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Lipopolysaccharide Structures in *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and *Vibrio cholerae* Are Immunologically Related to *Campylobacter* spp.

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To determine whether lipopolysaccharide (LPS) structures of *Campylobacter* species are immunologically related to those of 11 other gram-negative organisms, we immunoblotted from polyacrylamide gels the LPS of these strains with immune rabbit serum raised against six *Campylobacter jejuni* strains and two *Campylobacter fetus* strains. The LPS studied were from *Salmonella minnesota* wild type and Ra to Re mutants, *Salmonella typhi*, *Escherichia coli*, *Yersinia enterocolitica*, *Vibrio cholerae*, and *Pseudomonas aeruginosa*. None of the 11 LPS preparations was recognized by the eight antisera, but antisera to each of the *Campylobacter* strains recognized core determinants of some LPS preparations. Antiserum directed against the most serum-sensitive *C. jejuni* strain, 79-193, was the only antiserum sample that recognized core regions of the rough *Salmonella* mutants. In converse experiments, when LPS preparations from five *Campylobacter* strains were blotted with antiserum to *Salmonella* lipid A, recognition of core structures of each was shown; data from an enzyme-linked immunosorbent assay confirmed this result. In contrast, antiserum to *Salmonella typhimurium* Re LPS showed no reactivity. We conclude that LPS of *Campylobacter* strains share lipid A antigenic determinants with the core region of LPS of several other gram-negative organisms.

The lipopolysaccharide (LPS) of gram-negative organisms consists of the toxic lipid A moiety that is attached proximally to the bacterial cell envelope (12), a central core polysaccharide, and distal O-antigen polysaccharide groups which are highly diverse in composition and thereby in serologic specificity (14). The core polysaccharide is less variable, as evidenced by a limited number of distinct rough mutants and by being similar in large groups of bacteria such as members of the family *Enterobacteriaceae* (12, 15). Antibodies to core structures, prepared by immunization with rough mutant strains, interact with a variety of gram-negative bacteria (6), indicating that the core antigens are surface exposed in the intact cell (8). Lipid A, a common constituent of the LPS of gram-negative bacteria, is similar in structure and composition among *Enterobacteriaceae* and other families of bacteria (10, 12). Consequently, antibodies to lipid A exhibit wide serologic cross-reactions (7, 9, 16).

Recently, we have shown that LPS from most *Campylobacter jejuni* strains share antigenic determinants in the low-molecular-weight core region, as do *C. fetus* LPS preparations, but there is no interspecies cross-reactivity (19). Since LPS preparations from both species contain core antigens and gel *Limulus* amoebocyte lysates (18), we questioned whether immune sera against *Campylobacter* whole cells would be able to recognize LPS determinants from other gram-negative bacteria and whether lipid A antigenic determinants cross-react.

MATERIALS AND METHODS

Bacterial strains and antigen preparations. Characteristics of the *Campylobacter* strains used in this study have been described previously (18). Susceptibility to the bactericidal activity present in normal human serum was determined in a standard assay as described previously (5). The LPS preparations from wild-type *Salmonella minnesota*, *S. minnesota* Ra to Re chemotypes, *Salmonella typhi*, *Escherichia coli*, *Yersinia enterocolitica*, *Vibrio cholerae*, and *Pseudomonas aeruginosa* were obtained from List Biological Laboratories, Campbell, Calif. LPS was prepared from *C. jejuni* 79-193 and PEN1 and *C. fetus* 81-170 by the procedure described by Galanos et al. (8) and from *C. fetus* 82-40 by cold ethanol extraction as described previously (18).

Antisera. Adult New Zealand white rabbits were immunized with Formalin-killed suspensions of 10^8 *Campylobacter* cells per ml from 24-h cultures, and titers of serum were determined as described previously (4, 19). Rabbit antisera against purified *S. typhimurium* $LT_2G30/C21$ mutant Re LPS and lipid A were kindly provided by Charles A. McLaughlin.

Gel electrophoresis methods. Polyacrylamide gel electrophoresis was performed in slab gels by a modification of the system of Laemmli as described previously (2). After electrophoresis, gels were fixed and LPS was resolved with a silver stain as reported by Hitchcock and Brown (11). For immunoblotting we used previously described methods (3, 19).

