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Host-Vaginal Microbiota Interactions in the Pathogenesis of Bacterial Vaginosis

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Abstract

Purpose of Review—The etiology of BV, the most common cause of vaginal discharge in women, remains controversial. We recently published an updated conceptual model on BV pathogenesis, focusing on the roles of *G. vaginalis* and *Prevotella bivia* as early colonizers and *Atopobium vaginae* and other BVAB as secondary colonizers in this infection. In this paper, we extend the description of our model to include a discussion on the role of host-vaginal microbiota interactions in BV pathogenesis.

Recent Findings—Although *G. vaginalis* and *P. bivia* are highly abundant in women with BV, neither induce a robust inflammatory response from vaginal epithelial cells. These early colonizers may be evading the immune system while establishing the BV biofilm. Secondary colonizers, including *A. vaginae, Sneathia* spp., and potentially other BVAB are more potent stimulators of the host immune response to BV and likely contribute to its signs and symptoms as well as its adverse outcomes.

Summary—Elucidating the etiology of BV has important implications for diagnosis and treatment. Our current BV pathogenesis model provides a framework for key elements that should be considered when designing and testing novel BV diagnostics and therapeutics.

Keywords

Bacterial vaginosis; biofilm; *Gardnerella vaginalis*; *Prevotella bivia*; *Atopobium vaginae*; *Sneathia* species; host immune response

Introduction

Bacterial vaginosis (BV) is a vaginal dysbiosis resulting from displacement of lactic-acid producing *Lactobacillus* spp. with high concentrations of facultative and strict anaerobic bacteria including *Gardnerella vaginalis*, *Prevotella* spp., *Atopobium vaginae*, *Sneathia* spp., and other BV-associated bacteria (BVAB). A notable feature is the appearance of a polymicrobial biofilm on vaginal epithelial cells (1). BV is the most common cause of

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vaginal discharge globally, with an estimated annual economic burden of $4.8 billion dollars (2). Women with BV are at increased risk for adverse birth outcomes, gynecologic sequelae, and acquisition of HIV and other sexually transmitted infections (STIs) (3). BV is difficult to treat and has a high recurrence rate (4).

The epidemiology of BV strongly favors sexual transmission (5). However, the etiology remains controversial. *G. vaginalis*, a facultative anaerobe, is present in 95–100% of women with BV (6) and originally thought to be the single etiologic agent (7). *G. vaginalis* has unique characteristics compared to other BVAB that could allow it to function as an early colonizer on the vaginal epithelium (8, 9). *G. vaginalis* is able to effectively displace lactobacilli and adhere to vaginal epithelial cells (9) and has an increased propensity for biofilm formation compared to other BVAB (8). However, recent data suggest that *G. vaginalis* may be necessary, but not sufficient, for BV development (10), as colonization does not always lead to BV (11). Thus, other BVAB may also play an important role in BV pathogenesis.

Earlier genomic studies of *G. vaginalis* found that this genus consists of four non-recombining groups/clades of organisms with distinct gene pools and genomic properties (12). More recently, whole genome sequence analysis indicated 13 species exist within the genus *Gardnerella* (13). Correspondingly, genomic studies coupled with phenotypic characterizations have shown differences in pathogenic potential between *G. vaginalis* strains (14, 15). Healthy women may be colonized by *G. vaginalis* strains with lower pathogenic potential, while strains with higher pathogenic potential may be involved in BV development. This hypothesis is supported by data from a study investigating the association of behavioral practices and Nugent score with *G. vaginalis* clade distribution in women-who-have-sex-with-women (16). In this study, clades 1–3 and multi-clade (>2) communities were associated with BV by Nugent score. Clade 4 was neither associated with BV nor *Lactobacillus*-deficient microbiota. Clade 1 was associated with increasing number of recent sexual partners and clade 2 was associated with specific sexual behaviors. Overall, these data suggest that *G. vaginalis* clades have varying levels of pathogenicity, with acquisition through sexual activity.

Based upon our prospective study (10), a mouse model study (17), and an updated literature review (9, 18–20), we recently modified our prior conceptual model on BV pathogenesis which mainly involved *G. vaginalis* (21), to now focus on the roles of *G. vaginalis* and *Prevotella bivia* as early colonizers and *Atopobium vaginae* and other BVAB as secondary colonizers in BV (22). The proposed steps of BV development in this model include: (1) strains of *G. vaginalis* with higher pathogenic potential displace lactobacilli and initiate biofilm formation on the vaginal epithelium, (2) synergy between *G. vaginalis* and *P. bivia* (normally present in very low concentrations, acquired from maternal and environmental sources) occurs on the vaginal epithelium with production of metabolites facilitating their growth, (3) vaginal sialidase and other enzymes, produced by *G. vaginalis* and *P. bivia*, promote breakdown of the mucous layer of the vaginal epithelium, and (4) loss of the protective mucous layer leads to increased adherence of secondary colonizers, including *A. vaginae*, to the mature, polymicrobial BV biofilm (22).

