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# Antigenic and Physical Diversity of *Neisseria gonorrhoeae* Lipooligosaccharides†

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**We used mouse monoclonal antibodies (MAbs) to characterize *Neisseria gonorrhoeae* lipooligosaccharide (LOS). LOSs that bound two or more MAbs in a solid-phase radioimmunoassay usually bound them to different LOS components, as separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE); strains with multiple LOS components on SDS-PAGE usually bound more than one MAb. However, the LOS of some strains bound the same MAb to two LOS components with different relative molecular weights, and some individual LOS components bound more than one MAb. LOSs from different strains bound different amounts of the same MAb at saturation, reflecting differences in the quantitative expression of individual LOS components. Not all components recognized by MAbs were stained by silver after periodate oxidation. Treatment with NaOH variously affected epitopes defined by different MAbs. MAb 3F11 completely inhibited and MAb 2-1-L8 partially inhibited the binding of $^{125}$I-labeled 06B4 MAb to WR220 LOS and WR220 outer membranes in competitive binding studies. Other MAbs did not compete with the binding of $^{125}$I-labeled 06B4 to either antigen. We conclude that a strain of *N. gonorrhoeae* elaborates multiple LOSs that can be separated by SDS-PAGE and that are antigenically distinct. Epitope expression within these glycolipids is complex.**

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) separates the principle outer membrane glycolipids, or lipooligosaccharides (LOSs), of *Neisseria gonorrhoeae* into multiple components with $M_r$s between 3,000 and 7,000, as determined with isogenic *Salmonella* rough mutant LOS standards (25, 26). High $M_r$ components bearing polysaccharides composed of increasing numbers of oligosaccharide repeating units that are produced by smooth strains of members of the family *Enterobacteriaceae* (lipopolysaccharides [LPSs]) (7) are absent from gonococcal LOS. Gonococcal LOSs that migrate in the same molecular weight range as the bands observed with smooth enterobacterial LPS are LOS aggregates that diminish when treated with NaOH or when urea is included in the gel (16). For these reasons the term LOS has been used (8, 14, 24, 26, 33, 36) to distinguish the endotoxin moiety of *Neisseria* from the generally larger endotoxin molecules of members of the family *Enterobacteriaceae*.

Antigen expression within gonococcal LOS is heterogeneous, reflecting the heterogeneity of molecular size (26). The complex antigenic structures of their LOSs have made serological classification of gonococci difficult and thwarted efforts to understand human immunity (1, 3, 5, 9, 16–18, 23, 24). Monoclonal antibodies potentially could provide ideal tools for defining the physical basis of antigen expression within complex glycolipids (6).

Mouse monoclonal antibodies (MAbs) that bind to gonococcal LOSs have been described previously (2, 10, 14, 26). An immunoglobulin M (IgM) MAb, 3F11, recognizes a common LOS antigen and binds the LOS of all six gonococcal prototype serotype strains (2). We expected from these data that MAb 3F11 would also bind to all of the components of the LOS of a strain. On the other hand, an IgG MAb, 2-1-L8, bound to only one component of the LOS of several serum-resistant gonococcal strains (24, 26). We used these two and five additional MAbs to explore the organization of conserved and unique epitopes within gonococcal LOS components.

(Portions of this study were presented at the Fourth International Symposium on the Pathogenic *Neisseria*, Asilomar, Calif., 21 to 24 October 1984.)

## MATERIALS AND METHODS

**Microbiology.** *N. gonorrhoeae* strains were from the collections of the Walter Reed Army Institute of Research and have been extensively characterized (8, 22, 23, 25, 26, 30, 36). Strains were those used in previous studies by us and by others and are representative of strains commonly used for study. Cultural procedures have been described previously (26).

**LOSs.** We extracted LOS from acetone-dried organisms by the hot phenol-water method (25, 35). Stock solutions of LOS for solid-phase radioimmunoassay (SPRIA) studies were prepared by dissolving them in 50 mM NaOH, heating the solutions for 1 h at 37°C, and then carefully neutralizing them with 100 mM HCl. In one experiment fractions of F62 LOS were diluted in concentrations of NaOH up to 200 mM, heated at 56°C for 3 h, and then diluted in SDS-PAGE gel sample buffer (see below) prior to gel and immunoblot analysis.

