Bacteria in the Vaginal Microbiome Alter the Innate Immune Response and Barrier Properties of the Human Vaginal Epithelia in a Species-Specific Manner

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Background. Bacterial vaginosis increases the susceptibility to sexually transmitted infections and negatively affects women’s reproductive health.

Methods. To investigate host–vaginal microbiota interactions and the impact on immune barrier function, we colonized 3-dimensional (3-D) human vaginal epithelial cells with 2 predominant species of vaginal microbiota (Lactobacillus iners and Lactobacillus crispatus) or 2 prevalent bacteria associated with bacterial vaginosis (Atopobium vaginae and Prevotella bivia).

Results. Colonization of 3-D vaginal epithelial cell aggregates with vaginal microbiota was observed with direct attachment to host cell surface with no cytotoxicity. A. vaginae infection yielded increased expression membrane-associated mucins and evoked a robust proinflammatory, immune response in 3-D vaginal epithelial cells (ie, expression of CCL20, hBD-2, interleukin 1β, interleukin 6, interleukin 8, and tumor necrosis factor α) that can negatively affect barrier function. However, P. bivia and L. crispatus did not significantly upregulate pattern-recognition receptor–signaling, mucin expression, antimicrobial peptides/defensins, or proinflammatory cytokines in 3-D vaginal epithelial cell aggregates. Notably, L. iners induced pattern-recognition receptor–signaling activity, but no change was observed in mucin expression or secretion of interleukin 6 and interleukin 8.

Conclusions. We identified unique species-specific immune signatures from vaginal epithelial cells elicited by colonization with commensal and bacterial vaginosis–associated bacteria. A. vaginae elicited a signature that is consistent with significant disruption of immune barrier properties, potentially resulting in enhanced susceptibility to sexually transmitted infections during bacterial vaginosis.

Keywords. Atopobium vaginae; Prevotella bivia; Lactobacillus spp.; epithelial cell; vagina; barrier function; mucin; toll-like receptor; antimicrobial peptides; sexually transmitted infection; innate immunity; female reproductive tract; vaginal microbiota and bacterial vaginosis.

Bacterial vaginosis has been shown to be an independent risk factor for the acquisition of sexually transmitted infections (STIs), including human immunodeficiency virus (HIV) infection [1–3]. In addition, bacterial vaginosis is associated with complications during pregnancy, including preterm labor and birth, premature rupture of membranes, and postpartum endometritis [4–6]. Although bacterial vaginosis is highly prevalent, its etiology is poorly understood [7, 8]. Furthermore, it remains uncertain whether bacterial vaginosis can be sexually transmitted [9]. Available clinical data suggest that bacterial vaginosis is a multifactorial disease in which diverse bacterial species displace commensal lactobacilli [8, 9]. However, lactobacilli are the predominate genera of the vaginal microbiota and produce a variety of metabolites that competitively exclude the
growth of other microorganisms [10]. The displacement of lactobacilli is thought to disrupt the tight regulation of the vaginal homeostatic environment and may reduce immune barrier functions, resulting in the increased susceptibility to STIs during bacterial vaginosis [11].

The immune barrier of a healthy vaginal mucosa is composed of physicochemical properties that prevent the dissemination of pathogens, which include tight junctions, microvilli, and mucus production/secretion. Antimicrobial peptides, cytokines, and chemokines also play an immune barrier role following the recognition of pathogen-associated molecular patterns (PAMPs) [12, 13]. Host epithelial cell response to pathogenic or commensal bacteria is primarily mediated through pattern-recognition receptors (PRR), including Toll-like receptors (TLRs) [12, 14]. PAMP recognition through PRRs can result in NF-κB activation and, subsequently, the production of proinflammatory cytokines [14]. Studies have shown that specific vaginal microbiota modulate the host response in a distinct manner. However, the modulation of immune barrier function by vaginal microbiota, the relation of vaginal microbiota to acute and recurrent bacterial vaginosis, and the mechanism by which the host vaginal microbiota profile increases host susceptibility to STIs needs to be further studied [8].

