Heterogeneity and Variation Among \textit{Neisseria meningitidis} Lipopolysaccharides

CHAO-MING TSAI, ROBERT BOYKINS, AND CARL E. FRASCH

Office of Biologics, National Center for Drugs and Biologics, Bethesda, Maryland 20205

Received 28 January 1983/Accepted 14 May 1983

Eight immunotype lipopolysaccharides (LPSs) of \textit{Neisseria meningitidis} were prepared by the phenol-water procedure and characterized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and sugar analyses. By SDS-PAGE and a highly sensitive silver strain, \textit{N. meningitidis} LPSs from cells grown in tryptic soy broth were shown to contain one or two predominant components and a few minor, somewhat higher-molecular-weight components. The molecular sizes of the two predominant components were approximately the same as those of two \textit{E. coli} rough-type LPSs, one with a complete core and the other with an incomplete core. The molecular weight of the major LPS component varied somewhat among different immunotypes but was estimated to be in the range of 4,200 to 5,000. By sugar analyses, the eight immunotype LPSs were different in their monosaccharide compositions. All contained glucose, galactose, heptose, glucosamine, and 2-keto-3-deoxyoctonate, but in different molar ratios. The growth of \textit{N. meningitidis} in tryptic soy broth under different levels of aeration resulted in a change in the two major LPS components seen on the SDS-PAGE gel. High aeration increased the amount of the smaller component, whereas low aeration increased the amount of the larger component. Sugar analyses of LPSs from high and low aeration indicated that the larger LPS component contained more galactose residues per molecule. Use of different media for cell growth may also result in small, but noticeable, variations in the LPS components and in the galactose content of the LPS. The observed heterogeneity of \textit{N. meningitidis} LPS may explain why many strains of \textit{N. meningitidis} appear to possess more than one immunotype.

\textit{Neisseria meningitidis} is classified serologically into serogroups (3), protein serotypes (9, 10), and lipopolysaccharide (LPS) immunotypes (21). Immunotypes, rather than serotypes, will be used for serologically distinct LPS antigens to avoid confusion with outer membrane protein antigens, which have been called serotype antigens (8, 10). There are at least 11 LPS immunotypes in \textit{N. meningitidis} (21, 30, 31). There is no apparent correlation between the occurrence of an LPS immunotype and a polysaccharide serogroup or a protein serotype in \textit{N. meningitidis}, except that immunotypes 10 and 11 have been reported only in serogroup A organisms (31).

Based on the extensive immunochromatographic studies of enterobacterial LPSs (23), the serological specificities of \textit{N. meningitidis} LPS are probably due to structural differences in the carbohydrate chains of the LPS. Jennings et al. (15, 16), reported that the LPS of \textit{N. meningitidis} contains glucose, galactose, \textit{l}-glycerol-D-mannohep-tose (heptose), glucosamine, and 2-keto-3-deoxyoctonate (KDO) as the monosaccharide components. They also suggested that \textit{N. meningitidis} LPS is a rough-type LPS based on the size and composition of the carbohydrate chain obtained after mild acid hydrolysis of the LPS (15).

An increasing amount of evidence suggests that many LPS preparations contain a heterogeneous population of LPS molecules (13, 22, 26, 27). LPS heterogeneity may occur in the lipid A, core, and O side chain regions (12). Recently, more than 40 different size components in the smooth-type LPSs from \textit{Escherichia coli} O111 and \textit{Salmonella typhimurium} were demonstrated by autoradiography after sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (13, 22, 26). Compositional analysis of LPS will be more informative if the amount of each component or of each major component in LPS is known.

The reported chemical analyses of most \textit{N. meningitidis} LPSs are based on individual strains within the different serogroups (1, 15, 16, 19). Different strains within the same serogroup but different LPS immunotypes are expected to have different LPS sugar compositions. There-
fore, sugar composition analyses of LPS based on immunotypes (21, 30) rather than serogroups are required for meaningful immunochemical studies of *N. meningitidis* LPS. In this communication, the LPSs from eight LPS prototype strains and two additional strains were characterized by SDS-PAGE for their size components and by sugar analyses for their monosaccharide compositions. By SDS-PAGE, most LPSs contained two predominant low-molecular-weight components. The monosaccharide compositions of the eight immunotype LPSs were different. Variations in the sugar composition and the LPS components due to the difference in growth conditions were also observed.

