Molecular Analysis of Lipooligosaccharide Biosynthesis in Neisseria gonorrhoeae

EMANUEL F. PETRICOCIN III AND DANIEL C. STEIN*
Department of Microbiology, University of Maryland, College Park, Maryland 20742

Received 7 February 1989/Accepted 5 May 1989

A HindIII gene bank of Neisseria gonorrhoeae MUG116 was constructed in the cosmid vector pHC79. A cosmid (pSY81) was isolated that was able to convert N. gonorrhoeae FA5100 to reactivity with monoclonal antibody (MAb) 2-1-L8. Several MAB-reactive transformants were isolated and characterized with respect to lipooligosaccharide (LOS) production as analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, their ability to react with two other LOS-specific MAb (3F11 and 06B4), and Southern blot analysis. Escherichia coli containing the clone had altered lipopolysaccharide expression as determined by electrophoretic analysis; however, no reactivity was seen with gonococcus-specific MAb. The introduction of pSY81 into FA5100 had a pleomorphic effect, giving rise to transformants having the full parental phenotype or transformants lacking reactivity to a combination of LOS-specific MAb. Southern blot analysis indicated that the LOS biosynthetic mutation in FA5100 was not due to chromosomal rearrangement or large deletions.

Lipooligosaccharide (LOS) is an important virulence determinant in Neisseria gonorrhoeae. It mediates most of the toxic damage that occurs in the fallopian tube model (8), regulates complement activation on the bacterial cell surface (10, 19), and serves as a key target on the cell surface for bactericidal antibody (22). Gonococcal LOSs are heterogeneous glycolipids without repeating oligosaccharides (9) that show wide antigenic diversity among different strains (13). In studies using molecular sieve chromatography, fluorescence-activated cell sorter analysis, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) it was shown that, within a strain, LOS components differ in their relative concentrations and in the antigens they express (1, 21, 23, 24). Furthermore, LOS expression undergoes antigenic variation (24) similar to that seen for pilus and PII expression (27), although the mechanism that modulates this phenomenon is unknown. The LOS and the strains from which the LOS are isolated can be defined in terms of the monoclonal antibodies (MAb) they bind, the ability of pyocin to bind to the LOS and kill the cell, the SDS-PAGE banding patterns, and sensitivity to killing by normal human serum.

Pyocin, a bacteriocin produced by Pseudomonas aeruginosa, inhibits the growth of the gonococcus (16). Strains resistant to the killing action of pyocin have alterations in their LOS (5, 7, 15). FA5100, a spontaneous pyocin-resistant mutant of FA19, has been described previously (25). In addition to its pyocin-resistant phenotype, FA5100 does not react with any of the available MAb directed against gonococcal LOS. SDS-PAGE analysis indicates that LOS isolated from FA19 separates into five components, whereas LOS isolated from FA5100 runs as a small, single component and contains almost no detectable heptose, glucose, or galactose (25).

We describe in this paper the cloning of a DNA segment that is able to complement the LOS biosynthetic defect present in FA5100. When the cloned piece of DNA was introduced into FA5100, a pleomorphic effect was seen with respect to reactivity with LOS-specific MAb and LOS components produced. We propose a nomenclature which takes into consideration all genes involved in LOS biosynthesis. This nomenclature, which we will designate liposaccharide-involved (LSI) genes, allows us to establish a working nomenclature for describing our clones and mutants.

MATERIALS AND METHODS

Bacterial strains, plasmids, and culture conditions. N. gonorrhoeae FA5100 and FA19 were obtained from William Shafer (Emory University School of Medicine, Atlanta, Ga.), strain DOV was generously provided by Herman Schneider (Walter Reed Army Institute of Research, Washington, D.C.), and strain F62 was given to us by P. Frederick Sparling (University of North Carolina, Chapel Hill). MUG116 is a spontaneous rifampin-resistant, nalidixic acid-resistant derivative of WR302 and has been described previously (28). Escherichia coli HB101 and JM83 have been previously described (14). Strains constructed in this study and their respective MAb reactivities are listed in Table 1. Gonococci were grown in GCP broth and on GCK agar as previously described (29). E. coli HB101 and JM83 were grown in L broth (14) or on MacConkey or LB agar (14) containing 15 µg of tetracycline per ml, 30 µg of ampicillin per ml, 30 µg of chloramphenicol per ml, or 25 µg of kanamycin per ml as needed. The cloning vectors used in this study were pHC79 (12), pKan18 (18), and pACYC184 (2).

