

Adherence of *Burkholderia cepacia* to respiratory tract epithelial cells and inhibition with dextrans

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Adherence of *Burkholderia cepacia* to cells of the respiratory tract of patients with cystic fibrosis (CF) appears to be a necessary precondition for colonization and infection. To date, no effective anti-adhesive strategy has been devised for preventing *B. cepacia* infection in CF patients. It was found in this study that *B. cepacia* adhered to respiratory epithelial cells both *in vitro* and *in vivo*. However, strains with cable-like pili (Cbl) exhibited the typical clump formation on pneumocytes, whereas non-cable piliated strains predominantly showed single-cell adherence. Dextrans (nominally 4000–10 000 Da) significantly inhibited adhesion of *B. cepacia* to A549 pneumocytes. When compared on an equal weight basis, the nominally 10 000 Da dextran was most inhibitory. A dose-dependent inhibitory effect (up to 80 mg ml⁻¹) was observed for most strains. Dextran exerted less of an anti-adhesive effect on the two Cbl⁺ strains than on the others which were Cbl⁻. Dextrans appeared to block the adherence in a non-specific fashion, as shown by the observations that the inhibitory effect was readily reversible and oligosaccharides composed of 2–4 glucose units with the same α -1,6 linkage were not inhibitory. The mean molecular masses of dextrans used in this study, as determined by gel filtration and MS, were approximately 10-fold lower than those indicated by the manufacturers. Our data suggest that dextran of nominal molecular mass 4000 Da at a concentration of 40 mg ml⁻¹ (10 mM according to manufacturer's quoted molecular mass) or more may be useful in patients with CF to prevent colonization and infection with *B. cepacia*.

Keyword: cystic fibrosis

INTRODUCTION

Burkholderia cepacia is commonly found in soil and water and on plant surfaces. Its ubiquity in nature may contribute to its emergence as an opportunistic pathogen of humans, particularly those with cystic fibrosis (CF) (Isles *et al.*, 1984; Tablan *et al.*, 1985; Canadian cystic fibrosis foundation medical/scientific advisory committee, 1993). The clinical outcome for colonized CF patients is variable, but in about 30% of these patients, the clinical course is rapidly fatal, usually within a few months following acquisition of the organism (Isles *et al.*, 1984; Tablan *et al.*, 1985). Furthermore, in CF

patients who undergo lung transplantation, infection with *B. cepacia* enhances mortality by approximately fivefold (Snell *et al.*, 1993).

Recent taxonomic studies of isolates from human (CF and non-CF) and environmental origin indicate that the species *B. cepacia* is, in fact, highly heterogeneous, being composed of many subgroups, some of which might be reclassified as separate species (Govan *et al.*, 1996; Vandamme *et al.*, 1997). There are at least five distinct genotypic species in *B. cepacia*, referred to as genomovars I–V (Govan *et al.*, 1996; Vandamme *et al.*, 1997). All five genomovars have been isolated from CF patients; most of the epidemic strains are from genomovar III (Vandamme *et al.*, 1997).

Despite the progress in taxonomy and a better under-

Abbreviation: CF, cystic fibrosis.

standing of its evolving role in pulmonary infection in patients with CF, very little is known about the properties of *B. cepacia* that contribute to pulmonary infection. One of the most important microbial factors facilitating colonization and infection may be adhesion to host tissues, which seems to be mediated by bacterial pili (Kuehn *et al.*, 1992; Goldstein *et al.*, 1995). Recent molecular studies have confirmed at least five different structural pilus types in different *B. cepacia* strains; one pilus type has been implicated in the enhanced transmissibility of one clone of *B. cepacia* (Goldstein *et al.*, 1995). This unique clone, expressing the specific cable-like pilus (Cbl), has been recovered from CF patients in Canada and Great Britain, many of whom have rapidly deteriorated. This clone exhibits specific *in vitro* binding with high affinity to carbohydrates of respiratory mucins (Sajjan & Forstner, 1992); the mucin-binding adhesin is a 22 kDa protein present on the pili (Sajjan & Forstner, 1993; Goldstein *et al.*, 1995). This Cbl pilus is the only genetically well characterized putative virulence factor associated with an epidemic *B. cepacia* strain type (Sajjan & Forstner, 1992, 1993; Sajjan *et al.*, 1995). Whereas another genetic element, the *B. cepacia* Epidemic Strain Marker (BCESM), is also associated with epidemic spread among patients with CF, its mechanism of action remains unexplained (Mahenthiralingam *et al.*, 1997).

