Conservation of the Lipooligosaccharide Synthesis Locus *lgt* among Strains of *Neisseria gonorrhoeae*: Requirement for *lgtE* in Synthesis of the 2C7 Epitope and of the β Chain of Strain 15253

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Summary

The present study was undertaken to examine the extent to which the *lgt* locus varies among strains of gonococci. This locus encodes five glycosyl transferases involved in the synthesis of the lipooligosaccharide (LOS) of *Neisseria gonorrhoeae*. We examined seven gonococcal strains and found that the structure of the *lgt* locus is conserved among six of these strains. The locus is strikingly altered in strain 15253. This is one of the few strains where extensive structural analysis of its LOS is available, and therefore, we defined the altered *lgt* locus and focused on the reactivity of mAB 2C7. We found that strain 15253 contains only two *lgt* genes, *lgtA* and *lgtE*. As in F62, *lgtA* encodes a GlcNAc transferase and is subject to phase variation. In addition, by analysis of deletion mutants, we found that *lgtE*, which encodes a galactosyl transferase that is required for elongating the α-chain, is also necessary for completing the β chain.

The LPS of *Neisseria gonorrhoeae* and *N. meningitidis*, like those of *Haemophilus influenzae*, differ from the LPS of enteric bacteria in that they lack O antigen and possess relatively short oligosaccharides that are subject to phase variation. Because of their small size, these LPS are sometimes referred to as lipooligosaccharides (LOS)[1], the term that will be used in this report. The genetics of synthesis of LOS and of their phase variation is currently the subject of study in several laboratories. In neisserial LOS, the inner region, containing heptose and ketodeoxyoctulosonic acid (KDO) residues, is very similar to that of the *Salmonella* species, and this similarity allowed identification of both of the gonococcal heptosyl transferase genes by complementation of *Salmonella rfaC* and *rfaF* mutants (1, 2). The heptoses are substituted by three saccharide chains that have been designated α, β, and γ (see Fig. 1). The γ chain, α1→2–linked to heptose 2, is present in all LOS that have been characterized and consists of a single *N*-acetylglucosamine (GlcNAc). *rfaK*, the gene for the GlcNAc transferase that produces the γ chain, has recently been identified in the meningococcus (3, 4). The β chain consists of a lactosyl group linked α1→3 to heptose 2, and has been chemically identified in only one strain so far, 15253 (5). mAB 2C7, however, which is thought to recognize the β chain, binds to 95% of clinical gonococcal isolates (6), so it is possible that the β chain is present on a large proportion of gonococcal isolates. The genes responsible for the synthesis of the β chain have not been identified. The α chain, which substitutes heptose 1, is the part of the molecule that is subject to high frequency variation, and has also received the most attention. In gonococcal strain F62, the *lgt* locus contains five genes encoding glycosyl transferases that are responsible for the synthesis of the α chain, with the exception of the initiating glucose (7). Three of these genes undergo frequent on/off switching as a result of changes in the number of guanosine residues in poly-guanosine (G) tracts within their coding frames (8, 9). A similar locus that contains three genes has been described in the *N. meningitidis* strain MC58, and has been shown to undergo similar variation (10).

The heterogeneity of LOS seen by gel electrophoresis among different strains of gonococci suggested the possibility that the organization of the *lgt* locus might vary among different strains. The present study was undertaken to examine the extent to which the *lgt* locus varies among strains of gonococci. Our experiments showed that while the structure of the *lgt* locus is conserved among several gonococcal strains, it is strikingly altered in strain 15253. Since this strain is one of the few strains where extensive struc-

[1]*Abbreviations used in this paper:* G, guanosine; GlcNAc, *N*-acetylglucosamine; GSII, *Griffonia simplicifolia* II; Hep, heptose; KDO, ketodeoxyctulosonic acid; LOS, lipooligosaccharide.

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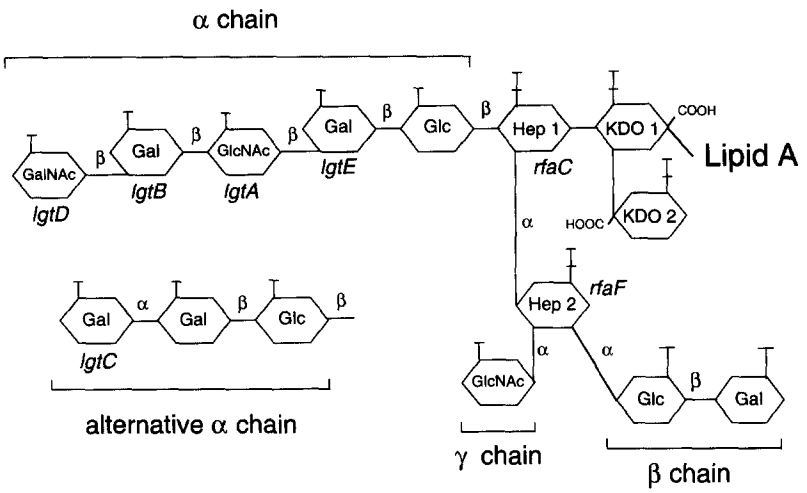

