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Serogroup Specificity of *Legionella pneumophila* Is Related to Lipopolysaccharide Characteristics

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We studied the lipopolysaccharide (LPS) of *Legionella pneumophila* and six other *Legionella* species to determine whether strain differences were apparent. The LPS was purified by a cold ethanol extraction procedure, and total carbohydrates represented 10 to 20% of LPS weight. 2-keto-3-deoxyoctonate represented 1 to 13% of the total carbohydrate present in the LPS. By sodium dodecyl sulfate-polyacrylamide gel electrophoresis, all strains except *L. dumoffi* showed smooth-type LPS with multiple high-molecular-weight complexes. Proteinase K-treated, whole-cell lysates showed profiles similar to those of purified LPS. Each serogroup of *L. pneumophila* and each *Legionella* species had a distinct sodium dodecyl sulfate-polyacrylamide gel electrophoresis profile. *L. pneumophila* lipid A is antigenically related to the lipid A of *Enterobacteriaceae*. In immunoblot assays with the LPS of *L. pneumophila* serogroups 1 to 6 as antigens, serogroup-specific immune monkey sera recognized homologous purified LPS, but not the LPS of the five heterologous serogroups. These studies indicate that LPS composition may be a determinant of serogroup specificity as defined by the immunofluorescence-based serogrouping schema for *L. pneumophila* and other *Legionella* species.

The etiologic agents of legionellosis, *Legionella pneumophila* and related species, have a cell envelope that is typical for gram-negative bacteria consisting of a cytoplasmic membrane, a peptidoglycan layer, and an outer membrane (11). The components of the outer membrane lie on the surface of the bacterial cell and thus mediate its interaction with the host. Lipopolysaccharide (LPS) is one of the most important outer membrane constituents and generally consists of three regions: lipid A, core oligosaccharides, and polysaccharide (O) side chains; O side chains are antigenic and highly diverse. It is this portion of the LPS molecule which determines serotype specificity for a wide variety of gram-negative organisms (5, 23).

The antigenic diversity in the genus *Legionella* first became apparent in 1979 when a second serogroup of *L. pneumophila* was isolated (25). To date, a total of nine serogroups of *L. pneumophila* (8) and 22 *Legionella* species have been identified through direct fluorescent-antibody staining (D. J. Brenner, A. G. Steigerwalt, G. W. Gorman, H. W. Wilkinson, W. F. Bibb, M. Hackel, R. L. Tyndall, J. Campbell, and J. C. Feeley, Program Abstr. 24th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 160, 1984). Several investigators have reported the isolation of serogroup-specific antigens for *L. pneumophila* (6, 12, 20, 37), but the exact chemical structure is not yet known. Some of these serogroup-specific antigens have been reported to possess properties similar to LPS, such as cell surface location (9), and chemical similarities (20). Endotoxin-like activity also has been described in *L. pneumophila* (13, 30, 36) which was similar but not identical to classical endotoxin, and recently the presence of LPS in *L. pneumophila* has been confirmed. Gabay and Horwitz (14) extracted LPS from the Philadelphia 1 strain and found that it was the major antigen recognized by patient sera in the indirect fluorescent-antibody assay.

In this report, we describe the characterization of LPS in *L. pneumophila* and other *Legionella* species and show its relationship to serogroup specificity.

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MATERIALS AND METHODS

Bacterial strains. The *Legionella* sp. strains used in this study were from the culture collection of the Denver Veterans Administration Medical Center microbiology laboratory or obtained from the Centers for Disease Control, Atlanta, Ga., or the Pittsburgh Veterans Administration Medical Center, Pittsburgh, Pa. The strains used are listed in Table 1; all had been identified according to standard criteria (4, 10). Most strains had been passaged multiple times on artificial media before storage at −70°C in brucella broth containing 15% glycerol. Working stocks of strains were obtained by culturing the freezer stock on buffered charcoal-yeast extract agar plates with 0.1% alpha-ketoglutaric acid (Pasco Labs Inc., Wheatridge, Colo.) followed by transfer to the same media. Cultures were incubated in a humidified atmosphere at 37°C for 72 h. Cells from plates were harvested in cold sterile distilled water and centrifuged twice at 3,000 × *g* for 15 min, and the cell pellet was lyophilized.

LPS preparation. The LPS from *L. pneumophila* serogroups 1 to 6 were prepared according to the cold ethanol extraction procedure of Darveau and Hancock (7) with the following modifications. For serogroups 3 to 6, 500 mg of dried bacterial cells was used, but for serogroups 1 and 2 1.0 and 1.5 g, respectively, were used. In brief, dried bacterial cells were suspended in water and then sonicated (Branson Instruments, Danbury, Conn.) for five 30-s bursts at a probe intensity of 75. After nucleic acid and protein digestions, cells were further incubated in Tris-sodium dodecyl sulfate (SDS)-EDTA as specified (7) and vortex mixed, and peptidoglycan was removed by centrifugation at 50,000 × *g* for 30 min. After clarification of the supernatant, LPS was precipitated when 2 volumes of 0.375 M $MgCl_2$ in 95% ethanol were

