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Electromorphic Characterization and Description of Conserved Epitopes of the Lipooligosaccharides of Group A *Neisseria meningitidis*†

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We studied the lipooligosaccharides (LOS) of 28 group A *Neisseria meningitidis* of epidemiologically diverse origins to investigate whether each of the LOS serotypes found in serogroup A could be identified physically as well as antigenically. Using a dot blot assay with LOS-specific monoclonal antibodies (MAbs), we identified four epitopes that were serotype specific. The LOS from strains of each serotype were electromorphically and antigenically distinct when analyzed by silver-stained sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting. The LOS of L8 strains contained a 3,600-M_r component that bound the L8 MAb. The LOS of L9 strains contained two major components of 4,500 and 4,200 M_r. They bound the L9 MAb to the larger component. The LOS of L10 strains had a single major component of 4,000 M_r that bound the L10 MAb. The LOS of L11 strains contained a major 3,600-M_r component that could not be distinguished from the 3,600-M_r LOS of L8 strains by SDS-PAGE but that bound the L11 MAb. LOS of group A strains contained a highly conserved epitope in addition to a serotype-specific epitope. This was identified by a MAb that bound to all the strains on dot-blots and to multiple LOS components of various M_rs on immunoblots. We conclude that the LOS which bear the L9, L10, and L11 determinants are physically distinct and can be identified by SDS-PAGE or MAb binding or both. L8 and L11 are both borne on a 3.6-kilodalton LOS and can only be distinguished serologically.

Early serologic studies showed that strains of *Neisseria meningitidis* are antigenically diverse (23). They can be separated into groups based on the chemical and antigenic structures of their capsular polysaccharides and can also be divided into types based on outer membrane protein and lipooligosaccharide (LOS) structures (7). The classification scheme for distinguishing capsular serogroups and protein and LOS serotypes has been recently reviewed (7).

Mandrell and Zollinger used hemagglutination and solid-phase radioimmunoassay inhibition to identify eight serologically distinct LOS classes (serotypes L1 to L8) among group B and C meningococci (14, 29). LOS of group A meningococci showed little cross-reactivity with LOS of group B and C strains, and additional serotypes, L9 to L11, were added (30). Serotypes L10 and L11 were uniquely associated with group A strains; serotype L9 cross-reacted with L7 and, to a lesser extent, with L3, L4, and L6 of groups B and C LOS. Type L8 was occasionally found on group A strains.

LOS serotyping has been used to follow the spread of group A disease (8), but its usefulness has been limited by uncertainty as to the structural basis of LOS antigenic diversity and the presence of multiple serotypes on the same organism (7, 29, 30). This multiplicity has necessitated the use of arbitrary criteria for the assignment of serotypes and has created a critical need for standardized sera. In addition, the assays used to assign LOS serotype are poorly suited for use in less-developed areas, where the bulk of meningococcal disease occurs (18).

In contrast, the antigens responsible for protein serotypes have been assigned to molecular species that are classified by their mobility in sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (7, 15). Many can now be assigned by using monoclonal antibodies (MAbs) with whole bacterial cells in simple and reproducible assays (1). This has improved the definition of these systems over that provided by polyclonal serum and made them efficient and available for field epidemiologic studies (19). Improved electrophoretic separation of LOS (21) and the availability of MAbs (24) have provided tools with which to study the molecular basis of LOS serotypes and to develop a more complete, rational, and efficient system.

The LOS of *Neisseria* strains are composed of from one to six components that can be separated by SDS-PAGE and whose relative molecular weights (M_rs) have been estimated to be between 3,150 and 7,100 (21, 26). The heterogeneous electrophoretic mobility of LOS components primarily reflects differences in the chemical compositions of their oligosaccharides (9). These oligosaccharide compositional differences also account for antigenic differences (9).

Studies with MAbs show that epitope expression within LOS oligosaccharides is quite complex, but that some epitopes are found only on LOS of certain M_r, as identified by SDS-PAGE mobility and immunoblot (13). Schneider et al. showed that a MAb raised against an L8 meningococcal LOS bound a 3.6-kilodalton (kDa) component (22). Similarly, Sugasawara identified a MAb (MCA14.2) which bound a

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TABLE 1. MAb binding as determined by dot blots of serotype L8 and L9 strains of *N. meningitidis*

Strain	Source (year)	Serotype[a]	MAb[b]					
			2-1-L8	I-9C4	MCA14.2	4C4	O6B4	D6A
2E	Würzburg, Federal Republic of Germany (1964)	L8, (L3)	+	−	−	+	−	+
6748	Manitoba, Canada (1971)	L7, L8, L9, (L6)	+	±	−	+	+	+
3844	Mali (1969)	L7, L8, L9, (L6)	+	−	±	+	+	+
120M	Pakistan (1967)	L7, L9, (L4)	+	+	−	−	+	+
3840	Mali (1969)	(L6), L7, [L9]	−	+	−	−	+	+
15236	PRC (1984)	L9	−	+	−	−	+	+
15237	PRC (1984)	L9	−	+	−	+	+	+
15238	PRC (1984)	L9	−	+	−	+	+	+

[a] Numbers in parentheses and brackets indicate weak inhibition of the indicated serotype reactions by the strain; ( ) denotes serotype inhibition by 50 to 75%, and [ ] denotes serotype inhibition by 25 to 49%.
[b] +, Positive; ±, weakly positive; −, negative.

