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Use of Pyocin To Select a Haemophilus ducreyi Variant Defective in Lipooligosaccharide Biosynthesis

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Haemophilus ducreyi, a cause of genital ulcer disease in developing countries, appears to facilitate the heterosexual transmission of the human immunodeficiency virus in Africa. Despite an increase in studies of this gram-negative human pathogen, little is known about the pathogenesis of chancroid. Our studies have shown that the lipooligosaccharides (LOS) of H. ducreyi may play an important role in ulcer formation. Monoclonal antibody and mass spectrometric analyses identified a terminal trisaccharide present on H. ducreyi LOS that is immunochemically similar to human paragloboside. This epitope is present on the LOS of Neisseria gonorrhoeae, and it may be the site of attachment for pyocin lysis. We have used pyocin, produced by Pseudomonas aeruginosa, to select LOS variants with sequential saccharide deletions from N. gonorrhoeae. On the basis of the similarities between N. gonorrhoeae and H. ducreyi LOS, we employed the same technique to determine if H. ducreyi strains were susceptible to pyocin lysis. In this study, we report the generation of a pyocin N-resistant H. ducreyi strain which synthesizes a truncated version of the parental LOS. Further studies have shown that this H. ducreyi variant has lost the terminal LOS epitope defined by monoclonal antibody 3F11. This report demonstrates that H. ducreyi is sensitive to pyocins and that this technique can be used to generate H. ducreyi LOS variants. Such variants could be used in comparative studies to relate LOS structure to biologic function in the pathogenesis of chancroid.

The resurgence in studies involving the gram-negative pathogen Haemophilus ducreyi has been stimulated by the fact that genital ulcer disease has been shown to be a significant contributor to human immunodeficiency virus transmission and seroconversion (12, 30, 32). Most of the recent work with H. ducreyi has characterized bacterial components such as outer membrane proteins, pili, and a cytotoxin (1, 23, 29, 31). However, the role of these components in infection remains unknown. The high frequency of chancroid in parts of Africa and Asia (24), where human immunodeficiency virus type 1 is epidemic, makes it imperative to elucidate the actual events involved in the pathogenesis of chancroid in vivo.

Our laboratory has shown that the lipooligosaccharides (LOS) expressed by H. ducreyi may be a significant factor in the initiation of ulcer formation (4). Immunochemical and physicochemical analyses indicate that H. ducreyi and Neisseria gonorrhoeae LOS share important components. The oligosaccharides from both organisms contain a terminal structure recognized by monoclonal antibody (MAb) 3F11, which also binds to human paragloboside (20).

Previous work from our laboratory has used pyocins, bac-
teriocins isolated from Pseudomonas aeruginosa, to select LOS variants from N. gonorrhoeae which have sequential deletions in their oligosaccharide (7, 11). We have shown that the probable LOS binding site for pyocin corresponds to the presence of the epitope reactive with MAb 3F11. Structural analyses have recently been completed on the LOS of the pyocin survivors, and this information will be critical in relating LOS structure to biologic function involving the pathogenesis of Neisseria species (11).

On the basis of the similarities between H. ducreyi and N. gonorrhoeae LOS, we sought to determine if H. ducreyi was pyocin susceptible and if pyocin selection could be used to generate stable LOS variants from these organisms. Such LOS variants would be useful in comparative experiments aimed at understanding the role of H. ducreyi LOS in the pathogenesis of chancroid.

MATERIALS AND METHODS

Bacteria. H. ducreyi 35000, 27722, 023233, 188, and CIP542 strains were obtained from our collection. The bacteria were cultured on chocolate agar plates at 34°C and in 5% CO2 as previously described (4). Any colonies resistant to pyocin lysis were confirmed by colony morphology. Gram stain, requirement for X but not V factor, oxidase positivity, catalase negativity, and the inability to ferment glucose, lactose, and sucrose (4). The P. aeruginosa strains used for the production of pyocins were obtained from our own collection and grown on supplemented GC medium base as described in the gono-
coccal studies (7).

Pyocin isolation. Pyocins were isolated from P. aeruginosa strains by the method described by Morse et al. (24).

