Microbial Pathogenesis 49 (2010) 381–387

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

# Invasion of *Burkholderia cepacia* complex isolates into lung epithelial cells involves glycolipid receptors

Tracy Mullen [a,b], Máire Callaghan [a,b], Siobhán McClean [a,b,*]

[a] Centre of Microbial Host Interactions, Institute of Technology, Tallaght, Dublin 24, Ireland
[b] National Institute of Cellular Biotechnology, Institute of Technology, Tallaght, Dublin 24, Ireland

ARTICLE INFO

*Article history:*
Received 12 January 2010
Received in revised form
14 July 2010
Accepted 16 August 2010
Available online 6 September 2010

*Keywords:*
Bacterial invasion
Cystic fibrosis
*Burkholderia cepacia* complex
Lung epithelial cells
Glycosphingolipids
Bacterial adherence

ABSTRACT:

*Burkholderia cepacia* complex (Bcc) is a group of opportunistic cystic fibrosis (CF) pathogens that invade lung epithelial cells. The mechanisms of invasion are poorly understood, in particular, the receptors utilised by this bacterium in the invasion process have not been identified. The aim of this study was to investigate the epithelial receptors involved in the invasion of Bcc isolates. We confirmed that invasion into two lung epithelial cell lines (16HBE14o- and CFBE41o-) which have a non-CF and CF phenotype, respectively, is receptor mediated and showed that pre-treatment of these epithelial cell lines with α- or β-galactosidase reduced invasion of isolates of two species of Bcc, *Burkholderia multivorans* and *Burkholderia cenocepacia*. In contrast, removal of mucin had no significant effect. Biotinylated Bcc strains were shown to bind to purified glycolipids separated by thin layer chromatography, albeit different patterns of binding were associated with different strains. Invasion of CF lung epithelial cells (CFBE41o-) by all three Bcc strains examined was significantly reduced by treatment of cells with inhibitors of glycolipid biosynthesis. Although the specific glycolipid involved in each case has not been elucidated, it is apparent that invasion of lung epithelial cells is mediated via binding to glycosphingolipid receptors.


## 1. Introduction

Cystic fibrosis (CF) is a genetically inherited disease caused by mutations in the cystic fibrosis transmembrane regulator (CFTR), a chloride channel. The main cause of morbidity and mortality in people with CF is the chronic respiratory infections that they encounter increasingly throughout their lives, resulting in lung damage and deterioration in lung function. *Burkholderia cepacia* is an opportunistic pathogen predominantly isolated from patients suffering from CF. To date at least fifteen species of this bacterium have been identified, collectively referred to as the *B. cepacia* complex (Bcc) [1,2]. Two of these species have been more predominant clinically, *Burkholderia multivorans* and *Burkholderia cenocepacia,* with 90% of Bcc isolates recovered from CF patients in Europe being attributed to these two species, [3,4] while the incidence of recovery of these isolates in the US is 35% and 49% respectively [5]. Bcc has become a persistent problem in the treatment of CF patients due to its ability to evade the host immune system and its inherent resistance to a number of antibiotics. Bcc strains have been found to invade epithelial cells [6,7], which enhances both evasion of the immune system and antibiotic resistance. Invasion into lung epithelial cells correlates with virulence in a mouse lung infection model [6]; however, the mechanisms by which invasion is mediated have yet to be fully explained. In particular, the epithelial receptors which facilitate invasion have not been elucidated [8].

Bcc strains have the ability to bind both intestinal and respiratory mucin [9]. Adherence to mucin and to cytokeratin 13 was found to be mediated by a 22 kDa protein expressed on piliated *B. cenocepacia* strains only [10,11]. The 22 kDa adhesin may have a role in pathogenesis, as blocking with antibodies reduced cytotoxicity and IL-8 secretion of bronchial epithelial cells [12]. More recently, a second 55 kDa protein has emerged to which piliated *B. cenocepacia* strain BC7, binds, TNF receptor1 (TNFR1). This interaction did not involve the 22 kDa protein as an adhesin, but was considered partly responsible for the potent pro-inflammatory response elicited by this virulent Bcc strain [13]. Overall, however, this mechanism of attachment is limited to certain piliated *B. cenocepacia* strains but is not present among all piliated *B. cenocepacia* isolates. Sphingolipids expressed on the surface of epithelial cells also provide receptor sites for multiple pathogens [14,15]. Bcc strains have been shown to bind to gangliosides asialo-GM1 (aGM1) and asialo-GM2 (aGM2), however, there is some disagreement in the published data. Sylvester et al., [16], showed preferential binding of Bcc strains to galactose-containing

