angiotensin II type 1 receptor inhibitors, ACE inhibitors, ACE2, and possibly angiotensins 1 to 7 in treating lung infections.

Interventions Targeting the Host and the Pathogen

Indoles

C-type natriuretic peptide is produced by lung epithelial, club, and endothelial cells and activates QS via the bacterial sensor protein, AmiC (237). The molecule AmiC has structural and pharmacological profiles similar to those of the human C-type natriuretic peptide receptor and is similarly inhibited by an interspecies indole called indole-2,3-dione or isatin, thus inhibiting QS-mediated virulence (237). The molecules 7-fluoroindole and curcumin are also able to inhibit *P. aeruginosa* QS signals *in vitro* and block pyocyanin production (238, 239). Indoles and curcumin are AHR ligands where dietary indole-3-carbinol and intraperitoneally administered curcumin have been shown to suppress LPS-induced lung injury in murine models (240, 241). If AHR ligands, particularly the indoles, also affect the C-type natriuretic peptide receptor AmiC is not clear. Nonetheless, nontoxic AHR ligands may be beneficial in targeting *P. aeruginosa* virulence factors and modulating the immune response to *P. aeruginosa*.

Cationic Molecules

Lactoferrin and LL-37 are both cationic molecules released from neutrophils that can then neutralize LPS but also function in the activation of TLR-4 (83, 242). Although the characterized functions of these molecules confound possible therapeutic approaches, they are pivotal to understanding pathogenesis. For example, lactoferrin also sequesters iron as a mechanism of host defense, where *P. aeruginosa* pyoverdine has been found to acquire the metal from lactoferrin (243). The peptide fragment of lactoferrin,

lactoferricin, also exhibits *P. aeruginosa* antimicrobial functions but lacks the ability to sequester iron, thereby suggesting an alternative antibacterial mechanism (244). LL-37 is an activator of the QS system (35) (Figure 2), suggesting that blocking LL-37 activity could be therapeutic. Continued exploration of lactoferrin, lactoferricin, and LL-37 may yield valuable insight into *P. aeruginosa* infections and treatment.

Conclusions

Current therapies are increasingly insufficient in the treatment of *P. aeruginosa* infections. The ability of the microbe to readily adapt to the host microenvironment, by modulating the expression of cell surface molecules and virulence factors, directly affects the efficiency of the host's innate and adaptive immune responses. This constant interplay between the microbe and host requires new and innovative approaches to effectively eradicate the pathogen from the most vulnerable populations. With increased resistance to antibiotics, effective treatments in the future may include combined therapies involving a QS inhibitor, pyocins, bacteriophages, an immunomodulatory agent, or neutralizing antibody or aptamer with conventional antibiotic therapy to increase the clinical cure rate and improve survival. ■

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