

Further Antigenic Similarities of *Neisseria gonorrhoeae* Lipooligosaccharides and Human Glycosphingolipids†

ROBERT E. MANDRELL‡

Centre for Immunochemistry, Veterans Administration Medical Center, 4150 Clement Street,
San Francisco, California 94121

Received 2 March 1992/Accepted 14 April 1992

Anticarbohydrate monoclonal antibodies were tested for their ability to bind to various strains of *Neisseria*. A monoclonal antibody that binds to the ganglio-series glycosphingolipid, ganglio-*N*-triaosylceramide, also bound to strains of *Neisseria gonorrhoeae* but not to other species of *Neisseria*. An antibody specific for the globo-series glycosphingolipid, globotriaosylceramide, also bound to strains of *N. gonorrhoeae*, *Neisseria meningitidis*, *Neisseria lactamica*, and *Branhamella catarrhalis* but not to any other strains of nonpathogenic *Neisseria*.

The lipooligosaccharides (LOS) of pathogenic and nonpathogenic *Neisseria* species are composed of multiple components that are different antigenically and chemically (5). A number of monoclonal antibodies (MAbs) that bind to epitopes in the LOS of *Neisseria gonorrhoeae* (17), *Neisseria meningitidis* (14, 17), *Neisseria lactamica* (13), and *Haemophilus influenzae* (21, 27) have been identified, and these epitopes are similar antigenically to epitopes on mammalian glycosphingolipids (GSL). One group of MAbs binds to lactoneo-series (Gal β 1 \rightarrow 4GlcNAc β 1 \rightarrow 3Gal-R) GSL (18). The LOS epitope bound by these MAbs can be sialylated in gonococci (20), meningococci (19), and *H. influenzae* (21). A second group of MAbs presumably binds to terminal Gal α 1 \rightarrow 4Gal residues in the LOS strains of *Haemophilus* and *Neisseria*. This conclusion was based on the specific inhibition of anti-LOS MAbs by Gal α 1 \rightarrow 4Gal disaccharides (27) and the binding of a MAb specific for the GSL antigen, P^k (Gal α 1 \rightarrow 4Gal β 1 \rightarrow 4Glc-ceramide), to strains of *H. influenzae* (21) and to a pyocin-resistant mutant strain of *N. gonorrhoeae* (8).

Recent chemical studies have confirmed that lacto-*N*-neotetraose (Gal β 1 \rightarrow 4GlcNAc β 1 \rightarrow 3Gal β 1 \rightarrow 4Glc) (8, 29) and globotriaose (Gal α 1 \rightarrow 4Gal β 1 \rightarrow 4Glc) (8) are part of the chemical structure of some gonococcal LOS components. These previous results suggested that there might be other examples of LOS antigenic mimicry. In an attempt to identify such antigens, a number of anticarbohydrate MAbs that bind to GSL have been analyzed for their ability to bind to strains of *Neisseria* and *Haemophilus*.

The MAbs were screened first in a solid-phase radioimmunoassay (17, 33) with whole bacteria as antigens. Strains of *Neisseria* were grown as described previously (23), and strains of *Haemophilus* were grown on plates of chocolate agar at 35°C (21). Bacteria were suspended in phosphate-buffered saline (PBS) to an optical density (at 620 nm) of 0.15. Polyvinyl microtiter wells were sensitized with the whole bacteria as described previously (1). Each anticarbohydrate MAb was tested at a range of concentrations with each bacteria. The MAbs tested were SH-34, 103HT30,

Gal-13, 3D9 (anti-P^k), 7B6 (anti-P₁), 5C11 (anti-Gal-Gal), 2D4, and MC-480. MAbs 3D9, 7B6, and 5C11 were purchased from Accurate Chemical Inc. (Westbury, N.Y.). MAbs 2D4 and MC-480 were purchased from the American Type Culture Collection (Rockville, Md.) and the Developmental Studies Hybridoma Bank (Camden, N.J.), respectively.

