Review

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Interactions of *Burkholderia cenocepacia* and other *Burkholderia cepacia* complex bacteria with epithelial and phagocytic cells

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Burkholderia cenocepacia is a member of the *B. cepacia* complex (Bcc), a group of opportunistic bacteria that infect the airways of patients with cystic fibrosis (CF) and are extraordinarily resistant to almost all clinically useful antibiotics. Infections in CF patients with Bcc bacteria generally lead to a more rapid decline in lung function, and in some cases to the 'cepacia syndrome', a virtually deadly exacerbation of the lung infection with systemic manifestations. These characteristics of Bcc bacteria contribute to higher morbidity and mortality in infected CF patients. In the last 10 years considerable progress has been made in understanding the interactions between Bcc bacteria and mammalian host cells. Bcc isolates can survive either intracellularly within eukaryotic cells or extracellularly in host tissues. They survive within phagocytes and respiratory epithelial cells, and they have the ability to breach the respiratory epithelium layer. Survival and persistence of Bcc bacteria within host cells and tissues are believed to play a key role in pulmonary infection and to contribute to the persistent inflammation observed in patients with CF. This review summarizes recent findings concerning the interaction between Bcc bacteria and epithelial and phagocytic cells.

Introduction

Cystic fibrosis (CF) occurs in individuals carrying mutations in a gene encoding a membrane-embedded chloride channel, named CF transmembrane regulator (CFTR), and it remains the most common lethal inherited disease in the Caucasian population (Riordan *et al.*, 1989). The CFTR abnormality is associated with defective mucociliary clearance and impaired innate immunity of the airways (Goldman *et al.*, 1997; Matsui *et al.*, 1998; Smith *et al.*, 1996), resulting in patients becoming susceptible to chronic respiratory infections and acute exacerbations, which in turn mediate progressive pulmonary deterioration, causing substantial morbidity and mortality. Traditionally, *Pseudomonas aeruginosa* is the most prevalent CF pathogen. However, recent evidence obtained using microbiological and molecular approaches underscores the polymicrobial nature of the flora in the lower airways of CF patients (Harris *et al.*, 2007; Rogers *et al.*, 2006; Tunney *et al.*, 2008), and the potential role of polymicrobial communities in disease progression (Sibley *et al.*, 2008). Infections with opportunistic non-fermentative Gram-negative species other than *P. aeruginosa* are also becoming

more common, particularly as the average survival of CF patients increases over time. Among them, a group of bacteria known as the *Burkholderia cepacia* complex (Bcc) is of major concern within the CF community. The Bcc comprises at least 15 species (Mahenthiralingam *et al.*, 2005; Vanlaere *et al.*, 2008); although all of them have been recovered from the sputum of CF patients (Coenye & Vandamme, 2003), *Burkholderia multivorans* and *Burkholderia cenocepacia* are particularly prevalent in CF infections (Mahenthiralingam *et al.*, 2005). Bcc members are highly resistant to antimicrobial peptides (Loutet *et al.*, 2006; Mahenthiralingam *et al.*, 2005) and to most clinically useful antibiotics, which complicates the effective treatment of respiratory infection in CF patients (Aaron *et al.*, 2000; Nzula *et al.*, 2002). The infecting bacteria can be transmitted between patients (Speert *et al.*, 2002) and once colonized some patients develop a rapid, fatal necrotizing pneumonia, sometimes associated with septicaemia, known as the cepacia syndrome (Govan & Deretic, 1996; Isles *et al.*, 1984; Mahenthiralingam *et al.*, 2005; Speert *et al.*, 2002). Although Bcc isolates predominantly grow extracellularly they can also survive intracellularly within free-living amoebae, phagocytes (Lamothe *et al.*, 2004; Landers *et al.*, 2000; Marolda *et al.*, 1999) and respiratory epithelial cells (Govan & Deretic, 1996). Survival and persistence within host cells is believed to play a key role in

Abbreviations: Bcc, *Burkholderia cepacia* complex; BcCV, Bcc-containing vacuole; CF, cystic fibrosis; CFTR, CF transmembrane regulator; DC, dendritic cell; ROS, reactive oxygen species; TLR, Toll-like receptor.

pathogenesis (Chiu *et al.*, 2001; Valvano *et al.*, 2005, 2006). In recent years, several studies have addressed the interactions of Bcc bacteria with host cells; these are the focus of this review article. Other aspects of the Bcc biology and epidemiology have been covered in recent reviews (Govan *et al.*, 2007; Mahenthiralingam *et al.*, 2008).

Bcc and epithelial cells

Airway epithelial cells play a preponderant role in maintaining mucosal integrity and are the first cells to be challenged by airborne pathogens. In healthy individuals, a mucus layer (containing antibacterial, antiproteolytic and antioxidant molecules) bathes the airway epithelium and traps particles and micro-organisms from the air, which are mechanically eliminated by mucociliary clearance (Zhou *et al.*, 2000). The absence of CFTR function leads to physiopathological changes resulting in a dehydrated airway liquid surface, increased mucus viscosity, and impaired mucociliary clearance, providing an ideal environment for bacterial colonization (Boucher, 2007). It is well established that the airway epithelium plays a central role in progression of CF lung disease via production of numerous cytokines, chemokines, inflammatory enzymes and adhesion molecules (Diamond *et al.*, 2000; Jacquot *et al.*, 2008).

Attachment to cell surfaces is one of the key steps in bacterial pathogenesis, and requires the specific interaction of bacterial surface molecules ('adhesins') with host cell membrane molecules or extracellular matrix proteins (Beachey, 1981; Karlsson *et al.*, 1992). To date, the cable (Cbl) pili and their associated 22 kDa adhesin are the only well-documented adhesins in Bcc species. They mediate bacterial adherence to mucin and cytokeratin 13 in epithelial cells, are required for bacterial transmigration across squamous epithelium, cause cytotoxicity and induce pro-inflammatory response by stimulating IL-8 production (Sajjan & Forstner, 1992, 1993; Sajjan *et al.*, 2000, 2002b; Urban *et al.*, 2005). However, only a subset of Bcc isolates produces cable pili and the associated adhesin (LiPuma *et al.*, 2001; McDowell *et al.*, 2004), suggesting that other uncharacterized bacterial adhesins may exist.

The pulmonary histopathology in CF patients is dominated by neutrophil infiltration, and there is evidence that subsequent epithelial damage associated with *B. cenocepacia* infection (Mahenthiralingam *et al.*, 2005; Speert *et al.*, 2002) is due to the marked inflammatory response elicited by the infecting bacteria (De Soyza *et al.*, 2004). Airway epithelial cells likely contribute to amplifying the inflammatory response associated with progressive deterioration of the airways (Jacquot *et al.*, 2008; Valvano *et al.*, 2005). However, the mechanisms by which the *B. cenocepacia*-epithelium interaction triggers the inflammatory process are yet to be fully characterized.