LOS component of the same M_r made by two different L10 strains (24).

In this study, we sought to identify serotype-specific LOS of group A meningococci by SDS-PAGE and by their binding of MAbs. We also identified LOS epitopes that appeared to be conserved, or common to, group A meningococcal strains.

(Portions of this study were presented at the Fifth International Symposium on the Pathogenic Neisseria, Amsterdam, the Netherlands, 14 to 18 September 1986 [11a].)

MATERIALS AND METHODS

Bacteriology. We studied 28 group A *N. meningitidis* strains representing the four LOS serotypes of this serogroup. They were of epidemiologically diverse origins. Their characteristics are listed in Tables 1 to 4. Sixteen isolates were from the People's Republic of China (PRC) (1980 to 1984) (11). One, 7889, was from Finland (1974) (17), and four (7880, 7883, 7908, and 7886) were isolated during the 1975 to 1976 Great Pacific Northwest (GPNW) outbreak (5). To these we added other group A meningococci from the collection of the Walter Reed Army Institute of Research. Most have been characterized previously (8, 16, 30). Epidemiologic and antigenic characteristics of 7889 and several GPNW strains have been reported previously by our laboratory (5, 9). Meningococci of groups B, C, and Y and a *Neisseria lactamica* from the Walter Reed collection were included for comparison.

Meningococci were cultured on Mueller-Hinton agar at 37°C in a humidified candle extinction jar. Strain 15247 and the *Neisseria lactamica* required GC agar base (Difco Laboratories) containing 1% (vol/vol) defined supplement (28) for optimum growth. Strains were determined to be *N. meningitidis* or *N. lactamica* by colony morphology, Gram stain reaction, production of cytochrome oxidase, and carbohydrate utilization (2). The strains were serogrouped by the slide agglutination technique (6). Strains from the PRC were LOS serotyped by hemagglutination inhibition with polyclonal rabbit serum (11, 14). They were examined only for serotypes L9, L10, and L11. The remaining strains were LOS serotyped by solid-phase radioimmunoassay inhibition with antisera against serotypes L1 to L11 (29, 30).

Preparation of proteinase K-treated whole-cell lysates. We prepared lysates used for SDS-PAGE analyses by the method of Hitchcock and Brown (10). Briefly, organisms were harvested from agar with a sterile cotton swab, suspended in 2 ml of cold Dulbecco phosphate buffered saline to an optical density of 0.8 to 1.0 at 650 nm, pelleted at 1,900 × *g* for 10 min, and suspended in 50 μl of dye solution (0.59 ml of sample buffer [2% sodium dodecyl sulfate, 60 mM Tris hydrochloride, 1 mM EDTA, pH 6.8], 0.08 ml of β-mercaptoethanol, 0.40 ml of glycerol, 0.04 ml of saturated bromphenol blue). Samples were heated at 100°C for 5 min and incubated at 60°C for 60 min with 25 μl of proteinase K dissolved in dye solution (1 mg/ml). Samples prepared in advance were stored at 4°C or frozen at −20°C.

TABLE 2. MAb binding as determined by dot blots of serotype L10 and L11 strains of *N. meningitidis*

Strain	Source (year)	Serotype	MAb[a]					
			2-1-L8	I-9C4	MCA14.2	4C4	O6B4	D6A
7880	GPNW (1976)	L10	±	−	+	−	−	+
7851	Brazil (1972)	L10	−	−	+	−	−	+
105M	Morocco (1967)	L10	−	−	+	−	−	+
15240	PRC (1984)	L10	−	−	+	−	−	+
15241	PRC (1984)	L10	−	−	+	−	−	+
7908	GPNW (1976)	L10, L11	−	−	+	+	−	+
7889	Finland (1973)	L11	−	−	−	+	−	+
7883	GPNW (1976)	L11	−	−	−	+	−	+
15242	PRC (1984)	L11	−	−	−	+	−	+
15243	PRC (1984)	L11	−	−	−	+	−	+
15244	PRC (1984)	L11	−	−	−	+	−	+
15245	PRC (1984)	L11	+	+	−	+	+	+
15247	PRC (1984)	L11	−	+	+	−	+	+

[a] +, Positive; ±, weakly positive; −, negative.