The carbohydrate sequence specificities of the MAbs are shown in Table 1. Five of the eight MAbs bound to at least one of the strains or antigens. These MAbs were 2D4 (binds to asialo-GM2: GalNAc β 1 \rightarrow 4Gal β 1 \rightarrow 4Glc-ceramide [cer]), anti-P^k (anti-Gal α 1 \rightarrow 4Gal β 1 \rightarrow 4Glc-cer), anti-P₁ (anti-Gal α 1 \rightarrow 4Gal β 1 \rightarrow 4GlcNAc-R), anti-Gal-Gal (anti-Gal α 1 \rightarrow 4Gal-R), and SH-34 (binds to asialo-GM1: Gal β 1 \rightarrow 3GalNAc β 1 \rightarrow 4Gal β 1 \rightarrow 4Glc-cer). The anti-P^k MAb had been shown previously to bind to the LOS of *H. influenzae* strains (21) and to an LOS of the gonococcal pyocin-resistant mutant strain 1291B (8). One of the two different anti-asialo-GM1 MAbs (SH-34 and 103HT30), MAb SH-34 bound to both gonococcal and meningococcal LOS, but 103HT30 did not bind to any strains tested. The terminal Gal β 1 \rightarrow 3GalNAc disaccharide presumably recognized by anti-asialo-GM1 was of interest, since this structure also can be O-linked to mucin glycoproteins (15) and is present in human cervical mucin (32). However, the results indicated that these two MAbs had very different specificities and suggested that MAb SH-34 was not specific only for ganglio-triaose. Also, MAb MC-480, specific for stage-specific embryonic antigen-3, which has a terminal Gal β 1 \rightarrow 3GalNAc disaccharide, did not bind to any of the strains. Because of its broad specificity, MAb SH-34 was not characterized further in this study. The variable amount of binding of some of the MAbs to different strains indicated that there was a wide range of epitope concentrations expressed among the strains (data not shown).

Table 2 shows the percentage of pathogenic and nonpathogenic *Neisseria* strains that bound MAb 2D4, anti-P^k, and anti-P₁. The anti-P^k MAb bound to strains of gonococci, meningococci, and *N. lactamica* and also to both strains of *Branhamella catarrhalis*. The largest percentage of neisserial strains binding the anti-P^k MAb (51%) were gonococci. The anti-Gal-Gal and anti-P₁ MAbs bound to only a few strains, and they bound to strains different from those that bound MAb anti-P^k, indicating that the bacterial epitopes binding the MAbs were different. The binding of anti-P₁

† Report no. 61 from the Centre for Immunochemistry of the University of California, San Francisco.

‡ Present address: Box 0868, University of California, San Francisco, San Francisco, CA 94143.

TABLE 1. Binding of anticarbohydrate MAbs to strains of *Neisseria* in solid-phase radioimmunoassay

MAb	Structure recognized ^a	Common name ^b	Reference	Binding of MAb to strains of ^c :	
				<i>Neisseria</i>	<i>Haemophilus</i>
3D9	Gal α 1 \rightarrow 4Gal β 1 \rightarrow 4Glc-cer	P ^k	2, 21	+	+
7B6	Gal α 1 \rightarrow 4Gal β 1 \rightarrow 4GlcNAc-R	P ₁	2	+	-
5C11	Gal α 1 \rightarrow 4Gal-R	Gal-Gal	22	+	NT
2D4	GalNAc β 1 \rightarrow 4Gal β 1 \rightarrow 4Glc-cer	Asialo-GM2	31	+	-
SH-34	Gal β 1 \rightarrow 3GalNAc β 1 \rightarrow 4Gal β 1-4Glc-cer	Asialo-GM1	25	+	-
103HT30	Gal β 1 \rightarrow 3GalNAc β 1 \rightarrow 4Gal β 1-4Glc-cer	Asialo-GM1	7	-	NT
MC-480	Gal β 1 \rightarrow 3GalNAc β 1 \rightarrow 3Gal α 1-R	SSEA-3	9	-	NT
Gal-13	Gal α 1 \rightarrow 3Gal β 1 \rightarrow 4GlcNAc β 1-R	CPH	4	-	NT

^a Only the terminal di- or trisaccharide of a GSL that bound the MAb is shown for MAbs 7B6, 5C11, MC-480, and Gal-13. Although this is a proposed specificity for the MAb, it is possible that the MAb binds to other similar structures with varying affinities.

