Gonococcal Opacity: Lectin-like Interactions between Opa Proteins and Lipooligosaccharide

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Previous evidence from our laboratory suggested that the tight intercellular adhesions between the outer membranes of gonococci displaying the opacity colony phenotype occurred because Opa proteins expressed on one gonococcus adhered to the lipooligosaccharide (LOS) of the opposing bacterium (M. S. Blake, p. 51–66, *in* G. G. Jackson and H. Thomas, ed., *The Pathogenesis of Bacterial Infections*, 1985, and M. S. Blake and E. C. Gotschlich, p. 377–400, *in* M. Inouye, ed., *Bacterial Outer Membranes as Model Systems*, 1986). A noncompetitive inhibition assay used previously to determine the carbohydrate structures recognized by the major hepatic asialoglycoprotein receptor was modified to determine the gonococcal LOS structures that bind Opa proteins (R. T. Lee, Targeted Diagn. Ther. Ser. 4:65–84, 1991). The LOS carbohydrates used in these assays were LOS structures purified from pyocin LOS mutants of *Neisseria gonorrhoeae* 1291 described by K. C. Dudas and M. A. Apicella (Infect. Immun. 56:499–504, 1988) and further characterized by C. M. John et al. (J. Biol. Chem. 266:19303–19311, 1991). Purified gonococcal Opa proteins were incubated with each of the parent and mutant LOS, and the amount of binding of Opa proteins was measured by a direct enzyme-linked immunosorbent assay using the Opa-specific monoclonal antibody 4B12. The affinities of the Opa proteins for each of the LOS were determined indirectly by measuring the concentrations of Opa proteins that noncompetitively inhibited 50% of the binding of LOS-specific monoclonal antibodies. This concentration is inversely proportional to the affinity of the inhibitor (R. T. Lee, Targeted Diagn. Ther. Ser. 4:65–84, 1991). Our data suggest that the gonococcal Opa proteins tested had the highest affinity for the Galβ1-4GlcNAc residue present on the gonococcal lactoneoseries LOS. This affinity was comparable to that reported for the binding of the major hepatic asialoglycoprotein receptor to glycoconjugates containing terminal galactose and *N*-acetylgalactosamine (R. T. Lee, Targeted Diagn. Ther. Ser. 4:65–84, 1991). After sialylation of the lactoneoseries LOS, presumably on the terminal galactose residue, the interaction with the Opa proteins was ablated. Therefore, the gonococcal Opa-LOS and mammalian epithelial cell asialoglycoprotein receptor-carbohydrate interactions have quite similar specificities.

The gonococcal colony opacity phenotype was first described by Swanson (26–30) and James and Swanson (12). Using electron microscopy, Swanson observed that gonococci displaying the opaque colony phenotype had extensive intercellular adhesions between the individual cocci (29) which were not seen with bacteria lacking this phenotype. These intercellular adhesions could be dissociated by the use of such proteolytic enzymes as trypsin and chymotrypsin, suggesting that these membrane-membrane interactions were due in part to proteins on the gonococcal outer membrane. In support of this observation, it was shown that opaque gonococci expressed a family of extra proteins on their surfaces which have since become known as the Opa proteins (32). Organisms which do not express this family of proteins are known as transparent gonococci. Most gonococci expressing Opa proteins exhibit some degree of colonial opacity, varying from very opaque to slightly opaque. This degree of opacity is somewhat correlated to the number of Opa proteins expressed (31). However, investigators have described some Opa-bearing gonococci lacking in opacity (11). Some gonococcal strains carry upwards of 11 different Opa genes whose expression is independently determined (3). However, even gonococci expressing one particular Opa protein can differ in colony opacity. Thus, it seemed that

another factor(s) was also involved in the determination of this colony phenotype.

