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Distribution of Porin and Lipopolysaccharide Antigens on a *Pseudomonas aeruginosa* Permeability Mutant

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***Pseudomonas aeruginosa* common surface antigens were compared in a permeability mutant (PCC118) and its parent (PAO503). The distribution of lipopolysaccharide and porin antigens in the mutant supports the conclusion that beta-lactam permeability was affected by lipopolysaccharide-side chain presentation rather than by a change in porin number.**

The outer membrane of gram-negative bacteria is a complex arrangement of proteins, lipopolysaccharide (LPS), and lipids (9, 10). To reach their targets, hydrophilic antibiotics must penetrate this region by concentration-dependent diffusion through hydrophilic pores within the outer membrane (1, 5, 10, 13). These pores are exposed at the surface of intact cells (7).

Pseudomonas aeruginosa mutants which are altered in their permeability characteristics for beta-lactam antibiotics have been described (3, 4). The defective permeability was found to correlate with a decrease in the amount of side-chain sugars present in the LPS of these strains. The conclusions drawn in these studies were based on the chemical composition of LPS isolated from the strains tested. Our study was an attempt to show a physical difference between these strains and their parent.

The bacterial strains used were described elsewhere (3). Before use, cultures were grown in brain heart infusion broth (Difco Laboratories, Detroit, Mich.) to an optical density at 650 nm of 0.5, collected by centrifugation, washed in phosphate-buffered (10 mM; pH 7.4) saline, and suspended in HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer at 100× the original concentration.

The protein-A-gold-conjugate-labeling procedure was a modification of that described by Robinson et al. (11). A 50-μl sample of the cell suspension was pelleted in a Beckman Airfuge (50,000 × *g*, 1 min) and suspended in 20 μl of antibody. After incubation for between 30 min and 1 h at 37°C, cells were collected, washed in 50 mM HEPES buffer (pH 7.5), and suspended in 20 μl of protein-A-gold conjugate (SPI Supplies, Toronto, Ontario, Canada). Interaction of protein-A-gold conjugate was allowed for 1 h at 37°C. In experiments in which single labeling only was used, free protein-A-gold conjugate was removed by washing. Cells were resuspended in HEPES buffer. A drop of this suspension was dried onto a coated electron microscope grid, washed by immersion in distilled water, dried, and examined directly in a Phillips 400 electron microscope. In double-labeling experiments, cells were again collected by centrifugation, washed extensively, and suspended in the second antibody. After incubation for 30 min to 1 h at 37°C, the unbound antibody was removed and the cells were suspended in protein-A-gold conjugate. Either 5 or 15 nm of protein-A-gold conjugate was used, depending on the size used to detect the primary antibody. After 1 h of incubation, the cells were collected, washed, and resuspended in HEPES buffer.

Each experimental run was repeated at least three times to exclude the possibility that random interaction accounted for the observations. Control procedures were repeated with each run, with the exception of the foreign antibody control (a monoclonal antibody specific for neisserial outer membrane; gift from E. Mackie), which was repeated twice only. Background binding of protein-A-gold conjugate or background of the O-specific antibody with protein-A-gold conjugate against a heterologous strain of *P. aeruginosa* (DG1) was minimal (data not shown).

Treatment of the mutant and parent strains with an antiporin monoclonal antibody (MCA 48H.3, one of the pan-reactive group described by Mackie et al. [6]) is shown in Fig. 1. MCA 48H.3 has been shown to interact specifically with the porin protein of all tested *P. aeruginosa* strains (H. R. Rabin, D. R. Cook, A. J. Godfrey, D. M. Mainland, and L. E. Bryan, Program Abstr. 22nd Int. Conf. Antimicrob. Agents Chemother., abstr. no. 252, 1982). The same degree of labeling was observed with both strains. Interaction of an anti-LPS monoclonal antibody (specific for the O side chain of PAO503 as determined by enzyme-linked immunosorbent assay reactions with all serotypes of *P. aeruginosa* and by Western blots [data not shown]) with the parent (Fig. 2a) and the mutant (Fig. 2b) showed a marked reduction in the intensity of label with PCC118. This observation verified the pseudorough phenotype, based on chemical composition of LPS, previously proposed for this strain (3).

