Exopolysaccharides from *Burkholderia cenocepacia* Inhibit Neutrophil Chemotaxis and Scavenge Reactive Oxygen Species

Johan Bylund1,2, Lee-Anna Burgess1, Paola Cescutti1, Robert K. Ernst1, and David P. Speert1,2

From the 1Department of Pediatrics, University of British Columbia, Child and Family Research Institute, Vancouver, British Columbia V5Z 4H4, Canada, the 2Department of Biochemistry, Biophysics and Macromolecular Chemistry, University of Trieste, 34127 Trieste, Italy, and the 3Department of Medicine, University of Washington, Seattle, Washington 98195

Bacteria belonging to the *Burkholderia cepacia* complex are important opportunistic pathogens in compromised hosts, particularly patients with cystic fibrosis or chronic granulomatous disease. Isolates of *B. cepacia* complex may produce large amounts of exopolysaccharides (EPS) that endow the bacteria with a mucoid phenotype and appear to facilitate bacterial persistence during infection. We showed that EPS from a clinical *B. cenocepacia* isolate interfered with the function of human neutrophils in vitro; it inhibited chemotaxis and production of reactive oxygen species (ROS), both essential components of innate neutrophil-mediated host defenses. These inhibitory effects were not due to cytotoxicity or interference with intracellular calcium signaling. EPS also inhibited enzymatic generation of ROS in cell-free systems, indicating that it scavenges these bactericidal products. *B. cenocepacia* EPS is structurally distinct from *Pseudomonas aeruginosa* alginate, yet they share the capacity to scavenge ROS and inhibit chemotaxis. These properties could explain why the two bacterial species resist clearance from the infected cystic fibrosis lung.

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The abbreviations used are: BCC, *Burkholderia cepacia* complex; CF, cystic fibrosis; CGD, chronic granulomatous disease; ROS, reactive oxygen species; EPS, exopolysaccharide(s); FA, fatty acid(s); GC, gas chromatography; MS, mass spectrometry; GLC, gas-liquid chromatography; KRG, Krebs-Ringer phosphate buffer; CL, chemiluminescence; FMLF, formyl-Met-Leu-Phe; PHPA, p-hydroxyphenylacetate; PMA, phorbol 12-myristate 13-acetate.

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2 This study was funded by grants from the Canadian Cystic Fibrosis Foundation (to J. B., L.-A. B., and D. P. S.), Cystic Fibrosis Foundation (to R. K. E.), National Institutes of Health (Grant U54AI057141) (to R. K. E.), the Swedish Society for Medical Research (to D. P. S.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

3 The authors would like to acknowledge Paola Cescutti for her help with data analysis. Portions of this work were done in the Department of Pediatrics, University of British Columbia, Child and Family Research Institute, Vancouver, British Columbia and the Department of Biochemistry, Biophysics and Macromolecular Chemistry, University of Trieste, Trieste, Italy.

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BCC consists of several different polysaccharides distinct from alginate. For example, the mucoid material produced by the B. cenocepacia strain, C9343, used in this study is a mixture of three different polysaccharides (17): polysaccharide-I (18) (constituted of a disaccharide repeating unit consisting of glucose and galactose, with the galactose residue substituted on C-4 and C-6 with a pyruvic acid); cepacian (19) (previously known as polysaccharide-II (20), composed of an acetylated heptasaccharide repeating unit containing galactose, mannose, rhamnose, glucose, and glucuronic acid); and dextran (α-1,6 glucan). We have previously shown that this EPS, produced by a mucoid B. cenocepacia isolate, interfered with phagocytosis of bacteria by human neutrophils and facilitated bacterial persistence in a mouse model (17). The aim of the present study was to expand on these observations and investigate whether B. cenocepacia EPS affects other functions of neutrophils. We showed that B. cenocepacia EPS inhibited chemotactic migration of human neutrophils and also scavenged ROS generated from activated cells. We concluded that despite the biochemical differences between alginate and B. cenocepacia EPS, these substances share functional properties that could be of relevance for CF. B. cenocepacia EPS could contribute to the inability of neutrophils to clear offending bacteria and may constitute an important virulence factor of relevance in the CF lung.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains and Purification of EPS—**B. cenocepacia strain C9343 is a mucoid isolate from a CF patient. The isolate is deposited in the Canadian BCC Research and Referral Repository (Vancouver, BC) and has been previously described (17). P. aeruginosa strain P1 is a mucoid CF isolate previously described (21). Both strains were routinely stored at −80 °C or maintained on Columbia Agar containing 5% sheep’s blood (PML Microbiologicals, Wilsonville, OR) for a maximum of two passages.