**Outer membrane complex.** We obtained the outer membrane complex (OMC) from *N. gonorrhoeae* WR220 by a

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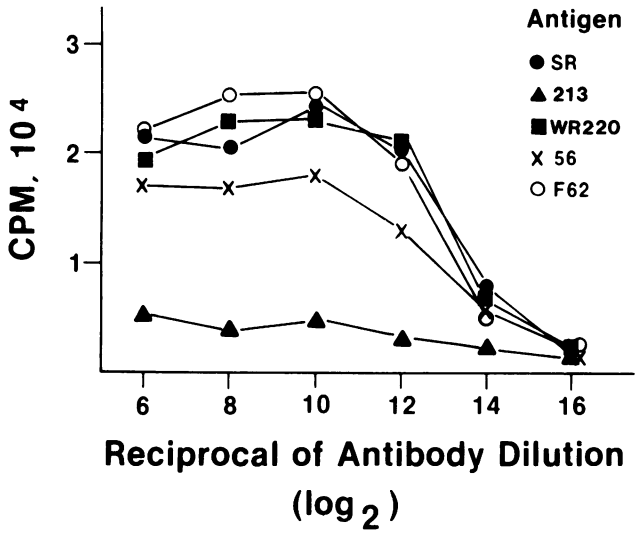


FIG. 1. SPRIA analysis of five gonococcal LOSs with 3F11 MAb. The following LOSs were used: SR (●), WR213 (▲), WR220 (■), GC56 (×), F62 (○). LOSs at 50 μg/ml were used for plate sensitization. Background binding was <1,000 cpm at all dilutions.

method described previously for the preparation of OMC from *N. meningitidis* (41).

**MAbs.** The preparation and characterization of MAbs 3F11, 06B4, 1-1-G, 1-1-M, and 2-1-L8 have been described previously (2, 14, 26, 40). Mouse MAbs 2C7 and 3G9 were prepared by the method of Kohler and Milstein (12), with the fusions performed as described by Kennett (11). We used the nonsecreting myeloma cell line Sp2/0-Ag14 (27). We harvested spleen cells from strain A mice that had been immunized with gonococcal outer membranes and boosted 3 days prior to the fusion. We identified hybridoma clones secreting gonococcal LOS antibodies by enzyme-linked immunosorbent assay (22) and cloned them by limiting dilution. We injected stable hybridoma clones into the peritoneum of CAF/1 mice. MAb was purified from ascitic fluid harvested from tumor-bearing mice by $(NH_4)_2SO_4$ precipitation followed by molecular sieve chromatography over Ultragel AcA-44 (LKB Instruments, Inc., Rockville, Md.).

**SPRIA.** We performed SPRIA as described previously (37). We diluted LOS to 50 μg/ml in Dulbecco phosphate-buffered saline (PBS; modified to contain 50 mM $MgCl_2$) and used it to sensitize polyvinyl plates. A filler of 0.5% casein–0.5% bovine serum albumin–0.1% sodium azide, all in Dulbecco PBS with $MgCl_2$, was used to block nonspecific binding. We estimated binding of each MAb to adsorbed LOS with use of $^{125}$I-labeled goat antibody against mouse IgG or IgM, as appropriate (Kierkegard and Perry, Gaithersburg, Md.). We plotted the counts per minute bound by each MAb against the reciprocal antibody dilution to determine the degree of binding at saturation and the titer at 50% saturation binding. We included wells that received all reagents except MAb or goat antibody as background controls. Comparisons among MAbs that bound to a particular LOS were limited to negative or positive because of differences in the concentration of each MAb and the concentration of goat antibodies against the different mouse immunoglobulin isotypes, including those against IgG isotypes. It was possible to make comparisons among LOSs bound by a particular MAb.

**MAb isotypes.** The isotypes of the IgG MAbs were determined by a modification of the SPRIA. Polyvinyl plates were sensitized with LOS. We added dilutions of ascites fluids containing MAbs and incubated the plates for 2 h. After unbound antibody was removed and washed, rabbit antibodies specific for a mouse IgG isotype (Litton Bionetics, Charleston, S.C.) and $^{125}$I-labeled goat anti-rabbit immunoglobulin were added in succession, with washing after each step. All incubations were carried out at room temperature.

**Competitive SPRIA binding.** We modified the SPRIA to examine the competitive binding of MAb to LOS. MAb 06B4, which binds goat anti-mouse IgM, was purified with protein A-Sepharose (Pharmacia Fine Chemicals, Piscataway, N.J.) by a procedure described previously (28). The antibody was concentrated by ammonium sulfate precipitation and then dialyzed versus Dulbecco PBS. The purified MAb was then labeled with $^{125}$I by the lactoperoxidase procedure, essentially as described previously (37), to a specific activity of approximately 400 cpm/ng of protein. We incubated 25 μl of twofold dilutions of each test MAb for 1 h in microtiter wells sensitized with either WR220 LOS or WR220 OMC. After unbound MAb was removed and the wells were washed, as described above, we added 25 μl of $^{125}$I-labeled 06B4 (approximately $8 \times 10^4$ cpm) and the wells were incubated again for 3 h. Unbound $^{125}$I-labeled 06B4 was removed, the wells were washed and cut out, and bound $^{125}$I was counted.