Combinations of the two antibodies were done with both strains. Interaction of the antiporin antibody followed by exposure to the anti-LPS antibody gave micrographs consistent with additive binding of both antibodies (data not shown). The lower degree of binding of the anti-LPS monoclonal antibody was again observed with the mutant strain. The order of antibody presentation may be of importance, because considerably less antiporin antibody was bound to

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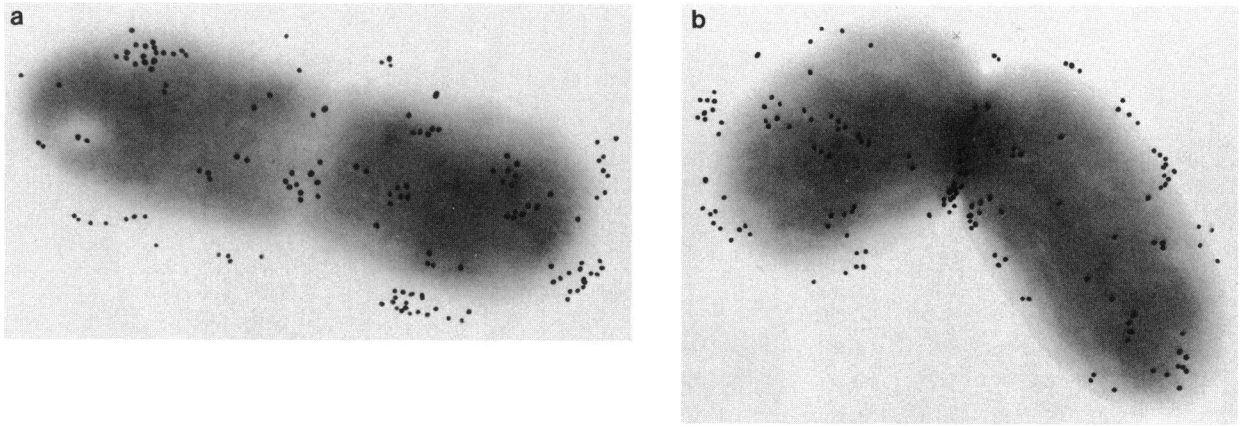

FIG. 1. Binding of an antiporin monoclonal antibody to cells of PAO503 (a) and PCC118 (b). Cells were exposed to excess antibody (titer, 1:50,000) and washed, and bound immunoglobulin G was detected by protein-A-gold-conjugate particles (size, 15 nm).

cells previously exposed to the LPS antibody (Fig. 3; cf. Fig. 3 and 1).

Binding of rabbit antibody derived against extracted LPS of a rough strain of PAO503 (PAO503-18 [2]) was observed to be similar in the two strains (Fig. 4). However, binding with antiporin antibody after treatment with anti-rough LPS was once again reduced. The reasons for the reduced binding of porin antibody to cells prelabeled with an anti-LPS antibody are not known. Steric hindrance by the first bound antibody may explain the reduction. However, binding of the O-specific antibody was markedly reduced in PCC118, suggesting that porin sites, if independent of the primary antibody, should have been exposed. Alternatively, the monoclonal antibody developed against the porin, although panreactive (6), was raised against heat-killed bacteria. The possibility that the antibody recognizes only a subclass of porins cannot be excluded. Phase of growth or growth conditions may influence the number of recognized epitopes (K. Williams, personal communication).