**MATERIALS AND METHODS**

Strains and growth conditions. The following LPS prototype strains of *N. meningitidis* (serogroups and immunotypes in parentheses) were examined: 126E (C, L1), 35E (C, L2), 6275 (B, L3), 891 (C, L4), M981 (B, L5), M992 (B, L6), 6155 (B, L7), and M978 (B, L8). Strains M986 (B, L3, L7) and M136 (B, L4) were also used in this study. Strains 126E, 35E, 6275, 891, and 6155 were kindly provided by W. D. Zollinger of the Walter Reed Army Institute of Research, Washington, D.C. All other strains were from the culture collection at the Office of Biologics, National Center for Drug and Biologics, Bethesda, Md. The normal growth conditions of the organisms in tryptic soy broth (TSB; Difco Laboratories, Detroit, Mich.), in modified Frantz medium B (MF-B) (11), in modified Frantz medium as used by the Walter Reed Army Institute of Research (MF-WR) (20, 30), and in Cattin medium (4) have been described previously (28). Briefly, an 8-h culture on brain heart infusion agar (Difco) containing 1% horse serum was heavily suspended in the medium, and 2 ml of the suspension was inoculated into 1.4 liters of medium contained in 2.8-liter baffled Fernbach flasks (Bellco Biological Glassware, Vineland, N.J.). The organisms were grown overnight at 37°C in a gyratory shaker at 130 rpm. The shaker speeds were adjusted to 140 and 90 rpm for high- and low-aeration experiments, respectively. The cells were harvested by centrifugation at 10,000 × g for 15 min.

Isolation of LPS. LPS was isolated from meningococcal cells by the hot phenol-water procedure of Westphal and Jann (29) as modified by Johnson and Perry (17) to include the pretreatment of the cells with lysozyme.

SDS-PAGE. LPSs were analyzed by SDS-PAGE with the Laemmli system (18), incorporating 4 M urea in 15% polyacrylamide slab gels. The running conditions and the method for silver staining LPSs in the gels have been described previously (27).

Monosaccharide analyses. For neutral sugars, samples (2 mg/ml) were hydrolyzed in 0.16 N methanesulfonic acid with 10 mg of Dowex 50 resin at 100°C for 72 h, and monosaccharides in the hydrolysates were quantitated with an automated neutral sugar analyzer as described by Boykins and Liu (2). A typical time course of neutral monosaccharide release from *N. meningitidis* LPS is shown in Fig. 1. Glucose and galactose were almost completely released in 24 h, but heptose required at least 72 h. Samples were routinely taken at 24 and 72 h for the composition analyses. Glucose, galactose, and heptose were stable for 3 days under these hydrolysis conditions. For amino sugars and ethanolamine, samples were hydrolyzed in 2 N methanesulfonic acid at 115°C for 12 h in evacuated tubes. Hexosamines and ethanolamine were quantitated with a Beckman model 121-M Amino Acid Analyzer. Quantitative estimation of KDO in LPS was made by the thioribarbituric acid method of Osborn (24).

KDO, other monosaccharides, and *E. coli* O111:B4 LPS prepared by the phenol-water procedure were obtained from Sigma Chemical Co., St. Louis, Mo. The rough (Re) LPS from *E. coli* J5 (a galE mutant of *E. coli* O111:B4) was obtained from List Biological Laboratories, Campbell, Calif. L-Glycero-d-manno-heptose and the rough (Ra) LPS from *E. coli* PL2 (a galE mutant of *E. coli* K-12 grown in a medium supplemented with galactose) (5) were provided by V. Ginsburg and W. G. Coleman, respectively, of the National Institutes of Health, Bethesda, Md.