In both prokaryotic and eukaryotic systems, there are three basic mechanisms by which cell surface molecules can mediate cell-cell adhesion: (i) homophilic binding, in which a molecule on one cell binds to an identical molecule on an adjacent cell; (ii) heterophilic binding, in which a molecule on one cell binds to a different molecule on the adjacent cell; and (iii) a model in which cell surface receptors on adjacent cells are linked to one another through a third secreted multivalent linker molecule (2). Several lines of evidence suggest that the opacity phenotype is not generated by the association between two Opa proteins (homophilic cell adhesion). Cross-linking studies performed by McDade and Johnston (21) give no evidence that Opa proteins are close to or can be cross-linked to any other protein, including Opa itself. Purified Opa proteins behave as monomers on molecular sieve columns (5). Heterophilic binding between Opa and another cell surface protein was also ruled out by these same studies. This led us to look for another constituent of the gonococcal outer membrane which might participate in a heterophilic cell adhesion mechanism and produce the intercellular adhesions described by Swanson (29).

The principal nonproteinaceous component of the gonococcal outer membrane is the lipooligosaccharide (LOS). These glycolipids are embedded in the outer membrane by the lipid A moiety. The carbohydrate portion of the LOS is an oligosac-

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TABLE 1. Characteristics of strains used in this study

Strain	Structure[a]	Reactive MAb(s)	I_{50} $(10^{-10}$ M$)$[b]	$-\log I_{50}$[c]
1291 wild type	Galβ1-4GlcNAcβ1-3Galβ1-4Glc-R	3F11 and 6B4	2.71 ± 0.81	9.57 ± 0.15
1291a	GlcNAcβ1-3Galβ1-4Glc-R	4G8	16.30 ± 0.15	8.79 ± 0.04
1291c	Galβ1-4Glc-R	L8	6.44 ± 0.46	9.19 ± 0.11
1291d[d]	Glc-R	4C4	59.32 ± 7.2	8.23 ± 0.06
1291e[d]	R	6E4	>100	<7

[a] Carbohydrate structure as determined by John et al. (13).
[b] Molar amounts of Opa added to obtain 50% inhibition of the various MAbs. Values are means ± standard deviations.
[c] Values are means ± standard deviations.
[d] 1291d and 1291e contained both species of LOS.

charide which lacks the repeating O antigen polysaccharide found in lipopolysaccharides of enteric bacteria. A common feature of gonococcal LOS is the presence of lacto-*N*-neotetraose (Galβ1-4GlcNAcβ1-3Galβ1-4Glc) as the major oligosaccharide (13). Studies suggest that this tetrasaccharide is synthesized distally, a single sugar residue at a time. Thus, at any given time, LOS molecules with nonreducing termini composed of any of the four possible sugar residues are present within the outer membrane of the gonococcus. Mutants which lack the enzymes necessary for the addition of each of the particular sugar residues have been identified (10), and the precise LOS carbohydrate structure produced by these mutants has been determined (13). Recently, Nairn et al. (22) showed that gonococci are sialylated when they are grown in the presence of CMP–*N*-acetylneuraminic acid (CMP-NANA), and others have shown that LOS is the only molecule modified (23), specifically the lactoneoseries LOS (18). The oligosaccharide portion of nonsialylated lactoneoseries LOS is identical to the terminal residue of the human i antigens (18, 35), and the sialylated lactoneoseries component is identical to human lactoneoseries gangliosides (25).

In this report, we present evidence that the intercellular adhesions formed by gonococci of the opaque colony phenotype might be of the heterophilic cell adhesion type and that the adhesions could consist of Opa proteins of one gonococcus adhering to the terminal carbohydrate residues of LOS on an adjacent gonococcus and vice versa. LOS purified from the pyocin-derived LOS mutants of strain 1291 (10) were used for these studies because the reported structures for the parent and mutant strain LOS represent the lactoneoseries LOS and each LOS successively truncated to the heptose core (13). These LOS and an anti-Opa monoclonal antibody (MAb), 4B12, provided an opportunity to characterize the interaction between purified gonococcal Opa proteins and different types of LOS, both sialylated and nonsialylated.

MATERIALS AND METHODS

Organisms. Strains were grown on agar medium (28) in a CO_2 incubator maintained at 37°C and were evaluated for the opacity phenotype as described previously (28).

