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Immunolabeling of Lipopolysaccharide Liberated from Antibiotic-Treated *Escherichia coli*

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Increased anti-core glycolipid antibody binding was visualized by immunoelectron microscopy after incubation of *Escherichia coli* K1:O7 cells with a bacteriolytic antibiotic and compared with binding in control cells. The findings suggest that the core glycolipid regions of the lipopolysaccharides of some gram-negative bacilli can be effectively sequestered until liberated by antibiotic-induced cell lysis.

Liberation of lipopolysaccharides (LPS) from bacterial cells after antibiotic-induced bacterial cell lysis has been demonstrated in vitro and in experimental gram-negative septicemia (3, 9, 12, 13). This phenomenon has also been demonstrated in patients with gram-negative bacterial sepsis (10). Antibiotic-induced release of LPS in patients being treated for gram-negative bacterial sepsis is thought by some investigators to evoke a multitude of toxic biological reactions, causing deterioration in some circumstances (D. A. B. Hopkin, Lancet ii:1193–1194, 1978; Anonymous, Lancet ii: 594, 1985). However, there is little evidence that directly demonstrates that the bacterial cell-bound LPS, compared with free LPS, is in any way constrained from interaction with the host.

In this study, visual confirmation of the antibiotic-induced liberation of endotoxin was attempted by using a monoclonal antibody directed to an epitope on the core glycolipid (CGL) region of *Escherichia coli* K1:O7. The epitope is not normally accessible to antibody on intact *E. coli* K1:O7 cells. The binding of this monoclonal antibody to its respective epitope was visualized by immunoelectron microscopy before and after exposure of the organism to a bacteriolytic antibiotic.

UDP-galactose-deficient mutant *E. coli* J5 (provided by Elizabeth Ziegler, School of Medicine, University of California, Davis) and *E. coli* K1:O7 strain C94 (provided by E. Richard Moxon, Oxford University, Oxford, United Kingdom) were stored in skim milk at −70°C and were recovered on tryptic soy agar. A representative colony was then inoculated into a flask of tryptic soy broth and incubated overnight at 37°C. After centrifugation at 600 × *g*, the cell pellet was washed twice and resuspended in broth.

The monoclonal antibodies used in this study have been previously described and characterized (1, 2). M1B1 was obtained after immunization of mice with boiled *E. coli* J5 cells and fusion of spleen cells with the non-immunoglobulin-producing mouse myeloma line P3-X63-Ag8.653. This antibody binds to the CGL region of purified LPS in both *E. coli* J5 and *E. coli* K1:O7, as well as to intact *E. coli* J5 cells but not to intact *E. coli* K1:O7 cells (1). M5B12, an irrelevant control monoclonal antibody, was produced after the immunization of mice with a homogenate of lungs obtained from rats with *Pneumocystis carinii* pneumonitis and fusion of spleen cells with the mouse myeloma line (2). Both antibodies are of the immunoglobulin G class.

The immunoelectron microscopy procedure was similar to that described by Murti et al. (4) and Shenep et al. (11). Bacterial suspensions were adsorbed on Formvar-coated copper grids that were previously glow discharged. The grids were then incubated for 30 min either with or without 0.125 μg of moxalactam (Eli Lilly & Co., Indianapolis, Ind.) per ml. Preliminary studies of bacterial cells incubated with moxalactam for variable lengths of time (15 to 60 min) and in an Eppendorf tube rather than affixed to the grid yielded similar results. The grids were washed with Tris-buffered saline (TBS) with 2% gelatin and incubated with either M1B1 or M5B12, both in TBS, for 20 min. After three washes with TBS–2% gelatin, a solution of goat anti-mouse immunoglobulin G bound to colloidal gold (Jannsen Life Sciences Products Division, Beerse, Belgium) in TBS was placed on the grid for 20 min. After three additional washes with TBS-2% gelatin, the grids were negatively stained with 2% phosphotungstic acid. All grids were reviewed with a Philips 301 electron microscope (Philips Electronic Instruments, Mahwah, N.J.). The bacterial suspension was centrifuged at 800 × g for 4 min to obtain sections of bacterial cells. The pellet was incubated with reagents for the bacteria-coated grids, as described above. After the final wash with TBS-2% gelatin, the sample was fixed with 2% glutaraldehyde in 0.1 M cacodylate buffer, postfixed with 1% osmium tetroxide in 0.1 M cacodylate buffer, stained en bloc with 1% uranyl acetate in 50% ethanol, dehydrated in graded ethanol (70% to absolute), and embedded in Spurr resin (Ladd Research Industries, Inc., Burlington, Vt.). Sections were also reviewed with a Philips 301 electron microscope.

