Enhanced factor H binding to sialylated Gonococci is restricted to the sialylated lacto-N-neotetraose lipooligosaccharide species: implications for serum resistance and evidence for a bifunctional lipooligosaccharide sialyltransferase in Gonococci

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Enhanced Factor H Binding to Sialylated Gonococci Is Restricted to the Sialylated Lacto-N-Neotetraose Lipooligosaccharide Species: Implications for Serum Resistance and Evidence for a Bifunctional Lipooligosaccharide Sialyltransferase in Gonococci

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We isolated serologically identical (by serovar determination and porin variable region [VR] typing) strains of Neisseria gonorrhoeae from an infected male and two of his monogamous female sex partners. One strain (termed 398078) expressed the L1 (Galα1→3Galβ1→4Glcβ1→4Hepl) lipooligosaccharide (LOS) structure exclusively; the other (termed 398079) expressed the lacto-N-neotetraose (LNT; Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→4Hepl) LOS structure. The strain from the male index case expressed both glycoforms and exhibited both immunotypes. Nuclear magnetic resonance analysis revealed that sialic acid linked to the terminal Gal of L1 LOS via an α2→6 linkage and, as expected, to the terminal Gal of LNT LOS via an α2→3 linkage. Insertional inactivation of the sialyltransferase gene (known to sialylate LNT LOS) abrogated both L1 LOS sialylation and LNT LOS sialylation, suggesting a bifunctional nature of this enzyme in gonococci.

Akin to our previous observations, sialylation of the LNT LOS of strain 398079 enhanced the binding of the complement regulatory molecule, factor H. Rather surprisingly, factor H did not bind to sialylated strain 398078. LOS sialylation conferred the LNT LOS-bearing strain complete (100%) resistance to killing by even 50% nonimmune normal human serum (NHS), whereas sialylation of L1 LOS conferred resistance only to 10% NHS. The ability of gonococcal sialylated LNT to bind factor H confers high-level serum resistance, which is not seen with sialylated L1 LOS. Thus, serum resistance mediated by sialylation of gonococcal L1 and LNT LOS occurs by different mechanisms, and specificity of factor H binding to sialylated gonococci is restricted to the LNT LOS species.

The lacto-N-neotetraose (LNT) lipooligosaccharide (LOS) of Neisseria gonorrhoeae can become sialylated when grown in medium supplemented with 5′-cytidinemonophospho-N-acetyleneuraminic acid (CMP-NANA) (20). LOS sialylation renders strains that are otherwise susceptible to complement-mediated killing, resistant to killing by nonimmune normal human serum (NHS) (26, 31). One explanation is the ability of gonococci bearing the sialylated LNT LOS to bind factor H (37), an important fluid-phase regulatory protein of the alternative pathway of complement (9, 29, 53, 56). Gonococcal LOS undergoes significant phase variability in vivo, which may result in a shift of expression away from the “conventional” (terminal lactosamine of LNT) sialylation site (3, 12). LOS phase variation that results in the loss of the ability of an otherwise serum-sensitive gonococcal strain to sialylate its LOS may render such a strain highly susceptible to complement-mediated killing and therefore may be disadvantageous to an organism, necessitating a redundant strategy to evade killing by comple-

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species). Sequence differences between gonococcal and bifunctional meningococcal sialyltransferases are detailed.

**MATERIALS AND METHODS**

Bacterial strains and growth. *N. gonorrhoeae* strain 398 was isolated from a male index case, and four additional strains (termed 398078 to 398081) were obtained from four of his female sex contacts. Strain 1291 b is a mutant derivative of strain 1291 described previously (15), which had been selected under pyrogen pressure and expresses L1 LOS. Bacteria were grown in standard gonococcal liquid medium supplemented with IsoVitalex equivalent. In order to sialylate LOS, CMP-NANA (Sigma Chemical Corporation, St. Louis, MO) was added to growth media (concentration specified for each experiment).

