Variation of Gonococcal Lipooligosaccharide Structure Is Due to Alterations in Poly-G Tracts in \textit{lgt} Genes Encoding Glycosyl Transferases

By Qing-Ling Yang and Emil C. Gotschlich

Summary

The lipooligosaccharide (LOS) expressed by gonococci spontaneously varies its structure at high frequency, but the underlying genetic mechanism has not been described. We have previously reported that the genes encoding the glycosyl transferases responsible for the biosynthesis of the variable \(\alpha\) chain of the LOS of \textit{Neisseria gonorrhoeae} are located in a locus containing five genes, \textit{lgtA}, \textit{lgtB}, \textit{lgtC}, \textit{lgtD}, and \textit{lgtE}. Sequence analysis showed that \textit{lgtA}, \textit{lgtC}, and \textit{lgtD} contained poly-G tracts within the coding frames, leading to the hypothesis that shifts in the number of guanosine residues in the poly-G tracts might be responsible for the high frequency variation in structure of gonococcal LOS. We now provide experimental evidence confirming this hypothesis.

Materials and Methods

\textbf{Colony Immunoblots.} GC on GC agar (12) were grown overnight, suspended and diluted in proteose peptone liquid medium
Genomic DNA was prepared from F62 Δ1 and variants 1-1, 2-1, and 1-6. 12-μg aliquots of DNA were digested with Clal, and the digests were separated by gel electrophoresis on 0.8% agarose (Seaplaque; FMC Corp., Rockland, ME), the region containing DNA around 2.3–3.0 kb excised, and the DNA purified using Gene Clean II (BIO 101, Inc., La Jolla, CA). The fragments were ligated into Clal-cut pBluescript KS+ and transformed into XL1-Blue MR F′-kompetent cells, and the desired clone isolated by plating on LB agar containing 50 μg/ml carbenicillin and 200 μg/ml erythromycin and incubating at 30°C for 2 n’rin at 94°C followed by 25 cycles of 60 s, 72°C for 30 s, and 94°C for 30 s. One additional cycle with a 4-min extension time at 72°C was performed. The agents used were part of a Gene Amp kit (Perkin Elmer Corp., Branchburg, NJ) with 0.5 μl of Taq polymerase per 100 μl reaction and primers at 0.1 μM. The primers are described by their position in the sequence deposited in Genbank under accession number U14554 (9). To amplify an 893-bp fragment containing the poly-G region of lgtA, a primer pair matching 309–328 and the reverse complement of 1182–1202 was used; the primer was biotinylated. To amplify a 467-bp fragment containing the poly-G region of lgtC, primers matching 2249–2275 and the reverse complement of 2693–2716 were used; the first primer was biotinylated.

DNA Sequencing. DNA sequencing was performed using the Sequenase II kit (United States Biochemical Corp., Cleveland, OH). The PCR products were purified by absorption to streptavidin-coated magnetic beads, and single-stranded template was eluted with NaOH and sequenced (14). Plasmid was purified by the spin-prep method (Qiagen Inc., Chatsworth, CA). The genomic DNA region in lgtA was sequenced in both directions using primers matching 2249–2275 and the reverse complement of 2693–2716; the first primer was biotinylated.

Cloning of lgtA and lgtC Region. Genomic DNA was prepared from F62 Δ1 and variants 1-1, 2-1, and 1-6. 12-μg aliquots of DNA were digested with Clal, and the digests were separated by gel electrophoresis on 0.8% agarose (Seaplaque; FMC Corp., Rockland, ME), the region containing DNA around 2.3–3.0 kb excised, and the DNA purified using Geneclean II (BIO 101, Inc., La Jolla, CA). The fragments were ligated into Clal-cut pBluescript KS+ and transformed into XL1-Blue MR F′-kompetent cells, and the desired clone isolated by plating on LB agar containing 50 μg/ml carbenicillin and 200 μg/ml erythromycin and incubating at 30°C for 2 n’rin at 94°C followed by 25 cycles of 60 s, 72°C for 30 s, and 94°C for 30 s. One additional cycle with a 4-min extension time at 72°C was performed. The agents used were part of a Gene Amp kit (Perkin Elmer Corp., Branchburg, NJ) with 0.5 μl of Taq polymerase per 100 μl reaction and primers at 0.1 μM. The primers are described by their position in the sequence deposited in Genbank under accession number U14554 (9). To amplify an 893-bp fragment containing the poly-G region of lgtA, a primer pair matching 309–328 and the reverse complement of 1182–1202 was used; the primer was biotinylated. To amplify a 467-bp fragment containing the poly-G region of lgtC, primers matching 2249–2275 and the reverse complement of 2693–2716 were used; the first primer was biotinylated.