TABLE 3. MAb binding as determined by dot blots of nontypeable strains of *N. meningitidis*

Strain	Source (year)	Serotype[a]	MAb[b]					
			2-1-L8	I-9C4	MCA14.2	4C4	O6B4	D6A
7886	GPNW (1976)	NT	−	−	−	−	±	+
15232	PRC (1976)	NT	−	−	+	−	±	+
15233	PRC (1984)	NT	−	−	+	−	+	+
15234	PRC (1984)	NT	−	−	+	−	+	+
15235	PRC (1984)	NT	−	−	−	−	−	+
15246	PRC (1985)		−	−	−	+	−	+
15249	PRC (1985)		±	−	+	±	−	+

[a] NT, Not typeable; strains 15246 and 15249 were not serotyped.
[b] +, Positive; ±, weakly positive; −, negative.

LOS extraction. We used a modification of the hot phenol-water method of Westphal and Jann (27) to extract LOS from meningococci grown overnight (ca. 18 h) at 37°C in modified Frantz liquid medium (3, 27). Strain 15247 and 15249 required GC liquid medium for optimum growth.

Physical characterization of LOS. We separated lysates or purified LOS by SDS-PAGE using a modification of the method of Laemmli (12, 21). We diluted LOS to ca. 1 to 2 mg of LOS per ml in sample buffer and then diluted them 1:1 in dye buffer. LOS or lysates were heated at 100°C for 5 min, and 5-μl portions were applied to discontinuous slab gels (3% acrylamide spacer gel, 14% acrylamide resolving gel). We electrophoresed samples at 10 mA of current per gel through the spacer gel and 15 mA per gel through the resolving gel. Total electrophoresis was for 5 to 6 h. We visualized LOS by the silver stain method of Tsai and Frasch (25). We determined the theoretical M_rs of individual LOS components by comparing their migration distances with those of a simultaneously electrophoresed LOS standard that was extracted from group Y *N. meningitidis* 8032. The M_rs of its six LOS components were estimated by coelectrophoresis with the LOS of *Salmonella minnesota* rough mutants whose structures and M_rs are known (21, 22). The M_rs of the LOS standard are 5,400, 5,100, 4,500, 4,000, 3,600, and 3,200.

Immunologic characterization of *N. meningitidis* LOS. (i) Mouse MAbs. We used mouse MAbs that were previously determined to bind purified neisserial LOS. The results of binding with six MAbs are detailed in this study. The preparation and characterization of MAbs O6B4, 2-1-L8, MCA14.2, MN8D6A (D6A), and I-9C4 have been described previously (13, 20, 22, 24, 31). MAb 4C4 was prepared as described by Apicella et al. (1a). MAbs 2-1-L8 (specific for L8) and MCA14.2 (previously reported to bind two L10 strains) were supplied by W. D. Zollinger, Walter Reed Army Institute of Research, Washington, D.C., and R. Sugasawara, IGEN Inc., Rockville, Md., respectively. MAb O6B4 was provided by M. A. Apicella, State University of New York, Buffalo. Schneider et al. reported that MAb 2-1-L8 binds an epitope on a 3.6-kDa LOS component made by certain strains of *Neisseria gonorrhoeae* and a serotype L8 *N. meningitidis* strain (22). MAb O6B4 binds many *N. gonorrhoeae*, *N. meningitidis*, and *N. lactamica* strains (11b, 13).

(ii) Dot blot assay. We took swabs of *N. meningitidis* grown on agar media and dotted them onto replicate pieces of nitrocellulose paper. We included swabs of sterile agar as controls. We recorded the results as positive if they were visually positive on autoradiographs compared with controls. The results were almost always clearly positive or negative. Rarely, we observed weakly positive dots that were consistently reproducible. The blotting technique was essentially the same as that used for immunoblotting (described below). Results of the dot blot assay agreed with those of a solid-phase radioimmunoassay used to characterize MAb binding to purified LOS of selected strains (13) (data not shown).

(iii) Immunoblot analysis of LOS components. We used a modification of the Western blot transfer method of Burnette to perform immunoblots of gels of electrophoresed lysates and LOS (4, 13). Transfer of separated samples from the gels to nitrocellulose paper was done at 30 V for 12 to 16 h. We first incubated the transblot in 1% casein dissolved in a solution of 0.01 M Tris hydrochloride–0.15 M NaCl–0.005 M $MgCl_2$–0.03 M NaN_3 (pH 7.4) for 30 min and then in MAb diluted in 1% casein for 1 to 2 h. We then washed the antibody-treated nitrocellulose papers and incubated them for 1 h with ^{125}I-labeled goat antibody against mouse immunoglobulin G or immunoglobulin M. The papers were washed, dried, and autoradiographed.