RESULTS

Analysis of eight immunotype LPSs by SDS-PAGE. When electrophoresed in polyacrylamide gels in the presence of SDS, the LPS components travel in the gel according to their molecular weights (26, 27). Eight immunotype LPSs from *N. meningitidis* grown in TSB, the smooth-type LPS of *E. coli* O111, and two rough-type LPSs of *E. coli* were analyzed by SDS-PAGE (Fig. 2A). The smooth *E. coli* LPS had over 30 orderly spaced components repre-
FIG. 2. SDS-PAGE of eight immunotype LPSs from *N. meningitidis*. Lanes 1 through 3 contain *E. coli* smooth and rough LPS; lane 1, *E. coli* O111; lane 2, *E. coli* PL2; and lane 3, *E. coli* J5. Lanes 4 through 11 contain eight immunotype LPSs of *N. meningitidis* (immunotypes in parentheses): lane 4, 126E (L1); lane 5, 35E (L2); lane 6, 6275 (L3); lane 7, 891 (L4); lane 8, M981 (L5); lane 9, M992 (L6); lane 10, 6155 (L7), and lane 11, M978 (L8). The sample load for each LPS, except for 5 μg for *E. coli* O111 smooth LPS, was 0.2 μg for (A) and 1 μg for (B).

senting from 0 up to 30 or more repeating units in the O side chains (13). In contrast, the two *E. coli* rough LPSs had a single fast-moving component. Most of the meningococcal LPSs contained two predominant components. Doublets in the LPS components were also occasionally observed. The LPSs of 126E (type L1) and M992 (type L6) appeared to contain one predominant component; however, the former split into two closely spaced components when less sample was loaded on the gel. The larger predominant component of an LPS was either similar or slightly larger in size than the *E. coli* Ra-type LPS, which has a complete core (Fig. 2A, lane 2). The smaller predominant component appeared to be slightly larger than the *E. coli* Rc-type LPS, which has an incomplete core terminated at glucose (7). The different mobilities on SDS-PAGE of the two predominant components of an LPS are probably due to the difference in the carbohydrate chain lengths of these two components. The molecular weight of the major LPS component varied somewhat among different immunotypes, but was estimated to be in the range of 4,200 to 5,000 (27). With a higher sample load (Fig. 2B), in addition to the predominant components each LPS had two or three minor higher-molecular-weight components which were not protein contaminants because they were not affected by the pretreatment of the LPS with proteinase K (14). These minor components accounted for approximately 3 to 5% of the LPS.

**Monosaccharide composition of eight immunotype LPSs.** Eight *N. meningitidis* prototype strains with different LPS immunotypes were grown in TSB. The monosaccharide compositions in mole ratios of the eight immunotype LPSs are presented in Table 1. The mole ratios were calculated based on two heptose residues per LPS molecule (15). All contained galactose, glucose, heptose, glucosamine, and KDO, and

<table>
<thead>
<tr>
<th>LPS immunotype</th>
<th>Prototype strain</th>
<th>Sero-group</th>
<th>Gal</th>
<th>Glc</th>
<th>Hep</th>
<th>GlcNH₂</th>
<th>KDO*</th>
<th>EtNH₂*</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>126E</td>
<td>C</td>
<td>2.8</td>
<td>1.7</td>
<td>2.0</td>
<td>3.7</td>
<td>0.9</td>
<td>2.6</td>
</tr>
<tr>
<td>L2</td>
<td>35E</td>
<td>C</td>
<td>1.9</td>
<td>2.6</td>
<td>2.0</td>
<td>3.2</td>
<td>1.1</td>
<td>1.6</td>
</tr>
<tr>
<td>L3</td>
<td>6275</td>
<td>B</td>
<td>2.9</td>
<td>2.0</td>
<td>2.0</td>
<td>4.3</td>
<td>0.9</td>
<td>3.2</td>
</tr>
<tr>
<td>L4</td>
<td>891</td>
<td>C</td>
<td>3.1</td>
<td>2.0</td>
<td>2.0</td>
<td>2.8</td>
<td>1.0</td>
<td>1.9</td>
</tr>
<tr>
<td>L5</td>
<td>M981</td>
<td>B</td>
<td>0.7</td>
<td>3.8</td>
<td>2.0</td>
<td>2.1</td>
<td>0.9</td>
<td>0.7</td>
</tr>
<tr>
<td>L6</td>
<td>M992</td>
<td>B</td>
<td>1.9</td>
<td>1.8</td>
<td>2.0</td>
<td>6.1</td>
<td>1.0</td>
<td>2.9</td>
</tr>
<tr>
<td>L7</td>
<td>6155</td>
<td>B</td>
<td>2.3</td>
<td>1.7</td>
<td>2.0</td>
<td>2.8</td>
<td>1.0</td>
<td>1.4</td>
</tr>
<tr>
<td>L8</td>
<td>M978</td>
<td>B</td>
<td>1.3</td>
<td>2.1</td>
<td>2.0</td>
<td>2.8</td>
<td>1.1</td>
<td>1.7</td>
</tr>
</tbody>
</table>

