Human Immunodeficiency Virus Type 1 Stimulatory Activity by Gardnerella vaginalis: Relationship to Biotypes and Other Pathogenic Characteristics

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Stimulation of human immunodeficiency virus (HIV) type 1 expression by Gardnerella vaginalis is one possible cause for an increase in the amount of virus in the genital tract. The ability of G. vaginalis to induce HIV expression in chronically infected U1 cells was investigated, along with its possible relationship to biotype, genotype, and resistance to metronidazole and bacteriocin. Significant HIV stimulatory activity was found in 5 (50%) lysates of G. vaginalis. The ability to induce HIV expression in U1 cells was statistically associated with G. vaginalis biotype (P = .048) but not with genotype or resistance to metronidazole and bacteriocin. Further studies to explore the in vivo relevance of HIV activation by G. vaginalis in the female genital tract are warranted, since prevention strategies of bacterial vaginosis and colonization by certain biotypes of G. vaginalis may be valuable in reducing the risk of sexual transmission of HIV.

Sexual transmission is the primary route of human immunodeficiency virus (HIV) type 1 infection worldwide. Women comprise a large number of the newly diagnosed AIDS patients in the United States [1, 2]. About 75% of the 13 million HIV-infected women in the world are infected through heterosexual transmission and most are of reproductive age [3].

Several population studies demonstrate that bacterial vaginosis (BV) is associated with an increased risk of HIV acquisition and transmission [4–6]. In fact, in one study, the rate of HIV infection among women with BV was 2-fold higher than that among women with normal vaginal flora [7]. The organisms associated with BV (e.g., Gardnerella vaginalis) may increase the incidence of sexual transmission of HIV by several direct and indirect mechanisms. One of these mechanisms is the change in the vaginal microflora during BV, including an increased vaginal pH and a decrease in the number of H2O2-producing lactobacilli. This change is thought to provide a favorable environment for HIV survival and replication [8]. Another possible explanation for the higher rates of HIV infection in women with BV is the increased HIV expression induced by BV-associated bacteria, as demonstrated in monocytoid and certain T cells [9]. In fact, among the BV-associated bacteria studied, G. vaginalis was the most potent inducer of HIV expression in vitro [10].

BV is the most common vaginal disorder among women of reproductive age and is defined as a clinical syndrome in which the normal vaginal flora is replaced by an overgrowth of G. vaginalis and anaerobic microorganisms [11, 12]. Most women with BV are colonized predominantly by G. vaginalis [11, 12]. Potentially pathogenic characteristics of G. vaginalis include the presence of pili, an exopolysaccharide coat, a lactoferrin-binding protein, and a hemolysin that lyse human red blood cells, neutrophils, and endothelial cells [13–15]. It also has been demonstrated that some G. vaginalis strains can exhibit resistance to metronidazole and to bacteriocin produced by vaginal lactobacilli species [16, 17].

Classification of G. vaginalis has been proposed as an important tool for etiopathogenic and epidemiologic studies. G. vaginalis has been classified into 8 biotypes on the basis of its metabolic property [18]. Several genotypes also were identified by polymerase chain reaction (PCR) amplification of the 16sRNA gene, with subsequent restriction enzyme analysis [19, 20]. To date, the association of specific G. vaginalis biotypes with BV remains controversial [18, 21]. Furthermore, BV has not been correlated with any genotypes of G. vaginalis. The relationship between biotypes and genotypes with different G. vaginalis pathogenic characteristics, such as HIV stimulatory activity (HIV-SA), clearly remains to be elucidated. The aim of this report was to investigate the ability of clinical G. vaginalis...
isolates to stimulate HIV expression and its relationship to biotypes and genotypes and resistance to metronidazole and to bacteriocin produced by vaginal lactobacilli.

Materials and Methods

Organisms and cells. Cultures of G. vaginalis used in this study included ATCC 14018, ATCC 49145, and 8 isolates from the genital tracts of women with and without BV, as diagnosed by the Nugent's criteria [22]. Three isolates were recovered from women with BV, and 4 were recovered from women without BV; source information was not available for 1 isolate. Presumptive identification consisted of gram-variable, small, pleomorphic rods representing catalase-negative and β-hemolytic colonies on human blood bilayer–Tween (HBT) agar plates (BBL Prepared Media; BD Microbiology Systems) after a 48-h incubation at 37°C in a humidified atmosphere with 5% CO₂. Identification was confirmed further by PCR amplification of the 16SRNA gene, as described below.

