The Structure of Lipooligosaccharide Produced by Neisseria gonorrhoeae, Strain 15253, Isolated from a Patient with Disseminated Infection

EVIDENCE FOR A NEW GLYCOSYLATION PATHWAY OF THE GONOCOCCAL LIPooligosaccharide*

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Ryohei Yamasaki†‡, Deborah E. Kerwood§, Herman Schneider, Kevin P. Quinn**, J. McLeod Griffiss††‡‡, and Robert E. Mandrell§§

From the °Department of Laboratory Medicine and the ‡Center for Immunochemistry, University of California, San Francisco, California 94121 and the ††Center for Immunochemistry, University of California, San Francisco and the IlWalter Reed Research Institute, Washington, D. C. 20307

We studied the structure of the lipooligosaccharide (LOS) that is produced by Neisseria gonorrhoeae, strain 15253. This strain, recovered from a patient with disseminated infection, produces predominately a single LOS component, and its lipooligosaccharide (OS) structure is different from those of previously studied LOSs. Definition of this OS structure provides additional information on the LOS biosynthesis. We determined that the 15253 OS has an unusual structure: 2 lactosyl residues at its nonreducing ends shown below,

Galβ1→4Glcβ1→4Hepα1→KDO

Galβ1→4Glcα1→3Hepα1

GlcNAcα1

where KDO is 2-keto-3-deoxy-manno-octulosonic acid and Hep is heptose. Comparison of this OS structure with those determined previously indicates the presence of a new glycosylation pathway for gonococcal OS biosynthesis: elongation of a GlcNAC-linked heptose, in contrast to elongation of the other heptose by sequential addition of glycoses which results in the antigenic similarity with human glycolipids. The current study provides not only additional structural information on LOS expressed during different clinical states of infection but also evidence for the diversity of gonococcal LOS biosynthesis. This evidence may be helpful in understanding the pathogenesis involving gonococcal LOS.

The lipooligosaccharides (LOSs) of Neisseria gonorrhoeae are important pathogenic and antigenic outer membrane components. LOSs cause damage on human fallopian tubes (1), and normal human serum contains anti-LOS bactericidal antibodies (2–4). Patients with disseminated infection produce anti-LOS antibodies whose specificities are different compared with normal human serum (5). Therefore, LOSs are important in understanding human immune responses against gonococci.

Each gonococcal strain may produce several antigenically different LOS components. Immunological (6–8) and structural (9–14) studies have shown that these antigenic differences of LOS components are due to their structural differences, primarily differences in the lipooligosaccharide moiety of the LOS. In addition, a recent study indicated that gonococcal LOS expression can shift with the stage of infection (15); a strain producing a single LOS component has the potential to produce multiple LOS components. Thus, LOS structure not only varies among strains, but within a single strain depending upon the environment.

Furthermore, gonococci can modify the LOS within the host, and such structural alteration can affect the interaction of gonococci with human immune defenses. Gonococci utilize exogenous cytidine monophospho-N-acetylmuramic acid and sialylate their LOSs in vivo, and, as a result of this sialylation, gonococci become resistant to complement-mediated killing (16, 17). Present data suggest that sialylation takes place in vivo (18, 19).

We have studied the structures and epitope expression of some prevalent LOS components produced by clinical gonococcal isolates (6, 7, 9, 12–14, 20). The 4.5-kDa LOS recognized by mAb 3F11 has a lactoneotetraosyl residue at the nonreducing end. We determined that the antigenic similarity between the 4.5-kDa LOS and a human glycosphingolipid, paragloboside (20), is due to an identical LOS structure (13). The 5.1-kDa LOS recognized by another mAb, mAb 1-1-M, has a N-acetylgalactosaminylated lactoneotetraose. We also showed that these higher molecular mass LOSs such as 4.5 and 5.1-kDa LOSs are produced by elongating the carbohydrate sequence of a small molecular mass LOS (14, 15).