Chemicals, reagents, and enzymes. Restriction enzymes were purchased from New England BioLabs (Beverly, Mass.). An in vitro packaging kit and T4 DNA ligase were purchased from Promega Biotech (Madison, Wis.). Enzymes were used as outlined in the product profiles. Chemicals used for transformation studies were reagent grade or better and were purchased from Sigma Chemical Co. (St. Louis, Mo.) unless otherwise specified. Chemicals used for SDS-PAGE were of molecular biology grade. Acrylamide and bisacrylamide were obtained from Boehringer Mannheim Biochemicals (Indianapolis, Ind.); ammonium persulfate was obtained from International Biotechnologies, Inc. (New Haven, Conn.); and N,N,N',N'-tetramethylmethylenediamine was obtained from Bio-Rad Laboratories (Richmond, Calif.). Silver nitrate, formaldehyde, and periodic acid were obtained from Fisher Scientific Co. (Silver Spring, Md.).

* Corresponding author.
TABLE 1. MAb reactivity patterns of strains used or constructed in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>MAb reactivity (2-1\text{-L8} )</th>
<th>O6B4</th>
<th>3F11</th>
</tr>
</thead>
<tbody>
<tr>
<td>MUG502(^b)</td>
<td>++ + +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MUG508(^c)</td>
<td>±</td>
<td>+ +</td>
<td></td>
</tr>
<tr>
<td>MUG518(^d)</td>
<td>±</td>
<td>+ +</td>
<td>+ +</td>
</tr>
<tr>
<td>MUG527(^b)</td>
<td>++ + +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FA19</td>
<td>++ + +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FA5100</td>
<td>--</td>
<td>+ +</td>
<td>+ +</td>
</tr>
<tr>
<td>MUG116</td>
<td>++ + +</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) The degree of reactivity on colony and Western blots is indicated as follows: ++ +, very strong; + +, strong; ±, weak; --, no reaction.
\(^b\) Constructs of strain FA5100 transformed with pSY81 and selected for reactivity with MAb 2-1-L8.
\(^c\) Construct of strain FA5100 transformed with pSY81 and selected for reactivity with MAb O6B4.
\(^d\) Construct of strain FA5100 transformed with pSY81 and selected for reactivity with MAb 3F11.

MAb 2-1-L8 was generously provided by Wendell Zollinger, Walter Reed Army Institute of Research, Washington, D.C. MAb O6B4 and 3F11 were gifts from Michael Apicella, University of Buffalo, Buffalo, N.Y.

Genetic transformations and transductions. *E. coli* HB101 and JM83 were transformed by the CaCl\(_2\) procedure (14). A cosmide gene bank representing the chromosome of MUG116 was prepared by partially digesting chromosomal DNA with HindIII and ligating this DNA into the HindIII site of the cosmide vector pHC79. Incorporation of the DNA into bacteriophage particles was carried out with an in vitro packaging kit. Transductions were carried out with *E. coli* HB101 as the host for the cosmide library. Transductants containing gonococcal chromosomal inserts were screened for ampicillin-resistant, tetracycline-susceptible phenotype. Cosmid DNA containing chromosomal inserts was isolated from each transductant and pooled. Pooled DNA was used to transform *N. gonorrhoeae* as described previously (29). Transforms were screened for reactivity with the MAb 2-1-L8 by plating cells on GCK agar and performing colony blot analysis as described previously (29).

Plasmid analysis and subclone construction. One cosmide, pSY81, was identified from the gene bank that could transform *N. gonorrhoeae* FA5100 to reactivity with MAB 2-1-L8 and was analyzed by restriction analysis. Various deletion plasmids were constructed by partial EcoRI and SmaI digestions followed by religation and transformation of HB101. Transforms were selected by growth on LB agar (14) plus ampicillin. Two subclones, pSY86 and pSY87, containing a 2.4-kilobase (kb) EcoRI fragment and retaining the ability to transform FA5100 to reactivity with MAB 2-1-L8, were made by inserting this fragment into the vectors pKan18 (pSY86) and pACYC184 (pSY87).