Interestingly, although *G. vaginalis* and *P. bivia* are highly abundant in women with BV (10), neither induce a robust inflammatory response from vaginal epithelial cells (17, 23). Other BVAB, including *A. vaginae* (24, 25), may be more potent stimulators of the host immune response to BV and contribute to its signs and symptoms in addition to adverse outcomes (26). Here we provide a discussion of our current opinions regarding the role of the host immune response in the pathogenesis of BV. We extend the description of our BV conceptual model to include a discussion of the immune barrier of an optimal vaginal microbiota and the role of host-vaginal microbiota interactions in BV pathogenesis, focusing on *G. vaginalis*, *P. bivia*, *A. vaginae*, and *Sneathia* spp. (Figure 1). Next, we discuss implications for BV diagnosis and treatment, outlining areas requiring additional research.

The Immune Barrier of an Optimal Vaginal Microbiota

Lactobacilli colonizing the lower female reproductive tract (FRT) play an important role in protection against invading pathogens through direct (production of bactericidal compounds and metabolites) and indirect (modulation of vaginal immune barrier) mechanisms (27). However, not all vaginal *Lactobacillus* species benefit the host equally. Data suggest that *Lactobacillis crispatus* is the optimal species associated with vaginal health whereas *L. iners* may be associated with dysbiosis and colonization with BVAB (25). The role of other common vaginal lactobacilli such as *L. jensenii* and *L. gasseri* is less studied and requires additional research.

A key protective mechanism of vaginal lactobacilli includes the production of lactic acid through fermentation of glycogen by-products, which acidify the cervicovaginal micro-environment (pH<4.5) (Figure 1A). Several *in vitro* studies have demonstrated that lactic acid is a potent antiviral and bactericidal compound, inhibiting replication/growth of genital STI pathogens and pathobionts, including *Chlamydia trachomatis* (28), *Neisseria gonorrhoeae* (29), group B *Streptococcus* (30), and HIV (31). These micro-organisms are more sensitive to lactic acid than hydrochloric acid, indicating that a low pH environment is necessary, but not sufficient, for this inhibitory effect. In the lower FRT, lactic acid exists in both D- and L-forms and the ratio of D- and L-isomers depends on the predominant *Lactobacillus* spp. (32). A recent study demonstrated that D-lactic acid, produced by *L. crispatus* but not *L. iners*, was more protective against chlamydia (28). This suggests that production of specific lactic acid isoforms may contribute to differential protective capacities of vaginal *Lactobacillus* spp. against STIs, including BVAB (e.g. *G. vaginalis*) that may be sexually transmitted. Other postulated protective mechanisms include hydrogen peroxide production by *L. crispatus*; however, it is still controversial whether this antimicrobial compound can be produced *in vivo* at inhibitory concentrations (33). Lastly, the protective effect of lactobacilli can be attributed to competitive exclusion, which is the ability to effectively compete with other micro-organisms for resources in the local micro-environment (34).

Colonization of the lower FRT with *L. crispatus* and other protective *Lactobacillus* spp. also results in low levels of vaginal epithelial cell activation. Multiple epidemiological studies have demonstrated that women with a *L. crispatus*-dominated vaginal microbiota exhibit low levels of pro-inflammatory cytokines [e.g. interleukin (IL)-1α, IL-1β] and chemokines [e.g.

IL-8, interferon gamma inducible protein (IP-10), macrophage inflammatory protein (MIP)-3α] in their cervicovaginal secretions (35, 36). In a cross-sectional study investigating the role of the vaginal microbiota in cervical carcinogenesis, women with a *Lactobacillus*-dominant vaginal microbiota showed less evidence of genital inflammation measured by genital inflammatory scores (e.g. elevated levels IL-1α, IL-1β, IL-8, MIP-1β, MIP-3α, RANTES, TNFα) compared to women with a non-*Lactobacillus*-dominant vaginal microbiota (37).

Interestingly, colonization with *L. iners* does not provide the same mucosal immune quiescence as *L. crispatus*. Two independent studies have shown that *L. iners* dominance is associated with elevated levels of chemokines, including IP-10, MIP-3α, and monokine induced by gamma interferon (MIG), which may result in mucosal recruitment of CD4+ cells and increased risk of HIV (35, 38). I*n vitro* studies, employing robust organotypic human three-dimensional (3-D) female reproductive tract epithelial cell models, validated these epidemiological findings and showed that *L. crispatus* does not significantly alter levels of pro-inflammatory cytokines (IL-1β, IL-6, TNFα), chemokines (IL-8, MIP-3α), or antimicrobial peptides [human β-defensin (hBD)-2 and secretory leukocyte protease inhibitor (SLPI)], resulting in low epithelial cell activation (25, 39, 40). Others have demonstrated that *L. crispatus* does not significantly change IL-8, RANTES, or SLPI levels in cervicovaginal monolayer cultures (41). In addition, metabolites produced by lactobacilli can modulate epithelial cell responses and stimulate secretion of anti-inflammatory cytokines, including IL-1 receptor antagonist (IL-1Ra), and low-level production of antimicrobial peptides (e.g. hBD-1, SLPI), resulting in mucosal homeostasis (42, 43).

