Commentary

The *Burkholderia cepacia* Complex and Cytokine Induction: An Inflammatory Tale

Commentary on the article by Reddi et al. on page 297

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At a time when the emergence of multiresistant “superbugs” is increasingly highlighted, it seems prudent to consider an alternative threat, namely the potential of inherently resistant plant pathogens to cause human infections and for pathogenic processes in both hosts to share common themes (1, 2). These challenging aspects of microbial evolution are exemplified by a group of organisms known as the *Burkholderia cepacia* complex (Bcc).

B. cepacia was first described in 1950 as the Gram-negative phytopathogen responsible for bacterial rot of onion bulbs (3), a plant host with formidable antibacterial defenses, illustrated by the *de novo* synthesis of the volatile disulfide compound allicin when onions, garlic, and other *alliums* are damaged during harvest or compromised (sliced) for culinary purposes. In the last decade, taxonomic studies have revealed that isolates previously identified as *B. cepacia* compose a group of closely related bacteria that comprise at least nine genomic species (genomovars), referred to collectively as the Bcc (4).

As human pathogens, the Bcc are a particular threat to individuals with chronic granulomatous disease (CGD) or cystic fibrosis (CF), causing life-threatening lung infections exacerbated by intrinsic resistance to antibiotic therapy and in CF the potential for epidemic spread (5, 6). In Europe and North America, *Burkholderia multivorans* (previously genomovar II) and *B. cenocepacia* (previously genomovar III) account for >90% of Bcc isolates from CF patients (4, 7, 8); these are also the two species most associated with patient-to-patient transmission and pulmonary deterioration, including complications after lung transplantation (9). Spread of individual Bcc strains can occur at local, national, and intercontinental levels. Enhanced transmissibility is particularly associated with the *B. cenocepacia* lineage known as ET12 (5, 10, 11). However, general statements on transmissibility and other biologic properties of individual Bcc species and strains must be made with caution. For example, minimum inhibitory concentrations (MICs) for β lactams, aminoglycosides, quinolones, and other

antibiotic groups vary widely within and between genomovars, challenging the dogma of the group’s inherent resistance, at least *in vitro* (12).

A striking feature of Bcc infection in individuals with CF is the pronounced inflammatory response associated with the fulminating pneumonia, sometimes referred to as cepacia syndrome (13, 14). Putative Bcc virulence determinants include lipopolysaccharide (LPS; endotoxin), protease, hemolysin, catalases, siderophores, quorum sensing, type III secretion, cytotoxins, exopolysaccharide, intracellularly, and two transmissibility determinants: unique cable pili and the epidemic strain marker bcesm (15, 16). The role of most of these factors in lung disease or patient-to-patient spread has not been formally proved. It is interesting, however, that the ET12 lineage alone seems to possess the bcesm and express cable pili. Host-pathogen interactions within individual patients seem crucial, as clinical outcome cannot be predicted even when a group of individuals are infected by the same strain. This phenomenon includes infection with ET12, which is represented in the article in this issue of *Pediatric Research* (17) by the Edinburgh isolate J2315/CF5610 (5, 11, 18). J2315 is also the Bcc candidate chosen for genome sequencing at the Sanger Institute UK (www.sanger.ac.uk/Projects/B_cepacia/).

As suggested in the opening paragraph of this commentary, it is possible that environmental phytopathogens, including Bcc, possess novel virulence mechanisms involving factors capable of multifunctional roles. For example, the small (<3000 Da) hemolytic pore-forming toxin produced by J2315 (19) resembles the amphipathic lipopeptide toxins produced by plant pathogenic pseudomonads. The properties of this toxin are particularly interesting in relation to human neutrophils, the primary agents of immune-mediated damage in CF airways. At concentrations above 100 ng/ml, the Bcc toxin causes degranulation of human neutrophils; in contrast, at lower concentrations, the same toxin induces an anti-inflammatory response, namely neutrophil apoptosis. Thus, host immune responses may be modulated during Bcc infection. In CF airways, a low population density of Bcc might avoid detection and initiate primary colonization; however, the result of subsequent bacterial growth and neutrophil degranulation would be self-inflicted immunopathology.