SDS-PAGE analysis. Proteinase K-treated whole-cell lysates were prepared from 18- to 20-h cultures by the procedure of Hitchcock and Brown (11). The lysates were diluted so that the apparent LOS concentration in 10 \(\mu\)l approximated 1 \(\mu\)g of purified LOS. Linear 10 to 17% polyacrylamide gradient gels were made, and the lysates were subjected to SDS-PAGE with a Tris-glycine buffer (0.025 M Tris base [pH 8.3], 0.192 M glycine, 0.1% SDS) at a constant current of 30 mA per gel for 5 h at 10°C. Gels were fixed overnight in 40% ethanol–5% acetic acid. Periodate-oxidized LOS was visualized by silver staining by the method of Tsaï and Frasch (30).

Southern hybridizations. Incorporation of \(^32\)P-labeled dCTP (DuPont, NEN Research Products, Boston, Mass.) into the subclones pSY86 and pSY87 was performed by nick translation with a commercial kit (Bethesda Research Laboratories, Inc., Gaithersburg, Md.). Chromosomal DNA was digested with EcoRI and electrophoresed overnight on a 0.8% agarose gel at 25 V. DNA was transferred from the gel onto a GeneScreen transfer membrane (DuPont, NEN) by the alkaline transfer method of Chomczynski and Qasba (3). Hybridization with labeled plasmid was carried out at 65°C by the method of Church and Gilbert (4). Wash conditions using salt concentrations equivalent to 0.2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at 65°C were used. Autoradiography was carried out overnight at -70°C with Kodak X-Omat AR X-ray film.

Immunological techniques. Binding of the MAbs 2-1-L8, O6B4, and 3F11 to LOS by Western blot (immunoblot) analysis was performed as described previously (26). To determine individual colony reactivities, colonies were blotted onto 82.5-mm nitrocellulose disks (Schleicher & Schuell Co., Keene, N.H.) and reacted with MAB 2-1-L8, 3F11, or O6B4 as previously described (28). Cells that bound antibody were detected by using goat anti-mouse immunoglobulin G or M (horseradish peroxidase-conjugated) antibodies (Fisher). A colorometric assay was used to detect bound secondary antibody with the following developing solution: 10 mg of 4-chloro-1-naphthol per ml in 0.86% hydrogen peroxide and 50 mM Tris hydrochloride (pH 8.0).

RESULTS

Isolation of LSI genes. To identify gonococcal genes that are involved in LOS biosynthesis, a cosmide gene bank was made. The cosmide vector pHC79 and gonococcal chromosomal DNA isolated from MUG116, a nalidixic acid-resistant, rifampin-resistant derivative of WR302, were digested with HindIII and ligated. After in vitro packaging of the DNA, *E. coli* HB101 was transduced with the ligation mix and plated on LB agar containing ampicillin. Cosmids containing gonococcal chromosomal DNA were identified by the inability of the ampicillin-resistant colonies to grow on medium containing tetracycline. Cosmid DNA was isolated from 250 individual colonies and pooled into 10 groups of 25. *N. gonorrhoeae* FA5100, which does not bind any MABs directed against gonococcal LOS, was transformed with the pooled cosmids and plated on nonselective medium at a dilution that gave approximately 3,000 colonies per plate. An LOS-specific MAb, 2-1-L8, was used to screen the FA5100 transformants for the ability to synthesize new LOS components. One pool had the ability to transform FA5100 to reactivity with MAB 2-1-L8. Each cosmid from this pool was tested for its ability to transform FA5100 as described above. One cosmid (pSY81) was identified which contained gonococcal DNA which could transform FA5100 to reactivity with MAB 2-1-L8.