Purification of the Opa proteins. The methods for the purification of the Opa proteins have been described previously (5). The paper describing the method, however, had a typographical error. After the ethanol precipitation, the proteins were solubilized in 5% Zwittergent instead of the 0.5% which was reported in the paper. The only difference in the present protocol by comparison with the previous protocol is the use of a fast protein liquid chromatography apparatus (Pharmacia-LKB, Piscataway, N.J.) equipped with a Mono S column for further purification of the Opa proteins. The purity of the Opa protein preparations was determined by silver staining of analytical sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels (Bio-Rad Laboratories, Richmond, Calif.) and by enzyme-linked immunosorbent assays (ELISAs) using MAbs to LOS. The enzymatic hydrolysis of the Opa proteins by chymotrypsin (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) and trypsin (Boehringer Mannheim Biochemicals) has been described previously (7). The proteolytic enzyme-generated Opa fragments were precipitated with ethanol, washed once, resuspended in

phosphate-buffered saline (PBS), and centrifuged at $100,000 \times g$ for 1 h. The Opa fragments that remained in the PBS supernatant were designated as the hydrophilic peptides.

SDS-PAGE and Western blotting (immunoblotting). The SDS-PAGE method used for the determination of Opa protein purity was a variation of Laemmli's method (14) as described previously (5). In some cases, the SDS-PAGE gels were silver stained (Bio-Rad Laboratories). Electrophoretic transfer to Immobilon P (Millipore Corp., Bedford, Mass.) was performed according to the methods of Towbin et al. (34), with the exception that the membrane was first wetted with methanol. The Western blots were processed according to the method of Mandrell et al. (20) and probed with phosphatase-conjugated reagents (8).

Purification of the LOS. The LOS was extracted and purified by a modified hot phenol-water method (24, 36). The purified LOS was extracted from strain 1291, and the pyocin-resistant mutants were selected from this strain by a method described by Dudas and Apicella (10). The chemical structures of the strain 1291 wild-type LOS and the pyocin-resistant LOS mutants, shown in Table 1, were determined by liquid secondary ion mass spectroscopy, tandem mass spectrometry, and methylation analysis as described by John et al. (13). The MAbs reactive with each of the parent and mutant LOS structures and used in the Opa protein inhibition ELISAs are also shown. The specificity and binding characteristics of these MAbs have been described previously (16, 20). Sialylated LOS was obtained by growing the parent strain 1291 in the presence of CMP-NANA as described previously (17), washing the bacteria to remove residual CMP-NANA and breakdown products, and then purifying the sialylated LOS by the hot phenol-water procedure (24, 36). Sialylated LOS bound to microtiter wells (at approximately 2 μg per well) was desialylated by the addition of 100 μl of 0.2 U of neuraminidase (Boehringer Mannheim) per ml diluted in 50 mM sodium acetate with 5 mM $CaCl_2$, pH 5.5. The microtiter plate was incubated overnight at 37°C and washed with buffer prior to the addition of the Opa proteins.

ELISAs and inhibition ELISAs. Microtiter plates (Nunc-Immuno Plate IIF; Vangard International, Neptune, N.J.) were sensitized by adding 0.1 ml of a solution containing each particular LOS (at 0.25 μg/ml of PBS with 0.02% azide) to each well. The plates were incubated overnight at 37°C. The plates were washed five times with 0.9% NaCl–0.05% Brij 35–10 mM sodium acetate (pH 7.0)–0.02% azide. The LOS MAbs were diluted in PBS with 0.5% Brij 35 and added to the plate and incubated for 2 h at room temperature. The plates were again washed as before, and the secondary antibody, alkaline phosphatase-conjugated goat anti-mouse immunoglobulin G (IgG) and IgM (Tago Inc., Burlingame, Calif.), was diluted in PBS-Brij, added to the plates, and incubated for 2 h at room temperature. The plates were washed as before, and *p*-nitrophenyl phosphate (Sigma 104 phosphatase substrate) (1 mg/ml) in 0.1 M diethanolamine–1 mM $MgCl_2$–0.1 mM $ZnCl_2$–0.02% azide (pH 9.8) was added. The plates were incubated at 37°C for 1 h, and the A_{405} was determined with an Elida-5 microtiter plate reader (Physica, New York, N.Y.). Wells lacking the primary and/or secondary antibody were included as controls for background binding. A dilution of MAb that resulted in 50% of the maximum optical density (OD) for that antibody in the ELISA was determined.