Toll-like receptors (TLRs) play a central role in innate immunity (Medzhitov, 2001). They recognize pathogen-conserved motifs and initiate a signalling cascade resulting

in the activation of NF- κ B and other transcription factors that regulate the expression of various host defence genes, including IL-8, IL-6, IL-1 and TNF α (Medzhitov, 2001). TLR4 and TLR5 are potential receptors for *B. cenocepacia* LPS and flagella respectively, and bacteria-receptor interactions result in both NF- κ B activation and IL-8 secretion in epithelial cells (Reddi *et al.*, 2003; Urban *et al.*, 2004). Others have reported that in bronchial epithelial cells the expression of TLR2 and TLR4 but not TLR5 is upregulated by *B. cenocepacia* infection (de C. Ventura *et al.*, 2008). However, Blohmke *et al.* (2008) showed that blood and airway cells from CF patients produce more TLR5-dependent pro-inflammatory cytokines than cells from non-CF controls following exposure to Bcc and *P. aeruginosa*. Inhibition of TLR5-mediated signalling abolished the inflammatory response, suggesting TLR5 as a novel target to reduce the damage caused by lung inflammation in CF patients (Blohmke *et al.*, 2008). Differences in the pro-inflammatory potential among Bcc isolates suggest that receptors other than TLRs must be involved in the inflammatory signalling activated by Bcc. Recently, Sajjan *et al.* (2008b) demonstrated that *B. cenocepacia* BC7, an isolate belonging to the transmissible ET12 lineage (Mahenthiralingam *et al.*, 2000), binds to tumour necrosis factor receptor-1 (TNFR1) and activates the TNFR1-related signalling pathway, leading to NF- κ B activation and IL-8 production. This interaction does not utilize the cable pili or the 22 kDa adhesin (Sajjan *et al.*, 2008b), suggesting the participation of an unidentified bacterial ligand. Activation of the pro-inflammatory signalling through NF- κ B may be critical in the context of CF patients. A recent study has demonstrated that CFTR is a negative regulator of NF- κ B-mediated innate immune response (Vij *et al.*, 2009). Also, defective CFTR function results in hyper-inflammatory signalling, chronic inflammation and lung disease (Jacquot *et al.*, 2008; Vij *et al.*, 2009).

Many studies have shown that Bcc can invade and survive within epithelial cells *in vitro* (Burns *et al.*, 1996; Caraher *et al.*, 2007; Cieri *et al.*, 2002; Duff *et al.*, 2006; Keig *et al.*, 2001, 2002; Martin & Mohr, 2000), but the ability to enter and survive within epithelial cells is predominantly strain dependent rather than species dependent. *In vivo* studies with a mouse model supported the concept that Bcc bacteria adhere to and invade respiratory epithelial cells (Chiu *et al.*, 2001), and demonstrated that the ability to invade lung epithelial cells *in vitro* correlates with the ability to infect *in vivo* (Cieri *et al.*, 2002). The histopathology of Bcc-infected lungs from CF patients reveals substantial numbers of bacteria between bronchial epithelial cells (Sajjan *et al.*, 2001). *In vitro* studies using well-differentiated primary human epithelial cell lines have facilitated investigations into the mechanisms by which Bcc isolates penetrate airway barriers and cause bacteraemia. Several routes of entry have been reported, depending on the specific isolate investigated, suggesting that Bcc bacteria have multiple mechanisms to interact with epithelial cells

(Schwab *et al.*, 2002). In one detailed study, a *B. cenocepacia* strain formed microcolonies at the apical cell surface, followed by entry into and destruction of epithelial cells, which also involved disruption of the glycocalyx and rearrangements of the actin cytoskeleton. In contrast, *Burkholderia stabilis* cells that penetrated the epithelium were located between epithelial cells, suggesting paracytosis. Finally, the *B. multivorans* strain penetrated the epithelium both by cell destruction and by paracytosis (Schwab *et al.*, 2002), which could be attributed to loss of occludin from tight junctions (Kim *et al.*, 2005). *B. multivorans* also promoted disruption of the actin filament network (Schwab *et al.*, 2003). The process of actin rearrangement by *B. cenocepacia* was confirmed in separate studies, but was common to both viable and nonviable bacteria (Sajjan *et al.*, 2006). Transmigration of *B. cenocepacia* can also occur across squamous epithelial cell cultures by paracellular and transcellular routes (Sajjan *et al.*, 2002a). Squamous metaplasia is frequently observed in both large and small airways of CF patients, probably as a consequence of continuing episodes of infection-related injury and repair (Simel *et al.*, 1984). This suggests that Bcc bacteria may be more adapted to colonize injured epithelial surfaces, as squamous metaplasia in the airways is rare in healthy individuals. Duff *et al.* (2006) have also proposed different mechanisms of invasion of Bcc species mediated by receptors at distinct locations within the polarized epithelial cells. Moreover, a potential role for lipases in Bcc epithelial cell invasion has been established (Mullen *et al.*, 2007). Together, these observations suggest that Bcc bacteria can employ a repertoire of strategies to breach the epithelial layer in the airways, and this may help to explain, at least in part, the different clinical outcomes of infection in patients with CF (reviewed by McClean & Callaghan, 2009).

In epithelial cells, *B. cenocepacia* is internalized via a membrane-bound vacuole and the bacterium interferes with the normal endocytic pathway. Vacuoles containing bacteria interact with early endosomes, but escape from late endosomes and lysosomes to enter autophagosomes and ultimately replicate within the endoplasmic reticulum (Sajjan *et al.*, 2006). Live *B. cenocepacia* are required for subverting the normal endocytic pathway; heat-killed bacteria are targeted to the lysosomes within 4 h post-infection (Sajjan *et al.*, 2006). Bacterial effectors of this process are unknown. A recent study suggested that a functional plasmid-encoded type IV secretion system contributes to the survival and replication of *B. cenocepacia* in eukaryotic cells (Sajjan *et al.*, 2008a). Also, Bcc species can induce apoptosis in airway epithelial cells (Cheung *et al.*, 2007; Moura *et al.*, 2008). Although the mechanisms accounting for cell apoptosis remain unknown, it has been shown that cell death in response to *B. cenocepacia* infection appears to be independent of the type III secretion system, biofilm formation and secreted bacterial cytotoxins and is mediated by Cbl pili (Cheung *et al.*, 2007).

Bcc and macrophages

Bcc isolates survive with minimal or no replication within murine and human macrophages (Lamothe *et al.*, 2007; Martin & Mohr, 2000; Saini *et al.*, 1999; Sajjan *et al.*, 2008a). Engulfed bacteria reside in spacious vacuoles termed Bcc-containing vacuoles (BcCVs), which exhibit a pronounced delay in fusion with lysosomes (Lamothe *et al.*, 2007) and in the assembly of a functional NADPH oxidase complex on their membrane (Keith *et al.*, 2009). The maturation delay of BcCVs is required for the intracellular survival of *B. cenocepacia* in macrophages (Lamothe *et al.*, 2007). Conceivably, such a delay enables the bacteria to activate genes that could confer resistance to the hostile environment of lysosomes, providing Bcc strains with an additional survival advantage in the intracellular environment. This notion is supported by the observation that mutants unable to mediate the maturation delay of the BcCV are not only rapidly cleared from macrophages but are also avirulent in the rat agar bead model of chronic lung infection (Aubert *et al.*, 2008; Hunt *et al.*, 2004; Maloney & Valvano, 2006).