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TABLE 1. Strains used in this study

Species	Strain designation	Source[a]	Serogroup
L. pneumophila	Philadelphia 1	CDC collection	1
L. pneumophila	Philadelphia 2	CDC collection	1
L. pneumophila	#35	Pittsburgh VA	1
L. pneumophila	JL	Clinical isolate, Denver VA	1
L. pneumophila	Detroit 1	CDC collection	1
L. pneumophila	IJ	Clinical isolate, Denver VA	1
L. pneumophila	3R	Environmental isolate, Denver VA	1
L. pneumophila	Flint 2	CDC collection	1
L. pneumophila	4R	Environmental isolate, Denver VA	1
L. pneumophila	Pontiac 1	CDC collection	1
L. pneumophila	Togus 1	CDC collection	2
L. pneumophila	Bloomington 2	CDC collection	3
L. pneumophila	Los Angeles 1	CDC collection	4
L. pneumophila	Dallas 1E	CDC collection	5
L. pneumophila	MP	Clinical isolate, Denver VA	6
L. gormanii	L_1 10	CDC collection	
L. feelei	W044-C	CDC collection	
L. longbeachae	LB-4	CDC collection	
L. dumoffi	TEX-KL	CDC collection	
L. bozemanii	WIGA	CDC collection	
L. micdadei	#147	Clinical isolate, Pittsburgh VA	
L. micdadei	#38	Clinical isolate, Pittsburgh VA	
L. micdadei	#67	Environmental isolate, Pittsburgh VA	
L. micdadei	#31	Environmental isolate, Pittsburgh VA	

[a] CDC, Centers for Disease Control; VA, Veterans Administration.

added, and the mixture was cooled to 0°C and centrifuged twice at 12,000 × *g* for 15 min at 0°C. After the final extraction was completed, the pellet was suspended in distilled water, dialyzed against distilled water for 24 h to remove salts, and lyophilized. This method worked well for serogroups 3 to 6 of *L. pneumophila*; however, for serogroups 1 and 2, a minimal amount of material was obtained and the starting cell volume had to be increased two- to threefold.

Whole-cell lysates and proteinase K digestion. Whole-cell lysates were treated with proteinase K digestion according to the method of Hitchcock and Brown (18), as previously described (27). Briefly, bacterial cells were solubilized in lysing buffer and then heated to 100°C for 10 min. Protein was digested by the addition of proteinase K (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) and incubated at 60°C for 60 min. Samples were heated to 100°C for 5 min prior to loading 10 μl of the preparations onto SDS-polyacrylamide gels as described below.

Analytical methods. Protein concentrations were measured by using the Markwell et al. modification (24) of the Lowry method. For determination of the 2-keto-3-deoxyoctonate (KDO) concentrations, the thiobarbituric acid method (34) with the modifications described by Keleti and Lederer (22) was used, with 3-deoxyoctulosonic acid ammonium salt (Sigma Chemical Co., St. Louis, Mo.) as the standard. Carbohydrate concentrations were determined by the phenol-sulfuric acid procedure, using glucose as the standard (16).

SDS-PAGE of LPS and immunoblot (Western) procedure. LPS preparations, whole-cell lysates, and LPS standards (List Biological Laboratories, Campbell, Calif.) were analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) in a modified Laemmli gel system (1), as previously described (2, 27). LPS from *Campylobacter jejuni* PEN 1 prepared by the Galanos procedure (15) and from *C. fetus* 82-40 prepared by the cold ethanol extraction of Darveau and Hancock (7) as modified (27) were obtained from Guillermo Perez Perez. Discontinuous SDS-PAGE was done in 1.5-mm-thick slab gels with a 4.5% stacking gel and separating gels ranging from 12 to 15% acrylamide; various acrylamide concentrations were used to optimize resolution. The LPS preparations were suspended in sample buffer containing SDS (5%), bromophenol blue (0.003%), glycerol (20%), dithiothreitol (0.5%), and Tris base (1.57%) at pH 6.8 and heated to 100°C for 5 min. Portions containing 100 to 200 μg of sample were applied to each gel lane. After electrophoresis, gels were fixed and LPS was resolved, using the silver stain of Tsai and Frasch (33) with the modifications of Hitchcock (17) and Hitchcock and Brown (18).