Enzyme-linked immunosorbent assay. The enzyme-linked immunosorbent assay (ELISA) used in this study was a modification of that described previously (1). In brief, purified LPS from four strains (*S. minnesota* Re 595, *S. minnesota* WT218, *C. jejuni* 79-193, and *C. fetus* 81-170) were dissolved in 0.1% triethylamine (Eastman Kodak Co., Rochester, N.Y.) and used as antigen to coat wells of polystyrene microtiter plates (Flow Laboratories, Inc., Hampden, Conn.) at LPS concentrations ranging from 0.0032 to 10 μg per well. Rabbit antiserum to the *Salmonella* lipid A and

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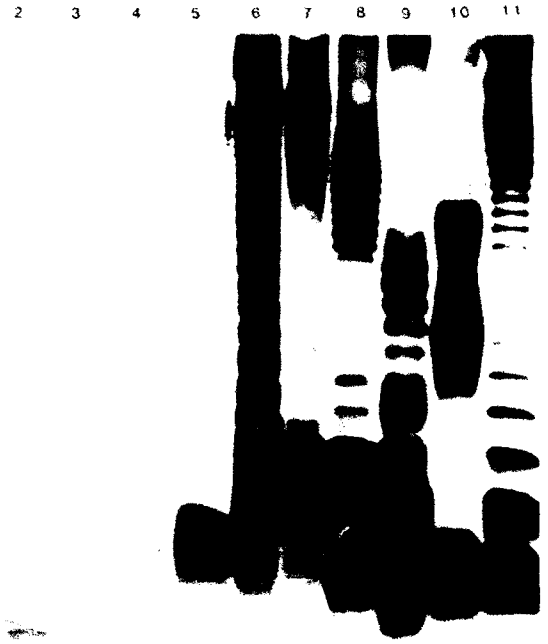

FIG. 1. Silver stain of 15% polyacrylamide gel with purified LPSs. Lanes 1 through 6, LPS from *S. minnesota* Re, Rd, Rc, Rb, Ra, and the wild type, respectively; lane 7, LPS from *S. typhi*; lane 8, LPS from *E. coli* O111; lane 9, LPS from *Y. enterocolitica*; lane 10, LPS from *V. cholerae*; lane 11, LPS from *P. aeruginosa*.

normal rabbit serum were diluted 1:400 in phosphate-buffered saline with 1% bovine serum albumin. A peroxidase conjugate of sheep anti-rabbit immunoglobulin, provided by Thomas Kloppel, was used at a 1:1,000 dilution. All assays were done in triplicate, and results were read on a Titertek Multiscan (Dynatech Laboratories, Inc., Alexandria, Va.) at 410 nm, as described previously (1).

RESULTS

Characterization of LPS preparations. Purified LPS from the 11 non-*Campylobacter* gram-negative organisms was subjected to polyacrylamide gel electrophoresis and visualized by silver staining. The LPS from the five defective rough mutants of *S. minnesota* had a zone of fast-migrating bands (Fig. 1), which represent the core and oligosaccharide regions (11). In contrast, the LPS from six gram-negative pathogens with smooth colonies (wild-type *S. minnesota*, *S. typhi*, *E. coli*, *Y. enterocolitica*, *V. cholerae*, and *P. aeruginosa*) showed classical smooth-type profiles with a fast-migrating core region and multiple higher-molecular-weight complexes. The profiles of each smooth LPS were different in the gel regions containing complete LPS with O-antigen side chains.

Western blots with *Campylobacter* immune serum. In Western blot procedures, the antisera to the *Campylobacter* strains recognized several antigens of the 11 non-*Campylobacter* enteric bacteria. When we used LPS from *S. minnesota* mutants Re to Ra and the wild type as antigens (data not shown), we found that only antiserum to *C. jejuni* 79-193 recognized the core regions. Antisera to other *C. jejuni* or *C. fetus* strains showed minimal recognition of mutant Re to Ra LPS. Several normal rabbit and human serum samples showed minimal or no recognition. Antiserum to *C. jejuni* 79-193 showed essentially no recognition of determinants of smooth LPS from several other gram-negative bacteria (Fig. 2 and 3). In contrast, antisera to *C. jejuni* PEN1, PEN2, and 83-85 and *C. fetus* 81-170 strongly recognized the core LPS region of *S. typhi* (Fig. 2A, lanes 2, 3, 5, and 7); and antisera to *C. fetus* 81-170 and 82-40 recognized a portion of the *S. typhi* LPS with O-antigen side chains. Antisera to *C. jejuni* PEN1 and PEN2 and *C. fetus* 81-170 recognized the core region of LPS from *E. coli* O111 (Fig. 2B, lanes 2, 3, and 7). Antisera to *C. jejuni* PEN1 and 83-85 (Fig. 3, lanes 2 and 5)

