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.
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 Ato PCBium vaginai 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

A. 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). Electrottransformation 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 (R&D Systems) in accordance with the manufacturer’s instructions, using 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 Bio-Plex 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 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), 1530 ± 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>
<thead>
<tr>
<th>Gene</th>
<th>Protein Description</th>
<th>mRNA Reference</th>
<th>Primer Sequences</th>
</tr>
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<tbody>
<tr>
<td>IFNB1</td>
<td>Interferon, β1, fibroblast</td>
<td>NM_002176.2</td>
<td>(R)GAACTCCTGGCTAATGCTATTC (R)TCCTTGGCCTTTCAGGAATTT</td>
</tr>
<tr>
<td>IL6</td>
<td>Interleukin 6</td>
<td>NM_000565.3</td>
<td>(R)GAGATGCAGGCTCAGGAGGTTTC (R)CCGTGAGGCTCAGGAGGTTTC</td>
</tr>
<tr>
<td>IRAK2</td>
<td>Interleukin 1 receptor–associated kinase 2</td>
<td>NM_000594.3</td>
<td>(R)CGTCGAGGATGTACCGAATTT (R)GTCAGGGATCAAAGCTGTAGG</td>
</tr>
<tr>
<td>TNF/TNFα</td>
<td>Tumor necrosis factor α</td>
<td>NM_000570.3</td>
<td>(R)CGTCGAGGATGTACCGAATTT (R)GTCAGGGATCAAAGCTGTAGG</td>
</tr>
<tr>
<td>IL12</td>
<td>Interferon regulatory factor 1</td>
<td>NM_002198.2</td>
<td>(R)CATCCTGACCGGCTAGCATAC (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>(R)CATCTGAAGGCTTACCAACTAC (R)TGACACGGAGGAGGAGGAGG</td>
</tr>
<tr>
<td>IRF1</td>
<td>Human interferon regulatory factor 1</td>
<td>NM_000565.3</td>
<td>(R)GAACTCCTGGCTAATGCTATTC (R)TCCTTGGCCTTTCAGGAATTT</td>
</tr>
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Abbreviations: F, forward; mRNA, messenger RNA; R, reverse.
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 TNFa (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, IFNB1, 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).
Infection With Bacterial Vaginosis–Associated Bacterial Strains and Exposure to Microbial Products Increases Expression of Membrane-Associated Mucins in 3-D Vaginal Aggregates

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*.

**Figure 3.** Antimicrobial peptides are most significantly induced by bacterial vaginosis–associated bacteria following 3-dimensional (3-D) vaginal epithelial cell aggregate colonization. Expression of selected antimicrobial peptides (A) was determined by real-time quantitative polymerase chain reaction analysis 24 hours following infection of 3-D vaginal epithelial cell aggregates with the indicated species (*Atopobium vaginae*, *Prevotella bivia*, *Lactobacillus iners*, or *Lactobacillus crispatus*) at a multiplicity of infection of 10 or following exposure to FSL-1 (5 μg/mL) or polyinosinic:cytidylic acid (poly I:C; 25 μg/mL). The fold-changes in expression of *hBD-2* (B), *CCL20* (C), and *SLPI* (D) are represented as mean values (±SD) from at least 3 independent experiments. To obtain the fold-change, the expression of each target gene was normalized to GAPDH and compared to basal transcript abundance in uninfected vaginal epithelial cells. Statistical significance was determined by a 2-tailed t test with the Welch correction. *P*<.05, **P**<.005.
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

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(Figure 4B and 4C). 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.
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 antiinflammatory 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 proportionate 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...
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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 effects on PRR signaling following 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-β, 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 pro-inflammatory 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 copyrighted. 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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