Pyocin selection. The pyocin assay was performed on a lawn of H. ducreyi by the procedure described previously (7). Briefly, the organisms were harvested from an overnight growth on chocolate agar and suspended in brain heart infusion broth and hemin, supplemented with IsoVitaleX and fetal bovine serum. The final concentration of bacteria was 107 CFU per ml. Approximately 100 μl of each bacterial suspension was plated onto the surface of a chocolate agar plate, and 10 μl of each purified pyocin preparation was spotted in a defined area on
each lawn. The plates were incubated at 35°C overnight in a 5% CO₂ incubator. After 18 to 24 h, the plates were observed for a zone of lysis where each pyocin spot was placed. In the initial screening for pyocin sensitivity (Table 1), each strain was scored as sensitive (S), resistant (R), or partially sensitive (T). Colonies that were present in a zone of lysis were individually picked and expanded for analyses.

**LOS preparations.** The LOS from parental and selected pyocin survivors were purified by a modification of the method described by Inzana (10). LOS analyzed by mass spectrometry were isolated by the phenol-water method described by Westphal and Jann (34).

**SDS-PAGE.** The LOS preparations were initially analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 14% acrylamide gels with 2.5% urea, as previously described (13). Subsequent analyses were performed by the tricine gel method, which provided better resolution of the LOS banding patterns (16).

**Colony blot assays.** Pyocin survivors were analyzed for the expression of the MAb 3F11 oligosaccharide epitope by using colony blots as previously described (3).

**Composition and linkage analysis.** Prior to composition analysis, oligosaccharide fractions from strain 188 and its pyocin variant, 188-2, were prepared by acetic acid hydrolysis of the LOS (~2 mg/ml) for 2 h at 100°C. Monosaccharide compositions of the released oligosaccharide fractions from the LOS of both strains were obtained after partial purification on two tandem Bio-Sil TSK-125 columns (60 by 0.75 cm) running with 50 mM pyridinium-acetate (pH 5.2) at a flow rate of 1 ml/min. Detection was carried out by on-line refractive index (Knauer). The partially purified oligosaccharide fractions were hydrolyzed in 2 M trifluoroacetic acid for 3 h at 100°C. The resulting monosaccharides were separated and quantified by high-pH anion exchange chromatography with pulsed amperometric detection as previously described (28). To determine linkages of the various sugars, partially methylated alditol acetates were prepared from the oligosaccharide fraction as described in detail elsewhere (28). Briefly, NaOH was used with dimethyl sulfoxide to prepare the corresponding aldoxide oligosaccharides, which were then methyalted with CH₃I, hydrolyzed, reduced with sodium borodeuteride (NaBD₄), and acetylated (17). The resulting partially methylated alditol acetates were analyzed by gas chromatography-mass spectrometry with a VG-70 mass spectrometer coupled to a 30-m DB-1 capillary column (J&W Scientific). A temperature program of 120 to 250°C at 4°C/min was used with electron impact detection (70 eV, 500 μA trap current, 2 mA emission current, and 180°C source temperature). A partially methylated alditol acetate mixture obtained from the purified major oligosaccharide of *Salmonella typhimurium* Ra lipopolysaccharide (Sigma) was used as a standard for correlating retention times.

**Mass spectrometry.** To determine the molecular weights of the LOS species and assess the heterogeneity of LOS glycoforms from both the wild type and pyocin variant, LOS were analyzed by electrospray ionization mass spectrometry (ESI-MS) after O-deacylation with mild hydrazine (9). We have found that O-deacylation of LOS greatly increases its water solubility and reduces its tendency to aggregate, making it more amenable to direct analysis by ESI-MS techniques. To prepare O-deacylated LOS, approximately 1 mg of LOS from strains 188 and 188-2 was suspended in 0.2 ml of hydrazine and heated at 37°C for 20 min. O-deacylated LOS was precipitated with cold acetone, washed three times, and taken up in 0.5 ml of water and lyophilized. For ESI-MS analysis, the lyophilized O-deacylated LOS samples were dissolved in water and injected into a stream of H₂O-CH₃CN (3:1) containing 1% acetic acid as previously described (8). Mass spectra were taken in the negative-ion mode on a VG/Fison BioQ triple quadrupole mass spectrometer operating in the negative-ion mode with a constant flow rate of 3 μl/min. Mass spectra were then averaged and masses were assigned via the VG/Fison data system by using an external calibrant. Average LOS molecular weights were calculated by adding the molecular weight of the conserved diphosphoryl diacetyl lipid A moiety, 953.0089, to the interval average mass values of the monosaccharide and phosphate constituents: hexose (Hex), 162.1424; heptose (Hep), 192.1687; N-acetylaminohexosamine (HexNAc), 203.1950, 3-deoxy-d-manno-octulosonic acid (Kdo), 220.1791; 5-N-acetylenuraminic acid (sialic acid or Neu5Ac), 291.2579; phosphoethanolamine (PEA), 123.0483; and phosphate (P), 79.9799.