Figure 1. Generalized structure for gonococcal LOS, incorporating features from several reported structures. The β chain has been reported only for strain 15253 (5). In the 15253 LOS studied by Yamasaki et al., the α chain was limited to Galβ1→4Glc (5). F62 wt LOS consists of two structures, one with the complete α chain shown here, and the other with an α chain lacking the terminal GalNAc (26). The α chain structure reported for 1291 wt also consists of a tetrasaccharide without a terminal GalNAc (27). The alternative α chain has been reported for 1291b (27) and for a mutant of F62 with a deletion in *lgtA* and *lgtB* (7). The α chain of MS11 variant A consists of the disaccharide Galβ1→4Glc, while MS11 variant C contains several LOS structures, the most prominent of which has a tetrasaccharide α chain (23, 24).

tural analysis of its LOS is available, we defined its altered *lgt* locus and focused on the reactivity of mAB 2C7. We found that strain 15253 contains only two *lgt* genes, *lgtA* and *lgtE*. As in F62, *lgtA* encodes a GlcNAc transferase and is subject to phase variation. In addition, by analysis of deletion mutants, we found that *lgtE*, which encodes a galactosyl transferase required for elongating the α chain, is also necessary for completing the β chain.

Materials and Methods

Bacterial Strains, Plasmids. The Neisserial strains and their sources are listed in Table 1, and the plasmids are depicted in Fig. 2. The plasmids used to generate mutations in the *lgt* locus were the same as those used previously (7). The plasmids used as probes contain the following inserts cloned into pBluescript (numbers refer to the sequence of the *lgt* locus deposited in GenBank/EMBL/DDBJ under accession number U14554 [7]): probe A/D, the RsaI fragment from 389 to 1043; probe B/E, the RsaI-BsaBI fragment from 1427 to 2346; probe C, the HinfI fragment from 2206 to 3241.

Southern Analysis of lgt Loci. Probes were labeled with fluorescein and used to probe restriction-digested genomic DNA by the enhanced chemiluminescence method (ECL) of Amersham Corp., as previously described (7, 11).

Construction of LOS Mutants. Piliated gonococci were transformed with plasmids I2, Δ4, or Δ5 by the method described previously (12), and transformants were identified by screening for erythromycin resistance.

Cloning of the lgt Locus of Strain 15253. Genomic DNA of strain 15253 was digested with ClaI and separated by gel electrophoresis, and DNA with a mobility corresponding to ~6 kb was excised from the gel. Using primers matching 309–328 and the reverse complement of 1182–1202 of the *lgt* locus (GenBank/EMBL/DDBJ accession number U14554 [7]), a 890-bp product was obtained by PCR amplification of the gel-purified DNA, indicating that it contained the *lgtA* sequence. The fragments were ligated into plasmid pSU39 (13) that had been cut with AccI and phosphatase treated, and the ligation was used to transform XL1-Blue MRF′ competent cells which were then plated on kanamycin plates containing X-Gal and IPTG. 20 clear colonies were isolated. Plasmids were prepared from each colony and pooled, and a PCR that was performed with the primers proved positive. This was repeated with pools containing five plasmids each, and then with each plasmid to identify the desired clone. Automated sequencing was performed by The Rockefeller University Protein/DNA Technology Center.

Table 1. *Bacterial Strains*

Strain	Characteristics	Source or reference
N. gonorrhoeae		
F62	Serum sensitive	(37)
F62Δ4	Deletion of *lgtA-lgtE*	(7)
F62Δ5	Deletion of *lgtA-lgtD*	(7)
FA1090	Serum resistant	Myron Cohen (University of North Carolina, Chapel Hill) and reference 37
FA1090Δ4	Deletion of *lgtA-lgtE*	This work
FA1090Δ5	Deletion of *lgtA-lgtD*	This work
FA1090*lgtE*	Insertion in *lgtE*	This work
15253	Serum resistant	(6, 28)*
15253*lgtE*	Insertion in *lgtE*	This work
1291		(37)
M94	AHU⁻; serum resistant	C.A. Ison (St. Mary's Hospital, London)
MS11	Serum resistant	John Swanson (Rocky Mountain Laboratory, NIH, Hamilton, MT) and reference 37
R10		(37)
N. meningitidis		
M1080		(38)

*Strain 15253 is designated in reference 6 as isolate 1 and in reference 28 as DGI 3.