Representative *E. coli* J5 and *E. coli* K1:O7 cells are depicted in Fig. 1. Monoclonal antibody M1B1 bound readily to the J5 cell (Fig. 1A), but there was no binding to the intact K1:O7 bacterial cell (Fig. 1B). After treatment with moxalactam, bacterial cell membranes appeared to be disrupted, with increased internal staining, and there was extrusion of cell wall material from the bacterium. Gold-labeled antibody was visualized while bound to both the remaining intact portions of the cell membrane and the extruding contents of the J5 cell (Fig. 2A). With the K1:O7 cell, there was little binding to the intact bacterial cell membrane, but binding was markedly increased between the gold-labeled antibody and the extruding K1:O7 cell contents (Fig. 2B and C). This

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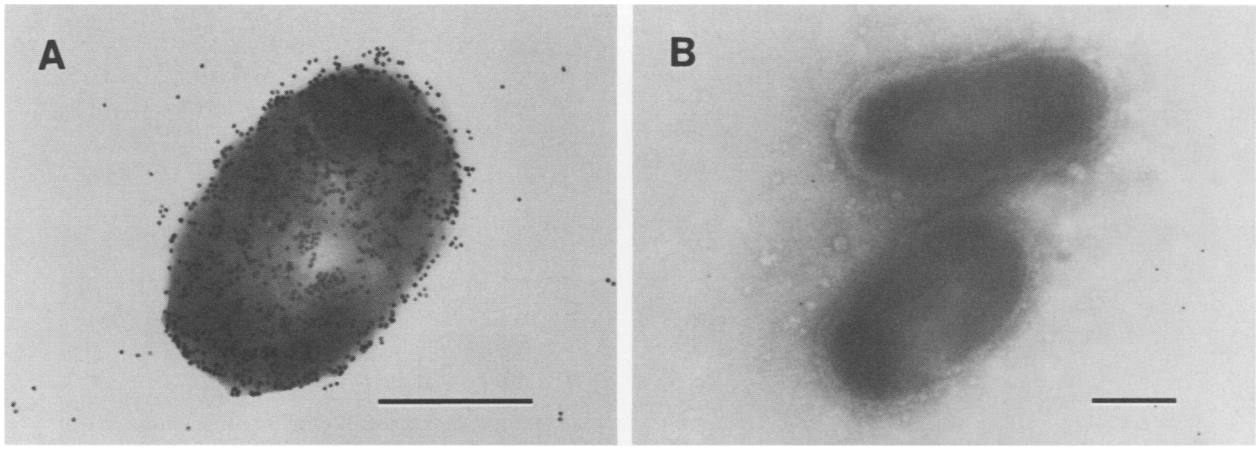

FIG. 1. *E. coli* J5 (A) and K1:O7 (B) incubated with monoclonal antibody M1B1 in the absence of antibiotic. The CGL region of intact *E. coli* K1:O7 is not accessible to antibody, in contrast to the accessibility of the CGL of intact *E. coli* J5. Bars, 0.5 μm.

finding is also evident on a cross section of *E. coli* K1:O7 incubated with moxalactam (Fig. 2D).

When each cell type was incubated with the irrelevant monoclonal antibody, M5B12, either with (Fig. 3A and B) or without (data not shown) moxalactam, no significant antibody binding was visualized. Thus, neither monoclonal antibody nor the label binds nonspecifically to intact cell membranes or extruded cell material.

