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***Pseudomonas aeruginosa*: So Many Virulence Factors, So Little Time**

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Despite 60 years of intensive antimicrobial development, bacterial infections remain an important cause of morbidity and mortality. The ever-increasing burden of resistance erodes the efficacy of conventional antibiotics, while critically ill patients frequently fail to respond to appropriate agents even when infected with susceptible organisms. To improve upon the current situation, attempts are being made to leverage our impressive knowledge of bacterial pathogenesis for development of novel therapeutics that block virulence mechanisms rather than simply kill or inhibit growth of bacteria. The thought is that such agents could be used by themselves or as adjuncts that would boost the efficacy of conventional antibiotics. But which virulence determinants should be targeted? A factor that is highly critical to the organism's pathogenesis, of course. In some cases, the choice is straightforward. For example, tetanus toxin is the major virulence determinant of *Clostridium tetani*, and administration of human tetanus immune globulin attenuates the course of tetanus [1]. In other cases, however, the choice is less clear. The problem is compounded by the current emphasis on reductionist approaches in microbiology. To test the importance of a particular bacterial factor in pathogenesis, scientists usually disrupt the gene encoding the factor in a well characterized laboratory strain, and the virulence of the mutant is compared to that of the parental strain in an established animal model of infection. This powerful approach has the benefit of demonstrating a causal relationship between the factor and disease in the model used and has become the gold-standard for identification of virulence factors [2]. However, it leaves unanswered two important questions: Is the factor critical for virulence in most clinical isolates? And how important is the factor relative to other virulence determinants made by the same bacterium? In this issue of Critical Care Medicine, Le Berre and colleagues address these questions in relation to *Pseudomonas aeruginosa* [3].

P. aeruginosa is an opportunistic pathogen familiar to every hospitalist and intensivist. It is a frequent cause of nosocomial infections, many of which are responsible for substantial mortality even when treated with appropriate antibiotics. Thus novel agents that target virulence determinants would be a welcome addition to our antipseudomonal armamentarium. But which virulence determinants should be targeted? *P. aeruginosa* produces a potpourri of pathogenic factors, any one of which might or might not be a worthy target for inhibition. Le Berre and colleagues focused on three extensively studied virulence determinants: quorum-sensing (QS), the type III secretion (TTS) system, and lipopolysaccharide (LPS) O-antigen. QS allows bacteria to respond to their own population

density by coordinately regulating their gene expression patterns [4]. For example, in the presence of high bacterial numbers, QS signaling leads to production of two *P. aeruginosa* toxins, elastase and pyocyanin. TTS is a mechanism by which bacteria inject a set of toxins directly into the cytosol of host cells. Two *P. aeruginosa* proteins secreted by this mechanism are ExoU and ExoS; both have been previously linked to virulence [5]. LPS O-antigen is a variable polysaccharide that decorates the outer surfaces of many bacteria and protects against complement-mediated lysis [6]; differences in O-antigens are the basis for serotyping *P. aeruginosa* strains. Each of these three factors has been studied extensively and conclusively shown to be pathogenic in animal models of infection. Importantly, *P. aeruginosa* strains also differ in their production of each of these factors.

Le Berre and colleagues characterized the QS, TTS, and LPS O-antigen properties of 56 non-clonal *P. aeruginosa* isolates from patients with ventilator-associated pneumonia. The severity of pneumonia caused by each isolate was quantified by measuring alveolar-capillary barrier permeability, lung wet/dry weight ratios, and bacterial dissemination from the lungs in a mouse model of pneumonia. The QS, TTS, and LPS O-antigen properties of each strain were then compared to the severity of pneumonia it caused. Univariate analysis indicated an association between lung injury and elastase production (and by inference QS), O11 O-antigen serotype, and TTS-positive phenotype. However, it had been shown previously that QS, TTS, and LPS O-antigen type are not independent variables. For example, O11 serotype strains more commonly secrete the potent TTS toxin ExoU [7], and QS regulates TTS [8]. To account for this, multivariate analysis was performed, which showed that TTS (especially secretion of ExoU) was most highly correlated with lung injury and that secretion of elastase was associated to a lesser degree.

The approach used by Le Berre and colleagues has several limitations. As acknowledged by the authors, the production of virulence factors in vitro may not mimic expression in vivo, and virulence measurements at a single time point may not adequately capture the true extent of disease. The measurement of QS was indirect, using elastase and pyocyanin secretion as surrogate markers. Also, although inbred mice offer the advantage of a uniform host model, their vulnerability to particular virulence determinants may not reflect that of humans. In this regard, it is reassuring that several studies have also found associations between *P. aeruginosa* TTS and poor outcomes in humans with acute respiratory infections [9–11]. Perhaps the most significant limitation is that this study does not show a causal relationship between severe disease and TTS or QS but only an association. However when viewed together with previously published reports describing conventional mutational approaches, the paper by Le Berre and colleagues provides a compelling argument in favor of an important role for these two factors in acute pneumonia.