*Lactobacillus iners*, *Lactobacillus crispatus*, *Prevotella bivia*, and *Atopobium vaginae* are prevalent species in the vaginal microbiota, and their presence is well correlated with a healthy microbiota (for *L. iners* and *L. crispatus*) or with bacterial vaginosis (for *P. bivia* and *A. vaginae*) in the clinic [7, 15]. While *L. crispatus* has not been associated with bacterial vaginosis, *L. iners* has been correlated with intermediate bacterial vaginosis. *P. bivia* and *A. vaginae* have been linked to bacterial vaginosis but are also found in healthy, asymptomatic women. These initial studies highlight the poorly defined roles of each bacterial species [15–17]. In addition, the contribution of each strain to the innate immune response in vaginal epithelial cells and their impact on immune barrier properties of the vaginal mucosa is still not understood [7, 8, 18]. This incomplete understanding of the impact of vaginal microbiota on barrier properties has been limited by a lack of model systems that collectively recapitulate these physiological features.

Previously, we have established and characterized a 3-dimensional (3-D) human vaginal epithelial cell model that fully recapitulates many physiologically relevant barrier properties of the vagina, including stratified squamous epithelium, microvilli, tight junctions, secretory vesicles, microridges, and mucus production [19–21]. Vaginal and endocervical epithelial cells derived from this bioreactor system serve as an integral platform to investigate host-microbe interactions [22] and mucus expression/regulation. We and colleagues have shown that microbial products induce the expression of membrane-associated mucins and antimicrobial peptides [13, 19, 21]. Here, we extend the use of vaginal epithelial cell aggregates to colonize this model with different species of vaginal microbiota and report unique innate immune signatures. Our study elucidates the effects of infection due to bacterial vaginosis-associated bacterial strains on vaginal epithelial cell–mediated NF-κB inflammation signaling pathways, mucus expression, and the induction of innate immune molecules, such as antimicrobial peptides and cytokines/chemokines. Although studies correlate the presence of *A. vaginae* with positive clinical scores of bacterial vaginosis, little is known about the innate host response to *A. vaginae* infection [23]. Our findings identify *A. vaginae* as a potent component of the vaginal microbiota that disrupts the vaginal immune barrier.

**MATERIALS AND METHODS**

**Generation of 3-D Human Vaginal Epithelial Cell Aggregates and TLR Stimulation**

Human primary vaginal epithelial cells (V19) were purchased from MatTek (Ashland, MA). These cells had been collected from a woman undergoing hysterectomy for nonneoplastic reasons and immortalized (V19I) by transduction with PA317/LXSN-16E6E7-conditioned medium as previously described [20]. V19I cells were grown on collagen-coated dextran microcarrier beads in a rotating wall vessel bioreactor to form differentiated 3-D vaginal aggregates as previously described [19–21]. Cell quantification and trypan blue exclusion staining were performed after 0.25% (v/v) trypsin dissociation (Mediatech, Manassas, VA) [19]. For experimental manipulations, 3-D vaginal epithelial cells were distributed into 24-well plates (1.5 × 10^5–4 × 10^5 cells/mL). 3-D vaginal epithelial cells were exposed to TLR agonists (InvivoGen, San Diego, CA), FSL-1 (5 μg/mL), and polyinosinic:cytidylic acid (poly I:C; 25 μg/mL).

**Bacterial Strains and Culture Conditions**

*Atroplus vaginae* (ATCC BAA-55TM), *L. iners* (ATCC 55195TM), and *P. bivia* (ATCC 29303TM) were cultured on tryptic soy broth (Becton Dickinson, Sparks, MD) supplemented with 5% (v/v) sheep blood (Nalgene, Ryegate, MT) and Bacto Agar, according to manufacturer’s instructions (BD). *L. crispatus* (ATCC 33820TM) was cultured on Difco Lactobacilli MRS Broth supplemented with Bacto Agar. Bacterial plates were incubated anaerobically for 48 hours at 37°C, using BD GasPak anaerobe container system (BD). Electrotransformation of *L. iners* and *L. crispatus* was performed as previously described [24]. Both strains were transformed with the pFVP25.1 plasmid (Addgene plasmid 20668) described previously [25].