For the Western blot procedure, samples containing (per lane) 260 μg of the purified LPS preparations or 10 μg of protein from whole-cell preparations were mixed with sample buffer and loaded onto 15% acrylamide gels as previously described. Electrophoresis was carried out as described above. After electrophoresis, gels were blotted onto nitrocellulose paper according to the method of Towbin et al. (32), as previously described (28). Briefly, the nitrocellulose paper was incubated with 1% bovine serum albumin at 42°C for 1 h and then incubated overnight at 25°C with a 1:128 dilution of immune serogroup-specific monkey sera (BioDx, Denville, N.J.). Monkeys were immunized by intramuscular injection of heat-killed strains of *L. pneumophila* serogroup 1 (Philadelphia 1), serogroup 2 (Togus 1), serogroup 3 (Bloomington 2), serogroup 4 (Los Angeles 1), serogroup 5 (Dallas 1e), and serogroup 6 (Chicago 2) mixed with complete Freund adjuvant. Subsequent indirect immunofluorescence assays on the antisera showed some cross-reactions among the serogroups, but the heterologous titers were always at least fourfold lower than the homologous titers (3). After washing, each strip was incubated with horseradish peroxidase-conjugated *Staphylococcus aureus* protein A (Amersham Corp., Arlington Heights, Ill.). After washing, the strips were developed with 3′,3′-diaminobenzidine

TABLE 2. Comparative analysis of LPS extracted from *L. pneumophila*[a]

Sero-group	Strain	% of dried cells by wt			Ratio A (μg of KDO/μg of protein)	% of LPS by wt			Ratio B (μg of KDO/μg of protein)	Ratio B/ ratio A	KDO/ CHO
		KDO	Protein	Yield of LPS		KDO	Protein	CHO[b]			
1	Philadelphia 1	0.33	57.5	0.4	0.0057	0.2	7.8	20.0	0.03	4.4	0.01
2	Togus 1	0.32	47	1.3	0.0068	0.11	5.3	10.2	0.21	30.9	0.01
3	Bloomington 2	0.26	65	3.7	0.0040	0.3	2.4	16.6	0.13	32.5	0.02
4	Los Angeles 1	0.29	49.5	4.4	0.0058	1.65	6.3	13.2	0.26	44.1	0.13
5	Dallas 1E	0.19	58	2.0	0.0033	0.68	2.6	12.8	0.26	78.8	0.05
6	Denver VA,[c] MP	0.20	71.6	0.8	0.0028	0.75	1.0	13.6	0.75	267.9	0.06

[a] All values shown are the means of at least two separate determinations.
[b] CHO, Total carbohydrate.
[c] VA, Veterans Administration.

(Sigma). Because of the potential for carcinogenicity of this compound, gloves were worn during this step. Rabbit immune serum to lipid A extracted from the Re mutant of *Salmonella typhimurium*, kindly provided by Charles McLaughlin, was used in other immunoblot analyses.

RESULTS

Yield of LPS. We attempted to purify LPS from six strains of *L. pneumophila* representing serogroups 1 to 6. Both the hot phenol-water (35) and the phenol-chloroform-ether (15) procedures for extraction of LPS were used on the Philadelphia 1 strain without success. Thus, we used the cold ethanol extraction procedure of Darveau and Hancock (7) with minor modifications to obtain LPS from each of the six *L. pneumophila* strains. The yield of LPS obtained from the six serogroups ranged from 0.4 to 4.4% of the dried cell weight (Table 2), similar to yields reported for other strains (7, 27). However, LPS yields ranged from 0.008 to 0.726 μg of KDO per mg of dry cell weight, with lowest results for the difficult-to-extract serogroup 1 and 2 strains.

Chemical composition of LPS preparations. The chemical composition of the purified LPS preparations obtained is shown in Table 2. Only one LPS preparation from each of the six serogroups was made, but each value represents the mean of at least two separate determinations. Protein contamination of preparations ranged from 1.0 to 7.8% (wt/wt). In all six strains we observed an increase in the ratio of KDO to protein in the LPS preparations compared with original values for the whole cells of 4- to 268-fold (ratio B/ratio A); lowest ratios were for the difficult-to-extract serogroup 1 and 2 LPS. The percentage of KDO in LPS was increased when compared with that in dried cells only for serogroups 4 to 6. The proportion of LPS represented by the carbohydrate fraction ranged from 10.2 to 20%. The proportion of the total carbohydrate represented by KDO ranged from 1 to 13%, suggesting that the LPS was the smooth type with multiple polysaccharide repeating units. In contrast, for the deep rough (Re) *Salmonella* sp. mutant KDO represented 99% of measured carbohydrate (data not shown).

Characterization of LPS. Extracted LPS was subjected to SDS-PAGE and visualized by the silver staining technique of Hitchcock and Brown (18). The migration characteristics of the LPS from the *L. pneumophila* strains were very distinctive when compared with the LPS of other gram-negative organisms (Fig. 1). Each of the six serogroups of *L. pneumophila* had a distinct profile and each showed a smooth-type LPS profile with multiple complexes. The core region usually could not be visualized on the photographed gels. In this gel, the core regions of most of the other gram-negative organisms were well visualized, whereas those of *L. pneumophila* were not at all. When staining these gels, the *Legionella* sp. core regions could eventually be visualized if left in the developer for more than 30 min, at which time the remainder of the gel developed significant background staining, making observation of the complete profile difficult.