For inhibition ELISAs, microtiter plates were sensitized with LOS and washed as described above. Purified Opa proteins or their enzymatically hydrolyzed products were added and diluted in the PBS-Brij solution, and the plates were incubated for 2 h at room temperature. The plates were then rewashed, and the MAbs were added at a dilution that was twice that resulting in 50% maximal binding. The plates were incubated for 2 h and washed, and then conjugated goat anti-mouse IgG and IgM (Tago Inc.) antibody was added to the appropriate wells. The wells were washed and substrate was added as described previously. The percentage of inhibition was calculated as follows: percent inhibition = [1 − (OD with the Opa protein added)/(OD without the Opa added)] × 100. The amount of the Opa protein bound to the strain 1291 LOS was determined by a direct ELISA run in parallel with anti-Opa MAb 4B12 and by detection with the secondary antibody as described above (MAb 4B12 has been shown to react with all gonococcal Opa proteins as well as with most meningococcal Class 5 proteins [1]). The relative amounts of MAb 4B12 bound were calculated by comparing the ODs for MAb bound to LOS-bound Opa and Opa bound directly to the microtiter wells. The amount of Opa bound to LOS was compared with the percentage of inhibition of anti-LOS MAb binding to the parent and mutant LOS. All assays
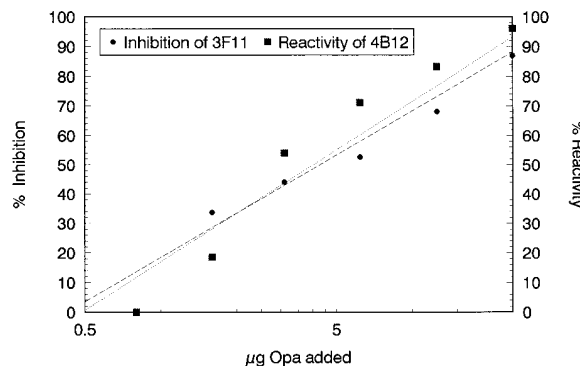
FIG. 1. A comparison of the inhibition (left *y* axis) of the LOS monoclonal antibody 3F11 by the addition of increasing amounts of R10 Opa protein and the positive detection (right *y* axis) of LOS-bound R10 Opa protein by the Opa monoclonal antibody 4B12, as described in Materials and Methods. Note that there is a direct correlation between the amount of inhibition of 3F11 LOS antibody and the amount of detectable Opa protein bound to the LOS.

were done in triplicate, with positive and negative controls included in each assay on at least three separate occasions.

RESULTS

Purification of Opa proteins. Opa proteins from several different gonococcal strains were isolated by the methods described previously (5). These purified proteins were analyzed by SDS-PAGE with silver staining, Western blotting, and ELISAs with specific anti-LOS MAbs. These analyses indicated that no other proteins or LOS contaminated the Opa preparations.

ELISAs and inhibition ELISAs. Figure 1 shows the results of a representative ELISA described in Materials and Methods that used an Opa protein isolated from strain R10, 1291 parent strain LOS, and MAb 3F11, which binds to the lactoneoseries LOS component of strain 1291. There was a direct correlation between the concentration of the Opa added to the 1291 LOS (reflected by the amount of MAb 4B12 binding) and the percentage of inhibition of the binding of MAb 3F11 to LOS. Similar results were obtained with MAb 6B4, which has a specificity for 1291 LOS similar to that of MAb 3F11 (results not shown). This suggested that Opa protein bound to the same epitope, or to a closely associated epitope, as that which binds MAb 3F11. A noncompetitive inhibition assay was used for these studies; thus, the amount of inhibition should not depend on the affinity of the MAb. Accordingly, after the Opa protein(s) was incubated with LOS, unbound Opa protein was removed by aspiration and washing prior to the addition of the MAb. To demonstrate this directly, Opa proteins were incubated with wild-type LOS, increasing amounts of 6B4 MAb were added, and Opa binding was detected as described above.