Gonococci produce several other LOS components whose structures are unknown, and these unidentified LOSs could be important for understanding the role and functions of the LOS in gonococcal disease. Recent studies suggest that the 4.5-kDa LOS, antigenically similar to human tissues (20), is associated with the experimental infection of mice (21) and the 5.1-kDa LOS is associated with the disseminated infection of rabbits (22).

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† To whom correspondence should be addressed: UCSF/VAMC 111W1, 4150 Clement St., San Francisco, CA 94121. Tel.: 415-476-9331; Fax: 415-221-7542
‡ Present address: NMR and Data Processing Laboratory, Syracuse University, Syracuse, NY 13244.
§ Present address: Athena Neuroscience Co., South San Francisco, CA 94080.
¶ Present address: Children’s Hospital Oakland, Research Institute, Oakland CA 94609.
** Present address: Athena Neuroscience Co., South San Francisco, CA 94080.
†† Present address: Children’s Hospital Oakland, Research Institute, Oakland CA 94609.
‡‡ Present address: NMR and Data Processing Laboratory, Syracuse University, Syracuse, NY 13244.
§§ Present address: Athena Neuroscience Co., South San Francisco, CA 94080.

The abbreviations used are: LOS(s), lipooligosaccharide(s); COSY, chemical shift correlation spectroscopy; DQF, double quantum filtered; ESI-MS, electrospray ion mass spectrometry; Hep, Heptose; HOHANA, homonuclear Hartman-Hahn spectroscopy; HPACE, high performance anion exchange chromatography; KDO, 2-keto-3-deoxy-manno-octulosonic acid; mAb, monoclonal antibody; MS, mass spectrometry; NOE, nuclear Overhauser effect; OS, lipooligosaccharide; PAGE, polyacrylamide gel electrophoresis.
with the gonococcal disease process (15) and that gonococcal LOS may play an important role in the interaction with human cell tissues. To understand the complex LOS biosynthesis that occurs both in vivo and in vitro, further structural determination of gonococcal LOSs of clinically relevant strains is essential.

This present paper describes the structure of the LOS produced by strain 15253. This strain was isolated from a patient who had developed disseminated gonococcal infection. mAb 3G9 binds to this LOS (7, 22), and its binding patterns to gonococcal LOS indicate that the 15253 LOS is structurally different from the LOSs studied previously (13, 14). This structural study of the OS derived from the 15253 LOS provided evidence for the presence of a novel glycosylation pathway for gonococcal LOS biosynthesis.

EXPERIMENTAL PROCEDURES

Materials—Disseminated gonococcal infection isolates of N. gonorrhoeae and mAb 3G9 (mouse IgG) were kindly provided by Dr. Peter Rice (Maxwell Finland Laboratory for Infectious Diseases, Boston University and Boston City Hospital). Other gonococcal strains used in this study were generously provided by Claudia Lammel and Dr. Geoff Brooks (Department of Laboratory Medicine, University of California, San Francisco). Other neisserial strains used are from our laboratory strain collections.

Neisserial strains were grown as described previously (23, 24). We measured the binding of mAb 3G9 to bacteria by using solid-phase radioimmunoassay (7, 24). Strains were designated as positive for the 3G9 epitope if they bound mAb >10% the amount of binding by a positive control strain, strain 15253. The 15253 LOS was purified using the hot phenol water extraction method (25) and analyzed by PAGE/immunoblot (14).

Oligosaccharides—The 15253 OS (dephosphorylated unless otherwise stated) was prepared as described previously (12–14): (a) hydrolysis of the LOS (1% AcOH, 2 h at 100°C); (b) Bio-Gel P-4 chromatography (Bio-Rad) (<400 mesh, 2.6 cm × 90 cm, 100 mM ammonium acetate) and desalting by Bio-Gel P-2 chromatography (<400 mesh, 2.6 cm × 90 cm, water); (c) dephosphorylation (48% HF, 48 h at 4°C); (d) final purification by the Bio-Gel P-2 chromatography.