In addition to the strains indicated above, *N. gonorrhoeae* strains F62 (40), FA1090 (54), 24-1 (6), WG (28), and 179088, 255034, 260041, 374073, 339063, 256036, 274045, and 252035 (36) were used for sequence analysis of the LOS sialyltransferase (*lst*).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blotting for LOS visualization and immunochromatographic characterization. Bacteria were digested with proteinase K (Sigma), and lysates were treated with NuPAGE LDS sample buffer (Invitrogen Life Technologies, Carlsbad, CA) and resolved on 12% T polyacrylamide gels. The Invitrogen proteinase K (mornorpholinepropanesulfonic acid) running buffer, according to the manufacturer’s instructions. LOS was visualized using a silver staining kit (Bio-Rad Laboratories, Hercules, CA). In some experiments, LOS was transferred to polyvinylidene fluoride membranes (Millipore) by Western blotting and probed with monoclonal antibodies (MAbs) 3F11 (2) and L1 (42) for immunochromatographic characterization of LOS.

LOS purification and analytical techniques. To sialylate LOS, bacteria were grown in 2 liters of gonococcal liquid medium supplemented with IsoVitalex equivalent (1% [vol/vol]) and CMP-NANA (10 μg/mL). Bacteria were harvested, and LOS was purified by the hot water-phenol extraction method (55). Sugars were determined as their alditol acetate derivatives by gas-liquid chromatography–mass spectrometry, and methylation analysis was carried out by the method of deuterated sodium dodecyl sulfate (10 mg/ml), and EDTA (1 mg/ml) was added for LOS visualization and immunochemical characterization.

**RESULTS**

Identification of epidemiologically related gonococcal strains bearing two different LOS sialylation sites and immunochromatographic characterization of LOS. Previously we have screened several strains of *N. gonorrhoeae* that were sensitive to the bactericidal action of 10% NHS in the native state but became serum resistant upon growth in media supplemented with CMP-NANA (22). We examined these strains initially by Western blotting for expression of the LNT LOS species (MAB 3F11 reactive) in the unsialylated state to identify 3F11-negative strains (i.e., strains that could become serum resistant upon sialylation, but lacked the well-characterized LNT LOS acceptor site). Using Western blotting with MAB 3F11 as the probe, we identified one such strain, 398078, isolated from a female contact of an index male with uncomplicated gonococcal infection (infected with strain 398). Three additional female sex partners were infected with strains 398079, 398080, and 398081. The five strains belonged to the PorB1-32 serovar (17) and were also identical by Por variable region (VR) typing (Por VR type B2,2,nt,1,3) (46), but showed dissimilar LOS migration patterns on sodium dodecyl sulfate-polyacrylamide gel electrophoresis and varied with respect to MAB 3F11 binding. Prior studies have shown that the L1 LOS in *N. meningitidis* can be sialylated (51), and thus we hypothesized that the sialylatable non-3F11 LOS species in strain 398078 was likely...
to be L1 LOS (Galα1 → 3Galβ1 → 4Glcβ1 → 4Hep1). Strain 398 expressed two distinct LOS species (Fig. 1A) and was found to react with both MAb 3F11 and a second MAb, L1, specific for the L1 LOS type that also defines the Pk blood group antigen (19) (Fig. 1B). Strain 398078 expressed only the lower-M, LOS species and reacted with MAb L1, while isolate 398079 expressed only the higher-M, LOS species, as seen on silver stain that reacted with 3F11 (Fig. 1A and B), and weak reactivity with MAb L1 by Western blotting (no corresponding band seen on silver stain).

Strains 398080 and 398081 had LOS migration patterns similar to those of the three strains examined (Fig. 1) and were not studied further. As seen in Fig. 1A, both LOS species migrated with slower velocities when strains were grown in media supplemented with CMP-NANA, suggesting that a sialic acid residue was added to both LOS species. Also shown in Fig. 1A are the migration patterns of the LOS sialyltransferase (lst) deletion mutants in the native (unsialylated) state and when grown in the presence of CMP-NANA (discussed in detail below). Collectively, these data suggested that the lower-M LOS species that could be sialylated was similar to the L1 LOS of N. meningitidis, and this was confirmed by mass spectrometry analysis (see below).