Cloning and Electrophoresis of LOS. LOS was prepared by a modification of the hot phenol extraction method and analyzed electrophoretically (9).

PCR Reactions. GC genomic DNA (usually 100 ng) was used as a template. The PCR conditions consisted of initial denaturation of DNA for 2 min at 94°C, followed by 25 cycles of 60°C for 60 s, 72°C for 30 s, and 94°C for 30 s. One additional cycle with a 4-min extension time at 72°C was performed. The reagents used were part of a Gene Amp kit (Perkin Elmer Corp., Branchburg, NJ) with 0.5 μl of Taq polymerase per 100 μl reaction and primers at 0.1 μM. The primers are described by their position in the sequence deposited in Genbank under accession number U14554 (9). To amplify an 893-bp fragment containing the poly-G region of lgtA, a primer pair matching 309–328 and the reverse complement of 1182–1202 was used; the primer was biotinylated. To amplify a 467-bp fragment containing the poly-G region of lgtC, primers matching 2249–2275 and the reverse complement of 2693–2716 were used; the first primer was biotinylated.

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Figure 1. Structures of gonococcal LOS. (A) The complete structure that has been reported for strain F62 (1) and MS11 variant C (2). (B) The alternative LOS structure, which has been observed in strain 1291b (3) and in the mutant strain F62 Δ1 (9). The second structure differs from the first in that it bears a terminal α-L-4-linked galactose instead of a β1-3-linked N-acetyl glucosamine. As indicated in the figure, this alternative LOS structure can be recognized by its reactivity with mAb 17-1-L1. The reactivities of a number of well-characterized mAbs are shown. The LOS glycolipid transferase gene (lgtA, B, C, D, E) responsible for the addition of each of the residues are indicated. The underlined lgt genes contain poly-G tracts in their coding frames. KDO, keto deoxyoctulosonic acid; HEP, heptose.

Results

Changes in the poly-G tract of lgtC should affect the addition of the terminal Gal in the LOS structure αGal1-4βGal1-4βGlc1-R. The α-Gal transferase encoded by lgtC appears to compete poorly with the GlcNac transferase for substrate; its activity is evident only if lgtA is silent (9). We therefore used mutant F62 Δ1 with a deletion in lgtA, which produces an LOS with the structure shown in Fig. 1 B (9). Colony immunoblots were performed with mAb 17-1-L1 and briefly stained with safranin to visualize the colonies that were not immunoreactive. We isolated three independent colonies that did not react with the antibody. LOS from these clones, designated as variants 1-1, 2-1, and 1-6, was prepared and compared by SDS-PAGE (Fig. 2) with the F62 Δ1 parent and F62 Δ5 mutant known to produce a LOS with an α chain limited to a lactosyl group (9).
Table 1. Number of G Residues Found in Poly-Tracts in Plasmid Clones

| Plasmid clone | lgtA | lgtC |
|---------------|------|------|
| F62 Δ1        | 10*  |      |
| Var 1-1       | 17   |      |
| Var 2-1       | 11   |      |
| Var 1-6       | 17   |      |
| MS11 Var A    | 12   | 8    |
| MS11 Var C    | 11†  | 8    |

*10 G is in frame with the remainder of the coding frame of lgtC.
†11 G is in frame with the remainder of the coding frame of lgtA.

We next wished to study the role of shifts in the poly-G region of lgtA. We took advantage of a closely studied example of antigenic variation observed in a study in which volunteers were infected with strain MS11, expressing a 3,600 mol wt LOS phenotype (2). Over the course of the infection, an increasing proportion of GC isolated from the volunteers were variants that produced a higher molecular weight LOS. Two variants, B and C, predominated (16). Nuclear magnetic resonance and immunochemical analysis showed that the α chain of variant A was a lactosyl group, whereas variant C produced a complete pentasaccharide α chain. These variants were kindly provided by Dr. Herman Schneider, Walter Reed Army Institute of Research, and Fig. 2 shows that the pattern of the LOS prepared in our laboratory is like that previously published (2). We sequenced the lgtA and the lgtC regions and found that lgtC contained 8 Gs in both strains (“OFF”). The lgtA region changed from 12 in variant A (“OFF”) to 11 Gs in variant C (“ON”) (see Fig. 4). Thus, the loss of a single G residue accounted for the regained ability to synthesize the full LOS. To be certain of the number of G residues in the lgtA gene in these two variants, we also cloned the relevant re-
gions and performed direct sequencing and obtained the same answer (see Table 1).