The results depicted in Fig. 2 demonstrate that the amount of inhibition by Opa is the same regardless of the concentration of the MAb added to detect LOS in the inhibition ELISA. Therefore, the binding of MAb reflects the number of binding sites remaining available and not the affinity of the MAb. Thus, in the equation [LOS] + [Opa] $\rightleftarrows$ [LOS-Opa], which is equal to $K_{(\text{binding})}$ = [LOS-Opa]/[LOS][Opa], the inhibition ELISA is an indirect measurement of the amount of LOS remaining free. To determine if the backward rate, i.e., [LOS-Opa] $\rightarrow$ [LOS] + [Opa], in the time frame of the assay influenced our results, we again allowed the LOS and Opa protein to interact. Free Opa protein was removed by washing, and the wells were filled with buffer. After the indicated times, the buffer was
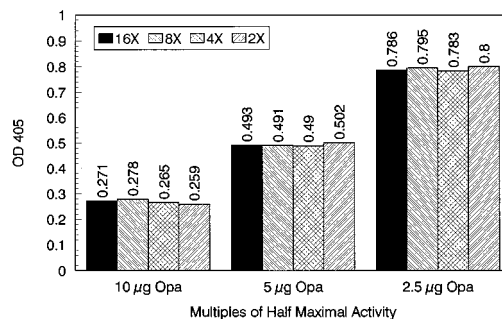

FIG. 2. A bar graph showing the results of an inhibition ELISA demonstrating that the assays are independent of the affinities of the MAbs to the LOS structures. Purified LOS from wild-type strain 1291 was used to sensitize the microtiter plates as described in Materials and Methods. Purified Opa protein from strain R10 was added in the indicated amounts and incubated for 2 h. The microtiter plate was washed, increasing multiples of the half-maximal titer of MAb 6B4 were added, and the assay was developed. Note that increasing the concentration of the MAb did not change the final OD of the assay and thus did not influence the percentage of inhibition. Thus, the extent of inhibition was dependent on the binding affinity of the Opa protein for the particular carbohydrate structure and the Opa concentration.

removed, the anti-LOS MAb was added, and the inhibition assay was completed as described in Materials and Methods. The data further substantiated that the inhibition ELISA data correlated with the affinities of the Opa proteins for the different LOS and therefore were not dependent on the affinities of the MAbs (data not shown). In addition, the data suggest that during the incubation, the rate of Opa dissociation from LOS was negligible. Similar experiments with other LOS and specific anti-LOS MAbs gave similar results (data not shown).

Opa proteins isolated from different gonococcal strains inhibited the binding of MAb 3F11 to 1291 parent LOS differently. Purified Opa proteins from gonococcal strains F62, MS11, and R10 each bound to 1291 LOS, but they inhibited the binding of MAb 3F11 to this LOS at different concentrations (Fig. 3). These minor variations seemed to be characteristics of the particular Opa protein, since an Opa protein demonstrating increased inhibition of the 3F11 antibody for the 1291 parent LOS also inhibited the reaction between MAb L8 and 1291c LOS at the same magnitude (data not shown). However, all Opa proteins could inhibit the 3F11 and 6B4 antibodies, but at slightly different concentrations. When the Opa proteins were cleaved with trypsin (Fig. 4) and chymo-
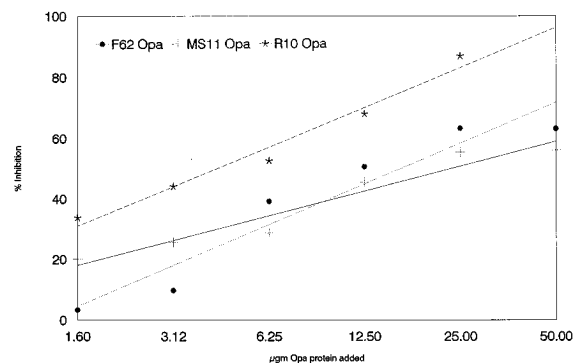