For EPS purification, bacteria were grown on modified yeast extract-mannitol agar consisting of 0.05% (w/v) yeast extract, 0.4% (w/v) mannitol, and 1.5% (w/v) yeast extract-mannitol agar at 37 °C for 46 h. EPS was recovered in 0.9% (w/v) NaCl, and liquefied phenol was added to a final concentration of 5% (v/v). This mixture was stirred at 4 °C for 8 h and then centrifuged to remove cells. The EPS was precipitated with 4 volumes of cold 95% ethanol, recovered by centrifugation for 10 min at 9600 × g, dialyzed against distilled water with a molecular mass cut-off of 12 kDa at 4 °C for 48 h, lyophilized, and resuspended in assay buffer.

**Endotoxin Purification—**Endotoxin was purified by suspending a freeze-dried pellet of B. cenocepacia strain C9343 in extraction mixture consisting of phenol (90%):chloroform:petroleum spirit in the proportions 2.5:8. The mixture was stirred on ice for 10 min and centrifuged (10,000 × g, 15 min), after which the supernatant was filtered. The filtrate was air-dried and repurified (22), washed in ice-cold ethanol, and resuspended in endotoxin-free water + 0.2% triethylamine after drying.

**Determination of Endotoxin Content in the EPS Preparation—**The amount of endotoxin was determined through direct transmethylation of the fatty acids (FA) and gas chromatography-mass spectrometry (GC-MS) analysis of the FA methyl ester derivatives obtained. A sample of C9343 EPS (10 mg) was placed in a Teflon-lined screw-capped tube. One ml of methanolic HCl, 1 ml of methanol, and 0.5 ml of hexane were added and heated at 100 °C for 1 h, with frequent shaking. After cooling, 2 ml of hexane and 2 ml of water were added, and the content was mixed by vortexing. The hexane layer was collected, and a known amount of C19:0 methyl ester (internal standard), previously treated in the same way, was added to the solution before drying it under nitrogen. The sample was dissolved in 30 μl of hexane and subjected to gas chromato
tography using a PerkinElmer Autosystem XL (PerkinElmer Life Sciences) gas chromatograph equipped with a flame ionization detector and an SP2330 capillary column (Supelco, 30 m), using helium as the carrier gas. The temperature program used was at 140 °C for 5 min, from 140 to 240 °C at 4 °C/min, at 240 °C for 10 min. GC-MS analyses were carried out on a Hewlett-Packard 5890 gas chromatograph coupled to a Hewlett-Packard 5971 mass selective detector. The determination of the endotoxin molecular weight was accomplished by separate analysis of the lipid A and the core oligosaccharide components. The lipid A was extracted from strain C9343, and its molecular weight determination was performed by matrix-assisted laser desorption ionization-MS and GC analysis. The matrix-assisted laser desorption ionization mass spectrum showed two major negative ions at 1670 and 1801 atomic mass units. The molecular weight of the core oligosaccharide was calculated from electrospray ionization-MS data obtained in our laboratory on the core extracted from a clinical strain of B. cepacia.4 Since both lipid A and core moieties showed a certain degree of structural variability, the mean molecular weight was evaluated, taking into account all the molecular masses obtained by MS analyses for both species. The mass spectra exhibited a single peak for each species present; the peak intensity was taken as the relative occurrence of each molecular species. The molecular mass of the endotoxin was then evaluated by summing the mass of each species multiplied by its relative occurrence, to give a value of 3380.