**Bacterial Colonization Assay**

3-D vaginal epithelial cell aggregates were seeded in 24-well plates and infected at a multiplicity of infection (MOI) of 10 bacteria/cell. After anaerobic incubation for 24 hours at 37°C to allow bacterial colonization, vaginal epithelial cell aggregates
were washed twice with Dulbecco’s phosphate-buffered saline. Colonized vaginal epithelial cells were then separated from microcarrier beads, using trypsin, and the bacterial cell/vaginal epithelial cell suspension was plated on selective media and incubated anaerobically for 48 hours at 37°C for quantification.

**Confocal Microscopy**

3-D vaginal epithelial cell aggregates were inoculated at a MOI of 10 bacteria/cell with green fluorescence protein–expressing *L. iners* or *L. crispatus*, incubated as above, rinsed twice in Dulbecco’s phosphate-buffered saline, and fixed in 4% paraformaldehyde for 30 minutes at room temperature. Samples were stained using ProLong Gold Antifade Reagent with DAPI (Invitrogen). Images were collected at 20 times or 63 times the original magnification, using a Zeiss Observer Zlaxio microscope, and were analyzed using Zeiss Zen2011 software.

**Chemokine/Cytokine Quantification**

Supernatants were collected from 3-D vaginal epithelial cell aggregates infected as described above and harvested 24 hours after infection or 24 hours following TLR agonist exposure (5 replicate wells). Cytokine and chemokine concentrations in supernatants were determined using a custom human cytokine kit (Bio-Rad) in accordance with the manufacturer’s instructions, with the following targets: interleukin 6 (IL-6), interleukin 8 (IL-8), interleukin 1β (IL-1β), tumor necrosis factor α (TNF-α), and interleukin 1 Ra (IL-1Ra). Data were collected on a BioPlex 200 and analyzed using 5.0 manager software (Bio-Rad).

**Reverse-Transcription Polymerase Chain Reaction (RT-PCR) and Real-Time Quantitative PCR (qPCR)**

RNA was extracted from 3-D vaginal epithelial cells, using the QIAgen RNeasy kit according to manufacturer’s instructions (QIAgen, Valencia, CA). All experiments to detect antimicrobial peptides and mucin expression, as well as detailed reaction setup and PCR parameters, accorded with methods described by Radtke et al [13]. Table 1 shows the list of primers for additional investigated target genes. Gene expression was measured by real-time qPCR analysis performed on an ABI 7500 system (Applied Biosystems, Foster, CA).

**Statistical Analysis**

All experimental analyses were performed in triplicate unless otherwise stated. An unpaired 2-tailed Student’s *t* test with the Welch correction was performed using Prism software (GraphPad, San Diego, CA). All experimental samples were compared to untreated control cells incubated under the same conditions. *P* values of < .05 were considered statistically significant, and *P* values of < .001 were considered highly significant.

## RESULTS

**Establishment of a 3-D Vaginal Epithelial Cell Colonization Model, Using Vaginal Microbiota**

We established a stable colonization model using 3-D human vaginal epithelial cell aggregates cultured with representative species of normal vaginal microbiota (*L. iners* and *L. crispatus*) or 2 prevalent bacterial vaginosis–associated bacterial strains; *A. vaginae* and *P. bivia* (Figure 1) [15]. *A. vaginae* and *P. bivia* colonized 3-D vaginal epithelial cell aggregates more efficiently than *L. iners* and *L. crispatus* (Figure 1A). *L. iners* showed the lowest affinity (mean ± SD), 1,530 ± 93 colony-forming units/mL) for colonization of 3-D vaginal epithelial cell aggregates 24 hours after infection at a MOI of 10. The quantity of each bacterial species was confirmed using 16S ribosomal RNA–specific primers and both RT-PCR and real-time qPCR technology (data not shown) [26]. Cytotoxic effects on 3-D vaginal epithelial cell aggregates following colonization with vaginal microbiota were measured by trypan blue exclusion, and we observed no difference in 3-D vaginal epithelial cell viability following