The following analyses were used to confirm the composition assignments of the oligosaccharide portions of these LOS, as suggested from monosaccharide and ESI-MS data. The oligosaccharide fractions from 188 and 188-2 LOS were separately analyzed by negative-ion liquid secondary ion mass spectrometry (LSIMS) on a Kratos MS50S mass spectrometer (28). LSIMS analysis of the oligosaccharides affords a higher degree of mass accuracy (~0.3 Da) than ESI-MS experiments and can also provide limited sequence information through the presence of fragment ions.

Oligosaccharides were dissolved in water, and small aliquots (~2 to 5 μg) were transferred to the LSIMS probe along with 1 μl of thioglycerol-glycerol (1:2, vol/vol). A Cs+ beam of 10 keV was used, and the resulting ions were accelerated at 8 keV. Spectra were taken at 300 s per decade and manually calibrated to an accuracy of better than ±0.2 Da with an external Ultramark calibrant. Since the MS50S mass spectrometer is run under conditions that resolve the isotopic distribution of the various ions (M/ΔM = 2,000), masses are reported as their most abundant isotopically pure component. To calculate the ¹³C-containing molecular ions, the following exact interval mass values were used: Hex, 162.0528; Hep, 192.0634; HexNAc, 203.0794, Kdo, 220.0583; *anhydro*Kdo, 202.0477; Neu5Ac, 291.0594; PEA, 123.0085; and H₂O, 18.0106.

**RESULTS**

**Pyocin selection and LOS analyses.** After initial determinations of pyocin sensitivity (Table 1), pyocin N was selected for use in these studies. Pyocin N was used on *H. ducreyi* 35000, 27722, 02323, CIP542, and 188 strains. Of these strains, 12 colonies were isolated from the zones of lysis: 3 from strain 35000, 2 from strain 02323, and 7 from strain 188. These colonies were expanded in culture, and the identities of the strains were confirmed as described in Materials and Methods. The LOS preparations from each colony were compared with

---

**TABLE 1. Pyocin susceptibilities of *H. ducreyi* strains used in this proposal**

<table>
<thead>
<tr>
<th><em>H. ducreyi</em> strain</th>
<th>Susceptibility to lysis by pyocin*†</th>
</tr>
</thead>
<tbody>
<tr>
<td>C D E F G H I J K L M N</td>
<td></td>
</tr>
<tr>
<td>CIP542</td>
<td>S R R R S S S S S S S S S S S S S</td>
</tr>
<tr>
<td>27722</td>
<td>S R R R S S S S S S S S S S S S S</td>
</tr>
<tr>
<td>188</td>
<td>S R R R S S S S S S S S S S S S S</td>
</tr>
<tr>
<td>35000</td>
<td>S R R R S S S S S S S S S S S S S</td>
</tr>
<tr>
<td>02323</td>
<td>S R R R S S S S S S S S S S S S S</td>
</tr>
</tbody>
</table>

* Abbreviations: R, resistant; S, sensitive; T, turbid (partially sensitive).
the parental LOS profile by SDS-PAGE and silver stain technique as described previously (10, 33). The results indicated that one isolated colony, from strain 188, appeared to assemble an LOS with a different SDS-PAGE banding pattern. Figure 1 is a silver stain of a tricine gel illustrating that the LOS from pyocin survivor isolate 2 (lane 2) had a more rapidly migrating electrophoretic banding pattern than the parental strain 188 (lane 1). It appears that the LOS from this pyocin N survivor, termed 188-2, is a truncated version of the major LOS species assembled by H. ducreyi 188.

**Colony blot studies with MAb 3F11.** Our previous studies have shown that MAb 3F11 reacts with a 4.8-kDa LOS band present on 97% of the H. ducreyi strains in our collection. On the basis of the LOS SDS-PAGE profile of pyocin survivor 188-2, we performed colony lifts and probed these with MAB 3F11. Figure 2 shows an example of a colony lift in which MAB 3F11 reacted to H. ducreyi 188 (panel A) but not to the pyocin-selected strain 188-2 (panel B). These data suggest that H. ducreyi 188-2 has lost all or part of the terminal trisaccharide present on the native LOS. Repeated passage and selection of H. ducreyi 188-2 have shown that this LOS variant appears to be stable.

