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MINIREVIEW

The Complex Link between the Female Genital Microbiota, Genital Infections, and Inflammation

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ABSTRACT The female genital tract microbiota is part of a complex ecosystem influenced by several physiological, genetic, and behavioral factors. It is uniquely linked to a woman's mucosal immunity and plays a critical role in the regulation of genital inflammation. A vaginal microbiota characterized by a high abundance of lactobacilli and low overall bacterial diversity is associated with lower inflammation. On the other hand, a more diverse microbiota is linked to high mucosal inflammation levels, a compromised genital epithelial barrier, and an increased risk of sexually transmitted infections and other conditions. Several bacterial taxa such as *Gardnerella* spp., *Prevotella* spp., *Sneathia* spp., and *Atopobium* spp. are well known to have adverse effects; however, the definitive cause of this microbial dysbiosis is yet to be fully elucidated. The aim of this review is to discuss the multiple ways in which the microbiota influences the overall genital inflammatory milieu and to explore the causes and consequences of this inflammatory response. While there is abundant evidence linking a diverse genital microbiota to elevated inflammation, understanding the risk factors and mechanisms through which it affects genital health is essential. A robust appreciation of these factors is important for identifying effective prevention and treatment strategies.

KEYWORDS cytokines, inflammation, female genital tract, immune response, vaginal microbiota

The human microbiota is an integral part of our immune system and plays an important role in the first line of defense in the female genital tract (FGT). Human immunity has evolved to live in symbiosis with beneficial bacteria to such an extent that our immune system recognizes commensal antigens in the body as self instead of foreign (1). Every woman has a unique genital microbiota, which can be broadly classified based on dominant bacterial taxa. These categories, generated using hierarchical clustering or nearest centroid classification of microbiota data, are defined as community state types (CSTs) or cervicotypes (2–4). While the distributions of dominant genital CSTs differ between studies, common clusters include a *Lactobacillus iners*-dominant CST, a *Lactobacillus crispatus*-dominant CST, and more diverse groupings, typically associated with the presence of bacterial vaginosis (BV), a clinical syndrome presenting with vaginal discharge and increased vaginal pH, characterized by a marked decrease in the abundance of *Lactobacillus* spp. and an overall increase in genital microbial diversity (5).

CHARACTERIZING THE FEMALE GENITAL TRACT MICROBIOTA

The vaginal microbiota is thought to play a crucial role in reproductive health, including the potential to protect against HIV and sexually transmitted infections (STIs), abnormal birth outcomes, and other pathogens (5–7). Its composition is

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dependent on several factors, including age, hormonal changes, and genital glycogen content (8), which, in the presence of the enzyme pullulanase or other amylases, can be used as an energy source for *Lactobacillus* spp., allowing increased colonization and the establishment of low-diversity CSTs (9–11). Typically, the cervicovaginal microbiota is characterized by a dominance of *Lactobacillus* species (12), a trait unique to humans (13), particularly *L. crispatus*, *Lactobacillus jensenii*, *Lactobacillus gasseri*, and *L. iners* (12, 14).

Although widely accepted, whether a low-diversity, *Lactobacillus*-dominated microbiota defines genital microbial health has been questioned in recent years. For example, a high relative abundance of *Bifidobacterium breve* can replace the production of lactic acid by lactobacilli (15). Furthermore, what is considered reproductively "optimal" has been associated with race in numerous analyses; however, race is a proxy for lived experiences that promote differences in biological and behavioral characteristics, including racism, and differences in microbiota should be attributed to these experiences rather than race itself. In a cohort of women living in North America, *Lactobacillus* species dominance was more common among white and Asian women, while Hispanic and black women had a more diverse microbiota, with a higher prevalence of communities not dominated by lactobacilli. This was seen in other studies where asymptomatic African American women were colonized with high proportions of *L. iners*, *Gardnerella vaginalis*, *Sneathia* spp., *Prevotella* spp., *Atopobium* spp., *Mycoplasma hominis*, *Aerococcus* spp., BV-associated bacterium 1 (BVAB1)/"*Candidatus* Lachnocurva vaginae" (16), BVAB2, and several other typically BV-associated anaerobes compared to white women, who were most likely colonized by *L. crispatus*, *L. jensenii*, *L. gasseri*, and *Staphylococcus* spp. (17). In studies of black women living in South Africa, the majority of women had a genital microbiota not defined by lactobacilli (2, 4, 18). Nonetheless, a low-diversity *Lactobacillus*-dominated vaginal environment (Fig. 1) is often associated with low inflammation, classically defined as having low levels of proinflammatory cytokines such as interleukin-1 (IL-1), IL-8, IL-16, tumor necrosis factor alpha(TNF-α), interferon gamma (IFN-γ), and monocyte chemoattractant protein 1 (MCP-1)/C-C motif chemokine ligand 2 (CCL2), among others, and high levels of anti-inflammatory/regulatory cytokines such as IL-1 receptor antagonist (IL-1RA) and IL-10 (19, 20). On the other hand, a higher-diversity microbiota is accompanied by higher inflammatory cytokine concentrations (2, 4, 21). The ratio of the BV-associated bacteria *G. vaginalis* and *Atopobium vaginae* to *Lactobacillus* spp. was associated with significantly elevated levels of IL-1α, IL-8, and IL-12(p70) and lower levels of IFN-γ-inducible protein 10 (IP-10) (22).

