INFECTION AND IMMUNITY, Mar. 1998, p. 923–926 Vol. 66, No. 3
0019-9567/98/$04.00+0

Structural Properties of Lipopolysaccharides from *Rickettsia typhi* and *Rickettsia prowazekii* and Their Chemical Similarity to the Lipopolysaccharide from *Proteus vulgaris* OX19 Used in the Weil-Felix Test

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Received 20 October 1997/Returned for modification 12 December 1997/Accepted 31 December 1997

The lipopolysaccharides (LPSs) isolated from typhus group (TG) rickettsiae *Rickettsia typhi* and *Rickettsia prowazekii* were characterized by chemical analysis and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by silver staining. LPSs from two species of TG rickettsiae contained glucose, 3-deoxy-D-manno-octulosonic acid, glucosamine, quinovosamine, phosphate, and fatty acids (β-hydroxylmyristic acid and heneicosanoic acid) but not heptose. The O-polysaccharides of these LPSs were composed of glucose, glucosamine, quinovosamine, and phosphorylated hexosamine. Resolution of these LPSs by their apparent molecular masses by SDS-PAGE showed that they have a common ladder-like pattern. Based on the results of chemical composition and SDS-PAGE pattern, we suggest that these LPSs act as group-specific antigens. Furthermore, glucosamine, quinovosamine, and phosphorylated hexosamine were also found in the O-polysaccharide of the LPS from *Proteus vulgaris* OX19 used in the Weil-Felix test, suggesting that they may represent the antigens common to LPSs from TG rickettsiae and *P. vulgaris* OX19.

The typhus group (TG) rickettsiae possess at least two different types of antigens. One type of antigen is sensitive to sodium metaperiodate, resistant to trypsin, stable in 0.2 M NaOH, and thermostable and includes erythrocyte-sensitizing substance (24), lipopolysaccharide (LPS) (26, 27), and OX19-like antigens (20). These antigens appear to be the group-specific antigens of TG rickettsiae. On the other hand, the species-specific antigens of both *Rickettsia typhi* and *Rickettsia prowazekii* are destroyed by incubation at 56°C for 45 min (15), suggesting that they are proteins.

LPS from *Coxiella burnetii* (3, 10, 14, 28, 29), a related species of rickettsia, has been identified and chemically described, but the exact structure of this LPS is unknown. Previously, Amano et al. also reported the chemical properties of the spotted fever group (SFG) rickettsial LPS, which contains 3-deoxy-D-manno-octulosonic acid (KDO), glucosamine, 6-deoxyglucosamine (quinovosamine), ribose, glucose, phosphate, and palmitic acid (2). On the other hand, endotoxic activity analogous to LPS endotoxin was found in *R. prowazekii* by Olitzki et al. (22, 23), and Schramek et al. (26, 27) extracted a hydrophobic LPS-like endotoxin from *R. typhi* and *R. prowazekii*. Smith and Winkler (30) have provided evidence that *R. prowazekii* contains KDO, a marker for LPS. Although these data suggest the presence of LPS in TG rickettsia, the structure of this LPS has remained obscure.

In attempt to ascertain the structure of LPS from TG rickettsiae, we extracted LPSs from *R. typhi* and *R. prowazekii* and analyzed their chemical components in terms of the heterogeneity of their migration on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). In addition, glucose, KDO, fatty acids, glucosamine, quinovosamine, and phosphate were all identified as components of the LPS from TG rickettsiae.

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MATERIALS AND METHODS

Organisms. *R. prowazekii* E and *R. typhi* Wilmington were grown in chicken yolk sacs, purified on Renografin density gradients, treated with formalin, and dialyzed against distilled water for 5 days as described previously (32). The nondialyzable fractions were lyophilized. *Proteus vulgaris* OX2 and OX19 and *Proteus mirabilis* OXK were obtained from the American Type Culture Collection, Rockville, Md. *Proteus* cells were grown in tryptic soy broth, harvested, centrifuged, washed several times, and lyophilized.