^b SSEA-3, stage-specific embryonic antigen-3; CPH, ceramide penta-hexa-oxide (for CPH, R = 3Gal β 1 \rightarrow 4Glc-cer).

^c Each MAb was tested with at least 20 strains, but not all of the same strains were tested with each MAb. +, at least one antigen bound the MAb; -, no antigens bound the MAb; NT, not tested.

suggested that a residue with galactose that was linked α to a Gal β 1 \rightarrow 4GlcNAc β 1 \rightarrow 3Gal residue might be present in some gonococcal LOS. Structural analysis of a gonococcal LOS that is strongly positive for the anti-P₁ MAb will confirm whether this structure exists. MAb 2D4 (anti-asialo-GM2) bound to 54% of the gonococcal antigens tested. It did not bind to any strain of meningococci, *N. lactamica*, nonpathogenic *Neisseria*, or *H. influenzae*. These results indicated that the LOS epitope defined by MAb 2D4 was specific for strains of *N. gonorrhoeae*.

The results of previous studies characterizing other anti-GSL and anti-LOS MAbs (18, 27) implied that MAbs 2D4 and anti-P^k had probably bound to LOS. To confirm this, LOS were extracted from two strains that had bound the MAbs in solid-phase radioimmunoassay, separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (16), and then either stained with silver (26) or transferred to nitrocellulose for analysis with MAbs (17). After being incubated with the appropriate MAb, the nitrocellulose was washed and incubated with an alkaline phosphatase-conjugated goat anti-mouse immunoglobulin secondary antibody (24).

Figure 1 shows the silver stain and immunoblot of the purified LOS from *N. gonorrhoeae* GC33 and *N. meningitidis* group Y 8032. The LOS of the gonococcal strain GC33 is heterogeneous and, therefore, migrated diffusely in SDS-PAGE (Fig. 1A, lane 1). The anti-P^k MAb bound two closely migrating bands within the diffuse mixture of LOS (Fig. 1B,

lane 1); these bands represented the more slowly migrating molecules shown in the diffuse silver-stained LOS (Fig. 1A, lane 1). Anti-P^k did not bind to 8032 LOS (Fig. 1B, lane 2), which was included as a molecular weight marker and as a negative control. MAb 3F11, which binds to a 4.5-kDa LOS band present in most gonococcal and group B and C meningococcal strains (17), bound to a component of GC33 LOS that migrated slightly ahead of the bands that bound anti-P^k (data not shown).

The binding of anti-asialo-GM2 (2D4) to the LOS of gonococcal strains 15373 and F62 is shown in Fig. 2. The LOS components of strains 15373 and F62 that bound anti-asialo-GM2 (Fig. 2A, lanes 2 and 3) had molecular masses of approximately 5.2 and 5.1 kDa, respectively. The MAb did not bind to meningococcal strain 8032 LOS (negative control). Anti-asialo-GM2 also bound to strain F62 LOS that migrated diffusely above the major 5.1-kDa band (Fig. 2B, lane 3).

Finally, since the anti-P^k and anti-asialo-GM2 MAbs were tested as ascites fluids, it was important to confirm that the antibody binding specifically to LOS was the same as the antibody binding to the carbohydrate of the GSL. For this analysis, purified asialo-GM2 was preincubated with anti-asialo-GM2 MAb and the inhibition of the binding of the antibody to purified F62 LOS was determined. Asialo-GM2 was diluted in a solution of chloroform-methanol-hexane (1:25:5.25) containing 0.08% polyisobutylmethacrylate (Polysciences Inc., Warrington, Pa.), and 10- μ l samples

TABLE 2. Activity of the anti-P^k, anti-P₁, and anti-asialo-GM2 MAbs with strains of various species of *Neisseria* and of *B. catarrhalis* in solid-phase radioimmunoassay

Species	Activity with ^a :								
	Anti-P ^k			Anti-P ₁			Anti-asialo-GM2		
	Total no. of strains	No. positive	% Positive	Total no. of strains	No. positive	% Positive	Total no. of strains	No. positive	% Positive
<i>N. gonorrhoeae</i>	70	36	51	70	8	11	44	24	54
<i>N. meningitidis</i>	63	6	9	12	0	0	12	0	0
<i>N. lactamica</i>	10	3	30	10	0	0	10	0	0
Other <i>Neisseria</i> spp. ^b	10	0	0	NT	NT	NT	10	0	0
<i>B. catarrhalis</i>	2	2	100	NT	NT	NT	2	0	0

^a A strain was considered positive if it bound >10% the amount of MAb that resulted in maximum binding to a positive control antigen. NT, not tested.