Analytical Methods—The molecular weight of the 15253 OS was estimated by electrospray ion mass spectrometry (ESI-MS) analysis; scans were taken in the negative-ion mode, and mass scales were calibrated using external carbohydrate standards as described previously (26, 27). Two-dimensional NMR data (the number of acquisitions >1024, 2000 data points, 2000 Hz (both dimensions); the 512 × 4096 real data points) were acquired at 19.8 and 50°C. Two-dimensional NMR scans were taken in the negative-ion mode, and mass scales were calibrated using external carbohydrate standards as described previously (26, 27).

RESULTS AND DISCUSSION

Strain 15253 (also designated as DGI3 in Ref. 22) produces predominately a single LOS (Fig. 1), and mAb 3G9 recognizes this LOS (7, 22). Table I summarizes the results of the binding of mAb 3G9 to strains of N. gonorrhoeae and other Neisseria species. In contrast to other mAbs such as 3F11 and 2-1-L8 studied previously (6, 7), this mAb does not bind to other Neisseria species and appears to be specific for gonococcal LOSs. In addition, mAb 3G9 bound all strains from patients with disseminated or pelvic inflammatory disease, whereas it bound only 60% of the strains causing uncomplicated disease (Table I). These preliminary results suggest a correlation of the mAb 3G9-defined epitope with gonococci causing systemic infections and prompted us to study the structure of a LOS that binds mAb 3G9. The LOS of strain 15253 from a patient with disseminated infection was purified, and the structure of its OS was investigated.

The results obtained in the compositional and NMR analysis indicated that 15253 OS is an octamer with a carbohydrate composition Gal:Glc:GlcNAc:Hep:KDO in a ratio of 2:2:1:2:1. This composition was also supported by molecular mass elucidation of the 15253 OS by ESI-MS (26, 27). The ESI-MS analysis indicated the presence of two major OS species whose molecular weights are 1456.8 and 1478.6. These weights were calculated from the two abundant doubly charged peaks (M+2H)2±, at m/z 737.0 and 738.3, respectively. The larger molecular weight species at m/z 738.3 is probably a sodiated form of the smaller one (1,456 + sodium). The calculated molecular weight for the 15253 OS is 1456.3, and therefore, the electrospray data confirm that this OS is an octamer of the carbohydrate composition described above.

Fig. 2A shows the one-dimensional spectrum of the dephosphorylated 15253 OS at 19.8°C together with its proposed structure. Seven anameric protons were confirmed: I (5.474 ppm); II (5.339 ppm); III (5.116 ppm); V (4.563 ppm); and VI (4.096 ppm).
Structural Analysis of N. gonorrhoeae Lipooligosaccharide

FIG. 2. Panel A, the one-dimensional spectrum (resolution enhanced) of the OS at 19.8 °C and the structure of the 1S253 OS. The Roman numerals refer to the seven different carbohydrate residues as shown above. Panel B, parts of the DQF-COSY spectrum which show the H-1/H-2 cross-peaks. The Roman numerals refer to the carbohydrate residue (see the structure), and the Arabic numerals refer to the proton in the respective carbohydrate residue. The subscript refers to the proton whose chemical shift is given on the \( \omega_2 \) axis, and the superscript refers to the proton whose chemical shift is given on the \( \omega_1 \) axis. The digital resolutions of the DQF-COSY spectrum were 0.78 and 1.56 Hz/point in the \( \omega_2 \) and \( \omega_1 \), respectively.

We determined the identity of each carbohydrate residue by comparatively analyzing DQF-COSY and HOHAHA spectra with those of the OSs studied previously (13, 14). This identification was also aided by NOE data analysis. Table II shows the chemical shifts and the coupling constant data of each carbohydrate residue except KDO.