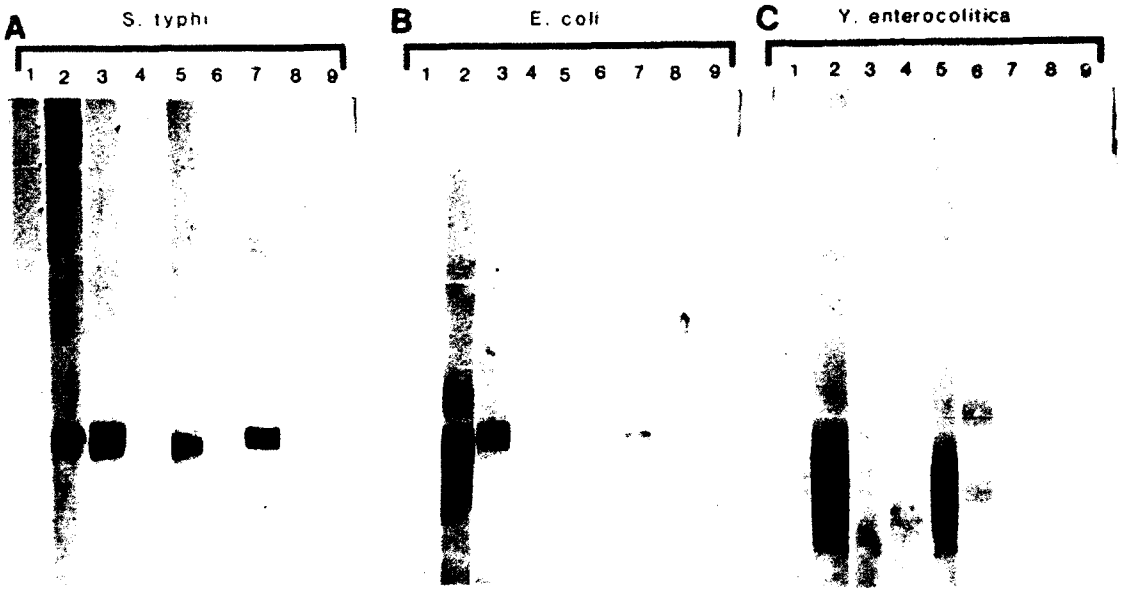

FIG. 2. Western blot of rabbit serum against *S. typhi* (A)-, *E. coli* (B)-, and *Y. enterocolitica* (C)-purified LPS preparations. Sera were from rabbits immunized with *C. jejuni* 79-193 (lanes 1), PEN1 (lanes 2), PEN2 (lanes 3), PEN3 (lanes 4), 83-85 (lanes 5), and 84-19 (lanes 6); *C. fetus* 81-170 (lanes 7) and 82-40 (lanes 8), and unimmunized rabbits (lanes 9). All sera were diluted 1:100.

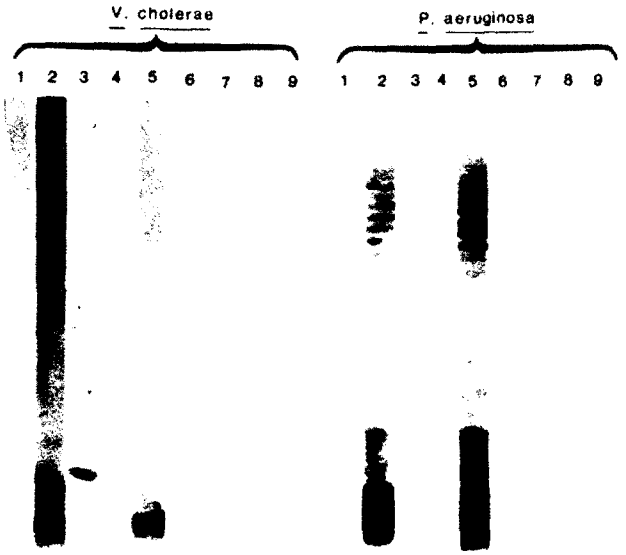

FIG. 3. Western blot of rabbit serum against *V. cholerae* (A)- and *P. aeruginosa* (B)-purified LPS preparations. Serum samples in each lane are as described in the legend to Fig. 2.

recognized core structures of LPS from *V. cholerae* and *P. aeruginosa* and complete LPS of *P. aeruginosa*, whereas antisera to *C. jejuni* PEN2 and *C. fetus* 81-170 recognized only some of the core regions (Fig. 3, lanes 3 and 7). Although all the LPS preparations used in this study had protein contaminations ranging from only 0.2 to 2.9% (dry weight), the higher-molecular-weight bands (Fig. 2A, lanes 7 and 8) could be proteins rather than LPS constituents.