**Concentration of MAb.** We determined the concentration of MAb in ascites fluid by a previously described method (37) that depends on the determination of two constants: (i) Rep, the ratio of secondary antibody to primary antibody at saturation binding of the former to the latter; and (ii) Fep, the fraction of primary antibody that remained bound after reaction with secondary antibody. We determined that these values for mouse IgG and IgM with affinity-purified secondary antibodies (Kierkegard and Perry) were as follows: Rep, IgG = 3.7, IgM = 2.6; Fep, IgG = 0.44, IgM = 0.48. (Two methods are described in reference 27 for the determination of Fep. The first method used radiolabeled primary antibody in a SPRIA to monitor the losses of primary antibody that occur during washing and binding of secondary antibody. This is the method used in this study.) We then determined the concentration of each McAb by using the following formula: MAb (μg/ml) = (Dep × cpm × 40)/(SA × Rep × Fep), where Dep is the reciprocal of the dilution of MAb at equivalence (endpoint); cpm is counts per minute of $^{125}$I-labeled secondary antibody (goat anti-mouse IgG or IgM) bound to primary antibody when 25 μl of primary antibody was used (the factor 40 adjusts the volume from 25 μl to 1 ml); and SA is specific activity of secondary antibody in counts per minute per microgram.

**PAGE and immunoblot analysis.** We separated LOS samples through polyacrylamide gels by the method of Laemmli (13), with minor modifications as described previously (26). Briefly, we diluted 0.1 to 2 μg of NaOH-treated (see above) LOS in a buffer consisting of 2% SDS in 60 mM Tris hydrochloride–1 mM EDTA–3.5% β-mercaptoethanol–18% glycerol–2% bromophenol blue (pH 6.8) (sample buffer) and heated it at 100°C for 5 min. We then applied the LOS samples to duplicate slab gels and electrophoresed them at 10 mA per slab for approximately 18 h. We stained LOS in one slab with silver (32) and electroblotted those in the duplicate gel onto nitrocellulose paper by applying 30 V for 5 to 18 h (4). We cut the paper, incubated it in a filler buffer consisting of 1% casein dissolved in 10 mM Tris hydrochloride–150 mM NaCl–5 mM $MgCl_2$–30 mM $NaN_3$ (pH 7.4) for 1 h, and reacted it with MAb diluted in filler buffer. We then

TABLE 1. MAb analysis of 20 gonococcal LOSs by SPRIA

| LOS | % Binding by the following MAbs[a] | | | | | | |
|---|---|---|---|---|---|---|---|
| | 3F11 IgM | 06B4 IgM | 1-1-M IgM | 2-1-L8 IgG3 | 1-1-G IgG2a | 2C7 IgG3 | 3G9 IgG2a |
| WR213 | + | +++ | ++ | − | − | − | − |
| GC56 | ++ | + | +++ | − | − | + | +++ |
| F62 | +++ | + | +++ | − | − | − | − |
| A9 | ++ | +++ | − | − | − | + | + |
| DC9 | ++ | ++ | − | − | + | + | +++ |
| P32 | + | + | − | − | + | + | + |
| GC39 | ++ | + | +++ | − | + | + | − |
| 8038-5 | ++ | ++ | + | + | − | − | − |
| DOV | ++ | ++ | +++ | − | − | + | − |
| DOV($OH^-$)[c] | + | +++ | +++ | − | − | + | − |
| JW31R | − | − | − | − | + | + | + |
| JW31 | + | ++ | + | − | + | + | +++ |
| DAV | ++ | +++ | − | − | − | − | − |
| GC13 | ++ | ++ | − | − | ++ | + | ++ |
| SR | +++ | +++ | +++ | − | + | + | +++ |
| WR302 | + | ++ | − | + | − | − | − |
| WR220 | +++ | ++ | − | +++ | − | + | + |
| 7134 | ++ | ++ | − | − | − | + | + |
| GC33 | ++ | ++ | − | + | − | − | − |
| 6611-33 | ++ | ++ | + | + | − | − | − |

[a] Symbols: +, 15 to 49% of maximum plateau binding; ++, 50 to 89% of maximum plateau binding; +++, 90 to 100% of maximum plateau binding.
[b] MAb isotyping (see text) of 2C7 showed partial reactivity of 2C7 with the anti-mouse IgG2a reagent (25% as high as the anti-mouse IgG3).
[c] LOS was treated with 50 mM NaOH, dialyzed, and lyophilized prior to retreatment with NaOH (see text) for SPRIA analysis.

washed the antibody-treated nitrocellulose papers and incubated them with $^{125}I$-labeled goat antibody against either mouse IgG or mouse IgM, which were diluted in blot filler as appropriate. The washed papers were then dried and autoradiographed.