Mass spectrometric analysis of the LOS of strain 398078, and characterization of the nature of the sialic acid linkage.

The MS spectra of LOS-OH isolated from strains 398, 398078, 398079, 398079-NANA, and 1291b-NANA were determined. As summarized in Table 1, it can be seen that 398078 LOS-OH elaborated only the L1-like extension, as evidenced by a single HexNAc known to be substituted on the distal HepII residue whereas 398079 LOS-OH elaborated only the 3F11-like (Galβ1 → 4GlcNAcβ1 → 3Galβ1 → 4Glcβ1 → 4Hep1) extension from Hep1 plus another HexNAc known to be substituted on the distal HepII residue. Strain 398 expressed both the L1- and 3F11-like extensions. It is worth noting that the epidemiologically linked strains (398, 398078, and 398079) display two...
phosphoethanolamine (PEtn) molecules in their core OS, and both of these PEtn moieties were localized to the distal heptose residue by NMR studies (HepII) of the inner core OS (data not shown). Control strain 1291b only elaborates one PEtn residue in the core OS also located at the HepII sugar (data not shown). 1291b also exhibits the L1-like extension. In order to elucidate the position of the sialic acid linkage in these strains, two approaches were adopted. For strain 398078-NANA, methylation analysis revealed the presence of 6-linked galactose residues, the amount being consistent with the level of sialylation in the molecule. Strain 1291b-NANA was also shown to elaborate sialic acid attached to the 6-position of a galactose residue by NMR methods, whereby the inter-nuclear Overhauser effect connectivities from the equatorial proton of the sialic acid residue identified signals for the 3-position of galactose as observed previously for N. meningitidis L1 LOS (51). Finally, strain 398079-NANA was shown to elaborate sialic acid at the 3-position of a galactose residue by virtue of inter-nuclear Overhauser effect connectivities from the equatorial proton of the sialic acid residue that identified a signal for the 3-position of galactose as observed previously for N. meningitidis L3 LOS (32).

As detailed above, strains 398078 and 1291b differed in their PEtn substitutions on HepII; the former strain had PEtn simultaneously at the 3- and 6-positions, while the latter had only a 3-PEtn. Therefore, these data suggest that PEtn substitutions on HepII did not influence the nature of the linkage of sialic acid to LOS.

** Interruption of lst results in the loss of the ability of gonococcal L1 LOS to sialylate.** To determine if the well-characterized lipooligosaccharide transferase, lst, was responsible for sialylation of the L1 LOS in gonococci, we constructed mutants using strains 398, 398078, and 398079 in which lst was interrupted. The LOS migration patterns of the mutants in the native (unsialylated) state and when grown in media supplemented with 100 μg/ml CMP-NANA are shown in Fig. 1A (lanes lst and lst-N, respectively). As expected, interruption of lst in 398079 (expresses predominantly the 3F11-like LOS) abrogated LOS sialylation in media containing CMP-NANA (Fig. 1A, upper panel, lane marked lst-N). Insertional inactivation of the same lst in 398078 resulted in loss of sialylated L1 LOS upon growth in CMP-NANA-containing media (Fig. 1A, lower panel, lane marked lst-N). The 398 lst mutant lacked the ability to sialylate both LOS glycoforms. Collectively, these data examined together with the mass spectrometric analysis strongly suggest that this lst in gonococci (i.e., one that is known to sialylate the LNT LOS [via an α(2,3) linkage] also mediates transfer of a sialic acid residue onto the terminal Gal in L1 LOS [via an α(2,6) linkage], thereby rendering it a bifunctional sialyltransferase.