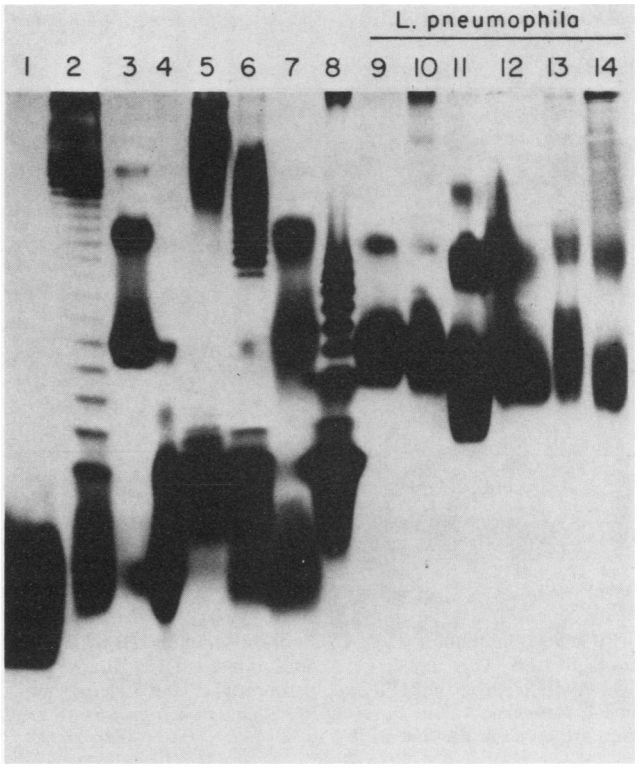

FIG. 1. Silver stain of 15% polyacrylamide gel of LPS of *L. pneumophila* and eight other gram-negative organisms. Lane 1, *S. minnesota* R60 (Ra); lane 2, *Pseudomonas aeruginosa* F-D type 1; lane 3, *C. fetus* 82-40; lane 4, *C. jejuni* PEN 1; lane 5, *S. typhimurium*; lane 6, *E. coli* O111:B4; lane 7, *Vibrio cholerae* Inaba 569B; lane 8, *Yersinia entercolitica*; lane 9, *L. pneumophila* serogroup 6 (strain MP); lane 10, *L. pneumophila* serogroup 5 (strain Dallas 1E); lane 11, *L. pneumophila* serogroup 4 (strain Los Angeles 1); lane 12, *L. pneumophila* serogroup 3 (strain Bloomington 2); lane 13, *L. pneumophila* serogroup 2 (strain Togus 1, proteinase K-treated whole-cell lysate); lane 14, *L. pneumophila* serogroup 1 (strain Philadelphia 1, proteinase K-treated whole-cell lysate).

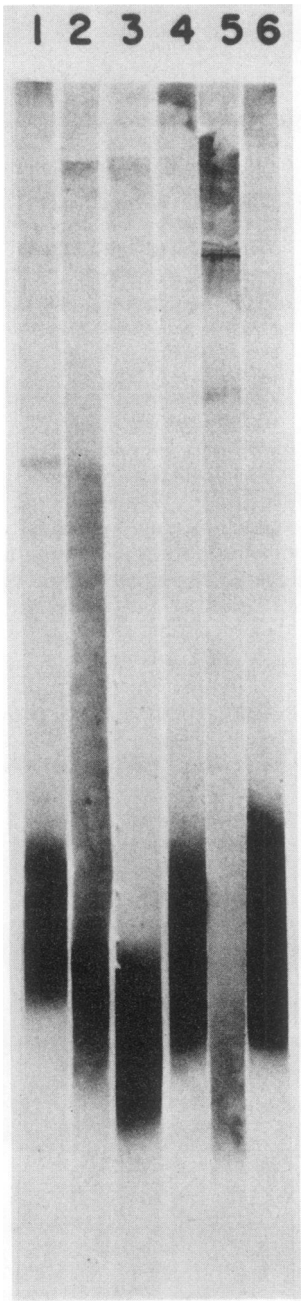

FIG. 2. Immunoblot (from 15% polyacrylamide gel) of *L. pneumophila* LPS with anti-lipid A sera diluted 1:100. Lane 1, *L. pneumophila* serogroup 1; lane 2, serogroup 2; lane 3, serogroup 3; lane 4, serogroup 4; lane 5, serogroup 5; lane 6, serogroup 6. Strains used are given in the legend to Fig. 1.

Low-molecular-weight cores were present, however, and could be resolved near the bottom of similar 15% polyacrylamide gels through immunoblots of *L. pneumophila* LPS with antiserum to *Salmonella* sp. lipid A (Fig. 2).

SDS-PAGE of proteinase K-treated whole-cell lysates. Whole-cell lysates treated with proteinase K also were used to assess the SDS-PAGE profiles of LPS from each of the six serogroups of *L. pneumophila* (Fig. 3). The profiles of LPS obtained from proteinase K-treated whole-cell lysates were similar to those from the purified LPS, but with better resolution. The washboard appearance of the high-molecular-weight complexes were more apparent than with the purified LPS (Fig. 1). Rather than a homogeneous distribution of complete LPS-complexes of varying molecular weights as illustrated by *S. minnesota* (see Fig. 4, lane a), LPS complexes were not evenly distributed by weight. For example, for serogroup 6 (Fig. 3, lane a), the distribution of complexes falls into nine different zones. LPS profiles of the proteinase K-treated whole-cell lysates of four strains of *L. micdadei* representing both clinical and environmental isolates all showed identical profiles (Fig. 4). Each strain had a smooth-type LPS with a large number of high-molecular-weight complexes similar in distribution to those of wild-type *S. minnesota* (lane A), but distinctly different from those seen in *L. pneumophila* (Fig. 3; and data not shown). Proteinase K-treated whole-cell lysate profiles from five other *Legionella* species are shown in Fig. 5. Each species had a very distinctive profile, each showing smooth-type LPS with high-molecular-weight complexes, with the exception of *L. dumoffi* (Fig. 5, lane b).