The bacterial determinants and the host cell targets involved in the mechanism underlying Bcc intracellular survival are not fully understood. Survival under extreme and changing conditions requires adaptive modulation of gene expression in response to environmental cues. These gene expression changes are often controlled by alternative sigma factors (reviewed by Kazmierczak *et al.*, 2005). Inactivation of the alternative sigma factors RpoN or RpoE in *B. cenocepacia* results in mutants unable to delay the maturation of the BcCVs in macrophages (Flannagan & Valvano, 2008; Saldías *et al.*, 2008), suggesting that the ability of Bcc to adapt to diverse environmental conditions allows these bacteria to survive intracellularly. Bcc strains can overcome oxidative damage, an ability that contributes to intracellular survival. In macrophages, Bcc bacteria survive despite the oxidative burst (Saini *et al.*, 1999). This requires bacterial production of a periplasmic superoxide dismutase (Keith & Valvano, 2007) and a melanin-like pigment that plays an important role in protecting the organism from oxidative damage (Keith *et al.*, 2007). *B. cenocepacia* also requires MgtC (a putative membrane transporter) for survival in the rat model of lung infection (Hunt *et al.*, 2004) and within macrophages (Maloney & Valvano, 2006). The function of MgtC currently remains unknown, but it is a virulence factor essential for survival within macrophages and animal models for infection in several bacterial pathogens such as *Salmonella enterica* serovar Typhimurium, *Mycobacterium tuberculosis*, *Brucella suis* and *Yersinia pestis* (Alix & Blanc-Potard, 2007).

Type III and type IV secretion systems are well-known mediators of virulence in Gram-negative bacteria, enabling the secretion of virulence factors directly into or in the proximity of host eukaryotic cells (Backert & Meyer, 2006; Galán & Wolf-Watz, 2006). *B. cenocepacia* has one type III and two type IV secretion systems. Inactivation of the type

III secretion system in *B. cenocepacia* J2315 does not abolish the BcCV maturation delay (Lamothe *et al.*, 2007), implying that type III secreted effectors are likely not involved in mediating the BcCV maturation delay. However, a *B. cenocepacia* J2315 type III mutant is cleared more rapidly from the lung of infected mice (Tomich *et al.*, 2003), suggesting that type III secretion is important in the pathogenesis of the bacterium, although it may not be essential for intracellular survival within macrophages. Recently, it has been reported that a plasmid-encoded type IV secretion system is required for the survival of *B. cenocepacia* within epithelial cells and macrophages (Sajjan *et al.*, 2008a), suggesting the possibility that an unidentified type IV secreted effector may be involved in the BcCV maturation delay. The type VI secretion system is a relatively newly described and highly conserved system in Gram-negative bacteria that closely interact with eukaryotic cells (Bingle *et al.*, 2008); it is now recognized as an important contributor to the virulence of several pathogens (Mougous *et al.*, 2006; Pukatzki *et al.*, 2006; Schell *et al.*, 2007; Suarez *et al.*, 2008; Zheng & Leung, 2007). Work in our laboratory has shown that *B. cenocepacia* mutants defective in type VI secretion are impaired for survival in a chronic lung infection model (Hunt *et al.*, 2004) and that the *B. cenocepacia* type VI secretion system contributes to host cytoskeletal rearrangements in macrophages (Aubert *et al.*, 2008), suggesting that the *B. cenocepacia* type VI secretion system could aid bacterial survival by targeting phagocytic cells. However, a role for this secretion system in the maturation delay of BcCVs has not been demonstrated.

Cellular biology of the BcCVs

Recent studies in murine macrophages have partially elucidated the biology of the BcCVs. Vacuoles containing live *B. cenocepacia* interact with early endosomes shortly after internalization. The early endosome autoantigen 1 (EEA1) protein, a marker for the sorting vesicles of the endocytic pathway (Scott *et al.*, 2002), is transiently recruited within minutes to the BcCVs but dissociates from these vacuoles after 30–45 min, demonstrating that Bcc does not alter the early events of vacuole maturation (Lamothe *et al.*, 2007). However, the interaction of BcCVs with the late endosomes and/or lysosomes is significantly altered. A delay of up to 6 h occurs before the accumulation of the late phagosome/lysosome marker LAMP-1 can be clearly detected in the BcCV membrane. During this time, the vacuoles maintain a luminal pH of 6.4, while vacuoles containing heat-inactivated bacteria rapidly reach a pH of 4.8 (Lamothe *et al.*, 2007).

NADPH oxidase is a membrane enzyme complex that plays a crucial role in host defence by professional phagocytes such as neutrophils and macrophages. In resting phagocytes the components of the NADPH oxidase are spatially segregated into the membrane and cytosol, but after activation by soluble agonist or phagocytosis the complex is assembled on the vacuolar membrane and the enzyme

becomes active in the production of potent microbicidal reactive oxygen species (ROS) (Nauseef, 2007). Localized production of ROS by the NADPH oxidase complex is spatially regulated and requires the recruitment of the membrane components to the phagosome and the assembly of cytoplasmic regulatory proteins on the phagosomal membrane (Minakami & Sumimoto, 2006). In macrophages infected with *B. cenocepacia*, the BcCVs exhibit a ~6 h delay in the normal assembly of a functional NADPH oxidase complex on their membrane, a delay that is associated with less superoxide production (Keith *et al.*, 2009). Thus, this delay may prevent the efficient clearance of this opportunistic pathogen from the infected airways of susceptible patients.

Using U937 monocyte-derived macrophages, Sajjan *et al.* (2008a) showed that at 24 h post-infection *B. cenocepacia* colocalized with the endoplasmic reticulum marker calnexin. The authors proposed that the bacteria escape from the classical endocytic pathway and traffic to the endoplasmic reticulum, where they replicate in a type IV secretion system-dependent fashion (Sajjan *et al.*, 2008a). We have not been able to demonstrate colocalization of calnexin and BcCVs in infected RAW264.7 macrophages (Lamothe, 2007). Thus, it is possible that the traffic of BcCVs may differ depending on the macrophage cell lines. Further studies are needed to address the maturation of BcCVs in macrophages.

Is there a role for CFTR in macrophages?

A recent study has suggested that phagosomes of CFTR-defective alveolar macrophages and neutrophils exhibit a constitutive acidification delay that could lead to impaired bactericidal activity (Di *et al.*, 2006). However, using fluorescence ratio imaging to measure the endosomal pH against an internal standard, Haggie & Verkman (2007) convincingly demonstrated that phagolysosomal acidification in alveolar macrophages is CFTR-independent, although these investigators did not examine directly the ability of CFTR macrophages to clear an intracellular infection. In agreement with this later study, we have shown that uninfected CFTR-defective macrophages or normal macrophages treated with a CFTR-specific inhibitor display normal acidification (Lamothe & Valvano, 2008). However, after infection with *B. cenocepacia*, BcCVs in CFTR-defective macrophages or macrophages pretreated with a CFTR functional inhibitor exhibit a more prolonged delay in acidification and phagolysosomal fusion than that usually observed in the control macrophages (Lamothe & Valvano, 2008). These results indicate that a dysfunctional CFTR enhances the *B. cenocepacia*-mediated maturation defect of the BcCVs. The CFTR-associated phagosomal maturation defect was absent in macrophages exposed to heat-inactivated bacteria or a non-CF pathogen such as *Salmonella enterica* (Lamothe & Valvano, 2008), suggesting that this process is specific to *B. cenocepacia* and perhaps other CF pathogens.