**Comparison of meningococcal and gonococcal lst gene products.** Our data suggest that the gonococcal Lst is a bifunctional enzyme capable of adding an α(2,3)-linked sialic acid onto LNT LOS and an α(2,6)-linked sialic acid onto L1 LOS. Wakarchuk et al. have shown that Lst from meningococcal strain 126E is also a bifunctional enzyme with similar substrate specificities (51). An isoleucine (Ile) residue at position 168 of the MC58 Lst resulted in a protein with only about 25% of the activity of an Lst with an Ile at the same position (52). It is worth noting that the FA1090 Lst also contained 7 additional amino acids at the C-terminal end due to a T-to-C substitution at the site of the 126E TAA stop codon.

To further examine gonococcal Lst sequence variation, we amplified and sequenced lst from 14 gonococcal strains (including FA1090). The region sequenced encompassed the putative 7-amino acid C-terminal extension found in FA1090. These sequences were aligned with six meningococcal Lst sequences available in GenBank (accession numbers U60662 through U60664 and NMB0922) using ClustalW. A representative alignment is shown in Fig. 2. We identified 21 amino acid positions that diverged between gonococci and meningococci. Within meningococci, Lst varied at 11 amino acid positions with a maximal diversity of 3%. The 7 C-terminal amino acids found in FA1090 were present at position 168 of the MC58 Lst resulted in a protein with only about 25% of the α(2,6) sialytransferase activity of an Lst with an Ile at the same position (52). It is worth noting that the FA1090 Lst also contained 7 additional amino acids at the C-terminal end due to a T-to-C substitution at the site of the 126E TAA stop codon.

**FIG. 2. Lst sequences from 6 meningococcal and 14 gonococcal strains were aligned using ClustalW. A representative alignment of the bifunctional Lsts from meningococcal strain 126E and gonococcal strain 398078 is shown.** Species-specific variation occurred at 21 separate amino acid positions (boxed) distributed across the protein. Strain variation within a species (shaded) was noted at 11 meningococcal (above) and 7 gonococcal (below) amino acid positions. The isoleucine (Ile) residue at position 168, shown to be critical for bifunctionality of 126E Lst, is marked with an asterisk.
position 168. All gonococcal \textit{lst} genes contained the 7 additional amino acids first noted in FA1090.

**Sialylation of the L1 LOS in \textit{N. gonorrhoeae} does not enhance factor H binding.** Sialylation of serum-sensitive gonococcal strains bearing the 3F11 LOS has been shown to enhance factor H binding and mediate serum resistance (37). We examined factor H binding to strains 398, 398079 (LNT LOS), and 398078 (L1 LOS) and their sialylated derivatives grown in media containing 0, 1, 10, or 100 $\mu$/ml CMP-NANA by flow cytometry (histogram for 10 $\mu$/ml CMP-NANA has been omitted to simplify the illustration). We observed that factor H binding was enhanced only when 3F11 LOS-bearing strains 398 and 398079, but not strain 398078 (L1 LOS), was sialylated (Fig. 3, upper panel). Maximal factor H binding was seen even at 1 $\mu$/ml CMP-NANA and was similar to binding observed with 100 $\mu$/ml CMP-NANA. The \textit{lst} mutant derivatives of these strains did not bind factor H when grown in CMP-NANA-containing media (Fig. 3, lower panel). These data suggest that enhancement of factor H binding to sialylated gonococci is restricted to strains that bear the LNT LOS species; enhancement was not seen in the strain that expressed only L1 LOS.