The coherence transfer of H-1 to the exocyclic protons (Fig. 3) and the coupling constants (Table II confirmed that residues II, III, and V have gluco configurations (13, 14). We assigned residue III as GlcNAc and both residues II and V as Glc. As will be described later, the Gal residue (VI) was linked to the 4-position of residue Glc. We did not detect the 4-linked GlcNAc in the methylation analysis, and therefore, GlcNAc is either II or III. Previous studies show that the anomeric proton of GlcNAc attached to a Hep is located at ~5.10 ppm (13, 14), and from this chemical shift comparison, the residue III whose anomeric proton is at 5.119 ppm was assigned as GlcNAc.

Similarly, the residues VII and VI were assigned as Gal, and residues I and IV assigned as Hep. The H-5 of the Gal residues, VI and VII, were assigned to be ~3.77 and 3.725 ppm, respectively, from the NOE analysis (Fig. 4) as will be described later. With the exception of H-2, the \(^1H\) chemical shifts of Hep(IV) were similar to those of the corresponding Hep of F62 and
Structural Analysis of N. gonorrhoeae Lipooligosaccharide

IV-5 and IV-6 were assigned based on our previous work (14), and IV-3 and -4 were also confirmed by the NOE analysis as shown below. Compared with the MS11mk OS, H-3 of the GlcNAc-linked Hep(1) was ~0.19 ppm shielded downfield, which is due to the substitution of 3-OH of the Hep(1) (Fig. 2A). This substitution contributed to the overlap of 1-2 and 1-3, and this overlap was indicated by the intensity and width of the 1-1:1-2 relay peak (Fig. 4). In contrast to the OSs studied previously (13, 14), the coherence of H-1 of the GlcNAc-linked Hep(1) was not transferred beyond H-3 (Fig. 4), which is possibly due to shorter $T_2$ times of the protons of Hep(1).

The structure of the 15253 OS was analyzed by methylation and two-dimensional NOE data analyses (13, 14). We determined that the 15253 OS with the addition of two glycoses was identical to the OS structure of the MS11mk LOS (14); another lactosyl residue, Gal(VII)-1-4Glc(II), was α1-5 linked to the Hep(1) to which GlcNAc(III) was attached (Fig. 2A).

Methylation results (Table III) and the interresidual NOEs (Fig. 4) confirmed the presence of 2 lactosyl residues, Gal(VI)-β1-4Glc(V) and Gal(VII)-β1-4Glc(II). Methylation analysis indicated the presence of α-Gal and 4-linked Glc. The interresidual NOE, Gal(VI)-1-Glc(V)-4, confirmed the presence of the first lactose structure (Fig. 4A). This NOE, indistinguishable from the intraresidual NOE $V_{VI}^I$ at $\tau_m = 200$ ms (Fig. 4B), became more distinct at $\tau_m = 250$ ms (Fig. 5A). The intraresidual NOEs between the 1,3-diaxially oriented protons were also detected with the β-anomers (13), and H-5 of Gal(V) was assigned to be at 3.775 ppm by this NOE analysis.

The interresidual NOE, Gal(VII)-1-Glc(II)-4 (Fig. 4A), confirmed the second lactose residue. Although the chemical shift difference between Gal(VI)-1 and Gal(VII)-1 was 0.01 ppm and the intraresidual NOEs partially overlapped, we were able to distinguish this interresidual NOE, Gal(VII)-1-Glc(II)-4, from the intraresidual ones, $V_{VI}^I$ and $V_{VII}^I$, by analyzing the 200- and 250-ms NOE data (Fig. 4, A and B). Similar to Gal(VI), the H-5 of Gal(VII) was assigned to be 3.725 ppm.