B. cepacia appears to establish colonization by adhering first to respiratory tract epithelial cells (Kuehn *et al.*, 1992). Once colonization with this pathogen is established, however, it is rarely if ever eradicated. Attempts to prevent bacterial colonization and infection in patients with CF by administering prophylactic antibiotics have been unsuccessful (Speert, 1989). A potential explanation is that epidemic *B. cepacia* strains have adapted to the role of human intracellular pathogen with the ability to invade and survive within respiratory epithelial cells (Burns *et al.*, 1996) and professional phagocytes (Saini *et al.*, 1999). This virulence phenotype could conceivably protect them from actions of extracellular antibiotics. Since infection of CF patients with *B. cepacia* is associated with an adverse prognosis, a novel strategy for preventing respiratory tract colonization is urgently needed.

Dextrans are α -1,6-linked homopolymers of glucose that have been used clinically as plasma volume expanders and antithrombotic agents. In previous studies, we have found that dextrans inhibit the adherence of *P. aeruginosa* to A549 pulmonary epithelial cells (Barghouthi *et al.*, 1996) and prevent infection in neonatal mice when administered by aerosol (Bryan *et al.*, 1999). We reasoned that these agents might also interfere with the adherence of *B. cepacia* to epithelial cells. The present study was undertaken to evaluate the capacity of dextrans to inhibit the binding of *B. cepacia* to A549 pneumocytes. We also explored the characteristics of binding of selected Cbl⁺ and Cbl⁻ *B. cepacia* strains *in vitro* to A549 cells and *in vivo* to murine respiratory tract epithelial cells to further elucidate the role for the cable-like pili in bacterial adherence.

METHODS

Bacterial strains and growth conditions. The description of strains used in this study is given in Table 1. All *B. cepacia* isolates recovered from patients with CF, patients without CF and the environment were received from the contributors acknowledged previously (Mahenthiralingam *et al.*, 1996, 1997). Culture and confirmation of identification of isolates were carried out as described previously (Mahenthiralingam *et al.*, 1996, 1997). *B. cepacia* was grown statically at 37 °C in Luria broth (L broth) overnight. Before use in the adherence assay, the unwashed bacterial suspension was diluted with L broth to an OD₆₀₀ of 0.5 (Spectronic 2000; Bausch and Lomb).

Cell culture. The A549 pneumocyte cell line (CCL165) was obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). The cells were grown to confluence in 25 cm² tissue culture flasks (Falcon), removed after treatment with 1 × trypsin/EDTA (0.25 % Trypsin, 1 mM EDTA.4Na) (Gibco BRL), enumerated and plated at 5 × 10⁴ cells per acid-washed, 12 mm coverslip and incubated overnight in F-12K medium (Gibco BRL) supplemented with 10 % fetal calf serum (FCS) before use in the adherence assay.

Adherence assay. Adherent cells were washed twice with warm PBS (pH 7.4) and incubated for 30 min in 0.5 ml binding buffer (138 mM NaCl, 8.1 mM Na₂HPO₄, 1.5 mM KH₂PO₄, 2.7 mM KCl, 1 mM MgCl₂, 0.25 mM CaCl₂ and 0.001 % phenol red, pH 7.4) with or without dextran or another binding inhibitor. An aliquot of 25 µl of bacterial suspension (OD₆₀₀ = 0.5) in L broth was then added and incubation was continued for 40 min at 37 °C. The coverslips were then washed four times with warm PBS and fixed with methanol for at least 15 min. The coverslips were mounted on slides, stained with fresh 3 % Giemsa stain for 15 min and examined microscopically. If bacteria showed clump formation on the surface of the epithelial cells when they bound, each clump was counted as one bacterium. Approximately 50 cells per coverslip were examined to calculate the number of adherent bacteria per epithelial cell. Data were expressed as mean number of bound bacteria per cell ± SEM. All experiments were done at least three times in duplicate.

Immunohistological staining. To characterize the adherence patterns of various *B. cepacia* strains to A549 pneumocytes, the following was done. After completion of the adhesion assay, as described above, coverslips were washed with PBS and incubated with polyclonal rabbit antiserum to *B. cepacia* strain JTC (dilution of 1:5000) at 25 °C for 0.5 h. Slides were then washed and incubated with biotinylated goat anti-rabbit IgG, followed by the avidin-biotin complex (Signet Laboratories). Finally, the substrate diaminobenzidine was applied and the specimens counterstained with haematoxylin. Sections were covered with aqueous mountant and dried on a warming plate at 60 °C until the mountant was polymerized. Slides were then examined by light microscopy.

Mice. Female C57BL/6 mice were purchased from Charles River Breeding Laboratories, St-Constant, Quebec, Canada. Mice were maintained in a specific pathogen-free environment until challenge with *B. cepacia*, after which they were housed in a biohazard room. Mice were used between 6 and 8 weeks of age. The animal procedures were approved by the University of British Columbia Committee on Animal Care, Vancouver, BC, Canada.