Generally, genital inflammation is crucial for mounting an effective host immune response against bacterial pathogens and other STIs. This cytokine response is generated as part of protective immunity. For example, the initial spike in IL-1β concentrations in the presence of pathogens, followed by IL-8, is thought to play a role in activating the vaginal innate and adaptive immune response against BV-associated bacteria (23). At the mucosa, Toll-like receptors (TLRs) are able to bind and recognize a broad range of bacterial pathogen-associated molecular patterns (PAMPs), including lipopolysaccharide (LPS), peptidoglycans, flagellin, and bacterial DNA, triggering a signaling cascade that typically leads to the clearance of bacterial infections (24, 25). However, sustained cytokine production can be detrimental to the FGT, associated with damage to the epithelial barrier and higher T-cell infiltration to the genital mucosa (26). Chronic inflammation caused by vulvovaginitis can lead to serious long-term obstetric and gynecological complications, including tubal infertility and pelvic inflammatory diseases (27). Persistent inflammation can also increase a woman's susceptibility to HIV (19).

This review focuses on the intrinsic relationship between a cisgender woman's genital microbiota and its surrounding inflammatory milieu.

BACTERIAL VAGINOSIS

BV is by far the most common and well-researched genital condition and affects 20 to 70% of women (28–31). Despite antibiotic treatment, BV recurs in up to 50% of

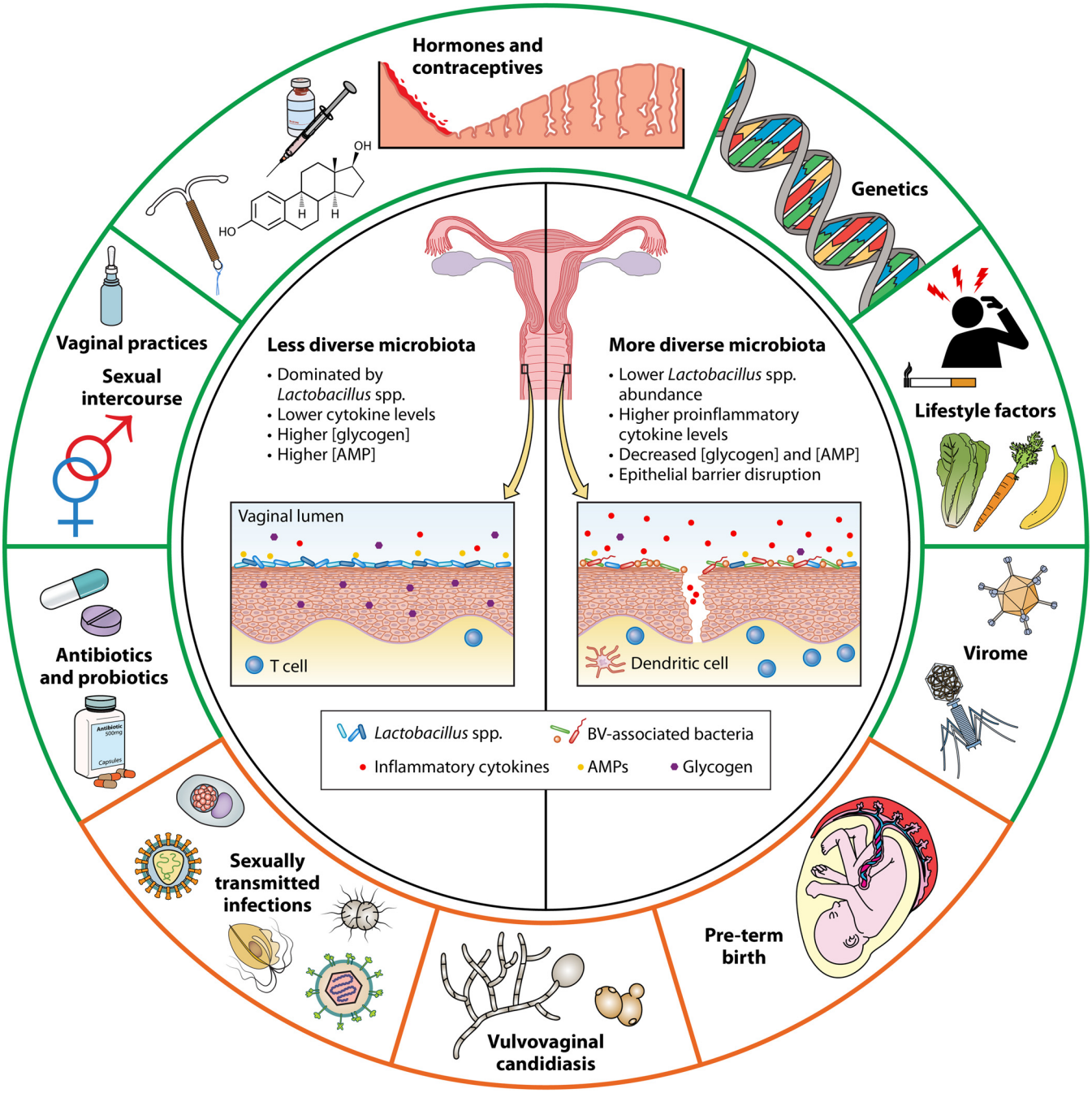

FIG 1 Common causes and consequences of a diverse, inflammatory genital microbiota. The shift to a more diverse genital microbiota is characterized by a marked decline in the abundance of *Lactobacillus* spp. and lower concentrations of glycogen and antimicrobial peptides, including lactic acid. This is accompanied by high levels of proinflammatory cytokines and chemokines and epithelial barrier damage. Several factors can potentially influence this transition to a higher inflammatory state, including sexual/reproductive practices, the use of antibiotics or probiotics, the vaginal virome, genetics, and lifestyle-related factors such as the woman's diet, stress, or smoking. This in turn leads to an increase in the risk of subsequent STI acquisition, vulvovaginal candidiasis infection, or preterm birth in some women.

