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MAJOR ARTICLE

Gardnerella vaginalis and *Prevotella bivia* Trigger Distinct and Overlapping Phenotypes in a Mouse Model of Bacterial Vaginosis

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(See the Editorial commentary by Randis and Ratner, on pages 1085–8.)

Background. Bacterial vaginosis (BV) is a common imbalance of the vaginal microbiota characterized by overgrowth of diverse *Actinobacteria*, Firmicutes, and Gram-negative anaerobes. Women with BV are at increased risk of secondary reproductive tract infections and adverse pregnancy outcomes. However, which specific bacteria cause clinical features of BV is unclear.

Methods. We previously demonstrated that *Gardnerella vaginalis* could elicit many BV features in mice. In this study, we established a BV model in which we coinfected mice with *G. vaginalis* and another species commonly found in women with BV: *Prevotella bivia.*

Results. This coinfection model recapitulates several aspects of human BV, including vaginal sialidase activity (a diagnostic BV feature independently associated with adverse outcomes), epithelial exfoliation, and ascending infection. It is notable that *G. vaginalis* facilitated uterine infection by *P. bivia.*

Conclusions. Taken together, our model provides a framework for advancing our understanding of the role of individual or combinations of BV-associated bacteria in BV pathogenesis.

Keywords. vagina; coinfection; exfoliation; sialidase.

One third of all women in the United States are affected by bacterial vaginosis (BV), a polymicrobial dysbiosis of the vaginal microbiota [1]. Bacterial vaginosis has been linked to increased risks of sexually transmitted infection, pelvic inflammatory disease, infertility, placental infection, and pregnancy complications, including preterm birth [2–4]. Since the first description of BV, both culture-dependent and -independent studies have identified multiple bacterial species as "BV-associated" [5–8]. An important challenge is determining the causative bacteria. Recent clinical studies have provided leads for which bacteria are associated with certain BV features [8, 9]. In this vein, experimental models addressing the sufficiency and causality of individual bacterial species in eliciting features and complications associated with BV are key. The Gram-variable bacterium *Gardnerella vaginalis* is commonly isolated from women with BV [10]. However, because *G. vaginalis* is found in some women who fail to meet the criteria for BV or do not report symptoms, whether this bacterium causes BV remains in debate [11–14].

To address this issue, we previously created a mouse vaginal infection model with an isolate of *G. vaginalis* from a woman with BV [15, 16]. *Gardnerella vaginalis* persisted in the mouse vagina for at least 72 hours, and the mice developed several features of BV, including vaginal epithelial exfoliation, sialidase activity in vaginal fluid, evidence of mucus degradation (sialic acid hydrolysis and depletion), and ascending uterine infection, while lacking histological inflammation. However, this simple model included only 1 bacterial species, whereas human BV is polymicrobial.

In this study, we sought to produce a more comprehensive model of human BV to determine whether *G. vaginalis* could cause BV-like phenotypes in the context of coinfection with another BV-associated bacterial species. We chose to incorporate the Gram-negative anaerobe *Prevotella bivia* into the model because it is a common isolate from the human vagina during BV and has been identified in infected uterine and placental tissues and amniotic fluid during pregnancy complications [17–21]. It is notable that vaginal *P. bivia* colonization has also been correlated with increased risk of preterm birth [22, 23]. Studies in vitro have suggested the potential for growth synergy between *G. vaginalis* and *P. bivia* [24], and a positive association between *G. vaginalis* and several *Prevotella* species, including *P. bivia*, has

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been noted in women with BV [25]. In addition, a recent report showed that the relative abundance of *G. vaginalis* and *P. bivia* increases in advance of incident BV [8]. In this study, we report that *G. vaginalis* and *P. bivia* together recapitulate several features of BV in a mouse model. Furthermore, *G. vaginalis* appears to enhance ascending uterine infection by *P. bivia*.

METHODS

Prevotella/Gardnerella Correlation in Human Samples

We examined the relationship between *Prevotella* and *Gardnerella* using previously collected microbiome data from 937 samples from 32 women [26]. Read data for the genus *Gardnerella* and the genus *Prevotella* were transformed using the formula log(reads +1) after rarefaction to 1000 total reads per sample. Data were plotted as a density plot using JMP Pro 13.0.0. Given the bimodal distribution of the resulting data, we generated a categorical variable in which the presence of either genus was considered to be valid with 3 or more rarefied reads in each sample (≥0.3% of the overall bacterial community).

Ethics Statement

Mouse experiments were carried out in strict accordance with the recommendations in the *Guide for the Care and Use of Laboratory Animals* and approved by the Animal Studies Committee of Washington University School of Medicine (Protocol nos. 20110149 and 20140114).