Studies such as this one are especially relevant given the current emphasis on translational research and the development of novel therapeutic agents. Since \$800 million is required to bring a new drug to market, it is essential that due diligence be performed to maximize the chances of success before costly clinical trials are begun. In this regard, the study by Le Berre and colleagues bodes well for ongoing preclinical and clinical trials examining the efficacy of inhibitors of TTS and QS in infections caused by *P. aeruginosa* [12–14]. When possible, this approach should be considered as well for other virulence factors that are candidate drug targets [15, 16].

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References

1. Blake PA, Feldman RA, Buchanan TM, Brooks GF, Bennett JV. Serologic therapy of tetanus in the United States, 1965–1971. *JAMA*. 1976; 235(1):42–44. [PubMed: 946000]
2. Falkow S. Molecular Koch's postulates applied to microbial pathogenicity. *Rev Infect Dis*. 1988; 10(Suppl 2):S274–S276. [PubMed: 3055197]
3. Le Berre R, Nguyen S, Nowak E, Kipnis E, Pierre M, Quenee L, Ader F, Lancel S, Courcol R, Guery BP, et al. Relative contribution of three main virulence factors in *P. aeruginosa* pneumonia. *Crit Care Med*. 2011
4. Juhas M, Eberl L, Tummeler B. Quorum sensing: the power of cooperation in the world of *Pseudomonas*. *Environ Microbiol*. 2005; 7(4):459–471. [PubMed: 15816912]
5. Hauser AR. The type III secretion system of *Pseudomonas aeruginosa*: infection by injection. *Nat Rev Microbiol*. 2009; 7(9):654–665. [PubMed: 19680249]
6. Pier GB. *Pseudomonas aeruginosa* lipopolysaccharide: a major virulence factor, initiator of inflammation and target for effective immunity. *Int J Med Microbiol*. 2007; 297(5):277–295. [PubMed: 17466590]
7. Faure K, Shimabukuro D, Ajayi T, Allmond LR, Sawa T, Wiener-Kronish JP. O-antigen serotypes and type III secretory toxins in clinical isolates of *Pseudomonas aeruginosa*. *J Clin Microbiol*. 2003; 41(5):2158–2160. [PubMed: 12734267]
8. Bleves S, Soscia C, Nogueira-Orlandi P, Lazdunski A, Filloux A. Quorum sensing negatively controls type III secretion regulon expression in *Pseudomonas aeruginosa* PAO1. *J Bacteriol*. 2005; 187(11):3898–3902. [PubMed: 15901720]
9. El Solh AA, Akinnusi ME, Wiener-Kronish JP, Lynch SV, Pineda LA, Szarpa K. Persistent infection with *Pseudomonas aeruginosa* in ventilator-associated pneumonia. *Am J Respir Crit Care Med*. 2008
10. Hauser AR, Cobb E, Bodí M, Mariscal D, Vallés J, Engel JN, Rello J. Type III protein secretion is associated with poor clinical outcomes in patients with ventilator-associated pneumonia caused by *Pseudomonas aeruginosa*. *Crit Care Med*. 2002; 30:521–528. [PubMed: 11990909]
11. Roy-Burman A, Savel RH, Racine S, Swanson BL, Revadigar NS, Fujimoto J, Sawa T, Frank DW, Wiener-Kronish JP. Type III protein secretion is associated with death in lower respiratory and systemic *Pseudomonas aeruginosa* infections. *J Infect Dis*. 2001; 183:1767–1774. [PubMed: 11372029]
12. Frank DW, Vallis A, Wiener-Kronish JW, Roy-Burman A, Spack EG, Mullaney BP, Megdoud M, Marks JD, Fritz R, Sawa T. Generation and characterization of a protective monoclonal antibody to *Pseudomonas aeruginosa* PcrV. *J Infect Dis*. 2002; 186:64–73. [PubMed: 12089663]
13. Hentzer M, Wu H, Andersen JB, Riedel K, Rasmussen TB, Bagge N, Kumar N, Schembri MA, Song Z, Kristoffersen P, et al. Attenuation of *Pseudomonas aeruginosa* virulence by quorum sensing inhibitors. *Embo J*. 2003; 22(15):3803–3815. [PubMed: 12881415]
14. Aiello D, Williams JD, Majgier-Baranowska H, Patel I, Peet NP, Huang J, Lory S, Bowlin TL, Moir DT. Discovery and characterization of inhibitors of *Pseudomonas aeruginosa* type III secretion. *Antimicrob Agents Chemother*. 2010; 54(5):1988–1999. [PubMed: 20176902]
15. Veessenmeyer JL, Hauser AR, Lisboa T, Rello J. *Pseudomonas aeruginosa* virulence and therapy: evolving translational strategies. *Crit Care Med*. 2009; 37(5):1777–1786. [PubMed: 19325463]
16. Kipnis E, Sawa T, Wiener-Kronish J. Targeting mechanisms of *Pseudomonas aeruginosa* pathogenesis. *Med Mal Infect*. 2006; 36(2):78–91. [PubMed: 16427231]