Reactivities of gonococcal strains with LOS-specific MABs show reversions from reactivity to nonreactivity and vice versa at a fairly high frequency (24). To demonstrate that the transformations of FA5100 to reactivity with MAB 2-1-L8 with chromosomal DNA and pSY81 were due to a DNA-dependent event and not to reversion, various concentrations of chromosomal DNA and pSY81 were used to transform FA5100. The concentration-dependent effect of the DNA on transformation was evident and not due to FA5100 reversion (Table 2). When 1.0 ng of DNA was used, with selection for MAB 2-1-L8 reactivity, a transformation frequency of \(1.6 \times 10^{-3}\) was obtained. When 10 times as
much DNA was used, the transformation frequency increased proportionately. When transformants were selected with O6B4 or 3F11, the same frequencies for transformation were obtained with the same amount of DNA (data not shown). Interestingly, FA5100 did not revert to reactivity with MAb 2-1-L8 at a detectable frequency. This is unusual, since phenotypic changes in LOS are seen in other gonococcal strains (1, 24). We have analyzed this strain extensively and never found an MAb-reactive revertant of FA5100 (unpublished observations).

**Immunoneutralization of the FA5100 transformants.** FA5100 was transformed with pSY81, and 15 colonies that reacted with MAb 2-1-L8 were isolated. These cells were tested for their ability to bind two other MAbs, 3F11 and O6B4; 14 of the 15 FA5100 colonies that were transformed to reactivity to MAb 2-1-L8 by pSY81 now reacted with these MAbs. The one transformant (MUG502) that did not react with the MAbs O6B4 and 3F11 seemed to hyperreact with MAb 2-1-L8.

FA5100 transformations were repeated with pSY81, and MAbs O6B4 and 3F11 were used for the initial selection of the transformants. Of 15 colonies that reacted with the MAb 3F11, 14 also reacted with MAb 2-1-L8. Of 15 colonies that reacted with the MAb O6B4, 14 also reacted with MAb 2-1-L8. All transformants selected with MAb 3F11 reacted to MAb 3F11 and vice versa. SDS-PAGE analysis of the 45 transformants described above indicated that 42 of the 45 transformants showed migration and banding patterns identical to those of FA19, the parent strain of FA5100 (data not shown). The three isolates that differed in banding pattern were the same isolates that gave the different MAb reactivity patterns (MUG502, MUG508, and MUG527).

MUG502, MUG508, MUG527, and MUG518 (a transformant that reacted with all three MAbs used in this study) were analyzed by SDS-PAGE and Western blot analysis. Figure 1A shows the SDS-PAGE profiles of FA5100 and its isogenic parent, FA19, along with MUG502, MUG508, MUG518, and MUG527. These transformants all have the same genetic background and were constructed by introducing a cloned fragment into their chromosomes. Since different banding patterns are seen in the transformants, these data indicate that the cloned DNA is involved in the biosynthesis of all of the LOS components present in FA19. Figure 1B shows the Western blot of the isolates with MAb 2-1-L8. All of the strains reacted with this MAb, but to varied degrees. This difference correlated with the amount of LOS made. MUG502 was an apparent overproducer of the LOS epitope that binds MAb 2-1-L8 when compared with FA19. The SDS-PAGE profile and Western blot analysis show that MUG502 produced much more of the LOS component that binds MAb 2-1-L8 than any other of the transformants or

![SDS-PAGE and immunoblot analysis of proteinase K-treated lysates](http://iai.asm.org/)

**FIG. 1.** SDS-PAGE and immunoblot analysis of proteinase K-treated lysates (A) silver stained after SDS-PAGE, (B) reacted with MAb 2-1-L8, or (C) immunoblot reacted with MAb 2-1-L8 and then with O6B4. The lanes represent lysates derived from the following strains: 1, FA5100; 2, FA19; 3, MUG502; 4, MUG508; 5, MUG518; 6, MUG527.

FA19. Figure 1C shows the Western blot used in Fig. 1B that was then reacted with MAb O6B4. All of the transformants made an LOS epitope which bound MAb O6B4, except for MUG502, the hyperproducer of the MAb 2-1-L8 component and MUG527. However, MUG527 reacted with O6B4 when these cells were analyzed by colony blotting, whereas MUG502 failed to react under the same conditions (unpublished data).

**Subcloning of pSY81.** To localize the region on pSY81 responsible for transforming FA5100 to reactivity with MAb, deletion plasmids were constructed. A 2.4-kb EcoRI fragment of gonococcal DNA was subcloned from pSY81 into both pACYC184 and pKanl8 and retained the ability to transform FA5100 to reactivity not only with the MAb 2-1-L8 but also with MAbs O6B4 and 3F11. Restriction analysis of pSY81, various deletion derivatives of it, and two subclones containing a 2.4-kb EcoRI fragment are shown in Fig. 2, along with the various MAb reactivity patterns obtained after transforming FA5100 with these plasmids. This indicates that this 2.4-kb EcoRI fragment complements the mutation in FA5100 that is essential for the biosynthesis of all LOS components made by FA19.