Bacterial Strains and Growth Conditions

To distinguish the introduced bacteria from endogenous mouse vaginal bacteria [16], all experiments used spontaneous streptomycin-resistant mutants (*Gv* JCP8151B-Sm^R and *Pb* ATCC 2903-Sm^R). The strains and mutants were obtained as previously described [15,16, 27]. *Gardnerella vaginalis* was grown in NYCIII media, and *P. bivia* was grown in CDC Anaerobe media supplemented with 5% laked and defibrinated sheep blood at 37°C in a Coy anaerobic chamber.

Mouse Vaginal Infection Model

Nine- to eleven-week-old female C57BL/6NCR mice were obtained from the National Cancer Institute (now Charles River, Frederick, MD) between January 2013 and February 2014. β-estradiol is often used to synchronize the estrous cycle in bacterial models of mouse vaginal infection [28–30]. A pilot experiment found that 0.5 mg of β-estradiol in 100-μL filter-sterilized sesame oil injected intraperitoneally 2 days before and on the day of bacterial inoculation was required for extended vaginal infection by *P. bivia* (Supplementary Figure 1). Thus, we estrogenized mice in all subsequent experiments.

When prepared in sterile phosphate-buffered saline (PBS) (OD_{600} = 5.0), *G. vaginalis* maintained viability outside of the anaerobic chamber, but *P. bivia* exhibited decreased viability. To limit oxygen exposure, *P. bivia* anaerobic CDC cultures were aliquoted into an individual tube for each mouse. Mice were anaesthetized with isofluorane and inoculated vaginally with 2 immediately successive 10-μL inoculations as follows: PBS and CDC media (mock controls); *G. vaginalis* and CDC media; PBS and *P. bivia*; *G. vaginalis* and *P. bivia*. The dose of *G. vaginalis* was ~8×10^7 colony-forming units (CFU) and *P. bivia* was 1–2×10^7 CFU.

Vaginal Wash Titers

Vaginal washes were collected using a P200 pipet (GeneMate) by gently inserting a tip containing 50-μL sterile PBS 2–5 mm into the mouse vagina and gently pipetting up and down. No additional measures were taken to disrupt epithelial cells or bacterial aggregates in vaginal washes before dilution plating because this could impact viability of these fastidious bacteria. Ten microliters of vaginal wash was immediately diluted into 90 μL anaerobic CDC blood media and transferred to the anaerobic chamber within 1 hour (time required to collect and return to laboratory). Washes were serially diluted in an anaerobic chamber and spot plated (5 replicates) onto 1 mg/mL streptomycin selection plates, incubated anaerobically at 37°C. Colonies were counted 48 hours later and reported as recovered CFU per milliliter of vaginal fluid.

Gram Staining of Vaginal Smears

Ten microliters of vaginal wash was dried on a glass slide, heat-fixed, stained with a BD BBL Gram stain kit, and visualized on an Olympus BX61 microscope.

Sialidase Activity Assays

A 25-μL aliquot of vaginal wash from each mouse was diluted into 50 μL of 100 mM sodium acetate, pH 5.5, containing 300 μM 4-methylumbelliferyl-Neu5Ac, and sialidase activity was measured using a Tecan M200 plate reader as we previously described [15, 16]. In brief, substrate hydrolysis was monitored as an increase in fluorescence (excitation 365 nm/emission 440 nm) that occurs when methylumbelliferyl is hydrolyzed from Neu5Ac.

Analysis of Epithelial Cell Exfoliation

Wet mounts of 5 μL vaginal wash from 1 day postinfection (dpi) were visualized by phase contrast microscopy on an Olympus BX61 microscope. Three representative images were captured from each specimen (1 per mouse), and epithelial cells were counted by a blinded observer to determine an average.

Tissue Collection

For bacterial recovery, 1 uterine horn was harvested from each mouse, weighed, homogenized in anaerobic CDC blood media, then transferred into an anaerobic chamber for serial dilution and plating (described above). Colonies were counted and reported as CFU per gram of tissue. The vagina and remaining uterine horn from each mouse were fixed in 10% buffered formalin phosphate at room temperature, then embedded in paraffin. Histological slide preparation and hematoxylin and

eosin (H&E) staining were performed by the Department of Developmental Biology Histology Core at Washington University.

Statistical Analysis

GraphPad Prism 7.0 software was used for all statistical analyses; tests used to analyze each dataset are indicated in the figure legends.