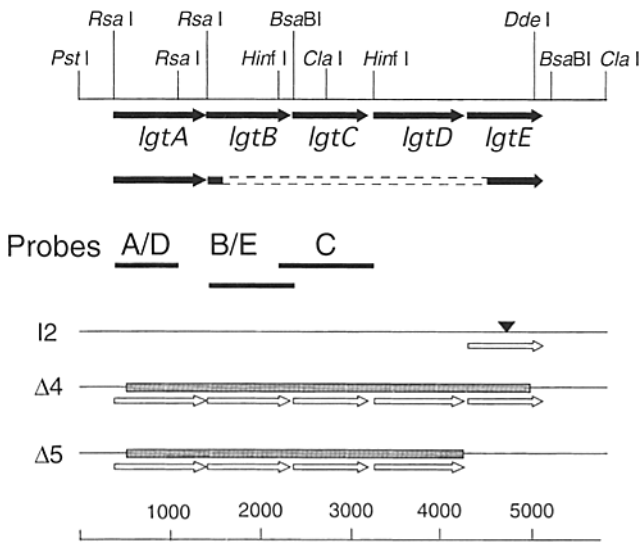

Figure 2. Map of the *lgt* locus. A restriction map is shown above the five genes of the *lgt* locus of strain F62. Below is depicted the structure proposed for the *lgt* locus of strain 15253 (see text). Probes A/D, B/E, and C were used for the Southern blots shown in Fig. 4, and are described in Materials and Methods. Plasmid I2 was used for insertional mutagenesis of *lgtE*, and plasmids Δ4 and Δ5 were used for making deletions of the regions shown. These plasmids include *ermC'* at the site of mutation to allow identification of transformants.

Purification of LOS. Bacteria were washed twice with acetone and dried under a vacuum. LOS was extracted from the acetone powder by hot phenol–water extraction (14), precipitated from the water phase with ethanol, and resuspended in water. Contaminating nucleic acids were removed by three or four cycles of ultracentrifugation (100,000 *g* for 4 h), followed by suspension in water. The final preparation was lyophilized and weighed, and a portion was suspended in water at 2 or 10 mg/ml.

Gel Electrophoresis and Immunoblotting. Gel electrophoresis (100–200 ng LOS per lane) was carried out using a tricine-SDS system modified from that described by Lesse et al. (15). The sample loading buffer consisted of 0.032 M Tris (pH 6.8), 1% SDS, 5% 2-ME, 1 mM EDTA, and 55% glycerol, with bromophenol blue added as tracking dye. An electrophoresis buffer consisting of 0.1 M Tris base, 0.1 M tricine, and 0.1% SDS was used for both the cathode and anode buffer. Gels were stained with silver (16). For immunoblotting, LOS were transferred by electrophoresis to polyvinyldifluoride membranes (Immobilon-P; Millipore Corp., Bedford, MA), as described by Towbin (17). Blocking (1 h), mAb treatment (overnight), washes (15 min three times), and second antibody treatment (3–4 h) were all carried out using a buffer consisting of 1% casein, 150 mM NaCl, 10 mM Tris (pH 7.5), and 30 mM NaN_3. mAB 2C7 (18, 19) was used at a dilution of 1:50. The second antibody was alkaline phosphatase–conjugated goat antibodies to mouse IgG, IgM, and IgA (Cappel, Organon Teknika Corp., West Chester, PA) used at a dilution of 1:2,000. After the second antibody treatment and washes, alkaline phosphatase activity was detected using 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium (20).

Screening Colonies with Lectin. GC agar plates were seeded with a dilution of strain 15253 containing 1,000 cfu and grown until the colonies were ~0.5 mm. A nitrocellulose filter was overlaid for 2 min, removed, and allowed to dry for 90 min. The plates were incubated at 30°C to allow regrowth of the colonies. The filter was placed into 2% gelatin dissolved in 10 mM Tris, pH 7.6, 150 mM NaCl, 1 mM $CaCl_2$, 0.02% NaN_3, and incubated for 60 min at 37°C. All subsequent steps were performed at room temperature in the same buffer containing 1% gelatin. The filter was incubated for 60 min with 20 μg/ml of biotinylated GSII lectin (*Griffonia simplicifolia II*) obtained from EY Laboratories Inc. (San Mateo, CA). The filter was washed four times for 10 min, incubated for 60 min with a 1/2,000 dilution of alkaline phosphatase–conjugated avidin (EY Laboratories). After washing the filter four times with buffer, it was stained for 20 min for phosphatase activity as described above. The gelatin or the filter itself appeared to react by this staining procedure, producing an overall background staining, while the bacterial colonies actually inhibited the reactivity and were negatively stained. There were four colonies that reacted strongly, however, and two of these were well separated from other colonies and were recovered after overnight growth at 30°C.

