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MAJOR ARTICLE

Desialylation of *Neisseria gonorrhoeae* Lipooligosaccharide by Cervicovaginal Microbiome Sialidases: The Potential for Enhancing Infectivity in Men

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(See the editorial commentary by Shafer on pages 1615–7.)

Previous studies have demonstrated that *Neisseria gonorrhoeae* sialylates the terminal *N*-acetyllactosamine present on its lipooligosaccharide (LOS) by acquiring CMP-*N*-acetyl-5-neuraminic acid upon entering human cells during infection. This renders the organism resistant to killing by complement in normal human serum. *N*-acetyllactosamine residues on LOS must be free of *N*-acetyl-5-neuraminc acid (Neu5Ac; also known as "sialic acid") in order for organisms to bind to and enter urethral epithelial cells during infection in men. This raises the question of how the gonococcus infects men if *N*-acetyllactosamine residues are substituted by Neu5Ac during infection in women. Here, we demonstrate that women with gonococcal infections have levels of sialidases present in cervicovaginal secretions that can result in desialylation of (sialylated) gonococcal LOS. The principle sialidases responsible for this desialylation appear to be bacterial in origin. These studies suggest that members of the cervicovaginal microbiome can modify *N. gonorrhoeae,* which will enhance successful transmission to men.

Keywords. *Neisseria gonorrhoeae*; sialidase; *N*-acetyllactosamine; cervicovaginal secretions; sialyltransferase; lipooligosacharide.

Neisseria gonorrhoeae uses different cellular receptors and signaling pathways to infect women and men [1–6]. In vitro and in vivo studies in men and with primary urethral epithelial cells indicate that infectivity is dependent on the availability of a terminal galactose residue on the gonococcal lipooligosaccharide (LOS) [7–9]. This is usually in the form of *N*-acetyllactosamine [7]. In contrast, gonococcal invasion of primary cervical epithelial cells is not affected by sialylation of the LOS structure [10]. Gonococci in male urethral exudates are naturally serum resistant and become serum susceptible on a single passage on complex medium [11] due to loss of the Neu5Ac present on LOS. Gonococci lack *siaB*, which is necessary to encode the enzyme that synthesizes the substrate for sialylation by sialyltransferases, cytidine-monophosphate-*N*-acetylneuraminic acid (CMP-Neu5Ac) [12]. Loss of LOS sialylation upon in vitro passaging occurs because the substrate is temperature labile and degraded during routine sterilization of growth medium used for culture. During human infection, the gonococcus circulates between extracellular and intracellular compartments, and when it is within cells, it acquires CMP-Neu5Ac present in the nucleus and the Golgi [13–15]. CMP-Neu5Ac can then be used by the gonococcal α2-3 sialyltransferase, and sialylation of LOS occurs, which, in large part, is responsible for serum-resistance of gonococci in exudates [16–19]. Neu5Ac incorporation into LOS can occur in vitro when CMP-Neu5Ac is added to medium after sterilization and cooling [16].

During infection, gonococci live within genital tract cells in both men and women [2] and have the ability to acquire CMP-Neu5Ac in both environments. Sialylation of the *N*-acetyllactosamine residue a priori on gonococcal LOS prior to instillation of organisms into the urethra significantly reduces urethral infection in the human male experimental model of gonococcal infection [9]. Men likely transmit sialylated organisms, the predominant LOS phenotype in urethral infection [20], and these LOS structures do not influence the frequency of cervical infection in women [10]. Removal of Neu5Ac from gonococcal LOS during the cervicovaginal phase of infection may occur independently of infectious potential. Cycle-dependent sialidase activity has been demonstrated in exomes from human cervical secretions [21], and sialidases are produced by bacteria found in the female genital tract, such as *Gardnerella vaginalis, Prevotella bivia*, and *Megasphaera* species [22].

These organisms are part of the normal female genital tract flora, and they flourish during certain vaginal infections [23]. This prompted us to examine whether cervicovaginal sialidases can remove Neu5Ac from the *N*-acetyllactosamine of gonococcal LOS prior to transmission to the male partner, potentially facilitating the infectious process.