RESULTS

Gardnerella and *Prevotella* Levels Are Correlated in Human Samples

To confirm the association between *Gardnerella* and *Prevotella* in human vaginal samples, we reanalyzed a published dataset [26] that evaluated the composition and stability of vaginal communities from 32 women over a 16-week period by sequencing the ribosomal 16S gene [26]. We used data from all independently collected samples available in the dataset and plotted log transformed 16S values for *Gardnerella* and *Prevotella* on an x/y axis (Figure 1A). Spearman correlation analysis showed a statistically significant association between titers of the 2 genera (Rho = 0.493). We also performed a categorical analysis based on the clear bimodal distribution of data for both organisms. It is interesting to note that a high proportion of samples without *Gardnerella* also lacked *Prevotella* (many of these were *Lactobacillus*-dominant microbiomes), and, conversely, the majority of samples containing high levels of *Gardnerella* also had high levels of *Prevotella* ($P < .0001$ by Fishers exact test) (Figure 1B). Although this analysis was done at the genus level, we acknowledge that different *Prevotella* species may have different relationships to *Gardnerella* and to BV in general.

Prevotella bivia Can Infect the Mouse Vagina on Its Own and in the Presence of *Gardnerella vaginalis*

To determine the cocolonization potential of *G. vaginalis* and *P. bivia*, we intravaginally infected mice with *G. vaginalis*, *P. bivia*, or both. Consistent with our previous report [16], *G. vaginalis* persisted for several days, both alone, or when coinfected with

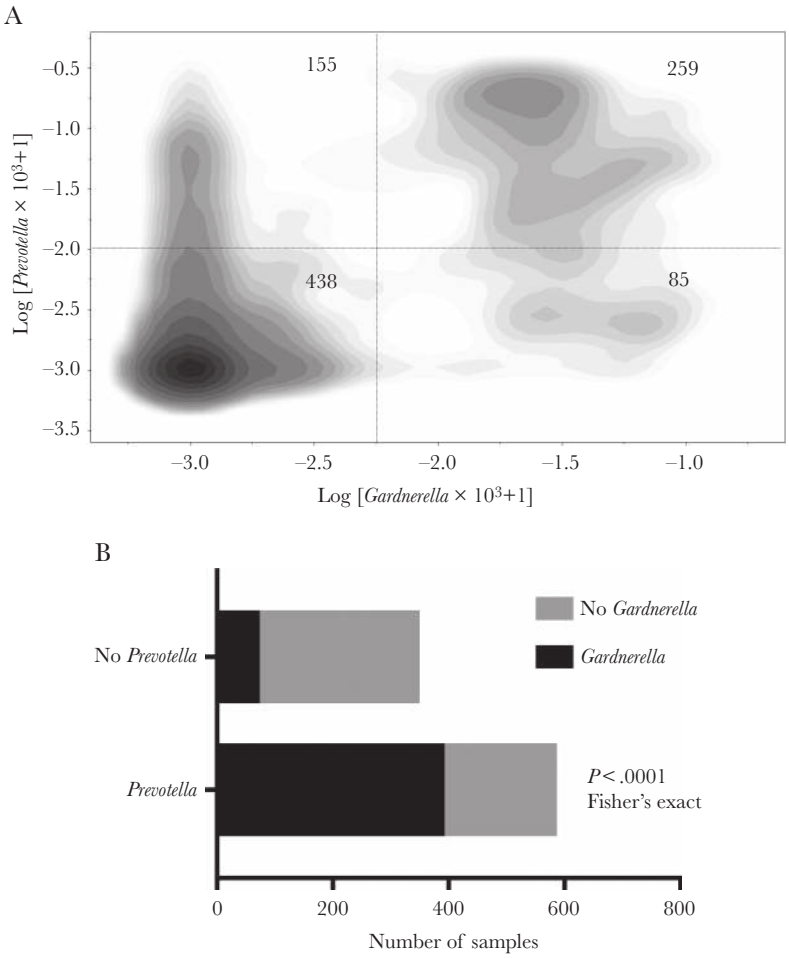

Figure 1. Correlation of *Prevotella* and *Gardnerella vaginalis* in the human vagina. (A) Density plot of log-transformed abundance of *Gardnerella* and *Prevotella* from N = 937 individual clinical samples were plotted (data were derived from previously published dataset from [26]). Spearman correlation was used to test for a correlation between *Gardnerella* and *Prevotella* (Rho = 0.493). L lines illustrate the threshold values used for the categorical analysis. (B) Samples were determined to be positive or negative for *Gardnerella* or *Prevotella* based on thresholds reported in A. Fisher's exact test was used to test statistical significance.