The referencing of components between silver stains and immunoblot autoradiographs was done by comparison of positive control components of known activity with MAbs and by reincubation of immunoblots with a second MAb that bound to a component with a different $M_r$.

## RESULTS

Dilutions of the seven MAbs were separately tested with each of 20 gonococcal LOSs by SPRIA. In Fig. 1 is depicted a representative SPRIA analysis, that of MAb 3F11 binding to five gonococcal LOSs. From the binding curves that were obtained a comparison was made of the maximum (plateau) binding levels of each of the gonococcal LOSs for a particular MAb. Table 1 provides a qualitative comparison of the saturation binding of seven MAbs to each of 20 LOSs. MAb 3F11 and 06B4, both IgM, had very similar binding patterns, binding all LOSs to some degree except that from JW31R. MAbs 2C7 and 3G9, of different IgG isotypes, had similar but not identical binding patterns. All gonococcal LOSs tested bound at least two different MAbs, but most bound three or more LOSs. A representative set of LOSs that bound multiple MAbs were further tested by SDS-PAGE and immunoblot analysis.

The silver-stained SDS-PAGE gels and immunoblot autoradiographs of six LOSs selected for binding of more than one antibody in the SPRIA are shown in Fig. 2. LOSs of WR213, F62, and JW31 (Fig. 2, lanes 1, 2, and 3, respectively) each bound 3F11 and 1-1-M to separate components. Because of the limited resolution of components with similar $M_r$s, this can be seen only when blots were sequentially exposed to MAbs, as were lanes 1, 2, and 3 in Fig. 2 (3F11 and then 1-1-M).

Three components of WR213 LOSs (Fig. 2, lane 1) stained with silver; 3F11 antibody bound to the top and bottom components, whereas 1-1-M bound to the middle component and to a component that appeared to migrate slightly slower than the slower migrating component bound by 3F11 and that did not stain silver. F62 LOS aggregates that did not stain with silver also bound 1-1-M, but not 3F11. WR220

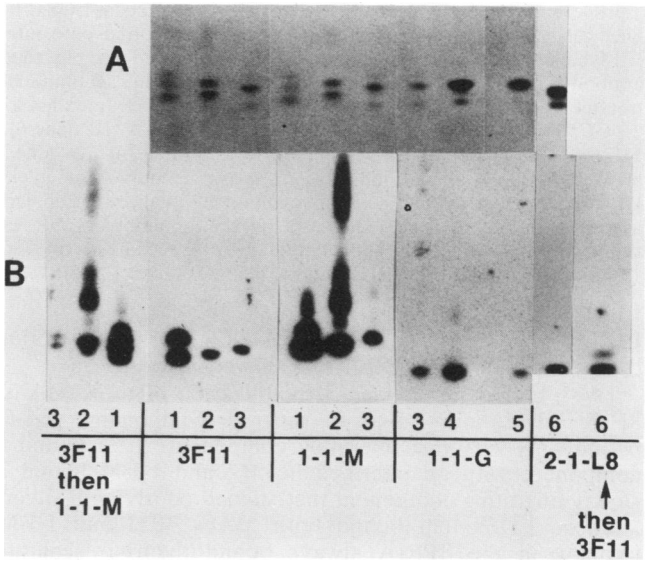


FIG. 2. PAGE and electroblot analysis of gonococcal LOSs. Six different gonococcal LOSs were separated by PAGE on duplicate slab gels. One gel was silver stained and the other was electroblotted onto nitrocellulose paper. The electroblots were tested with 3F11, 1-1-M, 1-1-G, or 2-1-L8 MAb and the appropriate $^{125}I$-labeled secondary antibody probe. After autoradiography of the immunoblots, the 3F11- and the 2-1-L8-treated papers were then tested with 1-1-M or 3F11 MAb, respectively; the immunoblots were again tested with $^{125}I$-second-labeled antibody and re-autoradiographed. (A) Silver stain. (B) Immunoblots. LOSs were as follows: WR213 (lane 1), F62 (lane 2), JW31 (lane 3), DC9 (lane 4), GC13 (lane 5), WR220 (lane 6).