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METHODS

Strain

N. gonorrhoeae strain 1291, a piliated clinical isolate with an LOS that expresses a single *N*-acetyllactosamine glycoform, was used in this study. The strain was reconstituted from frozen stock cultures and propagated at 37°C with 5% CO_2 on GC Agar (Becton Dickinson, Franklin Lakes, NJ) supplemented with 1% IsoVitaleX (Becton Dickinson). To sialylate LOS *N*-acetyllactosamine, strain 1291 was grown in supplemented GC broth that also contained 100 μM CMP-Neu5Ac (Sigma-Aldrich, St Louis, MO) added after the medium had been cooled to 50°C. To ensure complete sialylation of LOS, the strain was passaged 2 additional times in the presence of 100 μM CMP-Neu5Ac. After the final passage, organisms were heat killed at 65°C for 1 hour and washed in phosphate-buffered saline. These organisms were then used to demonstrate the effect of cervicovaginal secretions on removal of Neu5Ac.

Cervical and Vaginal Secretions

Eighty-four cervical or vaginal secretions or cervicovaginal lavage specimens [24] from 46 subjects were collected and archived from subjects enrolled in studies performed at the Boston Medical Center, the Medical College of South Carolina Sexually Transmitted Diseases Clinic (hereafter, the "STD clinic"), and the Indiana University Adolescent Primary Care Clinic (hereafter, the "adolescent health clinics"). Urethral lavage secretions [24] were also collected from 7 *N. gonorrhoeae*–infected men, five of whom were partners of infected women. Cervical secretions from 11 patients without gonorrhea who were attending a gynecology clinic at the University of Iowa Hospitals and Clinics for cervical cancer screening were included as control subjects. All of these samples were examined for sialidase activity. Table 1 lists the number of subjects and samples from each site and the tests, which were performed on these samples. Specimens were stored at −80°C prior to processing [25–27]; 50- or 100-μL aliquots were available for these studies. Fifty-eight specimens were from subjects at the STD and adolescent health clinics who were culture positive for *N. gonorrhoeae* at the time of collection, and findings for the remainder were recorded as either culture negative [18] or infection status unknown [8]. In one of the STD clinic subject groups, the presence of bacterial vaginosis was recorded, and 8 of 10 subjects had a clinical diagnosis of concurrent bacterial vaginosis. The collection of samples was approved by the University of Iowa Carver College of Medicine, Boston University Medical Center, Indiana University School of Medicine, the Medical College of South Carolina, and the Office of Clinical Regulatory Affairs and the National Institute of Allergy and Infectious Diseases, National Institutes of Health.

Sialidase Assay

Sialidase activity in all samples was measured using fluorescent changes based on the hydrolysis of 4-methyl-umbelliferyl-*N*-acetylneuraminic acid (MU-NANA, Toronto Research Chemicals) [28]. Methylumbelliferyl, when separated from the substrate by enzymatic activity, fluoresces in 0.6 M Na_2CO_3 at 390 nm, with emission at 450 nm. Standard curve analyses were performed with each assay, using sialidase from *Vibrio cholerae* (Roche Diagnostics). The dynamic range of the assay was 0.1–10 mU/μL. Specimens or sialidase standards were incubated overnight at 37°C with 100 nmoles of MU-NANA in NA-Nase buffer. The reaction was halted by the addition of Na_2CO_3 to 0.6 M, and fluorescence was measured using a fluorometer with the detection sensitivity set at 400 V.

Treatment of Organisms with Cervical Secretions

Digest mixtures that were used in the silver-stained gel were made with 10 μL of bacteria labeled with CMP-NANA in a buffer of 150 mM NaCl and 4 mM $CaCl_2$, (pH 5.6) plus 10 μL of secretion (boiled or unheated; 20-μL reaction mixtures). After incubation at 37°C for 24 hours, samples were centrifuged at 5000 × *g*, and bacterial cells were resuspended in a buffer of 60 mM Tris and 10 mM ethylenediaminetetraacetic acid (pH 6.8) containing 2% sodium dodecyl sulfate (SDS) and 50 μg of proteinase K/mL. A portion of each of these lysates was used as a sample in SDS–polyacrylamide gel electrophoresis (PAGE).

Table 1. Subject and Sample Data

Site[a]	Subjects, by Sex, No.	Samples, by Sex, No.	Positive Results by Assay, Samples, No.				
			Fluorescent Neu5Ac Assay	Sialyltransferase PCR	Immunodot Assay	SDS-PAGE	Confocal Study
IU	20 F/0 M	58 F/0 M	58	7	9	5	14
BMC	10 F/7 M	10 F/7 M	17	17	17	0	0
MCSC	16 F/0 M	16 F/0 M	16	2	2	2	2
UIHC	11 F/0 M	11 F/0 M	11	5	6	0	2
Total	57 F/7 M	95 F/7 M	102	31	34	7	18

Abbreviations: PCR, polymerase chain reaction; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

[a] Indiana University (IU), Boston Medical Center (BMC), Medical College of South Carolina (MCSC), and University of Iowa Hospitals and Clinics (UIHC).