In vivo adherence. Bacterial inocula were prepared by seeding five colonies of each *B. cepacia* strain to 5 ml Luria broth (L broth) and allowing them to grow for 18 h at 37 °C. Bacteria were collected by centrifugation and resuspended in 1 ml 1 %

Table 1. *B. cepacia* strains used in this study

Strain	Genomovar	Description*	<i>cblA</i> †	BCESM†
Cep31	I	ATCC 25416, environmental strain	–	–
Cep509	I	CF isolate, non-epidemic	–	–
JTC	II (<i>B. multivorans</i>)	CGD isolate	–	–
Cep54	III	CF epidemic strain	–	+
C1257	III	CF epidemic strain	–	+
C4455	III	CF epidemic strain	–	+
C6433	III	CF epidemic strain	–	+
C5424	III	CF epidemic strain	+	+
BC7	III	CF epidemic strain	+	+
Cep40	V (<i>B. vietnamiensis</i>)	CF isolate, non-epidemic	–	–
C2822	V (<i>B. vietnamiensis</i>)	CF isolate, non-epidemic	–	–

* CGD, Chronic granulomatous disease.

† Presence or absence of the cable pilus gene, *cblA*, and the *B. cepacia* Epidemic Strain Marker (BCESM) was determined by a dot-blot hybridization assay using specific DNA probes (Mahenthiralingham *et al.*, 1997). +, Present; –, absent.

gelatin-Hank's balanced salt solution (gel-HBSS; Gibco BRL). All mice were challenged intratracheally with approximately 5×10^8 bacteria in 50 μ l gel-HBSS and sacrificed by cervical dislocation 1 h after the infection. Immunohistologically stained lung sections were examined microscopically for bacterial adherence.

Dextran and oligosaccharides. Dextran of nominal molecular mass 4000 and 6000 Da were provided by Polydex Pharmaceuticals. Dextran of nominal molecular mass 10000 Da and three oligosaccharides, including isomaltose (Glc α 1 \rightarrow 6Glc), isomaltotriose (Glc α 1 \rightarrow 6Glc α 1 \rightarrow 6Glc) and isomaltotetraose [(Glc α 1 \rightarrow 6Glc) $_3$ Glc], were obtained from Sigma.

Gel filtration of dextrans. Dextran mixtures of each nominal molecular mass were separated first according to size by gel filtration chromatography using Bio-Gel P4 (Bio-Rad). Conditions were essentially as described by Ashford *et al.* (1987). Several milligrams of material was loaded on to a 1.5 \times 100 cm column. The solvent used was water. The eluant was monitored by changes in refractive index, the eluted fractions were combined into several pools and small amounts of these pools were analysed further by HPLC and MS.

HPLC and MS analysis of dextrans. Small samples from each pool were fluorescently labelled by reductive amination with 2-aminobenzamide, according to the method of Bigge *et al.* (1995), using a Signal Labelling Kit (Oxford GlycoSciences). HPLC analysis was then performed by procedures described by Guile *et al.* (1996) using a Waters 2690XE separations module and a Jasco FP-920 fluorescence detector. The mixtures of labelled dextran oligomers were separated by normal phase HPLC on a 4.6 \times 250 mm Oxford GlycoSciences Glycosep-N column. A binary gradient system using 50 mM ammonium formate, pH 4.4 (solvent A), and acetonitrile (solvent B) was used. Initial conditions of 35% solvent A and a flow rate of 0.4 ml min $^{-1}$ were followed by a linear gradient of 35–58% solvent A over the next 92 min. The flow rate was then increased to 1 ml min $^{-1}$ over the next 2 min and the column washed in 100% solvent A for 5 min before being re-equilibrated in 35% solvent A for the next injection. Column

temperature was maintained at 30 °C and total run time between samples was 120 min. The eluant was monitored by fluorescence (excitation at 330 nm, emission at 420 nm).

Matrix-assisted laser desorption/ionization MS was performed using a PerSeptive Biosystems Voyager elite reflection spectrometer as described by Kuster *et al.* (1997). Samples were loaded on to the mass spectrometer target in 1 μ l water, mixed with 1 μ l 2,5-dihydroxybenzoic acid (10 mg ml $^{-1}$ in acetonitrile) and allowed to dry. Oligosaccharides were observed as [M + Na] $^+$ ions accompanied by a smaller signal of the respective [M + K] $^+$ ions in the positive ion spectra.

RESULTS

Adherence of *B. cepacia* to respiratory tract epithelial cells *in vitro* and *in vivo*

B. cepacia strains showed different, but efficient levels of binding to A549 pneumocytes. Cbl $^+$ *B. cepacia* strains C5424 and BC7 exhibited clump formation when they were bound to A549 cells. Immunoperoxidase staining confirmed this specific binding pattern associated with Cbl; in contrast, Cbl $^-$ strains predominantly showed single-cell binding (Fig. 1). After intratracheal inoculation with approximately 5×10^8 bacteria, either Cbl $^-$ (strain C6433) or Cbl $^+$ (strain C5424) *B. cepacia* adhered to murine respiratory epithelial cells. Fig. 2 shows a representative *in vivo* adherence assay of *B. cepacia*. By the use of immunoperoxidase staining, *B. cepacia* diffusely attached to alveolar septa 1 h after infection; C5424 demonstrated the typical clump formation, while C6433 adhered to the epithelial cells individually.