Analysis of Sugar Composition. Approximately 200 μg LOS was hydrolyzed with acetic acid (1% final concentration in a total volume of 0.3 ml) for 2 h at 100°C, and the released lipid A was removed by centrifugation. The oligosaccharide solution was lyophilized and dissolved in water (0.3 ml). Monosaccharides were prepared by hydrolysis of 0.1 ml oligosaccharide solution with 4 M TFA for 4 h at 100°C in a total volume of 0.2 ml. The hydrolysate was dried in a Speed-Vac (Savant Instruments, Inc., Farmingdale, NY) and dissolved in 0.2 ml water. This step was repeated twice more, dissolving the final preparation in 100 μl water. Monosaccharides were analyzed by high pH anion exchange chromatography (Dionex Instruments, Sunnyvale, CA), as described (21, 22), with a modification to allow the determination of heptose. The solvent system used was 15 mM NaOH for 20 min, a linear gradient to 45 mM NaOH at 30 min, held at 45 mM for 5 min. A standard mixture of sugars contained 0.1 mM each fucose, galactosamine, galactose, glucosamine, glucose, mannose, rhamnose (all obtained from Sigma Chemical Company, St. Louis, MO), and glucoheptose (Pfanstiehl Laboratories, Inc., Waukegan, IL). LPS from *Escherichia coli* J5 and from *Salmonella minnesota* R60 (obtained from List Biological Laboratories, Campbell, CA) were analyzed in the same manner. In addition to the TFA hydrolysis, the oligosaccharide preparation from 15253 was also hydrolyzed in 4 N HCl (4 h, 100°C) to allow the determination of the proportion of hexosamines that were not recovered during TFA hydrolysis (22). The resulting correction factor, 0.62, was used in the calculations of molar ratios.

Results

Comparison of lgt Loci in Gonococcal Strains. We compared LOS from F62 with LOS from six other gonococcal strains by SDS-PAGE (Fig. 3, *upper panel*). 1291 LOS comigrated with the lower band of F62 LOS, consistent with the structures reported for these LOS. 15253 LOS migrated as a single low band, as reported (5). The LOS of other strains, however, had bands that could not be identified by comparison with LOS of known structure. As reported previously (23, 24), the LOS of MS11 (variant C) had three bands, two comigrating with F62 and a third higher band; M94 LOS had a similar pattern. FA1090 had three bands, the lowest of which comigrates with 15253 LOS. When immunoblotted using mAb 2C7, all three bands of FA1090 LOS reacted, as did 15253 LOS, while LOS from F62,

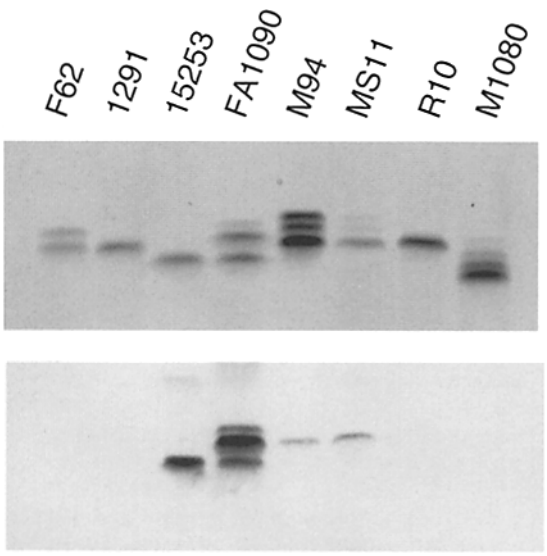

Figure 3. SDS–PAGE of LOS isolates. Lanes 1–7 contain LOS from gonococcal isolates, and the last lane contains LOS from meningococcal isolate M1080. LOS from F62, 15253, FA1090, and M94 were purified as described in Materials and Methods. LOS from 1291 and M1080 were prepared by small-scale phenol extraction (35), and LOS from MS11 and R10 were prepared by proteinase K digestion of whole-cell lysates (36). The upper panel shows a silver-stained gel, and the lower panel shows an immunoblot using mAB 2C7.

1291, R10, and M1080 did not (Fig. 3, *lower panel*). In LOS from MS11 and M94, a single narrow band reacted with antibody 2C7, while most bands that were visible by silver staining did not bind antibody.

To determine whether strains with LOS differing from F62 contained different glycosyl transferase genes in their *lgt* loci, we examined the genomic DNA of these strains by Southern blotting, using three probes derived from the *lgt* locus (Fig. 2). The three plasmids used as probes contained most of the coding regions of *lgtA*, *lgtB*, and *lgtC*, respectively. *lgtA* and *lgtD* are so similar that a probe derived from *lgtA* will hybridize with *lgtD* as strongly as with *lgtA* (7). The same is true of *lgtB* and *lgtE*. The three probes are therefore referred to as A/D, B/E, and C. As predicted from the restriction map (Fig. 2), a BsaBI digest of F62 DNA contained two bands of 10 and 2.8 kb that hybridized with all three probes (Fig. 4). The 10-kb band contains *lgtA*, *lgtB*, and 140 bp of *lgtC*, while the 2.8-kb band contains the remainder of *lgtC* and the entirety of *lgtD* and *lgtE*. Similarly, a ClaI digest of F62 DNA had bands of 5.5 and 4 kb hybridizing with all three probes (Fig. 4). All gonococcal strains, except 15253, had hybridization patterns similar to that of F62, with some variation in the sizes of the bands in the ClaI digests. In contrast, 15253 DNA did not hybridize at all with the probe derived from *lgtC*, and in BsaBI and ClaI digests, only a single band hybridized with probes A/D and B/E (Fig. 4).