**Analysis of LOS expression in E. coli.** To determine whether pSY81 could alter the LPS of E. coli, we analyzed HB101 containing this plasmid (Fig. 3). SDS-PAGE analysis indicated that E. coli containing pSY81 produced an altered LPS (Fig. 3; compare lanes 3 and 4). However, the Western blot indicated that this strain did not react with the gonococcus-specific MAb 2-1-L8 (Fig. 3B).

Alteration of E. coli LPS expression by a plasmid containing gonococcal DNA (pTME6) has been previously reported (17). To determine whether pTME6 contains DNA sequences found in our clone, we used pSY81 to probe pTME6; no hybridization was seen (data not shown). Therefore, we have concluded that pSY81 encodes different genes than pTME6.

**Transformation of other gonococcal strains with pSY81.** We have previously reported the ability of MUG116 chromosomal DNA to transform strain DOV to reactivity with MAb 2-1-L8 (28). To determine whether pSY81 contained gonococcal DNA that could transform DOV and other MAb 2-1-L8-nonreactive strains to reactivity, we transformed DOV and F62 with pSY81 and MUG116 chromosomal DNA.

---

**TABLE 2. Transformation**

<table>
<thead>
<tr>
<th>DNA used (ng)</th>
<th>Transformation frequenciesa</th>
</tr>
</thead>
<tbody>
<tr>
<td>pSY81 (100)</td>
<td>6.5 x 10^{-2}</td>
</tr>
<tr>
<td>pSY81 (10)</td>
<td>1.0 x 10^{-2}</td>
</tr>
<tr>
<td>pSY81 (1)</td>
<td>1.6 x 10^{-3}</td>
</tr>
<tr>
<td>MUG116 chromosomal DNA (100)</td>
<td>1.5 x 10^{-2}</td>
</tr>
<tr>
<td>MUG116 chromosomal DNA (10)</td>
<td>2.4 x 10^{-3}</td>
</tr>
<tr>
<td>MUG116 chromosomal DNA (1)</td>
<td>2.6 x 10^{-4}</td>
</tr>
</tbody>
</table>

a Transformation was performed by adding DNA to 2 ml of cells (5 x 10⁷ cells) incubating for 6 h, and plating on nonselective gonococcal growth agar. Colonies were screened for the ability to react with MAb 2-1-L8. Frequencies with MAbs O6B4 and 3F11 were approximately the same.

b Expressed as the number of transformants divided by the number of CFU.
pSY81 did not transform DOV or F62 to reactivity with MAb 2-1-L8, whereas positive transformants were observed when MUG116 DNA was used to transform DOV or F62 (data not shown). This indicates that LOS expression in FA5100 is not regulated by gross chromosomal rearrangements.

**DISCUSSION**

This study was undertaken to identify genes associated with LOS biosynthesis in *N. gonorrhoeae*. FA5100, the pyocin-resistant derivative of FA19, was chosen as the recipient strain for all transformations because of its inability to react with any MAb and its lack of any detectable LOS side chains. This strain appears to possess only a very small single-component LOS moiety when analyzed by SDS-PAGE and is nonreactive when tested for its ability to bind LOS-specific MAb.

A 13-kb region of DNA was cloned from MUG116 that possessed the ability to transform FA5100 to reactivity with several LOS-specific MAb (MUG116 reacts with all three of the MAb's used in this study). Restriction analysis and subcloning were performed, and a 2.4-kb *EcoRI* fragment was identified that retained the ability to transform FA5100 to reactivity with the MAb's isolated and analyzed by SDS-PAGE. The data indicated that the 45 FA5100 transformants possessed new LOS banding patterns when compared with FA5100.

Because pSY81 does not contain the DNA sequences necessary for replication in the gonococcus, a positive transformation event must arise due to homologous recombination between the cloned gonococcal DNA sequences and the recipient chromosome. Most of the FA5100 transformants that we obtained resembled the parent. However, three of the transformants had different LOS profiles as determined by SDS-PAGE and different reactivities with MAb's on Western blots. MUG102, an apparent hyperproducer for the 2-1-L8 epitope, did not react with MAb O6B4 on the Western blot and did not react with MAb 3F11 on the colony blot. MUG5108 and MUG527 reacted quite strongly with MAb's 3F11 and O6B4 but very weakly with MAb 2-1-L8.

