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Why is *Pseudomonas aeruginosa* a pathogen?

Joanna B Goldberg

Address: Department of Microbiology, University of Virginia Health System, Box 800734, Charlottesville, VA 22908-0734, USA

Email: jbg2b@virginia.edu

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Abstract

Despite the expression of a myriad of virulence factors, healthy individuals are generally able to resist infections with *Pseudomonas aeruginosa*. Polymorphonuclear leukocyte-dependent killing is the major mechanism responsible for resistance; dysregulation of host defense mechanisms in addition to expression of specific bacterial factors can result in life-threatening infections with this bacterium.

Introduction and context

Polymorphonuclear leukocytes (PMNs) (neutrophils) are phagocytic cells that are able to kill pathogens; elimination by these cells is a major innate host defense mechanism. In the case of healthy individuals, this host defense mechanism is usually effective at removing *Pseudomonas aeruginosa*. However, in hospitalized patients, particularly those with compromised immune systems, this bacterium is a major cause of morbidity and mortality. It is also the leading bacterial cause of acute ventilator-associated pneumonia and chronic lung infections in patients with cystic fibrosis (CF). Defining the dysfunction of neutrophils in susceptible individuals provides the basis for understanding normal host responses to *P. aeruginosa*. Furthermore, recognition of bacterial factors that promote resistance to PMN killing may help identify therapeutic targets for this important opportunistic pathogen.

Major recent advances

The breakdown of normal innate immune function results in increased susceptibility to *P. aeruginosa*. Unless there is compromise, either at a mucosal surface or by immune defects, healthy humans are generally able to resist becoming infected with this bacterium. Patients undergoing chemotherapy are particularly susceptible to *P. aeruginosa*, which can lead to life-threatening pneumonia and bacteremia. Mice have been used as a model organism for this type of compromise: treatment with the chemotherapeutic agent, cyclophosphamide (Cy) or an antibody generally specific for neutrophils, RB6-8C5 (also known as anti-Gr1), results in mice that are considered leukopenic or neutropenic, respectively. A lethal infection of these immunocompromised mice can be achieved by intranasal administration of a dose of bacteria that is more than 6 logs lower than that needed to cause a similar infection in mice with intact immune function [1]. The *P. aeruginosa* strain used in these studies did not contain the cytotoxic phospholipase, ExoU, and thus does not readily disseminate in normal mice except at very high doses [2] but was found in the liver and spleen of the immunocompromised mice at a very low dose [1]. This was not due to increased lung permeability by the immunocompromise itself but was a result of both Cy treatment and *P. aeruginosa* infection [1]. Similar dissemination from the gastrointestinal tract of *P. aeruginosa* colonized mice was seen after Cy or RB6-8C5 treatment [3]. This suggests that the combination of immunocompromise and *P. aeruginosa* promotes the dissemination that is characteristic of this devastating infection in patients undergoing chemotherapy.

While ExoU is apparently not required for infection in immunocompromised hosts, *P. aeruginosa* strains that express ExoU and the type III secretion system have been associated with poor outcomes in patients with hospital-acquired and ventilator-associated pneumonia [4-6]. In fact, it has been shown recently that ExoU itself interferes with the ability of recruited phagocytic cells to eradicate bacteria from the lung, thus promoting additional immunosuppression [7].

Recently, Koh *et al.* [8] repeated similar low challenges with multiple *P. aeruginosa* strains in Cy- or RB6-8C5-treated mice and observed the same increased susceptibility. Reconstitution of neutrophils by recombinant murine granulocyte colony-stimulating factor partially restored host resistance to infection, implicating these cells as critical immune effectors. MyD88$^{-/-}$ mice, which cannot recruit neutrophils to the lung, were also highly susceptible to *P. aeruginosa* infection. Interestingly, mice lacking functional lymphocytes (RAG$^{-/-}$) were only slightly more susceptible to infection than wild-type mice, but those depleted for resident alveolar macrophages did not show increased susceptibility. These findings suggest that the recruitment of neutrophils to the lung may be a reasonable approach to augment host resistance to *P. aeruginosa* infections. Other studies indicate that such recruitment must be carefully timed with respect to the infection process as the effects of neutrophils themselves can be detrimental if attuned inappropriately [9].

Patients with CF are particularly susceptible to infections with *P. aeruginosa* but are not generally considered immunocompromised. While a defective CF transmembrane conductance regulator (CFTR) gene can affect virtually all cell types, studies of neutrophils from patients with CF continue to implicate these cells as critical players in the susceptibility to chronic lung infection by *P. aeruginosa*.

Dysregulation of various neutrophil functions in CF results in decreased bacterial killing as well as persistent neutrophil influx to the airways, leading to increased lung damage (reviewed in [10-12]). It remains controversial whether abnormalities exist in the oxidative responses of these neutrophils compared with those from healthy individuals [13]. Microarray analysis of neutrophils from children with CF has indicated that these cells show a distinctive pattern of gene expression compared with cells from healthy young adults [14]; however, as none of the subjects in this study tested positive for *P. aeruginosa*, the effects in the context of infection were not considered.