The method used does allow observation of the spatial distribution of antigens on the surfaces of bacteria (11). It confirmed a difference in the quantity or availability of the O-specific epitope recognized by a monoclonal antibody in PCC118 when compared with PAO503. The similar distribution of porin-specific monoclonal antibody and core-LPS-specific polyclonal antibody was in full agreement with earlier studies (3) showing no differences in either of these components in PAO503 and PCC118. The number of observed porin epitopes on the surface of both strains correlated with the number of pores predicted by Nicas and Hancock (8). Thus, these data provided morphological support for the conclusion that the basis of the permeability change in PCC118 and its contribution to reduced beta-lactam susceptibility is due to the nature of the O-specific polysaccharide of PCC118. The mechanism by which this influence on beta-lactam permeability is manifested is unknown. It may be by virtue of a reduction in hydrophilicity of the bacterial surface or near the porin channels. Our results did not allow us to confirm either of these speculations or to provide a definite explanation.

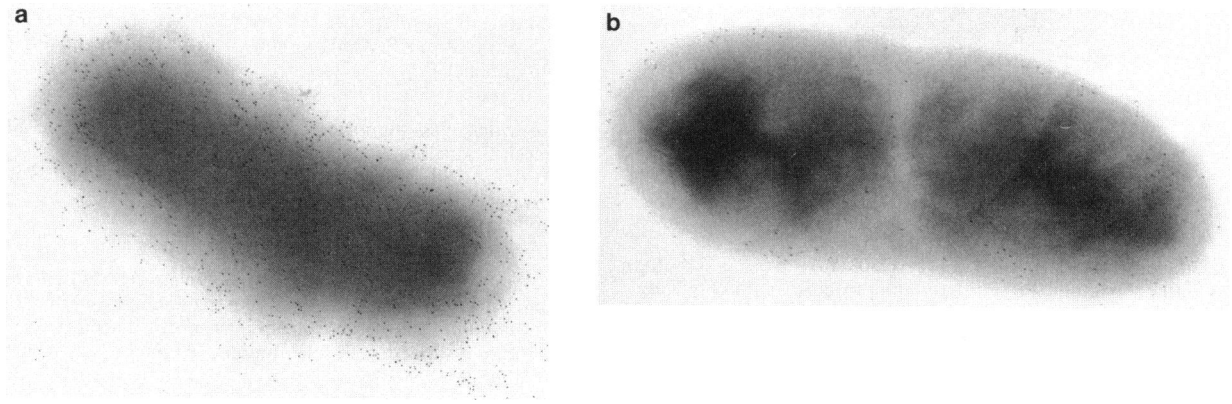

FIG. 2. Reaction of *P. aeruginosa* PAO503 (a) and mutant PCC118 (b) to monoclonal antibody raised against purified LPS from PAO503. Antibody titers used were 1:40,000 as determined by an enzyme-linked immunosorbent assay (12) using 10 μg of purified LPS. The protein-A-gold conjugate used for antibody detection contained colloidal gold as 5-nm particles.