### Table 1. Polymerase Chain Reaction Primers Used in This Study

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>mRNA Reference Sequence</th>
<th>Primer Sequences</th>
</tr>
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<tbody>
<tr>
<td>IRF1</td>
<td>Human interferon regulatory factor 1</td>
<td>NM_002198.2</td>
<td>(F)CATTCAACAGGGCGATACA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(R)GGCTGGACTTCGACTTTCTT</td>
</tr>
<tr>
<td>NFKBIA</td>
<td>Nuclear factor of κ light polypeptide gene enhancer in B cells inhibitor, α</td>
<td>NM_020529.2</td>
<td>(F)CATCTGAAGGCTACAACACTAC</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(R)TGACACAGGTGCAGGATT</td>
</tr>
<tr>
<td>IRAK2</td>
<td>Interleukin 1 receptor–associated kinase 2</td>
<td>NM_001570.3</td>
<td>(F)GGAATGAGTGGGGAGAAGAAAAG</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(R)AAAGAGCTCGAGGGAAGTAATG</td>
</tr>
<tr>
<td>IL6</td>
<td>Interleukin 6</td>
<td>NM_000565.3</td>
<td>(F)GAGATGCTGAGGCTCACTCTG</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(R)CTGAGGAGGGTGATACCAGGATT</td>
</tr>
<tr>
<td>TNF/TNFα</td>
<td>Tumor necrosis factor α</td>
<td>NM_000594.3</td>
<td>(F)CTCAGGCTCTTCTGCCTGCAA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(R)TGCAGGACATCGAAGGCTG</td>
</tr>
<tr>
<td>IFNB1</td>
<td>Interferon, β1, fibroblast</td>
<td>NM_002176.2</td>
<td>(F)GAACCTGTGCTAATGTCTATC</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(R)TCCCTGCACTTGAC</td>
</tr>
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Abbreviations: F, forward; mRNA, messenger RNA; R, reverse.

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colonization with the bacterial species tested (Figure 1B). Confocal microscopy of green fluorescent protein–expressing *L. iners* and *L. crispatus* was used to validate colonization through visualization of bacterial adherence to the 3-D vaginal epithelial cell surface (Figure 1C and 1D).

**Differential Induction of PRR Signaling and Downstream Inflammation Pathways in 3-D Vaginal Aggregates Following Colonization With Vaginal Microbiota**

To investigate the host-microbiota interaction and the global changes in host epithelial responses following vaginal microbiota colonization, we used a PCR array to assess expression of genes encoding 84 epithelial cell targets related to inflammation signaling. Additionally, we used purified microbial products as positive controls, because they trigger a robust TLR-mediated inflammatory response in 3-D vaginal epithelial cells. Specifically, we selected poly I:C (dsRNA) and FSL-1 (synthetic lipoprotein), which trigger TLR3 and TLR2/6, respectively. Target genes that were robustly upregulated (by >2.5-fold) in infected vaginal epithelial cell aggregates, compared with uninfected control cells, were validated by gene-specific real-time qPCR assays (Figure 2). Overall, *A. vaginae* infection increased expression in approximately 10% of 84 signaling genes analyzed. The most upregulated genes were either transcription factors (Figure 2A and 2C) or proinflammatory cytokines (Figure 2B, 2D, and 2F). *A. vaginae, L. iners,* and poly I:C significantly increased the expression of transcription factor *IRF1* (IFN regulatory factor 1) and *NFKBIA* (nuclear factor of κ light polypeptide gene enhancer in B cells inhibitor, α; Figure 2A and 2C). *A. vaginae, L. iners,* FSL-1, and poly I:C significantly upregulated the expression of the TLR signaling adaptor gene *IRAK2* (IL-1 receptor–associated kinase-like 2; *P* < .001 for all comparisons; Figure 2C). The same vaginal microbiota and microbial products were found to significantly increase the expression of *TNFα* (TNF-α; Figure 2D). However, the expression level of *IFNB1* (IFN-β1) and *IL6* (IL-6) in 3-D vaginal epithelial cell aggregates was only significantly increased after exposure to poly I:C (*P* < .001 for both comparisons). Notably, *P. bivia* and *L. crispatus* colonization did not result in a >2.5-fold increased expression of multiple targets (*IRF1, NFKBIA, IRAK2,*...
TNFα, and IL6). From these data, it appears that L. iners behaves more like a bacterial vaginosis–associated bacterial strain than a commensal with an expression profile similar to A. vaginae.