Inhibition by dextran of *B. cepacia* adherence to respiratory tract epithelial cells

Dextran (nominally 4000 Da) at a concentration of 20 mg ml $^{-1}$ inhibited adhesion of all *B. cepacia* strains of

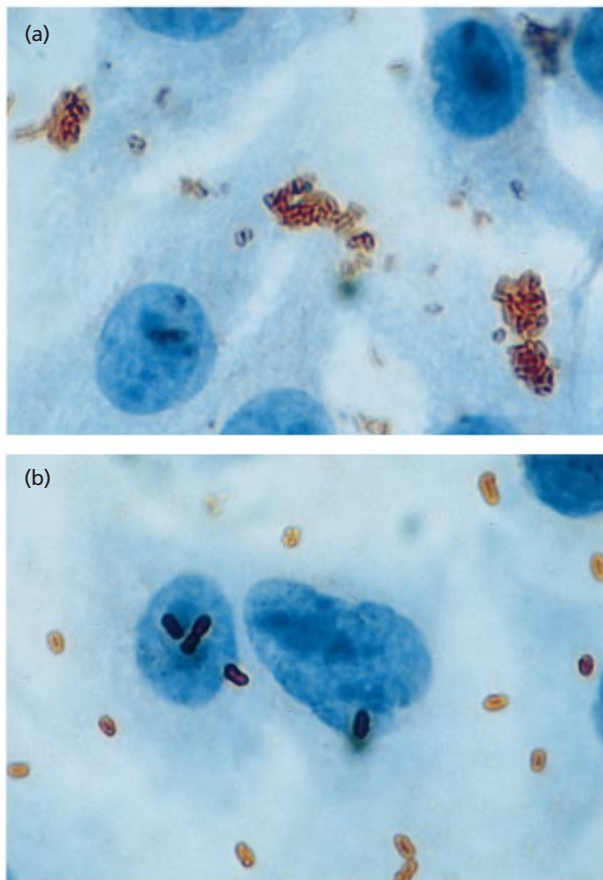


Fig. 1. *In vitro* adherence of Cbl⁺ and Cbl⁻ *B. cepacia* to A549 pneumocytes. *B. cepacia* were bound to A549 pneumocytes and visualized by immunoperoxidase staining as described in Methods. Cbl⁺ C5424 (a) shows clump formation, whereas Cbl⁻ strain C6433 (b) predominantly exhibits single bacteria adhering.

different genomovars to A549 pneumocytes. A dose-dependent inhibitory effect (up to 80 mg ml⁻¹) was observed for most strains (Fig. 3). Dextran exerted less of an anti-adhesive effect on the two Cbl⁺ strains (BC7 and C5424) than on the others which were all Cbl⁻ (Fig. 3). Nonetheless, profound inhibition of adherence was observed for all strains by dextran at a concentration of ≥ 40 mg ml⁻¹. The photomicrographs in Fig. 4 demonstrate the effect of dextran on Cbl⁺ and Cbl⁻ strains. *B. cepacia* also showed some non-specific binding to the glass coverslips, a phenomenon which was also inhibited by dextran. Adhesion was suppressed by all molecular mass preparations tested, but when they were compared on an equimolar basis, the nominally 10000 Da dextran was the most inhibitory (Fig. 5).

Reversible anti-adhesive effect of dextran

If the A549 pneumocytes were pretreated with dextran and then washed with PBS before adding bacteria