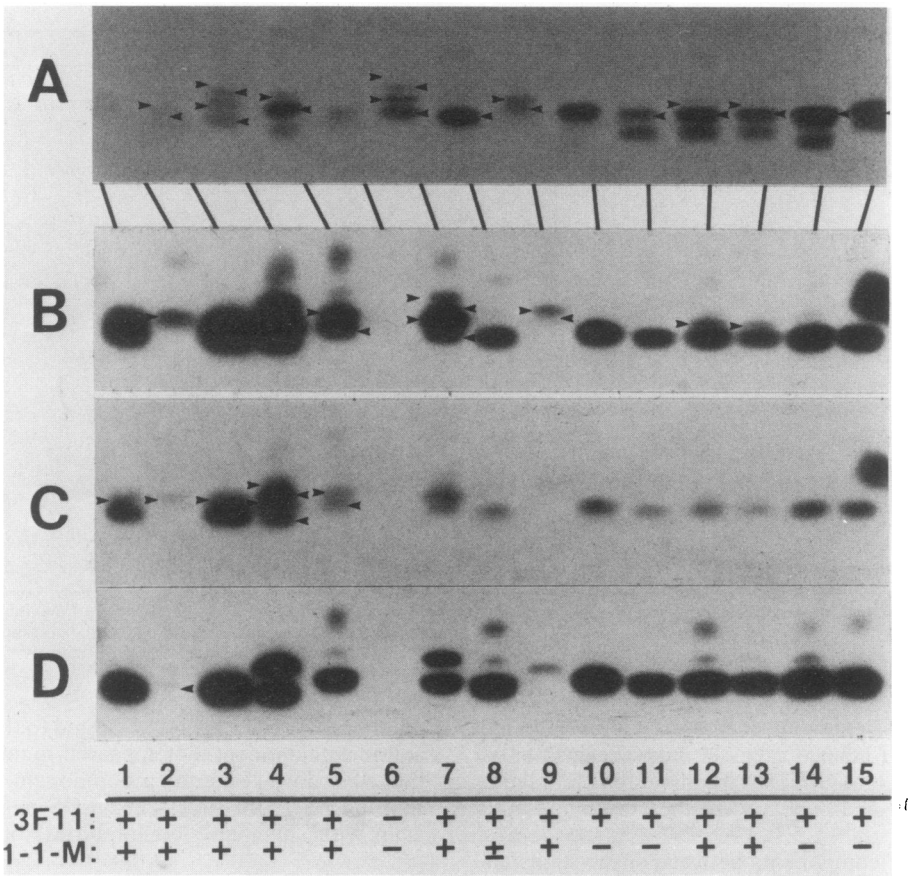


FIG. 3. PAGE and electroblot analysis of gonococcal LOSs. Fifteen gonococcal LOSs were separated by PAGE on duplicate slab gels. One gel was silver stained and one was electroblotted onto nitrocellulose paper. The electroblot was treated first with 3F11 MAb and $^{125}$I-labeled secondary antibody probe. After autoradiography the 3F11 blot was retreated with the 1-1-M MAb and $^{125}$I-labeled secondary antibody probe and autoradiographed. (A) Silver stain. (B) Immunoblot; treatment with 3F11 and then 1-1-M; 18-h exposure. (C) Immunoblot; treatment with 3F11 and then 1-1-M; 5-h exposure. (D) Immunoblot; treatment with 3F11 only. LOSs were as follows: SR (lane 1), F62 (lane 2), GC56 (lane 3), WR213 (lane 4), JW31 (lane 5), JW31R (lane 6), DOV (lane 7), GC33 (lane 8), GC39 (lane 9) GC13 (lane 10), DAV (lane 11), 6611-33 (lane 12), 8038-5 (lane 13), WR220 (lane 14), A9 (lane 15). Arrows pointing to the right indicate 1-1-M-positive components, and arrows pointing to the left indicate 3F11-positive components in either the silver-stained gels (panel A) or the immunoblots (panels B, C, and D). Arrows are provided for those components that are particularly difficult to distinguish. The reactivity of each LOS with each antibody as determined by SPRIA is shown below the immunoblots. SR, F62, and GC56 LOSs (lanes 1, 2, and 3, respectively) did not stain well in this gel but were present in sufficient quantity (especially SR and GC56) to bind both 3F11 and 1-1-M MAbs. The dark blotch above the sample 15 LOS component in panels B and C is an artifact.

LOS (Fig. 2 lane 6) bound MAb 2-1-L8 to a fast-migrating component but bound 3F11 to a slower migrating one.

JW31 LOS (Fig. 2, lane 3) bound each of three MAbs, 3F11, 1-1-M, and 1-1-G, to separate components. 1-1-G bound to a very fast migrating component; 3F11 bound a component with an intermediate $M_r$, and 1-1-M bound a slowly migrating component that stained poorly with silver.