women within a year (32). The exact causes are multifactorial but result in increased colonization of the lower genital tract by pathogenic bacteria such as *G. vaginalis*, *Prevotella* spp., *A. vaginae*, *Leptotrichia* spp., *Sneathia* spp., *Mobiluncus* spp., and *Megasphaera* spp., among others, and lower abundances of *Lactobacillus* spp. (4, 33–35) (Table 1). There is limited information about specific bacteria that trigger incident BV, but it has been suggested that *G. vaginalis*, *Prevotella bivia*, *A. vaginae*, and *Megasphaera* type I play an important role in initiating BV episodes (36–38). BV can be diagnosed using Amsel criteria

TABLE 1 Microbial and immunological changes associated with bacterial vaginosis

Microbiological or immunological change(s) (reference[s])
Increased microbial diversity
Transition to a Nugent score of 7–10; increased abundance of small Gram-variable rods or Gram-negative rods and curved Gram-variable rods (40, 211)
Diverse with low *Lactobacillus* abundance, typically characterized by decreased prevalence of CST I (*L. crispatus* dominant), CST II (*L. gasseri*), CST III (*L. iners*), and CST V (*L. jensenii*) and increased prevalence of CST IV (higher relative abundances of BVAB1, *G. vaginalis*, *A. vaginae*, and *Prevotella* spp., etc.) (2, 4, 212)
Soluble biomarkers
Increased vaginal pH (4, 213)
Lower L- and D-lactic acid concn (74)
Lower AMP (including α-defensins, HBD-2, and SLPI) concn (59)
Higher 12-hydroxyeicosatetraenoic acid (58) and SCFA (60, 61) levels
Polymicrobial biofilm formation by inflammatory bacteria such as *G. vaginalis*, *A. vaginae*, *P. bivia*, and/or *F. nucleatum* (113, 114, 119)
Changes in genital inflammation profile
Overall increase in cytokines and chemokines, with the exception of IP-10, MIG, GRO, CCL22, MIP-1α, and GM-CSF (2, 4, 42, 44)
Increased levels of MMPs (45–47)
Immune cells and humoral immunity
Phenotypic changes in APCs (2)
Maturation and activation of monocyte-derived DCs (CD40, CD83, and HLADR) (49)
Increased no. and activation of mucosal $CD4^+$ T cells (CD69 and CCR5) (26, 57)
IgA response to *G. vaginalis* cytolysin (53)
Metaproteomic changes
Decrease in epithelial barrier integrity, cytoskeletal alterations, and cell membrane biological processes; reduced cell wall organization and peptidoglycan biosynthesis (26, 112, 214)
Host genetics
Polymorphisms in inflammatory cytokines (e.g., IL-1β, IL-10, IL-5, IL-6, and TNF-α) and Toll-like receptor (e.g., TLR-2, -4, and -7) genes (107–110)

or Nugent scoring. Based on Amsel criteria, BV is defined as having three of the following four criteria: thin, white, yellow discharge; clue cells on wet-mount microscopy; pH $>$4.5; and fishy odor when adding a 10% potassium hydroxide solution to the wet mount (39). Nugent scoring, generally considered the gold standard for diagnosing BV, is a Gram stain scoring system that gives a score of 0 to 10 based on bacterial morphotype. A Nugent score of 7 to 10 indicates the presence of BV, while an intermediate genital microbiota is given a Nugent score of 4 to 6 (40). Women with highly diverse vaginal CSTs had an 18-fold-higher likelihood of being Nugent-defined BV positive (4). This microbial imbalance is often asymptomatic in women, with about 75% of women with Nugent-defined BV not experiencing any symptoms (41).

It is now well established that a BV-associated vaginal microbiota is linked to increased concentrations of several genital cytokines [proinflammatory markers such as IL-1α, IL-1β and TNF-α, TNF-β, IL-10, IL-8, IL-12(p70), IL-4, and FMS-related tyrosine kinase 3 ligand (FLT-3L)], while others are downregulated (chemokines such as IP-10/CXCL10, growth-regulated oncogene [GRO], macrophage-derived chemokine [MDC]/CCL22 and macrophage inflammatory protein 1α [MIP-1α]/CCL3, IL-7, and granulocyte-macrophage colony-stimulating factor [GM-CSF]) (2, 42) (Table 1). Genital inflammation is associated with high alpha (within-sample) bacterial diversity and low *L. crispatus* abundance (43). In a cohort of young South African women, higher abundances of *Prevotella* spp., *Dialister* spp., *Parvimonas micra*, and *Sneathia sanguinegens* and lower abundances of *L. crispatus* and *Lactobacillus johnsonii-L. gasseri* were predictive

of high levels of inflammatory markers (4). Among sub-Saharan African women, higher concentrations of human β-defensin 2 (HBD-2) were predictive of BV by Nugent scores, and lower IL-1RA/IL-1β ratios were predictive of intermediate Nugent scores (44). The presence of BV-associated bacteria or LPS was also associated with increased host matrix metalloproteinases (MMPs) as part of a negative-feedback loop aimed at dampening inflammation (45, 46). Cytokines can be cleaved by MMPs; in the case of IL-8, cleavage of the C terminus by MMP8, upregulated during BV (47), led to a 10-fold-higher potency (48), creating a stronger inflammatory response to bacterial infection. BV-associated bacteria induced the production of IL-1α, IL-1β, and IL-8 when cocultured with vaginal epithelial cells (2). *In vitro* and in germfree mice, vaginal introduction of *P. bivia* increased IL-6 and IL-8 production and recruited and/or activated $CD44^+$ $CD4^+$ T cells to the genital mucosa compared to vaginal exposure to *L. crispatus* (21), suggesting a causative role of these microbiota members in inflammation and cellular activation.