The Role of Host-Vaginal Microbiota Interactions in BV Pathogenesis

BV is not characterized as a neutrophilic disease (27). Vaginal white blood cells are uncommon in BV, unless concomitant vaginal (*Trichomonas vaginalis* or vulvovaginal candidiasis) and/or cervical infection (e.g. *C. trachomatis*) is present (44). BV is also not commonly associated with pain, redness, or swelling typical of gross tissue inflammation (27). However, studies have reported elevated cytokine and chemokine levels (e.g., IL-1β, TNFα, IL-6, and IL-8) in vaginal washes from women with BV (45). Early colonizers such as *G. vaginalis* and *P. bivia* may actively inhibit the host inflammatory response in the vaginal epithelium, evading the immune system while establishing the BV biofilm (Figure 1B). This is corroborated by data from a mouse model study where *P. bivia* alone or in combination with *G. vaginalis* did not cause an increase in histological inflammation in vaginal tissue (17). In contrast, secondary colonizers of the BV biofilm (e.g., *A. vaginae* and other BVAB) may stimulate the host immune response in vaginal epithelial cells and contribute to the symptoms (e.g., vaginal discharge and odor) and signs (e.g., homogeneous, white, vaginal discharge) of BV (22) (Figure 1C). Related to these symptoms of BV are the metabolites produced by BVAB, including biogenic amines (46, 47).

A limited number of studies have examined mechanisms of the host response and immunity to key BVAB using *in vitro* or animal models (17). A recent study utilizing EpiVaginal tissues demonstrated that apical infection with *G. vaginalis* does not induce significant changes in levels of pro-inflammatory immune mediators (48) in accordance with a previous

study showing that *G. vaginalis* does not induce IL-1β, IL-6 MIP-3α or TNFα in a human 3-D endometrial epithelial cell model (39). Regarding *P. bivia* and *A. vaginae*, Doerflinger et al demonstrated in a human 3-D vaginal epithelial cell model that *A. vaginae* induces a broad range of pro-inflammatory cytokines, chemokines, and antimicrobial peptides including IL-1β, IL-6, IL-8, MIP-3α, TNFα and hBD-2; whereas *P. bivia* significantly only induced IL-1β and MIP-3α (25). Subsequent unsupervised hierarchical clustering analysis of these data suggests that *A. vaginae* clusters separately from *P. bivia* based on their immune mediator profiles and are distinct from both *L. iners* and *L. crispatus* (23). Other reports have demonstrated that *A. vaginae* induces hBD-4, MIP-1β, Gro-α, and G-CSF and that the host immune response was Toll-like receptor (TLR) 2-dependent (24, 49). Interestingly, *G. vaginalis* and *A. vaginae* have also been found to amplify pro-inflammatory responses to *T. vaginalis*, whereas *P. bivia* suppressed these responses (50).

Other secondary BVAB besides *A. vaginae* may also stimulate the host immune response in vaginal epithelial cells. One report examining *Sneathia* spp. found that two species (*S. sanguinegens* and *S. amnii*) induced IL-1α, IL-1β, and IL-8, but not TNFα, in vaginal epithelial monolayer cultures (51). With regard to these microbes, *A. vaginae* or *S. amnii* alone and a polymicrobial cocktail of BVAB (*G. vaginalis* + *P. bivia* + *A. vaginae* + *S. amnii*) also induced IL-36γ, a novel cytokine belonging to the IL-1 superfamily, at a higher magnitude than other BVAB alone (*G. vaginalis* or *P. bivia*) in a human 3-D cervical model (23). Hierarchical clustering analysis revealed two main clusters. *G. vaginalis* and *P. bivia* did not exhibit robust inflammatory profiles and clustered with *L. crispatus*, while *A. vaginae*, *S. amnii*, and the polymicrobial cocktail formed a separate cluster defined by higher levels of inflammatory mediators (40). IL-36γ was recently shown to play a significant role in genital HSV-2 pathogenesis (40) and may be a key immune factor in the lower FRT and in BV (23).

Overall, these data support the hypothesis that *G. vaginalis* and *P. bivia* do not induce robust epithelial cell activation and that secondary colonizers, including *A. vaginae*, *Sneathia* spp., and other BVAB, are required to induce pro-inflammatory responses observed in women with BV. However, *G. vaginalis* and *P. bivia* might impact other components of the cervicovaginal epithelial barrier. A mouse model study revealed that *G. vaginalis* facilitates ascension of *P. bivia* to uterine horns (17). In addition, both bacteria contributed to sialidase activity, which plays an important role in mucus degradation. Interestingly, we have observed that not all *Prevotella* spp., similar to *G. vaginalis* strains, exhibit sialidase activity and that selected *Prevotella* spp. induced mucin production in our human 3-D endometrial epithelial model (manuscript in preparation). Furthermore, vaginal metabolites, such as lipids (e.g. long chain polyunsaturated fatty acids), are associated with both BVAB and genital inflammation, whereas anti-inflammatory nucleotides are associated with lactobacilli dominance (52). Interestingly, elevated levels of biogenic amines produced by BVAB do not correlate with genital inflammation (52). Overall, these data suggest that the pathogenic potentials of many BVAB are strain-specific. Robust longitudinal studies coupled with mechanistic studies utilizing human 3-D models are needed to identify unique mechanisms of pathogenesis for key BVAB and polymicrobial mixtures.