### Table II

| Chemical shifts and coupling constants of the carbohydrate components of the 15253 OS (the structure is shown in Fig. 2A) |
|---|---|---|---|---|---|---|
| Gal | Gal | Glc | GlcNAc | Gal | Hep |
| VI | VII | V | IV | II | I |
| H-3 | 3.672 | 3.696 | 3.647 | 4.144 | 3.792 | 3.816 | -4.16 |
| H-6 | 3.788 | -4.26 | -3.79 | 3.791 |
| H-6' | 3.997 | -4.33 | 3.890 |
| $\delta_{J_{12}}$ | 10 | 10 | 10 | 10 | 10 | 10 | 3 |
| $\delta_{J_{23}}$ | 4 | 4 | 10 | 9 | 9 |
| $\delta_{J_{34}}$ | 10 | -9 | -10 |
| $\delta_{J_{45}}$ | -6 | -5 |
| $\delta_{J_{56}}$ | -12 | -12 | -11 |

Fig. 3. Parts of the HOHAHA spectra of the 15253 OS at 19.8 °C. Panel A ($\tau_m = 120$ ms): Gal(VII), Gal(VI), Glc(V). Panel B ($\tau_m = 200$ ms): GlcNAc(III) and Hep(IV). Panel C ($\tau_m = 200$ ms): Hep(I) and Glc(II). Panel D ($\tau_m = 120$ ms): coherence relays of the KDO proton at 1.986 ppm (see Fig. 2B). The cross-peaks are labeled as explained in Fig. 2B. The digital resolutions of the spectrum were 0.78 and 3.12 Hz/pixel in the $\omega_1$ and $\omega_2$, respectively.
Structural Analysis of \( N. \) gonorrhoeae Lipooligosaccharide

**Fig. 4.** Parts of the pure absorption two-dimensional NOE spectra of the 15253 OS at 19.8 °C. They show the intra- and interresidual NOE cross-peaks of each anomeric proton. Panel A \((\tau = 250 \text{ ms})\): Gal(VII); Gal(VI); Glc(V). Panel B \((\tau = 200 \text{ ms})\): the NOEs of the two residues, Gal(VII) and Gal(VI), are shown for comparison with panel A. For the purpose of obtaining better resolution of the VII and VI residues, the NOEs for both A and B were plotted along the \( \omega_2 \) dimension. Therefore, substricpts and superscripts in A and B refer to the protons whose chemical shifts are given on the \( \omega_1 \) and \( \omega_2 \) axes, respectively. Panel C \((\tau = 200 \text{ ms})\): GlcNAc(III) and Hep(IV). Panel D \((\tau = 200 \text{ ms})\): Hep(I) and Glc(II). The digital resolutions of both spectra were 0.98 and 3.92 Hz/point in the \( \omega_2 \) and \( \omega_1 \) axes, respectively. Only some interresidual NOEs are labeled.

**Table III**

Methylation analysis of the dephosphorylated 15253 OS

Relative retention times \((t_r)\) are relative to 2,3,4,6-tetra-O-methylglycolactitol. The partially methylated alditol acetates in the table gave identical fragmentation patterns to those as reported previously (10, 13, 14). Trace amounts of t-Glc and 4-Gal were also detected in the analysis. No acetate derivatives of partially methylated deoxyoctitol were detected in this analysis.

<table>
<thead>
<tr>
<th>Detected as alditol acetates</th>
<th>( t_r )</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4,6-Tetra-O-Me-Gal</td>
<td>1.00</td>
</tr>
<tr>
<td>2,3,6-Tri-O-Me-Glc</td>
<td>1.08</td>
</tr>
<tr>
<td>3,4,6-Tri-O-Me-GlcNAc</td>
<td>1.27</td>
</tr>
<tr>
<td>2,6,7-Tri-O-Me-Hep</td>
<td>1.28</td>
</tr>
<tr>
<td>4,6,7-Tri-O-Me-Hep</td>
<td>1.33</td>
</tr>
</tbody>
</table>