**Isolation of Neutrophils—**Collection of blood from healthy adult volunteers was performed in accordance with University of British Columbia Research Ethics Board protocol C04-0193. Human neutrophils were purified using dextran sedimentation and Ficoll-Paque gradient centrifugation (23). The cells were washed and resuspended (107/ml) in Krebs-Ringer phosphate buffer (KRG, pH 7.3) containing glucose (10 mM), Ca2+ (1 mM), and Mg2+ (1.5 mM) and stored on melting ice until use. This protocol routinely produced a neutrophil population of ~95% purity as judged by visual inspection of Giemsa-stained slides.

**Neutrophil Chemotaxis—**Neutrophils were resuspended in KRG supplemented with 0.3% bovine serum albumin (to prevent adhesion to the plastic), and varying concentrations of EPS (or endotoxin) and 106 cells in 100 μl were placed in the upper compartment of a transwell system (Costar, Acton, MA) with a pore size of 3 μm. In the lower compartment was placed 600 μl of buffer with appropriate additions of EPS or (endotoxin) and/or chemotactant (formyl-Met-Leu-Phe (fMLF) at 10−8 M). The plates were incubated at 37 °C for 90 min, after which the upper compartments were removed, and the transmigrated cells were lysed with 0.1% Triton and quantified on the basis of lactate dehydrogenase content in the lysates using a lactate dehydrogenase kit (Roche Diagnostics). The data were expressed as the percentage of 106 cells that were lysed directly. Neither fMLF nor EPS affected the lactate dehydrogenase assay per se (not shown).

**Production of ROS—**Details about the various ROS detection systems are given in Ref. 24, and brief descriptions are given below. For cell-free systems, the xanthine/xanthine oxidase system was employed to generate ROS as described previously (25). The reaction mixture without cells (in the presence or absence of EPS) was supplemented with xanthine (2.5 mM) and ROS production was started by the addition of xanthine oxidase (12.5 milliunits/ml).

**Chemiluminescence (CL)—**An isoluminol-enhanced CL system was used with a Victor3 (PerkinElmer Life Sciences) plate reader and disposable 96-well plates containing 220-μl reaction mixtures. Each well con-

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4 A. Silipo, A. Molinaro, D. Comegna, R. Lanzetta, P. Cescutti, and R. Rizzo, unpublished results.
tained 10⁶ neutrophils, horseradish peroxidase (4 units/ml), and isoluminol (a cell-impermeable CL substrate; 2 × 10⁻⁵ M) in KRG. The cells were equilibrated in the Victor³ for 10 min at 37 °C, in the presence or absence of EPS, after which the stimulus (15–30 µl) was added using an automated internal injector (fMLF 10⁻⁷ M or PMA 100 ng/ml). The light emission was recorded continuously, and data are expressed as counts/s.

Cytochrome c Reduction—Neutrophils (5 × 10⁵/sample) were mixed with cytochrome c (1.5 mg/ml) and diluted in KRG (in the presence or absence of EPS) to 0.99 ml in a cuvette that was equilibrated at 37 °C for 10 min. The cuvette was then transferred to a spectrophotometer (Lambda 2; PerkinElmer Life Sciences), and absorbance measurements at 550 nm were started after the addition of 10 µl of PMA (100 ng/ml final) and continued for 30 min.

PHPA Oxidation—Neutrophils (5 × 10⁵/sample) were mixed with horseradish peroxidase (4 units/ml), p-hydroxyphenylacetate (PHPA; 0.5 mg/ml), and superoxide dismutase (50 units/ml) to ensure full conversion of O₂ to H₂O₂. The cuvettes were equilibrated at 37° for 10 min in the presence or absence of EPS before stimulation with PMA (100 ng/ml final). Emission was measured continuously at 400 nm with an excitation wavelength of 317 nm using a luminescence spectrometer (LS50B; PerkinElmer Life Sciences).