Components of neutrophils can directly influence *P. aeruginosa* infection. For example, CF neutrophils secrete increased levels of neutrophil elastase (NE) compared with cells from healthy subjects. While NE is generally considered a contributor to tissue damage associated with *P. aeruginosa* infection, neutrophils from NE-deficient mice are less able to kill *P. aeruginosa* than those from wild-type mice. Also, NE-deficient mice are more susceptible to infection, and as this is not due to a defect in recruitment, it suggests an important role for NE in innate defense [15]. Similar effects were seen with mice lacking the neutrophil serine protease inhibitor, *serpinb1*, which dampens the activity of NE and other proteases [16].

P. aeruginosa killing by neutrophils from normal donors and CF patients was recently measured by Painter *et al.* [17]. In addition to finding that CF neutrophils had a significantly lower rate of killing, these authors went on to show that inhibition of CFTR itself blocked *P. aeruginosa* killing, implicating this protein as an important component of neutrophil function.

Studies have shown that among the Toll-like receptors (TLRs) tested, only TLR-5 is surface-expressed and upregulated on the airway neutrophils of patients with CF compared with those of healthy individuals [18]. TLR-5 is the cellular receptor for bacterial flagellin. However, since chronic *P. aeruginosa* isolates from patients with CF show decreased flagella expression (one mechanism being the suppression of transcription of flagellin by NE [19]), there is ligand-receptor mismatch and therefore impaired neutrophil function (reviewed in [20]). The importance of other TLRs in neutrophil function and *P. aeruginosa* susceptibility has not been as thoroughly investigated.

A recent study by Hartl *et al.* [21] (reviewed in [22]) showed that interleukin-8 (IL-8) binds to CXCR1, a G protein-coupled chemokine receptor (also known as IL-8RA), to promote PMN-mediated killing of *P. aeruginosa*. However, CXCR1 on neutrophils from the airways of patients with CF was found to be proteolytically cleaved. This degraded receptor led to defects in the bactericidal capacity of neutrophils. In addition, the cleaved receptor fragments induced large amounts of IL-8 in the airways, which recruited more neutrophils, setting up a vicious cycle of airway inflammation in CF.

While numerous secreted and injected enzymes produced by *P. aeruginosa* can affect epithelial and other cells (reviewed in [23]), particular *P. aeruginosa* factors can protect the bacterium from neutrophil-mediated killing. While the polysaccharide alginate is well known for its anti-phagocytic properties, *P. aeruginosa* rhamnolipids have recently been shown to cause necrotic death of PMNs, resulting in reduced clearance of the bacteria [24]. The expression of rhamnolipids is controlled through the *P. aeruginosa* quorum-sensing system, and microarray studies have revealed that this system is itself activated in the presence of PMNs. Also, it was shown that biofilms formed in the presence of PMNs produced more rhamnolipids [25]. Thus, *P. aeruginosa* can provide itself with a protective shield from neutrophils (reviewed in [26]).

The persistent accumulation and lysis of neutrophils can lead to uncontrolled release of granule proteins, DNA, and actin from necrotic cells. These latter two factors have been shown to be important for biofilm development [27-29]. These findings suggest that these components could be important targets for anti-biofilm and anti-*Pseudomonal* therapy (reviewed in [30]).

Future directions

It is now appreciated that *P. aeruginosa* strains with distinct genotypes and phenotypes can be recognized in the natural environment and different types of infections [31-35]. In addition to recent *P. aeruginosa* transcriptome analysis (reviewed in [36]) and metabolic pathway studies [37], future investigations should be extended to include multiple *P. aeruginosa* strains as well as polymicrobial communities present in the environment and in infection.

The natural and acquired antibiotic resistance of *P. aeruginosa* makes infections by this bacterium extremely difficult to treat, and thus the search is on to identify mechanisms or molecules to increase sensitivity to antibiotics [38]. Also, there is a push to find inhibitors of the synthesis or regulation (or both) of factors critical for virulence.

It is a breakdown in the normal host response that is responsible for most of the susceptibility to infection with this bacterium. While the importance of alveolar macrophages and other cells remains controversial [39], the presence of neutrophils is required for protection from infection. However, the appropriate regulation of neutrophil function is critical for a productive response. Studies of neutrophils from CF and other immunodeficient patients have begun to define the pathways and molecules critical for an effective innate immune response. These studies have also shown that that there is a delicate interplay between *P. aeruginosa* and the host to resolve an infection. To define these interactions in more detail, microarray studies have been performed on neutrophils from animals infected with *P. aeruginosa* [40]. Future investigations using human neutrophils [41] in the context of *P. aeruginosa* infection could be even more informative. Such studies may help identify mechanisms to promote a more effective innate host response in susceptible individuals and therefore provide protection from infections by this important pathogen.

Abbreviations

CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; Cy, cyclophosphamide; IL-8, interleukin-8; NE, neutrophil elastase; PMN, polymorphonuclear leukocyte; TLR, Toll-like receptor.

Competing interests

The author declares that she has no competing interests.

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