We determined that the Gal(VII)\(1\rightharpoonup4\)Glc(II) and Gal(VI)-\(\beta1\rightharpoonup4\)Glc(V) were, respectively, \( \alpha1\rightharpoonup3 \) linked to the Hep(I) and \( \beta1\rightharpoonup4 \) linked to the Hep(IV), and we confirmed the presence of GlcNAc(III)\(1\rightharpoonup2\)Hep(III)\(1\rightharpoonup3\)Hep(IV)\(1\rightharpoonup1\rightharpoonup1\)KDO. The proposed structure was supported by the presence of 2,3-linked Hep, 3,4-linked Hep, and \( \gamma \)-GlcNAc in the methylation analysis (Table IV) and by the following interresidual NOE data (Fig. 5): (a) Glc(V)-1:Hep(IV)-4; (b) Glc(II)-1:Hep(I)-3; (c) GlcNAc(III)-1:Hep(I)-2. We were not able to assign the cross-peak due to \( \omega_1 \)-1 and a proton centered at 4.135 ppm; (d) Hep(I)-1:Hep(IV)-3. This NOE analysis also confirmed the assignments of some protons described earlier.

The Hep(IV) is suggested to be linked to the 4- or 5-position of the KDO. The cross-peaks due to Hep(IV)-1 and proton(s) at ~4.2 ppm may be the interresidual NOEs; IV-1 and the KDO proton(s), possibly H-4 or H-5; the NOE cross-peaks matched with the HOHAHA relay peaks from one (at 1.895 ppm, Fig. 4D) of the KDO deoxy protons. Further studies to identify this NOE together with the unresolved structure of the KDO at the reducing end (13, 14) will be published elsewhere.

From the data presented above, we propose the structure of the 15253 OS as shown in Fig. 2A. The OS moiety of 15253 LOS has 2 lactosyl residues at nonreducing ends; Gal(VII)\(\beta1\rightharpoonup4\)Glc(V) is \( \beta1\rightharpoonup4 \)-linked to the Hep(IV) residue and Gal(VII)-\(\beta1\rightharpoonup4\)Glc(II) is \( \alpha1\rightharpoonup3 \)-linked to the Hep(I) residue. This work is the first structural evidence to describe the modification of a GlcNAc-linked Hep residue (I in Fig. 2A) by gonococci. Previously, it has been proposed that GlcNAc and Glc are \( \beta \)-linked to the 2- and 3-hydroxy groups of this Hep residue, respectively. However, to date, no data have been available to support these proposed structures.

The mobility of the 15253 LOS in PAGE and the retention time of its OS in the HPAEC are noteworthy. Since the OSs derived from 15253 LOS and the 4.5-kDa LOS recognized by mAb 3F11 are both octamers, the PAGE mobility of the 15253 LOS might be expected to be similar to the 4.5-kDa LOS. However, the 15253 LOS migrated just above the 3.6-kDa LOS recognized by mAb 2-1-L8 (Fig. 1) and therefore would be assigned a molecular mass of 3.7–3.9 kDa. Similarly, the retention time of the 15253 OS by HPAEC was longer than expected based on the elution patterns of F62 and MS11mk OS (Table...
Further elongation? Galβ1→4Glcβ1→4Hepα1→KDO

Further elongation? Galβ1→4Glcβ1→4Hepα1→KDO

Galβ1→4Glcβ1→3Hepα1

GlcNAcα1

15253 OS

MS11mk OS

(3.6 KDa LOS)

GlcNAβ1→3Galβ1→4Glcβ1→4.2 KDa LOS

Galβ1→4GlcNAβ1→3Galβ1→4Glcβ1 (paragloboside structure)

GlcNAβ1→3Galβ1→4GlcNAβ1→3Galβ1→4Glcβ1→4.8 KDa LOS

Table IV

HPAEC analysis of gonococcal OSs

Retention times are relative to the OS derived from the 3.6-kDa LOS (MS11mk LOS). The structures of the OSs derived from the 3.6-4.8-kDa LOSs are shown in Fig. 5.