In human neutrophils, CFTR channel dysfunction affects neutrophil chlorination of engulfed bacteria (Painter *et al.*, 2006, 2008), raising the possibility that CFTR contributes to bacterial clearance rather than phagosomal acidification. Our experiments with *B. cenocepacia*-infected CFTR-defective macrophages, showing an extended delay in the trafficking of BcCVs to lysosomes, do indeed support a role for CFTR in the mechanism of clearance of the intracellular infection, as we have shown before that *B. cenocepacia* localized to the lysosome rapidly loses cell envelope integrity (Lamothe *et al.*, 2007). Furthermore, we have recently shown that the delayed NADPH oxidase assembly phenotype observed upon infection with live *B. cenocepacia* is enhanced in the presence of a dysfunctional CFTR (Keith *et al.*, 2009). These experiments also support a role for CFTR in the clearance of the intracellular infection by *B. cenocepacia* and could help in understanding the molecular basis of the persistence of the bacteria within CF patients compared to healthy individuals.

Bcc and neutrophils

Neutrophils play a vital role in lung defence against bacteria and are a fundamental component of the innate immune response. Upon infection of the respiratory system, neutrophils are rapidly recruited from the peripheral circulation to the site of infection, where they ingest and destroy the invading micro-organisms using a combination of oxidative and non-oxidative mechanisms. Neutrophils are short-lived cells and normally, after phagocytosis, they undergo apoptosis and are disposed of

by other cell types, most commonly macrophages (Downey *et al.*, 2009). Neutrophils are not only important effectors of bacterial phagocytosis but are also at the centre of the inflammatory process in CF. One of the most salient characteristics of the chronic inflammation in CF lung disease is the predominant neutrophilic infiltration. Indeed, neutrophils are considered responsible for the onset and promotion of inflammation within the CF lung (Downey *et al.*, 2009), and because of ineffective bacterial clearance multiple rounds of activation and destruction of neutrophils in the CF lung may lead to a vicious cycle that appears to be a major contributor to tissue damage (Fig. 1). The abnormally thickened mucus in the airways of CF patients may impede neutrophil motility and impair both bacterial capture and killing (Matsui *et al.*, 2005). Bcc species are also highly resistant to cationic antimicrobial peptides, and thus resistant to non-oxidative phagocytic killing (Speert *et al.*, 1994). Moreover, Bcc strains express a series of virulence factors that protect them from oxidative killing. *B. cepacia*, *B. multivorans* and *B. cenocepacia* produce superoxide dismutase, catalase and a melanin pigment, which scavenge superoxide *in vitro* (Charalabous *et al.*, 2007; Keith *et al.*, 2007; Keith & Valvano, 2007; Lefebvre & Valvano, 2001; Zughaier *et al.*, 1999). Some isolates of *B. cenocepacia* also express haem-binding proteins in their outer membrane which may help detoxify ROS (Smalley *et al.*, 2001). In addition, Bylund *et al.* (2006) showed that the exopolysaccharide produced by a clinical *B. cenocepacia* isolate interfered with the function of human neutrophils *in vitro* by inhibiting chemotaxis and ROS production.

Fig. 1. Proposed model depicting the interactions of Bcc bacteria with epithelial and immune cells in the airways of CF patients. The two types of transepithelial migration (paracellular and intracellular) are shown, and the effects of intracellular bacteria in macrophages, dendritic cells and neutrophils are also indicated. The model predicts that the clearance of intracellular bacteria is inefficient and leads to host cell death and the subsequent release of bacteria to the extracellular space, which promotes bacterial persistence, as the infection cannot be resolved. Cell death and ineffective resolution of the infection account for a protracted inflammatory response that damages the host tissue.

Apoptosis is an essential mechanism for the regulation of neutrophil homeostasis and inflammation. Therefore, alteration of neutrophil apoptosis in CF could have significant effects on the inflammatory response and resolution of infection (Downey *et al.*, 2009). Exposure to live *B. cenocepacia* enhances apoptosis in neutrophils compared with untreated cells (Bylund *et al.*, 2005; Hutchison *et al.*, 1998). Furthermore, *B. cenocepacia* can induce neutrophil necrosis when ROS production is compromised (Bylund *et al.*, 2005). This may be important in the context of CF infection, as it has been shown that intracellular *B. cenocepacia* delays the assembly of a functional NADPH oxidase. This delay is associated with reduced superoxide production and is enhanced in the presence of a dysfunctional CFTR (Keith *et al.*, 2009). Neutrophils that die by necrosis, instead of apoptosis, release their toxic contents in an uncontrolled fashion, and macrophages ingesting necrotic cells may become activated to secrete high levels of proinflammatory cytokines (Haslett, 1999). Thus, the compromised ability of neutrophils to clear bacteria from the lungs and airways in CF patients compounded with neutrophil necrosis may play a key role in persistent inflammation and tissue destruction.

Bcc and dendritic cells

Dendritic cells (DCs) are crucial in regulating the immune response by bridging innate and adaptive immunity. DCs are specialized to capture and process antigens and then display them on their surface. These antigens are then 'presented' to cells of the innate immune system (lymphocytes) (Suzuki *et al.*, 2008). DCs are found in all tissues, including blood and lymphoid organs. In peripheral tissues, DCs are found in an immature stage specialized in the capture of antigens. In response to microbes, DCs undergo a complex process of maturation into antigen-presenting cells (Blanco *et al.*, 2008). Recently, it was shown that Bcc bacteria alter the normal function of DC necrosis (Macdonald & Speert, 2008). Although DCs bind and internalize *B. multivorans* and *B. cenocepacia*, and both Bcc species induce cytokine release from DCs, *B. cenocepacia* but not *B. multivorans* interferes with the normal functioning of DCs by inhibiting upregulation of co-stimulatory molecules and inducing necrosis (Macdonald & Speert, 2008). However, there is no information on the bacterial factors responsible for apoptosis of dendritic cells. We have recently observed that type VI secretion is required at least in part to induce cell death in infected macrophages (D. W. Hynes & M. A. Valvano, unpublished). It is tempting to propose that this secretion system may also be involved in inducing cell death in dendritic cells.

Concluding remarks

In this review, we have described current research on the interaction of Bcc bacteria with host cells encountered during lung infection in CF patients. The ability of Bcc

species to gain access to mucosal tissues in the airways across the respiratory epithelium provides a mechanism for dissemination of the infection to extra-pulmonary sites, whereas intracellular survival of Bcc bacteria within host cells may contribute to bacterial persistence within the CF lungs and airways and to the sustained tissue inflammation and destruction that is characteristic of CF lung infections, as represented in the model shown in Fig. 1. Unfortunately, the pathogenesis of Bcc infections in CF patients is still not well understood. The variety of strategies employed by Bcc bacteria to successfully colonize their host may explain, at least in part, the different clinical outcomes of infection in patients with CF. There is increasing evidence indicating that the presence of a non-functional CFTR affects the outcome of Bcc–eukaryotic cell interactions, suggesting that normal CFTR function is important for the cell homeostasis, although the mechanistic details of CFTR involvement have not yet been elucidated.