Immunoblot of proteinase K-treated whole-cell lysates. Im-

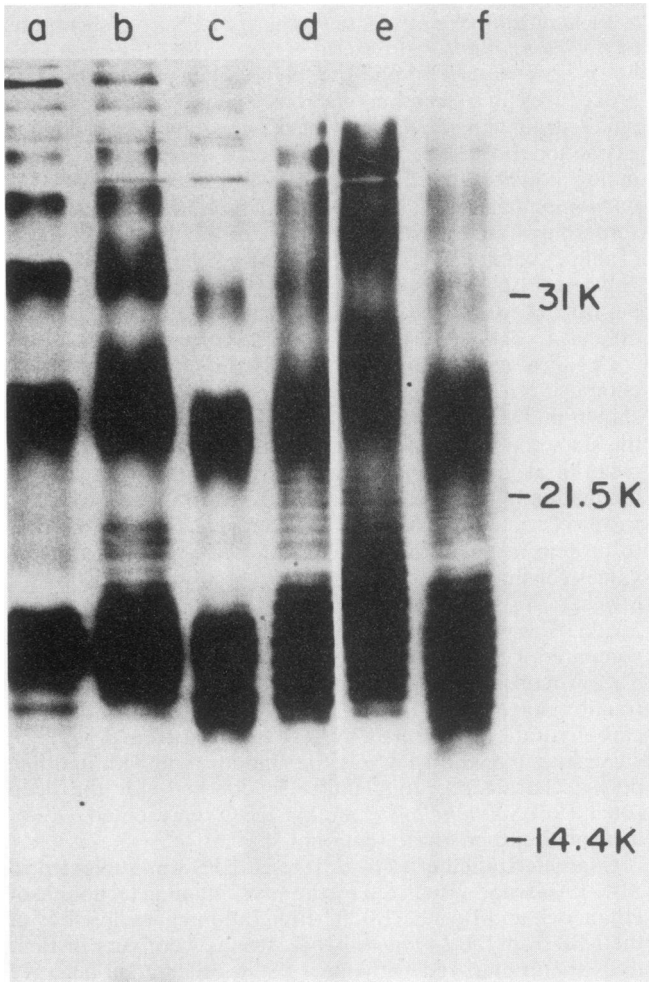

FIG. 3. Silver stain of 15% polyacrylamide gel with proteinase K-treated whole-cell lysates of *L. pneumophila*. Lane a, Serogroup 6; lane b, serogroup 5; lane c, serogroup 4; lane d, serogroup 3; lane e, serogroup 2; lane f, serogroup 1. Strains used are given in the legend to Fig. 1.

munoblots of proteinase K-treated whole-cell lysates of *L. pneumophila* serogroups 1 to 6 were done by using serogroup-specific immune monkey sera to determine whether LPS is a determinant in the serogroup-specific antigen as recognized by the direct fluorescent-antibody typing schema which is standard for *Legionella* spp. (Fig. 6). The immune monkey sera recognized homologous LPS, but not the LPS of the five heterologous serogroups. There are some cross-reactions, generally of minor intensity. However, antisera to serogroup 5 recognized some low-molecular-weight antigens of serogroup 6 LPS but the converse reaction was not seen. When these same Western blots were done with whole-cell preparations instead of whole-cell lysates treated with proteinase K, the major antigen resolved still was LPS, but there were many other minor cross-reacting bands, probably representing proteins; no common (species) antigen was seen (data not shown). This differs from the reports of other investigators who found *L. pneumophila* species-specific protein antigen using *L. pneumophila* serogroup 1 (14; E. Pearlman, N. C. Engleberg, and B. I. Eisenstein, Program Abstr. 24th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 158, 1984). However, our results may not be comparable due to differences in both antigen preparation and species of animal in whom the antisera were prepared (29). In addition, we used sera with indirect fluorescent-antibody titers of 1:128 which may have been too low for the specific protein bands to be resolved.