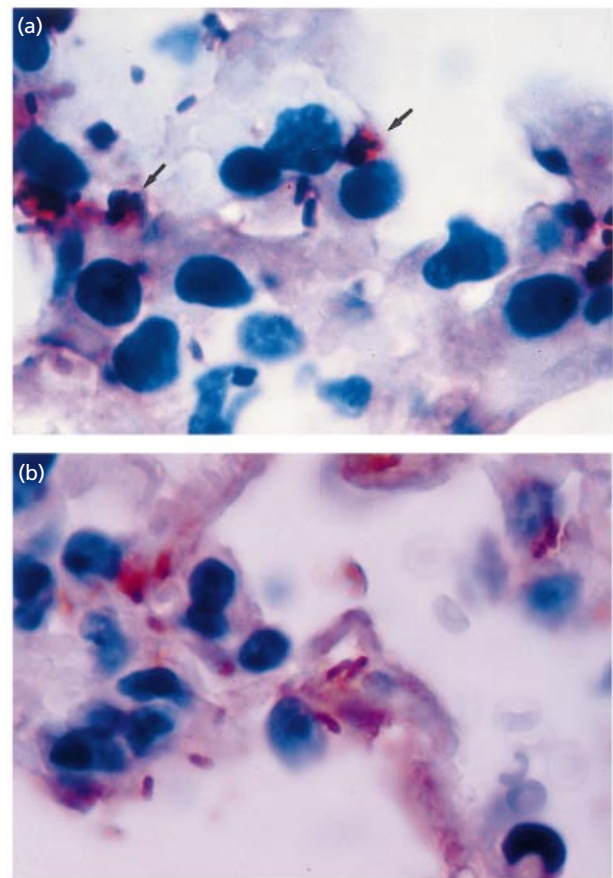


Fig. 2. *In vivo* adherence of Cbl⁺ and Cbl⁻ *B. cepacia* to murine respiratory tract epithelial cells. C57BL/6 mice were injected intratracheally with approximately 5×10^8 *B. cepacia* C5424 (a) and C6433 (b) in 50 μ l gel-HB55 and sacrificed by cervical dislocation 1 h after infection. Lung tissues were removed and immunoperoxidase-stained as described in Methods. Cbl⁺ C5424 (a) shows clump formation (arrows), whereas Cbl⁻ strain C6433 (b) predominantly exhibits single bacteria adhering.

(C5424), the inhibitory effect was largely abrogated: 6.9 ± 0.5 bound bacteria per washed cell without dextran; 1.6 ± 0.1 bound bacteria per unwashed cell with dextran (nominally 4000 Da, 80 mg ml⁻¹); 7.4 ± 1.7 bound bacteria per cell washed after dextran treatment. Similar results were seen if the bacteria were pretreated with dextran, then centrifuged once and resuspended in binding buffer before adding them to the epithelial cells. Under such conditions, the dextran-pretreated bacteria bound to epithelial cells as well as the PBS-pretreated bacteria (5.2 ± 0.4 vs 6.1 ± 0.7 bound bacteria per cell).

HPLC and MS

Dextran, nominally 4000 Da, was actually a mixture of glucose and its oligomers, ranging in size from 1 to 19 glucose units with the majority of the sugars in the very low molecular mass range: 3–6 glucose units (527–

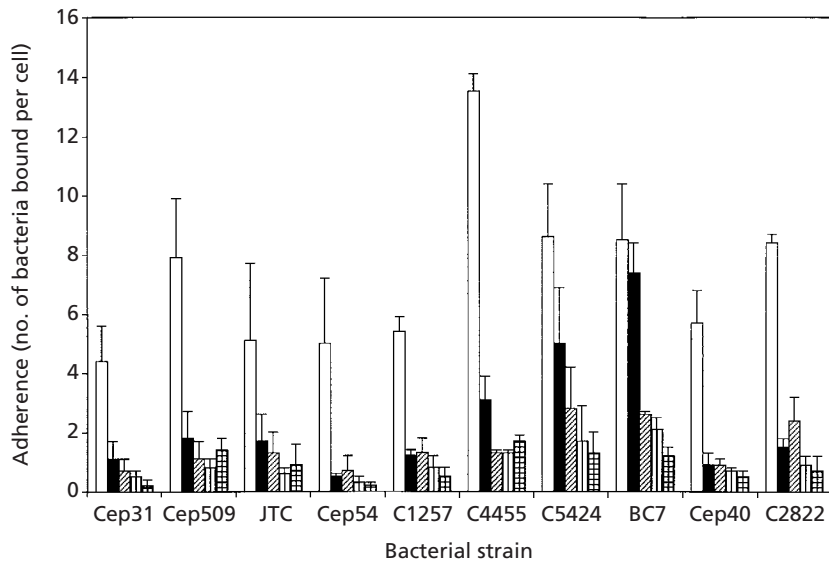


Fig. 3. The effect of dextran (20–80 mg ml⁻¹) on binding of a number of *B. cepacia* strains to epithelial cells. A549 pneumocytes were pretreated with 0 (□), 20 (■), 40 (▨), 60 (▩) or 80 (▧) mg nominally 4000 Da dextran ml⁻¹ and then *B. cepacia* was added. Cell-associated bacteria were enumerated visually. Results are mean values ± SEM from three experiments, each done in duplicate.