TABLE 4. Electromorphic criteria for group A LOS serotypes and properties of two conserved LOS epitopes

LOS serotype	MAb	M_r[a]	Comments
L8	2-1-L8	3,600	L8 strains express the 2-1-L8 and 4C4 epitopes on a 3.6-kDa LOS
L9	I-9C4	4,500	L9 strains contains multiple LOS components and serotypes; also binds O6B4 strongly
L10	MCA14.2	4,000	Single major LOS component
L11	4C4	3,600	Single major LOS component
NA[b]	O6B4	≥4,500	Antibody binds to same LOS component as I-9C4[c]; binds weakly to a higher M_r LOS (5,300) in many nontypeable strains
NA	D6A	Variable	Antibody binds to all group A strains; epitope expressed on many but not all LOS components

[a] MAb associated with this serotype binds an LOS component of the specified M_r.
[b] NA, Not applicable.
[c] Except in strain 15247 (see text).

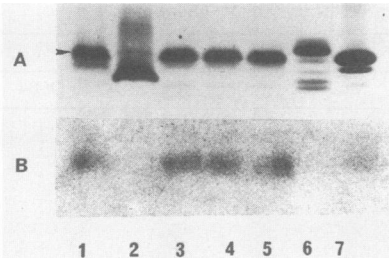

FIG. 1. SDS-PAGE and immunoblot of L9 lysates. (A) Silver-stained electrophoresis gel. (B) Autoradiograph of the immunoblot with MAb I-9C4. The arrow points to the LOS component that binds the L9 MAb (M_r, 4,500). Lysates were from the following strains: 3840 (lane 1), 3844 (lane 2), 15236 (lane 3), 15237 (lane 4), and 15238 (lane 5). LOS samples of strains 8032 (reference) (lane 6) and 15238 (lane 7) are also shown.

RESULTS

Identification of LOS MAbs that bind group A *N. meningitidis*. Using the dot blot assay, we looked for a consistent pattern of MAb binding with strains of a designated LOS serotype (as determined using polyclonal sera). We identified one MAb that appeared to be associated specifically with each of the four LOS serotypes that occur among group A strains (Tables 1 to 3). MAb 2-1-L8 bound 3 of 3 strains which were identified as serotyped L8 with polyclonal sera. MAb I-9C4 bound 5 of 7 L9 strains. The MAb MCA14.2 bound 5 of 5 L10 strains and a single L10,11 isolate. MAb 4C4 bound the L10,11 isolate and 6 of 7 L11 strains. All strains of serotype L8 also bound 4C4 (L11). Several strains differed in their MAb-binding pattern when compared with other strains of the same serotype. These will be discussed below. LOS from nontypeable strains were heterogeneous and had no distinct MAb-binding pattern.

We identified two additional MAbs that bound strains regardless of their LOS serotype. One, D6A, bound the LOS of all 28 group A strains. Another, MAb O6B4, bound L9 and 4 of 7 nontypeable strains.

In immunoblots, these MAbs bound LOS components of the following M_rs from reference strain 8032: MAb O6B4, M_r 5,100 (see Fig. 5, lane 7); MAb MCA14.2, M_r 4,000 (see Fig. 2); and MAb D6A, primarily M_rs 4,000, 3,600, and 3,200 (see Fig. 5). By using immunoblots of LOS of strain 8032 electrophoresed in adjacent lanes, we determined the M_rs of the serotype-associated LOS components described below.

Electromorphic identification of L8 LOS. All group A meningococcal strains whose serotype by polyclonal sera included L8 had a major LOS component of M_r 3,600 that bound the L8-specific MAb (data not shown). Strain 2E expresses only serotype L8; it made a single LOS of M_r 3,600, confirming previous observations that the L8 epitope is borne on this molecule (22).

Electromorphic identification of L8,9 and L9 LOS. LOS from L8,9 and L9 strains were heterogeneous (Fig. 1). Lysates of the four L9 strains that did not express the L8 serotype had two major LOS components of 4,500 and 4,200 M_r. These were best delineated in Fig. 1, lanes 1 and 7. By reference to immunoblots of simultaneously electrophoresed LOS of strain 8032, we could assign the binding of MAb I-9C4 to the 4.5-kDa LOS (see arrow, Fig. 1). The SDS-PAGE result of LOS of strain 120M (not shown) was similar to that of strain 3840.

The two strains that expressed both L8 and L9, 3844 (Fig. 1, lane 2) and 6748, had faintly staining components of 4,500 and 4,200 M_r and a darkly staining component of 3,600 M_r that bound MAb 2-1-L8. The other L9 strains made comparatively little of the 3,600-M_r component and variously bound 2-1-L8.

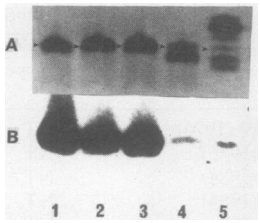

FIG. 2. SDS-PAGE and immunoblot of L10 and L10,11 lysates. (A) Silver-stained electrophoresis gel. (B) Immunoblot with MAb MCA14.2. Lysates were from the following strains: 15240 (lane 1), 15241 (lane 2), 7880 (lane 3), and 7908 (lane 4). Strain 7908 is type L10,11. Lane 5 contains the LOS reference (8032). The arrowheads denote the LOS component that binds the L10 MAb.