The above data show that levels of factor H binding to the LNT LOS-bearing strains were similar over CMP-NANA concentrations ranging from 1 to 100 $\mu$/ml. We next quantified the proportion of LOS of strains 398078 (L1) and 398079 (mostly 3F11) that was sialylated at the different CMP-NANA concentrations to ensure that differences in the level of sialylation between the two LOS species did not account for the inability of sialylated L1 LOS to bind to factor H. Direct mass spectrometry showed that 5%, 25%, and 30% of glycoforms were sialylated when strain 398079 was grown in 1, 10, and 100 $\mu$/ml CMP-NANA, respectively. The corresponding percentage of sialylated glycoforms in strain 398078 grown in the three concentrations of CMP-NANA (in order of increasing concentration) were 10%, 40%, and 55%, respectively. In a separate experiment, we repeated the sialic acid estimation in samples grown in 1 and 10 $\mu$/ml of CMP-NANA using precursor ion scanning for the $m/z$ 951 species, which represents the basic O-deacylated lipid A structure without additional phosphorylation. The percentages of 398079 LOS (predominantly 3F11) that was sialylated were 30% and 50%, and the percentages of sialylated L1 LOS in 398078 were 15 and 62.5 when organisms were grown in 1 and 10 $\mu$/ml CMP-NANA, respectively. These data show that the two glycoforms were sialylated to similar extents and that only a small proportion of LOS sialylation was necessary for maximal factor H binding to the strain bearing the LNT LOS. Proportionally higher sialylation of L1 LOS did not result in factor H binding.

**Differences in the efficiency of serum resistance conferred by sialylation of the 3F11 and L1 LOS.** We examined the ability of strains 398079 (LNT LOS) and 398078 (L1 LOS) to resist the bactericidal action of 10% NHS when grown in media containing increasing concentrations of CMP-NANA. Figure 4A shows that LNT LOS-expressing strain 398079 demonstrated ~88% survival when grown in media containing 1 $\mu$/ml CMP-NANA, while MAb L1-positive strain 398078 showed only ~17% survival under similar growth conditions. Survival of 398078 gradually increased with increasing CMP-NANA concentrations in the media. Strain 398, which expressed both LOS species, showed an intermediate level of serum resistance. The \textit{lst} mutants of all three strains were completely killed in
DISCUSSION

In order to survive the milieu of the genital tract and establish infection, *N. gonorrhoeae* must evade the innate immune system at the level of the genital mucosa. Naturally occurring antibody and complement are important effector arms of the immune response at the cervical mucosal level (33). Gonococcal LOS undergoes considerable phase variation in vivo (3, 12, 41), which could be advantageous in evading complement-mediated killing. For example, alteration in LOS structure may decrease binding of preexisting natural anti-LOS antibody to the bacterial surface. However, this could occur at the expense of the loss of a mechanism to evade complement.

Gonococci have developed a variety of mechanisms to evade complement-mediated killing. Sialylation of the lacto-N-neotetraose LOS of otherwise serum sensitive strains renders them resistant to bactericidal killing by NHS (31). This is termed “unstable” serum resistance, because it is a property that is lost when organisms are subcultured onto media that lack the donor molecule for sialic acid, CMP-NANA (30). Certain gonococcal strains are intrinsically resistant to killing by NHS, independent of LOS sialylation, a property termed “stable” serum resistance (38, 39). Stable serum resistance may in part be mediated by the ability of gonococcal porin to bind complement regulatory molecules such as factor H and/or C4b-binding protein (C4bp) (35, 36).

Prior work in our laboratory has shown that sialylation of gonococcal 3F11 (LNT) LOS enhances factor H binding, which results in regulation of the alternative pathway of complement (37). Factor H is a key soluble phase regulator of the alternative pathway of complement and acts as a cofactor in the factor I-mediated cleavage of C3b to the hemolytically inactive molecule, iC3b (29, 44, 56). Factor H also limits the amount of C3b deposited on the bacterial surface by virtue of its decay accelerating function, in which the factor Bb is irreversibly dissociated from C3-convertase, C3b,Bb (10, 29, 44, 53, 56).