Those LOSs that bound both MAbs 3F11 and 1-1-M strongly in the SPRIA always bound them to separate components (Fig. 3). The weak positivity of 1-1-M for GC33 LOS (Fig. 3, lane 8) was due to high background binding. A component that bound 3F11 was present in every LOS tested except JW31R. MAb 06B4 bound either to the same component or to a component with the same $M_r$. MAbs 2C7 and 3G9 bound to the 3F11- and 06B4-binding components of some LOSs (e.g., JW31) but to components with different $M_r$s of other LOSs (e.g., WR220) (unpublished data). With all LOSs tested so far with both 2C7 and 3G9 (GC56, WR213, JW31) the two antibodies bound to a component with the same $M_r$. WR213 and DOV LOS bound 3F11 and 1-1-M each to multiple and separate components with different $M_r$s (Fig. 3, lanes 4 and 7).

Purified LOS requires NaOH hydrolysis for optimal expression of the 3F11- and 1-1-M-defined epitopes, but the epitopes were affected differently (Fig. 4). The fast-migrating 3F11-binding LOS component was optimally expressed after treatment with 50 mM NaOH and was weakly expressed after treatment in concentrations of >100 or <12.5 mM. The 1-1-M-binding component was less affected, but it showed some loss of expression in NaOH concentrations of >100 mM. Decreased silver staining of components occurred at concentrations of NaOH that resulted in increased antigenic expression of the MAb-defined epitope on that component (>12.5 mM NaOH; Fig. 4B and C). Aggregated LOSs bound 1-1-M MAb but not 3F11 (Fig. 4C).

MAbs 3F11 and 06B4 had a similar binding pattern with the gonococcal LOSs tested (Table 1). Also, by SDS-PAGE and immunoblot analysis each gonococcal LOS tested to date (F62, WR213, DOV, WR220, WR302, SR) bound 3F11 and 06B4 to a component with an indistinguishable $M_r$

(unpublished data). Competitive binding studies were performed to further characterize this similarity. The ability of five MAbs to compete with $^{125}$I-labeled 06B4 for binding to LOS and OMC from WR220 is compared in Fig. 5. As expected, 3F11 competed to a level equivalent to that of the homologous 06B4 MAb for both OMC and LOS. MAb 2-1-L8 competed to a lesser extent; it competed slightly better when 06B4 bound to LOS than to OMC. MAbs 1-1-M, 1-1-G, 2C7, and 3G9 did not compete with $^{125}$I-labeled 06B4 for binding to either antigen; MAbs 1-1-M and 1-1-G were included as negative controls in that they exhibited no binding to 220 LOS in SPRIA (Table 1).

## DISCUSSION

Wild-type members of the family *Enterobacteriaceae* have long-chain polysaccharides built up from repeating units of oligosaccharides. During growth these bacteria can undergo a spontaneous loss of O antigen (R mutant), resulting in a loss of pathogenicity, enhanced susceptibility to complement lysis, and phagocytosis.

Because of the absence of O-repeating units, the LOS of *N. gonorrhoeae* has been considered to be an R-type LPS (19). The antigenic heterogeneity which has been identified among the LOSs of the gonococcus (1, 3) and the meningococcus (15, 20, 21, 31, 33, 38, 39) and the variable susceptibility of gonococcal strains to complement lysis suggests that the *Enterobacteriaceae* model does not apply to the LOSs of neisserial strains. Results of the studies presented here demonstrate that gonococcal LOS consists of individual LOS components that are separable by SDS-PAGE and antigenically discrete and that do not represent minor modifications of one another.

Exact assignment of immunoblotted components to silver-stained components was difficult because of the lateral diffusion of energy released by decay of $^{125}$I and because some components that bound MAb failed to stain with silver. We tried labeling LOS extrinsically with $^{125}$I (34) and using it as a reference in immunoblot analysis, but it proved unsuitable because of the resulting change in $M_r$ of each of the components and the excessive labeling of some components relative to others. An enzyme-linked second antibody may be more useful for particularly difficult reference problems. Extending the time of development did not improve silver staining of these components. Failure of an LOS to stain with silver could result from a number of factors: (i) the presence in the LOS of glycoses, linkages insensitive to periodate oxidation, or both; (ii) the presence of the LOS component in very low quantity; (iii) selective elution of some LOS components during staining; or (iv) the fact that the LOS component was conformed such that expression of the epitope, but not to periodate oxidation, was permitted. The poor silver staining of certain LOSs (Fig. 3A, lanes 1, 2,