Electromorphic identification of L10 LOS. The LOS of the five L10 strains had similar electromorphologic patterns. Lysates of three representative L10 strains and an L10,11 strain are shown in Fig. 2. Each lysate, as well as the reference LOS 8032, contained a major component of 4,000 M_r that bound MAb MCA 14.2. Purified LOS from strain 7880 also contained a minor amount of a 3,600-M_r component that bound MAb 2-1-L8 (Fig. 5, lane 9).

Electromorphic identification of L11 LOS. The one L10,11 strain (7908) contained LOS components of 4,000 and 3,600 M_rs (Fig. 2, lane 4), and the L10 MAb bound the 4.0-kDa component. This suggested that L11 was borne on the 3.6-kDa LOS.

Lysates from five of the seven L11 strains contained a single LOS component of 3,600 M_r that bound the L11-associated MAb, 4C4 (Fig. 3). The L10,11 strain, 7908, bound MAb 4C4 to its 3,600-M_r component (Fig. 3; lane 8). None of the five L11 strains that made only the 3.6-kDa LOS bound 2-1-L8 or expressed serotype L8. In immunoblots all of the L8 strains bound MAb 4C4 to the 3.6-kDa LOS that also bound 2-1-L8 (data not shown). Therefore, LOS of strains designated L8 by using polyclonal sera express both the 4C4 and 2-1-L8 epitopes.

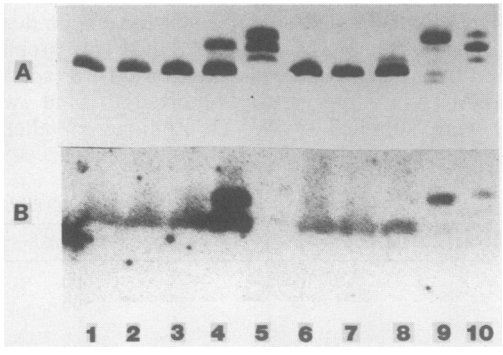

FIG. 3. SDS-PAGE and immunoblot of L11 lysates. (A) Silver-stained electrophoresis gel. (B) Reblot with MAb 4C4 after blotting with MAb O6B4. Lysates were from the following strains: 15242 (lane 1), 15243 (lane 2), 15244 (lane 3), 15245 (lane 4), 15247 (lane 5), 7883 (lane 6), 7889 (lane 7), and 7908 (lane 8). LOS samples of 8032 (reference) (lane 9) and 15247 (lane 10) are also shown. The L11 MAb 4C4 binds the low-molecular-weight LOS component (M_r, 3,600) in lanes 1 to 8. MAb O6B4 binds the top LOS components in lanes 4, 5 (faint), 9, and 10.

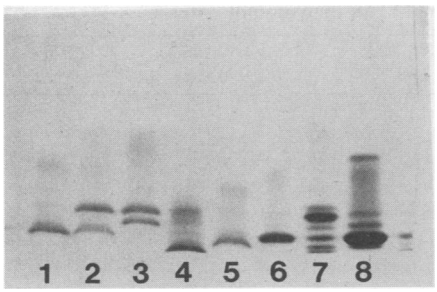

FIG. 4. Silver-stained SDS-PAGE of nontypeable group A meningococci. Lysates were from the following strains: 15232 (lane 1), 15233 (lane 2), 15234 (lane 3), 15235 (lane 4), 15246 (lane 5), and L5249 (lane 6). LOS samples of 8032 (reference) (lane 7) and 15249 (lane 8) are also shown.

Two strains serotyped L11 in their laboratory of origin gave results that differed from other L11 strains. The lysate of L11 strain 15245 contained LOS of 4,500 and 3,600 M_r and bound the L9-, L8- and L11-specific MAbs in dot blots. The latter two MAbs bound the 3.6-kDa LOS (Fig. 3, lane 4). This MAb-binding pattern is characteristic of L8,9 strains; however, the additional 4.2-kDa component that other L9 strains make was not apparent in lysates. Also, the quantity of the 4.5-kDa LOS made by this strain was nearly equivalent to that of the 3.6-kDa component as judged by density of silver stain, in contrast to the other L8,9 strains. The lysate of L11 strain 15247 contained 5.2-kDa, 4.5-kDa, and 4.0-kDa LOS components. As expected from these M_rs, it bound the L9- and L10-specific MAbs on dot blots. The lysate did not contain a 4.2-kDa LOS and weakly bound MAb O6B4 to the 5.2-kDa LOS (see below).

Electromorphic characterization of nontypeable LOS. LOS of nontypeable strains were heterogeneous. They often contained multiple components and bound several MAbs (Table 3; Fig. 4). Two group A strains, 15249 and 15246, were not serotyped previously. The lysate of strain 15246 (Fig. 4, lane 5) contained a 3.6-kDa LOS and bound MAb 4C4, and we conclude that it is an L11 strain. Strain 15249 and four additional nontypeable strains bound the L10-specific MAb in dot blots and made an LOS of 4,000 M_r that bound MAb MCA14.2. (The 4.0-kDa component in lysates of strain 15234 [Fig. 4, lane 3] stained faintly.) This pattern is consistent with their being of type L10, but these strains also made other LOS components of higher M_rs that are variably imaged by silver stain. Lysates of the nontypeable strain 7886 (not shown) contained LOS of 5,600 and 5,200 M_r; both LOS bound MAb D6A (see below).