Antimicrobial Peptides Are Most Significantly Induced by A. vaginae Infection

To assess the impact of vaginal microbiota colonization on antimicrobial peptides, hBD-1, hBD-2, CCL20, and SLPI expression were measured 24 hours after infection with vaginal microbiota or following exposure to microbial products. The expression of hBD1 did not change under any experimental condition (Figure 3A). The expression of hBD2 and CCL20 in 3-D vaginal epithelial cell aggregates was most significantly upregulated following A. vaginae and P. bivia infection but also increased following L. iners colonization (Figure 3B). Notably, CCL20 expression was upregulated 10–20-fold following bacterial vaginosis–associated bacterial infection of 3-D vaginal epithelial cell aggregates (Figure 3B). However, there was no significant change in the CCL20 expression level following colonization with Lactobacillus species. Vaginal microbiota did not alter the expression of SLPI, whereas the exposure of 3-D vaginal epithelial cell aggregates to poly I:C or FSL-1 resulted in significantly increased expression (Figure 3D).
Next, we determined the impact of infection with bacterial vaginosis–associated bacterial strains on mucin expression, a critical component of the mucosal immune barrier. We evaluated the membrane-associated mucins most highly expressed by 3-D vaginal epithelial cell aggregates (Supplementary Figure 1) and observed that *A. vaginae* and *P. bivia* elicited increased expression of *MUC1*, *MUC3*, and *MUC4* but not *MUC16*. The basal expression level of *MUC3* in 3-D vaginal epithelial cell aggregates was higher in 3-D vaginal epithelial cell aggregates than in 3-D endocervical epithelial cell aggregates (Supplementary Figure 1). The expression of the gene encoding gel-forming secreted mucin 5AC (*MUC5AC*) did not change following infection with any bacterial species or after microbial product exposure (Figure 4A). Specifically, *A. vaginae* infection resulted in upregulated expression of membrane-associated mucins and, most significantly, increased expression levels of *MUC1* and *MUC3*.
Colonization with Lactobacillus species resulted in slightly increased levels of expression of membrane-associated mucins relative to findings for controls, but differences were not statistically significant. Consistent with previously published findings that used 3-D endocervical epithelial cell aggregates, poly I:C treatment of vaginal epithelial cell aggregates increased expression of MUC1, MUC4, and MUC16. In addition, we showed that poly I:C induced a significant induction of MUC3 in 3-D vaginal epithelial cell aggregates.

A. vaginae but Not Commensal Vaginal Microbiota Elicit Increased Proinflammatory Cytokine Secretion From 3-D Vaginal Aggregates

To determine the impact of bacterial vaginosis–associated bacterial strains and commensal colonization on the downstream PRR signaling pathways, we measured the cytokine secretion from 3-D vaginal epithelial cell aggregates 24 hours after infection. The effects of bacterial vaginosis–associated bacterial strains and commensal colonization on proinflammatory...
cytokine secretion from 3-D vaginal epithelial cell aggregates are shown in Figure 5. A. vaginae significantly increased the secretion of IL-6, IL-8, and TNF-α but did not influence the secretion of anti-inflammatory IL1-Ra. However, the colonization of vaginal epithelial cell aggregates with P. bivia or Lactobacillus species did not increase the secretion of IL-6, IL-8, or TNF-α (Figure 5). Microbial product exposure of 3-D vaginal epithelial cell aggregates resulted in the robust secretion of proinflammatory cytokines. Poly I:C and FSL-1 showed a high potential to increase IL-6 and TNF-α secretion from 3-D vaginal epithelial cells. The secretion of IL-1β and IL-1Ra in vaginal epithelial cell aggregate supernatants was inversely proportional between vaginal microbiota and microbial products. Microbial product treatment did not result in increased IL-1β secretion from vaginal epithelial cells. However, all vaginal microbiota significantly increased IL-1β secretion (Figure 5D). The treatment of 3-D vaginal epithelial cell aggregates with FSL-1 and poly I:C resulted in increased IL-1Ra secretion. In addition, IL-1Ra levels were not altered following vaginal microbiota colonization. Overall, A. vaginae was the most potent activator of epithelial cell–mediated inflammation of the vaginal microbiota studied, whereas P. bivia and L. iners elicited an intermediate-to-mild level of inflammation, mostly through the induction of IL-1β.