Conclusion: Implications for BV Diagnosis and Treatment

Elucidating the etiology of BV has important implications for diagnosis and treatment. In addition to the commonly used Amsel criteria and Nugent score, five highly sensitive and specific multiplex PCR tests are available for the diagnosis of BV in symptomatic women (BD Max™ Vaginal Panel (53), Hologic Aptima® BV (54), LabCorp NuSwab® VG (55), Quest Diagnostics™ SureSwab® Bacterial Vaginosis, and Medical Diagnostics Laboratory (MDL) OneSwab® (56)). These assays include various combinations of *Lactobacillus* spp. in addition *G. vaginalis*, *A. vaginae*, BVAB2, and *Megasphaera*-1 and −2. Based upon our conceptual model (Figure 1), it may be prudent to determine the level to which the addition of *P. bivia* and *Sneathia* spp. contributes to the sensitivity and specificity of these assays.

With regards to treatment, one aspect of the high rate of BV recurrence after therapy could be due to biofilm persistence (57). Biofilm-disrupting agents such as TOL-463 (intra-vaginal boric acid enhanced with ethylenediaminetetraacetic acid) (58) are being investigated to determine their role in BV treatment (NCT03930745). Use of biofilm-disrupting agents could increase the susceptibility of key BVAB to commonly used antibiotics when they are disassociated from the biofilm (4). Additionally, future studies focusing on interventions that modify or block the synergistic relationship between key BVAB and host response mechanisms are needed (22). Studies on the role of secondary colonizers in BV symptomatology should also be conducted. Finally, clinical trials evaluating the treatment of *P. bivia* or *A. vaginae* colonization in women without BV are needed. Of note, we are conducting a clinical trial of treatment of *G. vaginalis* colonization with amoxicillin among women without BV (NCT03211156). Vaginal microbiota transplant from a donor with optimal vaginal microbiota is a new provocative treatment option for women with BV. A universal donor screening approach was recently implemented in a pilot study (59). An inherent risk of this procedure, however, is transmission of a sexually transmitted pathogen. The baseline infection status of the recipient should be known in addition to that of the donor. Collectively, our model provides a framework for key elements that should be considered when designing and testing novel diagnostics and therapeutics.

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Conflicts of Interest

CAM has received research funding from Lupin Pharmaceuticals. She is also a consultant for Lupin Pharmaceuticals, BioFire Diagnostics, and Cepheid and has received honoraria from Roche Diagnostics and Becton Dickinson. JRS has received research funding from Becton Dickinson, Hologic, Talis, Toltec, Lupin Pharmaceuticals, and StarPharma. MMHK has been a consultant for Lupin Pharmaceuticals and Becton Dickinson. PŁ has no reported conflicts of interest.

