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Bacterial vaginosis and the cervicovaginal immune response

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Abstract

Bacterial vaginosis (BV) is a common cause of vaginal discharge in reproductive age women around the world, and is associated with several poor reproductive health outcomes, including HIV-1 acquisition. One possible mechanism for this association is the inflammatory immune response induced by BV in the cervical and vaginal mucosae. There is significant heterogeneity in reports of markers of cervicovaginal inflammation in women with bacterial vaginosis, likely due to microbial and host diversity, as well as differences in study design. In this article we review the characteristics of the mucosal immune response in BV, the potential role of lactobacilli in modulating that response, and the impact of individual BV-associated bacterial species on mucosal immunity. We focus on inflammatory markers that are proposed to increase the risk of HIV-1 acquisition.

Introduction

Bacterial vaginosis (BV) is the most prevalent cause of vaginal discharge in reproductive age women,[1] is present in ~29% of women in the United States,[2] and is characterized by vaginal colonization with anaerobic bacterial species and a loss of normal lactobacilli. Moreover, BV is even more common in women who live in areas of the world where HIV-1 seroprevalence is highest, particularly sub-Saharan Africa.[3] The clinical presentation of BV is characterized by an odorous discharge (or no symptoms at all), without the redness, swelling or pain typical of inflammation. However, at the mucosal level this condition has a significant pro-inflammatory impact that is associated with several poor clinical outcomes, including a nearly 2-fold increased risk of HIV-1 acquisition[4,5] as well as a 3-fold increased risk of HIV-1 transmission to a male partner.[6]

There are several hypotheses for mechanisms that link genital mucosal inflammation and increased HIV-1 acquisition, including disruption of mucosal integrity, alteration of protective innate immunity, and increased numbers of HIV-1 target cells at the mucosal surface.[7,8] The early events in HIV-1 acquisition in the female genital tract appear to include[9] uptake of the virus by CD4+ T cells or Langerhans cells located in the stratified squamous epithelium of the vagina and/or ectocervix, which then transfer the virus to CD4+ T cells.[10,11] It is also possible that HIV transmission occurs across the upper reproductive

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tract epithelia, which are single-layer columnar structures in the endocervix and endometrium, though this has been difficult to evaluate.

While the potential for BV to cause genital mucosal inflammation is not in dispute, many questions remain as to how and when this actually happens, and how the effects can be mitigated. The association between BV and HIV-1 acquisition may be mediated by many different factors (Figure 1). The field is in desperate need of well-designed, longitudinal studies to provide a better understanding of the mechanisms by which the vaginal microbial community regulates and alters the host reproductive mucosal immune response.

Mucosal inflammatory markers associated with HIV-1 transmission

In the HPTN 035 Study, which evaluated two vaginal microbicides for efficacy in preventing HIV-1 acquisition, women who acquired HIV-1 were found – prior to HIV-1 seroconversion -- to have higher levels of human beta-defensin 2 (HBD2 – a cationic antimicrobial peptide) in vaginal secretions and more *E. coli* bactericidal activity of their vaginal fluid than non-seroconverters.[12] Several studies of highly HIV-1 exposed but persistently seronegative sex workers have shown lower levels of inflammatory cytokines such as IL1α, IL1β, IL8 and RANTES in genital secretions compared to those in HIV-1 positive and HIV-1 negative controls.[13,14] Analysis of cervical samples from a study of HIV acquisition in hormonal contraceptive users showed higher levels of RANTES and lower levels of SLPI in women who acquired HIV.[15] In vitro, a TLR 1/2 agonist (PAM3CSK4) and TNFα increased HIV-1 transmission by Langerhans cells.[16] Together, these data suggest that higher levels of vaginal inflammation, and lower levels of anti-inflammatory factors are associated with increased HIV-1 transmission across the genital mucosa.

What is a healthy vagina?