<table>
<thead>
<tr>
<th>Parent LOS (kDa)</th>
<th>Retention times</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.6</td>
<td>1.00</td>
</tr>
<tr>
<td>4.2</td>
<td>0.98</td>
</tr>
<tr>
<td>4.5</td>
<td>0.84</td>
</tr>
<tr>
<td>4.8</td>
<td>0.68</td>
</tr>
<tr>
<td>15253 LOS</td>
<td>1.78</td>
</tr>
</tbody>
</table>

The present study reveals a newly recognized glycosylation pathway for gonococcal LOS. Our previous studies indicated that higher molecular mass LOS components elongate from a LOS precursor represented by a LOS similar to MS11mk (13, 14). For example, elongation of the lactosyl moiety of the MS11mk LOS with presumably β-N-acetylglucosaminyltransferase followed by the sequential addition of Gal leads to synthesis of a 4.5-kDa LOS that shares the same OS moiety at the nonreducing end as the mammalian glycosphingolipid paragloboside (Fig. 5). The 4.5-kDa LOS is modified further in some strains by the addition of N-acetylgalactosamine (12, 13) or, in other instances, by sialic acid (35). In addition, a recent study reported that a pyocin-resistant mutant strain synthesizes an OS tentatively proposed as Galα1→4Galβ1→4Glc (Pk antigen) at its nonreducing end (11). In contrast to these two different types of elongation of the lactosyl residue described previously, strain 15253 modifies the GlcNAc-linked heptose of the LOS, producing a second lactosyl residue.

It is also possible that 15253 gonococci may further elongate one or the other, or both, of the lactose residues (Fig. 5). Previous immunochemical analysis indicated that mAb 3G9 binds to other LOSs larger than the molecular mass of the 15253 LOS (22). In addition, strain 15253 gonococci grown in the presence of cytidine monophospho-N-acetylneuraminic acid can sialylate its LOS, although much lower than the gonococci that produce the 4.5-kDa LOS (36). Further structural and immunochemical analysis of other LOSs recognized by mAb 3G9 and the sialylated 15253 LOS should provide a definitive answer regarding the specificity of mAb 3G9 and the characteristics of this new type of OS elongation.

The lactosylation of a GlcNAc-linked heptose residue described above not only provides evidence for a new glycosylation pathway of gonococcal LOS biosynthesis, but also further insight into the immunogenicity and the role of the gonococcal LOS in the disease process. Strain 15253 was isolated from the nasopharynx of a patient with disseminated gonococcal infection (7, 22, 37), and the binding of mAb 3G9 to 100% of the available strains causing systemic disease suggested that this epitope might be associated with dissemination. In addition to this possible link with the disease process, the LOS structure of strain 15253 suggests a possible interaction with human tissues. Either of the Galβ1→4Glc residues of the 15253 LOS could be acceptor substrates in vivo for human glycosyltransferases that recognize similar carbohydrates (38). Also, human lectins may bind to the 15253 LOS via either lactosyl residue (21). These possible in vivo interactions may play some role in the disease process.

The novel structure represented by 2 lactosyl residues in the same LOS may be related to the antigenic specificity of mAb 3G9 for gonococci. mAb 3G9 binds to >60% of the gonococci or gonococcal LOS that have been tested, but it does not bind to other neisserial species, including LOSs on strains of Neisseria meningitidis and nonpathogenic Neisseria (7, 22) (Table I). Studies by us and others indicate that the antigenic similarity between meningococci and gonococci is indeed due to identical OS structures expressed by these two species (7, 11, 13, 14, 20, 39–42). However, to date, no meningococcal strains that produce 2 Galβ1→4Glc residues at the nonreducing ends of LOS have been identified. mAb 3G9 does not bind to meningococcal LOSs of serotype L10 and L11 whose complete structures have yet to be determined (27). These preliminary results support the antigenic evidence obtained to date (Table I) that the mAb 3G9-defined epitope is specific for gonococci.

Strain 15253 produces a novel LOS structure that has 2 lactosyl residues at its nonreducing ends. This new glycosyla-
tion pathway not only demonstrates additional complexity in gonococcal LOS biosynthesis but also provides additional structural information on LOS expressed during different clinical states of infection.

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