References

1. Swidsinski A, Mendling W, Loening-Baucke V, et al. Adherent biofilms in bacterial vaginosis. Obstet Gynecol 2005;106(5 Pt 1):1013–23. [PubMed: 16260520]
2. Peebles K, Velloza J, Balkus JE, et al. High Global Burden and Costs of Bacterial Vaginosis: A Systematic Review and Meta-Analysis. Sex Transm Dis 2019;46(5):304–11. [PubMed: 30624309] * This article details the global prevalence of BV and estimates the direct medical costs of treating symptomatic BV.
3. Hillier SL, Marrazzo J, Holmes KK. Bacterial vaginosis In: Holmes KK, Sparling PF, Mardh PA, et al., editors. Sexually transmitted diseases. 4th edition. New York: McGraw-Hill; 2008 p. 737–68.
4. Bradshaw CS, Sobel JD. Current Treatment of Bacterial Vaginosis-Limitations and Need for Innovation. J Infect Dis 2016;214 Suppl 1:S14–20. [PubMed: 27449869]
5. Muzny CA, Lensing SY, Aaron KJ, Schwebke JR. Incubation period and risk factors support sexual transmission of bacterial vaginosis in women who have sex with women. Sex Transm Infect 2019, e-published online 3/14/19.
6. Hill GB. The microbiology of bacterial vaginosis. Am J Obstet Gynecol 1993;169(2 Pt 2):450–4. [PubMed: 8357043]
7. Gardner HL, Dukes CD. Haemophilus vaginalis vaginitis: a newly defined specific infection previously classified non-specific vaginitis. Am J Obstet Gynecol 1955;69(5):962–76. [PubMed: 14361525]
8. Alves P, Castro J, Sousa C, et al. Gardnerella vaginalis outcompetes 29 other bacterial species isolated from patients with bacterial vaginosis, using in an in vitro biofilm formation model. J Infect Dis 2014;210(4):593–6. [PubMed: 24596283]
9. Castro J, Alves P, Sousa C, et al. Using an in-vitro biofilm model to assess the virulence potential of bacterial vaginosis or non-bacterial vaginosis Gardnerella vaginalis isolates. Sci Rep 2015;5:11640. [PubMed: 26113465]
10. Muzny CA, Blanchard E, Taylor CM, et al. Identification of Key Bacteria Involved in the Induction of Incident Bacterial Vaginosis: A Prospective Study. J Infect Dis 2018;218(6):966–78. [PubMed: 29718358] * This article studies the sequence of microbiological events prior to incident BV, finding that G. vaginalis, P. bivia, and A. vaginae may play a significant role in its pathogenesis.
11. Hickey RJ, Forney LJ. Gardnerella vaginalis does not always cause bacterial vaginosis. J Infect Dis 2014;210(10):1682–3. [PubMed: 24855684]
12. Ahmed A, Earl J, Retchless A, et al. Comparative genomic analyses of 17 clinical isolates of Gardnerella vaginalis provide evidence of multiple genetically isolated clades consistent with subspeciation into genovars. J Bacteriol 2012;194(15):3922–37. [PubMed: 22609915]
13. Vaneechoutte M, Guschin A, Van Simaey L, et al. Emended description of Gardnerella vaginalis and description of Gardnerella leopoldii sp. nov., Gardnerella piotii sp. nov. and Gardnerella swidsinskii sp. nov., with delineation of 13 genomic species within the genus Gardnerella. Int J Syst Evol Microbiol 2019;69(3):679–87. [PubMed: 30648938] * This article describes 13 species within the genus Gardnerella.
14. Janulaitiene M, Gegzna V, Baranauskiene L, et al. Phenotypic characterization of Gardnerella vaginalis subgroups suggests differences in their virulence potential. PLoS One 2018;13(7):e0200625. [PubMed: 30001418] This study suggests that G. vaginalis subgroups with different virulence potential might play distinct roles in the vaginal microbiota and the pathogenesis of BV.
15. Harwich MD Jr., Alves JM, Buck GA, et al. Drawing the line between commensal and pathogenic Gardnerella vaginalis through genome analysis and virulence studies. BMC Genomics 2010;11:375. [PubMed: 20540756]
16. Plummer EL, Vodstrcil LA, Murray GL, et al. Gardnerella vaginalis clade distribution is associated with behavioural practices and Nugent Score in women who have sex with women. J Infect Dis 2019, e-published online 9/23/19.* This study suggests that G. vaginalis clades have varying levels of pathogenicity in women who have sex with women, with acquisition occurring through sexual activity.

17. Gilbert NM, Lewis WG, Li G, et al. Gardnerella vaginalis and Prevotella bivia Trigger Distinct and Overlapping Phenotypes in a Mouse Model of Bacterial Vaginosis. J Infect Dis 2019;220(7):1099–108. [PubMed: 30715405] * This mouse model study suggests that G. vaginalis and P. bivia play a very important role in the pathogenesis of BV.
18. Castro J, Machado D, Cerca N. Unveiling the role of Gardnerella vaginalis in polymicrobial Bacterial Vaginosis biofilms: the impact of other vaginal pathogens living as neighbors. ISME J 2019;13(5):1306–17. [PubMed: 30670827] * This study analyzed the ecological interactions between G. vaginalis and 15 BVAB using a dual-species biofilm model, finding that some BVAB enhanced G. vaginalis virulence while others had a lower or no impact on G. vaginalis virulence.
19. Janulaitiene M, Paliulyte V, Grinceviciene S, et al. Prevalence and distribution of Gardnerella vaginalis subgroups in women with and without bacterial vaginosis. BMC Infect Dis 2017;17(1):394. [PubMed: 28583109]
20. Castro J, Franca A, Bradwell KR, et al. Comparative transcriptomic analysis of Gardnerella vaginalis biofilms vs. planktonic cultures using RNA-seq. NPJ Biofilms Microbiomes 2017;3:3. [PubMed: 28649404]
21. Schwebke JR, Muzny CA, Josey WE. Role of Gardnerella vaginalis in the pathogenesis of bacterial vaginosis: a conceptual model. J Infect Dis 2014;210(3):338–43. [PubMed: 24511102]
22. Muzny CA, Taylor CM, Swords WE, et al. An Updated Conceptual Model on the Pathogenesis of Bacterial Vaginosis. J Infect Dis 2019;220(9):1399–405. [PubMed: 31369673] * This review presents an updated conceptual model of the pathogenesis of BV, focusing on the roles of G. vaginalis, P. bivia, and A. vaginae.
23. Gardner J, Laniewski P, Knight A, et al. IL-36 gamma is elevated in cervicovaginal epithelial cells in women with bacterial vaginosis and in vitro after infection with microbes associated with bacterial vaginosis. J Infect Dis 2019 In Press.* The findings from this study suggest that IL-36 gamma may exhibit an important function in the host response to BV and other sexually transmitted infections.
24. Libby EK, Pascal KE, Mordechai E, et al. Atopobium vaginae triggers an innate immune response in an in vitro model of bacterial vaginosis. Microbes Infect 2008;10(4):439–46. [PubMed: 18403235]
25. Doerflinger SY, Throop AL, Herbst-Kralovetz MM. Bacteria in the vaginal microbiome alter the innate immune response and barrier properties of the human vaginal epithelia in a species-specific manner. J Infect Dis 2014;209(12):1989–99. [PubMed: 24403560]
26. Ramsey PS, Lyon MD, Goepfert AR, et al. Use of vaginal polymorphonuclear to epithelial cell ratios for the prediction of preterm birth. Obstet Gynecol 2005;105(1):139–44. [PubMed: 15625155]
27. Onderdonk AB, Delaney ML, Fichorova RN. The Human Microbiome during Bacterial Vaginosis. Clin Microbiol Rev 2016;29(2):223–38. [PubMed: 26864580]
28. Edwards VL, Smith SB, McComb EJ, et al. The Cervicovaginal Microbiota-Host Interaction Modulates Chlamydia trachomatis Infection. MBio 2019;10(4).
29. Foschi C, Salvo M, Cevenini R, et al. Vaginal Lactobacilli Reduce Neisseria gonorrhoeae Viability through Multiple Strategies: An in Vitro Study. Front Cell Infect Microbiol 2017;7:502. [PubMed: 29270390]
30. Marziali G, Foschi C, Parolin C, et al. In-vitro effect of vaginal lactobacilli against group B Streptococcus. Microb Pathog 2019;136:103692. [PubMed: 31445119]
31. Tyssen D, Wang YY, Hayward JA, et al. Anti-HIV-1 Activity of Lactic Acid in Human Cervicovaginal Fluid. mSphere 2018;3(4).* This study found that potent and irreversible anti-HIV-1 activity is significantly associated with the concentration of the protonated (acidic, uncharged) form of lactic acid.
32. Witkin SS, Mendes-Soares H, Linhares IM, et al. Influence of vaginal bacteria and D- and L-lactic acid isomers on vaginal extracellular matrix metalloproteinase inducer: implications for protection against upper genital tract infections. MBio 2013;4(4).
33. Tachedjian G, O'Hanlon DE, Ravel J. The implausible "in vivo" role of hydrogen peroxide as an antimicrobial factor produced by vaginal microbiota. Microbiome 2018;6(1):29. [PubMed: 29409534]