Western blots with immune sera to *S. minnesota* Re and lipid A. We next wanted to determine whether the recognition detected was because the antisera to *Campylobacter* species reacted with the oligosaccharide portion or the lipid A portion of the core region. For this study, we used purified LPS preparations from seven *Salmonella*, five *Campylobacter*, and four other gram-negative organisms (Fig. 4). Antiserum to purified *Salmonella* lipid A recognized core regions of the Re mutant strain and, to various degrees, core regions of all other strains tested (Fig. 4a). When these same antigens were blotted with antisera to the Re mutant of *S. typhimurium*, best recognition was of the LPS from the Re mutant strain, the *S. minnesota* wild-type strain, and *C. jejuni* PEN1 (Fig. 4b). There was virtually no recognition of the *S. minnesota* Rd to Ra mutants, *S. typhi*, all three *C. fetus* strains, and the *V. cholerae* strain. In other studies, core LPS from the *S. minnesota* Ra to Rd mutants was recognized by anti-lipid A serum, although to a lesser degree than that from the Re mutant. These LPS preparations were virtually unrecognized by the antiserum to mutant Re LPS (data not shown).

ELISA. Control wells incubated with normal rabbit serum, no antiserum, no second antibody, or without LPS showed uniformly low-level reactivity in this assay. For the LPS from the wild-type *S. minnesota* strain, reactivity was at the same low level as that of the controls at every concentration (data not shown), indicating that the method used does not result in LPS binding to the polystyrene plate or that anti-lipid A does not react with this LPS on polystyrene

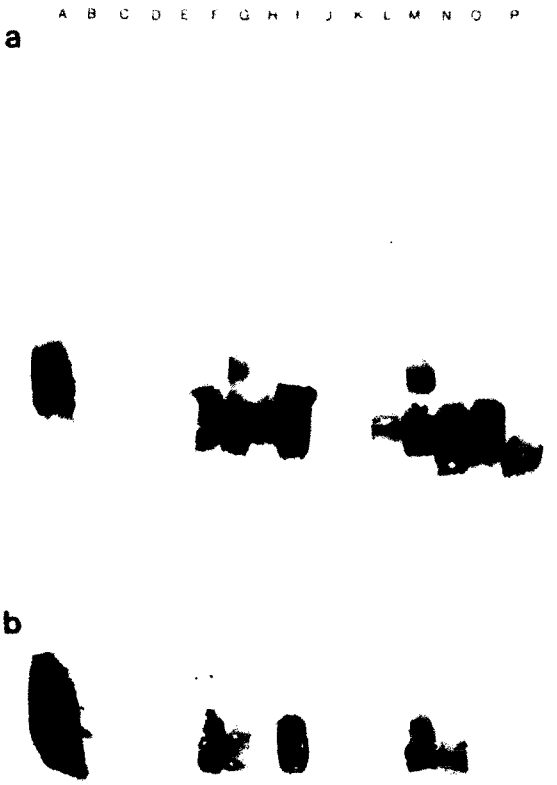

FIG. 4. Western blot of purified LPSs of members of the family *Enterobacteriaceae* and other gram-negative organisms with antisera to purified lipid A from *S. typhimurium* Re (a) or LPS from *S. typhimurium* Re (b). Lanes A through F, LPS from *S. minnesota* Re, Rd, Rc, Rb, Ra, and wild-type, respectively; lane G, LPS from *S. typhi*; lanes H and I, LPS from *C. jejuni* 79-193 and PEN1, respectively; lanes J, K, and L, LPS from *C. fetus* 81-170, 82-40, and 80-109, respectively; lane M, LPS from *E. coli* O111; lane N, LPS from *Y. enterocolitica*; lane O, LPS from *V. cholerae*; lane P, LPS from *P. aeruginosa*.

plates (Charles A. McLaughlin, personal communication). Increasing concentrations of LPS from the *S. minnesota* Re mutant resulted in increasing antibody binding (Fig. 5). The dose response to equal concentrations of LPS from a *C. jejuni* and a *C. fetus* strain were quite similar to that for the *S. minnesota* Re mutant strain.