Lysates and purified LOS gave comparable results on gels and immunoblots, except that minor components in purified LOS stained more distinctly with silver than did the same components in lysates (compare LOS and lysates in Figs. 1 and 2 with Fig. 5). Some strains very weakly bound a MAb specific for a second serotype. Examination of the silver-stained gel showed that purified LOS from these strains contained a minor amount of the appropriate LOS on SDS-PAGE (Fig. 5).

Electromorphic identification of common and conserved LOS epitopes. Two additional LOS-specific MAbs provided interesting binding patterns. MAb O6B4 was known to bind an epitope expressed by most gonococcal, many group B and C meningococcal, and many *Neisseria lactamica* strains (11b, 13). SDS-PAGE profiles of LOS of representative meningococci and of a *N. lactamica* are shown in Fig. 5; the lower panels are the corresponding immunoblots with MAbs

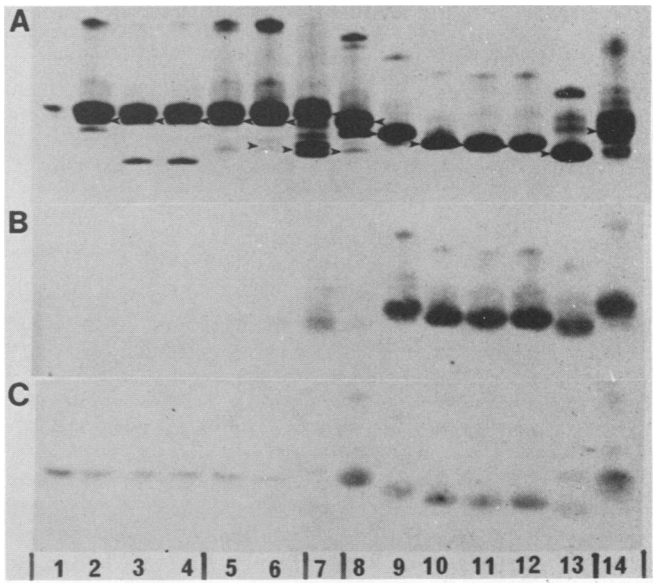

FIG. 5. Silver-stained SDS-PAGE (A) and immunoblots (B and C) of purified LOS from group C (lanes 1 to 4), B (lanes 5 and 6), Y (lane 7), and A (lanes 8 to 13) *N. meningitidis* and from a *N. lactamica* strain (lane 14). Lanes 1, 2, 3, and 4 are from LOS serotypes L2, L2, L2,4, and L2,4, respectively, and lanes 5 and 6 are from LOS serotype L3. The group Y strain is 8032 (reference) (lane 7). Lane 8 contains LOS from serotype L9; lane 9 contains LOS from serotype L10; lane 10 contains LOS from serotype L10,11; and lanes 11 and 12 contain LOS from serotype L11. Lane 13 contains LOS from strain 15235. Lane 14 contains LOS from a *N. lactamica* strain. Panel B contains the immunoblot with MAb D6A, and panel C contains a reblot with MAb O6B4 after D6A. The right-pointing arrowheads denote the LOS component(s) that binds MAb D6A. The left-pointing arrowheads denote the LOS component that binds MAb O6B4.

D6A and O6B4. MAb O6B4 bound the 4.5-kDa component made by L9 group A meningococci (Fig. 5, lane 8) and an LOS of the same M_r made by some group B and C strains (lanes 1 to 5). This is the same LOS component on the L9 strains that bound MAb I-9C4. MAb O6B4 bound to a slightly larger component (M_r, 5,100) of the group Y strain, 8032 (Fig. 3, lane 9).

Strain 15247 (L11) and two of the nontypeable strains, 15233 and 15234, also bound MAb O6B4, but they bound it weakly to a 5.2-kDa LOS component (e.g., Fig. 3, lane 10).

MAb D6A bound all group A meningococcal strains. The D6A-defined epitope was expressed on many, but not all, LOS components (Fig. 5). L10 strains bound D6A to the 4.0-kDa LOS that also bore the L10 epitope; L8, L8,9, and L11 strains bound D6A to the 3.6-kDa LOS component that also bore the L8 or L11 (or both) epitopes. L9 strains that did not express the L8 epitope bound MAb D6A to minor components of low molecular weight (M_r, 3,200 to 3,600). These components were not imaged by silver staining of lysates, but were faintly visible in silver-stained gels of purified LOS (Fig. 5, lane 8). MAb D6A bound all the LOS components of nontypeable strains of reference strain 8032 that were well visualized by silver staining. Reference strain 8032 LOS bound MAb D6A mainly to lower components (Fig. 5, lane 7).