Intracellular Calcium Measurements—Freshly isolated neutrophils (10⁶/ml) in KRG buffer without calcium, supplemented with 0.1% bovine serum albumin, were labeled with the fluorescent calcium indicator Fluo-4 AM (2 µg/ml; Molecular Probes, Eugene, OR) for 30 min at room temperature in the dark with occasional shaking. The labeled cells were then washed twice and resuspended in KRG with calcium in the absence of EPS) to 0.99 ml in a cuvette that was equilibrated at 37 °C for 10 min. The cuvette was then transferred to a spectrophotometer (Lambda 2; PerkinElmer Life Sciences), and absorbance measurements at 550 nm were started after the addition of 10 µl of PMA (100 ng/ml final) and continued for 30 min.

Endotoxin can affect neutrophil chemotaxis, although both inhibitory and enhancing effects have been reported (26, 27). To rule out the possibility that contaminating endotoxin was responsible for the inhibition of chemotaxis, we analyzed the endotoxin content of the purified EPS preparations. The endotoxin produced by strain C9343 is rough, lacking the O side chain (17), and the contaminating amount was calculated indirectly, through transmethylation of the FA present in the C9343 EPS sample using C19:0 as an internal standard. Two independent derivatizations and measurements were performed and were consistent with one another. Four different FA were identified by comparison with retention times of standards in the GLC chromatogram and by electron impact mass spectrometry (Table 1). Three additional peaks in the GLC chromatogram did not correspond to any of the available standards, whereas their e.i mass spectra suggested the presence of two unsaturated FA and one 3-hydroxyl FA, all in lesser amounts than C18:1 ω7c.

RESULTS

B. cenocepacia EPS Inhibits Neutrophil Chemotaxis—We investigated whether B. cenocepacia EPS inhibits neutrophil chemotaxis in vitro as has been described for alginate from various P. aeruginosa strains (14). The formylated peptide fMLF is a potent chemoattractant, with maximal effect at a concentration of 10⁻⁸ M. Chemotaxis toward fMLF was inhibited in a dose-dependent manner by B. cenocepacia EPS (Fig. 1A). At high concentrations of EPS (>1 mg/ml), spontaneous (random cell movement in the absence of fMLF) neutrophil migration was also inhibited (not shown). This could probably be attributed to the viscous nature of the EPS. However, significant inhibition of fMLF-directed chemotaxis was observed with EPS concentrations in the µg/ml range (at which random migration was not affected), indicating that the inhibition of directed migration was not simply based on viscosity.


**TABLE 1**

| FA content of EPS from *B. cenocepacia* C9343 | Average % (w/w) |
|-----------------------------------------------|-----------------|
| C14:0                                         | 0.0161          |
| C16:0                                         | 0.0120          |
| C18:0                                         | 0.0056          |
| C18:1(9)                                      | 0.0029          |

**EPS Inhibits fMLF-induced ROS Production**—Apart from chemotaxis, fMLF also governs a number of other neutrophil effector functions, such as activation of the NADPH-oxidase that results in the production of ROS. When cells were stimulated with fMLF, a robust production of ROS ensued. This response was abrogated in the presence of *B. cenocepacia* EPS (Fig. 2). The inhibition of ROS production was probably not due to contaminating endotoxin since purified *B. cenocepacia* endotoxin enhanced (rather than diminished) the levels of ROS produced after fMLF stimulation (Fig. 2, inset).

*B. cenocepacia* EPS Does Not Interfere with Cell Signaling and/or Viability—Binding of fMLF to its receptor, the formyl peptide receptor, induces various intracellular signaling events, of which a transient increase in the cytosolic calcium concentration is one of the most prominent (28). We measured fMLF-induced calcium flux in the presence or absence of EPS to investigate whether the inhibitory effects of EPS on fMLF-induced activities were due to inactivation/immobilization of the agonist or interference with intracellular signaling events. Even in the presence of EPS concentrations as high as 2.5 mg/ml, fMLF induced a swift and transient increase in cytosolic calcium concentrations (Fig. 3), indistinguishable from the calcium response in the absence of EPS (Fig. 3, inset). Furthermore, incubation of neutrophils in the presence of EPS (at concentrations ranging from 100 ng/ml to 2 mg/ml) for up to 18 h was not cytotoxic, as determined by lactate dehydrogenase release or trypan blue exclusion experiments (not shown).