Several of the cytokines upregulated with BV likely work in tandem to modulate mucosal immunity (Table 1). Cervicovaginal lavage fluids from women with BV induced the maturation and activation marker expression (CD40, CD83, and HLADR) of monocyte-derived dendritic cells (DCs) and the production of IL-12, IL-23, and p40 by these DCs (49). The maturation of DCs is brought about by the secretion of the growth factor GM-CSF and IL-4, a hallmark T-helper 2 (Th2) cytokine, while TNF-α and the combination of IL-1β and IFN-γ can cause DCs to produce the inflammatory cytokine IL-12 and the regulatory cytokine IL-10 (50). DCs are important for activating naive T cells and for the development of a Th immune response (51). This cytokine interplay could potentially play a role in cellular activation and recruitment in the presence of BV. The inflammatory cytokine IL-1β produced by activated macrophages leads to cellular proliferation, differentiation, and apoptosis (52). Similarly, Cauci et al. found that genital IL-1 concentrations, especially IL-1β, were almost 13-fold higher in women with BV and were essential in triggering an immunoglobulin A response to a hemolysin produced by *G. vaginalis* (53). Mitchell et al. showed that IL-1β concentrations were inversely associated with H_2O_2-producing *Lactobacillus* spp. in women with BV (54). Dysbiosis is associated with increased genital IL-1β concentrations and increased neutrophils or potentially other leukocytes (15, 55). It has also been hypothesized that microbially induced inhibition of TLR activation by certain pathogenic bacteria could lead to BV, including the downregulation of host heat shock protein production, which induces a proinflammatory response against pathogens (8, 56). Treatment of BV with metronidazole (an antibiotic that targets anaerobic bacteria and some protozoans) led to reductions in IL-1β, IL-8, and RANTES (regulated upon activation, normal T-cell expressed and secreted) as well as in the expression of the activation markers CD69 and CCR5 in mucosal $CD4^+$ T cells (57).

In addition to influencing the secretion of cytokines, lactobacilli acidify the vagina and produce other metabolites and antimicrobial molecules that protect against pathogens. Genital metabolites can clearly delineate women with/without BV, with BV-positive women having higher levels of 12-hydroxyeicosatetraenoic acid, an inflammatory biomarker (58). Women with BV have significantly lower levels of antimicrobial peptides (AMPs), including α-defensins (produced by neutrophils), HBD-2 (produced by epithelial cells), and secretory leukocyte protease inhibitor (SLPI), in cervical mucus (59). After treatment, these AMP levels were comparable to those of women without BV, confirming that this was indeed due to BV. While *Lactobacillus* species are associated with low short-chain fatty acid (SCFA) levels, which can regulate host cytokine production, SCFAs are found at higher concentrations in women with BV, leading to the production of IL-8, IL-6, IL-1β, and TNF-α, some of them in a dose-dependent manner (60, 61).

CONTRIBUTORS TO VAGINAL MICROBIOTA DIVERSITY AND INFLAMMATION

Several physiological and behavioral factors may increase the risk of vaginal microbial dysbiosis and inflammation, including sexual intercourse, reproductive and

contraceptive hormones, menstruation, use of antibiotics, vaginal insertion practices, and lifestyle habits (Fig. 1).

Sexual intercourse and exposure to semen. Although the exact etiology of BV is unclear, higher total numbers of and new sexual partners put women at a higher risk of BV, regardless of partner gender. Condomless sex with a regular male partner after treatment for BV was associated with the presence of a diverse, inflammatory vaginal microbiota, with a higher prevalence of BV-associated bacteria, in particular *Gardnerella*, *Atopobium*, and *Sneathia* spp., and a decrease in *Lactobacillus* spp. (62). Semen is known to contain a complex community of microbes, dominated by *Ralstonia* spp., *Lactobacillus* spp., *Corynebacterium* spp., *Staphylococcus* spp., *Prevotella* spp., *Finegoldia* spp., *Ureaplasma* spp., *Clostridiales* spp., and several other bacterial taxa present during BV (63). The presence of spermatozoa in vaginal samples was a strong predictor of incident BV (64). A study involving heterosexual couples found *Gardnerella* biofilms on desquamated semen epithelial cells, suggesting that exposure to semen could be a cause of BV in women (65). Furthermore, a partner's penile microbiota composition at the meatal opening and coronal sulcus was highly predictive of incident BV (sensitivity, specificity, and accuracy of $\geq$74.6%), with *Parvimonas*, *L. iners*, *L. crispatus*, *Dialister*, *S. sanguinegens*, and *G. vaginalis* being among the most predictive of incidence (66). In African women, reporting of recent unprotected sex was associated with decreased *L. crispatus*, *Lactobacillus vaginalis*, and other *Lactobacillus* species concentrations and increased *G. vaginalis* and *L. iners* concentrations (67), which would potentially lead to higher levels of genital inflammation. Among women who have sex with women (WSW), sexual contact with a partner with BV or with new partners was associated with an increased risk of BV (68) or a shift toward a more diverse vaginal CST dominated by *G. vaginalis* (69).

Menstrual cycle. The vaginal microbiota can fluctuate rapidly throughout the menstrual cycle, with menses accompanied by decreased concentrations of *L. jensenii* and *L. crispatus* and increased concentrations of *L. iners* and *G. vaginalis* irrespective of the initial Nugent-defined BV status (36, 70, 71). Irregular menstrual cycles could render the vaginal microbiota more unstable and lead to a higher incidence of BV (70). Srinivasan et al. proposed that low estrogen levels during menses result in a decreased glycogen content in the vaginal epithelium, which in turn restricts the growth of *Lactobacillus* spp. that depend on glycogen as a critical nutrient. In contrast, the increase in *G. vaginalis* seen with menstruation could be due to menstrual blood providing an iron source supporting its growth (36).