^b Other nonpathogenic *Neisseria* spp. tested were *Neisseria sicca* (two strains), *Neisseria cinerea* (two strains), *Neisseria mucosa* (two strains), and *Neisseria perflava* (four strains).

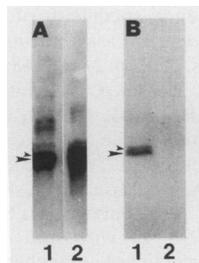


FIG. 1. Binding of anti- P^k MAb to LOS. LOS of *N. gonorrhoeae* GC33 (lane 1) and *N. meningitidis* 8032 (lane 2) were separated by SDS-PAGE, and then the LOS were silver stained (A) or transferred to nitrocellulose and immunostained with anti- P^k (B). The arrowheads point to the closely migrating major and minor bands positive for anti- P^k . Anti- P^k does not bind to strain 8032 LOS (negative control).

containing 40 to 2,500 ng of GSL were added to microtiter wells. After the solvent evaporated, the wells were washed with a blocking reagent (1% casein–10 mM Tris–150 mM NaCl–30 mM sodium azide, pH 7.5), MAb 2D4 (anti-asialo-GM2) was added to each well (approximately 20 ng), and the wells were incubated for 14 h at room temperature. The liquid was removed from each well and transferred to a second well sensitized with purified F62 LOS (50 μ g/ml), and the wells were incubated for 2 h at room temperature. The liquid was removed, each well was washed with blocker and PBS (50 mM, pH 7.2), and then the amount of MAb bound was detected as described previously (17). In this assay, 50% of anti-asialo-GM2 was inhibited from binding to purified F62 LOS by approximately 200 ng of purified asialo-GM2 (data not shown). There was no inhibition with asialo-GM1 in a similar assay. MAb 2D4 bound only to a single band of the purified, and thin-layer-chromatography-separated, asialo-GM2; it did not bind to asialo-GM1 (data not shown) (3). In a similar assay, the anti- P^k MAb could be prevented from binding to purified GC33 LOS by Gal α 1 \rightarrow 4Gal disaccharide linked to bovine serum albumin (BSA) by a cross-linker (Carbohydrates International, Arlöv, Sweden). The Gal α 1 \rightarrow 4Gal-BSA at a concentration of 50 μ g/ml inhibited 50% of the maximal binding of approximately 2 μ g of MAb anti- P^k per ml to a microtiter well sensitized with LOS (50 μ g/ml). These results confirmed that the antibodies binding to the LOS were the same as those specific for the carbohydrate epitopes of the GSL.

The anti-asialo-GM2 MAb bound only to gonococci (Table 2) and only to strains possessing an LOS component with a molecular mass of \geq 5.1 kDa. This LOS component is

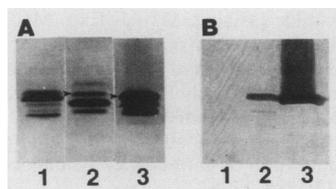


FIG. 2. Binding of anti-asialo-GM2 MAb (2D4) to LOS. LOS of *N. meningitidis* 8032 (lane 1) and *N. gonorrhoeae* 15373 (lane 2) and F62 (lane 3) were separated in SDS-PAGE and silver stained (A) or transferred to nitrocellulose and immunostained with anti-asialo-GM2 (B). The arrowheads point to the bands that bind anti-asialo-GM2. Note the diffusely migrating LOS in lane 3 (A) that binds MAb 2D4 (B).