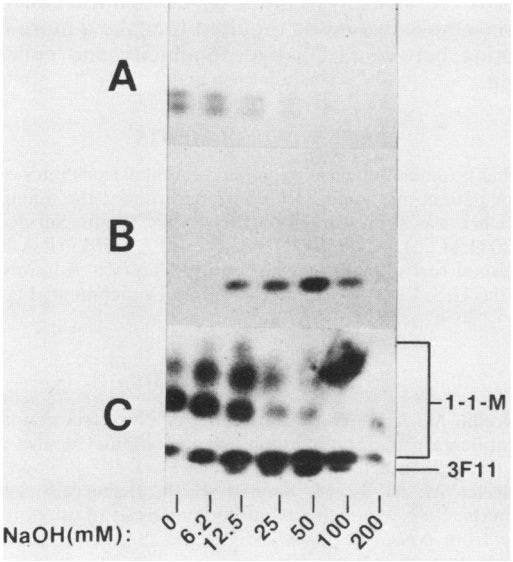


FIG. 4. Effect of NaOH treatment on the antigenicity of electroblotted F62 LOS. LOS was heated at 56°C for 3 h in various concentrations of NaOH (from 0 to 200 mM). LOSs were separated by PAGE and then electroblotted. The electroblot was incubated with 3F11 MAb and then $^{125}$I-labeled secondary antibody probe before autoradiography. After autoradiography the 3F11-treated blot was retreated with 1-1-M MAb and then $^{125}$I-secondary antibody probe. (A) Silver stain. (B) Immunoblot; treatment with 3F11 MAb. (C) Immunoblot; treatment with 3F11 MAb and then 1-1-M MAb. The diffuse smear observed above the 100 mM NaOH sample is an artifact.

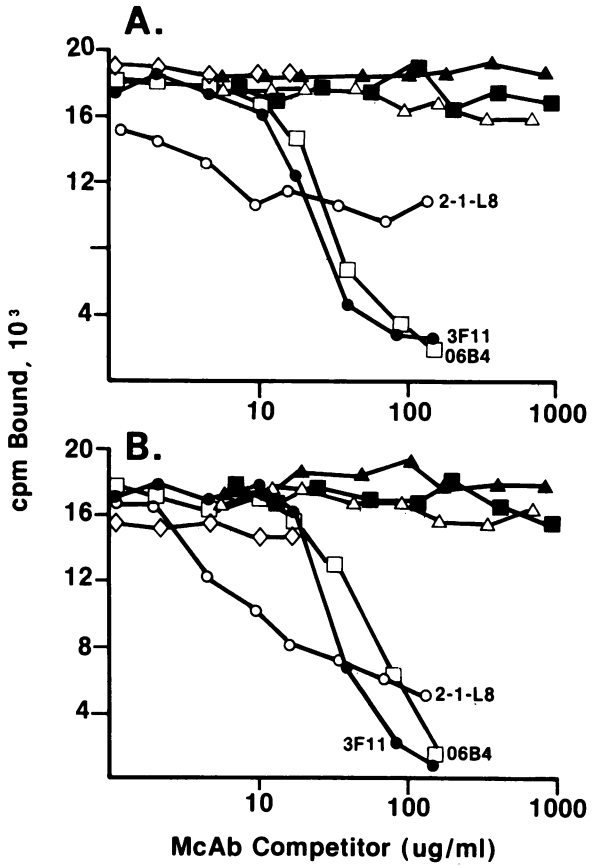


FIG. 5. Competitive inhibition of $^{125}$I-labeled 06B4 binding to WR220 OMC and WR220 LOS by various MAbs. Twofold dilutions of different MAbs were incubated in plastic wells sensitized with either WR220 OMC or WR220 LOS. An equal volume (25 μl) of a dilution of $^{125}$I-labeled 06B4 containing approximately $2 \times 10^4$ cpm of specific antibody was added, and the mixture was incubated for 3 h. All incubations were carried out at room temperature. The antibody mixture was removed; the wells were washed with filter and then PBS; and then the wells were dried, cut, and then counted. The $^{125}$I-labeled 06B4 cpm bound were plotted versus the competitor MAb concentration at each dilution. The antigens used to sensitize the wells were WR220 OMC (A) and WR220 LOS (B). Competitive MAbs were 3F11 (●), 2-1-L8 (○), 1-1-M (▲), 2C7 (△), 3G9 (■), 06B4 (□), 1-1-G (◇).

and 3) and LOS components (Fig. 3A, lanes 4, 7, and 12) may also reflect the effect of NaOH hydrolysis (Fig. 4A).

For the most part, each LOS expressed at least one epitope recognized by one of the MAbs, and those LOSs that expressed multiple epitopes generally had components with different $M_r$s positive for MAbs. The relative proportion of components may vary among LOSs (21, 24, 26, 33); and expression of individual components, identified by both similar $M_r$s and MAb-binding patterns, quantitatively differed among LOSs from different strains. The differences in saturation binding to different LOSs by MAbs (Fig. 1) may reflect variations in the quantitative expression of LOS components that bear the respective epitopes or differences in avidity of the MAbs for an epitope shared among different strains but modified by small variations in chemical structures.