NMR analysis of the sialylated LOS of 398078 showed an α(2,6)-linked sialic acid residue to the terminal Gal on HepI. This contrasted with the sialylation observed on the serologically identical and epidemiologically related strain, 398079 (LNT LOS), which as expected, possessed an α(2,3)-linked sialic acid. Inactivation of *lst* in 398078 and 398079 resulted in an inability of both strains to sialylate their LOS and suggests that the same Lst enzyme was capable of adding sialic acid in two distinct configurations on two different glycoforms. Bifunctionality of Lst has previously been described in meningococcal *lst* donors for sialic acid. Inactivation of donor molecule for sialic acid, CMP-NANA (30). Certain gonococcal strains are intrinsically resistant to killing by NHS, independent of LOS sialylation, a property termed “stable” serum resistance (38, 39). Stable serum resistance may in part be mediated by the ability of gonococcal porin to bind complement regulatory molecules such as factor H and/or C4b-binding protein (C4bp) (35, 36).

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In a separate experiment (Fig. 4B), we examined the degree of serum resistance that was conferred by sialylation of the two LOS species. LOS was fully sialylated by growth in media containing 100 μg/ml CMP-NANA, and bacteria were subjected to the effects of 10%, 25%, and 50% NHS in a serum bactericidal assay. We observed that sialylation of 398 and 398079 resulted in a higher degree of serum resistance (both strains fully resisted even 50% NHS), compared to sialylated 398078, which fully resisted only 10%, but was killed 75% and 99.5% in 25% and 50% NHS, respectively.

![Graph showing serum resistance](image-url)
Meningococci that cause invasive disease are encapsulated, and LOS sialylation does not appear to impact virulence at least in animal models and may play only a minor role in meningococcal serum resistance (49, 50). In contrast, gonococci may depend more on LOS sialylation to survive the effects of complement, and this may be reflected in the maintenance of a relatively highly conserved and bifunctional enzyme among isolates. The Lst enzyme has recently been localized to the bacterial outer membrane (43), but the kinetics of the Lst enzymes of the two pathogenic neisserial species were not compared in situ; likewise, the differences in sialylation rates of the 3F11 and L1 LOS species are unknown.

We compared the abilities of strains bearing sialylated L1 LOS (NANAα2 → 6Galβ1 → 3Galβ1 → 4Glcβ1 → 4Hepl) and predominantly sialylated 3F11 LOS (NANAα2 → 3Galβ1 → 4GlcNAcβ1 → 3Galβ1 → 4Glcβ1 → 4Hepl) to bind factor H. As expected, the latter strain bound factor H well, but the sialylated L1 LOS did not enhance factor H binding to the gonococcal surface (Fig. 3). These data suggest that the binding of factor H to sialylated gonococci is highly specific and restricted to strains that possess sialylated lacto-N-neotetraose LOS. Specificity of factor H binding to polyanions is illustrated by the observation that factor H binds to heparin, sialic acid, dextran sulfate, chondroitin sulfate A, and type III and IV carrageenan, while little or no binding occurs to colominic acid, chondroitin sulfate C, keratan sulfate, hyaluronic acid, or polyaspartic acid (24). Ongoing work in our laboratory has shown that only sialylation of gonococcal, but not meningococcal, LNT LOS augments factor H binding. Preliminary evidence suggests that a cooperative mechanism between gonococcal porin and sialylated LNT LOS is required for factor H binding in this neisserial species (18).

Factor H binding to strain 398079 was not augmented as the proportion of sialylated LOS increased, possibly because the bacterial surface has already been “saturated” with factor H. Approximately 50,000 factor H molecules have been shown to bind to a single group A streptococcus (14). The factor H binding and serum bactericidal data obtained at lower CMP-NANA concentrations are likely to be biologically relevant because the amount of CMP-NANA obtained from cell lysates from whole blood is <1 µg/ml (26). Shifting of serum-sensitive N. gonorrhoeae to a serum-resistant phenotype was noticeable when bacteria were grown in media containing as little as 2 × 10⁻² nM (~0.01 µg/ml) CMP-NANA (8).