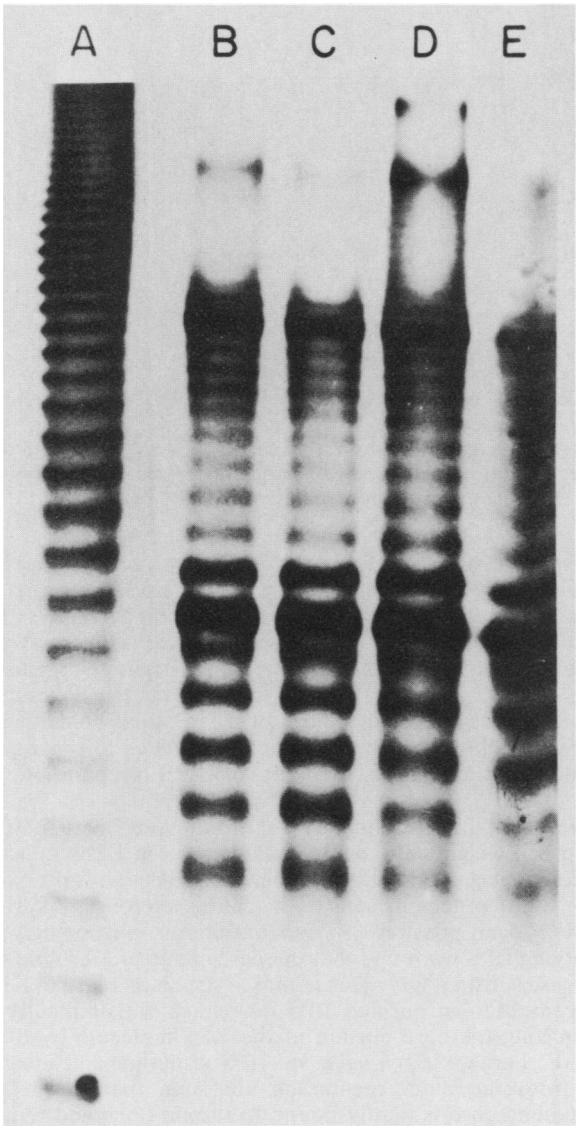

FIG. 4. Silver stain of 12% polyacrylamide gel of proteinase K-treated whole-cell lysates of four strains of *L. micdadei* and LPS of *S. minnesota*. Lane A, *S. minnesota* wild-type strain; lane B, *L. micdadei* #147, lane C, *L. micdadei* #38; lane D, *L. micdadei* #67; lane E, *L. micdadei* #31.

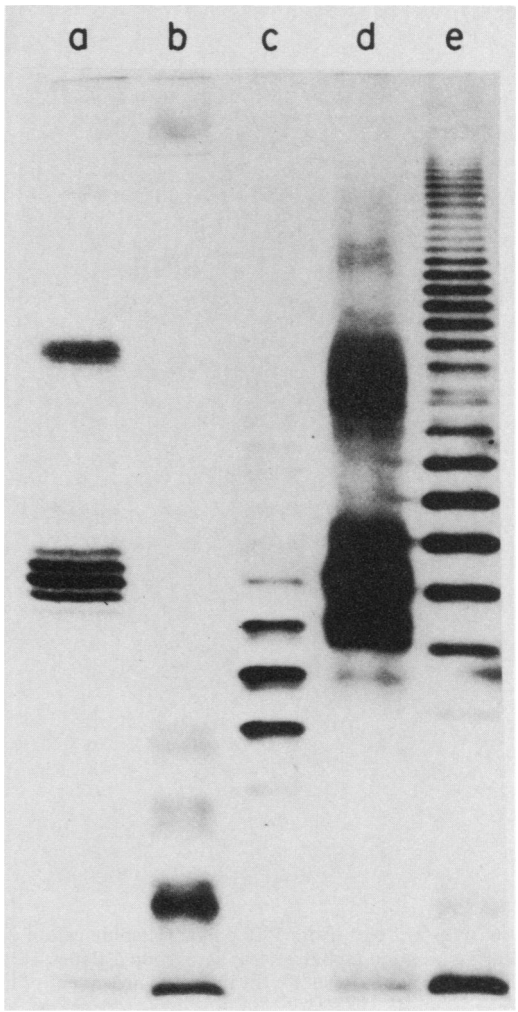

FIG. 5. Silver stain of 15% polyacrylamide gel with proteinase K-treated whole-cell lysates of five *Legionella* species. Lane a, *L. gormanii* (strain L_1 10); lane b, *L. dumoffi* (strain TEX-KL); lane c, *L. feelei* (strain WO44-C); lane d, *L. bozemanii* (strain WIGA); lane 3, *L. longbeachae* (strain LB-4).

DISCUSSION

As expected for gram-negative organisms and as indicated by earlier reports (13, 14, 20, 30, 36), LPS are present in *Legionella* sp. organisms. Wong et al. demonstrated that the LPS of *Legionella* spp. may be different from that of other gram-negative organisms; although *L. pneumophila* endotoxin was active in gelating *Limulus* lysate in vitro, it had low pyrogeneity in rabbits (36). Other in vivo biologic tests, including Shwartzman reaction, actinomycin D potentiation,