**Electrospray analysis of O-deacylated LOS.** The ESI-MS spectra of the O-deacylated LOS from strains 188 and 188-2 shown in Fig. 3 indicated that a substantial change to a much simpler and less heterogeneous LOS mixture had occurred in the pyocin survivor. In contrast, the spectrum of the parent strain 188 contained many of the same peaks previously reported for H. ducreyi 35000 (22) but was considerably more heterogeneous. For example, the ESI-MS spectrum of O-deacylated LOS from the parental H. ducreyi strain (Fig. 3A) clearly shows two dominant triply charged peaks at m/z 943.6 and 1,041.6, as well as their anhydro counterparts at m/z 938.2 and 1,034.7, corresponding to the average molecular weights of 2,833.4 (LOS L) and 3,127.8 (LOS N), respectively (Table 2). These are similar to the two major components observed earlier for strain 35000 (22), which were found to differ by the presence or absence of sialic acid (Δm ~ 291 Da). However, a multitude of less abundant secondary peaks, the bulk of which appear also to be triply charged ions, were also present. These peaks can mostly be assigned as LOS components containing an additional PEA moiety, and/or sequential saccharide deletions down to a conserved Hep Kdo(PS) O-deacyl lipid A core (i.e., LOS A, Mr of 1,830.3) (Table 2).

The ESI-MS spectrum of the O-deacylated LOS from H. ducreyi 188-2 contained peaks which correspond to a relatively simple series of related LOS components with mostly smaller molecular weights. The most abundant LOS form is evident as a triply charged ion, (M-3H)3 at m/z 822.3, and much less abundant is a doubly charged ion, (M-2H)2 at m/z 1,233.6. Taken together, this pair of ions yields an average Mr of 2,469.5 for this major component, LOS C. Curiously, this spectrum is essentially devoid of peaks originating from a loss of water, i.e., anhydro LOS forms, which mostly dominated the spectrum of the parental LOS mixture. In addition to the dominant LOS C from 188-2, there are two other LOS species of lower mass whose molecular weights are consistent with the loss of a single heptose (LOS A, Mr of 2,276.8) or hexose (LOS B, Mr of 2,307.3) and two species of higher masses consistent with the addition of PEA (LOS D, Mr of 2,592.0) or Hex and HexNAc (LOS E, Mr of 2,712.7). This latter component may represent a very small amount of full-length parental LOS (<3%) in the total LOS preparation. Despite the complexity and heterogeneity observed in the wild-type strain, if one considers only the difference between the most abundant LOS forms in each preparation, i.e., parental LOS N and the pyocin variant LOS C, it is evident that the pyocin variant has undergone a
trisaccharide deletion consisting of hexose, N-acetylhexosamine, and sialic acid but has picked up an additional PEA moiety.

**Structural analysis of oligosaccharides.** To obtain additional structure information supporting this conclusion, the oligosaccharide fractions from the LOS of both the parental strain and the pyocin N survivor were subjected to LSIMS analysis. As shown in Fig. 4A for the parental *H. ducreyi* strain, the base peak appears as the singly deprotonated molecular ion peak, (M-H)− at m/z 1,676. (Masses for LSIMS are reported as their nominal mass equivalents unless otherwise noted, i.e., m/z 1,676 is 1,676.5.) A second molecular ion peak of less
abundance is also observed at m/z 1,514, which is consistent with the loss of a (terminal?) hexose from the more abundant molecular ion at m/z 1,676. These masses are in excellent agreement with the expected compositions of Hex$_x$HexNAc$_x$Hep$_y$anhydroKdo and Hex$_x$HexNAc$_x$Hep$_y$anhydroKdo. These generic composition assignments are also supported by monosaccharide composition analyses as listed in Tables 3 and 4. For the parent strain 188, relative compositions of approximately 2:1:1 for Gal/Glc/GlcNH$_2$ were found, in addition to an estimated relative composition of approximately 2:1 for 1-glycero-D-manno-heptose and 1-glycero-D-manno-heptose. As previously observed, Kdo was not seen in the composition analysis because of its conversion to anhydroKdo from phosphorylated Kdo (9, 22, 26, 27). The assignment of phosphorylated Kdo is also supported by the molecular weight data and the triply charged states observed under negative-ion ESI-MS (Table 2). These data can only be explained through the presence of three unsubstituted phosphates (ζ = -3), two of which can be accounted for in the conserved diphosphoryl lipid A, and the remaining phosphate on the oligosaccharide portion. Since neither phosphate nor Kdo was found in the hydrolyzed oligosaccharides, and the mass of the oligosaccharide was 18 Da lower than would be expected if unmodified Kdo were present, we can conclude that Kdo was initially present in its phosphorylated form. The conversion of phosphorylated Kdo to anhydroKdo during acetic acid hydrolysis has been previously noted by ourselves and others in the LOS from several Haemophilus strains (22, 26).