Hormones and contraception. Endogenous hormones can influence the vaginal microbiota, for example, at puberty, when hormonal changes influence shifts in the microbiota from one dominated by anaerobic bacteria to a more *Lactobacillus* species-dominated one (72). Estrogen increases vaginal epithelial thickness and glycogen availability (11). In turn, this state allows colonization by *Lactobacillus* spp. dependent on the metabolism of glycogen, which is converted to maltose, maltotriose, and α-dextrines, with lactic acid (a metabolite known to have antimicrobial and anti-inflammatory properties [73, 74]) being the by-product of this metabolic pathway (11, 75). This was seen in a cohort of young women where a higher-diversity, low-*Lactobacillus* inflammatory microbiota was associated with lower estrogen levels (4). This higher rate of colonization by lactobacilli has been associated with a lower prevalence of BV and lower cytokine levels. However, *Gardnerella* species is also capable of using products of glycogen metabolism by secreting its own α-glucosidase, thus potentially generating more nutrients for its colonization (76). Conversely, postmenopausal women experience a reduction in estrogen levels, leading to reduced colonization by *Lactobacillus* spp. and increased microbial diversity, although it does not necessarily lead to a higher prevalence of BV (77–79).

Observational data suggest that hormonal contraceptive use may affect the genital microbiota and immunity, although studies are flawed due to bias introduced by different condom use patterns in contracepting versus noncontracepting women or different behaviors in women who self-select various contraception methods. Some studies report a positive association between the use of various contraceptive methods and

the prevalence of BV. Twelve months of intramuscular depot medroxyprogesterone acetate (DMPA-IM) use resulted in a lower proportion of women having *Lactobacillus*-dominant vaginal biomes (53% compared to 27% at baseline) (80). In women with BV, using DMPA-IM appeared to exacerbate inflammation, with elevated IL-8, MCP-1, and IP-10 concentrations (81). In randomized trials, Balle et al. found the combined contraceptive vaginal ring to be highly inflammatory compared to combined oral contraceptive pills (COCP) or injectable norethisterone enanthate (Net-EN) (82), and Brown et al. found that copper intrauterine device (copper-IUD) use led to increases in BV-associated bacteria and several inflammatory cytokines and chemokines compared to DMPA-IM or the levonorgestrel implant (B. P. Brown, R. F. Tanko, S. Z. Jaumdally, R. Bunjun, S. Dabee, A.-U. Happel, M. Onono, G. Nair, T. Palanee-Phillips, C. W. Scoville, K. Heller, D. D. Nyangahu, J. M. Baeten, S. E. Bosinger, A. Burgener, J.-A. S. Passmore, R. Heffron, and H. B. Jaspan, submitted for publication). Similarly, African women initiating copper-IUD experienced increases in Nugent scores and concentrations of BV-associated organisms by quantitative PCR (qPCR) (83, 84). Since menstruation has been linked to decreases in *Lactobacillus* numbers and increases in BV-associated organisms (36), it is possible that these effects are indirectly associated with amenorrhea and menorrhagia often experienced by women using DMPA-IM and copper-IUD, respectively.

In contrast to the studies described above, several studies have indicated that some hormonal contraceptives have a "protective" effect against BV (82, 85–89). Among black Kenyan women, consistent DMPA-IM use was accompanied by a significant decrease in *G. vaginalis* numbers and an overall decrease in bacterial loads, together with no change in *Lactobacillus* species numbers (90). In the randomized Evidence for Contraceptive Option and HIV Outcomes (ECHO) trial, DMPA-IM initiation was associated with an increase in *Lactobacillus* species relative abundance (Brown et al., submitted). A systematic review and meta-analysis of the effects of hormonal contraceptives showed that women using COCP or DMPA experienced BV less frequently (effect sizes of 0.68 for prevalent BV and 0.82 for incident BV), with similar results seen with estrogen-containing contraceptives in a randomized trial relative to no contraception (89, 91). Estradiol-progestin COCP users were more likely to be colonized by *Lactobacillus* spp. than women using condoms only but not DMPA-IM users in an observational study (92). Among South African young women randomized to various hormonal contraceptives, COCP initiation resulted in an increased relative abundance of *L. iners* (82). These findings could be due to a higher level of glycogen accumulation triggered by estrogen. Interestingly, in a recent study in women using COCP, transitioning from a normal to an intermediate microbiota (Nugent score of 4 to 6) was preceded by a general immunosuppressive profile with decreased IL-1β, MIP-3α, IL-6, vascular endothelial growth factor (VEGF), and BD-2 concentrations (44).

Vaginal insertion practices. Certain vaginal insertion practices, particularly vaginal douching, have been linked to a higher prevalence of BV (93, 94). Vaginal douching after menses increased the BV risk 5-fold, and women who had douched in the past week before sampling were twice as likely to have BV (95). In a study of women engaging in transactional sex, using a cloth to clean inside the vagina was associated with a hazard ratio (HR) of 1.58 for BV (86). In a meta-analysis, cleaning with soap intravaginally led to the development of BV or intermediate vaginal flora in women who had a normal vaginal microbiota at baseline (96).

Lifestyle-related factors: diet, stress, and smoking. A woman's diet appears to influence not only her gut microbiota but also her genital microbiota. Dietary fat intake has been associated with a heightened BV risk, while diets rich in or supplemented with folate, vitamin A, and calcium may be protective (97). Among pregnant adolescents, low vitamin D levels were associated with a higher prevalence of BV and higher vaginal TNF-α concentrations (98). "Naturally nutrient-rich" and glycemic load dietary scores were predictive of BV progression and persistence, which could be due to hyperglycemia-related oxidative stress, leading to impaired immune function, or via colonization from the rectum (99). In South African adolescents, body mass index positively correlated with

an inflammation-associated vaginal microbiota (4), whereas Lokken et al. instead found a 20% lower risk of incident BV in obese than in normal-weight women (100).