The combination of SDS-PAGE and MAb-binding patterns provide electromorphic criteria that can be used to assign LOS serotypes (Table 4). Using these criteria, we

TABLE 5. Assignment of serotypes by electromorphic and MAb immunoblot criteria

Strain	Nominal serotype[a]	3.6-kDa LOS[b]	MAb 2-1-L8[c]	MAb 4C4[c]	4.0-kDa LOS[b]	MAb MCA14.2[c]	4.5-kDa LOS[b]	MAb I-9C4[c]	Revised serotype[b]
2E	L8	+	+	+					L8
6748	L8, 9	+	+	+			+	+	L8, 9
15245	L11	+	+	+			+	+	L8, 9[d]
3844	L8, 9	+	+	+		±	+		L8, 9
120M	L9	±	+				+	+	L8, 9
3840	L9	±					+	+	L9
15236	L9	±					+	+	L9
15237	L9	±		+			+	+	L9, 11
15238	L9	±		+			+	+	L9, 11
15247	L11				+	+	+	+	L9, 10[d]
105M	L10				+	+			L10
7851	L10				+	+			L10
15240	L10				+	+			L10
15241	L10				+	+			L10
15232	NT[e]				+	+			L10[d]
15233	NT				+	+			L10[d]
15234	NT				+	+			L10[d]
15249		±	±	±	+	+			L10[d]
7880	L10	±	±	±	+	+			L10
7908	L10, 11	+		+	+	+			L10, 11
7889	L11	+		+					L11
15242	L11	+		+					L11
14243	L11	+		+					L11
15244	L11	+		+					L11
7883	L11	+		+					L11
15246		+	±	+					L11
7886	NT								NT
15235	NT								NT

[a] Determined by using polyclonal sera (see text).
[b] Presence of LOS determined by SDS-PAGE and silver staining: +, present; ±, weakly present.
[c] Binding (+, strong; ±, weak) of indicated antibody as determined by dot blot.
[d] Incomplete serotype assignment (see text).
[e] NT, Not typeable.

were able to assign LOS serotypes to 26 of 28 group A strains from different epidemiologic settings (Table 5), including 5 that were nontypeable by polyclonal sera or not specified. We also revised the serotypes of two strains, 15245 and 15247, and added additional minor types to three strains, 120M, 15237, and 15238.

The electromorphic patterns of several lysates suggested that the revised serotypes were incomplete for several strains (Table 5). Strains were considered to have a serotype if they bound the serotype-associated MAb strongly. Because MAb I-9C4 was of low affinity on dot blots and immunoblots, strains with weak binding to this L9-associated MAb were included in the revised serotype assignments for L9. Strain 3844 did not bind MAb I-9C4 but was electromorphically similar to another L8,9 strain (6748). We therefore retained the original serotype assignment for strain 3844.

DISCUSSION

From our studies we conclude that the LOS of group A meningococci have distinct electromorphic and MAb-binding (epitopic) patterns for each serotype (Table 4). The lysates of L10 and L11 strains each contained a single major LOS component of 4,000 and 3,600 M_r, respectively, that bound their serotype-specific MAb. Lysates of L9 group A strains were composed of a mixture of components, and each strain expressed multiple LOS serotypes (26, 29, 30). Their LOS were comprised primarily of components of 4,500 and 4,200 M_r but also possessed variable amounts of the 3.6-kDa component with its epitopes (defined by MAbs 4C4 and 2-1-L8). Strains of serotype L8,9 contained the 3.6-kDa LOS component (L8) and relatively small amounts of the 4.5- and 4.2-kDa components.

The MAb I-9C4 bound the 4.5-kDa component of L9 lysates. Since serotyping was developed by using polyclonal rabbit sera, the original L9 determinant(s) could reside on either the 4.5-kDa LOS or the 4.2-kDa LOS or could represent a combination of both components. In the absence or precise information about the structural basis of the polyclonally defined L9 determinant, we assigned the L9-associated epitope to the higher component. It is important to note that the 4.2-kDa LOS may have other L9-associated epitopes, but we do not have MAbs that identify these at present.

Both L8 and L11 group A strains express a 3.6-kDa LOS component but could be distinguished by their MAb-binding pattern. The L8 strain may contain two separate LOS components that comigrate by SDS-PAGE (one that bears the 2-1-L8 epitope and another that bears the 4C4 epitope). Alternatively, L8 and L11 strains may have a single 3.6-kDa LOS which may have subtle structural differences in the oligosaccharide portion of the LOS which is associated with the 2-1-L8 epitope.