Samples used in an immunodot assay (described below) were made from digest mixtures that used 15 μL of bacteria (labeled with CMP-NANA) in a buffer of 150 mM NaCl and 4 mM $CaCl_2$ (pH 5.6) plus 15 μL of secretion (boiled or unheated; 30-μL reaction mixtures). Boiling inactivates all enzyme activity in the samples. The mixtures were incubated for 48 hours at 37°C and then briefly centrifuged, and 10 μL (one third of the mix, or approximately 5×10^6 colony-forming units [CFU]) was spotted onto nitrocellulose membranes.

Proteinase K Extracts

Proteinase K LOS extracts were prepared according to the method of Hitchcock and Brown [29] from samples that were mixtures of sialylated *N. gonorrhoeae* strain 1291 incubated with cervical secretions.

SDS-PAGE and Silver Staining

Seven samples containing sialylated organisms that had been incubated with cervical secretions were electrophoresed in SDS-PAGE (17% acrylamide followed by silver staining [8]) to determine whether LOS band shifting had occurred after treatment of LOS sialylated organisms with secretions.

Immunodot Assay

Thirty-four samples were examined in an immunodot assay, using monoclonal antibodies 6B4 or 3F11; both monoclonals react with terminal *N*-acetyllactosamine on LOS [30] that is unoccupied by Neu5Ac. The binding of either antibody is prevented by the presence of Neu5Ac on the *N*-acetyllactosamine.

Confocal Microscopy

Confocal microscopy was performed using a BioRad 600 laser scanning confocal microscope (BioRad Laboratories, United Kingdom) housed at the University of Iowa Central Microscopy Research Facility. Cervicovaginal secretions from 13 gonococcal infected subjects and 5 uninfected subjects were stained with ethidium bromide to visualize eukaryotic cells and then were counterstained with monoclonal antibody (MAb) 6B4 (murine immunoglobulin M [IgM]) that recognizes the nonsialylated *N*-acetyllactosamine epitope [20]; fluorescein-labeled goat anti-mouse IgM (Sigma-Aldrich Co, St Louis, MO) was used as secondary antibody. To ensure specificity of the *N*-acetyllactosamine epitope for *N. gonorrhoeae*, microscopy samples were also labeled with MAb 2C3, a murine IgG1 isotype, which binds to the H.8 outer membrane protein [31]; Texas red-labeled goat anti-mouse IgG (Molecular Probes, Eugene, OR) was used as a secondary antibody, and overlapping binding was seen as a merged image (yellow).

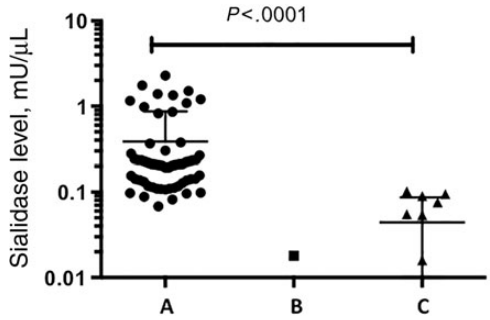

Figure 1. Sialidase activity (fluorescent changes based on the hydrolysis of 4-methyl-umbelliferyl-*N*-acetylneuraminic acid; Toronto Research Chemicals) [28]) in genital secretions; threshold for detection, <0.1 mU/μL (baseline). Group A denotes fluorescent readings for 84 cervicovaginal samples from women attending sexually transmitted diseases or adolescent health clinics. Group B denotes fluorescent readings in urethral exudates from 7 men, 5 of whom were sex partners of women represented in group B. Group C denotes fluorescent readings in samples from 11 gonococci-uninfected women attending a gynecology clinic for cervical cancer screening. Statistical analysis was performed comparing groups A and C was performed using the Mann–Whitney test.