*Gal, Galactose; Glc, glucose; Hep, heptose; GlcNH₂, glucosamine; KDO, 2-keto-3-deoxyoctonate; EtNH₂, ethanolamine.

*KDO was quantitated by the thiobarbituric acid method, which will not detect KDO residue when its C-4 or C-5 position is linked to another LPS component.

*Ethanolamine was determined simultaneously with glucosamine in amino sugar analysis.
the mole ratios in each immunotype were different. The molar sugar content in the different immunotype LPSs ranged from 0.7 to 3.1 for galactose, from 1.7 to 3.8 for glucose, and from 2.1 to 6.1 for glucosamine. The KDO and heptose contents remained rather constant. LPS immunotypes L2, L3, and L4 were indistinguishable by SDS-PAGE, but could be differentiated by their sugar compositions. The LPSs probably have more than one KDO residue per molecule (Table 1) because quantitation of KDO by the thiobarbituric acid method will not detect KDO residues that have either the C-4 or C-5 position linked to another LPS component (15).

The large variation in the monosaccharide compositions of different LPS immunotypes (Table 1) may indicate that there are several different types of oligosaccharides in N. meningitidis LPS.

LPS obtained from different strains of one immunotype would be expected to have the same monosaccharide composition. In fact, the LPS compositions of strains M136 (L4) and M986 (L3 with L7 as a minor type) from high aeration (see Table 3) are the same as those of prototype strains 891 (L4) and 6275 (L3) (Table 1), within experimental errors.

Of the eight prototype strains (Table 1), five belong to serogroup B, and three belong to serogroup C. Thus LPSs from different strains of any one serogroup have very different monosaccharide compositions.

**Influence of growth media on LPS components and sugar composition.** Different laboratories have used various media for the growth of N. meningitidis (4, 11, 15, 21). We observed small, but noticeable, variations in the LPS components seen on SDS-PAGE and in the galactose content of the LPS when different media were used for the growth of the organisms. N. meningitidis M986 was propagated in TSB (11), chemically defined Catlin medium (4), MF-B (11), and MF-WR (20). Among these media, TSB provided the best cell yield (see Table 2, column 2). LPSs prepared from strain M986 grown in these media were analyzed for their components by SDS-PAGE (Fig. 3). The major component in these LPSs appeared to be the same. However, the low-molecular-weight component was present in different amounts; TSB had the highest amount, and Catlin medium had the lowest (almost none). The monosaccharide compositions are presented in Table 2. When the composition of the LPS from cells grown in Catlin medium was compared with that of cells grown in TSB, the galactose content showed an increase of more than 20%, which was higher than the 5 to 10% error obtained in the sugar analysis.

**Effect of high and low aeration on LPS components and sugar composition.** Obvious changes in the LPSs were observed due to differences in aeration during the growth of the organisms. N. meningitidis M986 (L3, L7), M136 (L4), and M981 (L5) were grown in TSB under high and low aeration. The cell yield under high aeration was three- to fivefold higher than that under low aeration (Table 3, column 4). Analysis of the LPSs by SDS-PAGE (Fig. 4) showed that they had two predominant components and that the proportion of the higher- and lower-molecular-weight components changed with aeration. High aeration resulted in an increase in the amount of the lower-molecular-weight component, which was the minor component in M986 and M136 LPS but was the major component in M981 LPS. The degree of LPS homogeneity obtained under high aeration increased for M981 LPS but decreased for M986 and M136 LPS. The monosaccharide composition of LPS from these three strains grown under high and low aeration is

---

**FIG. 3.** SDS-PAGE of N. meningitidis LPS from strain M986 grown in different media. Lanes 1 and 2 contain E. coli smooth and rough LPS: lane 1, E. coli O111, 5 μg; and lane 2, E. coli PL2, 0.5 μg. Lanes 3 through 6 contain N. meningitidis M986 LPS, 0.5 μg each, obtained from organisms grown in one of four media: lane 3, tryptic soy broth; lane 4, MF-B; lane 5, MF-WR; and lane 6, Catlin medium.