Some epitopes appeared to be expressed on more than one LOS component. WR213 and DOV LOSs each bound 3F11 and 1-1-M to duplicate but alternating LOS components with different $M_r$s on SDS-PAGE (Fig. 3, lanes 4 and 7). We presume that these two LOSs contain common glycose sequences, since antigenicity appeared to reside in the oligosaccharide moiety of gonococcal LOS (36). In addition, the alternating component binding suggested that if multiple components of gonococcal LOS represent sequential steps in the synthesis of a final product (the highest $M_r$ component), then epitopes within these components are not always expressed.

Some LOS components appeared to express more than one epitope. MAbs 3F11 and 06B4 had very similar binding patterns for the tested LOS (Table 1) and appeared to bind to a component with the same $M_r$, estimated as 4,800 (24), within these LOSs. However, saturation binding to some gonococcal LOSs and the patterns of binding to meningococcal LOSs (unpublished data) are different for these two MAbs, indicating that they do not recognize identical epitopes.

Because the appearance of binding of an MAb to the same LOS component could have resulted from the fact that we failed to resolve separate components with similar or identical $M_r$s by SDS-PAGE, we performed competitive binding experiments to determine if the 3F11 and 06B4 MAb-defined epitopes were on a single component. The epitopes they defined appeared to be closely associated in both extracted LOSs and native LOSs present in the OMC of WR220 (Fig. 5). However, MAb 2-1-L8 also competed with 06B4 for binding to WR220 LOS, albeit to a lesser degree, and yet the former bound a WR220 LOS component that had a different $M_r$ than that which bound 3F11 and 06B4 (Fig. 2, lane 6). Thus, steric hindrance, or negative cooperativity, can occur in SPRIAs among antibodies that bind to different but closely associated LOS components (29).

Epitopes may be artificially adjacent in purified LOS micelles, compared with their positions when the LOSs are associated within the membrane with proteins, phospholipids, and other LOS components. MAbs 2C7 and 3G9 bound to the same LOS components but to different components than 06B4 and did not compete with 06B4 binding to WR220 LOS. The epitopes that 2C7 and 3G9 recognized in WR220 LOS were on different components (4,300 and 4,900 $M_r$s) from the 06B4 epitope (4,800 $M_r$) (data not shown). The lack of competition by 2C7 and 3G9 MAbs indicated that their epitopes must be spatially well separated within the outer membrane from the 06B4-defined epitope. The less effective competition by MAb 2-1-L8 for the 06B4-defined epitope when WR220 OMC was used as antigen than when purified LOS was used may reflect increased spatial separation of the two epitopes when the LOSs that express them are in their native conformation within the membrane.

Whereas MAbs 2C7 and 3G9 recognized epitopes on a component of WR220 LOS that did not bind 06B4, they bound to epitopes on the same LOS component of JW31 as did 06B4 (data not shown). If these data do not reflect incomplete separation of JW31 LOS components, they imply that glycose sequences are independently assembled by different strains. This has not been the case for the 2-1-L8 MAb-defined epitope that has been expressed within a 3.6-kilodalton LOS of all strains that bind this MAb that we have tested to date (24).

All the strains we tested except the pyocin-resistant mutant JW31R elaborated the 4.8-kilodalton LOS that expresses the epitopes recognized by 3F11 and 06B4. The failure of JW31R to express an epitope recognized by 3F11 has been reported previously (17). JW31R LOS consists of a major 4.5-kilodalton component with an intermediate $M_r$ compared with the multiple LOS components of its parent strain JW31 (5, 26), and smaller than the 4.8-kilodalton component that binds 3F11 and 06B4.

The 1-1-M-defined LOS epitope was more stable to alkaline hydrolysis than the 3F11-defined epitope (Fig. 4). Although mild NaOH treatment (12.5 to 100 mM) was required for optimal expression of the 3F11-defined epitope, high concentrations of NaOH (200 mM) decreased expression. Alkaline hydrolysis deacylates glycolipids, removing fatty acids and acetyl groups. It also reduces aggregation (16), presumably because of diminished hydrophobicity because of the loss of acyl-linked fatty acids. These results indicate that the expression of the epitope defined by 3F11 is more dependent on the presence (and absence) of undefined acyl-linked constituents, whereas that defined by 1-1-M is not. However, an analysis of the exact chemical constituents of these samples would be required to make a more definite correlation between LOS hydrophobicity and epitope expression.

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