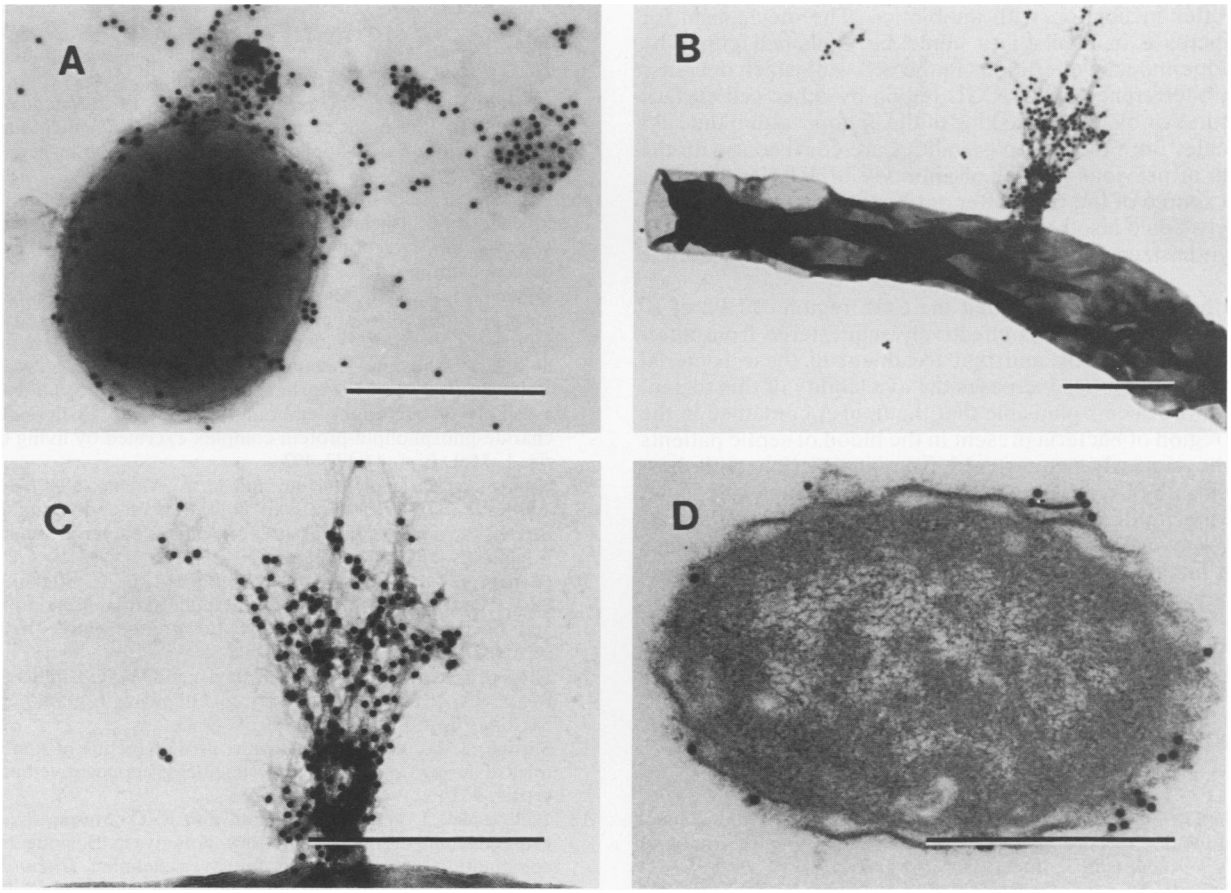

FIG. 2. *E. coli* J5 (A) and K1:O7 (B, C, and D) cells exposed to moxalactam and incubated with monoclonal antibody M1B1. Increased internal staining and breaks in the outer cell membrane were present after antibiotic exposure in both strains. (C) Detailed view of the cell wall pictured in panel B, demonstrating gold-labeled antibody bound to escaping cellular material. (D) Cross section of *E. coli* K1:O7 demonstrating irregularities in the cell membrane, with gold beads adhering to the material escaping from the cell. Bars, 0.5 μm.