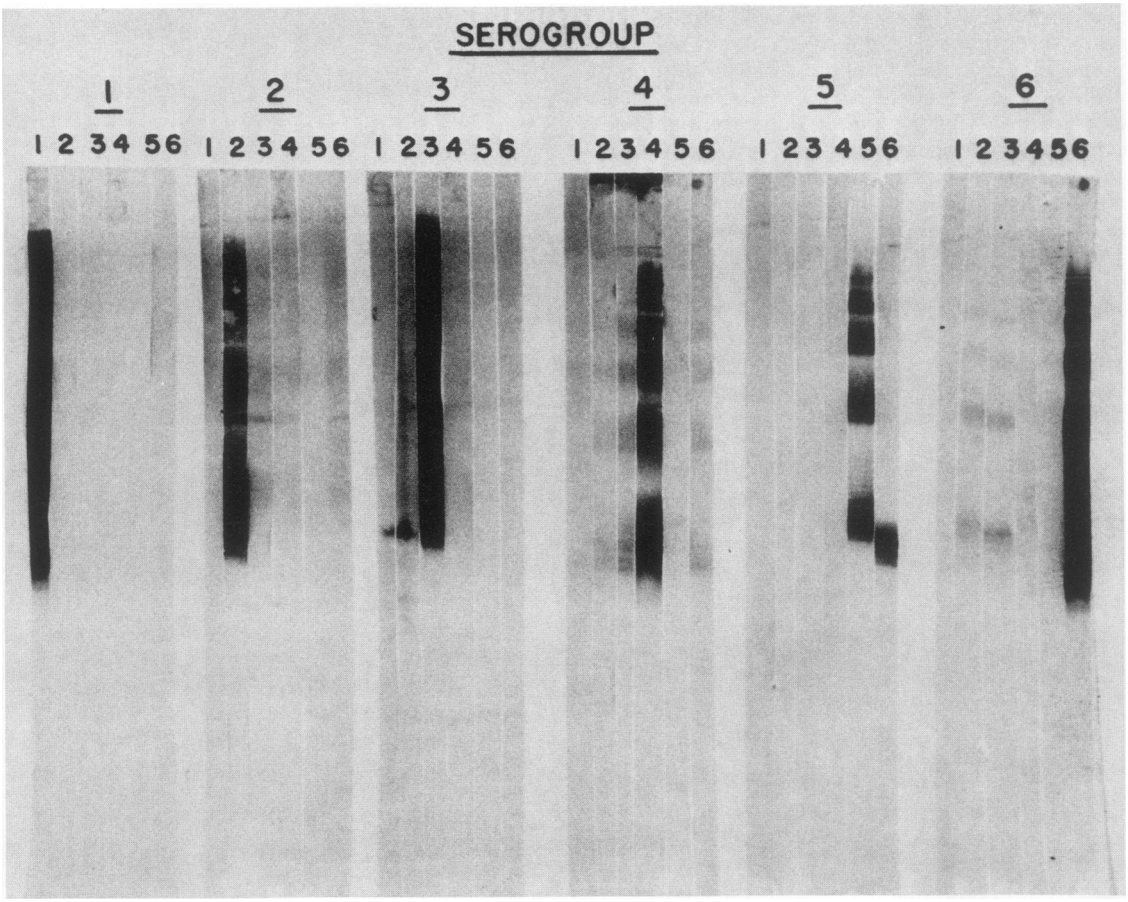

FIG. 6. Western blot (from 15% polyacrylamide gel) of proteinase K-treated whole-cell lysates of *L. pneumophila* serogroups 1 to 6 with the following immune monkey sera: serogroup 1 (lanes 1), serogroup 2 (lanes 2), serogroup 3 (lanes 3), serogroup 4 (lanes 4), serogroup 5 (lanes 5), serogroup 6 (lanes 6). Strains used are given in the legend to Fig. 1. All sera were diluted to a titer of 1:128.

weight gain in mice, and polymyxin B inactivation, indicated a relatively weak endotoxin. Small quantities of KDO (approximately 0.6% of dried cell weight) were present in the two strains tested but not the hydroxy fatty acids that generally are associated with lipid A in the classic *Enterobacteriaceae* endotoxin molecule. Using a phenol extraction method, Schramek et al. (30) isolated from serogroup 1 *L. pneumophila* a lipid A-type material which also showed low lethality for mice. Serologic studies showed that this compound cross-reacted with antisera to lipid A isolated from other gram-negative organisms including *S. minnesota*, *Coxiella burnetti*, and *Chlamydia psittaci*. These results are confirmed by our western blot of the purified LPS with antisera to *Salmonella* sp. lipid A; in each of the six preparations, a strong reaction was seen, suggesting close antigenic similarities between *Enterobacteriaceae* lipid A and the lipid in the core region of the LPS molecule from *L. pneumophila*.

The low LPS yields in this study may have been due to inadequate cell breakage, although the sonication level used should leave <1% of the cells intact (7). Gabay and Horwitz (14), using essentially the same extraction procedure as used in our study but producing higher yields, found that the LPS SDS-PAGE profile of the Philadelphia 1 isolate was different from those of two *Escherichia coli* strains studied. The profile they observed was identical to the one seen in Fig. 1, although their yield of LPS was much higher than ours. These investigators began the extraction procedure with previously prepared cell membrane preparations, while we began with intact bacterial cells, which could account for the increased yield. The differences in PAGE profiles between the *Legionella* sp. strains and other gram-negative organisms (Fig. 1) are unlikely to be due to differences in extraction technique used, since in studies of other strains cold ethanol extractions produced nearly identical profiles to those produced by the classic methods of LPS preparation (7, 27).