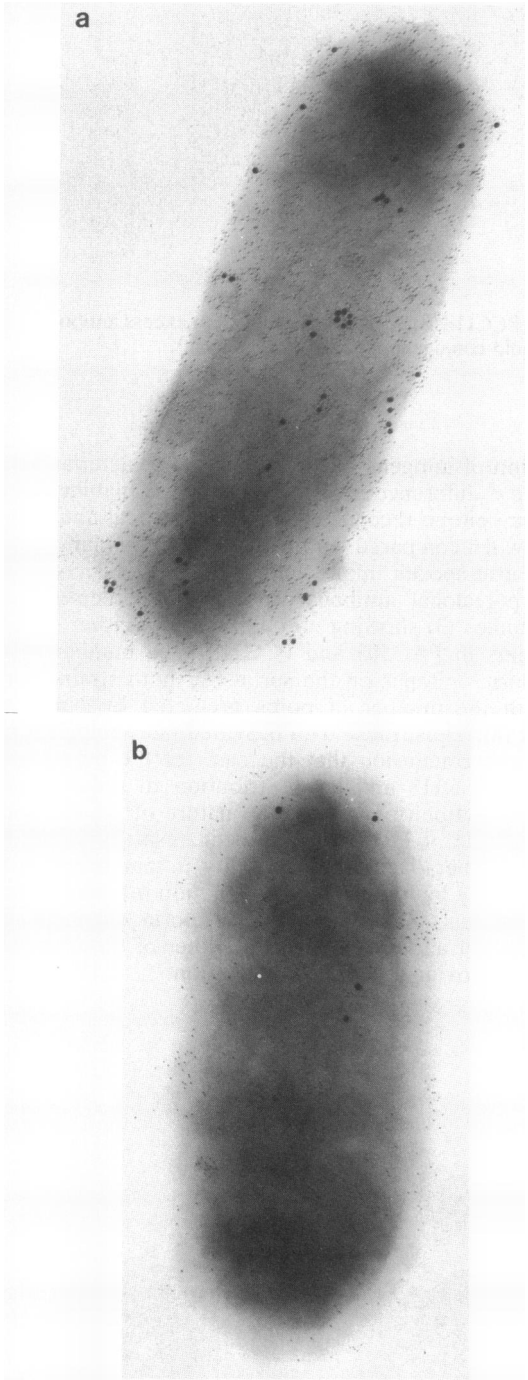

FIG. 3. Cells of *P. aeruginosa* PAO503 (a) and PCC118 (b) reacted with O-specific LPS antibody and protein-A-gold-conjugate particles (5 nm) and then treated with porin antibody and protein-A-gold-conjugate particles (15 nm).

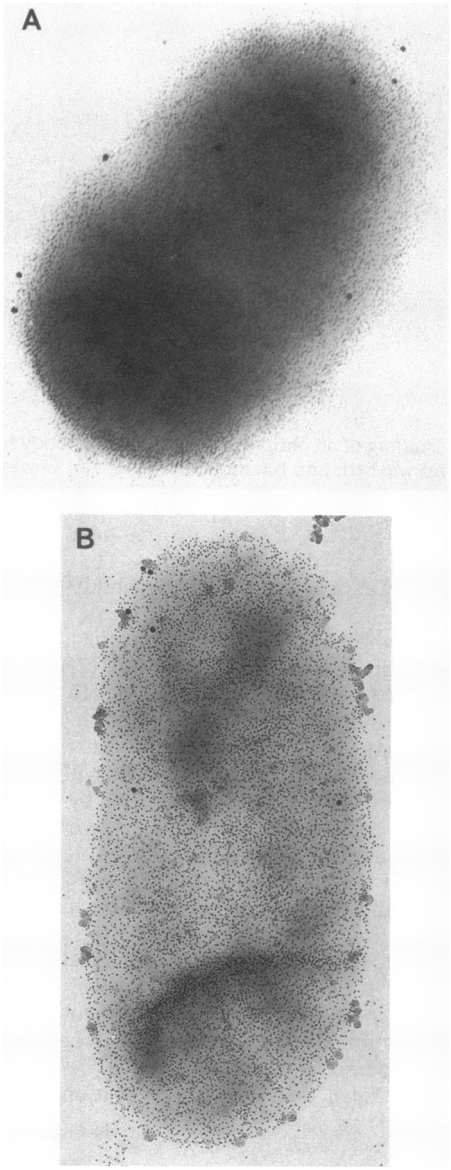

FIG. 4. Binding of antiporin antibody (gold particle size, 15 nm) after treatment of cells with polyclonal rabbit antibody (titer, 1:52,000) raised against core LPS isolated from a rough variant of *P. aeruginosa* PAO503 (PAO503-18 [2]). Rabbit immunoglobulin attached to cells was detected with protein-A-gold conjugate (particle size, 5 nm). Strain PAO503 is shown in panel A, and PCC118 is shown in panel B.

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