Polymerase Chain Reaction (PCR) Analysis for DNA Encoding Microbial Sialidases

PCR analyses for detection of DNA encoding sialidases from *G. vaginalis* (accession WP_032836912), *P. bivia* (accession WP_036886553), and *Megasphaera* species (accession EPP10089) were

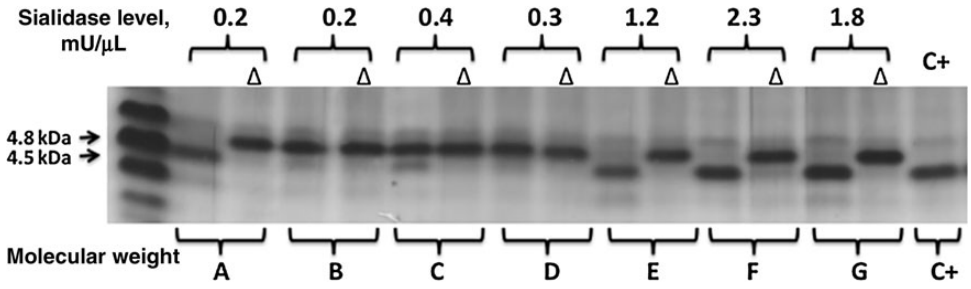

Figure 2. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of lipooligosaccharide (LOS) from *Neisseria gonorrhoeae* strain 1291 after incubation with sialidase containing secretions. LOS migration patterns: 4.8 k, fully sialylated; 4.5 k, desialylated. *A–G*, Heat-killed sialylated *N. gonorrhoeae* strain 1291 incubated with unheated or boiled (Δ) secretion samples, extracted with protease, and electrophoresed. LOS in unboiled secretion samples present in lanes *A*, *E*, *F*, and *G* (left-side of brackets) that migrated to 4.5 k indicated complete desialylation of LOS; *C* showed partial desialylation and *B* and *D* minimal desialylation. Controls included LOS in boiled secretion samples (*A–G*; right-side of brackets [Δ]) where sialylated LOS migrated to 4.8 k. Controls: C+, positive control demonstrating sialylated1291 LOS desialylated using *Vibrio cholera* sialidase. The respective sialidase assay values obtained for the corresponding sample (taken from Figure 2) are indicated above the gel image.

performed on secretions from 24 female subjects and urethral lavages from 7 *N. gonorrhoeae*–infected male subjects [22]. Primers used in these reactions are indicated in Supplementary Table 1.

Statistical Analysis

Statistical comparisons between the 3 groups of subjects in Figure 1 were performed using the Mann–Whitney test, assuming a nonparametric distribution.

RESULTS

We hypothesized, based on our preliminary confocal microscopy studies, that sialidases in female genital tract secretions might remove Neu5Ac from the *N*-acetyllactosamine on *N. gonorrhoeae* and indeed may also desialylate host surfaces. Using the methylumbelliferyl-*N*-acetylneuraminic acid assay, we measured sialidase activity in 84 female secretions obtained from STD and adolescent health clinics, 11 normal secretions from women attending a cervical cancer screening gynecology clinic, and urethral secretions from 7 men (Figure 1). Sixty of 84 cervical secretions (71%) from women at the STD and adolescent health clinics had measurable levels of enzyme (mean ± SEM, 1.4 ± 0.4 mU/μL; >10-fold the limit of detection); 7 women from the gynecology clinic had low levels of sialidase (between 0.2 and 0.02 mU/μL). The remainder of the sample from women had no detectable sialidase activity. Only one of the men has detectable level of sialidase (0.02 mU/μL). The remainder of the sample from men had no detectable sialidase activity.

Seven cervicovaginal secretions with sialidase levels of 0.3–2.2 mU/μL were selected and their ability to remove Neu5Ac from sialylated gonococcal LOS examined. Sialylated organisms (10^8 CFU) were heat killed (at 65°C for 1 hour) and mixed with either unheated secretion samples or the same samples heated to 100°C (ie, boiled) to inactivate any sialidase. Proteinase K extracts from the samples were electrophoresed on SDS-PAGE and silver stained. In this gel system, sialylated LOS runs to 4800 Da; desialyated LOS runs faster, to approximately 4500 Da. Figure 2 shows that samples A, E, F, and G completely removed Neu5Ac from LOS of sialylated *N. gonorrhoeae* strain 1291; samples B, C, and D partially removed Neu5Ac. Thus, 6 of 7 cervical secretions with measurable levels of sialidase removed all or a portion of Neu5Ac from sialylated gonococcal LOS. Neu5Ac was not removed from LOS in the corresponding boiled samples.