**DISCUSSION**

We used a 3-D human organotypic vaginal epithelial cell model colonized with highly prevalent vaginal microbiota [15] to study host-microbiota interactions and the impact of particular species on innate immune barrier properties of vaginal epithelial cells. Previously, we demonstrated that 3-D human vaginal epithelial cells accurately reflect physiologically relevant barrier properties, including the formation of tight junctions, microvilli, microridges, and secretory vesicles [19]. In addition, fully differentiated vaginal epithelial cell aggregates express functional innate immune receptors and cell-associated mucins [19–21].

We exploited these characteristics to investigate how vaginal microbiota regulate these epithelial innate immune responses in a species-specific manner. Others have shown that L. crispatus induces NF-κB signaling activity and increased IL-8 secretion in vitro [18]. Despite increased PRR signaling after colonization with L. crispatus or L. iners, we did not detect increased IL-8, IL-6, or TNF-α concentrations in vaginal epithelial cell supernatants. The contribution of L. iners to vaginal health is controversial, because it is commonly identified as a dominant component of the normal flora, but its presence has also been associated with a disturbed vaginal microflora [26, 27]. This study shows an increased expression of multiple transcription factors...
factors and proinflammatory cytokines related to PRR signaling in 3-D vaginal epithelial cell aggregates colonized with *L. iners*. Notably, this induction of proinflammatory immune pathways did not result in proinflammatory cytokine secretion. In comparison, microbiota of gut and lung epithelial cells trigger TLR signaling activity as well, contributing to mucosal homeostasis, immune responsiveness, and differentiation [28, 29]. Similar to our findings, commensal bacteria of the lung were shown to increase PRR-mediated NF-κB signaling, but nuclear translocation NF-κB and proinflammatory cytokine secretion was prevented [29]. Further studies are required to investigate whether vaginal microbiota are preventing NF-κB-mediated proinflammatory response of vaginal epithelial cells to *P. bivia*. Strong evidence suggests a correlation between *P. bivia* and bacterial vaginosis diagnosis [15]. However, there are ethnicity-dependent variations in the composition of the vaginal microbiota of women, suggesting that *P. bivia* does not necessarily correlate with a disturbed microbiota [15]. The ability of *P. bivia* to evoke IL-8 and TNF-α secretion in vaginal epithelial cells has been shown previously [18] and is thought to derive from the expression of lipopolysaccharide-triggering TLR4 [30]. Notably, highly invasive *P. bivia* strains have been shown to elicit low cytokines levels [31]. Our study shows no significant proinflammatory effects on PRR signaling following *P. bivia* infection, which may be due to low levels of TLR4 expression. These differential findings may result from the use of a different cell culture model and *P. bivia* strains [18, 31].

Over 19 mucin genes have been identified, and their role in health and disease has gained much attention [32, 33]. Mucins lubricate mucosal surfaces and can trap pathogens through mucooadhesion, thereby altering the vaginal epithelial cell barrier [32, 34, 35]. These mucooadhesive properties potentially influence the acquisition of bacterial vaginosis and STIs. Given the importance of membrane-associated mucins in pathology and in numerous disease processes [33], as well as the lack of data on mucin expression in bacterial vaginosis, we assessed whether bacterial vaginosis–associated bacterial strains altered the expression of mucins in 3-D vaginal epithelial cells. In bacterial vaginosis, a thin, homogenous vaginal discharge is a common symptom and diagnostic indicator and is characterized by high concentrations of mucin-degrading enzymes and a watery consistency [36]. The altered composition of mucins may affect mucus-virus interaction at the vaginal mucosa [37, 38]. Similar to the ocular surface, endocervical epithelial endocervical epithelial cell membrane–associated mucin expression increased in response to TNF-α elicited by poly I:C [13, 39]. Poly I:C increased TNF-α gene expression and secretion and upregulated the expression of genes encoding all membrane-associated mucins (ie, *MUC1*, *MUC3*, *MUC4*, and *MUC16*) in 3-D vaginal epithelial cell aggregates. In accordance, *A. vaginae* elicited significant levels of secreted TNFα and increased expression of mucin-encoding genes (ie, *MUC1*, *MUC3*, and *MUC4*). We demonstrated that *A. vaginae* and a viral PAMP (dsRNA) increase the expression of membrane-associated vaginal mucins in a differential manner. In turn, this alters the conditions for bacterial adherence and colonization, as well as the vaginal epithelial cell microenvironment and, thus, might contribute to a disruption of vaginal immune barrier properties and enhanced STI acquisition.