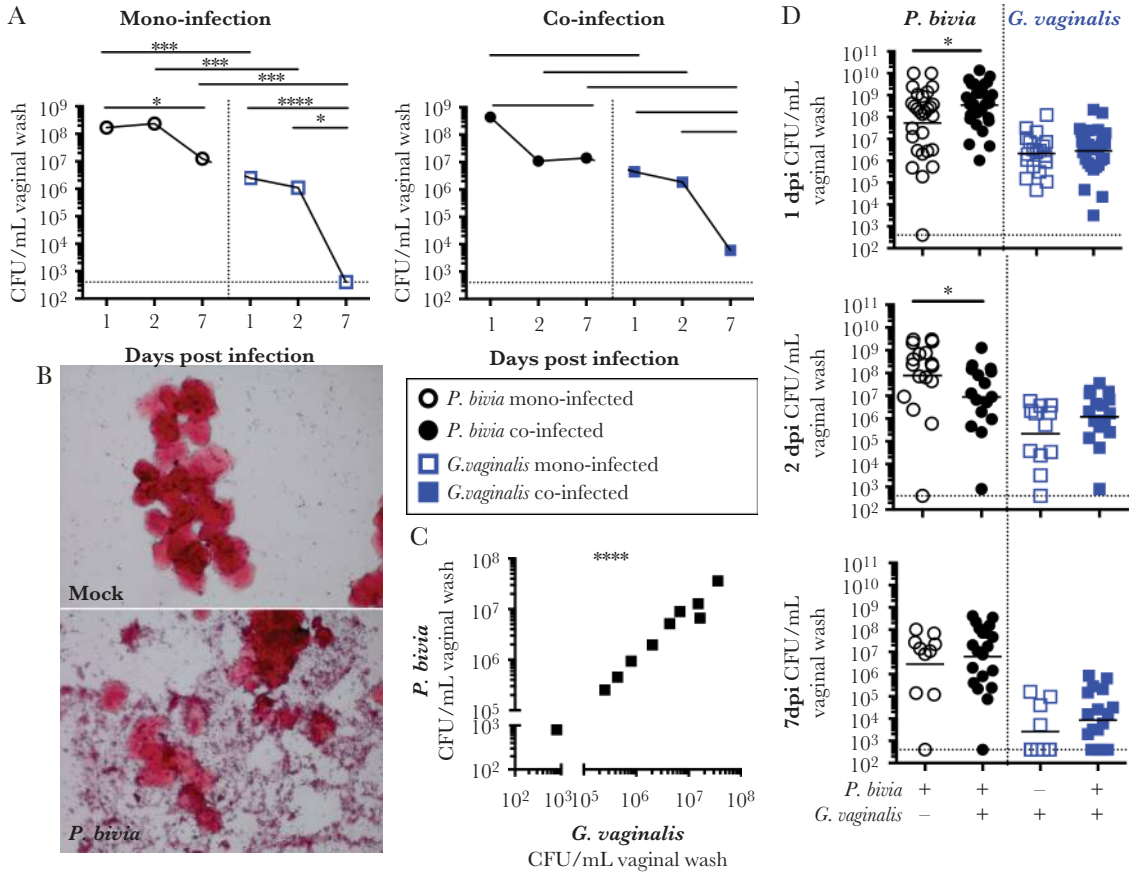

Figure 2. Mouse model of *Gardnerella vaginalis* and *Prevotella bivia* vaginal colonization. (A) Time course of mean vaginal *P. bivia* and *G. vaginalis* titers during monoinfection (left) and coinfection (right). For samples containing no colonies, the limit of detection (400) for each was plotted. (B) Gram-stained smears of vaginal fluid collected at 2 days postinfection (dpi). (C) Correlation between *G. vaginalis* and *P. bivia* vaginal wash titers at 2 dpi in coinfected mice. Spearman r = 0.9636; ****$P < .0001$. (D) Data from (A) have been replotted to compare bacterial titers between mono- and coinfected mice. Results in A and D are combined data from 2 independent experiments, each with 5–10 mice per infection group. For statistical analyses, a Kruskal-Wallis test was performed followed by pairwise Mann-Whitney tests, with uncorrected *P* values indicated on graphs: *$P < .05$; **$P < .01$; ***$P < .001$; ****$P < .0001$.

P. bivia (Figure 2A). *Prevotell bivia* could also colonize alone and in the presence of *G. vaginalis*. Despite being inoculated at a lower dose than *G. vaginalis*, *P. bivia* achieved a higher density in the vagina (often by 1–2 orders of magnitude) and persisted for longer (Figure 2A). Gram staining of vaginal smears illustrated an increased Gram-negative bacterial burden (a hallmark feature of BV) in *P. bivia*-infected animals compared with mock-infected mice, which were colonized primarily by Gram-positive bacteria (Figure 2B). Consistent with the data from human samples (Figure 1), *G. vaginalis* and *P. bivia* titers in mouse vaginas were positively correlated with one another at 2 dpi (data from 2 independent experiments in Figure 2C and Supplementary Figure 2). At both time points examined, vaginal *G. vaginalis* titers were similar between mono- and coinfected mice (Figure 2D), suggesting that *P. bivia* did not have a significant effect on *G. vaginalis* in this model. In contrast, at 1 dpi, vaginal *P. bivia* titers were higher in coinfected mice than those receiving *P. bivia* alone ($P = .04$) (Figure 2D). Although the exact mechanism driving this boost in *P. bivia* titers is unknown, previous in vitro studies have suggested that *G. vaginalis* growth produces amino acids that could fuel *P. bivia* growth [24, 31]. The benefit of *G. vaginalis* for *P. bivia* vaginal colonization was transient in this model; by 2 dpi, vaginal *P. bivia* titers were lower in the vaginas of coinfected mice than in monoinfected mice ($P = .02$) (Figure 2D).