LPS preparation. LPSs were extracted from formalin-treated rickettsia and *Proteus* with hot phenol-water as described previously (10). The crude extracts were purified by ultracentrifugation (100,000 × *g*, 15 h). LPSs from the wild type and Re mutant of *Salmonella typhimurium* were purchased from Ribi ImmunoChem, Hamilton, Mont. LPS from *Vibrio cholerae* 569B (INABA) was purchased from Sigma Chemicals, St. Louis, Mo.

Analytical methods. Neutral sugars, heptose, KDO, and total phosphate content were determined by a method described previously (2). Amino acids and amino compounds were analyzed in a Beckman model 121-M amino acid analyzer after hydrolysis of the samples in 6 N HCl at 100°C for 15 h in sealed glass ampoules (9). Fatty acids were analyzed as methyl esters (9) in a Varian Vesta 6000 gas chromatograph equipped with a DB Wax capillary column (30 m; Supelco). Quantitative and qualitative analyses of neutral sugars were achieved after their conversion to alditol acetates as described previously (10). An SP-2330 capillary column (10 m; Supelco) was used for the detection of alditol acetates.

SDS-PAGE. Samples were analyzed by SDS-PAGE as described by Amano et al. (4) but with a 12.5% separating gel. Each sample (2 to 8 μg) was boiled for 5 min in sample buffer and applied to a slot on the gel. Silver staining was as described previously by Hitchcock and Brown (18).

Amino sugars. Quinovosamine and 6-deoxygalactosamine (fucosamine) were generously provided by S. Kaya and Y. Araki of Hokkaido University, Sapporo, Japan (33). 3-Amino-3-deoxyglucose, 6-amino-6-deoxyglucose, 3-amino-3-deoxymannose, 2-amino-2-deoxyallose, 3-amino-3-deoxyallose, and 3-amino-2,3,6-trideoxylyxlose were all obtained from Sigma.

TABLE 1. Chemical composition of LPSs from *R. typhi* and *R. prowazekii*

LPS	Component (nmol/mg)							
	Glucose	Heptose	KDO	Phosphate	Glucosamine	Hexosamine-phosphate	Compound Y	Ethanolamine
R. typhi	950 (4.3)[a]	Trace	223 (1)	709 (3.2)	721 (3.2)	669 (3.0)	1,382 (6.2)	107 (0.5)
R. prowazekii	878 (3.7)	Trace	240 (1)	720 (3.0)	753 (3.1)	663 (2.8)	1,345 (5.6)	92 (0.4)

[a] The values in parentheses are the molarities of the components relative to that of KDO, which is equal to 1.

RESULTS

Isolation and chemical analysis of TG rickettsial LPSs. The yields of *R. typhi* and *R. prowazekii* LPSs from the formalin-treated whole cells (each, 120 mg [dry weight] of purified cells) were 2.3 mg (1.9%) and 1.7 mg (1.4%), respectively. These yields were a little lower than those of LPSs from *Coxiella burnetii* phases I and II (1.9 and 4.3%, respectively) (10) but higher than those of LPSs from SFG rickettsiae (0.9 to 1.3%) (2). The amounts and molar ratios of neutral sugar, KDO, phosphate, total glucosamine, and unknown amino compounds (denoted as compound Y) for *R. typhi* and *R. prowazekii* LPSs were not significantly different, except for the fatty acid contents (Table 1). These data suggest that the structures of both LPSs are very similar or the same. Both LPSs contained only glucose as the neutral sugar, as determined by gas chromatography (data not shown). The molar ratio of glucose, KDO, phosphate, glucosamine, hexosamine-phosphate, and compound Y in both LPSs was approximately 4:1:3:3:3:6. The structures of TG rickettsial LPSs seem to be slightly different from those of enterobacterial LPSs in that the former LPSs contained trace amounts of heptose and small amounts of KDO. On the other hand, *P. vulgaris* OX19 LPS contained glucosamine, hexosamine-phosphate, galactosamine, and compound Y as the amino sugars of its polysaccharide chain (data not shown).