In separate experiments, all three probes hybridized with a single 8-kb band in DdeI digests of DNA from all gonococcal strains except 15253, where a 6-kb band hybridized with probes A/D and B/E (not shown). HindII digests of gonococcal DNA also contained a single band hybridizing with the *lgt* probes, ranging from 6 to 8 kb in various strains, but measuring only 4 kb in the 15253 HindII digest (not shown).

These data suggested that the organization of the *lgt* locus in 15253 differed from that in all other strains studied. First, 15253 contained no DNA hybridizing with *lgtC*. Second, in four different enzyme digests, 15253 was either missing a band or had a much smaller band than was seen in DNA from other strains.

Identification of 15253 Variants Binding Lectin GSII. We screened colonies of 15253 for the ability to bind the lectin GSII, which has specificity for nonreducing terminal GlcNAc. We isolated two colonies that appeared to bind this lectin and named them variants GSII 1 and 2. By SDS-PAGE, the LOS of these two variants was perceptibly larger than that of 15253 wild type, and it matched in mobility the LOS derived from the previously characterized F62 insertion mutant I3, which carries an α chain consisting of GlcNAcβ1-3Galβ1-4Glcβ1-R (reference 7 and data not shown). The presence of an additional GlcNAc unit is supported by the chemical data presented below. Using primers designed to amplify a portion of the *lgtA* sequence and sequencing the area containing the poly-G region, we established that the 15253 parent contained the *lgtA* sequence and that the poly-G regions of the two mutants clearly contained 1 G less than the parent strain (not shown). To determine accurately the number of Gs, the *lgt* locus of 15253 was cloned and its sequence revealed 15 Gs in *lgtA*. This puts the gene out of frame. The loss of 1 G seen in both GSII variants would render the gene active. The sequence obtained indicated that the *lgt* locus of 15253 has undergone an internal recombination between homologous regions of *lgtB* and *lgtE*, resulting in the presence of a phase-variable *lgtA* and a functional *lgtE*, but the loss of *lgtC* and *lgtD*. The results of this recombination are depicted in Fig. 2.

Comparison of Meningococcal and Gonococcal lgt Loci. Jennings et al. reported that the *lgt* locus of meningococcal strain MC58 contains only three genes, *lgtA*, *lgtB*, and *lgtE* (10). In contrast, DNA from the meningococcal strain M1080 hybridized with all three of our probes and thus clearly contains *lgtC* (Fig. 4). Bands in the BsaBI digest of M1080 were similar in size to those for the gonococcal strains, but the ClaI digest had only one band. It is possible that M1080 lacks *lgtD*, as reported for strain MC58 (10). Consistent with this, the DdeI band in M1080 DNA was 4.5 kb, smaller than in the gonococcal strains. The HindII band, however, was similar in size to that of F62 (not shown). It is possible that *lgtD* is present in strain M1080, but that minor differences in nucleotide sequence result in the smaller DdeI band and in the absence of the ClaI site in the middle of the locus.

Mutation of the lgt Locus of 15253 and FA1090: Effects on 2C7 Binding. We constructed *lgt* mutants of strains FA1090 and 15253 that were analogous to the F62 mutants previously described (7). Purified LOS were examined by SDS–PAGE and immunoblotting, using mAB 2C7 (Fig. 5). 2C7