**EPS Inhibits PMA-induced ROS Production**—EPS also inhibited neutrophil ROS production in response to the phorbol ester PMA, a very powerful activator of the NADPH-oxidase. The inhibitory effect of EPS was dose-dependent, and near complete inhibition of ROS production was obtained at an EPS concentration of 1 mg/ml (Fig. 4). When EPS was added to cells after PMA stimulation, an abrupt decline in ROS production was observed (Fig. 4B).

To determine whether the EPS inhibition of ROS production was due to interference with the CL system, such as interference with the luminescence (light quenching), we evaluated the EPS by an optical scan spanning the visible spectrum. EPS did not show any significant absorbance at wavelengths greater than 300 nm (not shown); we therefore concluded that it was highly unlikely that any light quenching properties of the EPS were responsible for the decreased CL signals observed in its presence. We also performed ROS measurements using the cytochrome c reduction assay (24) to rule out the possibility of EPS interference with the components of the CL system, e.g. the peroxidase used (horseradish peroxidase). Using this assay, *B. cenocepacia* EPS markedly inhibited ROS production in response to PMA (Fig. 5A). Both the CL and the cytochrome c reduction assays detect O₂⁻, which is the ROS primarily produced by the activated NADPH-oxidase. O₂⁻ is rapidly converted to H₂O₂, which can be detected using the PHPA oxidation assay in the presence of superoxide dismutase (to ensure a full conversion of O₂⁻ to H₂O₂). EPS markedly decreased the ROS response of PMA stimulated neutrophils (Fig. 5B), as observed with this assay. Inhibition of ROS by *B. cenocepacia* EPS was similar in dose dependence to that of alginate from *P. aeruginosa*, regardless of which technique was used to measure ROS production (CL results are shown in Fig. 4A).

**EPS Scavenge ROS in Cell-free Systems**—Since we ruled out likely artificial effects of EPS on the ROS measuring systems employed (such as light quenching/absorption) and inhibition of cell signaling upstream of the NADPH-oxidase (such as calcium flux), we reasoned that the inhibitory effect on ROS production could be based on two different mechanisms. Either the effect could be explained by scavenging/quenching of the ROS once formed, or the effect could be due to interference with the activation/assembly of the NADPH-oxidase. The former model has been presented as an explanation for the effect of alginate since this polysaccharide could effectively neutralize ROS generated in the absence of cells by the xantine/xantine oxidase system (25, 29). The xantine oxidase catalyzes transfer of electrons from xantine to molecular oxygen-generating superoxide anions. This simple chemical reaction is often used to mimic the generation of phagocyte-derived ROS in the absence of phagocytes (25, 29).

*B. cenocepacia* EPS inhibited the ROS production obtained by adding xantine oxidase to xantine in the absence of cells, as assessed by CL (not shown) and reduction of cytochrome c (Fig. 6). The complete inhibition of ROS production in this cell-free system was apparent at EPS concentrations around 1 mg/ml, concentrations similar to those required in the cell-based systems. The enzymatic action of xantine oxidase on xantine results in the generation of ROS and uric acid (30). To ensure that the inhibition of ROS production was not due to inhibition of the enzymatic activity of xantine oxidase, we measured the production of uric acid from xantine in the presence or absence of EPS. We found that 1 mg/ml EPS had no effect on uric acid production (not shown). These results, that EPS inhibits ROS production in the absence of cells, supported a model in which EPS scavenges existing ROS after...
these are formed, rather than interfering with the cellular processes leading up to ROS production, e.g. assembly of the NADPH-oxidase components.