There were few discrepancies between assignment of LOS serotypes by polyclonal sera and by electromorphic and epitopic profiles. Using the epitopic criterion, we could predict the LOS serotype based on polyclonal sera of 18 of 21 isolates for which a serotype was known (Table 5). The L9-associated MAb bound relatively weakly. Although strain 3844 (serotype L8,9) has a SDS-PAGE pattern similar

to another L8,9 strain (6748), it did not bind the L9-associated MAb (Table 1). Strains 15245 and 15247 were originally typed L11 by polyclonal sera and had electromorphic and epitopic properties that differed from other L11 strains. These isolates had MAb-binding patterns characteristic of L8,9 and L9,10, respectively. However, neither made the 4.2-kDa LOS found on other L9 strains in our collection. Strain 15247 also bound MAb O6B4 to the 5.2-kDa LOS rather than the 4.5-kDa component. Thus, the LOS of these strains have some but not all of the morphologic and antigenic characteristics of the other L9 LOS that were described in Results.

The dot blot assay with MAbs was more sensitive at identifying minor LOS components than conventional serotyping with polyclonal sera. These minor components were faintly visible on silver-staining samples containing higher concentrations of LOS (e.g., Fig. 5, lanes 8 and 10). Minor LOS components may represent phase variation (i.e., a change in expression of LOS molecules by a single strain) (20). They have an uncharacterized role in the pathogenicity of the meningococcus and may be important in describing the full repertoire of LOS.

The LOS of nontypeable strains were heterogeneous both morphologically and by MAb-binding pattern. They often contained the L10 LOS component but also contained other LOS that were not identified by our MAb library.

Thus, we have provided guidelines for determining the LOS serotypes of group A meningococci (Table 4). If a strain binds a serotype-specific MAb, we can reliably predict the presence of that serotype-specific LOS component by SDS-PAGE. Conversely, we can predict whether strains are serotype L9 or L10 by SDS-PAGE pattern alone. MAbs or polyclonal sera are required to distinguish L8 from L11 strains. Our MAb library is not complete enough to fully describe the LOS of nontypeable group A meningococci.

The LOS serotype-associated epitopes described above are restricted to LOS components of a given molecular weight. The D6A-defined epitope was found on LOS of various M_rs and is present on all group A meningococci studied (as well as some strains of non-group A meningococci and other neisserial species). For serotypes L8, L10, and L11, the D6A epitope was expressed on the LOS component that bears the serotype marker. In contrast, for L9 strains, D6A was not found on the major components but appears to be expressed on several low-molecular-weight components.

Differences in the antigenic profiles and electromorphologic mobilities reflect, in part, structural differences in the oligosaccharide portion of the LOS (9). The D6A epitope was found primarily on lower-molecular-weight LOS components of group A and on some group B, C, and Y strains that we studied. It may represent a common structural portion of the oligosaccharide side chain found on all LOS components of group A meningococci. Addition or modification of certain glycose moieties may alter or occlude the D6A epitope on certain LOS components.

The O6B4 epitope was also found on LOS components of various molecular weights (M_rs: 4,500 for L9 strains, 5,300 for nontypeable group A strains, and 5,200 for 8032, group Y). We found that MAb O6B4 recognized a LOS epitope found on L9 and some nontypeable group A meningococci. This epitope is also found on many group B and C meningococci (including L2,4 and L3,7 strains) and on many *N. gonorrhoeae* and *N. lactamica* (11b, 13).

In addition to the O6B4 epitope, the L9-associated epitope is also found on L3,7 meningococcal strains (unpublished observations). Mandrell and Zollinger and subsequently Tsai et al. observed that there are antigenically similar LOS components between group B and C strains of different serotypes (14, 26, 29). Our observations suggest that these epitopes may provide some of the molecular and antigenic basis for the cross-reactivity between the group A L9 strains and the L1-8 serotypes (Fig. 5) (30).

Cross-reactive (shared) and minor epitopes are more easily identified by MAb analysis. Zollinger and Mandrell originally devised the LOS serotyping system to classify meningococci according to those containing similar LOS properties on the basis of reactivity with polyclonal antisera raised against organisms chosen as LOS prototype strains (10, 29, 30). Since a single strain frequently reacted with antisera from multiple serotypes, clear separation into distinct serotypes was often difficult. Minor serotyping cross-reactions between strains were observed. Therefore, in assigning serotypes it was necessary to establish arbitrary cutoff criteria. Strains having minor amounts of a serotype epitope insufficient to achieve the cutoff value could be designated nontypeable.

Besides the inherent difficulty of this serotyping system due to cross-reactivity, the variability of an individual rabbit's LOS antibody response to immunization with the prototype strain may make the LOS serotyping system difficult to reproduce in different laboratories (R. E. Mandrell, unpublished observations). Furthermore, one might fail to identify widely shared LOS epitopes if these were not among the most immunogenic epitopes in the immunized rabbit.

Therefore, we conclude that electromorphic typing augmented by MAb-binding pattern (Table 4) is a more sensitive and accurate method of discriminating among the LOS of group A *N. meningitidis* strains. This more precise characterization may be helpful in understanding the role of LOS in the pathogenesis of meningococcal disease.

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