The definition of vaginal health includes both absence of symptoms and lack of risk for poor outcomes such as infertility, infection, pregnancy loss or preterm delivery. The vaginal microbiota in many women is dominated by certain *Lactobacillus* species, which have been associated with lower rates of these reproductive health complications.[4,17] It is not certain whether the presence of these *Lactobacillus* species is simply a proxy for the absence of BV-associated bacterial species, or whether they themselves have immunomodulatory effects. Ravel et al performed deep sequencing on vaginal samples from reproductive age women and grouped the genital microbiota into four *Lactobacillus*-dominant “community state types” (CST) and one non-*Lactobacillus*-dominant CST.[18] The presence of a diverse, heterogenous microbiota in ostensibly healthy women was used to argue that *Lactobacillus*-dominance is not necessarily universal and should not be used to define health. However, participants in this study were not screened for BV by either Amsel’s or Nugent’s criteria, and indeed, the majority of participants in the non-*Lactobacillus*-dominant CST had Nugent scores consistent with BV. As we try to understand how the presence of *Lactobacillus* species and the diagnosis of BV interact with the reproductive mucosal immune response to impact adverse outcomes such as HIV-1 acquisition, it is important to use consistent definitions of “health” and “disease.” A 2008 NIH-sponsored workshop on research on BV

recommended consistently using Nugent score plus modified Amsel's criteria to diagnose BV, to allow better standardization across studies.[19]

At the gastrointestinal mucosal surface, the presence of lactobacilli is associated with downregulation of immune response to inflammatory stimuli,[20,21] but a similar process has not been defined in the genital tract. Because most of the adverse health outcomes associated with BV are presumably related to inflammatory complications of BV, understanding whether the presence of (or introduction of) *Lactobacillus* species could mitigate those complications is important. In a study of Swedish women without BV, the presence of *L. iners* by culture was negatively correlated with IL1β levels, but positively with the anti-inflammatory molecule secretory leukocyte protease inhibitor (SLPI), while *L. gasseri* was positively associated with IL1β.[22] In a group of U.S. women, where pyrosequencing of the 16S rRNA gene was used to determine the dominant microbial species, those with vaginal microbiota dominated by *L. crispatus* had the lowest levels of IL1β and highest levels of SLPI.[23] In pregnant Japanese women, those with *Lactobacillus* spp detected by culture had the lowest levels of IL8 – whether or not anaerobic species were also detected.[24] In a cohort of 30 asymptomatic Belgian women, quantities of *L. iners* measured by qPCR were also associated with lower levels of IL8.[25] This is in contrast to a study of primarily African American adolescents, where those who had *Lactobacillus* spp detected by culture (73% of the 89-person cohort) had no difference in cytokine concentrations compared to those that did not have *Lactobacillus* spp detected.[26] The species of lactobacilli involved were not identified, making these results difficult to interpret. Further evaluation of the immunologic and reproductive health implications of variations in the microbial communities of women without BV would greatly advance the field.

BV is not the same microbiologic syndrome in all women

In recent years we have come to understand that BV is not the same in all women; the composition of the vaginal microbiota and the dominant microbial species vary from woman to woman.[27] In fact, some species are more associated with one or the other of the clinical signs of BV, such as altered pH or clue cells.[27] While investigators have long tried to identify a profile of more "severe" BV (such as a higher Nugent score or presence of *Mobiluncus* morphotypes)[28,29] the molecular techniques available now allow for a broad characterization of the microbial community and potentially a better assessment of what types of communities have the most significant immunologic impact. Srinivasan et al recently used molecular techniques to demonstrate that the *Mobiluncus* morphotypes that make up part of the Nugent scoring criteria for vaginal fluid Gram stains are more often the Clostridia-like fastidious species Bacterial Vaginosis Associated Bacterium 1 (BVAB1) than *Mobiluncus* species.[30]

The vaginal microbiota in some women is a dynamic community that changes on a daily basis.[31–34] Additional studies are starting to demonstrate differential expression of genes in species such as *L. iners* in women with and without BV,[35] though it is not clear if this is due to strain differences or if some genes are upregulated due to change in the microbial environment. Finally, although it has long been known that short-chain fatty acids such as butyrate and succinate are present in BV,[36] recently it has been shown that these molecules

can have an impact on the mucosal immune response.[37] These data make it clear that to understand BV and its interaction with the vaginal immune response, longitudinal samples and a holistic evaluation of the vaginal environment are necessary.