34. Laniewski P, Herbst-Kralovetz MM. Vagina In: Skinner M, editor. Encyclopedia of Reproduction, 2nd Edition. Academic Press: Elsevier; 2018 p. 353–9.
35. Shannon B, Gajer P, Yi TJ, et al. Distinct Effects of the Cervicovaginal Microbiota and Herpes Simplex Type 2 Infection on Female Genital Tract Immunology. J Infect Dis 2017;215(9):1366–75. [PubMed: 28201724]
36. Masson L, Barnabas S, Deese J, et al. Inflammatory cytokine biomarkers of asymptomatic sexually transmitted infections and vaginal dysbiosis: a multicentre validation study. Sex Transm Infect 2019;95(1):5–12. [PubMed: 30018088]
37. Laniewski P, Cui H, Roe DJ, et al. Features of the cervicovaginal microenvironment drive cancer biomarker signatures in patients across cervical carcinogenesis. Sci Rep 2019;9(1):7333. [PubMed: 31089160]
38. Joag V, Obila O, Gajer P, et al. Impact of Standard Bacterial Vaginosis Treatment on the Genital Microbiota, Immune Milieu, and Ex Vivo Human Immunodeficiency Virus Susceptibility. Clin Infect Dis 2019;68(10):1675–83. [PubMed: 30407498] * This study found that BV treatment reduced genital CD4+ T-cell HIV susceptibility and IL-1 levels, but dramatically increased the genital chemokines that may enhance HIV susceptibility.
39. Laniewski P, Gomez A, Hire G, et al. Human Three-Dimensional Endometrial Epithelial Cell Model To Study Host Interactions with Vaginal Bacteria and Neisseria gonorrhoeae. Infect Immun 2017;85(3).
40. Gardner J, Swaims-Kohlmeier A, Herbst-Kralovetz MM. IL-36g Is a Key Regulator of Neutrophil Infiltration in the Vaginal Microenvironment and Limits Neuroinvasion in Genital HSV-2 Infection. J Immunol 2019 In Press.* This study found that IL-36γ plays a significant role in genital HSV-2 disease pathogenesis and may be a key immune factor in the lower female reproductive tract and in BV.
41. Fichorova RN, Yamamoto HS, Delaney ML, et al. Novel vaginal microflora colonization model providing new insight into microbicide mechanism of action. MBio 2011;2(6):e00168–11. [PubMed: 22027006]
42. Hearps AC, Tyssen D, Srbinovski D, et al. Vaginal lactic acid elicits an anti-inflammatory response from human cervicovaginal epithelial cells and inhibits production of pro-inflammatory mediators associated with HIV acquisition. Mucosal Immunol 2017;10(6):1480–90. [PubMed: 28401934]
43. Yarbrough VL, Winkle S, Herbst-Kralovetz MM. Antimicrobial peptides in the female reproductive tract: a critical component of the mucosal immune barrier with physiological and clinical implications. Hum Reprod Update 2015;21(3):353–77. [PubMed: 25547201]
44. Geisler WM, Yu S, Venglarik M, Schwebke JR. Vaginal leucocyte counts in women with bacterial vaginosis: relation to vaginal and cervical infections. Sex Transm Infect 2004;80(5):401–5. [PubMed: 15459411]
45. Hedges SR, Barrientes F, Desmond RA, Schwebke JR. Local and systemic cytokine levels in relation to changes in vaginal flora. J Infect Dis 2006;193(4):556–62. [PubMed: 16425135]
46. Srinivasan S, Morgan MT, Fiedler TL, et al. Metabolic signatures of bacterial vaginosis. MBio 2015;6(2).
47. Nelson TM, Borgogna JL, Brotman RM, et al. Vaginal biogenic amines: biomarkers of bacterial vaginosis or precursors to vaginal dysbiosis? Front Physiol 2015;6:253. [PubMed: 26483694]
48. Garcia EM, Kraskauskiene V, Koblinski JE, Jefferson KK. Interaction of Gardnerella vaginalis and Vaginolysin with the Apical versus Basolateral Face of a Three-Dimensional Model of Vaginal Epithelium. Infect Immun 2019;87(4).** Results from this study suggest that while G. vaginalis may grow on the apical face of the vaginal epithelium, its VLY toxin does not target these cells in this model; this phenomenon could have important implications regarding colonization of the vagina by G. vaginalis and may suggest an explanation for the lack of an overt immune response to this organism.
49. Eade CR, Diaz C, Wood MP, et al. Identification and characterization of bacterial vaginosis-associated pathogens using a comprehensive cervical-vaginal epithelial coculture assay. PLoS One 2012;7(11):e50106. [PubMed: 23166828]