Lipopolysaccharides of Actinobacillus actinomycetemcomitans (57), Klebsiella pneumoniae (1), and Salmonella enterica serovar Montevideo (16) have been shown to bind to C3b. Edwards et al. have identified gonococcal LOS as a target where C3b is converted to iC3b (7). Recently, we have identified N. meningitidis LOS as a target for complement C4b and have shown that alterations in Hepl hexose substitutions and phosphoethanolamine residues on Hepl can modulate the nature of the linkage (i.e., amide versus ester) of C4b with LOS (34). Sialylation of both 398078 and 398079 decreased C4 binding to a similar extent in a flow cytometry assay (data not shown), suggesting that LOS sialylation may also diminish or prevent C4b binding to LOS. The differences in the level (or degree) of serum resistance between 398078 and 398079, when grown in the presence of increasing concentrations of CMP-NANA (Fig. 4A), reflect the importance of factor H bound to sialylated LNT LOS on 398079 in down-regulating complement. Therefore any alternative pathway amplification that is initiated by the classical pathway is regulated by factor H bound directly to 398079. On the other hand, 398078 must rely on classical pathway regulation by obscuring targets for C4b. Such a mechanism would likely require a greater proportion of LOS to be sialylated and may be less efficient than a mechanism that involves binding of a complement regulator (in addition to obscuring targets for C3b and C4b).

In conclusion, we have demonstrated sialylation of the L1 (or P₅⁻like) LOS in gonococci and defined the nature of the sialic acid linkage. Although the chemistry of the sialylation of gonococcal LOS is similar to that seen in meningococci, we have noted differences in amino acid sequences of the bifunctional Lst enzymes in these two neisserial species. Our observations suggest that the bifunctionality of gonococcal Lst is not related to the Ile at position 168 as was reported in meningococci. Determining the requirements for the ability of gonococcal Lst to add sialic acid in two distinct configurations may shed light on the structure-function relationships of this enzyme. The ability of gonococci to sialylate two distinct LOS species would enable strains to undergo a phase variation of C4b. Such a mechanism would likely require a greater proportion of LOS from whole blood is NANA concentrations are likely to be biologically relevant.

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ERRATUM

Enhanced Factor H Binding to Sialylated Gonococci Is Restricted to the Sialylated Lacto-\(\begin{array}{c}N\end{array}\)-Neotetraose Lipooligosaccharide Species: Implications for Serum Resistance and Evidence for a Bifunctional Lipooligosaccharide Sialyltransferase in Gonococci

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Volume 73, no. 11, p. 7390–7397, 2005. Page 7390, abstract: “L1 (Gal\(_\alpha1\)→3Gal\(_\beta1\)→4Glc\(_\beta1\)→4HepI)” should read “L1 (Gal\(_\alpha1\)→4Gal\(_\beta1\)→4Glc\(_\beta1\)→4HepI).”

Column 2, lines 1 and 2: “L1 LOS (Gal\(_\alpha1\)→3Gal\(_\beta1\)→4Glc\(_\beta1\)→4HepI)” should read “L1 LOS (Gal\(_\alpha1\)→4Gal\(_\beta1\)→4Glc\(_\beta1\)→4HepI).”

Page 7392, column 2, line 1: “L1 LOS (Gal\(_\alpha1\)→3Gal\(_\beta1\)→4Glc\(_\beta1\)→4HepI)” should read “L1 LOS (Gal\(_\alpha1\)→4Gal\(_\beta1\)→4Glc\(_\beta1\)→4HepI).”

Column 2, line 7 from the bottom: “the Gal\(_\alpha1\)→3Gal\(_\beta1\)→4Glc\(_\beta1\)→4HepI” should read “the Gal\(_\alpha1\)→4Gal\(_\beta1\)→4Glc\(_\beta1\)→4HepI.”

Page 7396, column 1, lines 13 and 14: “L1 LOS (NANA\(_\alpha2\)→6Gal\(_\alpha1\)→3Gal\(_\beta1\)→4Glc\(_\beta1\)→4HepI)” should read “L1 LOS (NANA\(_\alpha2\)→6Gal\(_\alpha1\)→4Gal\(_\beta1\)→4Glc\(_\beta1\)→4HepI).”