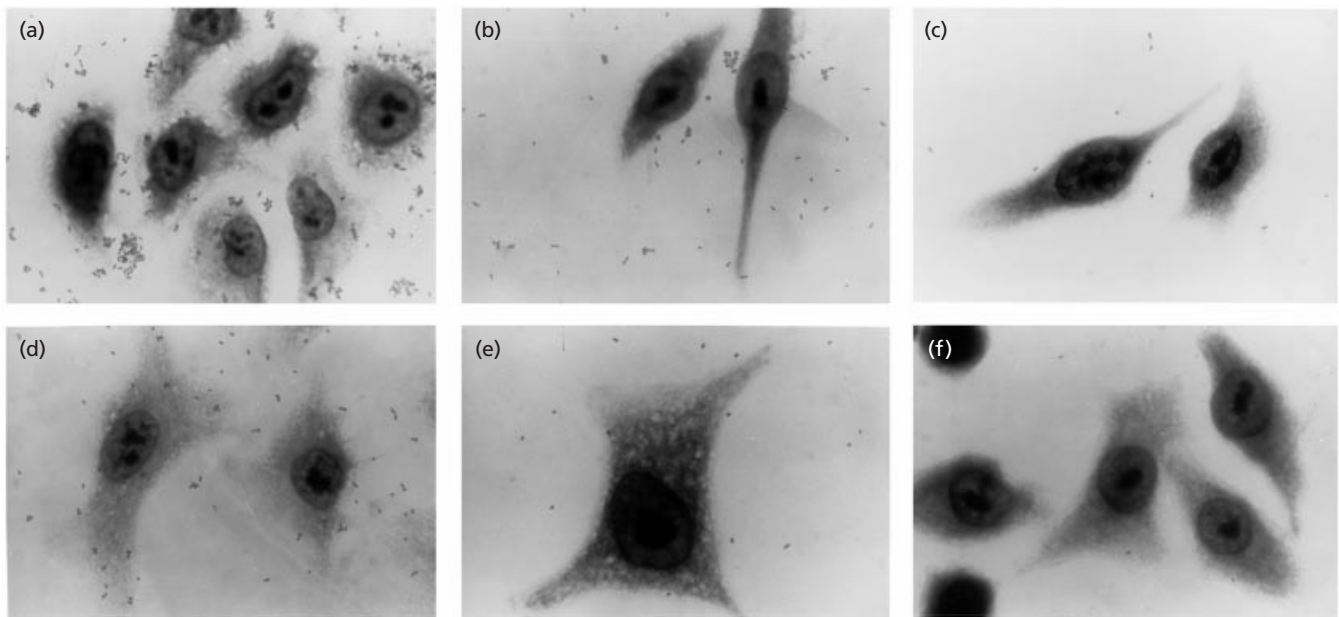


Fig. 4. Adherence of Cbl⁺ and Cbl⁻ strains of *B. cepacia* to epithelial cells and inhibition by dextran. Cbl⁺ *B. cepacia* strain C5424 (a, b, c) and Cbl⁻ strain C6433 (d, e, f) were bound to A549 cells without any inhibitor (a, d) or in the presence of dextran (nominally 4000 Da) at 20 mg ml⁻¹ (b, e) or 40 mg ml⁻¹ (c, f), then Giemsa-stained and enumerated visually as described in Methods.

1013 Da) (Fig. 6). The expectation of a peak corresponding to 23 glucose units for nominally 4000 Da dextran was not found by normal phase HPLC, nor by MS of underivatized dextran. Moreover, dextran, nominally 6000 Da, appeared very similar to dextran of 4000 Da, with a mean size of approximately 5 glucose units (828 Da). Dextran, nominally 10000 Da, was more uniform, but the mean molecular mass was low and far less than 10000 Da; the mean size detected was 12 glucose units (1962 Da).

Effect of isomaltose, isomaltotriose and isomaltotetraose on binding

To determine the dextran moiety which was most anti-adhesive, experiments were performed with three α -1,6-linked di- to tetrasaccharides to examine their influence on binding of strain C5424 to A549 cells. Compared with nominally 4000 Da dextran (80 mg ml⁻¹), the three saccharides at a concentration of 20 mM had a modest effect on binding (Table 2); they appeared to cause

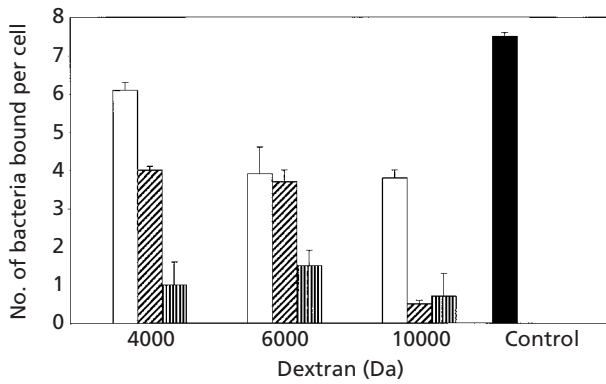


Fig. 5. The effect of dextrans of different nominal molecular mass on the binding of *B. cepacia* C5424 to epithelial cells. A549 pneumocytes were pretreated with 5 (□), 10 (▨) or 20 (▩) mM dextrans of different molecular mass or buffer control (■) and then *B. cepacia* C5424 was added. Cell-associated bacteria were enumerated visually. Results are mean values \pm SEM from three experiments, each done in duplicate.

a molecular-mass-dependent inhibition of binding of C5424 to A549 cells with the isomaltotetraose exerting the greatest anti-adhesive effect (Table 2).