Fatty acid analysis of the TG rickettsial LPSs was carried out by methyl ester derivatization of acid hydrolysates from LPSs. Both *R. typhi* and *R. prowazekii* LPSs consisted of two kinds of fatty acids, namely, β-hydroxymyristic acid ($\beta OHC_{14:0}$) and heneicosanoic acid ($C_{21:0}$), at a molar ratio of 1:1. The $\beta OHC_{14:0}$ component is a constituent of lipid A of the enterobacterial LPSs, while the $C_{21:0}$ component is an uncommon fatty acid that has never before been reported to be a constituent of the LPS fraction of any gram-negative bacteria.

SDS-PAGE pattern of TG rickettsial LPSs. SDS-PAGE patterns of *R. typhi* and *R. prowazekii* LPSs (Fig. 1, lanes 3 and 4, respectively) showed a mixture of ladder structures that resembled those of wild-type *S. typhimurium* LPS (Fig. 1, lane 1) and Re mutant *S. typhimurium* LPS because of the presence of a fast-migrating band (Fig. 1, lane 2). Close examination of the gels revealed that rickettsial LPSs were composed of closely spaced ladder-like bands, the distance between these bands being narrower than that of *Salmonella* LPS, suggesting that the number of saccharide residues of one repeating unit of the TG rickettsial LPSs is smaller than that of *Salmonella* LPSs. Thus, because of the similar migration patterns between *R. typhi* and *R. prowazekii* LPSs, except for the presence of a band at about 14 kDa in *R. typhi* LPS, these LPSs seem to be group-specific antigens.

Characterization of compound Y. When *R. typhi* and *R. prowazekii* LPSs were hydrolyzed in 6 N HCl at 100°C for 15 h, amino compounds including compound Y were detected with an amino acid analyzer (Table 1). *R. typhi* LPS was hydrolyzed in 6 N HCl and dried; after being applied to a Dowex 50Wx4 column, the hydrolysate was eluted stepwise with H_2O, 0.5 N HCl, 1 N HCl, and 2 N HCl solutions and lyophilized, and each eluate was applied to an amino acid analyzer. The major part of compound Y was eluted with 0.5 N HCl along with glucosamine and neutral amino acids, suggesting that compound Y is an amino sugar having one amino group or neutral amino acid. Because the reducing group of the 0.5 N HCl eluate was 365 nmol/0.25 mg of LPS and the contents of glucosamine plus glucosamine phosphate were 205 nmol/0.25 mg of LPS, the remaining reducing group (160 nmol/0.25 mg of LPS) might be due to compound Y if it were an amino sugar (data not shown). Furthermore, when several amino sugars and compound Y were analyzed on an amino acid analyzer, compound Y and quinovosamine showed the same retention times (Table 2). This result was supported by amino acid analysis of an HCl hydrolysate of *V. cholerae* 569B LPS which contains quinovosamine (data not shown) (17, 19, 25).

Separation of polysaccharide from LPS. We tried to separate the polysaccharide portion of *R. typhi* LPS by hydrolysis in 2% acetic acid at 100°C for 2 h. The supernatant fraction (SUP) of the acetic acid hydrolysate was further hydrolyzed with 0.1 N HCl at 100°C for 1 h. The precipitate fraction (PPT) of the acetic acid hydrolysate contained small amounts of glucosamine, hexosamine-phosphate, and quinovosamine in comparison to the amounts in two other fractions (PPT and SUP of HCl hydrolysates) (Table 3). The PPT/SUP ratios of the contents of glucosamine and hexosamine-phosphate of the HCl hydrolysates were about 4:5, while the PPT/SUP ratios of the quinovosamine and ethanolamine contents were approximately 1:9. However, we could not determine whether an amino sugar