To confirm desialylation of gonococcal LOS by using an alternative method, we used a sensitive immunochemical assay to ascertain exposure of the *N*-acetyllactosamine epitope on 34 specimens. We prepared samples as described above, probed with MAb 6B4 by using an immunodot assay, and observed almost complete removal of Neu5Ac in 5 of 7 secretions shown in Figure 3. One secretion (455-096) was grossly contaminated with blood and could not be evaluated because MAb 6B4 binds to human desialylated glycosphingolipids from erythrocytes in the sample [32]. Another sample, shown in Figure 3 (543-113), with a sialidase level below the limit of sensitivity of the assay, failed to desialyate LOS. Immunodot studies with either MAb 6B4 or MAb 3F11 on the remaining 27 samples indicated that 6 showed complete loss of Neu5Ac, 11 (with little to no measurable sialidase activity) showed no loss of Neu5Ac, and 3 indicated partial removal of Neu5Ac (data not shown). Seven samples were contaminated with blood and could not be evaluated.

PCR analysis for DNA encoding microbial sialidase was performed on 24 cervicovaginal and 7 urethral lavage secretions

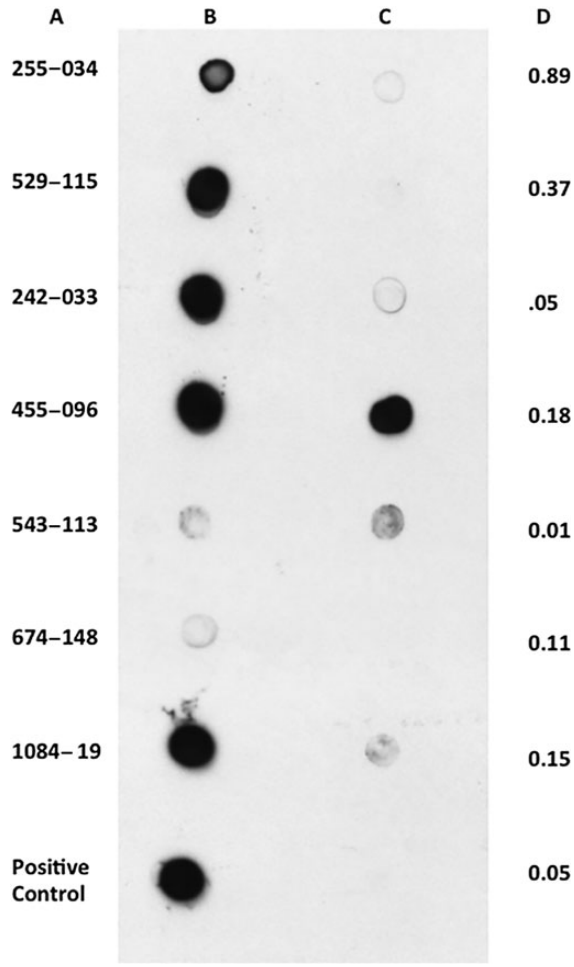

Figure 3. Immunodot assays using monoclonal antibody (MAb) 6B4, which recognizes the unsialylated *N*-acetyllactosamine terminus of *Neisseria gonorrhoeae* strain 1291 lipooligosaccharide (LOS). *A*, Cervicovaginal secretions used to treat sialylated *N. gonorrhoeae* 1291. *B*, Binding of MAb 6B4 (or lack thereof) to *N. gonorrhoeae* 1291 LOS following treatment with unboiled cervicovaginal secretions as a potential source of sialidase; samples 543-113 and 674-148 showed no/minimal sialidase assay values (indicated in lane D [see below also]). *C*, Binding (or lack thereof) of MAb 6B4 to sialylated *N. gonorrhoeae* 1291 LOS following treatment with boiled cervicovaginal secretions; sample 455-096, which contained blood, was positive following treatment with both boiled and unboiled secretions (see text). Lane D shows the respective sialidase assay values in milliunits per microliter obtained for the corresponding samples (taken from Figure 2). The immunodots labeled "positive control" demonstrate the effect of *V. cholerae* sialidase treatment (0.05 mU/μL) on sialylated *N. gonorrhoeae* 1291.