50. Fichorova RN, Buck OR, Yamamoto HS, et al. The villain team-up or how Trichomonas vaginalis and bacterial vaginosis alter innate immunity in concert. Sex Transm Infect 2013;89(6):460–6. [PubMed: 23903808]
51. Anahtar MN, Byrne EH, Doherty KE, et al. Cervicovaginal bacteria are a major modulator of host inflammatory responses in the female genital tract. Immunity 2015;42(5):965–76. [PubMed: 25992865]
52. Ilhan ZE, Laniewski P, Thomas N, et al. Deciphering the complex interplay between microbiota, HPV, inflammation and cancer through cervicovaginal metabolic profiling. EBioMedicine 2019;44:675–90. [PubMed: 31027917]
53. Gaydos CA, Beqaj S, Schwebke JR, et al. Clinical Validation of a Test for the Diagnosis of Vaginitis. Obstet Gynecol 2017;130(1):181–9. [PubMed: 28594779]
54. Hologic Aptima® BV Assay. Available from: https://www.hologic.com/sites/default/files/2019-05/AW-18811_001_01%20BV%20IVD%20assay_0.pdf. Accessed on 9/23/19.
55. Cartwright CP, Lembke BD, Ramachandran K, et al. Development and validation of a semiquantitative, multitarget PCR assay for diagnosis of bacterial vaginosis. J Clin Microbiol 2012;50(7):2321–9. [PubMed: 22535982]
56. Hilbert DW, Smith WL, Chadwick SG, et al. Development and Validation of a Highly Accurate Quantitative Real-Time PCR Assay for Diagnosis of Bacterial Vaginosis. J Clin Microbiol 2016;54(4):1017–24. [PubMed: 26818677]
57. Muzny CA, Schwebke JR. Biofilms: An Underappreciated Mechanism of Treatment Failure and Recurrence in Vaginal Infections. Clin Infect Dis 2015;61(4):601–6. [PubMed: 25935553]
58. Marrazzo JM, Dombrowski JC, Wierzbicki MR, et al. Safety and Efficacy of a Novel Vaginal Anti-infective, TOL-463, in the Treatment of Bacterial Vaginosis and Vulvovaginal Candidiasis: A Randomized, Single-blind, Phase 2, Controlled Trial. Clin Infect Dis 2019;68(5):803–9. [PubMed: 30184181] * This study found that a novel boric acid-based vaginal anti-infective with enhanced antibiofilm activity (TOL-463) was effective in treating BV and VVC in women.
59. DeLong K, Bensouda S, Zulfiqar F, et al. Conceptual Design of a Universal Donor Screening Approach for Vaginal Microbiota Transplant. Front Cell Infect Microbiol 2019;9:306. [PubMed: 31555606] * A universal donor screening approach for vaginal microbiota transplant, implemented in a small study of 20 women, is described in this paper.