The U1 cell line was provided by Thomas Folks (AIDS Research and Reference Reagent Program, Division of AIDS, National Institute for Allergy and Infectious Diseases, National Institutes of Health [NIH]) [23]. The cell line is chronically HIV infected and has been used as a model to test the effects of various substances on HIV replication in monocytoid cells [24, 25]. The U1 cells were maintained in RPMI 1640 medium supplemented with 10 mM HEPES, 2 mM l-glutamine, and 10% fetal bovine serum (BioWhittaker). All cells were negative for Mycoplasma species, as determined by PCR [26].

Isolation of G. vaginalis DNA and PCR amplification of the 16SRNA gene fragment. All isolates were confirmed as G. vaginalis by PCR amplification of the 16SRNA gene fragment. The reactions were done in a thermocycler (model 2400; Perkin-Elmer). G. vaginalis DNA was isolated according to a rapid, salt-based isolation procedure [27]. The primers were derived from sequences of conserved regions at the 5’t and 3’ ends of G. vaginalis ATCC 14018 (Integrated DNA Technologies). The amplified products were electrophoresed on a 1.2% agarose gel, and the presence of the 1.5-kb DNA fragment of the 16SRNA gene of G. vaginalis was detected by ethidium bromide staining. The 2 ATCC G. vaginalis strains (14018 and 49145) were used as positive controls. Negative controls included a DNA sample from a clinical strain of Escherichia coli and water.

Restriction enzyme analysis of PCR products. To recognize differences in genotypes of the G. vaginalis isolates, we digested 12 μL of each PCR-amplified 16SRNA gene product with restriction endonuclease HpaII (New England BioLabs), and the fragment patterns were analyzed on a 1.8% agarose gel. This enzyme was chosen on the basis of its ability to generate different fragment patterns [19, 20].

Preparation of lysates from G. vaginalis. Culture plates of G. vaginalis were harvested, were washed with PBS, and were centrifuged (700 g) for 10 min at 24°C. The pellet then was resuspended in 5 mL of PBS, and lysates were prepared as described elsewhere [10].

Measurement of HIV-SA. To test HIV-SA, we incubated 4 × 10⁴ U1 cells with 5 μg/mL lysate protein of G. vaginalis isolate in microtiter plate wells at 37°C. After a 3-day culture, supernatants were collected and mixed with Triton X-100 (final concentration, 0.1%), and the p24 concentration was measured by an ELISA (Fredrick Cancer Research Institute, National Cancer Institute, NIH). RPMI 1640 medium was used as the control. Lysates were empirically defined as HIV-SA positive when they enhanced HIV p24 production 5-fold over base levels.

Determination of biotypes. Biotypes of G. vaginalis were determined on the basis of 3 biochemical tests: hippurate hydrolysis, lipase, and β-galactosidase activity [18]. Each isolate then was classified into 1 of the 8 possible biotypes.

Bacteriocin susceptibility test. Bacteriocin susceptibility of the G. vaginalis isolates was tested against a bacteriocin-producing strain of Lactobacillus acidophilus, using a modification of Kecessy’s method [28]. A plug of Mann-Rogosa-Sharp (MRS) agar from a plate with an overnight uniform growth of Lactobacillus species at the surface was placed onto an HBT agar plate freshly inoculated with the G. vaginalis isolate to be tested. A similar-sized plug of 0.8% plain agar was interposed between the MRS plug and the HBT agar. After a 48-h incubation, the growth-inhibition zone surrounding the plug was measured by use of a vernier caliper. Isolates were classified as bacteriocin resistant when no growth-inhibition zone was observed surrounding the plug and as bacteriocin susceptible when a growth-inhibition zone was observed.

Metronidazole susceptibility test. Metronidazole disk susceptibility testing was done according to the methods described by the National Committee for Clinical Laboratory Standards [29]. A 0.5 McFarland standard inoculum (1–2 × 10⁷ cfu/mL) was used to inoculate an HBT agar plate. One 80-μg metronidazole disk was placed on the surface of the inoculated agar plate and then was incubated for 24 h. The zones of growth inhibition were measured with a vernier caliper. G. vaginalis isolates were classified as metronidazole resistant when there was no growth inhibition surrounding the disk.

Statistical analysis. Fisher’s exact test was used to investigate possible associations between biotype, genotype, HIV-SA, and resistance to metronidazole and bacteriocin. Nonsignificant results of this test do not indicate absence of association, since the power was low because of the small sample size.

Results

The levels of HIV p24 produced by U1 cells in response to G. vaginalis lysates are shown in figure 1. Although untreated U1 cells expressed low levels of HIV, U1 cells with added lysates from 5 of the 10 G. vaginalis isolates exhibited positive HIV-SA. The highest increase was 20-fold greater than base levels. However, the other 5 G. vaginalis isolates did not significantly enhance the HIV expression and were considered to be HIV-SA negative.