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References

- Aaron, S. D., Ferris, W., Henry, D. A., Speert, D. P. & Macdonald, N. E. (2000). Multiple combination bactericidal antibiotic testing for patients with cystic fibrosis infected with *Burkholderia cepacia*. *Am J Respir Crit Care Med* **161**, 1206–1212.
- Alix, E. & Blanc-Potard, A. B. (2007). MgtC: a key player in intramacrophage survival. *Trends Microbiol* **15**, 252–256.
- Aubert, D. F., Flannagan, R. S. & Valvano, M. A. (2008). A novel sensor kinase-response regulator hybrid controls biofilm formation and virulence in *Burkholderia cenocepacia*. *Infect Immun* **76**, 1979–1991.
- Backert, S. & Meyer, T. F. (2006). Type IV secretion systems and their effectors in bacterial pathogenesis. *Curr Opin Microbiol* **9**, 207–217.
- Beachey, E. H. (1981). Bacterial adherence: adhesin-receptor interactions mediating the attachment of bacteria to mucosal surface. *J Infect Dis* **143**, 325–345.
- Bingle, L. E., Bailey, C. M. & Pallen, M. J. (2008). Type VI secretion: a beginner's guide. *Curr Opin Microbiol* **11**, 3–8.
- Blanco, P., Palucka, A. K., Pascual, V. & Banchereau, J. (2008). Dendritic cells and cytokines in human inflammatory and autoimmune diseases. *Cytokine Growth Factor Rev* **19**, 41–52.
- Blohmke, C. J., Victor, R. E., Hirschfeld, A. F., Elias, I. M., Hancock, D. G., Lane, C. R., Davidson, A. G., Wilcox, P. G., Smith, K. D. & other authors (2008). Innate immunity mediated by TLR5 as a novel antiinflammatory target for cystic fibrosis lung disease. *J Immunol* **180**, 7764–7773.
- Boucher, R. C. (2007). Cystic fibrosis: a disease of vulnerability to airway surface dehydration. *Trends Mol Med* **13**, 231–240.
- Burns, J. L., Jonas, M., Chi, E. Y., Clark, D. K., Berger, A. & Griffith, A. (1996). Invasion of respiratory epithelial cells by *Burkholderia (Pseudomonas) cepacia*. *Infect Immun* **64**, 4054–4059.

- Bylund, J., Campsall, P. A., Ma, R. C., Conway, B. A. & Speert, D. P. (2005). *Burkholderia cenocepacia* induces neutrophil necrosis in chronic granulomatous disease. *J Immunol* **174**, 3562–3569.
- Bylund, J., Burgess, L. A., Cescutti, P., Ernst, R. K. & Speert, D. P. (2006). Exopolysaccharides from *Burkholderia cenocepacia* inhibit neutrophil chemotaxis and scavenge reactive oxygen species. *J Biol Chem* **281**, 2526–2532.
- Caraher, E., Duff, C., Mullen, T., Mc Keon, S., Murphy, P., Callaghan, M. & McClean, S. (2007). Invasion and biofilm formation of *Burkholderia dolosa* is comparable with *Burkholderia cenocepacia* and *Burkholderia multivorans*. *J Cyst Fibros* **6**, 49–56.
- Charalabous, P., Risk, J. M., Jenkins, R., Birss, A. J., Hart, C. A. & Smalley, J. W. (2007). Characterization of a bifunctional catalase-peroxidase of *Burkholderia cenocepacia*. *FEMS Immunol Med Microbiol* **50**, 37–44.
- Cheung, K. J., Jr, Li, G., Urban, T. A., Goldberg, J. B., Griffith, A., Lu, F. & Burns, J. L. (2007). Pilus-mediated epithelial cell death in response to infection with *Burkholderia cenocepacia*. *Microbes Infect* **9**, 829–837.
- Chiu, C. H., Ostry, A. & Speert, D. P. (2001). Invasion of murine respiratory epithelial cells *in vivo* by *Burkholderia cepacia*. *J Med Microbiol* **50**, 594–601.
- Cieri, M. V., Mayer-Hamblett, N., Griffith, A. & Burns, J. L. (2002). Correlation between an *in vitro* invasion assay and a murine model of *Burkholderia cepacia* lung infection. *Infect Immun* **70**, 1081–1086.
- Coenye, T. & Vandamme, P. (2003). Diversity and significance of *Burkholderia* species occupying diverse ecological niches. *Environ Microbiol* **5**, 719–729.
- de C. Ventura, G. M., Le Goffic, R., Balloy, V., Plotkowski, M. C., Chignard, M. & Si-Tahar, M. (2008). TLR5, but neither TLR2 nor TLR4, is involved in lung epithelial cell response to *Burkholderia cenocepacia*. *FEMS Immunol Med Microbiol* **54**, 37–44.
- De Soya, A., Ellis, C. D., Khan, C. M., Corris, P. A. & Demarco de Hormaeche, R. (2004). *Burkholderia cenocepacia* lipopolysaccharide, lipid A, and proinflammatory activity. *Am J Respir Crit Care Med* **170**, 70–77.
- Di, A., Brown, M. E., Deriy, L. V., Li, C., Szeto, F. L., Chen, Y., Huang, P., Tong, J., Naren, A. P. & other authors (2006). CFTR regulates phagosome acidification in macrophages and alters bactericidal activity. *Nat Cell Biol* **8**, 933–944.
- Diamond, G., Legarda, D. & Ryan, L. K. (2000). The innate immune response of the respiratory epithelium. *Immunol Rev* **173**, 27–38.
- Downey, D. G., Bell, S. C. & Elborn, J. S. (2009). Neutrophils in cystic fibrosis. *Thorax* **64**, 81–88.
- Duff, C., Murphy, P. G., Callaghan, M. & McClean, S. (2006). Differences in invasion and translocation of *Burkholderia cepacia* complex species in polarised lung epithelial cells *in vitro*. *Microb Pathog* **41**, 183–192.
- Flannagan, R. S. & Valvano, M. A. (2008). *Burkholderia cenocepacia* requires RpoE for growth under stress conditions and delay of phagolysosomal fusion in macrophages. *Microbiology* **154**, 643–653.
- Galán, J. E. & Wolf-Watz, H. (2006). Protein delivery into eukaryotic cells by type III secretion machines. *Nature* **444**, 567–573.
- Goldman, M. J., Anderson, G. M., Stolzenberg, E. D., Kari, U. P., Zasloff, M. & Wilson, J. M. (1997). Human β -defensin-1 is a salt-sensitive antibiotic in lung that is inactivated in cystic fibrosis. *Cell* **88**, 553–560.
- Govan, J. R. W. & Deretic, V. (1996). Microbial pathogenesis in cystic fibrosis: mucoid *Pseudomonas aeruginosa* and *Burkholderia cepacia*. *Microbiol Rev* **60**, 539–574.
- Govan, J. R., Brown, A. R. & Jones, A. M. (2007). Evolving epidemiology of *Pseudomonas aeruginosa* and the *Burkholderia cepacia* complex in cystic fibrosis lung infection. *Future Microbiol* **2**, 153–164.
- Haggie, P. M. & Verkman, A. S. (2007). Cystic fibrosis transmembrane conductance regulator-independent phagosomal acidification in macrophages. *J Biol Chem* **282**, 31422–31428.
- Harris, J. K., De Groote, M. A., Sagel, S. D., Zemanick, E. T., Kapsner, R., Penvari, C., Kaess, H., Deterding, R. R., Accurso, F. J. & Pace, N. R. (2007). Molecular identification of bacteria in bronchoalveolar lavage fluid from children with cystic fibrosis. *Proc Natl Acad Sci U S A* **104**, 20529–20533.
- Haslett, C. (1999). Granulocyte apoptosis and its role in the resolution and control of lung inflammation. *Am J Respir Crit Care Med* **160**, S5–S11.
- Hunt, T. A., Kooi, C., Sokol, P. A. & Valvano, M. A. (2004). Identification of *Burkholderia cenocepacia* (formerly *Burkholderia cepacia* genomovar III) genes required for bacterial survival *in vivo*. *Infect Immun* **72**, 4010–4022.
- Hutchison, M. L., Poxton, I. R. & Govan, J. R. W. (1998). *Burkholderia cepacia* produces a hemolysin that is capable of inducing apoptosis and degranulation of mammalian phagocytes. *Infect Immun* **66**, 2033–2039.
- Isles, A., Maclusky, I., Corey, M., Gold, R., Prober, C., Fleming, P. & Levison, H. (1984). *Pseudomonas cepacia* infection in cystic fibrosis: an emerging problem. *J Pediatr* **104**, 206–210.
- Jacquot, J., Tabary, O., Le Rouzic, P. & Clement, A. (2008). Airway epithelial cell inflammatory signalling in cystic fibrosis. *Int J Biochem Cell Biol* **40**, 1703–1715.
- Karlsson, K. A., Angstrom, J., Bergstrom, J. & Lanne, B. (1992). Microbial interaction with animal cell surface carbohydrates. *APMIS Suppl* **27**, 71–83.
- Kazmierczak, M. J., Wiedmann, M. & Boor, K. J. (2005). Alternative sigma factors and their roles in bacterial virulence. *Microbiol Mol Biol Rev* **69**, 527–543.
- Keig, P. M., Ingham, E. & Kerr, K. G. (2001). Invasion of human type II pneumocytes by *Burkholderia cepacia*. *Microb Pathog* **30**, 167–170.
- Keig, P. M., Ingham, E., Vandamme, P. A. & Kerr, K. G. (2002). Differential invasion of respiratory epithelial cells by members of the *Burkholderia cepacia* complex. *Clin Microbiol Infect* **8**, 47–49.
- Keith, K. E. & Valvano, M. A. (2007). Characterization of SodC, a periplasmic superoxide dismutase from *Burkholderia cenocepacia*. *Infect Immun* **75**, 2451–2460.
- Keith, K. E., Killip, L., He, P., Moran, G. H. & Valvano, M. A. (2007). *Burkholderia cenocepacia* C5424 produces a pigment with antioxidant properties using a homogentisate intermediate. *J Bacteriol* **189**, 9057–9065.
- Keith, K. E., Hynes, D. W., Sholdice, J. E. & Valvano, M. A. (2009). Delayed association of the NADPH oxidase complex with macrophage vacuoles containing the opportunistic pathogen *Burkholderia cenocepacia*. *Microbiology* **155**, 1004–1015.
- Kim, J. Y., Sajjan, U. S., Krasan, G. P. & LiPuma, J. J. (2005). Disruption of tight junctions during traversal of the respiratory epithelium by *Burkholderia cenocepacia*. *Infect Immun* **73**, 7107–7112.
- Lamothe, J. (2007). *Characterization of the strategy used by Burkholderia cepacia complex bacteria to survive within phagocytic cells*. PhD thesis, Microbiology and Immunology, Faculty of Medicine and Dentistry, University of Western Ontario.
- Lamothe, J. & Valvano, M. A. (2008). *Burkholderia cenocepacia*-induced delay of acidification and phagolysosomal fusion in cystic fibrosis transmembrane conductance regulator (CFTR)-defective macrophages. *Microbiology* **154**, 3825–3834.
- Lamothe, J., Thyssen, S. & Valvano, M. A. (2004). *Burkholderia cepacia* complex isolates survive intracellularly without replication