Both *Gardnerella vaginalis* and *Prevotella bivia* Contribute to Vaginal Sialidase Activity In Vivo

Women with BV, but not those with a lactobacilli-dominated vaginal microbiota, have sialidase activity in their vaginal fluids [32–34]. Sialidase activity independently associates with adverse outcomes [35, 36]. The genomes of many *Prevotella* and *Gardnerella* strains encode known or putative sialidase genes and thus could contribute to vaginal sialidase activity in BV [15, 34]. We previously demonstrated that the *G. vaginalis* strain we use for our mouse infections produced sialidase both in vitro and in vivo [15, 16]. Likewise, in this study, we found that our *P. bivia* strain also produced sialidase when grown in vitro (Figure

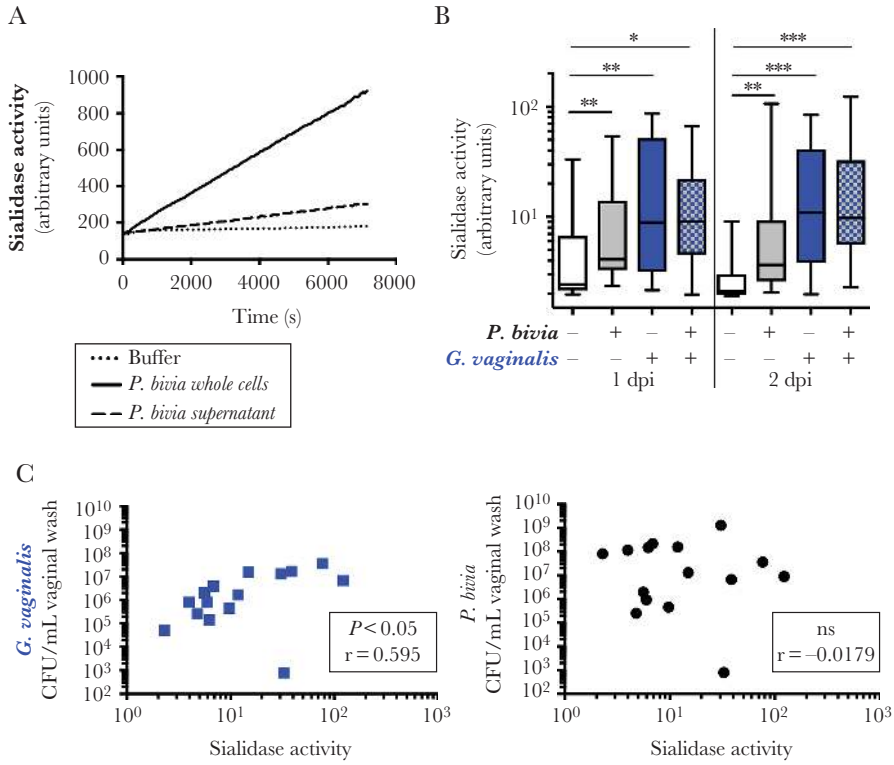

Figure 3. Sialidase activity during *Gardnerella vaginalis* and *Prevotella bivi*a mono- and coinfections in mice. (A) Sialidase activity of *P. bivia* in vitro. (B) Sialidase activity in vaginal washes at 24 and 48 hours postinfection (hpi). Results are meta data from 2 independent experiments (mock, n = 17; *P bivia*, n = 25; *G vaginalis*, n = 18; *P. bivia* + *G. vaginalis*, n = 25). For statistical analyses, a Kruskal-Wallis test (separately for each time point in A) was performed followed by Dunn's multiple comparisons test; ***$P < .0001$; **$P < .01$; *$P < .05$). (C) Bacterial titers in vaginal washes from coinfected animals at 48 hpi for *G. vaginalis* and *P. bivia* were plotted in relation to sialidase activity levels. Significant correlation was determined by Spearman test; *$P < .05$.