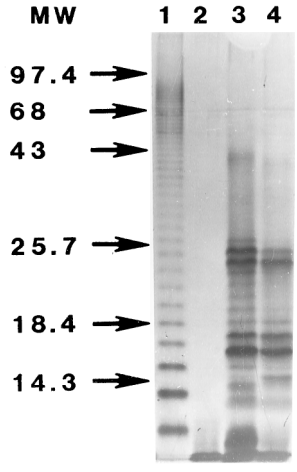

FIG. 1. Comparison of LPSs from TG rickettsiae and *S. typhimurium* by SDS-PAGE with a 12.5% acrylamide gel concentration. After electrophoresis, the gels were stained with silver. Lanes: 1, LPS from *S. typhimurium* wild-type strain LT2; 2, LPS from *S. typhimurium* Re mutant G30/C21; 3, *R. typhi* LPS; 4, *R. prowazekii* LPS. Molecular mass markers: 97.4 kDa, phosphorylase *b*; 68 kDa, bovine serum albumin; 43 kDa, ovalbumin; 25.7 kDa, α-chymotrypsin; 18.4 kDa, β-lactoglobulin; 14.3 kDa, lysozyme.

TABLE 2. Retention times of standard amino sugars and compound Y in the amino acid analyzers[a]

Amino sugar	Retention time[b]	
	121-M	120-B
Compound Y	1.30	
Glucosamine	1.00	1.00
Mannosamine	1.06	
Galactosamine	1.12	
3-Amino-3-deoxyglucose	1.01	
6-Amino-6-deoxyglucose	1.02	
3-Amino-3-deoxymannose	1.11	
2-Amino-2-deoxyallose	1.11	
3-Amino-3-deoxyallose	1.20	
2-Amino-2,6-dideoxymannose (rhamnosamine)		1.22
2-Amino-2,6-dideoxyglucose (quinovosamine)	1.30	1.29
2-Amino-2,6-dideoxygalactose (fucosamine)	1.45	1.48
2-Amino-2,6-dideoxytalose (pneumosamine)		1.72
3-Amino-2,3,6-trideoxylyxose (daunosamine)	1.85	

[a] The amino acid analyzer used in this study was the Beckman model 121-M; the values from using model 120-B were taken from reference 19.
[b] Retention times are relative to that for glucosamine.

in hexosamine-phosphate is glucosamine or quinovosamine because of the small amounts of RT rickettsial LPSs available for further analysis. The results described above indicate at least two important possibilities: (i) that the linkage between the polysaccharide and lipid portions of rickettsial LPS is resistant to 2% acetic acid and (ii) that quinovosamine might be a constituent of the polysaccharide moiety. On the other hand, glucosamine and hexosamine-phosphate may be distributed on both the polysaccharide and lipid moieties. Glucose was detected in the polysaccharide moiety (data not shown).

DISCUSSION

Previously, Amano et al. reported that LPSs from the Japanese and TT-118 strains of SFG rickettsiae contained KDO, glucosamine, quinovosamine, ribose, phosphate, and palmitic acid but neither heptose nor β-hydroxy fatty acid (2) and that the sera from patients infected with Japanese spotted fever reacted with SFG rickettsial LPSs and *P. vulgaris* OX2 LPS (4). Amano et al. further showed that the sera of the Japanese spotted fever patients reacted with the polysaccharide moiety of strain OX2 LPS and with the core saccharide or lipid A moiety of OX19 LPS (1). On the other hand, *Orientia tsutsugamushi* has no LPS (8), while the sera from patients infected with scrub typhus reacted with *P. mirabilis* OXK LPS (7).

TABLE 3. Chemical composition of acid-treated *R. typhi* LPS[a]

Hydrolysate fraction	Component (nmol/fraction)			
	Hexosamine-phosphate	Glucos-amine	Quinovos-amine	Ethanol-amine
PPT of acetic acid treatment	6.1 (8)[b]	11.4 (8)	6.5 (3)	0 (0)
PPT of HCl treatment	29.4 (41)	52.0 (38)	25.9 (10)	2.1 (9)
SUP of HCl treatment	36.8 (51)	73.0 (54)	221 (87)	21.2 (91)

[a] *R. typhi* LPS (0.25 mg) was hydrolyzed with 2% acetic acid at 100°C for 2 h and centrifuged at 1,500 × *g* for 20 min. The supernatant (SUP) of the hydrolysate was subsequently treated with 0.1 N HCl at 100°C for 1 h and centrifuged at 1,500 × *g* for 20 min. Each fraction was hydrolyzed with 4 N HCl at 100°C for 16 h, dried in vacuo, and applied to the amino acid analyzer.
[b] Values in parentheses are percentages representing the amount of each compound relative to the amount of the total contents.