**DISCUSSION**

BCC is a family of environmental bacteria that are becoming increasingly recognized as opportunistic pathogens in compromised patients, in particular patients suffering from CGD or CF. Innate resistance to antibiotics (31) and an ability to spread from patient to patient (32) make these bacteria especially problematic clinically. As of yet, little is known about virulence determinants that make BCC particularly virulent for CF and CGD patients, whereas posing little or no threat to the population in general (2). In this study, we show that *B. cenocepacia* EPS, isolated from a mucoid isolate from a CF patient, could inhibit neutrophil chemotaxis and scavenge neutrophil-derived ROS. Similar findings have been presented for alginate from *P. aeruginosa* (14, 25, 29, 33). It is interesting to note that despite structural differences, the mucoid material from *P. aeruginosa* and *B. cenocepacia* share many properties that could profoundly interfere with the functions of immune cells. Most notably, they both have the ability to scavenge ROS, which is normally of utmost importance for proper antimicrobial action of neutrophils (11). In effect, if the mucoid material of these bacteria can neutralize ROS, the antimicrobial action of neutrophils would be totally dependent on non-oxidative means of killing. These non-oxidative mechanisms of killing largely consist of cationic, antibacterial peptides (34); although *P. aeruginosa* are susceptible to the action of such peptides, BCC are resistant (6). This would make the ROS scavenging capacities substan-

![Image](362x26 to 389x38)

**FIGURE 4. Inhibition of PMA-induced ROS production.** Isoluminol-enhanced CL was used to monitor extracellular release of ROS. A, neutrophils were stimulated with PMA (100 ng/ml) and extracellular release of ROS in the presence of varying concentrations of *B. cenocepacia* EPS (solid line) or *P. aeruginosa* alginate (dotted line) was measured. Peak CL values were compared with control peak CL values (100%) and are expressed as the mean percentage of inhibition ± S.E. from 3 independent experiments. B, neutrophils were stimulated with PMA (left arrow) and subjected to subsequent addition (right arrow) of EPS (1 mg/ml final concentration; dotted line) or KRG (solid line). A representative experiment is shown (n = 3).

![Image](362x26 to 389x38)

**FIGURE 5. Inhibition of neutrophil ROS production.** Neutrophils were stimulated with PMA (arrows; 100 ng/ml), and the generation of O$_2^*$ (A) or H$_2$O$_2$ (B) was followed kinetically in the presence (dotted lines) or absence (solid lines) of EPS (1 mg/ml). Representative experiments of cytochrome c reduction (A; n = 4) and PHPA oxidation in the presence of superoxide dismutase (B; n = 3) are shown.

![Image](362x26 to 389x38)

**FIGURE 6. Inhibition of ROS production in a cell-free system.** O$_2^*$ production from the reaction of xanthine with xanthine oxidase was measured by cytochrome c reduction in the presence (dotted line) or absence (solid line) of EPS (1 mg/ml). A representative experiment is shown (n = 5). Abs, absorbance.
described. It is likely that even higher alginate concentrations could be found in CF lungs and that sputum samples represent underestimations due to dilutions with host secretions (14). In lung homogenates from infected CF mice, alginate concentrations of above 900 μg/ml have been described (36). Regarding B. cenocepacia, the EPS is very tightly associated to the bacterial cells (17), making the EPS concentrations very high at the site where ROS scavenging would be most important, at the bacterial surface. In light of this, it is likely that mucoid material from both P. aeruginosa and BCC could play important roles in pulmonary pathology during infection.

In contrast to P. aeruginosa, in which alginate production and conversion to mucoid phenotypes are clearly associated with virulence in CF patients, mucoid BCC isolates have been considered relatively rare (12). However, 80–90% of the BCC isolates recovered from respiratory infections in CF patients in Portugal were shown to produce large amounts of EPS (37, 38). In addition, several studies employing mouse models describe more persistent infections with mucoid BCC than with non-mucoid strains (17, 39, 40). The mucoid material of BCC consists of several different polysaccharide species, the most common of which is called cepacian (19). This EPS species is composed of a branched acetylated heptasaccharide repeating unit and is produced by strains isolated called cepacian (19). This EPS species is composed of a branched acetylated heptasaccharide repeating unit and is produced by strains isolated in different parts of the world (20, 41–43). Cepacian appears to be a BCC specific EPS species; together with two other polysaccharides, dextran and polysaccharide-I (18), cepacian makes up the EPS used in this study (17).