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The role of pulmonary neutrophils in the susceptibility of individuals with CF and CGD is interesting. In CF, the innate resistance of Bcc to neutrophil antimicrobial peptides could combine with catalase production to resist killing by neutrophil oxidative mechanisms (20). In CGD, susceptibility to Bcc infection can be explained by peptide resistance combined with inherited loss of neutrophil oxidative killing (21). Clearly, identification of the bacterial and host factors responsible for life-threatening pulmonary inflammation in susceptible humans is vital.

The paper by Reddi *et al.* in this issue confirms the inflammatory potential of the Bcc and provides important novel data revealing the mechanisms that lead to IL-8 gene expression in human lung epithelial (HLE) cells *via* CD14 receptors and mitogen-activated protein kinase. The authors justify use of alveolar epithelial cells on reports that Bcc (including J2315) is capable of invading and surviving within A549 pulmonary epithelial cells and primary type II pneumocytes (22, 23). Although respiratory epithelial cells are one of the main cell types encountered by Bcc in CF airways, it could be argued that pulmonary monocytes are the key players in the synthesis of excess proinflammatory cytokines and subsequent immunopathology.

The novel data in the study relate primarily to the cellular mechanisms involved in IL-8 induction in HLE cells after exposure to the Bcc isolate ATCC 700070, identified as genomovar III (*B. cenocepacia*). Reddi *et al.* show that induced IL-8 secretion in HLE involves *de novo* IL-8 protein synthesis at the level of gene transcription and that the p38 mitogen-activated protein kinase pathway and the transcriptional element NF- κ B are key factors.

The data presented by Reddi *et al.* relating to the Bcc component(s) responsible for IL-8 induction in HLE cells are also important. The authors implicate bacterial LPS as the inflammatory stimulant by noting reduction of IL-8 induction in the presence of human MAb to CD14, and the enhancing effect of serum lipopolysaccharide binding protein. Although, these results are novel with respect to HLE cells, they essentially confirm previous direct evidence of the inflammatory effects of Bcc LPS (including strain J2315) on other key players in pulmonary defense. For example, Shaw *et al.* (24) showed that LPS extracted from clinical and environmental isolates of Bcc elicited a strain-dependent tumor necrosis factor- α (TNF- α) response from human mononuclear leukocytes that in some strains (including the ET12 strain C1359) was nine times more potent than *P. aeruginosa* PAO1. Similar LPS-induction of TNF- α was subsequently reported by Zughaier *et al.* (25). LPS from ET12 (J2315) has also been reported to increase neutrophil surface expression of the β 2 integrin, to prime neutrophils for a potent respiratory burst response to *P. aeruginosa* and other stimuli (26) (J.E. Hughes and Govan JRW, unpublished observations) and to up-regulate inducible nitric oxide synthase and proinflammatory cytokine mRNA in both CF and non-CF leukocytes (27). It is interesting that LPS from *Ralstonia* and other potential CF pathogens can also induce IL-6, IL-8, and TNF- α from human blood and the human-derived monocyte cell line (THP-1) to a degree comparable with J2315 (28).

The high prevalence and enhanced transmissibility associated with *B. cenocepacia* contrasts with the lack of transmission of some strains between CF siblings (5) and serves to emphasize the danger of making general statements on epidemiology, pathogenesis, and management of Bcc infections. In their article, Reddi *et al.* include the statement, "*B. cepacia* ATCC strain 700070 induces A549 cells to secrete IL-8 in a similar manner to *B. cepacia* strain J2315." This was an important experiment to show that IL-8 induction by ATCC 700070 is shared by other Bcc isolates. Unfortunately, the authors did not report the pulsed-field gel electrophoresis or random amplified polymorphic DNA typing profile of ATCC 700070. This could have excluded the possibility that these *B. cenocepacia* isolates are clonal—a reasonable precaution given the Canadian origin of ATCC 700070 and the widespread Canadian prevalence of the ET12 (random amplified polymorphic DNA type 2) lineage (29).

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