FIG. 3. Individual Opa proteins from selected opaque colony phenotypes of strains F62, MS11, and R10 were purified, and their abilities to bind to gonococcal 1291 wild-type LOS and inhibit the LOS 3F11 MAb were compared by the inhibition ELISA. Note that each Opa protein could inhibit the LOS MAb, but to a different degree.
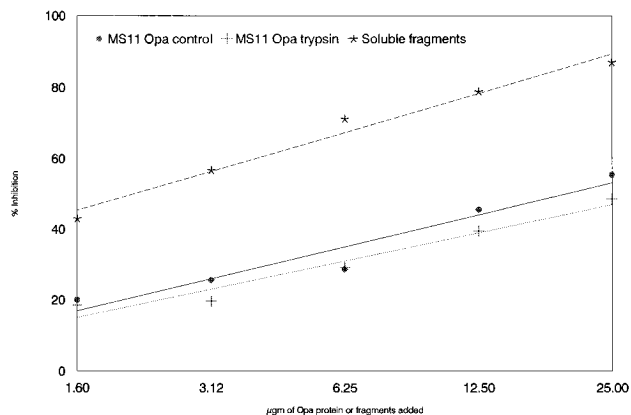
FIG. 4. Purified Opa protein from strain MS11 was digested with trypsin, and the resulting products were examined for their abilities to inhibit the 3F11 LOS MAb from reacting with the purified 1291 wild-type LOS. As can be seen, trypsinization had no effect on this inhibition. The trypsin-generated Opa fragments were precipitated with ethanol, washed, resuspended in PBS, and centrifuged at $100,000 \times g$ for 1 h. The supernatant was removed, quantified, and tested by the inhibition ELISA as described before. Most of the 3F11 inhibition seemed to be in the aqueous hydrophilic Opa peptides.
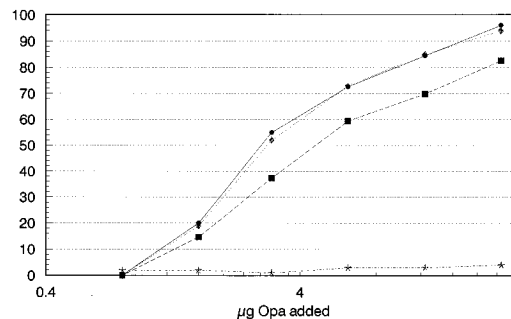

FIG. 5. Purified 1291 sialylated (star) and wild-type (circle) LOS were compared for their abilities to bind purified R10 Opa protein. The sialylated LOS bound the Opa protein very poorly, whereas after it was treated with neuraminidase (square), the binding activity was regained and was comparable to that of the wild-type LOS treated with neuraminidase (diamond).

trypsin (data not shown), the ability of the trypsin and chymotrypsin fragments to bind and inhibit the LOS MAbs was not diminished. The majority of the inhibiting activity of the Opa proteins remained in the aqueous soluble fraction after ultracentrifugation, whereas no activity could be detected in the resuspended pellet.

Characterization of LOS oligosaccharides to which Opa proteins bind. In order to more clearly define the carbohydrate structure to which the Opa proteins bound, parent strain 1291 LOS and LOS from each of four 1291 mutants were characterized in the ELISAs. The LOS structure of each of these LOS had been determined previously, and MAbs that reacted with each of these structures were available, as shown in Table 1. To compare the abilities of the Opa proteins to bind to each of these structures, the molar amount of Opa needed to produce a 50% inhibition of each of the various MAbs was determined in separate assays and has been included in Table 1 as a 50% inhibition value (I_{50}). These results indicate that the Opa proteins adhere most avidly to the LOS expressing Galβ1-4GlcNAc and, therefore, inhibit MAbs that bind to this epitope. The I_{50} for this interaction is comparable to that reported for the major hepatic asialoglycoprotein receptor binding to asialoorosomucoid (15). Removal of a terminal galactose, as has occurred in 1291a LOS, markedly decreases the binding of the Opa proteins to this LOS. This is reflected by an increased I_{50}. A comparison of the I_{50} values for Opa binding to parent 1291 LOS and to 1291c LOS indicates that the N-acetyl group on the glucosyl residue has less of an effect on Opa binding than removal of the terminal galactose. It is interesting to note that the reverse is true for the asialoglycoprotein receptor, which binds more avidly to lactosyl groups than to N-acetyllactosamine (15). In a manner similar to that of the hepatic asialoglycoprotein receptor, the Opa proteins also bound to terminal glucose (e.g., 4C4-1291d LOS reaction), although binding was a log lower compared with Opa binding to lactosyl LOS.