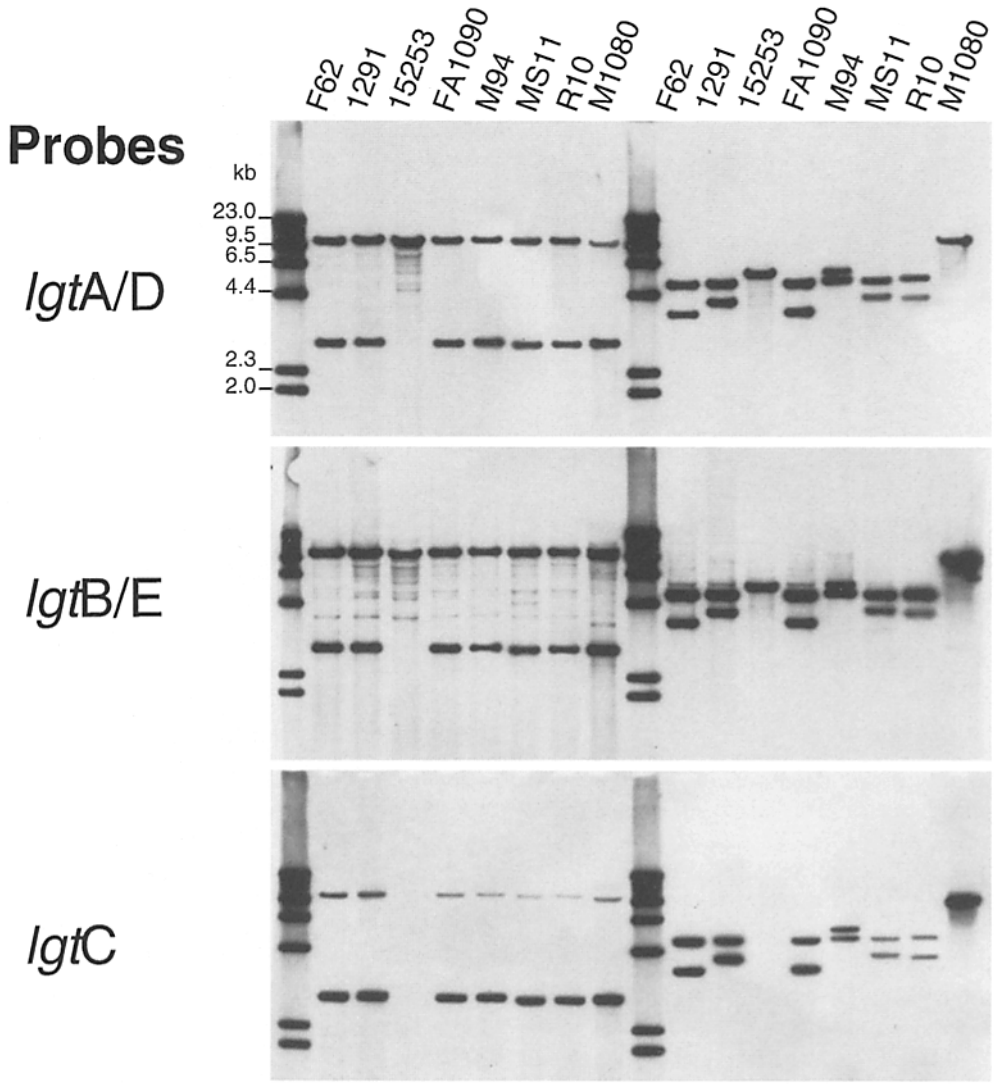

Figure 4. Southern hybridization of probes from the *lgt* locus. Genomic DNA from the same strains used for LOS analysis (Fig. 3) was digested with BsaBI (*left half of each panel*) or ClaI (*right half of each panel*) and subjected to electrophoresis through agarose gels. Identical blots were hybridized with three different probes from the *lgt* locus, the locations of which are shown in Fig. 2. Fluorescein labeled HindIII-digested λ DNA was used as size markers, with bands of 23,130, 9,416, 6,557, 4,361, 2,322, 2,027, 564, and 125 bp.

reacts with LOS of strain 15253, but weakly or not at all with the LOS of F62, MS11, or 1291. This has led to the idea that this antibody is specific for the β chain. We found that FA1090 LOS also binds 2C7 (Fig. 5). Deletion of *lgtA* through *lgtD* (Δ5) of FA1090 resulted in production of an LOS with mobility very similar to that of 15253, and this LOS retained 2C7 reactivity. LOS of the corresponding mutant of F62 (F62Δ5) had a slightly greater electrophoretic mobility than LOS of FA1090Δ5 or 15253, consistent with the lack of a β chain in F62. Deletion of the whole *lgt* locus (Δ4) in F62 or FA1090 produced LOS with an even greater mobility. Unexpectedly, the LOS of FA1090Δ4 failed to bind antibody 2C7. *lgtE* mutants of FA1090 and 15253 produced LOS with similar mobilities to the Δ4 mutants, and the LOS of the *lgtE* mutants also did not bind antibody 2C7.

We considered two possibilities. The 2C7 epitope may be the β chain, and mutation of *lgtE* prevents synthesis of the β chain. Alternatively, the 2C7 epitope may not be the β chain, but may be some other structure that is present in LOS of 15253 and of LOS from both FA1090wt and FA1090Δ5, but not present in F62 wt or F62Δ5. In that case, the first galactose of the α chain (added by the *lgtE* product) must be necessary but not sufficient for the 2C7 epitope. We addressed these possibilities by determining the hexose and hexosamine composition of these LOS.

Monosaccharide Analysis. TFA hydrolysis of the oligosaccharide prepared from LOS of 15253 released glucosamine, galactose, and glucose (Fig. 6) in molar ratios of 1:1.8:2 (Table 2), as predicted by the structure determined by Yamasaki et al. (5). KDO is destroyed under these hydrolysis conditions. Only a small amount of heptose was released, consistent with previous observations that the heptose–heptose bond is resistant to TFA hydrolysis (25). The heptose seen in the gonococcal preparations eluted slightly later than the glucoheptose in the standard mixture, but coeluted with the heptose in hydrolysates from *E. coli* J5 and *S. minnesota*. Yamasaki et al. reported that the heptose in F62 LOS comigrated with L-gluco-D-mannoheptose (26). A similar analysis of oligosaccharide sugars from the *lgtE*