The amounts of HIV p24 produced by U1 cells in response to G. vaginalis culture supernatants are shown in figure 2. The thioglycolate medium alone, compared with RPMI medium, increased HIV expression in U1 cells. Slight enhancement of HIV expression was found in 3 of the supernatants from G. vaginalis isolates. The greatest enhancement was 3-fold that of thioglycolate medium alone.
Figure 1. Levels of human immunodeficiency virus (HIV) p24 produced by U1 cells stimulated with Gardnerella vaginalis lysates. Data are mean ± SD HIV p24 concentration after 3-day incubation of U1 cells with 5 μg/mL of lysate. Dashed line, RPMI medium alone (control). *Levels enhanced >5-fold over base level and considered to be positive for HIV stimulatory activity.

Of the 3 G. vaginalis isolates from women with BV, 2 were positive for HIV-SA. Only 1 of the 4 G. vaginalis isolates obtained from women without BV was positive for HIV-SA (table 1). Biotype 1 was statistically associated with the G. vaginalis isolates positive for HIV-SA (P = .048; table 1).

Figure 3 illustrates the 2 different fragment patterns of the amplified 16SRNA gene after digestion with HpaII. The patterns were labeled as genotypes A and B. Three (30%) G. vaginalis isolates were classified as genotype A, and 7 (70%) were classified as genotype B. Genotype B was found in 4 (80%) isolates among the 5 G. vaginalis isolates that were positive for HIV-SA (table 1). No statistically significant association between HIV-SA and genotype was found (P = 1.00).

Eight (80%) G. vaginalis isolates showed resistance to metronidazole, and 4 (40%) showed resistance to bacteriocin. Table 2 depicts the distribution of metronidazole and bacteriocin sus-
Table 1. Comparison of Gardnerella vaginalis isolates on the basis of human immunodeficiency virus (HIV) stimulatory activity (HIV-SA), Gram’s stain status, biotype, and genotype.

<table>
<thead>
<tr>
<th>G. vaginalis isolate</th>
<th>HIV-SA</th>
<th>Gram’s stain</th>
<th>Biotype</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>4F</td>
<td>+</td>
<td>BV</td>
<td>1</td>
<td>B</td>
</tr>
<tr>
<td>1F</td>
<td>+</td>
<td>NA</td>
<td>1</td>
<td>B</td>
</tr>
<tr>
<td>ATCC 14018</td>
<td>+</td>
<td>NA</td>
<td>1</td>
<td>B</td>
</tr>
<tr>
<td>411F</td>
<td>+</td>
<td>BV</td>
<td>1</td>
<td>A</td>
</tr>
<tr>
<td>26</td>
<td>+</td>
<td>Normal</td>
<td>5</td>
<td>B</td>
</tr>
<tr>
<td>ATCC 49145</td>
<td>–</td>
<td>NA</td>
<td>4</td>
<td>B</td>
</tr>
<tr>
<td>619</td>
<td>–</td>
<td>Normal</td>
<td>5</td>
<td>B</td>
</tr>
<tr>
<td>610</td>
<td>–</td>
<td>Normal</td>
<td>5</td>
<td>B</td>
</tr>
<tr>
<td>422</td>
<td>–</td>
<td>Normal</td>
<td>5</td>
<td>A</td>
</tr>
<tr>
<td>624</td>
<td>–</td>
<td>BV</td>
<td>7</td>
<td>A</td>
</tr>
</tbody>
</table>

NOTE: BV, bacterial vaginosis; NA, information not available.

a Determined on the basis of the levels of HIV p24 produced in response to
G. vaginalis lysates (figure 1). –, negative for HIV-SA; +, positive for HIV-SA.

b Determined on the basis of Nugent’s criteria [22].

c Biotypes classified as described by Piot et al. [18].

d Genotypes classified as the fragment patterns after HpaII digests (figure 3).

Discussion

The ability of G. vaginalis to stimulate HIV expression has been proposed to contribute to the high risk of HIV sexual transmission by increasing the amount of virus in the genital tract [9]. However, it has not been determined whether such ability is present in all G. vaginalis strains. In the present study, HIV-SA was found in 5 (50%) of 10 lysates from G. vaginalis isolates. Such variation in HIV-SA among G. vaginalis isolates suggests that some might be more important than others in facilitating HIV transmission. It is possible that the G. vaginalis HIV-SA depends on the presence of other BV-related anaerobes or on other environmental factors.

Furthermore, we cannot ignore the possibility of other factors involved in the high risk of sexual transmission of HIV among women with BV. Both the amount of virus and the survival of the virus in the female genital tract are important [8]. The stimulatory activity of G. vaginalis on HIV replication may interplay with other effects of BV, such as the changes in the vaginal pH itself. In particular, regarding HIV acquisition, it is possible that, in women with BV, the hydrogen ion concentration may have an important role in the survival of the virus.