The pleomorphic effect on the LOS production in the transformants generated with our clone was surprising. The smallest subclone contained only 2.4 kb of gonococcal DNA yet was able to transform FA5100 to reactivity with a number of MAb's. Clearly, there is not enough DNA on this fragment to encode enough genes to synthesize a complete LOS molecule. Three possible models that can explain the data are as follows: (i) we have cloned a single gene that encodes a transferase that adds the first sugar to the KDO, and without this enzyme the further addition of sugar resi-
dues is impossible; (ii) we have cloned a regulatory gene for LOS biosynthesis; or (iii) the pleomorphic effect is the result of differential recombination between the incoming cloned fragment and the resident chromosome.

If we have cloned a single transferase gene required for the addition of the first sugar to the LOS chain, then the first sugar added to the 3-deoxy-manno-2-keto-octulosonic acid residue in all of the components of FA19 should be the same. JW31R, a pyocin-resistant derivative of JW31, has recently been chemically and structurally characterized (7a). This analysis revealed that of the four major oligosaccharides and seven minor oligosaccharides produced by this strain, all contained a core region of 3-deoxy-manno-2-keto-octulosonic acid linked to two heptose molecules and an N-acetylglu- cosamine residue. The outer components of the chain varied in numbers but not types of sugars; each progressively longer LOS chain contained within it the same LOS sequence of the shorter chain (7a). All seven LOS molecules could be produced on the surface of the same cell, yet all had a core region present. The addition of any of the subsequent sugars to the growing LOS chain would require that the core region be synthesized first. If a block occurred in one necessary for the biosynthesis of this core region, then no other sugars could be added on to the core. The mutation present in FA5100 appears to render it incapable of expressing sugar moieties in its LOS chain, yet when our 2.4-kb cloned fragment was introduced into it, the transformants produced a number of LOS components.

Plasmid pSY81 expressed a gene that was able to effect the type of LPS made by E. coli, as seen by its altered LPS profile. However, E. coli HB101 was unable to make a complete gonococcal LOS, because the new LPS that it made failed to react with any of the MAbs used in this study. The altered E. coli LPS would be expected if pSY81 encoded at least one sugar transferase gene and when this sugar was added to the E. coli core, it would give rise to two different LPS molecules (Fig. 3). This observation differs from that of Palermo et al. (18), who identified a clone containing gonococcal DNA (pTME6) which, when transformed into E. coli, expressed an antigen on its surface that was resistant to proteinase K digestion and that reacted with anti-gonococcal antiserum. Because our cloned piece of DNA failed to hybridize with pTME6, and pTME6 failed to transform FA5100 and several other MAb 2-1-L8-nonreactive gonococcal strains (data not shown), we have determined that our clone is different. pSY81 contains DNA that produces a specific change in the gonococcus that can be examined with LOS-specific MAbs.