In vivo measurements of cytokines and antimicrobial peptides

Many authors have measured cervicovaginal cytokine levels in BV, with disparate results. In most studies IL1β is elevated in women with BV, while SLPI is decreased (Table 1). IL6 and IL8 have also been measured in multiple studies, with much more variable results. The heterogeneity in the measurements is likely due to several factors: variation in composition of the microbial community, small sample sizes and variable methods between studies. It is difficult to say whether studies showing no difference are simply underpowered. Most critically, the cross-sectional nature of many of these studies introduces considerable uncertainty as to how representative a single "snapshot" of the vaginal microbiota and associated immune milieu is at any given time. Moreover, many of these studies evaluated pregnant women, in whom vaginal cytokines are significantly elevated compared to non-pregnant women.[38,39] Longitudinal studies where cytokines are measured before and after treatment of BV provide a more dynamic look at the inflammatory response, and in the 6 studies we reviewed, IL1β decreased after treatment in five, while IL8 decreased in three and increased in two.[40–45]

Marconi et al[46] evaluated the association of individual species with vaginal cytokines and found that higher quantities of *G. vaginalis, A. vaginae* and total 16S bacterial rRNA gene copies measured by qPCR were associated with higher IL1β levels, while levels of *Megasphaera spp.* were inversely associated with IL8 quantity. Although IL6 and IL8 levels were correlated with IL1β, they did not also correlate with bacterial quantities. In that study, quantities of lactobacilli were not measured. In a study of pregnant women, all nine BV-associated bacterial species measured by qPCR were associated with lower quantities of HBD3.[47] The variation seen between these studies suggests that in addition to the broad diagnosis of BV, the specific composition of the vaginal microbial community may have a significant impact on mucosal inflammation and the risk of poor reproductive health outcomes

In vitro studies demonstrate higher pro-inflammatory potential in some BV-associated bacterial species compared to others

Progress in understanding the mucosal immune response to BV and BV-associated bacterial species has been slow in part due to the lack of an animal model to test mechanistic hypotheses. However, *in vitro* experiments using co-cultured genital epithelial cells and common vaginal bacteria species offer some insight into the simplest host-microbe interactions. In models using a monolayer culture of immortalized vaginal epithelial cells, co-culture with *Atopobium vaginae or Gardnerella vaginalis* induce significantly higher levels of IL6 and IL8 than *Lactobacillus species*.[48,49] In another study that compared immortalized cell lines from endocervix, ectocervix and vagina, BV-associated bacterial species such as *G. vaginalis, A. vaginae, Mobiluncis curtisii,* and *Prevotella bivia* induced IL6, IL8, G-CSF, IP-10, MIP-1β, RANTES and Gro-α from all three cell types, while

Lactobacillus species did not.[50] These results confirm that BV-associated species can induce an innate immune response from the genital epithelium characterized by upregulation of cytokines associated with increased risk of HIV-1 transmission, while commensal lactobacilli do not. In addition, these in vitro studies show differences in stimulatory potential between individual species, which may account for some of the heterogeneity observed in the studies summarized in Table 1.

In the gastrointestinal tract, the presence of commensal *Lactobacillus* and *Bifidobacteria* species are associated with decreased immune response to inflammatory stimuli, in part through activation of Toll-like receptor pathways.[51] In a multilayer culture of immortalized vaginal epithelial cells *L. jensenii* suppressed the epithelial cell response to both Toll-receptor agonists FSL-1 (TLR 1/2) and PIC (TLR3), while *L. crispatus* did not.[52] In a separate model using 3D bead-based vaginal epithelial cell aggregates, *L. crispatus* induced minimal epithelial immune response, while *P. bivia* and *L. iners* upregulated PRR-signalling.[53] When co-cultured with HeLa cells, *L. crispatus* diminishes the IL-8 response to challenge with *Candida* species.[54] While these results suggest that *Lactobacillus* species are an anti-inflammatory influence on the genital epithelia, the more interesting question is how the presence of lactobacilli alters (or does not alter) the mucosal inflammatory response to BV-associated species, and whether individual *Lactobacillus* species have different effects.