identical to (or comigrates in the same region as) the LOS that binds MAb 1-1-M (17, 30). Both MAbs bind only to LOS of gonococci, indicating that they recognize strain-specific epitopes; however, their binding patterns with different gonococcal strains indicate that the MAbs bind to separate epitopes (data not shown). In contrast, the anti- P^k MAb bound to both pathogenic and nonpathogenic strains of *Neisseria* (Table 2) and, as shown in previous studies, to strains of *H. influenzae* (21). However, a structure apparently similar to P^k and present on *H. influenzae* LOS (digalactoside; 4C4 epitope) (27) has been implicated in virulence. These results suggest either that the epitopes are different or that the epitopes play a different role on different species of mucosal pathogens. Possibly, the P^k -like antigen on *Neisseria* strains is related to the organism's ability to exist as a commensal and/or in a state of temporarily asymptomatic infection. The P^k antigen is present in a number of human tissues, including cells of the uroepithelial system (12).

The binding of these anticarbohydrate MAbs to LOS indicates only that the bacterial LOS are similar antigenically to human GSL. However, recent studies have shown conclusively that a pentaose in the larger component of strain F62 LOS is as follows: GalNAc β 1 \rightarrow 3Gal β 1 \rightarrow 4GlcNAc β 1 \rightarrow 3Gal β 1 \rightarrow 4Glc, which is V³-(β -N-acetylgalactosaminyl)lactoneotetraose (29), a structure which is identical to pentaoses of the X₂ (10) and asialo-G₃ (28) GSL present in human erythrocyte membranes. This would be consistent with the results of subsequent studies in which MAb 2D4 bound to the X₂ GSL as well as to asialo-GM2 (11). Thus, it is probable that MAb 2D4 bound to the GalNAc β 1 \rightarrow 3Gal residue at the terminus of the large mass component of F62 LOS.

Galactose that is linked α 1 \rightarrow 4 to galactose of lactosylceramide (Gal β 1 \rightarrow 4Glc-cer) defines the globo-series GSL present in mammalian cells; Gal β 1 \rightarrow 4GlcNAc that is linked β 1 \rightarrow 3 to galactose of lactosylceramide defines the neolacto-series GSL (6). The presence of multiple LOS structures that mimic human GSL would provide the gonococcus with molecular diversity within the varied environments encountered in humans. MAb-defined LOS differences among strains of *Neisseria* and *Haemophilus* that can be related to differences in the glycoconjugate network on the surface of cells in these environments might be helpful in achieving a better understanding of the mechanisms of pathogenesis of these bacteria.

I thank M. Apicella for providing strains of *Neisseria* and *Haemophilus* and T. Higgins (SH-34), B. Zalc (103HT30), U. Galili (Gal-13), and H. Leffler (asialo-GM1 and asialo-GM2) for supplying samples of MAbs or GSL. I also thank J. M. Griffiss and the members of the Centre for Immunochemistry for their support and encouragement during these studies.

This work was supported by the Research Service of the Department of Veterans Affairs and by Public Health Service grants AI21620 and AI24616 from the National Institutes of Health.

REFERENCES

1. Abdillahi, H., and J. T. Poolman. 1987. Whole-cell ELISA for typing *Neisseria meningitidis* with monoclonal antibodies. *FEMS Microbiol. Lett.* **48**:367–371.
2. Brodin, N. T., J. Dahmén, B. Nilsson, L. Messeter, S. Mårtensson, J. Heldrup, H. O. Sjögren, and A. Lundblad. 1988. Monoclonal antibodies produced by immunization with neoglycoproteins containing Gal α 1 \rightarrow 4Gal β 1 \rightarrow 4Glc β -O and Gal α 1 \rightarrow 4Gal β 1 \rightarrow 4GlcNAc β -O residues: useful immunochemical and