within acidic vacuoles of *Acanthamoeba polyphaga*. *Cell Microbiol* **6**, 1127–1138.

Lamothe, J., Huynh, K. K., Grinstein, S. & Valvano, M. A. (2007). Intracellular survival of *Burkholderia cenocepacia* in macrophages is associated with a delay in the maturation of bacteria-containing vacuoles. *Cell Microbiol* **9**, 40–53.

Landers, P., Kerr, K. G., Rowbotham, T. J., Tipper, J. L., Keig, P. M., Ingham, E. & Denton, M. (2000). Survival and growth of *Burkholderia cepacia* within the free-living amoeba *Acanthamoeba polyphaga*. *Eur J Clin Microbiol Infect Dis* **19**, 121–123.

Lefebvre, M. D. & Valvano, M. A. (2001). Catalases and superoxide dismutases in strains of the *Burkholderia cepacia* complex and their roles in resistance to reactive oxygen species. *Microbiology* **147**, 97–109.

LiPuma, J. J., Spilker, T., Gill, L. H., Campbell, P. W., Liu, L. & Mahenthiralingam, E. (2001). Disproportionate distribution of *Burkholderia cepacia* complex species and transmissibility markers in cystic fibrosis. *Am J Respir Crit Care Med* **164**, 92–96.

Loutet, S. A., Flannagan, R. S., Kooi, C., Sokol, P. A. & Valvano, M. A. (2006). A complete lipopolysaccharide inner core oligosaccharide is required for resistance of *Burkholderia cenocepacia* to polymyxin B and bacterial survival in vivo. *J Bacteriol* **188**, 2073–2080.

Macdonald, K. L. & Speert, D. P. (2008). Differential modulation of innate immune cell functions by the *Burkholderia cepacia* complex: *Burkholderia cenocepacia* but not *Burkholderia multivorans* disrupts maturation and induces necrosis in human dendritic cells. *Cell Microbiol* **10**, 2138–2149.

Mahenthiralingam, E., Coenye, T., Chung, J. W., Speert, D. P., Govan, J. R., Taylor, P. & Vandamme, P. (2000). Diagnostically and experimentally useful panel of strains from the *Burkholderia cepacia* complex. *J Clin Microbiol* **38**, 910–913.

Mahenthiralingam, E., Urban, T. A. & Goldberg, J. B. (2005). The multifarious, multireplicon *Burkholderia cepacia* complex. *Nat Rev Microbiol* **3**, 144–156.

Mahenthiralingam, E., Baldwin, A. & Dowson, C. G. (2008). *Burkholderia cepacia* complex bacteria: opportunistic pathogens with important natural biology. *J Appl Microbiol* **104**, 1539–1551.

Maloney, K. E. & Valvano, M. A. (2006). The *mgtC* gene of *Burkholderia cenocepacia* is required for growth under magnesium limitation conditions and intracellular survival in macrophages. *Infect Immun* **74**, 5477–5486.

Marolda, C. L., Hauröder, B., John, M. A., Michel, R. & Valvano, M. A. (1999). Intracellular survival and saprophytic growth of isolates from the *Burkholderia cepacia* complex in free-living amoebae. *Microbiology* **145**, 1509–1517.