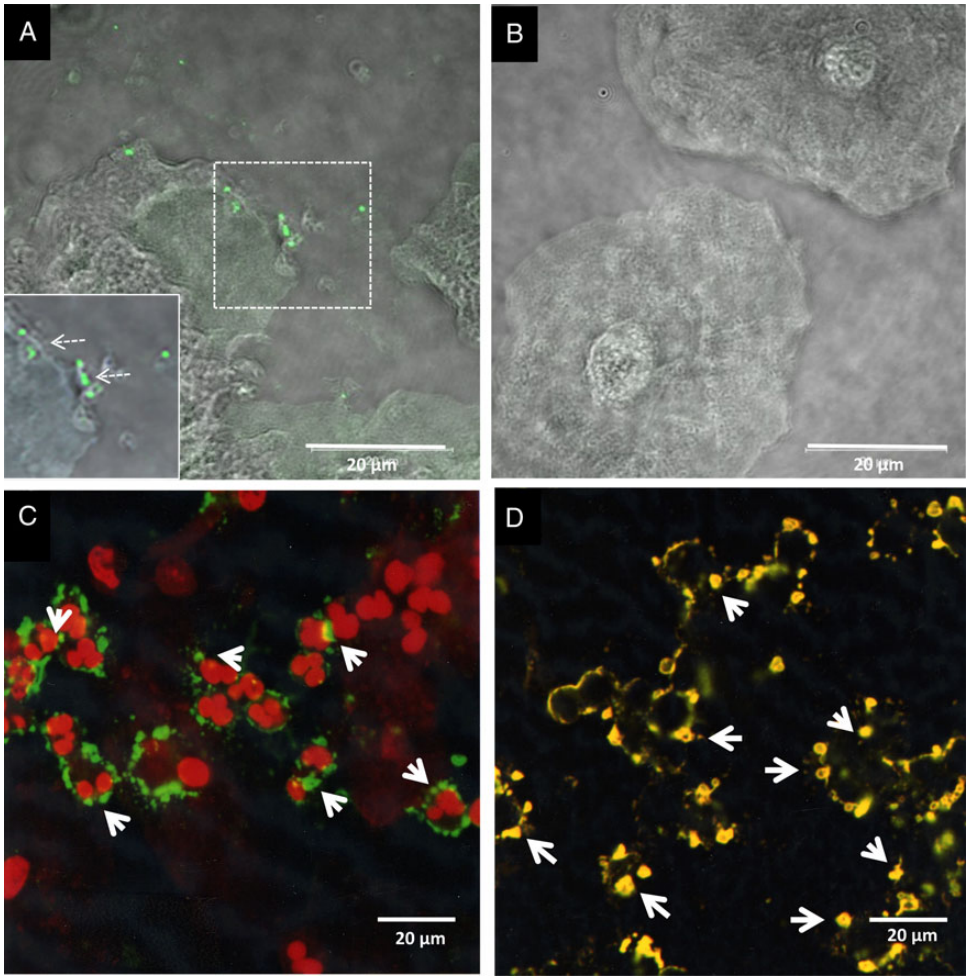

Figure 4. Representative confocal microscopic image of a *Neisseria gonorrhoeae*–infected genital (vaginal) secretion. *A* and *B*, Confocal images at 400× original magnification with phase contrast and stained with monoclonal antibody (MAb) 6B4 and FITC. *A*, A secretion from an individual with a sialidase level of 1.20 mU/µL in her secretion sample. MAb 6B4–stained organisms (white arrows) can be seen associated with the cells in the secretion, indicating the presence of the *N*-acetyllactosamine epitope on the surface. In the lower left hand corner, the dotted box is enlarged 2-fold to demonstrate the biscuit-like diplococcic characteristic of *N. gonorrhoeae*. *B*, Findings from a similar study of another subject also infected with *N. gonorrhoeae* but with a sialidase level of approximately 0.10 mU/µL. No organisms with a free *N*-acetyllactosamine epitope on the surface were observed upon careful scanning of the samples from 2 subjects. These studies suggest that, as we show in the laboratory studies, the level of sialidase in the secretion is critical with regard to whether desialylation of the gonococcus occurs. *C*, Results of confocal studies from another subject in which the eukaryotic cell nuclei (PMN and epithelial cell) were stained with ethidium bromide (red) and then counterstained with MAb 6B4 (murine immunoglobulin M [IgM]) that recognizes the gonococcal *N*-acetyllactosamine epitope; fluorescein-labeled goat anti-mouse IgM (green) was used as secondary antibody. White arrows designate some of the *N. gonorrhoeae* in the field. *D*, An infected vaginal-secretion specimen showing colocalization of gonococcal MAb 6B4 and MAb 2C3 (murine immunoglobulin G [IgG]) specific for the gonococcal H.8 protein, to ensure that MAb 6B4 staining was specific for *N. gonorrhoeae*. Texas red–labeled goat anti-mouse IgG was used as secondary antibody for labeling of MAb 2C3, and fluorescein-labeled goat anti-mouse IgM (green) was used as secondary antibody for MAb 6B4. The merged label on some of the stained gonococci is yellow and designated by white arrows showing the MAbs 6B4 and 2C3 colocalization.

from men to determine a possible source of the enzyme. Nine samples (from 7 women and 2 men) were positive for *G. vaginalis* sialidase DNA, and a secretion sample from 1 female was positive for *P. bivia* sialidase DNA. Twenty-one samples (from 16 women and 5 men) were positive for *Megasphaera* species sialidase DNA.