3A). Consistent with our previous report [16], mice infected with only *G. vaginalis* had approximately 4-fold higher levels of vaginal sialidase activity than mock-infected mice at both 1 and 2 dpi (Figure 3B). Mice infected with only *P bivia* had approximately 2-fold higher levels of vaginal sialidase activity compared with mock-infected mice (Figure 3B). Mice coinfected with both *G. vaginalis* and *P. bivia* had 4-fold higher levels of sialidase activity than mock-infected mice (Figure 3B). At 2 dpi, vaginal sialidase activity correlated directly with *G. vaginalis* but not with *P. bivia* titers (Figure 3C). Taken together, these data indicate that both of these organisms likely contribute to the vaginal sialidase activity seen in women with BV.

Gardnerella vaginalis, but not *Prevotella bivia*, Induces Epithelial Exfoliation

We previously demonstrated that women with BV had more exfoliated vaginal epithelial cells than women with a lactobacilli-dominated microbiota [37]. Furthermore, we showed that *G. vaginalis* was sufficient to cause vaginal exfoliation in the mouse model [16]. In this study, we tested whether *G. vaginalis* could induce exfoliation in the presence of *P. bivia* and whether *P. bivia* could cause exfoliation. Microscopic evaluation of vaginal fluid wet mounts (Figure 4A) revealed that vaginal fluid from mice monoinfected with *P. bivia* contained similar numbers of epithelial cells as vaginal fluid from mock-infected mice ($P = .141$) (Figure 4B). In contrast, vaginal fluid from mice monoinfected with *G. vaginalis* or coinfected with *G. vaginalis* and *P. bivia* contained significantly more epithelial cells (Figure 4B). These data show that the host responds differently to these 2 bacterial species. These findings are consistent with a report that *G. vaginalis*, but not *P. bivia*, was associated with the "clue" cell clinical criteria in women [9]. Because epithelial exfoliation has been regarded as host response to eliminate bacteria from mucosal surfaces in other niches, we speculate that exfoliation in coinfected mice could explain, at least in part, the decrease in vaginal *P. bivia* titers at 2 dpi (Figure 2D).

Neither *Gardnerella vaginalis* nor *Prevotella bivia* Induce Vaginal Inflammation

In women, BV is not commonly associated with the pain, redness, or swelling typical of gross tissue inflammation, unless there are coinfections present (for example, *Trichomonas*, *Candida*, or human papillomavirus [38, 39]). In one study, flow cytometry analysis of vaginal lavages showed that women with BV had lower levels of neutrophils than women with normal lactobacillus-dominated microbiotas [40]. Another study found a negative

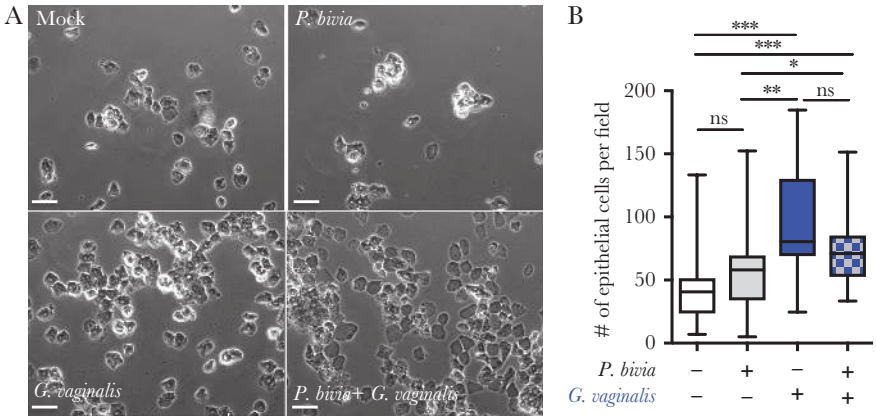

Figure 4. Epithelial exfoliation during *Gardnerella vaginalis* and *Prevotella bivia* mono- and coinfections in mice. (A) Representative images of vaginal fluid wet mounts at 1 day postinfection (dpi). Scale bars = 50 μM. (B) Epithelial exfoliation in vaginal washes at 1 dpi. Epithelial cells were counted in vaginal washes by wet mount microscopy. Results are meta-data from 2 independent experiments (mock, n = 17; *P. bivia*, n = 25; *G. vaginalis*, n = 18; *P. bivia* + *G. vaginalis*, n = 25). For statistical analyses, a Kruskal-Wallis test was performed followed by Dunn's multiple comparisons test; ***$P < .0001$; **$P < .01$; *$P < .05$). ns, not significant.