The 3.6-kilodalton LOS that reacts with MAb 2-1-L8 could be considered a core LOS structure. Additions to this structure would result in the appearance of larger components, as seen in FA19, DOV and F62, and as measured by reactivity with MAbs 3F11 and O6B4. All gonococcal strains would produce a certain amount of LOS, and the amount of the 3.6-kilodalton LOS that is not converted to higher-molecular-mass forms would be regulated by the level of the various sugar transferases that are synthesized. If none of these enzymes were produced, a strain would have the appearance of overproducing the 3.6-kilodalton LOS component. We have been able to construct just such a strain (MUG502). Antigenic variation in LOS biosynthesis would be manifest in strains producing LOS components greater than 3.6-kilodalton, with the amount of the various components varying depending on strain. Our cloning studies have shown that the amount of the various LOS components present in a single strain varies at a high frequency but that, over time, the relative proportions of the various LOS components remain constant (1, 24). This steady state would reflect the biosynthetic capabilities of a given strain, whereas the differences seen among different colonies of the same strain would reflect a pathogenic property of the various LOS components, as selected by the host. Alternatively, the pleomorphic effect of the cloned piece of DNA on FA5100 may be due to the fact that the gene(s) cloned are regulatory gene(s) of LOS biosynthesis and that, depending on the recombination event, different biosynthetic genes are turned on or off. Regulatory genes of LPS biosynthesis have been described both in E col i (the sfrB locus) (20) and Salmonella typhimurium (the rfaH locus) (6), and the effects of mutations in these regulatory genes reveals a pleomorphic effect similar to what we observed with our clone. MUG508 and MUG527 weakly react with MAb 2-1-L8, and the SDS-PAGE profiles reveal that the 3.6-kilodalton component that is overproduced in MUG502 is not present (MUG527) or is slightly apparent (MUG508). The fact that the core structure that binds the MAb 2-1-L8 in the gonococcus is not made, or is masked by the addition of terminal sugars, in these transformants indicates that a regulation of gene expression may be occurring. If the DNA cloned in pSY81 encodes a transferase for the first sugar of the LOS chain of FA19, then one would expect to generate transformants that produced a core region. The additional sugars comprising the epitopes recognized by MAb 3F11 and O6B4 would be added on but would vary in their expression levels; the antigenic variation seen and recently chemically and structurally characterized (7a) should be seen in these transformants. However, this was not apparent, because most of the transformants gave rise to the parental FA19 LOS compliment, and the transformants that did differ had unexpectedly varied degrees of expression of the core region. Regulation of the expression of isi genes by our cloned piece of DNA could be similar to regulation of the rfaH or sfrB genes of the enteric bacteria, which act as presumptive antiterminators to several gene loci.

The genetic mechanisms behind the antigenic variation and switching that occur in most of the gonococcal outer-membrane components has revealed a complex picture of chromosomal rearrangements involving multiple copies of genes and silent loci that are expressed when rearranged (27). The heterogeneity of the numbers and types of antigens produced by the gonococcus points to control mechanisms that orchestrate this overall production. However, our Southern blot data indicated that there were no such rearrangements or deletions responsible for the lack of LOS biosynthesis in FA5100. Because FA5100 is an isogenic derivative of FA19, the loss of LOS expression by FA5100 due to a change at the DNA level should have been seen. A possible explanation for this observation is that the mutation in FA5100 is a point mutation, small deletion, or inversion that was not detected on the Southern blot. However, most point mutations show a detectable reversion frequency. We have studied FA5100 quite extensively, and we have never seen a spontaneous reversion to reactivity with any MAbs used.

Our data indicate that we have cloned a gene or genes involved in the biosynthesis of LOS in N. gonorrhoeae. The mutation that caused FA5100 to lose the capability to produce any oligosaccharide side chains was corrected by our cloned piece of DNA. Our clone failed to transform DOV and F62, two other MAb 2-1-L8-nonreactive gonococcal strains which produce a variety of LOS molecules, to reactivity with MAb 2-1-L8. Southern hybridization data with pSY86 as a probe revealed no apparent differences.
between DOV and the other strains analyzed in this study (Fig. 4). DOV was not transformed to MAb 2-1-L8 reactivity with the cloned pSY1, but could transform FA5100 to reactivity with pSY1, yet Southern blot data revealed common DNA sequences. We conclude that DOV contains the locus that is defective in FA5100. Since pSY1 does not convert DOV to reactivity with MAb 2-1-L8 and is able to repair the defect in FA5100, the genetic locus that we reported earlier that is able to convert DOV to reactivity with the MAb 2-1-L8 (28) must be different from the locus encoded by our clone.

We have described two genetic loci involved in LOS expression in MUG116, one by molecular cloning and one by DNA-mediated transformation (28). The mutation found in MUG116 DNA that is able to transform DOV to reactivity with MAb 2-1-L8 will be called Isi-1, and the MUG116 DNA that is able to transform DOV to reactivity with MAb 2-1-L8 will be called Isi-2.

ACKNOWLEDGMENTS

We thank Mike Apicella and Wendell Zollinger for supplying the MAb used in this study. We also thank Herman Schneider and Mac Griffith for stimulating discussion.

This work was supported by a grant from the Programme on Vaccine Development from the World Health Organization and by Public Health Service grant IR29AI24452 from the National Institutes of Health.

LITERATURE CITED