Published in final edited form as:

Crit Care Med. 2012 August ; 40(8): 2503–2504. doi:10.1097/CCM.0b013e318258e5bb.

Ventilator-Associated Pneumonia Caused by *Pseudomonas aeruginosa*: Cap Your Needles!

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Keywords

Pseudomonas aeruginosa; virulence; PcrV, type III secretion; KB001, ventilator-associated pneumonia, treatment

The advent of the recombinant DNA revolution, which made available powerful techniques such as cloning and mutational analyses, has been a boon for the field of bacterial pathogenesis. We now know the mechanistic details of many bacterial toxins, including their enzymatic activities, multimeric states, processing, cofactors, and host cell substrates. In many cases, the exact amino acids necessary for each step of the pathogenic mechanism have been determined. However, the torrent of advances made in our understanding of molecular pathogenesis has led to only a trickle of novel therapies to help clinicians combat these bacterial infections. Vaccine development has been arguably quite successful, but most new antibiotics are still found by screening compound libraries and modifying existing antibiotic scaffolds. For physicians treating bacterial infections, was the promise of the recombinant DNA revolution merely a mirage, always receding just beyond the horizon of the clinic?

Fortunately, recent events suggest that the fruits of the recombinant DNA revolution are not truant but merely tardy to the field of bacterial infections. The translational research spigot is slowly opening, allowing for the preclinical development of an array of compounds that inhibit virulence factors discovered and characterized with the aid of recombinant DNA technology. For example, in the *Pseudomonas aeruginosa* field alone, anti-infective agents targeting quorum-sensing, biofilm development, flagella, and exotoxin A are under development [1, 2].

Another *P. aeruginosa* virulence determinant that has garnered much recent attention is type III secretion [3]. This complex system forms needle-like structures on the bacterial surface. Upon contact, these needles inject toxic factors called effector proteins into human cells. The effector proteins then subvert these cells by cleaving the phospholipids in their membranes, disturbing their actin cytoskeletons, and otherwise disrupting signaling pathways. The clinical importance of type III secretion has been confirmed by studies

Conflicts: The author collaborates on two grants with Microbiotix, Inc., on the development of inhibitors of *Pseudomonas aeruginosa* type III secretion.

showing that patients have worse outcomes when infected with strains producing functional type III secretion systems compared to strains that do not secrete these proteins [4-7]. As a consequence, investigators have focused on developing agents that block type III secretion [8, 9]. One particularly promising approach has been to use antibodies that recognize PcrV, a protein exposed at the tip of the type III secretion needle [10]. These antibodies block translocation of effector proteins into host cells, in essence forming a cap on the type III needle [11]. Such antibody-based approaches have attenuated disease severity in animal models of acute pneumonia, sepsis, and burn infections [12-15].

In this issue of Critical Care Medicine, François and colleagues present the results of a phase 2a clinical trial examining the efficacy of KB001, a recombinant, PEGylated human Fab' fragment that binds to PcrV [16]. The trial was sponsored by KaloBios Pharmaceuticals, which has developed KB001. The multicenter, placebo-controlled, double-blind trial consisted of 39 mechanically ventilated patients colonized with *P. aeruginosa* randomized to receive a single intravenous infusion of low dose KB001 (3 mg/kg), high dose KB001 (10 mg/kg), or placebo. The primary goals of the study were to determine the safety and pharmacokinetics of the agent. In this regard, KB001 was well tolerated and associated with the same incidence of adverse events as placebo. Likewise, the human Fab' fragment did not appear to be immunogenic, as no anti-KB001 antibodies were detected in any of the patients. KB001 was detected in the endotracheal fluid of all patients who received it and persisted in some patients out to 28 days. Although not designed to determine efficacy, the trial does give an enticing glimpse into this important aspect of KB001. Whereas 60% of patients receiving placebo developed *P. aeruginosa* pneumonia, only 32% of those treated with KB001 did. This difference, however, did not reach statistical significance due to the small number of patients in each arm. Nevertheless, the results support moving forward to a phase 3 trial to examine efficacy.

The trial has several limitations, most of which are discussed by the authors. First, the small sample size precludes definitive conclusions with regards to associations between protection from pneumonia and administration of KB001 or protection from pneumonia and KB001 levels. Second, the patients in the study had relatively severe lung compromise, with prolonged mechanical ventilation and high disease severity scores, which may have facilitated penetration of KB001 from the blood into pulmonary secretions. Thus similar results may not be observed in patient populations characterized by less severe disease. Third, the type III secretion phenotypes of the *P. aeruginosa* strains were not measured. Isolates were tested for the genes encoding type III secretion proteins PcrV, ExoU, and ExoS, but the presence of these genes does not ensure that a functional type III secretion apparatus is produced. Approximately 10-25% of *P. aeruginosa* strains cultured from patients with ventilator-associated pneumonia do not have functioning type III secretion systems [4, 5]; KB001 would not be expected to be effective against these strains. It would be interesting to know whether pneumonia that developed in KB001-treated patients was caused by *P. aeruginosa* strains that lacked functioning type III secretion systems.

The study by François and colleagues is an important step in establishing the potential efficacy of virulence-directed therapies targeting *P. aeruginosa*. It lays the foundation for future studies to determine whether this antibody fragment directed against the tip of the

type III secretion needle can prevent ventilator-associated pneumonia. And as any ICU nurse can tell you, capping your needles is always a good idea.

Acknowledgments

This work was supported by grants R01AI053674 and R01AI075191 from the National Institutes of Health.

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