Martin, D. W. & Mohr, C. D. (2000). Invasion and intracellular survival of *Burkholderia cepacia*. *Infect Immun* **68**, 24–29.

Matsui, H., Grubb, B. R., Tarran, R., Randell, S. H., Gatzky, J. T., Davis, C. W. & Boucher, R. C. (1998). Evidence for periciliary liquid layer depletion, not abnormal ion composition, in the pathogenesis of cystic fibrosis airways disease. *Cell* **95**, 1005–1015.

Matsui, H., Vergheze, M. W., Kesimer, M., Schwab, U. E., Randell, S. H., Sheehan, J. K., Grubb, B. R. & Boucher, R. C. (2005). Reduced three-dimensional motility in dehydrated airway mucus prevents neutrophil capture and killing bacteria on airway epithelial surfaces. *J Immunol* **175**, 1090–1099.

McClellan, S. & Callaghan, M. (2009). *Burkholderia cepacia* complex: epithelial cell-pathogen confrontations and potential for therapeutic intervention. *J Med Microbiol* **58**, 1–12.

McDowell, A., Mahenthiralingam, E., Dunbar, K. E., Moore, J. E., Crowe, M. & Elborn, J. S. (2004). Epidemiology of *Burkholderia*

cepacia complex species recovered from cystic fibrosis patients: issues related to patient segregation. *J Med Microbiol* **53**, 663–668.

Medzhitov, R. (2001). Toll-like receptors and innate immunity. *Nat Rev Immunol* **1**, 135–145.

Minakami, R. & Sumimoto, H. (2006). Phagocytosis-coupled activation of the superoxide-producing phagocyte oxidase, a member of the NADPH oxidase (Nox) family. *Int J Hematol* **84**, 193–198.

Mougous, J. D., Cuff, M. E., Raunser, S., Shen, A., Zhou, M., Gifford, C. A., Goodman, A. L., Joachimiak, G., Ordoñez, C. L. & other authors (2006). A virulence locus of *Pseudomonas aeruginosa* encodes a protein secretion apparatus. *Science* **312**, 1526–1530.

Moura, J. A., Cristina de Assis, M., Ventura, G. C., Saliba, A. M., Gonzaga, L., Jr, Si-Tahar, M., Marques Ede, A. & Plotkowski, M. C. (2008). Differential interaction of bacterial species from the *Burkholderia cepacia* complex with human airway epithelial cells. *Microbes Infect* **10**, 52–59.

Mullen, T., Markey, K., Murphy, P., McClean, S. & Callaghan, M. (2007). Role of lipase in *Burkholderia cepacia* complex (Bcc) invasion of lung epithelial cells. *Eur J Clin Microbiol Infect Dis* **26**, 869–877.

Nauseef, W. M. (2007). How human neutrophils kill and degrade microbes: an integrated view. *Immunol Rev* **219**, 88–102.

Nzula, S., Vandamme, P. & Govan, J. R. W. (2002). Influence of taxonomic status on the in vitro antimicrobial susceptibility of the *Burkholderia cepacia* complex. *J Antimicrob Chemother* **50**, 265–269.

Painter, R. G., Valentine, V. G., Lanson, N. A., Jr, Leidal, K., Zhang, Q., Lombard, G., Thompson, C., Viswanathan, A., Nauseef, W. M. & other authors (2006). CFTR expression in human neutrophils and the phagolysosomal chlorination defect in cystic fibrosis. *Biochemistry* **45**, 10260–10269.

Painter, R. G., Bonvillain, R. W., Valentine, V. G., Lombard, G. A., Laplace, S. G., Nauseef, W. M. & Wang, G. (2008). The role of chloride anion and CFTR in killing of *Pseudomonas aeruginosa* by normal and CF neutrophils. *J Leukoc Biol* **83**, 1345–1353.

Pukatzki, S., Ma, A. T., Sturtevant, D., Krastins, B., Sarracino, D., Nelson, W. C., Heidelberg, J. F. & Mekalanos, J. J. (2006). Identification of a conserved bacterial protein secretion system in *Vibrio cholerae* using the *Dictyostelium* host model system. *Proc Natl Acad Sci U S A* **103**, 1528–1533.

Reddi, K., Phagoo, S. B., Anderson, K. D. & Warburton, D. (2003). *Burkholderia cepacia*-induced IL-8 gene expression in an alveolar epithelial cell line: signaling through CD14 and mitogen-activated protein kinase. *Pediatr Res* **54**, 297–305.

Riordan, J. R., Rommens, J. M., Kerem, B., Alon, N., Rozmahel, R., Grzelczak, Z., Zielenski, J., Lok, S., Plavsky, N. & other authors (1989). Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science* **245**, 1066–1073.

Rogers, G. B., Carroll, M. P., Serisier, D. J., Hockey, P. M., Jones, G., Kehagia, V., Connett, G. J. & Bruce, K. D. (2006). Use of 16S rRNA gene profiling by terminal restriction fragment length polymorphism analysis to compare bacterial communities in sputum and mouthwash samples from patients with cystic fibrosis. *J Clin Microbiol* **44**, 2601–2604.

Saini, L. S., Galsworthy, S. B., John, M. A. & Valvano, M. A. (1999). Intracellular survival of *Burkholderia cepacia* complex isolates in the presence of macrophage cell activation. *Microbiology* **145**, 3465–3475.

Sajjan, S. U. & Forstner, J. F. (1992). Identification of the mucin-binding adhesin of *Pseudomonas cepacia* isolated from patients with cystic fibrosis. *Infect Immun* **60**, 1434–1440.

Sajjan, U. S. & Forstner, J. F. (1993). Role of a 22-kilodalton pilin protein in binding of *Pseudomonas cepacia* to buccal epithelial cells. *Infect Immun* **61**, 3157–3163.