**TABLE 2.** Monosaccharide composition of N. meningitidis M986 LPS obtained from cells grown in different media

<table>
<thead>
<tr>
<th>Medium</th>
<th>Cell yield (g [wt wt]/liter)</th>
<th>Mole ratio of monosaccharide&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Gal</th>
<th>Glc</th>
<th>Hep</th>
<th>GlcNH&lt;sub&gt;2&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSB</td>
<td>5.7</td>
<td>3.2</td>
<td>2.0</td>
<td>2.0</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>MF-B</td>
<td>4.7</td>
<td>3.6</td>
<td>2.0</td>
<td>2.0</td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td>MF-WR</td>
<td>4.7</td>
<td>3.9</td>
<td>1.9</td>
<td>2.0</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>Catlin</td>
<td>4.0</td>
<td>3.9</td>
<td>1.8</td>
<td>2.0</td>
<td>4.2</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Media are defined in the text.

<sup>b</sup> Gal, Galactose; Glc, glucose; Hep, heptose; GlcNH<sub>2</sub>, glucosamine.
presented in Table 3. The LPS from cells grown under high aeration had much lower galactose contents than did the LPS from cells grown under low aeration for all three strains examined. By comparison, the glucose and glucosamine contents in LPSs grown under high and low aeration varied only slightly. Changes in the galactose content in these LPSs indicate that the lower-molecular-weight component observed by lower SDS-PAGE in Fig. 4 contained less galactose since the decrease in the galactose content was directly related to the amount of the lower-molecular-weight component in the LPS.

The efficiency of extraction of rough LPS from E. coli or S. typhimurium depends on the method of extraction and the size of the core oligosaccharide (12). N. meningitidis LPS has oligosaccharide chains equivalent in size to those of rough E. coli LPS. The possibility therefore exists that N. meningitidis LPS prepared by the phenol-water procedure (29) may be preferentially enriched in the LPS component with the longer oligosaccharide chain. N. meningitidis M986 was therefore grown overnight in TSB under high and low aeration. The SDS-PAGE patterns of the LPS samples obtained from the phenol-water procedure were compared with those from whole-cell lysates digested with proteinase K (14). No differences were observed between their SDS-PAGE patterns, indicating that the phenol-water-purified N. meningitidis LPS did not differ in composition from the LPS in the cells. This result suggests that both large- and small-size LPS molecules of N. meningitidis have long enough oligosaccharide chains such that there was no preferential extraction of the larger LPS molecules by the phenol-water procedure.

**DISCUSSION**

In the process of defining the optimal hydrolysis conditions for determining the monosaccharide composition of meningococcal LPS, we observed that the heptose residues in the LPS were quite resistant to mild acid hydrolysis. Although most galactose and glucose in the LPS were hydrolyzed within 24 h, only 25% of the heptose in M986 LPS was released (Fig. 1). The maximum release of heptose required 72 to 96 h. In contrast to N. meningitidis LPS, heptose and other monosaccharides in E. coli O111 and S. typhimurium LPSs were released in 24 h (C. M. Tsai, unpublished data). This may well explain the low heptose content of N. meningitidis LPS reported in the literature (16, 19). In agreement with our results, a recent study by Jennings et al. (15) indicated that the glycosidic linkage between the contiguous glucosamine and heptose unit in N. meningitidis LPS is relatively stable to acid hydrolysis. They suggested that N. meningitidis LPS contains two heptose residues per molecule (15), based on sugar composition and methylation analysis.