* Corresponding author. Centre of Microbial Host Interactions, Institute of Technology, Tallaght, Dublin 24, Ireland. Tel.: +353 1 4042794; fax: +353 1 4042404.
*E-mail address:* siobhan.mcclean@ittdublin.ie (S. McClean).

0882-4010/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.micpath.2010.08.003

globosides Gb2 and Gb3 while Krivan et al., showed binding to asialo-GM1 and asialo-GM2 [16,17]. No role for these receptors in invasion was established in either case.

The main aim of this study was to investigate the Bcc receptors on epithelial cells which mediate invasion. We have examined two different lung epithelial cells, the CFTR expressing 16HBE14o- cells and another cell line, CFBE41o-, which is homozygous negative for the ΔF508 mutation, the most common CFTR mutation, responsible for 75% of CF cases. We selected three strains, one *B. multivorans* strain and two *B. cenocepacia strains*, one which was piliated (BC7) and one non-piliated (C1394). Determining the receptor to which the bacteria binds prior to invasion, will allow the further development of treatments which can prevent or inhibit binding of the Bcc, thereby inhibiting the potential for invasion.

## 2. Results

### 2.1. The effect of multiplicity of infection on invasion

A panel of isolates representing five species of the Bcc was examined initially for their ability to invade the two different lung epithelial cell lines at various MOI in order to monitor the effect of available bacteria on uptake. In both cell lines the % of invasion decreased from an optimum starting MOI of 5 bacteria/cell and reached a plateau at >100 bacteria/cell (Fig. 1A and B). In 16HBE14o- cells, the invasion of strains from the two clinically significant species, *B. multivorans* and *B. cenocepacia*, were comparable with each other, and significantly more invasive than the *B. cepacia* environmental isolate ATCC24516 or the non-invasive *Escherichia coli* strain NCIB 9454 (Fig. 1A). Both of these strains showed invasion of <0.01% at all MOI examined. Plots for the *Burkholderia stabilis* isolate LMG14294 (CF patient), and *Burkholderia vietnamiensis* isolate (recovered from rice), LMG10929, were comparable to that of *B. cepacia* strain ATCC24516 (data not shown). In contrast, there was a dramatic difference between invasion efficiency of the *B. cenocepacia* isolate BC7, in the CFTR negative cells, CFBE41o-, compared to the *B. multivorans* isolate LMG13010 (Fig. 1B). Overall, 16HBE14o- cells were more readily invaded by all Bcc isolates examined than CFBE41o- cells.