- Sajjan, U. S., Sylvester, F. A. & Forstner, J. F. (2000). Cable-piliated *Burkholderia cepacia* binds to cytokeratin 13 of epithelial cells. *Infect Immun* **68**, 1787–1795.
- Sajjan, U., Corey, M., Humar, A., Tullis, E., Cutz, E., Ackerley, C. & Forstner, J. (2001). Immunolocalisation of *Burkholderia cepacia* in the lungs of cystic fibrosis patients. *J Med Microbiol* **50**, 535–546.
- Sajjan, U., Ackerley, C. & Forstner, J. (2002a). Interaction of CblA/adhesin-positive *Burkholderia cepacia* with squamous epithelium. *Cell Microbiol* **4**, 73–86.
- Sajjan, U., Liu, L., Lu, A., Spilker, T., Forstner, J. & LiPuma, J. J. (2002b). Lack of cable pili expression by *cblA*-containing *Burkholderia cepacia* complex. *Microbiology* **148**, 3477–3484.
- Sajjan, U. S., Yang, J. H., Hershenson, M. B. & LiPuma, J. J. (2006). Intracellular trafficking and replication of *Burkholderia cenocepacia* in human cystic fibrosis airway epithelial cells. *Cell Microbiol* **8**, 1456–1466.
- Sajjan, S. U., Carmody, L. A., Gonzalez, C. F. & LiPuma, J. J. (2008a). A type IV secretion system contributes to intracellular survival and replication of *Burkholderia cenocepacia*. *Infect Immun* **76**, 5447–5455.
- Sajjan, U. S., Hershenson, M. B., Forstner, J. F. & LiPuma, J. J. (2008b). *Burkholderia cenocepacia* ET12 strain activates TNFR1 signalling in cystic fibrosis airway epithelial cells. *Cell Microbiol* **10**, 188–201.
- Saldías, M. S., Lamothe, J., Wu, R. & Valvano, M. A. (2008). *Burkholderia cenocepacia* requires the RpoN sigma factor for biofilm formation and intracellular trafficking within macrophages. *Infect Immun* **76**, 1059–1067.
- Schell, M. A., Ulrich, R. L., Ribot, W. J., Brueggemann, E. E., Hines, H. B., Chen, D., Lipscomb, L., Kim, H. S., Mrázek, J. & other authors (2007). Type VI secretion is a major virulence determinant in *Burkholderia mallei*. *Mol Microbiol* **64**, 1466–1485.
- Schwab, U., Leigh, M., Ribeiro, C., Yankaskas, J., Burns, K., Gilligan, P., Sokol, P. & Boucher, R. (2002). Patterns of epithelial cell invasion by different species of the *Burkholderia cepacia* complex in well-differentiated human airway epithelia. *Infect Immun* **70**, 4547–4555.
- Schwab, U. E., Ribeiro, C. M., Neubauer, H. & Boucher, R. C. (2003). Role of actin filament network in *Burkholderia multivorans* invasion in well-differentiated human airway epithelia. *Infect Immun* **71**, 6607–6609.
- Scott, C. C., Cuellar-Mata, P., Matsuo, T., Davidson, H. W. & Grinstein, S. (2002). Role of 3-phosphoinositides in the maturation of *Salmonella*-containing vacuoles within host cells. *J Biol Chem* **277**, 12770–12776.
- Sibley, C. D., Parkins, M. D., Rabin, H. R., Duan, K., Norgaard, J. C. & Surette, M. G. (2008). A polymicrobial perspective of pulmonary infections exposes an enigmatic pathogen in cystic fibrosis patients. *Proc Natl Acad Sci U S A* **105**, 15070–15075.
- Simel, D. L., Mastin, J. P., Pratt, P. C., Wisseman, C. L., Shelburne, J. D., Spock, A. & Ingram, P. (1984). Scanning electron microscopic study of the airways in normal children and in patients with cystic fibrosis and other lung diseases. *Pediatr Pathol* **2**, 47–64.
- Smalley, J. W., Charalabous, P., Birss, A. J. & Hart, C. A. (2001). Detection of heme-binding proteins in epidemic strains of *Burkholderia cepacia*. *Clin Diagn Lab Immunol* **8**, 509–514.
- Smith, J. J., Travis, S. M., Greenberg, E. P. & Welsh, M. J. (1996). Cystic fibrosis airway epithelia fail to kill bacteria because of abnormal airway surface fluid. *Cell* **85**, 229–236.
- Speert, D. P., Bond, M., Woodman, R. C. & Curnutte, J. T. (1994). Infection with *Pseudomonas cepacia* in chronic granulomatous disease: role of non-oxidative killing by neutrophils in host defense. *J Infect Dis* **170**, 1524–1531.
- Speert, D. P., Henry, D., Vandamme, P., Corey, M. & Mahenthalingam, E. (2002). Epidemiology of *Burkholderia cepacia* complex in patients with cystic fibrosis, Canada. *Emerg Infect Dis* **8**, 181–187.
- Suarez, G., Sierra, J. C., Sha, J., Wang, S., Erova, T. E., Fadl, A. A., Foltz, S. M., Horneman, A. J. & Chopra, A. K. (2008). Molecular characterization of a functional type VI secretion system from a clinical isolate of *Aeromonas hydrophila*. *Microb Pathog* **44**, 344–361.
- Suzuki, T., Chow, C. W. & Downey, G. P. (2008). Role of innate immune cells and their products in lung immunopathology. *Int J Biochem Cell Biol* **40**, 1348–1361.
- Tomich, M., Griffith, A., Herfst, C. A., Burns, J. L. & Mohr, C. D. (2003). Attenuated virulence of a *Burkholderia cepacia* type III secretion mutant in a murine model of infection. *Infect Immun* **71**, 1405–1415.
- Tunney, M. M., Field, T. R., Moriarty, T. F., Patrick, S., Doering, G., Muhlebach, M. S., Wolfgang, M. C., Boucher, R., Gilpin, D. F. & other authors (2008). Detection of anaerobic bacteria in high numbers in sputum from patients with cystic fibrosis. *Am J Respir Crit Care Med* **177**, 995–1001.
- Urban, T. A., Griffith, A., Torok, A. M., Smolkin, M. E., Burns, J. L. & Goldberg, J. B. (2004). Contribution of *Burkholderia cenocepacia* flagella to infectivity and inflammation. *Infect Immun* **72**, 5126–5134.
- Urban, T. A., Goldberg, J. B., Forstner, J. F. & Sajjan, U. S. (2005). Cable pili and the 22-kilodalton adhesin are required for *Burkholderia cenocepacia* binding to and transmigration across the squamous epithelium. *Infect Immun* **73**, 5426–5437.
- Valvano, M. A., Keith, K. E. & Cardona, S. T. (2005). Survival and persistence of opportunistic *Burkholderia* species in host cells. *Curr Opin Microbiol* **8**, 99–105.
- Valvano, M. A., Maloney, K. E., Lamothe, J. & Saldías, S. (2006). Intracellular survival of *Burkholderia cepacia* complex isolates. In *Burkholderia: Molecular Biology and Genomics*, pp. 283–300. Edited by T. Coeyne & P. Vandamme. New York: Horizon Scientific Press.
- Vanlaere, E., Lipuma, J. J., Baldwin, A., Henry, D., De Brandt, E., Mahenthalingam, E., Speert, D., Dowson, C. & Vandamme, P. (2008). *Burkholderia latens* sp. nov., *Burkholderia diffusa* sp. nov., *Burkholderia arboris* sp. nov., *Burkholderia seminalis* sp. nov. and *Burkholderia metallica* sp. nov., novel species within the *Burkholderia cepacia* complex. *Int J Syst Evol Microbiol* **58**, 1580–1590.
- Vij, N., Mazur, S. & Zeitlin, P. L. (2009). CFTR is a negative regulator of NF κ B mediated innate immune response. *PLoS One* **4**, e4664.
- Zheng, J. & Leung, K. Y. (2007). Dissection of a type VI secretion system in *Edwardsiella tarda*. *Mol Microbiol* **66**, 1192–1206.
- Zhou, T., Daugherty, M., Grishin, N. V., Osterman, A. L. & Zhang, H. (2000). Structure and mechanism of homoserine kinase: prototype for the GHMP kinase superfamily. *Structure* **8**, 1247–1257.
- Zughaier, S. M., Ryley, H. C. & Jackson, S. K. (1999). A melanin pigment purified from an epidemic strain of *Burkholderia cepacia* attenuates monocytic respiratory burst activity by scavenging superoxide anion. *Infect Immun* **67**, 908–913.