DISCUSSION

Our data indicate that dextran reduced the adherence of all strains of *B. cepacia* tested, including Cbl⁺ and Cbl⁻ strains, to A549 immortalized pneumocytes. Adherence was suppressed by all molecular mass preparations tested, but when they were compared on an equal weight basis, the nominally 10000 Da dextran was most inhibitory. A differential inhibitory effect was exerted by dextran of nominal molecular mass 4000 Da on Cbl⁺ vs Cbl⁻ *B. cepacia*. These observations have important clinical implications: the Cbl⁺ clone of *B. cepacia* appears to be highly contagious and pathogenic for CF patients so that any *in vivo* studies in the future attempting to prevent *B. cepacia* colonization in CF patients using dextran should utilize the optimal mol-

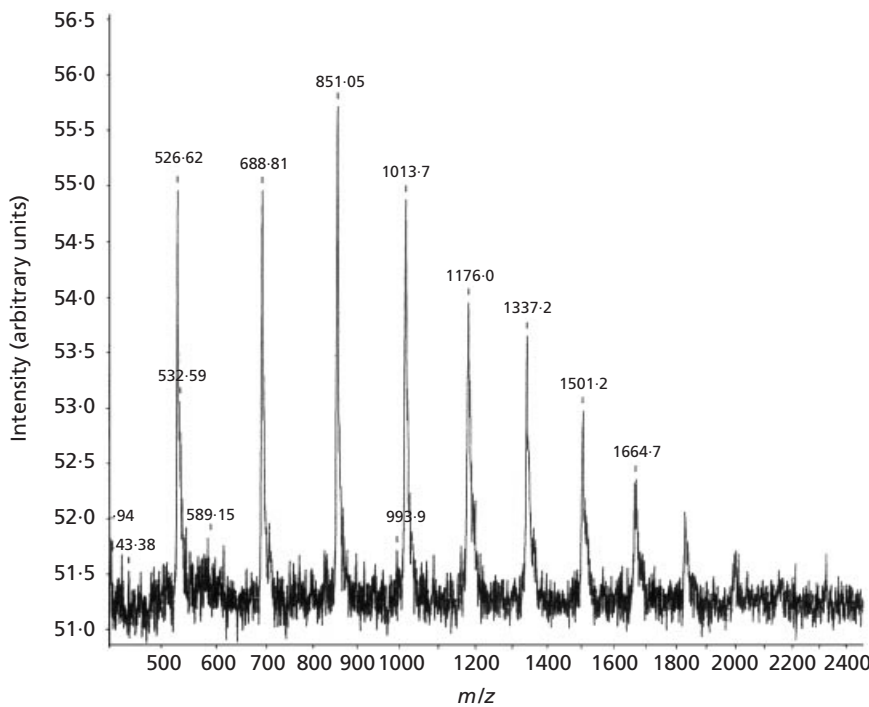


Fig. 6. MS of dextran. Dextran, nominally 4000 Da (Polydex Pharmaceuticals), contained a glucose oligomer mixture, ranging in size from 3 to 12 oligomers with 11 and 12 oligomers in much less abundance than the rest. Peaks for glucose monomer and dimer could not be discerned from the matrix peaks and are not shown. Underivatized dextran was analysed by MS by using the method described in the text.

Table 2. The effect of isomaltose, isomaltotriose and isomaltotetraose on binding of *B. cepacia* strain C5424 to A549 epithelial cells

Treatment*	Cell-associated bacteria (mean \pm SEM)	Inhibition (%)
Buffer control	8.2 \pm 2.8	
Dextran (nominal molecular mass 4000 Da)	1.3 \pm 0.1	84.1
Isomaltose	8.0 \pm 1.2	2.4
Isomaltotriose	6.4 \pm 0.5	22.0
Isomaltotetraose	5.2 \pm 0.5	36.6

* 20 mM, according to the nominal molecular masses of the sugars tested.

ecular mass of dextran. If dextran of nominal molecular mass 4000 Da is used, a concentration of ≥ 40 mg ml⁻¹ or more would have to be applied. A concern with this concentration of dextran is that the administration of such a large dose to the airway may cause osmotic injury to the epithelial cells; however, recent murine studies (Bryan *et al.*, 1999) demonstrated that aerosol delivery of high dose nominally 4000 Da dextran did not result in any histopathology.