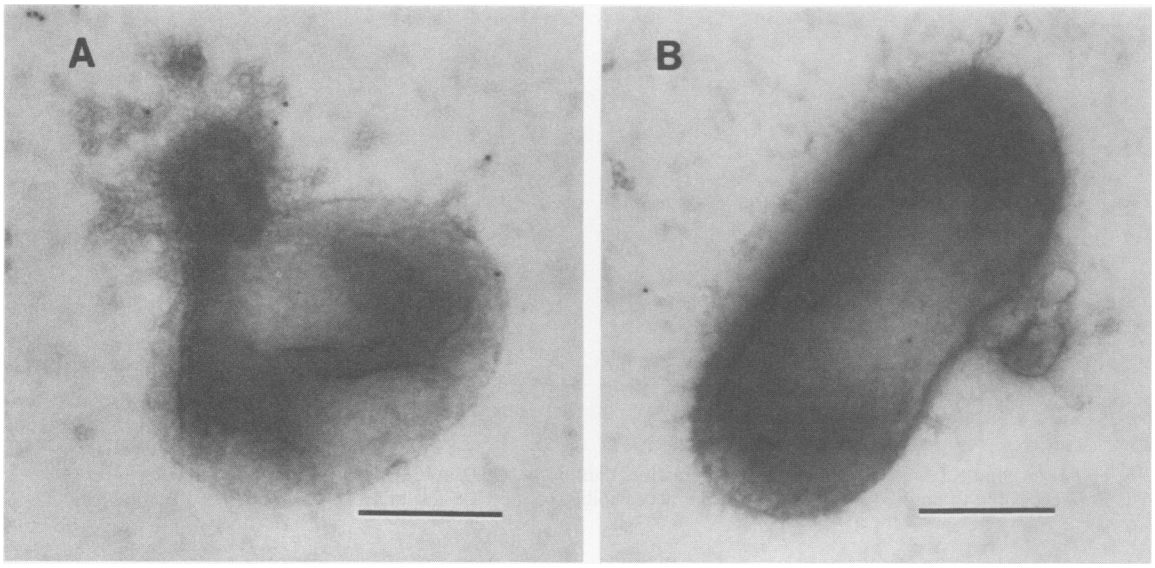

FIG. 3. *E. coli* J5 (A) and K1:O7 (B) exposed to moxalactam and incubated with an irrelevant monoclonal antibody, M5B12. Binding of labeled antibody is not seen with an irrelevant antibody, confirming the specificity of the labeled antibody to CGL. Bars, 0.5 μm.

The binding of anti-CGL antibody to antibiotic-treated *E. coli* K1:O7 cells but not to intact cells suggests an increase in the availability of the corresponding CGL epitope of K1:O7 LPS after incubation with antibiotics. The mechanism for this increase in availability could be explained either by antibiotic-induced alterations in the cell wall which decrease steric interference in the CGL region by other cell surface structures or by the unmasking of the epitope within the LPS molecule. Both of these possibilities are consistent with the results of previous studies of antibody M1B1 (1). An additional source of the CGL after antibiotic treatment of bacterial cells could also be the release of LPS precursors as they are synthesized and translocated to the outer cell membrane (5–8).

We have demonstrated that the CGL region of LPS of *E. coli* K1:O7 strain C94 is effectively sequestered from interaction with antibody and that treatment of these bacterial cells with antibiotics increases the availability of this region. It therefore seems plausible that the lipid A contained in the CGL region of bacteria present in the blood of septic patients may be similarly sequestered from interaction with host elements so that the full toxicity of the LPS molecule is not expressed until after liberation by the action of antibiotics. Further investigation is needed to determine the significance of our findings in reference to the antibiotic-treated patients with gram-negative bacterial sepsis.

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