Key Points

1. The etiology of BV, the most common vaginal infection, remains controversial.
2. *G. vaginalis* and *P. bivia* (early colonizers) in addition to *A. vaginae*, *Sneathia* spp. (secondary colonizers) may be key bacteria in the pathogenesis of BV.
3. *G. vaginalis* and *P. bivia* appear to actively inhibit the host inflammatory response in the vaginal epithelium, potentially evading the immune system while setting up infection and establishing the BV biofilm.
4. Secondary colonizers, including *A. vaginae*, likely contribute to the signs and symptoms of BV, as they have been found to stimulate the host immune response in vaginal epithelial cells.
5. Elucidating the exact etiology of BV has important implications for diagnosis and treatment moving forward.

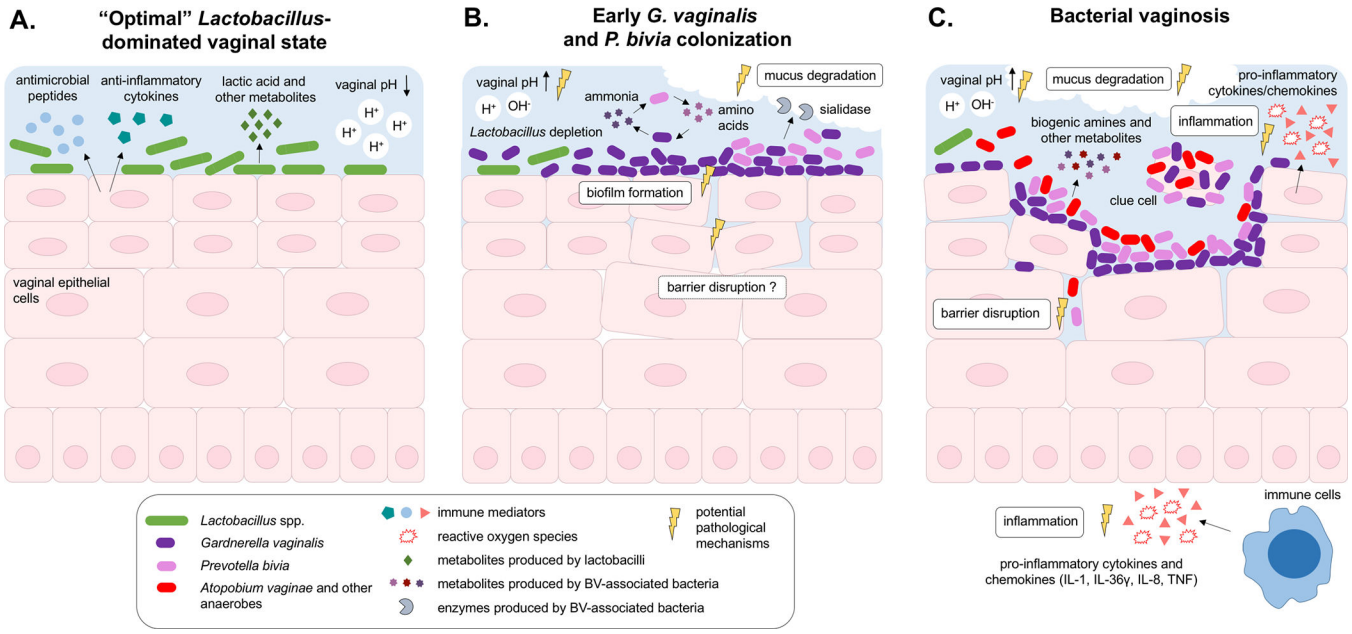

Figure 1. Depiction of putative model for the establishment of BV and immunologic/physiological changes related to host-microbe interactions.

A. In a healthy state the vaginal microbiome is dominated by *Lactobacillus* species. Lactobacilli produce lactic acid, which lowers the vaginal pH and protects against invading pathogens and pathobionts. Epithelial cells constitutively produce low levels of antimicrobial peptides (e.g. SLPI) and cytokines. Additionally, epithelial cells and immune cells contribute to homeostasis by producing anti-inflammatory cytokines (e.g. IL-1RA). **B.** Vaginal dysbiosis begins with initial colonization with the facultative anaerobe, *Gardnerella vaginalis*, usually following a sexual exposure. *G. vaginalis* colonizes the vaginal epithelial cells, replaces lactobacilli, and provides scaffolds for biofilm formation. Following *G. vaginalis* colonization, the strict anaerobe, *Prevotella bivia*, is recruited to the biofilm. *G. vaginalis* and *P. bivia* support each other's growth through ammonia and amino acid metabolism. Both *G. vaginalis* and *P. bivia* are capable of producing enzymes, e. g. sialidase, which may contribute to mucus degradation and barrier disruption. No overt inflammation is observed, which suggest that these bacterial species are able to evade host immune response through unknown mechanisms. **C.** Other secondary colonizers, e.g. *Atopobium vaginae* and *Sneathia* spp., are recruited to the biofilm. At this stage, exfoliation of epithelial cells coated with the polymicrobial biofilm occurs. These "clue cells" can be detected in wet mounts of vaginal fluid and are included in the Amsel criteria. Production of biogenic amines and other metabolites produced by BV-associated bacteria contribute to elevated vaginal pH and BV symptoms such as fishy odor. Epithelial cells and recruited immune cells produce pro-inflammatory cytokines and chemokines, which could lead to genital inflammation.