Growth of N. meningitidis in TSB under different aeration conditions may cause changes in the sugar composition of the LPS, especially in the galactose content. Use of different media for the growth of N. meningitidis may also cause

**TABLE 3. Monosaccharide composition of N. meningitidis LPS obtained from cells grown in TSB under high and low aeration**

<table>
<thead>
<tr>
<th>Strain</th>
<th>LPS immunotype</th>
<th>Aeration</th>
<th>Cell yield (g [wet wt]/liter)</th>
<th>Mole ratio of monosaccharidea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Gal</td>
</tr>
<tr>
<td>M986</td>
<td>3, 7</td>
<td>High</td>
<td>7.1</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Low</td>
<td>2.0</td>
<td>4.7</td>
</tr>
<tr>
<td>M136</td>
<td>4</td>
<td>High</td>
<td>8.0</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Low</td>
<td>1.5</td>
<td>4.0</td>
</tr>
<tr>
<td>M981</td>
<td>5</td>
<td>High</td>
<td>5.8</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Low</td>
<td>1.7</td>
<td>1.4</td>
</tr>
</tbody>
</table>

a Gal, Galactose; Glc, glucose; Hep, heptose; GlcNH₂, glucosamine.
b ND, Not done.
small variations in the LPS composition. However, under the same growth conditions the LPSs from the eight prototype LPS immunotype strains had very different monosaccharide compositions (Table 1). Compositional and structural analyses of N. meningitidis LPS should therefore be carried out with LPS from strains of known LPS immunotypes grown under well-defined conditions. The growth conditions to obtain a more homogeneous LPS are strain dependent, since low aeration increased the homogeneity of M986 and M136 LPSs but decreased the homogeneity of M981 LPS, and high aeration decreased the homogeneity of M986 and M136 LPSs but increased that of M981 LPS (Fig. 3). The sugar compositions of N. meningitidis LPS reported in the literature (15, 16, 19) are mostly based on the serogroups of the organisms, and the reported compositions will become fully informative when the LPS immunotypes of the organisms used for these studies are established.

The apparent molecular weight of an LPS may be estimated by comparing its mobility in SDS-PAGE with that of other LPSs whose molecular weights can be calculated from their chemical structures (27). The molecular sizes of eight N. meningitidis immunotype LPSs were similar to those of rough E. coli LPS, and their molecular weights were estimated to be in the range of 4,200 to 5,000. The sugar composition data in Table 1 predict that the oligosaccharides released from these LPSs by mild acid hydrolysis would have 7 to 10 monosaccharide residues, assuming 2 glucosamine residues in the lipid A region (12), whereas the complete core of E. coli LPS would have 9 residues (12). The SDS-PAGE results and the sugar compositions of the N. meningitidis LPSs were consistent for most immunotypes. There was, however, a deviation in the mobilities in SDS-PAGE for immunotypes L1 (126E), L5 (M992), and L7 (6155), based on the numbers of their sugar residues. These three LPSs may have somewhat different amounts of lipid or intrinsic charge in their molecules, causing slightly different mobilities in SDS-PAGE (13, 26).

Most N. meningitidis LPS had two predominant components. The LPS from the cells grown under high aeration tended to have an increased amount of the smaller component. This may be due to the incomplete biosynthesis of the LPS oligosaccharide (6, 25) when cell division is more rapid under high aeration (11). LPSs used in this study were isolated from the organisms at the stationary phase. Whether there is any difference in the sugar composition or in the components of LPS between log-phase and stationary-phase organisms requires further investigation.

The observed heterogeneity of N. meningitidis LPS may explain why many strains possess more than one LPS immunotype (21, 30). These different immunotypes may reside in the different LPS molecules resolved by SDS-PAGE, although the possibility that they reside in different portions of an LPS molecule cannot be excluded. The variation in the sugar composition of LPS from a single strain grown under high and low aeration suggests that growing N. meningitidis under different conditions may result in the appearance of a secondary immunotype in addition to the original immunotype. The correlation of sugar composition (Table 3) with SDS-PAGE patterns of LPS (Fig. 4) showed that the slower-migrating components had more galactose residues than did the faster-migrating components. These additional galactose residues are either at or near the nonreducing ends and may therefore play a role in the antigenic determinants of certain immunotypes.

LITERATURE CITED