

CLINICAL IMPLICATIONS OF BASIC RESEARCH

A Critical New Pathway for Toxin Secretion?

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Vaccines that are composed of inactivated bacterial toxins work well for diseases such as diphtheria and tetanus, in which a single secreted toxin is the primary mediator of disease. Pathogens that rely on multiple secreted toxins, however, render vaccines impractical, partly owing to the fact that some toxins are directly injected into host cells and are therefore inaccessible to antibody. An alternative approach that is gaining momentum is to target the critical pathways required for toxin secretion.¹ Five distinct protein secretion pathways have been known to contribute to the virulence of bacterial pathogens. Recent studies by the Mekalanos group (Pukatzki et al.² and Mougous et al.³) have identified a sixth secretion pathway, present in both *Vibrio cholerae* and *Pseudomonas aeruginosa*, that is a potential target for vaccine development and for therapeutic intervention.

The classic diarrheal disease resulting from colonization of the small intestine by *V. cholerae* is mediated by and dependent on the secretion of cholera toxin. Some strains of *V. cholerae*, such as strain V52, lack the cholera toxin genes but still cause sporadic episodes of cholera-like and nondiarrheal diseases through poorly defined mechanisms. To gain further insight into the pathogenic mechanisms of strain V52, Pukatzki and colleagues² established an infection system using amoebae. Amoebae have macrophage-like properties and can be used to model interactions between bacteria and macrophages.

With the use of a high-throughput screen, the investigators isolated *V. cholerae* mutants lacking the ability to kill amoebae. Many of these organisms carried mutations in a cluster of genes that the investigators designated the virulence-associated secretion (VAS) genes, which together encode a new protein secretion system (designated "type VI") that is distinct from the previously described secretion systems known as types I through V (Fig. 1).² Four proteins are secreted

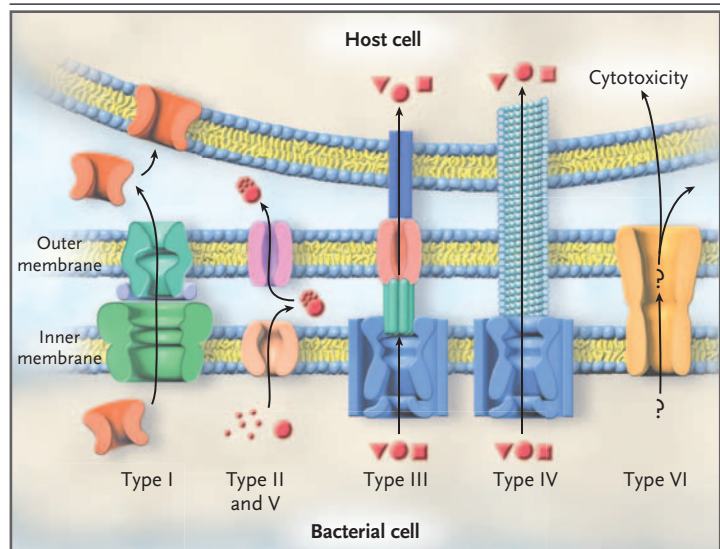


Figure 1. Protein Secretion Pathways of Gram-Negative Bacteria.

Many pathogenic bacteria rely on specialized pathways to secrete proteins that are important for virulence. Each pathway has distinct properties that profoundly influence the manner in which the secreted products interact with host cells. Pore-forming toxins are commonly secreted into the extracellular milieu by the type I secretion pathway. Protein secretion by the type II pathway occurs in two steps: transport across the inner membrane, followed by transport across the outer membrane. Although the type I and II pathways facilitate protein secretion, they have no role in targeting toxins to host cells. However, the type III and type VI secretion pathways are involved in both toxin secretion and targeting to host cells. Each of these systems consists of a multiprotein complex that spans the bacterial-cell envelope and penetrates the host-cell plasma membrane, allowing the injection of toxins directly into the host cell while limiting antigen exposure to host defense mechanisms. The type V pathway represents a minor variation of the type II secretion pathway. Recent studies by Pukatzki et al.² and Mougous et al.³ provide evidence of the existence of a type VI secretion pathway. Although mechanistic details are lacking, virulence factors may be secreted by the pathway directly into host cells.

by the VAS system into cell-culture supernatant fluid, and several of these proteins are essential for killing amoebae and for VAS-dependent cytotoxic effects on mammalian macrophages.

Cell-free culture supernatant containing all the known VAS-secreted proteins lacked cytotoxic-

ic activity when mixed with macrophages — suggesting that physical contact between bacteria and host cells is necessary for the delivery of the VAS-related cytotoxin to host cells. This finding also suggests that the type VI secretion system injects cytotoxins directly into host cells.

The VAS genes of *V. cholerae* are highly conserved in several gram-negative pathogens, including *P. aeruginosa*, a particularly insidious bacterium for patients with cystic fibrosis. Such patients are unusually susceptible to chronic colonization of the airways by the bacterium and, once colonized, remain so for life. Mougous and colleagues³ have recently reported that the VAS-like genes of *P. aeruginosa* also encode a functional type VI secretion system. More important, they provide data suggesting that the type VI secretion system contributes to the pathogenesis of chronic *P. aeruginosa* infection in patients with cystic fibrosis. First, expression of the *P. aeruginosa* VAS-like genes is coordinately regulated with other factors thought to contribute to the pathogenesis of chronic infections. Second, a protein secreted by the *P. aeruginosa* VAS system was present in sputum samples from patients with cystic fibrosis. Third, immunoreactivity to that protein was detected in serum from patients who had been colonized by *P. aeruginosa* for more than 10 years. Immunoreactivity to the same protein was absent or dramatically reduced in serum from patients who had been colonized by *P. aeruginosa* for less than 4 years. Although the sample was too small to yield significant results, this observation suggests that the expression of the VAS genes may increase over the course of chronic infections in patients with cystic fibrosis. Finally, another group had previously isolated *P. aeruginosa* mutants with attenuated pathogenicity in a rat model of chronic lung

infection,⁴ and some of these organisms carried mutations that specifically disabled the type VI secretion pathway.

Specialized protein secretion systems are essential for the virulence of many bacterial pathogens. They have therefore become attractive therapeutic targets. The identification of the type VI secretion pathway is important for several reasons. Its discovery provides a target for therapeutic interventions aimed at disrupting protein secretion. This may be especially relevant for *P. aeruginosa* infections in the airways of patients with cystic fibrosis, in whom the elimination of the bacteria is all but impossible, despite aggressive antibiotic treatment. The mechanism by which *P. aeruginosa* persists in the airways of such patients is poorly understood. A deeper understanding of the type VI system may provide invaluable insight into the nature of persistence. Finally, the presence of VAS-like genes in *Salmonella enterica*, *Yersinia pestis*, and *Escherichia coli* O157:H7 suggests that the inhibition of type VI secretory activity may have wide use as a potential therapeutic approach.

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