We found that KDO made up approximately 0.2 to 0.3% of dried cell weight. The onefold differences in KDO concentration between our study and that of Wong et al. is probably due to differences in sensitivity of the microassay system used (21) and possibly to strain variability. Surprisingly, in purifying LPS we found that the percentage of KDO did not increase substantially. This failure to see an increase in KDO concentration in purified LPS correlates with difficulty in visualizing the core portion of the LPS molecule by SDS-PAGE. Perhaps *Legionella* sp. LPS structure and integration into the outer membrane are such that the KDO-containing core is tightly bound to the proteins and thus is partially lost in the purification procedure. This hypothesis also may explain the inability to obtain higher yields of LPS and may reflect some peculiarities of *L. pneumophila* LPS-protein-branched chain fatty acid outer membrane interactions. *L. pneumophila* contains LPS that is structurally

different by SDS-PAGE from that of a wide variety of gram-negative organisms, as is demonstrated in Fig. 1.

Several investigators have reported the isolation of serogroup-specific, and recently species-specific, antigens from *Legionella* spp., but there is no agreement on the complete chemical structure or composition. Johnson et al. (20) isolated serogroup-specific antigens from *L. pneumophila* serogroups 1 to 4 which were 35% carbohydrate, 2.6% protein, and 1% KDO, which is similar to the values in our purified LPS preparations. This antigen induced a localized Shwartzman reaction and a biphasic fever response in rabbits and has been shown to be located on the bacterial cell surface (9), all of which suggest the presence of an endotoxin-like material. The antigenic activity was believed to reside in the polysaccharide portion of the antigen, which may correspond to the O-side chains on the LPS molecule.

Although the serogroup-specific antigen isolated from outer membrane material by Flesher et al. (12) did not contain KDO, the lipid portion of the preparation contained fatty acids and glucosamine, both of which are found in lipid A. Periodate oxidation destroyed the serologically active site, confirming the polysaccharide nature of the antigen. Collins et al. (6) identified a heat-stable serogroup-specific surface antigen which was highly reactive with *Limulus* amoebocyte lysates and formed a precipitin resembling LPS on crossed immunoelectrophoresis. Although the complete chemical structure of the serogroup- and species-specific antigens of *Legionella* spp. are unknown, the reports by these investigators suggest that some may have similarities to LPS.

Our studies provide further evidence that the serogroup-specific antigen of *L. pneumophila* is contained in the LPS portion of the bacterial cell surface. Each of the six serogroups were shown to possess different LPS profiles, and on immunoblotting, essentially only homologous antisera reacted with the LPS. Our conclusions are limited as we have not used our LPS preparations to absorb the reactivity of the monkey antisera. However, in a recent study, absorption of convalescent sera from patients with serogroup 1 *L. pneumophila* disease with serogroup 1 purified LPS reduced the indirect fluorescent-antibody titer 99.2 to 99.8% (14). Additional evidence includes the distinctive LPS by SDS-PAGE in each of the *Legionella* species studied. Each showed a smooth-type profile with multiple complexes representing complete LPS molecules with repeating O antigenic polysaccharides. That *Legionella* spp. should possess smooth-type LPS correlates with the finding that these strains are serum resistant (19). Smooth-type LPS is a characteristic closely correlated with serum resistance in other gram-negative bacterial genera (31). An exception to this finding was the *L. dumoffi* strain we studied, which did not show high-molecular-weight complexes; however, this strain too was serum resistant (unpublished data).

The silver-stained SDS-PAGE profiles of the purified LPS and the profiles of the proteinase K-treated whole-cell lysates were similar in the six serogroups of *L. pneumophila* we studied. This correlation also has been described in *Salmonella* (17), *Pseudomonas*, *E. coli*, *Haemophilus*, *Neisseria* (26), and *Campylobacter* spp. (27). The finding of additional regions of banding in the higher-molecular-weight region of the proteinase K-treated serogroup 5 whole-cell lysates, but not in the purified LPS, may be due to differences in concentration. The cold ethanol procedure developed by Darveau and Hancock for the extraction of both smooth- and rough-type LPS was useful in purifying the LPS of *L. pneumophila*, although the method was less efficient in our study for serogroups 1 and 2. The hot phenol-water method (35) for the extraction of smooth-type LPS was very inefficient in our one attempt to apply this method for *L. pneumophila* and confirms a previous report (14). This may be due to differences in the LPS of *Legionella* spp. and the *Enterobacteriaceae* already discussed.

Legionnaires disease is a multisystem illness and the clinical manifestations, including high fevers, acute renal deterioration, and septic shock, may be endotoxin mediated. Further investigations into the biologic and biochemical properties of *Legionella* sp. LPS are necessary to further characterize their role in human disease and their possible roles as antigens for disease detection.

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