The authentic molecular masses of dextrans utilized in this study were at variance with those reported by the manufacturer. The concentration of dextran preparations used in our previous studies were based on the manufacturer's quoted molecular mass (Barghouthi *et al.*, 1996; Bryan *et al.*, 1999). The mean molecular masses of dextrans used, as determined by gel filtration and MS, were approximately 10-fold lower than that indicated. Thus the reported apparent molarities must be adjusted according to the authentic molecular mass determined. It is not practical to determine the molecular mass of all dextrans used in studies such as these (Barghouthi *et al.*, 1996; Bryan *et al.*, 1999), but our observations should provide a note of caution in interpreting results.

All data from the present as well as previous studies (Barghouthi *et al.*, 1996) support the conclusion that dextran blocks the adherence of *B. cepacia* in a non-specific fashion; that is, it does not interfere with a single type of receptor–ligand interaction. Observations in favour of this conclusion are: the inhibitory effect was readily reversible; oligosaccharides composed of 2–4 glucose units with the same α -1,6 linkage were only minimally inhibitory; dextran did not bind specifically to either *P. aeruginosa* or epithelial cells (Barghouthi *et al.*, 1996); and dextran blocked attachment of other respiratory tract pathogens (*Staphylococcus aureus*, group A streptococcus and *Haemophilus influenzae*) as well (Barghouthi *et al.*, 1996).

The mechanism by which dextran inhibits adhesion of bacteria to epithelial cells remains incompletely understood. However, it has been recognized for years that dextran has pervasive effects on cell–cell interactions. For instance, it enhances erythrocyte clumping and is used as an agent for facilitating sedimentation *in vitro*. Furthermore, it inhibits platelet adhesiveness and has anticoagulant activity (Cronberg *et al.*, 1966). Dextran could have exerted its inhibitory effect by coating both the epithelial cells and the bacteria. One possible target for this antiadhesive effect was the Cbl pilus on the bacteria.

After their discovery in 1995, Cbl pili have been identified as one of the adhesins of a specific epidemic *B. cepacia* clone (Goldstein *et al.*, 1995; Sajjan *et al.*, 1995). Sajjan *et al.* (2000) recently found that cytokeratin 13 (CK13) may be the target for the binding of cable-piliated *B. cepacia*; however, the expression of CK13 in normal human bronchial epithelial cells is low. In the current study, we demonstrated that epidemic *B. cepacia* isolates, irrespective of cable piliation, are capable of

attaching both *in vitro* and *in vivo* to respiratory tract epithelial cells. These data suggest that the Cbl pilus is not the only factor required for adherence of epidemic *B. cepacia* strains to respiratory tract epithelial cells. Furthermore, we found that the Cbl⁺ strains of *B. cepacia* formed clumps when they bound to A549 pneumocytes, whereas the Cbl⁻ strains bound as single bacteria. It was also the case when *B. cepacia* were bound to murine respiratory tract epithelial cells *in vivo*. A previous study indicated that most Cbl⁺ *B. cepacia* isolates from CF patients exhibited a rough morphotype that were subject to autoagglutination (Butler *et al.*, 1994). This rough LPS morphotype could contribute to clump formation when bacteria bind to epithelial cells. The observations of this study strongly support the speculation that the Cbl⁺ strains are able to co-aggregate via tangling with similar fibres from neighbouring bacteria, thus enhancing the attachment and the survival of the bacterial microcolonies on the respiratory tract epithelial cells. This autoaggregation may play a role in enhancing the virulence of *B. cepacia* in the lung of patients with CF. As shown in this study, dextran can effectively inhibit the bacterial binding as well as change the specific binding pattern associated with the Cbl pili.

Dextran has several features that make it an attractive candidate therapeutic agent for preventing respiratory tract infections in patients with CF. It is inexpensive, non-toxic and, most importantly, of low viscosity, even at a high concentration. It therefore can be aerosolized readily (Bryan *et al.*, 1999; Finlay *et al.*, 2000). The ability of aerosolized dextran to protect mice from pneumonia due to *P. aeruginosa* has been documented (Bryan *et al.*, 1999). Our data further confirmed the *in vitro* effect of dextran in another important CF pathogen, *B. cepacia*. Other studies have demonstrated that dextran improves mucociliary clearance of CF sputum as monitored using a frog palate mucociliary transportability assay (Feng *et al.*, 1996). The combined effect on sputum rheology and inhibitory effect on bacterial adherence together suggest that dextran delivered by aerosol may be useful in patients with CF to prevent colonization and infection with *P. aeruginosa* and *B. cepacia*.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the assistance of Geoffrey Guile from the Edward Jenner Institute for Vaccine Research in the separation and analysis of dextran preparations. This work was supported by Canadian Cystic Fibrosis Foundation SPARXI Programme and the Canadian Bacterial Diseases Network (both to D.P.S.).

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Received 12 April 2001; revised 19 June 2001; accepted 27 June 2001.