Recently, Amano et al. also reported reactivity between LPSs from TG rickettsiae and from *P. vulgaris* OX19 (5); however, a chemical study of LPSs from TG rickettsiae has never been done.

Previously, Amano et al. and Mizushiri et al. described the chemical compositions of LPSs from *Proteus* strains OX2, OX19, and OXK, all of which are used as antigens for the Weil-Felix test (6, 21). The polysaccharide moiety of strain OX2 LPS contained glucose, glucosamine, and quinovosamine, and the polysaccharide moiety of strain OXK LPS contained glucose, uronic acid, and galactosamine, whereas OX19 LPS seemed to lack the O-polysaccharide. Furthermore, Cedzynski et al. (12) and Swierzko et al. (31) determined the structures of the polysaccharide repeating units of OX2 LPS and OXK LPS, respectively. The polysaccharide repeating unit of OX2 LPS was composed of glucose, *N*-acetylglucosamine, and *N*-acetylquinovosamine in a molar ratio of 1:2:1, and the O-acetyl group was bound to about 70% of *N*-acetylglucosamine. The polysaccharide repeating unit of OXK LPS consisted of glucose, glucuronic acid, galacturonic acid, *N*-acetylgalactosamine, and lysine in a molar ratio of 1:1:1:2:1. More recently, Ziolkowski et al. (34) determined the structure of the repeating unit in the polysaccharide of OX19 LPS, which contains galactose, glucosamine, galactosamine, quinovosamine, and phosphorylated quinovosamine in a molar ratio of 1:1:1:1:1.

In this communication, we have presented the chemical composition of LPSs from *R. typhi* and *R. prowazekii* and demonstrated that they contain glucose, KDO, glucosamine, quinovosamine, phosphate, and fatty acids (β-hydroxymyristic acid and heneicosanoic acid). Heptose, which is a component of enterobacterial LPSs, was present at a very low level in TG rickettsial LPSs. Based on the mild acid hydrolysis of *R. typhi* LPS, quinovosamine was found to be distributed on the polysaccharide moiety of the LPS, while glucosamine and hexosamine-phosphate appear to be components of both the polysaccharide and lipid portions. This LPS was relatively resistant to acid hydrolysis, because after 2% acetic acid hydrolysis, only minor parts of both glucosamines were present in the hydrophobic portion (PPT). This resistance suggests that the linkage between the core saccharide and lipid A moieties in the TG rickettsial LPSs is different from that of the enterobacterial LPSs. Brade et al. (11), and Chaby and Szabo (13) reported that KDO substituted at position C-4 or C-5 was resistant to acid hydrolysis and that liberation of KDO required conditions of at least 1 M HCl at 100°C for 1 h. These reports suggest the presence of C-4- or C-5-substituted KDO between the lipid and saccharide moieties of typhus LPSs. As described by Brade et al. (11), the determination of KDO content was extremely difficult. At this point, we can only say that KDO is present. On the other hand, heneicosanoic acid may link the hydroxy groups of β-hydroxymyristic acid, if lipid A of rickettsial LPSs is similar to enterobacterial lipid A.

Based on the chemical compositions of the polysaccharides of LPSs from the TG rickettsiae and *P. vulgaris* OX19, these two organisms were shown to have glucosamine, quinovosamine, and phosphorylated hexosamine as common components of their LPSs. Although at this time we have no precise knowledge of the nature of the common epitopes, we are continuing to investigate the structures of the epitopes responsible for the common antigenicity of LPSs from TG rickettsiae and *P. vulgaris* OX19.

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Editor: J. T. Barbieri