Host genotype can influence the mucosal immune response to BV

In several studies, the reproductive health risks associated with BV are modified by the presence of genetic polymorphisms in genes associated with the inflammatory response. Genc et al showed that in women heterozygous for an allele of the IL1ra gene associated with less gene function no change in vaginal IL1β when anaerobic gram negative rods or *G. vaginalis* were present, but women homozygous for the wildtype allele showed increased levels.[55] Women carrying a TLR4 polymorphism associated with lower response to LPS had no change in IL1β when colonized with BV-associated bacterial species, and 10-fold higher quantities of *G. vaginalis* colonization compared to women without the polymorphic allele.[56] Goepfert et al showed that women with IL1β and IL8 gene polymorphisms associated with increased cytokine response had lower prevalence of BV, while women with an IL6 gene polymorphism associated with less response had a higher prevalence of BV.[57] While these types of polymorphisms have been linked to differences in risk of preterm birth in women with BV,[58] their impact on HIV-1 acquisition risk has not been evaluated.

Cellular immunity

In addition to increasing levels of pro-inflammatory cytokines like IL1β, BV has been associated with an increase in HIV-1 target cells in the genital mucosa. In a cohort of Kenyan sex workers, treatment of BV was associated with a decrease in numbers of CD4+, CD4+CCR5+ and CD4+CD69+ T cells in the cervix.[43] In a cohort of Brazilian women, those with BV had fewer CD4+ T cells in cervicovaginal fluid than women with no vaginal infections.[59] Conversely, in 30 healthy Belgian women, the presence of both *L. crispatus* and *L. jensenii* by qPCR was associated with lower numbers of cervical CD3+HLADR+ or CD3+CD4+CCR5+ cells.[25] These results suggest that further evaluation of cellular

immunity in BV will be important. Although BV is not associated with clinical inflammation in the lower genital tract, it has been associated with inflammatory clinical sequelae in the upper genital tract. Both cervicitis[60] and pelvic inflammatory disease[61] are linked to bacterial vaginosis, and could increase the risk of HIV-1 acquisition.

Conclusion

Studying BV and the mucosal immune response is challenging: there is no animal model, there are multiple factors that could alter the vaginal microbial and immune environments in a highly dynamic fashion over time, and previous literature has used a range of samples and assays, making generalization difficult. However, understanding how the reproductive mucosa interacts with and responds to the vaginal microbial community is an important component of developing strategies to prevent reproductive health complications, particularly HIV-1 acquisition.

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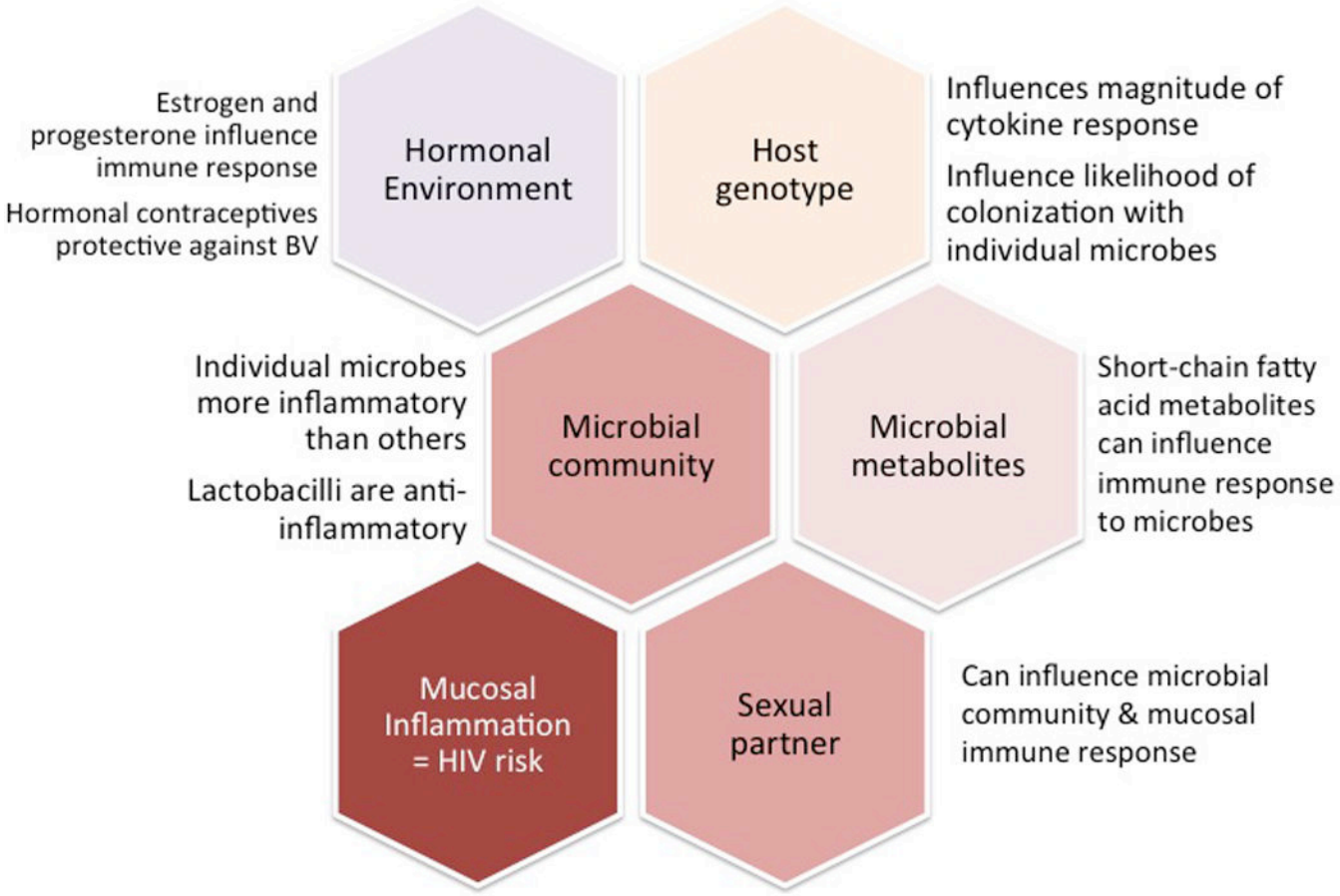

