The best defense is a good (Protease) offense: How *Pseudomonas aeruginosa* evades mucosal immunity in the lung

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In the field of critical care, the requirement for mechanical ventilation in patients presents a double-edged sword for clinicians because the lung is a highly susceptible site of infection. Medical indications that necessitate assisted breathing are commonly presented in subjects already compromised in respiratory health, yet this procedure greatly increases the potential for introduction of opportunistic bacterial pathogens into the airways. Ventilator-associated pneumonia is a significant source of morbidity in medical intensive care units and contributes to extended days in the hospital, resulting in substantial economic burden as well as higher mortality rates in afflicted patients. Moreover, increased antibiotic use for the treatment of ventilator-associated pneumonia is problematic in health care settings due to the potential for enhanced development of non-antibiotic responsive bacterial strains. The gram-negative bacterium *Pseudomonas aeruginosa* is the most common etiologic agent of ventilator-associated pneumonia, and is frequently associated with other nosocomial infections. Management of patients with ventilator-associated pneumonia as a result of *P. aeruginosa* infection is particularly challenging from a clinical perspective as this microbe is often multi-drug resistant. While drug design strategies for treatment of *P. aeruginosa* infection have emphasized new antibiotics, development of compounds that are both efficacious and low in toxicity for critically ill patients remains a challenge for biomedical research.

Alternative and adjunctive therapies for antibiotic treatment of ventilator-associated pneumonia have been directed toward pathogenic mechanisms of *P. aeruginosa*, including motility and adherence (i.e. flagella, pili). Targeting of virulence factors for *P. aeruginosa* such as secretory proteins, quorum sensing, and biofilm formation has been evaluated for potential efficacy as a substitute for traditional antibiotics. The article by Guillon, et. al. in this issue proposes a new immunotherapeutic approach for attenuation of *P. aeruginosa* pathogenicity, centered upon identification of a virulence factor that may allow *P. aeruginosa* to evade mucosal host-pathogen defense mechanisms. The basis for the Guillon, et. al. study originally stems from a report by Ader, et. al, in which prior airway exposure to *Candida albicans* was shown to impair survival of *P. aeruginosa* in a murine model. *C. albicans* has been isolated in tracheal aspirates of patients with ventilator-induced pneumonia and may affect the clinical outcome of patients who are simultaneously infected with *P. aeruginosa*. A follow-up study by Mear, et. al. using a similar murine model demonstrated that the protective mechanism of *C. albicans* against lung injury by *P. aeruginosa* infection was dependent upon the induction of IL-22 in the lung; introduction of neutralizing antibodies against IL-22 following *C. albicans* airway instillation resulted in increased mortality from *P. aeruginosa* infection. IL-22 is a member of the IL-10 cytokine family, and is primarily expressed by T lymphocytes and innate lymphoid cells. IL-22 binds to a heterodimeric receptor comprised of IL-22R1 and IL-10R2, with downstream signaling mediated by STAT3. While IL-10R2 is ubiquitously expressed, IL-22R1 is localized to epithelial surfaces of skin, gut, and lung, consistent with the known epithelial cell repair properties of its ligand IL-22. IL-22 has been shown to support host-pathogen defense mechanisms against gram-negative bacteria at pulmonary mucosal sites by promoting epithelial cell production of antimicrobial peptides, thereby maintaining barrier integrity of epithelium. Because of the known
protective immune functions of IL-22 for epithelia, particularly with respect to the pathogenicity of *P. aeruginosa* in the murine lung, the authors of the Guillon, et. al. study hypothesized that the human host may be limited in ability to combat *P. aeruginosa* infection if the immunomodulatory properties of IL-22 are compromised, possibly by direct action from bacterial products.\(^{13}\) Through a series of elegant *in vitro* studies, Guillon, et. al. test their hypothesis by first assessing the cytokine modifying properties of *P. aeruginosa*. They initially focused on biochemical evaluation of the *P. aeruginosa* secretome, using mutant strains in order to segregate individual virulence factors for ability to degrade IL-22 protein.

A major outcome of the Guillon et. al. study was the definitive identification of *P. aeruginosa*-derived protease IV as an enzymatic mediator of IL-22 degradation.\(^{13,23}\) As a corollary to their *in vitro* results, the authors reported that protease IV activity could not be inhibited by anti-proteases found in the lung and also demonstrated protease IV activity in tracheal aspirates obtained from *P. aeruginosa*-infected patients. While the findings from the Guillon et. al. study are intriguing, there some caveats to be considered before immediately embarking on IL-22 therapy in clinical trials for ventilator-induced pneumonia. First, it should be noted that direct evidence of IL-22 degradation by *P. aeruginosa*-derived protease IV was limited in *in vitro* assessments in the study; confirmation of attenuated IL-22 levels in tracheal aspirates from *P. aeruginosa*-infected patients would have strengthened the correlation of *in vitro* and *in vivo* protease IV activity. An additional limitation to the Guillon et. al. study is that degradation of cytokines other than IL-22 was not tested to confirm specificity of the *P. aeruginosa*-derived protease IV response. Given the high levels of myeloperoxidase detected in subjects afflicted with ventilator-associated pneumonia regardless of *P. aeruginosa* status, verification that the neutrophilic cytokine IL-17 (often co-expressed with IL-22) is unaffected by protease IV would lend further support for the primary role of IL-22 in *P. aeruginosa* pathogenicity within the lung.

Further research with more comprehensive immune profiling of ventilator-associated pneumonia patients as well as testing of mechanisms using appropriate *in vivo* models is needed to strengthen the functional link between *P. aeruginosa* protease IV activity and IL-22. Regardless, the identification of a novel immune evasion tactic mediated by a *P. aeruginosa* virulence factor provides an important clue as to how this opportunistic pathogen takes advantage of the lung environment to promote colonization. The observations by Guillon et. al. further suggests that airway supplementation with IL-22 may enhance recovery of the injured lung following *P. aeruginosa* infection.\(^{13}\) Reduced levels of airway IL-22 expression has been documented for patient populations outside of those afflicted with ventilator-induced pneumonia, including subjects with acute respiratory distress syndrome and sarcoidosis.\(^{24}\) It would be expected that cystic fibrosis patients, who are highly susceptible to *P. aeruginosa*, would have reduced IL-22 levels, but there is currently no evidence of quantitative differences in this cytokine from nasal lavages.\(^{25}\) Despite these inconsistencies, immunotherapy to enhance local IL-22 production has been explored for lung diseases, as well as chronic conditions outside of the respiratory system such as ulcerative colitis and pancreatitis.\(^{26}\) While it remains to be seen whether IL-22 immunotherapy will be successful for ventilator-induced pneumonia in the future, it would be of interest to explore whether other pathogens similarly utilize virulence factors as an offensive strategy to subvert mucosal immune responses at the site of infection.

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No potential conflicts of interest were disclosed.

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