Effect of sialylation of LOS on Opa binding. The MAb inhibition data, taken together, suggest that the gonococcal Opa proteins bind best to either a Galβ1-4GlcNAc or a Galβ1-4Glc structure and that the N-acetyl group contributes significantly to the binding. The majority of the lactoneoseries LOS

in a population of gonococci become sialylated when the gonococci are provided with CMP-NANA, the nucleotide sugar for sialic acid (22). Sialylation of the lactoneoseries LOS blocks the binding of MAbs 3F11 and 6B4 (18), and it is presumed that the loss of binding is due to sialylation of the terminal Galβ1-4GlcNAc residue. A similar pattern of MAb 3F11 binding occurred with mammalian nonsialylated lactoneoseries glycosphingolipids and lactoneoseries glycosphingolipids sialylated on the terminal galactose (i.e., NeuNAcα2-3Galβ1-4GlcNAc) (18). To determine whether sialylation of LOS affects its binding of Opa proteins, sialylated gonococcal LOS was isolated and used in a direct ELISA to detect bound Opa proteins. As can be seen from Fig. 5, purified gonococcal Opa proteins were unable to bind to sialylated LOS, as determined by the lack of 4B12 reactivity. The same wells which had been coated with sialylated LOS were subsequently treated with neuraminidase and washed, then the Opa was added again, and binding was detected with MAb 4B12. The results shown in Fig. 5 demonstrate that the lack of binding of Opa to sialylated LOS was fully restored by treating the sialylated LOS with neuraminidase. These data were consistent with the concept that at least some gonococcal Opa proteins bind preferentially to terminal Galβ1-4GlcNAc carbohydrate residues of the lacto-N-neotetraose that is present in LOS expressed by most clinical strains of gonococci. In contrast, the lactosyl residue (Galβ1-4Glc), a precursor of the terminal lactosamine residue of most gonococci, is a poorer ligand for Opa.

DISCUSSION

Zones of adhesion between gonococci were first shown in electron micrographs of gonococcal pili by Swanson et al. in 1971 (33). These adherent areas "resemble the gap junctions that have been seen in several animal systems," with a 20-Å (2.0-nm) gap separating the outer membranes of two gonococci (33). Swanson later found that these zones of adhesion were more extensive in gonococci that expressed what are now known as Opa proteins on their surface and displayed the observable opacity colonial phenotype (26–29). These intercellular adhesions could be easily disrupted by the addition of proteolytic enzymes to the bacterial culture, which suggested that they were, in part, made up of protein, more specifically Opa proteins. It was further shown that the addition of these proteolytic enzymes to opaque gonococci would cleave the surface-exposed region of Opa proteins, leaving a fragment still embedded in the outer membrane (6, 7). Thus, most gonococci expressing Opa proteins exhibit various degrees of colony

opacity, from very opaque to slightly opaque (31). Some of this variation in colony opacity is correlated with the number and/or amount of Opa proteins being expressed. However, in other cases, gonococci expressing similar amounts and numbers of Opa proteins vary in opacity. Thus, it seemed that colony opacity was also influenced by another gonococcal component.