Figure 1.

The microbial community is one component of a complex set of interactions that may influence a woman's risk of HIV-1 acquisition.

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Table 1

Summary of studies comparing concentrations of cytokines and anti-microbial peptides in genital secretions of women with and without BV, showing the number and type of studies reviewed and how many showed an increase, decrease or no difference in the analyte of interest.

		Increase		No difference		Decrease	
		Cross-sectional	**Longitudinal**	**Cross-sectional**	**Longitudinal**	**Cross-sectional**	**Longitudinal**
IL1b[a]	CVL	7[26,46,55,62–64]	3[4344, 67]		1[41]		
	VS	1[38]	1[42]				
	CS	2[65,66]	2[40,45]				
IL2	CVL			1[69]			
	CS	1[68]	1[40]	1[70]			
IL4	CVL			2[26,69]			
	CS		1[40]	1[70]			
IL6	CVL			4[26,46,38,71]	3[41,67,73]		
	VS				1[42]		
	CS	1[68]	1[45]	2[70, 72]	2[40]		
IL8[b]	CVL	1[73]	3[41,43,67]	4[26,46,6364]			1[44]
	VS	1[38]		1[76]	1[42]		
	CS	2[74,75]	1[45]	2[70,72]			1[40]
IL10	CVL	1[77]		2[26,71]		1[62]	
	CS	1[78]	1[40]	3[70,72,75]			
IL12[c]	CVL			2[26,71]			
	CS	1[68]		2[70,75]			

		Increase		No difference		Decrease	
		Cross-sectional	Longitudinal	Cross-sectional	Longitudinal	Cross-sectional	Longitudinal
TNFa[d]	CVL			1[26]			
	CS	1[66]	1[40]	2[68,70]			
IFNg	CVL			1[26]		1[69] (trend)	
	CS		1[40]	3[68,70,75]			
RANTES	CVL		1[43]				
	CS		1[79]	1[70]			
SLPI	CVL						1[79]
	VS					2[38,80]	1[42]
HNP1–3	CVL						1[44]
	VS	1[e 81]			1[47]	1[e 81]	
	CS			1[82]			
HBD2	CVL	1[69]					1[44]
	VS				1[47]		
HBD3	CVL						1[44]
	VS						1[47]

[a] 3 studies showed increase with abnormal Nugent score, not BV[23, 64,65]

[b] 1 study showed an increase with abnormal Nugent score, not BV[74]

[c] Not detected: 1 study[77]

[d] Not detectable: 2 studies[46,73]

[e] HNP 1–3 was elevated in white participants with BV, and lower in black participants with BV, compared to women without BV

CVL = Cervicovaginal lavage, VS = vaginal swab, CS = cervical swab or cytobrush

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