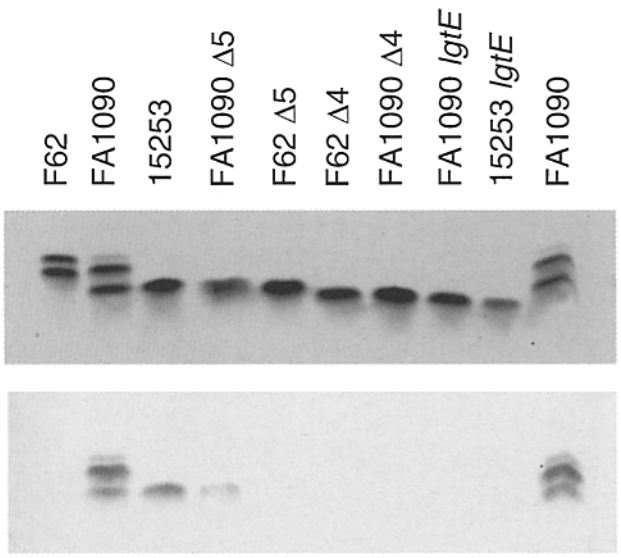

Figure 5. Analysis by SDS–PAGE and immunoblotting of LOS from *lgt* mutants. Lanes *1–3* contain LOS of wild-type strains F62, FA1090, and 15253, respectively; lanes *4* and *5*, Δ5 mutants of FA1090 and F62; lanes *6* and 7, Δ4 mutants of F62 and FA1090; lanes *8* and *9*, *lgtE* mutants of FA1090 and 15253; lane *10*, wild-type FA1090. *Upper panel*, silver-stained gel; *lower panel*, immunoblotting using mAb 2C7.

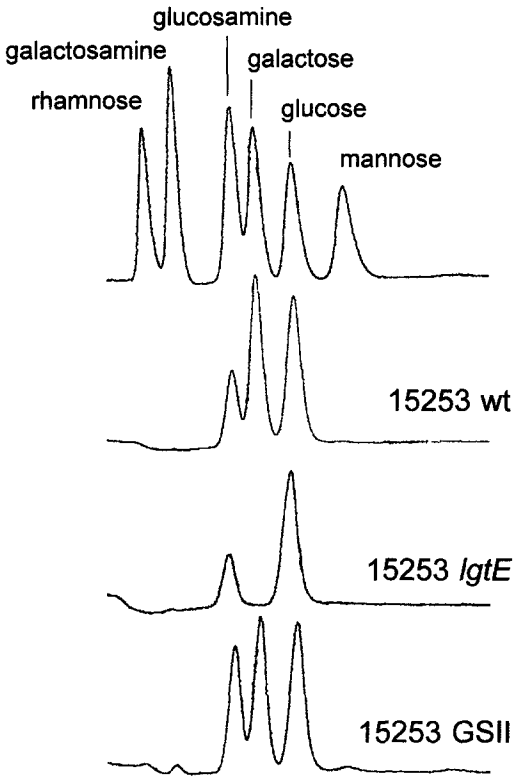

Figure 6. Composite of HPLC tracings from the monosaccharide analysis of 15253 wt, lgtE mutant, and variant GSII.

mutant of 15253 showed that while the ratio of glucosamine to glucose was essentially unchanged, galactose was completely absent (Fig. 6 and Table 2). The same was true for FA1090: while for FA1090 Δ5 the GlcNAc/Gal/Glc ratio was 0.7:1.2:2, galactose was not seen in either FA1090 Δ4 or the *lgtE* mutant of FA1090 (Table 2). In the 15253 GSII variant identified by binding of lectin GSII, the proportion of glucosamine was increased substantially (Fig. 6), which is in accord with the DNA sequence data suggesting that *lgtA* had shifted to the active form. The reactivity with mAB 2C7 of the LOS of GSII mutants was unaltered.

Discussion

The heterogeneity of gonococcal LOS, as seen by SDS-PAGE, has long been noted. Part of the heterogeneity is caused by phase variation of the α chain. For example, a strain expressing a lactosyl α chain may contain variants expressing a lacto-*N*-neotetraose α chain; this variation has now been shown to result from variation in numbers of Gs in the poly-G region of *lgtA* (7, 8). It is not certain whether all strains possess the ability to produce all known structural variants of LOS. Wild-type strain 1291 produces a lacto-*N*-neotetraose α chain (27), and expression of a pentasaccharide α chain has not been reported. Our analysis of genomic DNA from 1291 indicates that *lgtD* is present in this strain; however, it may be mutated or may contain a poly-G region that puts it out of frame. Some strains express two or more LOS structures that are seen as multiple bands on a gel. For example, F62wt typically produces two LOS, one with a pentasaccharide α chain and the other with a tetrasaccharide α chain (26), the latter presumably resulting from translocation of LOS to the bacterial surface before completion of the chain. Other aspects of heterogeneity have not yet been explained. For example, the LOS of strains MS11, M94, and FA1090 all contain bands that do not comigrate on gels with LOS structures that have been chemically characterized.