The low HIV-SA seen in culture supernatants from most G. vaginalis isolates supports an earlier report that suggests that most HIV-SA is cell associated [9]. This further supports the notion that only a minority of G. vaginalis isolates secrete the G. vaginalis stimulatory substances responsible for HIV-SA. Even though the cell-associated HIV-SA substances are not released in cultures of some G. vaginalis strains, it is possible that the cell-associated HIV-SA from these strains could affect HIV replication in the genital tract. For example, some of the HIV-SA substances could be released when bacteria die and degrade.

It has not been determined whether HIV-SA is associated with certain biotypes of G. vaginalis or with other pathogenic characteristics. In the present report, only 1 isolate did not belong to biotype 1 among the G. vaginalis isolates that were positive for HIV-SA. It is possible that this biotype might be more pathogenic, at least with respect to its ability to stimulate HIV expression. For a limited number of isolates, this is the first report that addresses the association of HIV-SA by G. vaginalis and biotype. Other studies, however, have examined the relationship between G. vaginalis biotypes and the presence of BV [18, 21]. Briselden and Hillier [21] demonstrated that biotypes 1–4 were associated significantly with the occurrence of BV [21]. Studies relating biotypes of G. vaginalis to genotypes and pathogenic characteristics have not shown any association.

The genotype data in this study show 2 fragment patterns of the amplified 16S RNA gene that were identified after HpaII enzyme digestion. Only amplified ribosomal DNA restriction analysis has allowed for the subdivision of G. vaginalis strains into a limited number of different genotypes [19, 30]. The empirically labeled genotype B was found in 4 of the 5 G. vaginalis isolates positive for HIV-SA in the current study. On the other hand, the same genotype was found in 3 of the 5 isolates that were negative for HIV-SA. It did not support an association between G. vaginalis genotypes and HIV-SA. These data are consistent with a previous report that did not find a relationship between G. vaginalis genotypes and other pathogenic characteristics, such as the occurrence of BV itself [19]. However, the lack of an association in our data may be because of low power.

Another pathogenic characteristic of G. vaginalis is its resistance to bacteriocin produced by vaginal lactobacilli [17]. In the present study, 40% of the G. vaginalis isolates were bacteriocin resistant. Thus, the association between these 2 pathogenic characteristics appears to be unlikely, at least in the isolates evaluated in this study. However, only 1 of the 5 isolates was both positive.
for HIV-SA and bacteriocin-resistant. Because our power was low, we cannot be certain that there is no association between these 2 pathogenic characteristics. The high rate of resistance to metronidazole found in this study is consistent with a previous report [12]. However, no association between HIV-SA and metronidazole resistance was found. Again, the failure to find an association may be because of low power.

It is intriguing that, with the exception of one isolate, all G. vaginalis isolates that were positive for HIV-SA were recovered from patients with BV. We can, therefore, hypothesize that HIV-SA might play an important role in BV pathogenesis. A study involving a larger number of isolates is warranted to address this possible association.

In conclusion, our results indicate that the ability to induce HIV expression in U1 cells may not be present in all G. vaginalis isolates. Such variation in HIV-SA among G. vaginalis isolates suggests that women who are colonized with certain G. vaginalis isolates might facilitate sexual transmission of HIV more efficiently than do women colonized with other G. vaginalis isolates. In addition, HIV-SA was significantly associated with biotype 1 but not with other G. vaginalis pathogenic characteristics investigated. We acknowledge that this report has a limitation in its power because of the number of isolates tested. However, the results demonstrate a correlation worth considering and highlight the need for further studies with larger samples to explore the in vivo relevance of HIV activation by G. vaginalis in the female genital tract. Furthermore, the results suggest that strategies for BV prevention and colonization by certain biotypes of G. vaginalis may be valuable in reducing the risk of sexual transmission of HIV.

Acknowledgments

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References


Table 2. Relationship between human immunodeficiency virus (HIV) stimulatory activity (HIV-SA) and metronidazole and bacteriocin susceptibility among Gardnerella vaginalis isolates.

<table>
<thead>
<tr>
<th>HIV-SA</th>
<th>Metronidazole susceptibility</th>
<th>Bacteriocin susceptibility</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Susceptible</td>
<td>Resistant</td>
</tr>
<tr>
<td>Positive</td>
<td>1 (20)</td>
<td>4 (80)</td>
</tr>
<tr>
<td>Negative</td>
<td>1 (20)</td>
<td>4 (80)</td>
</tr>
<tr>
<td>Total</td>
<td>2 (20)</td>
<td>8 (80)</td>
</tr>
</tbody>
</table>

NOTE: Data are no. (%) of isolates. HIV-SA is based on the levels of HIV p24 produced in response to G. vaginalis lysates (figure 1).