DISCUSSION

Results of our previous studies showed that *Campylobacter* species possess a core LPS structure (18, 19). By immunoblotting, we found from the results of this study that *C. jejuni* strains share core antigens with several gram-negative strains, a phenomenon recently reported from other gram-negative organisms (7, 17). We found that antiserum directed toward a very serum-sensitive strain, *C. jejuni* 79-193 (5), recognized the core region of *S. minnesota* rough chemotypes Ra to Re, suggesting that there are structural similarities. That other *C. jejuni* or *C. fetus* sera minimally recognized or failed to recognize the rough but heterologous *S. minnesota* LPS preparations agrees with the suggestion that the presence of core oligosaccharides and O-antigen polysaccharides adjacent to the 2-keto-3-deoxyoctulosonic acid di- or trisaccharide shields the core components from

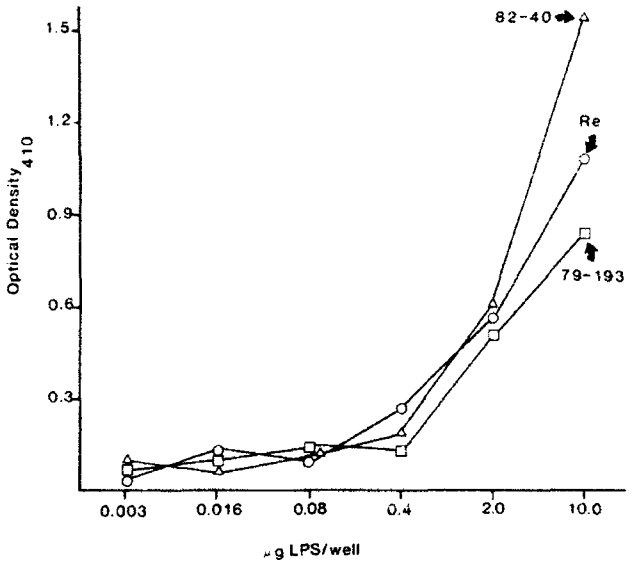

FIG. 5. ELISA of rabbit anti-*Salmonella* lipid A serum with purified LPSs of *Salmonella* and *Campylobacter* strains. LPSs used are from *S. minnesota* mutant Re (○), *C. jejuni* 79-193 (□), and *C. fetus* 82-40 (△). The methods used are as described previously (3) and in the text; each point represents the mean of triplicate determinations. Mean optical density was less than 0.1 for all preparations of LPS from *S. minnesota* WT218 (data not shown).

reacting with antibodies (13) or, as we observed previously, from inducing the formation of antibodies to core structures (19). This hypothesis also correlates with the fact that antibodies to lipid A bind only a relatively small portion of the *Campylobacter* LPS and not the entire ladder of the smooth LPS as observed for *Chlamydia* species (7). Each of the other *Campylobacter* antisera recognized core regions of some of the LPS preparations from other gram-negative organisms, suggesting that there are cross-reacting oligosaccharide regions. The nature of the high-molecular-weight immunoreactive components present in some immunoblots is unknown (7), but they may be aggregated LPS. The purified LPS preparations (*S. minnesota* mutants Ra and Rb) showing the high-molecular-weight bands had low protein concentrations (less than 1.3%), but we cannot rule out the possibility that such contamination is immunoreactive.

Recognition of high-molecular-weight regions in *P. aeruginosa* and *V. cholerae* by *C. jejuni* antisera may represent the uncommon cross-reactions that occur despite the great diversity in O-antigen polysaccharide structure (14). Alternatively, the high-molecular-weight determinants that were recognized may be core antigens that are present in the complete LPS as well as in the low-molecular-weight core region.

That the fast-migrating zones which correspond to core regions of LPS (11) are recognized predominantly by *Campylobacter* antisera and that lipid A is broadly antigenically conserved in gram-negative bacteria (16) favor the hypothesis that the widely shared cross-reactivities observed among LPS from gram-negative organisms are due, in part, to antigenic similarities among the lipid A regions. Results of our Western blot with antiserum to *Salmonella* lipid A confirm that lipid A is a common constituent of the LPS of several gram-negative species and shows antigenic similarity among members of the family *Enterobacteriaceae* and other bacterial families, including members of the genus *Campylobacter*. The ELISA data further show the antigenic similarities of the lipid A regions of a *C. jejuni*, a *C. fetus*, and a *Salmonella* strain. In contrast, we did not find any cross-reactivity with *Campylobacter* LPS when *S. typhimurium* mutant Re antiserum was used. This observation suggests that the relatively small 2-keto-3-deoxyoctulosonic acid trisaccharide or some other yet unidentified moiety or structural configuration present on the mutant Re strain exerts significant impact on the antigenicity of the molecule, concealing the cross-reacting antigenic determinants of lipid A.

The presence of cross-reacting antibodies between *Campylobacter* LPS and enterobacterial lipid A could explain why bactericidal antibodies to *C. jejuni* are present in normal human serum (5) and helps define the core LPS antigens of *Campylobacter* for the purposes of immunodiagnosis and vaccine development.

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