- cytochemical reagents for blood group P antigens and a differentiation marker in Burkitt lymphoma and other B-cell malignancies. *Int. J. Cancer* 42:185-194.
3. Buehler, J., and B. A. Macher. 1986. Glycosphingolipid immunostaining: detection of antibody binding with an avidin-biotin enzyme system. *Anal. Biochem.* 158:283-287.
 4. Galili, U., C. B. Basbaum, S. B. Shohet, J. Buehler, and B. A. Macher. 1987. Identification of erythrocyte Gal α 1 \rightarrow 3Gal glycosphingolipids with a mouse monoclonal antibody, Gal-13. *J. Biol. Chem.* 262:4683-4688.
 5. Griffiss, J. M., H. Schneider, R. E. Mandrell, R. Yamasaki, G. A. Jarvis, J. J. Kim, B. Gibson, R. Hamadeh, and M. A. Apicella. 1988. Lipooligosaccharides: the principal glycolipids of the neisserial outer membrane. *Rev. Infect. Dis.* 10:S287-S295.
 6. Hakomori, S. I. 1981. Glycosphingolipids in cellular interaction, differentiation, and oncogenesis. *Annu. Rev. Biochem.* 50:733-764.
 7. Jacquemart, F., G. Millot, C. Goujet-Zalc, G. Mahouy, and B. Zalc. 1988. Production and characterization of a mouse monoclonal antibody to the glycolipid asialo-GM1. *Hybridoma* 7:323-331.
 8. John, C. M., J. M. Griffiss, M. A. Apicella, R. E. Mandrell, and B. W. Gibson. 1991. The structural basis for pyocin-resistance in *Neisseria gonorrhoeae* lipooligosaccharides. *J. Biol. Chem.* 266:19303-19311.
 9. Kannagi, R., N. A. Cochran, F. Ishigami, S. I. Hakomori, P. W. Andrews, B. B. Knowles, and D. Solter. 1983. Stage-specific embryonic antigens (SSEA-3 and -4) are epitopes of a unique globo-series ganglioside isolated from human teratocarcinoma cells. *EMBO J.* 2:2355-2361.
 10. Kannagi, R., M. N. Fukuda, and S. I. Hakomori. 1982. A new glycolipid antigen isolated from human erythrocyte membranes reacting with antibodies directed to globo-N-tetraosylceramide (globoside). *J. Biol. Chem.* 257:4438-4442.
 11. Kannagi, R., and S. I. Hakomori. 1986. Monoclonal antibodies directed to carbohydrate antigens, p. 117.1-117.20. *In* D. M. Weir (ed.), *Handbook of experimental immunology*, 4th ed., vol. 4. Blackwell Scientific Publications Ltd., Oxford.
 12. Kasai, K., J. Galton, P. I. Terasaki, A. Wakisaka, M. Kawahara, T. Root, and S. I. Hakomori. 1985. Tissue distribution of the P^x antigen as determined by a monoclonal antibody. *J. Immunogenet.* 12:213-220.
 13. Kim, J. J., R. E. Mandrell, and J. M. Griffiss. 1989. *Neisseria lactamica* and *Neisseria meningitidis* share lipooligosaccharide epitopes but lack common capsular and class 1, 2, and 3 protein epitopes. *Infect. Immun.* 57:602-608.
 14. Kim, J. J., R. E. Mandrell, H. Zhen, M. A. J. Westerink, J. T. Poolman, and J. M. Griffiss. 1988. Electromorphic characterization and description of conserved epitopes of the lipooligosaccharides of group A *Neisseria meningitidis*. *Infect. Immun.* 56:2631-2638.
 15. Kobata, A. 1984. The carbohydrates of glycoproteins, p. 87-161. *In* V. Ginsburg and P. W. Robbins (ed.), *Biology of carbohydrates*, vol. 2. John Wiley and Sons, New York.
 16. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* 227:680-685.
 17. Mandrell, R., H. Schneider, M. Apicella, W. Zollinger, P. A. Rice, and J. M. Griffiss. 1986. Antigenic and physical diversity of *Neisseria gonorrhoeae* lipooligosaccharides. *Infect. Immun.* 54:63-69.
 18. Mandrell, R. E., J. M. Griffiss, and B. A. Macher. 1988. Lipooligosaccharides (LOS) of *Neisseria gonorrhoeae* and *Neisseria meningitidis* have components that are immunologically similar to precursors of human blood group antigens. Carbohydrate sequence specificity of the mouse monoclonal antibodies that recognize crossreacting antigens on LOS and human erythrocytes. *J. Exp. Med.* 168:107-126.