Psychosocial stress can also lead to a higher prevalence and incidence of BV (101). Cortisol release during chronic stress can modulate estrogen production, leading to the inhibition of vaginal glycogen deposition and shifts to low-*Lactobacillus* states (102). Smoking has independently been associated with an increased risk of BV (103, 104), which the authors suggest could possibly occur through the exclusion of *Lactobacillus* spp. other than *L. iners* due to their lower stability in the FGT, facilitating the transition to BV. Smoking has been associated with low *Lactobacillus* abundance in the FGT, and smoking cessation led to a shift from a *Lactobacillus*-deficient CST to a *Lactobacillus*-dominated one (104).

Host genetics. Although the effect of host genetics on gut microbiota composition has been well described (105, 106), evidence of a possible relationship between host genetics and the vaginal microbiota is scarcer (Table 1). Those factors that have been identified either modulate the risk of BV or modulate the inflammatory response to vaginal microbes (107–111). In a study of polymorphisms in inflammatory cytokines and TLR genes in pregnant women, Goepfert et al. found that women with BV were less likely to have polymorphisms at IL-1β exon 5 +3954, IL-10 −1082, and TLR4 399 loci, irrespective of race. In the same study, among black women, a polymorphism at the IL-6 −174 locus was associated with an increased risk of BV (107). Among HIV-negative women, the single nucleotide polymorphisms (SNPs) *TLR7 rs5743737* and *TLR7 rs1634323* were associated with a decreased BV risk, and *TLR7 rs179012* was associated with an increased risk, while *TLR2 rs3804099* was associated with a lower BV risk in women living with HIV (108). Polymorphisms in the TLR4 gene (896A>G polymorphism) led to a more subdued inflammatory response to LPS from *G. vaginalis* and other anaerobic Gram-negative rods among pregnant women (reduced production of IL-1 and IL-1RA), potentially allowing greater colonization of the vagina by these bacteria (109). A high relative abundance of *Prevotella* spp. was associated with increased vaginal cytokine levels and the activation of TLR and NF-κB pathways: Si et al. found a strong association between the polymorphism *rs2069812* in the IL-5 gene and genital *Prevotella melaninogenica* abundance (110). A polymorphism in the TNF-α gene (TNFA-208G>A) led to higher vaginal fluid TNF-α concentrations in women with BV (111). These studies highlight the potential importance of host genetics and gene polymorphisms in modulating both the vaginal microbiota and the immune response to BV-associated bacteria in women.

INFLAMMATORY EFFECTS OF SPECIFIC BV-ASSOCIATED BACTERIA

There is a multitude of bacteria found in BV, yet studies in different populations have found various bacteria to be most inflammatory, likely due to their frequent cooccurrence. For example, even in the absence of symptomatic BV, Anahtar et al. found that higher relative abundances of *Fusobacterium*, *Aerococcus*, *Sneathia*, *Gemella*, *Mobiluncus*, and *Prevotella* were associated with the highest levels of inflammation in young South African women in the FRESH cohort, while Lennard et al. found higher relative abundances of BVAB1, *S. sanguinegens*, *A. vaginae*, BVAB2, *G. vaginalis*, *Prevotella amnii*, and *Megasphaera*, among others, in women with high inflammation levels as part of the WISH cohort (2, 4). Based on community functional inference (determined using phylogenetic information derived from 16S marker gene sequences, and a database of reference genomes, to predict functional potential), BVAB1 was found to be the strongest contributor to BV persistence and inflammation (4). Using metaproteomic techniques, Alisoltani et al. found that the molecular functions of bacterial cell wall, peptidoglycan, and cell membrane biological processes and cellular components were more predictive of genital inflammation than the presence of specific "nonoptimal" bacteria. Overall, however, higher levels of microbial proteins associated with *G. vaginalis*, *Prevotella* spp., *Megasphaera* spp., *Sneathia amnii*, and *A. vaginae* and lower levels of proteins of *Lactobacillus* origin were most strongly associated with high inflammation levels (112).

One of the characteristics of persistent BV is the formation of polymicrobial biofilms, thought to be initiated by *G. vaginalis* or *A. vaginae* (113, 114). These biofilms can provide bacteria with protection against antibiotics and antimicrobial compounds produced by *Lactobacillus* spp. (115, 116). In turn, BV-associated organisms, particularly *G. vaginalis*, secrete toxins such as vaginolysin and sialidase, which have cytolytic activity and induce host epithelial cells to produce cytokines such as IL-8 (117), thus creating a chronically highly inflamed milieu. It is also thought that colonization with *G. vaginalis* may initiate the transition of the vaginal microbiota to a more dysbiotic state because of its biofilm-forming ability, which allows symbiotic BV-associated bacteria like *P. bivia*, *A. vaginae*, or *Fusobacterium nucleatum* to adhere and colonize (118, 119).

A. vaginae is found in up to 55% of BV-positive women, as opposed to only 8% in Nugent-defined BV-negative women (120, 121), and higher concentrations are more likely to be found in women experiencing persistent BV (122). *A. vaginae* strongly induces the production of IL-6, IL-8, and HBD-4 *in vitro* via TLR2-to-NF-κB signaling, although the specific TLR2 agonist involved is yet to be defined (123). In a three-dimensional (3D) vaginal epithelial cell model, *A. vaginae* caused the production of molecules such as MIP-3α, HBD-2, IL-1β, IL-6, IL-8, and TNF-α, which have been shown to be associated with damage of epithelial barrier function (124).