In addition to the two molecular ions identified in the LSIMS spectrum of H. ducreyi 188, some limited fragmentation is present in the form of the glycosidic Y- and Z-type fragment ions (6). For instance, fragment ions arising from cleavage at either side of the glycosidic oxygen with charge retention at the reducing terminus are seen at m/z 1,311 and 1,293 (loss of HexNAc$_x$Hex$_y$, m/z 1,149 and 1,131 (loss of HexNAc$_x$Hex$_y$), and m/z 957 and 939 (loss of HexNAc$_x$Hex$_y$)). These fragment ions allowed the partial sequence of the main nonreducing branch to be assigned as Hex$_x$HexNAc$_x$→Hex$_y$→Hep$_z$→Hep$_y$→anhydroKdo. Overall, this leaves Hex$_x$P, and anhydroKdo as the remaining undetermined core oligosaccharides in the parent strain. Tandem mass spectrometry of the most abundant parent ion at m/z 1,676 yielded a spectrum that was identical to the one previously reported for the major oligosaccharide from strain 35000 (data not shown) (22). These data, therefore, allowed a more complete structural assignment for this oligosaccharide:

\[
\text{Hex}_x\text{HexNAc}_x\leftrightarrow\text{Hex}_y\leftrightarrow\text{Hep}_z\rightarrow\text{Hep}_y\rightarrow\text{anhydroKdo}
\]

Hep$_y$→Hep$_z$

Linkage analysis of strain 188 revealed eight major sugars eluting in the following order: terminal Gal, 3-Gal, 6-Glc, terminal Hep, 4-Hep, 2-Hep, 3,4-Hep, and 4-GlcNAc (Tables 3 and 4). These sugars were the same as those previously found in the major oligosaccharide from H. ducreyi 35000 (22). Moreover, the presence of a terminal galactose and a 4-linked N-acetylgalactosamine can be unambiguously assigned to the terminal branch, on the basis of the LSIMS oligosaccharide fragment data, as Gal$_1$→GlcNAc$_1$. Given the previously reported high level of binding of MAb 3F11 to the LOS of this parent strain (3), this terminal structure is likely to be Gal$_1$→4GlcNAc$_1$ or terminal lactosamine. If H. ducreyi 188 LOS were to contain the same oligosaccharide as that previously proposed for H. ducreyi 35000 (22), the full structure would be:

\[
\text{Gal}1\rightarrow4\text{GlcNAc}1\rightarrow3\text{Gal}1\rightarrow4\text{Hep}1\rightarrow6\text{Gal}1\rightarrow4\text{Hep}1\rightarrow\text{anhydroKdo}
\]

Hep$_1$→2Hep$_1$
The LSIMS spectrum of the oligosaccharide from the pyocin survivor, strain 188-2, contains a much smaller oligosaccharide of $M_r$ 1,434, as shown in Fig. 4B. This mass is in agreement with an oligosaccharide composition of Hex$_2$Hep$_2$PEA$_1$anhydroKdo$_1$. PEA had previously been assigned to the composition of this LOS on the basis of the ESI-MS molecular weight data (Table 2). Treatment of the oligosaccharide with aqueous hydrogen fluoride (HF) to remove phosphoester moieties and analysis by LSIMS gave the spectrum shown in Fig. 4C. Fragment ions in the LSIMS spectrum at $m/z$ 1,119 and $m/z$ 1,149 and 957 can be interpreted as arising from glycosidic bond cleavages originating from two separate nonreducing terminal branches, Hep and Hex$\rightarrow$Hep, respectively. Therefore, the LOS of the *H. ducreyi* pyocin variant 188-2 clearly lacks the terminal lactosamine structure that endows the parent strain with the acceptor for sialylation, as well as the epitope for MAB 3F11 binding (Fig. 5). Tandem mass spectrometry data on this oligosaccharide supported the argument for this overall structure, as well as determining the linkage of PEA to heptose, but otherwise did not contain any additional information (data not shown). These structural data strongly suggest that *H. ducreyi* 188-2 synthesizes an LOS with a truncated oligosaccharide.