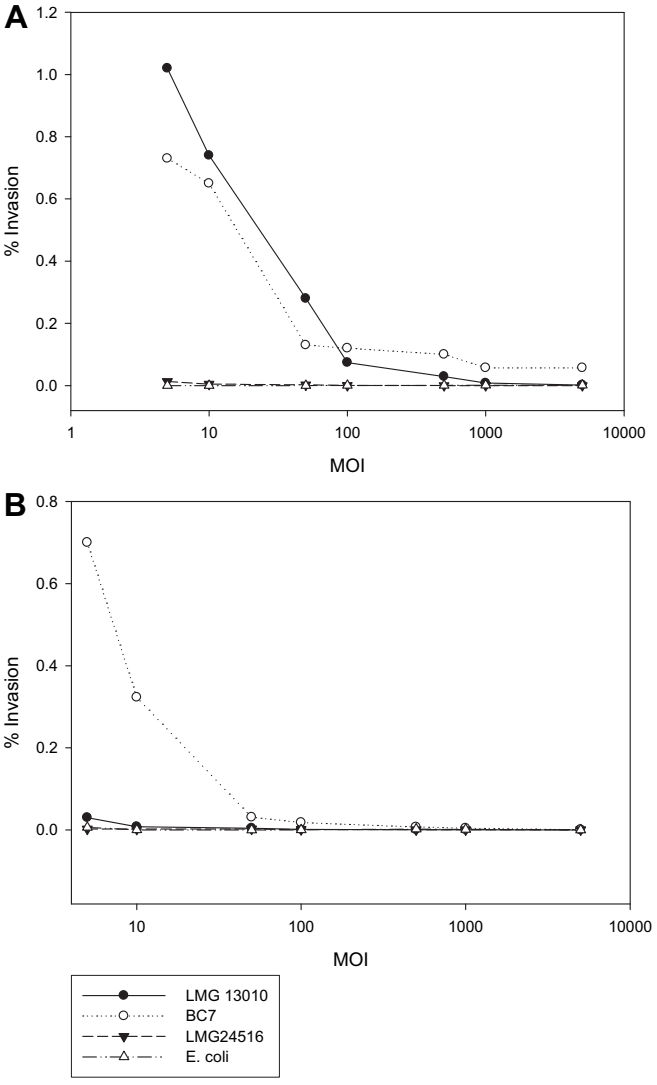


**Fig. 1.** Comparative study showing the effect of Bcc MOI on invasion efficiency versus the percentage of total bacteria internalised into lung epithelial cell lines, 16HBE14o- (A) and CFBE41o- (B). Results represent mean % invasion of each *B. cepacia* strain ATCC24516, *B. multivorans* strain LMG13010, *B. cenocepacia* strain BC7 and *E. coli* strain NCIN9485, from three independent experiments.

### 2.2. Invasion of both CF and non-CF cell lines by the Bcc following the treatment with surface modifying enzymes and N-acetylcysteine

In order to investigate the potential receptor(s) involved in mediating cellular invasion, the epithelial cells were pre-treated with enzymes and invasion of strains from the two most invasive species, *B. multivorans* isolate LMG 13010 and two *B. cenocepacia* isolates, the piliated BC7 and non-piliated C1394, were examined. Preincubation of lung epithelial cells with α-galactosidase caused a significant reduction in invasion by the *B. multivorans* isolate LMG13010 and *B. cenocepacia* isolates, BC7 and C1394, into the CFBE41o- cells (Fig. 2A) ($p < 0.05$) suggesting that attachment to moieties containing α(1-3)- or α(1-6)-linked galactoses were involved in invasion of these bacteria into these cells. Pretreatment with β-galactosidase also resulted in a reduction in CFBE41o- invasion by all isolates examined, indicating that receptors containing β(1-3)-, β(1-4)-, or β(1-6)-linked galactoses also play a role in adhesion prior to invasion (Fig. 2A). Again, this was most prominent for *B. multivorans* strain LMG13010 and *B. cenocepacia* strain BC7, where invasion was completely inhibited ($p < 0.05$, for all isolates invading CFBE41o- cells). In contrast, treatment with neuraminidase, which removes terminal sialic acid from glycoconjugates, did not have any significant effect on invasion of any of the isolates into CFBE41o- cells. None of the enzymes had any significant effect on the uptake of the negative control NCIB 9485 *E. coli* strain.

Pretreatment of 16HBE14o- cells with either α- or β-galactosidase significantly reduced the invasiveness of *B. multivorans*, LMG 13010 and *B. cenocepacia*, BC7 ($P < 0.05$), but surprisingly had little effect on *B. cenocepacia* strain C1394 (Fig. 2B). Pretreatment of these cells with α-neuraminidase, again had no significant effect on the level of invasion *B. multivorans* isolate LMG13010 or on *B. cenocepacia* isolate BC7 (Fig. 2A). Removal of sialic acid reduced the invasiveness of non-piliated C1394 into 16HBE14o- cells, however. The mucolytic agent, N-acetylcysteine reduced the invasion of isolates into both of the cell lines, however, this was not statistically significant for any isolate examined, indicating that attachment to mucus does not play a major role in their invasion.

In order to examine the effect of enzyme pre-treatment of epithelial cells on surface mucins, the Alcian blue-PAS stains were used. Confluent 16HBE14o- and CFBE41o- cells were treated with the surface modifying enzymes and stained to visualise the levels of mucin present in both of the cell lines. Untreated 16HBE14o-