Prevotella spp. can be found at low concentrations in the genital tract even in the absence of BV (125). However, a higher abundance of *P. bivia* is strongly associated with increased genital cytokine levels and inversely associated with *Lactobacillus* species abundance (2, 110). In *in vitro* cultures of vaginal epithelial cells, *Prevotella* induced high concentrations of IL-1α, IL-1β, IL-8, IL-6, MIP-2α, MIP-3α, MIP-2β, and RANTES compared to *L. crispatus* (2, 21, 124, 126). In a cohort of adult women from KwaZulu-Natal, South Africa, women with a higher relative abundance of *P. bivia* were 19 times more likely to have high genital tract inflammation levels and an increased risk for later HIV seroconversion. Here, LPS biosynthesis abundance was highly associated with inflammation (127). While other BV-associated bacteria also produce LPS (such as *G. vaginalis*) with differing inflammatory capabilities (128), *P. bivia* appears to contribute the highest LPS concentrations in vaginal secretions (129). In turn, LPS leads to the activation of the NF-κB pathway by binding to TLR4 and CD14 on genital epithelial cells, monocytes, and macrophages (24). Phenotypic and transcriptional analyses of antigen-presenting cells (APCs) from women with *Prevotella*-dominant vaginal communities showed an enrichment of genes related to NF-κB, TLR, nucleotide-binding oligomerization domain (NOD)-like receptor, and TNF-α signaling pathways (2).

Prevotella timonensis and *Megasphaera elsdenii* are associated with BV and, *in vitro*, induce a strong DC-mediated genital inflammatory response, including IL-1β, IL-6, IL-8, IL-12, and TNF-α, unlike *L. crispatus*, which induced neither Th differentiation nor a DC-mediated response (130). In a study of Kenyan women, the BV-associated bacteria *Dialister micraerophilus*, *Eggerthella* species type 1, *M. hominis*, *Parvimonas* species type 2, *Gemella asaccharolytica*, *Sneathia* spp., and *Megasphaera* spp., linked to an increased risk of HIV infection in this cohort, were associated with the upregulation of TNF-α and IL-1β (131).

Pathogenic bacteria can directly modulate inflammatory markers. As mentioned above, although BV is generally considered inflammatory, some chemokines, including IP-10, appear to be actively decreased in women with BV (132, 133). The mechanisms or bacteria mediating this effect are unclear. *P. bivia* can have a suppressive effect on IP-10 production by epithelial cells *in vitro* (134). Clinical strains of *Finegoldia magna*, a BV-associated pathobiont (135), express a serine proteinase, which degrades the chemokine monokine-induced gamma interferon (MIG) and suppresses its antimicrobial effect (136).

LACTOBACILLUS SPECIES ARE ASSOCIATED WITH LOW-INFLAMMATION STATES

Lactobacillus species contribute to host defenses by producing antimicrobial compounds such as lactic acid and bacteriocins. Lactic acid (both L- and D-isoforms), a key *Lactobacillus* species metabolite produced during anaerobic glucose metabolism, had

a direct anti-inflammatory effect in a 3D human vaginal epithelial tissue model, where it triggered the production of the anti-inflammatory cytokine IL-1RA; suppressed the production of IL-6, IL-8, TNF-α, RANTES, and MIP-3α after stimulation by TLR2, -3, and -4 agonists; and inhibited the production of IL-6 and IL-8 upon exposure to seminal plasma (74). Lactobacilli can also physically protect the FGT in a nonspecific way by forming colonies on epithelial cells or by causing coaggregation between bacterial species and preventing the movement of pathogenic bacterial species (137). *L. crispatus* and *L. iners* are the two major *Lactobacillus* species colonizing the genital microbiota in women, although *L. gasseri* and *L. jensenii* are present at lower concentrations (138).

L. crispatus is known for its anti-inflammatory effect, and lower abundances of this species tend to be associated with higher inflammatory cytokine levels (124, 139). Compared to gut strains, genital *L. crispatus* have evolved to be more adapted to their local milieu, with a higher abundance of genes related to acid tolerance, redox reactions, pullulanase, and carbohydrate-binding molecules (140). In a vaginal epithelial cell culture system, *L. crispatus* as well as *L. jensenii* had a dampening effect on inflammation after TLR stimulation (139). *L. crispatus* and *L. jensenii* proteins in vaginal fluid were found to inhibit *Escherichia coli* growth (141, 142). *L. crispatus*, which is considered more beneficial, is more dependent on glycogen than *L. iners* (11, 143). Thus, circumstances where glycogen levels are low could potentially select for less optimal lactobacillus species, leading to lower lactic acid levels and higher pH.

While *L. crispatus* is rarely found in women with BV (144), *L. iners* can be present in the vaginas of women with and without BV (145, 146). *L. iners* has the smallest genome size of the vaginal lactobacilli (1.3-Mbp single chromosome) and has evolved to be highly specialized to the genital mucosa, with the majority of its genes encoding core metabolic proteins found among all lactobacilli (147). *L. iners*, the most commonly found bacterial species in the female genital tract (148), has been proposed as a "transitional" species that increases the likelihood of a shift to a more diverse microbiota than an *L. crispatus*-dominated microbiota (149). *L. iners* persists irrespective of BV status, suggesting that it is uniquely adapted to dynamic changes in the FGT (145). One possible reason is that *L. iners* possesses the gene for inerolysin, a pore-forming toxin that increases its adhesion capability and allows it to consistently take up nutrients from the host despite fluctuations in the genital milieu (150, 151). In addition, *L. iners* gene expression is increased up to 10% in women with BV, which included higher expression of a cytolysin, eight proteins for the CRISPR antibacteriophage defense system, mucin, and glycerol transport and metabolic enzymes (152). *L. iners*, found most often in African women with low-diversity microbiota and often associated with low inflammation (4), can also have an immune-dampening or probiotic effect, shown to be capable of disrupting *G. vaginalis* biofilms and reducing the risk of developing BV (153).