 19. Mandrell, R. E., J. J. Kim, C. M. John, B. W. Gibson, J. V. Sugai, M. A. Apicella, J. M. Griffiss, and R. Yamasaki. 1991. Endogenous sialylation of the lipooligosaccharides of *Neisseria meningitidis*. *J. Bacteriol.* 173:2823-2832.
 20. Mandrell, R. E., A. J. Lesse, J. V. Sugai, M. Shero, J. M. Griffiss, J. A. Cole, N. J. Parsons, H. Smith, S. A. Morse, and M. A. Apicella. 1990. *In vitro* and *in vivo* modification of *Neisseria gonorrhoeae* lipooligosaccharide epitope structure by sialylation. *J. Exp. Med.* 171:1649-1664.
 21. Mandrell, R. E., R. McLaughlin, Y. A. Kwaik, A. Lesse, R. Yamasaki, B. Gibson, S. M. Spinola, and M. A. Apicella. 1992. Lipooligosaccharides (LOS) of some *Haemophilus* species mimic human glycosphingolipids, and some LOS are sialylated. *Infect. Immun.* 60:1322-1328.
 22. Mårtensson, S., T. Brodin, A. S. Carlström, J. Dahmén, T. Frejd, A. Gunnarsson, U. Jansson, G. Magnusson, and A. Lundblad. 1986. A monoclonal antibody specific for non-reducing terminal Gal α 1 \rightarrow 4Gal residues. *Glycoconjugate J.* 3:163-174.
 23. Schneider, H., J. M. Griffiss, G. D. Williams, and G. B. Pier. 1982. Immunological basis of serum resistance of *Neisseria gonorrhoeae*. *J. Gen. Microbiol.* 128:13-22.
 24. Sidberry, H., B. Kaufman, D. C. Wright, and J. Sadoff. 1985. Immunochemical analysis by monoclonal antibodies of bacterial lipopolysaccharides after transfer to nitrocellulose. *J. Immunol. Methods* 76:299-305.
 25. Soloman, F. R., and T. J. Higgins. 1987. A monoclonal antibody with reactivity to asialo GM1 and murine natural killer cells. *Mol. Immunol.* 24:57-65.
 26. Tsai, C. M., and C. E. Frasch. 1982. A sensitive silver stain for detecting lipopolysaccharides in polyacrylamide gels. *Anal. Biochem.* 119:115-119.
 27. Virji, M., J. N. Weiner, A. A. Lindberg, and E. R. Moxon. 1990. Antigenic similarities in lipopolysaccharides of *Haemophilus* and *Neisseria* and expression of a digalactoside structure also present on human cells. *Microb. Pathog.* 9:441-450.
 28. Watanabe, K., and S. I. Hakomori. 1979. Gangliosides of human erythrocytes. A novel ganglioside with a unique N-acetylneuraminosyl-(2 \rightarrow 3)-N-acetylgalactosamine structure. *Biochemistry* 18:5502-5504.
 29. Yamasaki, R., B. E. Bacon, W. Nasholds, H. Schneider, and J. M. Griffiss. 1991. Structural determination of oligosaccharides derived from lipooligosaccharide of *Neisseria gonorrhoeae* F62 by chemical, enzymatic, and two-dimensional NMR methods. *Biochemistry* 30:10566-10575.
 30. Yamasaki, R., W. Nasholds, H. Schneider, and M. Apicella. 1991. Epitope expression and partial structural characterization of F62 lipooligosaccharide (LOS) of *Neisseria gonorrhoeae*. IgM monoclonal antibodies (3F11 and 1-1-M) recognize non-reducing terminus of F62 LOS components. *Mol. Immunol.* 28:1233-1242.
 31. Young, W. W., Jr., E. M. S. MacDonald, R. C. Nowinski, and S. I. Hakomori. 1979. Production of monoclonal antibodies specific for two distinct steric portions of the glycolipid ganglio-N-triosylceramide (asialo GM2). *J. Exp. Med.* 150:1008-1019.
 32. Yurewicz, E. C., F. Matsuura, and K. S. Moghissi. 1982. Structural characterization of neutral oligosaccharides of human midcycle cervical mucin. *J. Biol. Chem.* 257:2314-2322.
 33. Zollinger, W. D., J. M. Dalrymple, and M. S. Artenstein. 1976. Analysis of the parameters affecting the solid phase radioimmunoassay quantitation of antibody to meningococcal antigens. *J. Immunol.* 117:1788-1798.