correlation between sialidase activity and levels of neutrophils in vaginal fluid of women with BV, but without coinfections present [41]. This lack of inflammatory cell infiltration is puzzling given that the levels of bacteria and lipopolysaccharide are dramatically higher in the vaginas of women with BV than in healthy women [25, 42, 43]. We previously reported that *G. vaginalis* monoinfection did not cause histologically evident inflammation [16]. Here, our wet mount microscopy analysis did not detect significant leukocytes in vaginal washes in any infection group at 1 dpi (Figure 4A and Supplementary Figure 3A) or 7 dpi (data not shown). In addition, histological analysis of H&E-stained vaginal tissue confirmed this finding and demonstrated that *P. bivia* alone or in combination with *G. vaginalis* did not cause an evident increase in gross histological inflammation in the vaginal tissue (Supplementary Figure 3B). To further substantiate this finding, we performed flow cytometry analysis of the innate immune cells that occupy the female reproductive tract during acute infection and corroborated the lack of higher infiltrating numbers of neutrophils, natural killer cells, or macrophages infiltrate into the vagina in infected mice (Supplementary Figure 4A and B). Infection did not result in a change in the numbers of neutrophils, natural killer cells, or macrophages in the spleen (Supplementary Figure 4C). The lack of inflammation was not simply an artifact of the estrogenized mouse model, because vaginal inoculation with uropathogenic *Escherichia coli* resulted in robust levels of polymorphonuclear leukocytes in vaginal washes (Supplementary Figure 3C). Thus, in our coinfection mouse model of BV, *G. vaginalis* and *P. bivia* evade detection by the immune system as they take residence in the reproductive tract.

Gardnerella vaginalis Fosters Ascending Uterine Infection by *Prevotella bivia*

The most severe adverse outcomes associated with BV occur when bacteria ascend from the vagina to the uterus. In this study, we used the mouse model to evaluate (1) the causal influence of *Prevotella* and *Gardnerella* on one another's capacity to reach uterine tissue and (2) whether leukocyte infiltrates were evident in tissue by flow cytometry. Consistent with our previous report [16], we detected *G. vaginalis* in the uterus after vaginal infection. However, neither the proportion of animals affected nor the level *G. vaginalis* in uterine tissue was influenced by the presence of *P. bivia* (Figure 5). Although *P. bivia* vaginal titers decreased approximately 10-fold in coinfected mice from 1 to 2 dpi (Figure 2), *P. bivia* titers in uterine horn tissue were significantly higher (by ~20-fold) in coinfected versus monoinfected animals at 2 dpi (Figure 5A). In both mono- and coinfected mice, the level of *P. bivia* ascending infection at 2 dpi was significantly correlated with *P. bivia* vaginal titers at the same time point (Figure 5B), but not with *P. bivia* vaginal titers at 1 dpi. These data suggest that the higher uterine *P. bivia* in coinfected animals was not solely a consequence of the boost in vaginal titers seen at 1 dpi (Figure 2D). It is interesting to note that despite the higher levels of *P. bivia*, the uterine tissue failed to mount an evident inflammatory response at 2 dpi (Supplementary Figure 4). These results demonstrate that *G. vaginalis* enhances the ability of *P. bivia* to cause ascending uterine infection.

DISCUSSION

In this study, we expanded our previous model of *G. vaginalis* vaginal infection to a more complex "BV-like" setting by including *P. bivia* as a representative BV-associated Gram-negative anaerobe. Clinical features of human BV that are reproduced in this coinfection model include a high vaginal bacterial burden with 2 prominent BV-associated bacteria (*G. vaginalis* and *P. bivia*), vaginal sialidase activity, epithelial exfoliation, and absence of a purulent inflammatory response in

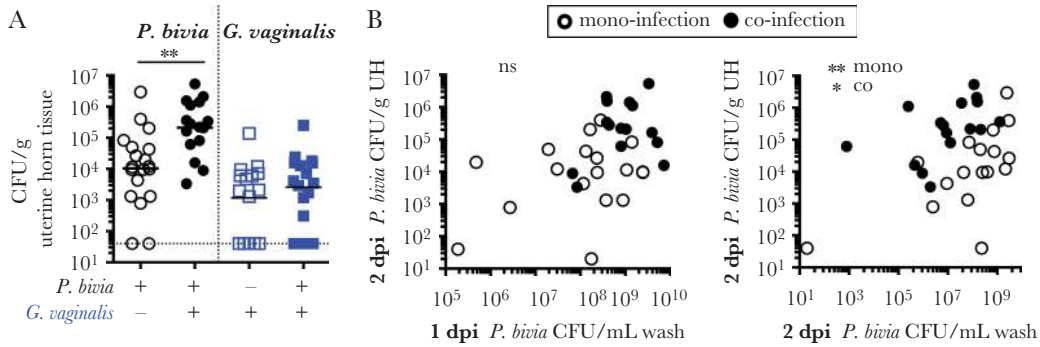

Figure 5. Ascending uterine infection in vaginally inoculated mice. (A) *Prevotella bivia* and *Gardnerella vaginalis* titers were determined in uterine horn homogenates at 2 days postinfection (dpi). For samples with no colonies, the limit of detection (40) was plotted. Statistical analysis was performed by Mann-Whitney *U* test (**P = .0017). (B) Relationship between *P. bivia* titers in uterine horn homogenates at 2 dpi in relation to vaginal washes at 1 dpi (left) and 2 dpi (right). **P = .0082, Spearman r = 0.6278; *P = .0248, Spearman r = 0.5647; ns, not significant. Results in (A) and (B) are combined data of 2 independent experiments, each with 6–10 mice per group.