An initial aim of this work was to examine the extent to which heterogeneity among strains was reflected in differences in the *lgt* locus. In general, we found no evidence that the *lgt* locus differed substantially from one strain to another. Of the seven gonococcal isolates examined, only one was clearly different. In the remainder of this work, we focused on this strain, 15253, comparing it with prototypic F62 and also with FA1090, which like 15253, is serum resistant and binds antibody 2C7.

Southern analysis of the 15253 *lgt* locus suggested that it contained only two genes. It has been noted previously that *lgtA* and *lgtD* are strongly homologous, particularly in their 5′ ends; the same is true of *lgtB* and *lgtE* (7). The altered structure of the 15253 *lgt* locus is readily explicable if a recombination between one of these pairs of homologous regions occurred at some time in the past. Recombination between *lgtA* and *lgtD* would produce a hybrid gene similar to *lgtD*, while leaving *lgtE* intact. In contrast, recombination between *lgtB* and *lgtE* would leave *lgtA* intact and produce a hybrid that would probably function as *lgtE*.

The LOS structure determined by Yamasaki et al. is consistent with either of these two possibilities, since the only *lgt* function required for this structure is that of the

Table 2. *Sugar Compositions of Oligosaccharides Derived from LOS—Molar Ratios*

		GalNAc	GlcNAc	Gal	Glc
F62	Wild type	0.62*	1.70	1.53	1
	Δ5	—	0.89	0.76	1
	Δ4	—	0.34	—	1
15253	Wild type	—	1.00	1.75	2
	GSII	—	1.88	1.62	2
	IgtE	—	0.78	—	2
FA1090	Wild type	0.27	1.72	2.16	2
	Δ5	—	0.68	1.16	2
	Δ4	—	0.80	0.06	2
	lgtE	—	0.64	—	2

*The data are molar ratios and are the averages of the ratios determined in analyses of two monosaccharide preparations from each LOS. —, none detected.

lgtE product, which transfers galactose onto the initial glucose of the α chain. Determination of which of the two possible recombinations had occurred depended on the realization that if *lgtA* were intact in 15253, its gene product would modify the LOS by the addition of a GlcNAc residue to the lactosyl group of its α chain. In contrast, the *lgtD* gene product normally adds GalNAc to a lacto-*N*-neotetraose group and would probably not be able to use the lactosyl α chain as a substrate. We hypothesized that *lgtA* was intact, but failed to produce enzyme because its poly-G region put it out of frame. Screening for colonies reactive with lectin GSII allowed us to identify variants in which *lgtA* was active. Chemical analysis of the LOS from such variants confirmed the presence of an additional glucosamine residue, and analysis of the DNA sequence of the *lgt* locus confirmed the presence of *lgtA* followed by *lgtE*.

Strain 15253 was first reported in 1983 and had been isolated from the pharynx of a patient with disseminated gonococcal infection in 1975 (28). In a report published in 1988 (6), 15253 was described as failing to bind to several mAbs that recognize the LOS α chain. Although the strain used in this study had undergone only three to five passages in vitro, it cannot be concluded that the strain is currently pathogenic, since the recombination may have occurred during laboratory passage.

Mutagenesis of the *lgt* locus of 15253 provided us with unexpected information about the role of *lgtE* in LOS structure. Previous study of strain F62 had identified the role of the *lgt* locus in α-chain synthesis. We anticipated that the interruption of *lgtE* in 15253 would prevent addition of galactose to its α chain without affecting its β chain. However, the *lgtE* mutant of 15253 produced an LOS lacking galactose entirely. The same was true for FA1090, either if *lgtE* was interrupted or if the whole *lgt* locus was deleted. The simplest explanation is that the *lgtE* product has two functions and is able to add galactose onto the initial glucose of the β chain, as well as onto the initial glucose of the α chain. It is also possible, however, that the β chain galactose is added by a different unknown transferase that is not able to function unless the lactosyl group of the α chain is already present. At the present time, we cannot distinguish experimentally between these two possibilities.

There is precedent that the steps in LPS synthesis occur in a certain order. It appears that addition of the α chain requires the presence of heptose 2 and its substituent γ chain, since the α chain was absent from LOS of a meningococcal *rfaK* mutant (3) and of a gonococcal *rfaF* mutant (1). Studies of LPS synthesis in other species have demonstrated that such requirements may differ from one species to another. In *S. typhimurium*, transfer of at least one KDO residue to the lipid A precursor must occur before the addition of lauroyl and myristoyl residues (29). In contrast, *Pseudomonas aeruginosa* does not have this requirement, and at least one secondary fatty acid can be added to the lipid A precursor before the addition of core sugars (30).

Several reports have shown that LOS structure varies during naturally acquired (31) and experimental human (23, 24) and animal (32) neisserial infections, and that LOS structure affects serum resistance (33) and the interaction of neisseriae with cultured cells (2, 34). The ability to generate mutants with defined, fixed LOS structures will allow further study of this important area. Our findings that the *lgt* locus is highly conserved among gonococcal strains and that *lgtE* is involved in synthesis of the β chain and of the 2C7 epitope add to the knowledge that is required to generate such mutants.

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