Previous studies of urethral secretions from *N. gonorrhoeae*–infected men showed that Neu5Ac was covalently linked to *N*-acetyllactosamine on LOS in the majority of organisms seen in the secretions [20]. Sialylation of LOS took place intracellularly while organisms trafficked through urethral epithelia [20]. In this study, confocal microscopy of 18 secretion samples (representative examples from 2 subjects are shown in Figures 4 and 5) from *N. gonorrhoeae*–infected women demonstrated that the *N*-acetyllactosamine epitope was devoid of Neu5Ac substitution in many organisms seen in these secretions. The in vivo desialylation appeared to correlate with the level of sialidase in the respective secretion, similar to our laboratory studies with secretions. In 2 *N. gonorrhoeae*–infected individuals with very low levels of sialidase activity (≤0.1 mU/µL),

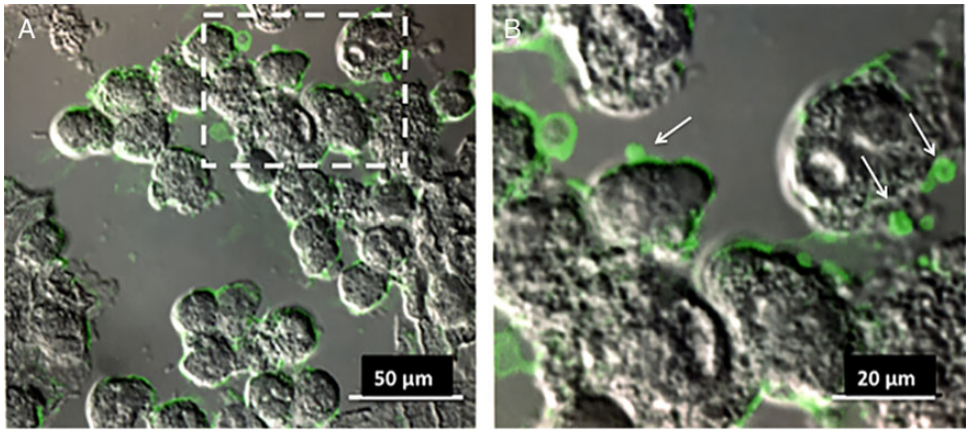

Figure 5. Figure 5 shows results of a confocal study of a subject with sialidase levels in secretions (0.22 mU/µL) in whom the desialylation of the genital tract epithelial cells can be seen after staining with monoclonal antibody (MAb) 6B4. *A*, An image (400× original magnification) of a secretion sample stained with MAb 6B4 with phase contrast. *B*, An image (630× original magnification) of the area enclosed in the dashed box in panel *A*. As can be seen, the surface of a number of cells in both images is stained by MAb 6B4, indicating desialylation of the cell surface. White arrows designate the putative gonococci in the image.

we were unable to identify MAb 6B4–staining organisms in these secretions (Figure 4). This suggests that the level of sialidase in the secretion plays a role in the loss of Neu5Ac as the terminal sugar on LOS. In addition, MAb 6B4, which binds to a similar epitope on certain human glycosphingolipids [32], also reacted with host cells in secretions from some of the patients (green stain on the surface of eukaryotic cells in the left-hand panels in Figures 4 and 5), indicating that desialylation of human cervicovaginal tissue and polymorphonuclear leukocytes was also occurring in individuals with high levels of sialidase (>1 mU/µL).

DISCUSSION

It has been known for approximately 20 years that sialidases primarily of bacterial origin were present in vaginal secretions of women with bacterial vaginosis [33]. This enzyme has been shown to be one of many enzymes (glycolytic and proteolytic) present in bacterial vaginosis, which can degrade the mucus gel. Recently, Lewis and Lewis showed that, in patients with bacterial vaginosis, vaginal sialidases resulted in removal of sialic acid from secretory immunoglobulin A (IgA) and the O-glycans of mammalians mucins [34]. In this article, we have shown that many of the secretions obtained from subjects attending STD clinics contain sufficient sialidase to remove sialic acid from the LOS of sialylated *N. gonorrhoeae,* thus exposing a terminal asialo-lactosamine on the LOS necessary for infection of the male urethral cell [4, 7].