the vaginal mucosa. The relative simplicity of the vaginal inoculation model and the availability of myriad knockout strains in the C57BL/6 background make it a potentially valuable tool for future studies investigating basic disease mechanisms from both sides of the host-pathogen interaction.

We acknowledge the limitation that mice do not have dominant *Lactobacillus* microbiomes in the vagina as do many women. Therefore, this model cannot reflect aspects of human vaginal physiology that accompany a shift away from a lactobacillus-dominant microbiome (eg, reduced lactic acid levels, increased pH). Other attributes of the vagina that differ between human and mouse (eg, preference of vaginolysin for human cells) may also be a limitation of the murine model.

The findings presented here have 3 specific implications for our understanding of BV pathogenesis. First, *G. vaginalis* and *P. bivia* both result in measurable levels of vaginal sialidase activity in the mouse model, further implicating these organisms as the cause of sialidase activity in BV. It is interesting to note that although *P. bivia* produced sialidase in vivo, it appeared to have a lower capacity to do so, because *P. bivia* required ~100-fold higher infection titer to produce similar levels of vaginal sialidase activity as *G. vaginalis*. Sialidase has been implicated in multiple aspects of host-pathogen interaction, including mucosal barrier degradation, bacterial attachment, and release of carbon sources to facilitate bacterial growth. Our work here confirmed our previous finding that *G. vaginalis* produced sialidase in vivo [16]. Understanding the bacterial origin of sialidase activity is important because its presence, or recurrence after treatment, is an independent risk factor for adverse pregnancy outcomes [33, 35, 36].

Second, results from this model shed light on one of the biggest puzzles of BV, that Gram-negative bacteria (such as *P. bivia*) can reach high concentrations without resulting in overt inflammation. Although it has been suggested that this lack of inflammation in response to BV bacteria might be due to a defect in the inflammatory response in some women, the model presented here suggests instead that BV bacteria may actively inhibit inflammatory responses. The lack of evident inflammation in infected uterine tissue in our model is also consistent with a study in which *Prevotella* or other BV bacteria (by quantitative reverse transcription-polymerase chain reaction) in uteri of nonpregnant women undergoing hysterectomy was "not" associated with higher levels of soluble proinflammatory mediators [44]. BV-associated microorganisms are commonly isolated from the upper reproductive tracts of women with pelvic inflammatory disease [45]. In addition, *Prevotella* and *Gardnerella* have both been isolated from placenta, amniotic fluid, and tubo-ovarian abscesses, often in the context of polymicrobial infection [17–21, 46, 47]. In an intrauterine inoculation model in pregnant New Zealand White rabbits, *G. vaginalis* infection was associated with increased amniotic fluid tumor necrosis factor-α and altered fetal brain histology [48]. In the same rabbit model, 33% of *P. bivia*-infected animals delivered preterm but with no significant differences in histologic inflammation scores [49]. These findings coupled with the data presented here warrant future investigations into the potential immunomodulatory mechanisms of *G. vaginalis* and *P. bivia* and whether pregnancy or other host factors shift the balance of host-microbe equation.

Third, results from our model address the historical argument in the literature regarding whether *G. vaginalis* is an inciting factor in the features and complications associated with BV. We present in vivo evidence that directly implicates *G. vaginalis* as a source of vaginal sialidase and as a trigger for epithelial exfoliation. Furthermore, the presence of *G. vaginalis* enhanced the invasive potential of *P. bivia*, facilitating its ascension into the uterus. Degradation of mucus barriers has long been hypothesized as a potential explanation for the enhanced susceptibility to ascending infections in women with BV and will be pursued in future experiments as a potential mechanism for these observations. Taken together, this model provides strong additional evidence that *G. vaginalis* is a direct contributor in the features and complications associated with BV.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Potential conflicts of interest. Although not directly related to the work, during the conduct of the study, A. L. L. and W. G. L. received personal (consulting) fees from companies involved in making diagnostics or treatments for bacterial vaginosis (Talis Biomedical Corporation, Tennor Therapeutics, and Toltec Pharmaceuticals) and performed contract research for Metis Therapeutics. 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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