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Original article

Differential interaction of bacterial species from the *Burkholderia cepacia* complex with human airway epithelial cells

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Abstract

To increase knowledge of the pathogenic potential of the *Burkholderia cepacia* complex (BCC), we investigated the effects of reference strains of the nine BCC species on human bronchial epithelial cells *in vitro*. *B. multivorans* exhibited the highest rates of adherence to and internalization by host cells. Two out of three clinical isolates recovered from cystic fibrosis patients confirmed the *B. multivorans* high adhesiveness. All four *B. multivorans* isolates exhibited an aggregated pattern of adherence but any of them expressed cable pili. When bacteria were centrifuged onto cell cultures to circumvent their poor adhesiveness, *B. pyrrocinia* exhibited the highest internalization rate, followed by *B. multivorans*. The percentages of apoptotic cells in cultures infected with *B. cepacia*, *B. multivorans*, *B. cenocepacia* (subgroups IIIA and IIIB), *B. stabilis* and *B. vietnamiensis* were significantly higher than in control non-infected cultures. All nine BCC species triggered a similar release of the inflammatory cytokine IL-8, that was not reduced by cell treatment with cytochalasin D. Hence, our data demonstrate, for the first time, that all BCC species exhibit a similar ability to induce the expression of host immune mediators whereas they differ on their ability to adhere to, invade and kill airway epithelial cells.
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Keywords: *Burkholderia cepacia* complex; Bacterial adherence; Bacterial internalization; Bacterial cytotoxicity; Pro-inflammatory activity

1. Introduction

During the past decades, bacteria identified as "*Burkholderia cepacia*" has emerged as problematic pulmonary pathogens in patients with cystic fibrosis (CF). The clinical outcome of "*B. cepacia*" colonization/infection is variable and unpredictable, ranging from maintenance or chronic decline in the respiratory function to a progressive, invasive and fatal bacteremic disease known as cepacia sindrome [1]. This wide variation in disease severity reflects the complex nature of the bacterium-host relationship and suggests diversities in the pathogenic potential among the microorganisms.

In recent years, taxonomic studies have shown that bacteria previously classified as "*B. cepacia*" actually comprise a group of nine closely related species referred to as the *B. cepacia* complex (BCC) [1]. Initially identified as genomovars, each species has received a formal binomial designation (Table 1). Whether the heterogeneity in bacterial species originally identified as "*B. cepacia*" holds the key to understanding the variable pathogenic potential of these microorganisms for CF patients remains to be determined.

All BCC species are potentially capable of airway infections in CF patients, but *B. cenocepacia* and *B. multivorans* predominate in every country where the prevalence of BCC species has been monitored [1]. The reasons for this

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Table 1
Bacterial strains

BCC species (genomovars)	Strain	Origin
B. cepacia (I)	ATCC 17759	T. Pitt*
B. multivornas (II)	ATCC 17616	T. Pitt
B. multivornas (II)	EAM 522; CF clinical isolate	E.A. Marques**
B. multivornas (II)	EAM 532; CF clinical isolate	E.A. Marques
B. multivornas (II)	EAM 1654; CF clinical isolate	E.A. Marques
B. cenocepacia (IIIA)	BC 818	T. Pitt
B. cenocepacia (IIIB)	BC 842	T. Pitt
B. cenocepacia (IIIA) − ET12 clone	J2315	Institut Pasteur microorganisms depository
B. stabilis (IV)	C7322	E. Mahenthiralingam***
B. vietmaniensis (V)	LMG 10929	E. Mahenthiralingam
B. dolosa (VI)	LMG 18943	E. Mahenthiralingam
B. ambifaria (VII)	ATCC 53266	E. Mahenthiralingam
B. anthina (VIII)	LMG 16670	E. Mahenthiralingam
B. pyrrocinia (IX)	ATCC 39227	E. Mahenthiralingam

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predominance, despite the very close phylogenetic and phenotypic relatedness of the species within the BCC, are unknown. Reports that *B. cenocepacia* and *B. multivorans* are not more abundant in the environment [2] suggests that the disproportionate representation of these two species in CF patients depends on their enhanced capacity for causing human infection.

Several virulence factors have been defined for "*B. cepacia*" prior to the classification of these microorganisms as a complex or for *B. cenocepacia* (reviewed in Ref. [3]) but knowledge of the disease mechanisms employed by the other BCC species is still limited [4]. To address this deficiency, in this study, we investigated the effects of reference strains of each BCC species on human bronchial epithelial cells. Our data represent the first demonstration that all species exhibit a similar ability to trigger the release of the inflammatory cytokine IL-8 by host cells. In contrast, bacteria differed markedly on their ability to adhere to, invade and kill airway cells.

2. Materials and methods

2.1. Airway epithelial cell culture and infection

Transformed human bronchial epithelial cell line BEAS-2B was cultured in M-199-HEPES medium (Gibco BRL) containing 10% fetal calf serum (FCS), glutamine and antibiotics. Two days before each assay, cells were trypsinized, resuspended in culture medium and seeded in uncoated 96- (2.5×10^4 cells/well) or 24-well (2.0×10^5 cells/well) microtitration plates, to obtain confluent cell cultures. Cells were infected at a multiplicity of infection (MOI) of 10 bacteria per epithelial cell.

2.2. Bacteria

Bacteria used in this study (shown in Table 1) were cultured in Trypticase Soy Broth (Difco Laboratories) for 16–18 h at 37 °C, to obtain late logarithmic growth phase cultures, harvested by centrifugation and resuspended in culture medium containing 10% FCS to $A_{660nm} = 0.1$, corresponding to about 10^8 colony forming units/mL.

2.3. Light microscopy analysis of BCC interaction with airway cells

Confluent cells cultured on glass coverslips in 24-well microplates were infected with the different bacterial species for 1 or 3 h. Cells were then rinsed 3 times with 0.01 M PBS, fixed and stained with May-Grunwald-Giemsa stain.

2.4. Assessment of cable pili gene expression

PCR amplification was used to detect the cblA gene in *B. multivorans* isolates and in the ET12 *B. cenocepacia* (J2315) strain, used as a positive control. Bacteria were lysed by two freeze-thaw cycles followed by boiling. The cblA gene was amplified by PCR using GoTaq DNA polymerase (Promega) and oligonucleotide primers previously described [5]. The PCR reactions were carried out by 35 cycles at 94 °C (1 min), 50 °C (1 min) and 72 °C (1 min), followed by an additional 5 min extension time at 72 °C. Amplicons were analysed by electrophoresis on 1.2% agarose gels.

2.5. Bacterial internalization

Cell cultures were exposed for 1 h at 37 °C to suspensions of the different BCC species and then rinsed with PBS. In some assays, bacteria were centrifuged ($1000 \times g$ for 10 min) onto cultured cells prior to incubation for 1 h. Since BCC are resistant to gentamicin, cells were exposed to ceftazidime (1 mg/mL) in combination with gentamicin (1 mg/mL) for 2 h, to kill extracellular microorganisms [6]. Cells were then rinsed, lysed with 0.05% Triton X100 in PBS, and cell lysates were diluted and plated to determine the concentration of intracellular bacteria. Preliminary assays showed that bacterial killing by the antibiotics at these concentrations was greater than 99.99% (data not shown).

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