In early studies, *A. vaginae* was considered a common component of the vaginal microbiota, but there has been increasing evidence indicating that *A. vaginae* has an important role in bacterial vaginosis pathogenesis [16, 23]. The presence of *A. vaginae* is correlated with positive clinical scores, but little is known about the impact of this bacterium on properties of the vaginal epithelial cell immune barrier [23]. Our study reveals the unique potential of *A. vaginae* to evoke a proinflammatory immune response by increased levels of IL-1β, IL-6, IL-8, and TNF-α. Our findings are related to the clinical picture of bacterial vaginosis, characterized by high IL-6, IL-8, and IL-1β cervicovaginal secretions, but they also elucidate changes in TNF-α secretion by 3-D vaginal epithelial cells [30, 40]. Increased TNF-α levels have been shown to decrease cell integrity in intestinal epithelial cells and to increase susceptibility to HIV infection by enabling the virus to penetrate the host immune barrier through alteration of tight junction complexes [41]. The integrity of vaginal epithelium constitutes an important mechanical barrier property, with sensitivity to a variety of cytokines, including TNF-α, and this barrier may be damaged during bacterial vaginosis. In accordance with our findings, IL-1β has been shown in clinical samples to play a role in the sustained immune activation that mediates adverse outcomes during bacterial vaginosis [40, 42]. We also observed differential ratios of IL-1β to IL-1Ra, and this alteration has been shown to affect the susceptibility to STIs, including HIV infection [43].

Cytokine secretion by vaginal epithelial cells functions as a host defense response but under certain conditions could be detrimental to the host through disruption of the barrier function. We found that *A. vaginae* infection of 3-D vaginal epithelial cell increases expression of TNF-α, IL-1β, hBD2, and CCL20. Notably, CCL20 and hBD2 both encode antimicrobial peptides and ligands of chemokine receptor 6 (CCR6), a receptor specifically expressed on CD4+ T cells, leukocytes, and dendritic cell populations that regulates the migration of these cell types during inflammation. CCL20 production has been shown to be regulated by TNF-α and IL-1β [44]. We hypothesize that...
leukocytes may be recruited to the site of bacterial vaginosis as a result of increased CCL20 and hBD2 levels and may promote STIs, including HIV infection. SLPI expression following infection with bacterial vaginosis–associated bacterial strains was not changed in our study, which is consistent with clinical data showing either decreased or unchanged SLPI levels [45].

Our findings highlight the unique species-specific innate immune signatures elicited by vaginal microbiota. L. crispatus colonization resulted in low epithelial cell activation and minimal disruption of immune barrier properties, in turn supporting its role as a beneficial species of the vaginal microbiota. P. bivia also induced minimal epithelial cell activation, but infection resulted in changes in antimicrobial peptide and mucin expression, suggesting that this organism does not induce significant vaginal inflammation but may still alter barrier properties. To our knowledge, we are the first to describe that L. iners significantly induces PRR signaling. This finding suggests that L. iners may exhibit more-proinflammatory qualities and act less like a commensal. Alternatively, A. vaginae induces a robust inflammatory profile that disrupts physicochemical barrier properties of the vaginal mucosa. Host epithelial cells are tasked with controlling the innate immune responses to microbiota and effectively discriminating between commensal and pathogenic bacteria [46]. The emerging concept that the line distinguishing commensals from pathogens is not as clearly defined as previously appreciated [7, 46] is highlighted by the data presented here. We plan to evaluate clinical samples from women with and those without bacterial vaginosis to further investigate species- and strain-specific differences in barrier function and host response signatures to vaginal microbiota. Overall, epithelial host cell interaction with vaginal microbiota are relevant factors of bacterial vaginosis pathogenesis and may influence the acquisition, development, and progression of reproductive disease through disruption of the immune barrier.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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