For all specific molecules isolated from Gram-negative bacteria, endotoxin is a potential contaminant. This is an especially problematic issue with BCC since polymyxin B, a substance normally used to abrogate endotoxic activity (44), lacks affinity for BCC endotoxin (45). To ensure that contaminating endotoxin was not responsible for the effects we used purified endotoxin as a control. Neither the inhibition of chemotaxis nor the inhibition of ROS release could be explained by the presence of endotoxin contamination. With regard to ROS production, endotoxins in general prime the neutrophil response to FMLF (46) and other chemotacticants (47) as has been described previously for B. cepacia endotoxin (48). Thus, EPS has the opposite effect of endotoxin in this respect, in that it markedly decreased the ROS response after stimulation.

The decreased ROS response was apparent also when ROS were generated enzymatically in the absence of neutrophils. This implied that EPS was able to scavenge ROS regardless of how these were generated and that the effect was not mediated by interference with cellular processes leading up to assembly of the active NADPH-oxidase. The finding that EPS did not affect the uric acid production in the xanthine/xanthine oxidase system means that the enzymatic activity was intact, supporting the model in which EPS scavenge ROS. The fact that EPS was not restricted to neutralization of phagocyte-derived ROS could mean that this mucoid material protects bacteria from ROS damage regardless of their source. Although the phagocyte NADPH-oxidase is probably the most important source of ROS in an infectious setting, other ROS-producing cells/systems exist in humans (49).

As described, both P. aeruginosa alginate and B. cenocepacia EPS interfere with effector functions of neutrophils (ROS production and chemotaxis). In addition, alginate is also directly linked to the ability of P. aeruginosa to form biofilms (12). This mode of growth, in which the bacteria form multicellular communities embedded in alginate, is thought to further facilitate bacterial evasion of various immune systems. Biofilm growth has also been observed for BCC (2), and there are even examples of CF patients simultaneously infected with both P. aeruginosa and BCC, in which the two bacterial species form mixed biofilms (50). However, EPS production is not required for BCC biofilm formation, although it may play a role in the establishment of thick biofilms (51). Furthermore, the mucoid B. cenocepacia isolate used in this study, C9343, does not form biofilms in vitro, whereas its non-mucoid counterpart does (17). Despite the lack of biofilm formation, the vast amount of EPS produced by B. cenocepacia C9343 appears to facilitate bacterial survival in a murine model (17). The very close association of EPS to the bacterial cell surface could protect the bacteria from toxicity of ROS before they can damage the cells. Such a layer, masking various surface ligands, could also explain the very poor association with host defense cells displayed by this mucoid strain (17). Although further studies regarding the in vitro significance of BCC EPS are needed, we concluded that despite the biochemical differences between P. aeruginosa alginate and B. cenocepacia EPS, these two substances share functional properties that could be of relevance for CF. The presence of EPS could render neutrophils unable to kill the bacteria by oxidative means since these antibacterial effectors are neutralized by the mucoid material. This leaves the neutrophils dependent on their non-oxidative arsenal, i.e. effectively like the situation in CGD. We have previously shown that non-oxidative mechanisms are unable to kill BCC (6); a mucoid exolayer, capable of disarming oxidative killing, would thus leave neutrophils without means to clear the offending bacteria. This could have grave consequences in the CF lung. EPS production could be as important for B. cenocepacia virulence as alginate production is for P. aeruginosa (12).

Acknowledgments—We thank Rebecca Ma, Veronica Johansson, and Deborah Henry for expert technical assistance.

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B. cenocepacia EPS Inhibit Neutrophil Function

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