N. gonorrhoeae may be unique among human-specific pathogens because mechanisms involved in pathogenesis differ between men and women [3]. A major difference appears to be that human cervical cells are indifferent to LOS sialylation, while in men, urethral cell infection is reduced substantially if gonococcal LOS is sialylated, owing to Neu5Ac substitution of *N*-acetyllactosamine at the LOS terminus. Experimental infection studies in men also showed that instilling sialylated gonococci into the urethra significantly reduced infectivity [9]. A free terminal galactose is required for engagement of the asialoglycoprotein receptor on the male urethral cell surface [3, 4]. *N. gonorrhoeae* cannot synthesize the substrate necessary for LOS sialylation, CMP-Neu5Ac, and acquires this substrate as part of its intracellular life cycle during natural infection in both men and women [35, 36]. In women, LOS sialylation might be expected to hinder transmission of gonococci to men; nevertheless, transmission of gonorrhea from women to men approaches 25% per episode of sexual intercourse with an uninfected man [37]. Reduction of gonococcal LOS sialylation in women would allow effective transmission of infection to men.

The studies presented herein demonstrate that, during cervical infection, removal of Neu5Ac from the gonococcal LOS can occur. Most secretion samples (71%) from women from STD and adolescent health clinics had measurable sialidase activity in their cervical secretions. Our studies showed that women with levels of >0.15 mU/µL of sialidase activity could remove a portion or all of Neu5Ac present on a representative gonococcal LOS (Figures 3 and 4). Confocal microscopy findings mirrored the results seen in the laboratory studies: in subjects with high levels of sialidase, gonococci could readily be found (Figures 4 and 5), but they were not evident in subjects with low sialidase levels (Figure 4). These sialidases could originate from several sources, such as endogenous sources from the host [21], if the subject had an concomitant bacterial vaginosis infection [38], and/or from the cervicovaginal microbiome. Cervical sialidases are released during the ovulatory phase of the estrous cycle [21] in normal women; 7 subjects from a gynecologic cancer-screening clinic had positive results of the sialidase

assay at the limit of sensitivity of the test. Sperm, which contains several different sialidases [39], or infection with a microbe that expresses a sialidase could engender a positive test result in a male. We found no sialidase activity in the urethral exudate of any of the 7 men. Two *N. gonorrhoeae*–infected male subjects had PCR evidence of *G. vaginalis* sialidase DNA, and 5 had evidence of *Megasphaera* species sialidase DNA in their exudates. Our studies suggest that microbial members of the cervicovaginal microbiome play a major role in desialylation by supplying the necessary sialidases, as 23 of 24 cervicovaginal samples (96%) tested by PCR were positive for DNA encoding sialidases from *G. vaginalis, Megasphaera* species, or *P. bivia*. Elevated levels of sialidase activity (tested using a predecessor [40] of the assay used in this report) were detected in 42 of 50 vaginal fluid specimens (84%) from women with bacterial vaginosis [33]. Many of our patients may have concurring bacterial vaginosis, as has been reported from other STD centers [41, 42].

Previous studies by Shakhnovich et al demonstrated that pneumococcal sialidases present in culture medium desialylated LOS from *N. meningitidis* and *Haemophilus influenzae* [43]. Loss of surface Neu5Ac (sialylation) may not be advantageous for gonococcal survival in the human environment per se, and like other pathogenic bacteria, such as nontypeable *H. influenzae* and *Campylobacter jejuni*, gonococci have evolved other mechanisms to evade human defenses, such as phase and antigenic variation of important virulence structures [44]. However, a successful microbe must also be capable of infecting a new host (perhaps) to a new host, to ensure/expand its survival. *N. gonorrhoeae*, by interacting with sialidases from other organisms present in the female genital tract, takes advantage of Neu5Ac surface modification to enhance infectivity when the organism is transmitted to the male. Transfer of unsialylated gonococci to men is followed by engagement of unsialylated organisms with asialoglycoprotein receptors on urethral cells and by entry, where intracellular organisms can (only) be resialylated and then released back into the urethral lumen [20].

Our studies emphasize the importance of the accompanying microbial environment surrounding gonococcal infection in women and how even a single biochemical event that changes the gonococcal surface may potentially influence transmission to men. This represents a tangible example of an interaction that results in successful and productive symbiosis of different microbial populations.

Supplementary Data

Supplementary materials are available at http://jid.oxfordjournals.org. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

Notes

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Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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