**TABLE 3. Oligosaccharide composition**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Mole ratio relative to glucose*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Galactose</td>
</tr>
<tr>
<td>188</td>
<td>1.5</td>
</tr>
<tr>
<td>188-2</td>
<td>0.8</td>
</tr>
</tbody>
</table>

* Kdo was not detected in the hydrolyzed oligosaccharide fraction because of its conversion to anhydroKdo forms during acetic acid hydrolysis of LOS.

* This second heptose has been tentatively identified as d-glycero-d-manno-heptose on the basis of earlier data (22), as opposed to the major core heptoses which are L-glycero-d-manno-heptoses.

**TABLE 4. Oligosaccharide methylation analysis**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relative peak area*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>t-Gal</td>
</tr>
<tr>
<td>188</td>
<td>1.0</td>
</tr>
<tr>
<td>188-2</td>
<td>1.0</td>
</tr>
</tbody>
</table>

* Partially methylated alditol acetates are abbreviated according to their substitution patterns as follows: t-Gal is 1,3-di-O-acetyl-2,4,6-tetra-O-methylgalactitol; 3-Gal is 1,3,5-tri-O-acetyl-2,4,6-tri-O-methylgalactitol; 6-Glc is 3,4,6-tri-O-acetyl-d-glucitol; and 4-Hep is 1,3,5-tri-O-acetyl-2,4,6-tri-O-methylgalactitol.

* Two terminal heptoses were found, the ratio stated is the unusual d-glycero-d-manno-heptose to the expected terminal core L-glycero-d-manno-heptose.
branch terminating prior to the formation of the lactosamine disaccharide. However, the LOS of the pyocin N survivor also contain at least one PEA group in the core heptose region and therefore differs further from the parent strain, which contains either one or none at all. Composition and methylation analyses of *H. ducreyi* 188-2 also support these interpretations (Tables 3 and 4). Further structural studies will be needed, however, to fully determine the complete LOS structures present in both the parent and pyocin-variant strains.

**DISCUSSION**

Although the exact mechanism by which pyocin acts remains undefined, previous studies with *N. gonorrhoeae* have suggested that pyocin binds to LOS on the surface of the outer membrane (7, 24). Moreover, the electrospray mass spectral analysis of the pyocin-derived gonococcal LOS variants 1291 through 1291c has shown that pyocins can select for organisms which express a truncated LOS structure (11). The generation of LOS variants from other enteric and nonenteric human pathogens has also been shown to be extremely useful in delineating the structure of these complex glycolipids and can be an effective means to relate structure to biologic function (18, 19). In this study, we have used the same strategy to isolate *H. ducreyi* 188-2, a pyocin N survivor, from the parent strain 188. Moreover, repeated passage of strain 188-2 on chocolate agar has not demonstrated any reversion back to the parental LOS phenotype, suggesting that *H. ducreyi* 188-2 is stable.

Biochemical and mass spectral analyses demonstrated that *H. ducreyi* 188-2 assembles a set of truncated LOS structures. Mass spectrometric, composition, and linkage studies of this structure revealed that the variant LOS no longer expresses the terminal lactosamine present on the LOS of *H. ducreyi* 188. These data were confirmed by immunologic studies which show that colonies of *H. ducreyi* 188-2 have lost reactivity with MAb 3F11 (Fig. 2).

The epitope recognized by MAb 3F11 has important pathogenic implications for the success of *N. gonorrhoeae*. Previous studies by Mandrell and colleagues have shown that the epitope recognized by this antibody is identical to that found on the tetrasaccharide of paragloboside, a glycosphingolipid precursor of the major human blood group antigen which terminates in the disaccharide, N-acetyllactosamine (20). Pathogens and coworkers have shown that gonococci grown in the presence of CMP-N-acetyleneuraminic acid, modify their LOS structure by the addition of N-acetyleneuraminic acid (or sialic acid) and consequently become more serum resistant (25). Subsequent studies have demonstrated that the terminal galactoside residue of the gonococcal LOS epitopes serves as the acceptor for sialic acid (21).

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**REFERENCES**

glycolipids are adhesion receptors for *Neisseria gonorrhoeae*. J. Biol. Chem. 265:12774–12777.