We had reported previously that purified Opa proteins were able to bind to purified gonococcal LOS, a process that appeared to result in cell-cell adhesion (4, 6). The purified LOS used in these initial studies was representative of the total population of LOS present in the gonococcal outer membrane, a population composed of LOS with heterogeneous molecular weights and chemical structures. By the use of purified, well-characterized LOS from defined gonococcal LOS mutants and MAbs to these defined LOS carbohydrate structures, we have been able to more precisely define these interactions. These results also add support to the notion that, of the three basic types of cell-cell adhesion mechanisms (i.e., homophilic, heterophilic, and extracellular linkage), gonococci of the opaque colony phenotype bind by heterophilic binding. This mechanism of binding might involve an Opa molecule on one cell binding to the nonreducing terminal portion of an LOS molecule on the adjacent cell. The carbohydrate structure on gonococcal LOS that binds Opa proteins most avidly is similar or identical to the structural epitope defined by MAbs 3F11 and 6B4.

MAb 3F11 binds to LOS and glycosphingolipids that terminate in Galβ1-4GlcNAcβ1-3Gal residues at their nonreducing ends (38). Approximately 98% of gonococcal strains isolated from the genital tract bind MAb 3F11, indicating that a terminal lactoneoseries LOS is expressed on these strains (16). The lactoneoseries LOS are immunochemically similar to glycosphingolipid precursors of the major human blood group antigens, notably paragloboside, which contain lacto-*N*-neotetraose (16). Lacto-*N*-neotetraose is present in gonococcal LOS that bind MAb 3F11 (37, 38).

The epitope defined by MAb 6B4 is similar to that defined by MAb 3F11, but a comparison of the binding characteristics of the two antibodies for glycosphingolipids (16), sialylated and nonsialylated LOS (17, 18), and the LOS of different strains of *Haemophilus influenzae* (19) and *Haemophilus ducreyi* (9) indicates that the two antibodies have distinct specificities. MAb 3F11 binds better to branched-chain *N*-acetyllactosamine glycosphingolipids than MAb 6B4, whereas MAb 6B4 prefers linear *N*-acetyllactosamine molecules (16). MAb 3F11 binding is decreased more by LOS sialylation than that of 6B4, presumably because MAb 3F11 recognizes an epitope at the terminus of the lactoneoseries LOS (17, 18).

The presence of identical carbohydrates on gonococci and on human cells and the data presented in this study suggest that the gonococcal Opa proteins might also bind to eukaryotic cells expressing the same carbohydrate structures at the surface of cells. Currently, we are trying to determine whether this type of interaction is occurring.

In addition to the binding of Opa to Galβ1-4GlcNAc residues, we found that the purified Opa proteins could also bind to LOS having terminal Galβ1-4Glc, GlcNAcβ1-3Gal, and Glc residues in descending order of affinity. The nonreducing terminus of the 4.5-kDa component of most wild-type gonococcal LOS consists of the tetrasaccharide Galβ1-4GlcNAcβ1-3Galβ1-4Glc, which is bound to a heptose residue. This tetrasaccharide is synthesized by the gonococcus by the addition of single sugars to the growing terminus. This biosynthesis is regulated by the expression of the specific enzymes (presumably glycosyltransferases) involved and by the availability of the nucle-

otide sugar substrates. Thus, at any given period of time, any of these four residues could be the terminal sugar for an LOS molecule. In addition, depending on the enzymes involved, any one of these terminal structures could be over- or underrepresented in the outer membrane by comparison with another terminus. The gonococcal LOS mutants of strain 1291 are obvious examples of this concept, because each of the mutants has a defect(s) in a particular enzyme(s) important in LOS biosynthesis. Thus, this defect results in the absence of the LOS acceptor substrate necessary for further synthesis and the overrepresentation of the LOS in a population of gonococci. The results presented in this study suggest that gonococci expressing similar amounts of Opa proteins could still vary in their colony opacity, depending on the structure of the LOS. Thus, Opa-expressing gonococci having the majority of their LOS molecules terminating in GlcNAcβ1-3Galβ1-4Glc, which has less affinity for the Opa proteins, would have fewer intercellular adhesions and be less opaque than gonococci expressing the same amount of Opa but having a majority of their LOS molecules terminating in Galβ1-4Glc. Our observations also suggest that most Opa proteins contain a common LOS binding domain which might be influenced in minor ways by surrounding variable domains. Recently, we identified one such carbohydrate binding region on Opa proteins, located in a conserved area of the molecule (unpublished data). The complete characterization of this LOS binding domain awaits further studies.

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