We were unable despite repeated colony blots to isolate from variant A variants producing the 1-17-L1 epitope. Such variants should have been easy to detect because we were looking for gain in reactivity with the mAb. The failure to isolate antibody-reactive variants suggests that the run of 8 Gs in lgtC of that strain is not sufficiently long to expand or contract at a perceptible frequency.

Discussion

We have described the molecular mechanism for the LOS phase variations that have been observed by previous investigators. However, the selective advantage to the organism of this genetic mechanism is unclear. The correlation of LOS structure with function is in its early stages. A large proportion of human bactericidal antibodies are to LOS epitopes, and its structure influences the bacteriolytic effects of human serum on GC. Some GC strains when grown in vitro are not killed by human serum (SerR) and are common among isolates from patients with disseminated GC infection (17). However, most GC strains are sensitive (SerS). Many SerS strains become phenotypically SerR when incubated in the presence of cytidine monophosphate N-acetyl neuraminic acid (CMP-NANA). GC possess a sialyl transferase that is capable of using exogenous CMP-NANA to sialylate its LOS (18). Concentrations of CMP-NANA in vivo are sufficient to support this reaction (19). The reaction depends on the LOS being a competent substrate. It is well established that the lacto-N-neotetraose chain is the substrate for the sialylation leading to the SerR phenotype, and that the lactosyl α chain is not modified (20). The resistance may be due to inability of antibodies to bind to the modified LOS, but there also appears to be a general defect of effective complement deposition (21, 22).

The ability of GC to adhere to epithelial cells is a fundamental attribute of their virulence. Preeminent among GC adhesins are pili. A 110-kd protein, pilC, present in small amounts in pili, is responsible for adherence to epithelial cells. The expression of this protein is subject to high frequency variation by a frame-shift mechanism due to a stretch of G residues early in the coding frame (10, 11). The opacity proteins (opa) are also important adhesins. This is a family of 11 outer membrane proteins that are subject to high frequency variation by a frame-shift mechanism that involves a variable number of repeats of the pentameric sequence CTCTT (23, 24). Most of the opa proteins promote adherence to polymorphonuclear phagocytes, and sialylation of the LOS strongly inhibits this opa protein-mediated adherence to polymorphonuclear phagocytes (25). One particular opa protein in strain MS11 not only promotes adherence to epithelial cells, but is essential for invasion of the cells (26). van Putten (6) has shown that sialylation of LOS markedly inhibits epithelial cell invasion without greatly altering adhesion. His studies suggest that in the mucosal infection, LOS that cannot be sialylated may be important for cell invasion. On the other hand, Griffiss et al. (27) reported that sialylation enhanced invasiveness in HEC-1-B cells.

GC has clearly evolved in this locus alone a very elegant system to shift readily between four different LOS structures, and at least one of these is subject to further modification by sialylation. That each of the various α chain structures that the GC can produce is a mimic of a host carbohydrate structure has raised the question of what the role of LOS may be in the mucosal infection. The host uses a large number of ligand-binding proteins recognizing the rich array of carbohydrate structures on glycolipids and glycoproteins for its own homeostatic purposes. The C-lectins, the S-lectins, and the sialoadhesins have binding specificities for structures mimicked by LOS. Thus, it is likely that these structures on the GC would also be recognized and may contribute in important ways to the mucosal infection.

This ability to shift the expression among a number of different LOS structures is not peculiar to the GC, but exists also in the meningococcus, where the genetics is likely to be very similar. The laboratories of Moxon and Hansen (28-30) have shown that this also occurs in another mucosal pathogen, Haemophilus influenzae, where at least four genes are subject to phase variation. In this organism, the mechanism is also by slipped-strand mispairing due to repeated tetrameric sequences, which can be either CAAAT or GCAA. H. influenzae produces chemically similar LOS, and also sialylates its LOS (31). The existence of this genetic capability in H. influenzae and the pathogenic Neisseria spp. argues that organisms with phase-variable LOS have a selective advantage. It strongly suggests that specific LOS structures afford an advantage in one biological niche, but a disadvantage in another host environment, and that these mucosal pathogens negotiate this dilemma by phase variation.

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