Studies investigating the direct interaction between *Lactobacillus* spp. and BV-associated bacteria have mostly been in oversimplified *in vitro* systems lacking immune cells and mucus. In a CaSki epithelial cell culture model, a range of vaginal *L. crispatus*, *L. jensenii*, *L. mucosae*, and *L. gasseri* strains dampened the inflammatory response to *G. vaginalis* in a coculture model (154). However, lactobacilli isolated from women with a more diverse vaginal microbiota tended to independently induce higher concentrations of IL-6, IL-8, IL-1α, IL-1β, MIP-1α, and MIP-1β from CaSki cells than those from women with a *Lactobacillus*-dominant microbiota, and inflammation induction was inversely associated with their ability to adhere to epithelial cells (155). In coculture experiments, lactobacillus strains (particularly *L. crispatus* strains) inhibited the growth of *Prevotella* species, while only a few strains inhibited *G. vaginalis* growth. In addition, all the lactobacillus strains increased the production of IL-1RA and IL-10 by vaginal epithelial cell lines, which modulated the production of proinflammatory cytokines, including MIP-1α, MIP-1β, and IP-10, previously associated with a heightened risk of HIV acquisition (19, 156).

ANTIBIOTICS AND PROBIOTICS TO OPTIMIZE THE VAGINAL ECOSYSTEM

BV is currently treated with antibiotics that target anaerobic organisms (157–159). In U.S. and Kenyan cohorts, metronidazole has a profound short-term effect on the genital microbiota and BV prevalence, where bacteria such as *G. vaginalis* or other BV-associated bacteria become depleted within hours, to be replaced by less-antibiotic-sensitive lactobacilli such as *L. iners* posttreatment (160, 161). Clearance of BV was associated with significant decreases in concentrations of the proinflammatory cytokines IL-1α and IL-1β, although several chemokines were upregulated (including IP-10, MIG, MIP-3α, MCP-1, and MIP-1α), which could indicate that while treatment leads to a drastic reduction in BV-associated bacteria, it does not completely eradicate them (161). Unfortunately, these effects seem to be short-lived, with more than half of the women successfully treated presenting with recurrent BV at 12 weeks posttreatment and experiencing an increased prevalence of CST IV (*G. vaginalis* dominant) (A. Mtshali, S. Ngcapu, S. E. James, F. Osman, N. J. Garrett, C. Balle, J. Giandhari, K. Mngomezulu, G. Mzobe, T. de Oliveira, A. M. Rompalo, A. Mindel, S. S. Abdool Karim, J. A. S. Passmore, Q. Abdool Karim, H. B. Jaspan, and L. J. P. Liebenberg, submitted for publication).

Although findings are conflicting, studies have investigated the use of probiotic or biotherapeutic lactobacilli administered in conjunction with antibiotics to lower BV recurrence rates (162–165). A recent groundbreaking randomized controlled trial found that *L. crispatus* CTV-05, administered through vaginal application for 11 weeks following BV treatment with metronidazole gel, reduced the risk of BV recurrence by 34% (165). Certain lactobacilli, such as *Lactobacillus reuteri* RC-14 and *L. rhamnosus* GR-1, are able to dislodge *G. vaginalis* and *A. vaginae* and disrupt their biofilms through the production of bacteriocins or biosurfactant-like molecules and have been tested in clinical trials (166–170). The direct administration of *Lactobacillus* metabolites or prebiotics has also been explored: a study showed that the use of a combination of metronidazole and a vaginal gel containing lactic acid and glycogen was more effective in *Lactobacillus* colonization than metronidazole alone (171). *In vitro* studies have shown that lactic acid had an anti-inflammatory effect on cells in culture, upregulating the anti-inflammatory cytokine IL-1RA and inhibiting the production of IL-6, IL-8, TNF-α, RANTES, and MIP-3α when stimulated with TLR agonists (74).

CONSEQUENCES OF A HIGHLY DIVERSE MICROBIOTA AND INFLAMMATION

Having a highly inflammatory, diverse genital microbiota has been associated with an increased risk of genital infections, including several sexually transmitted infections (STIs), among others (Fig. 1).

Sexually transmitted infections. Highly diverse microbiota and STIs have agonistic relationships in women. Having BV (Nugent score of 7 to 10) or an intermediate vaginal microbiota (Nugent score of 4 to 6) is associated with a higher rate of incident infections such as *Trichomonas vaginalis*, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, or human papillomavirus (HPV), among others (7, 172–174), independent of sexual behavior. BV leads to chronic inflammation, which is associated with increased susceptibility to other genital infections, possibly via barrier disruption. Other possible mechanisms include the production of specific metabolites that enhance the growth or infectivity of pathogens (175). The fact that metronidazole treatment for BV leads to a significant decrease in the subsequent acquisition of STIs is further evidence of the key role of the genital microbiota in modulating STI risk (176, 177).

A recent systematic review linked vaginal microbiota with low *Lactobacillus* relative abundance to higher *C. trachomatis* incidence (7). Although the mechanism is unclear, commensal lactobacilli can inhibit the pathogenic effect of *C. trachomatis*, possibly via the activation of the innate immune response (124). *In vitro*, *L. crispatus* and its culture supernatant led to decreases in IL-6, IL-8, and TFN-α and an increase in IL-10 production by *C. trachomatis*-infected HeLa and J774 cells (178), while lactic acid potentially deactivated chlamydial elementary bodies, the infectious form of the pathogen, by

inactivating their surface molecules, damaging their outer membrane, or disrupting chlamydial metabolic activity by hydrogen ions (179).

Higher susceptibility to *N. gonorrhoeae* is evident in women with *Lactobacillus*-deficient microbiota (180). *In vitro* and in a porcine vaginal mucosal model, several *Lactobacillus* spp. were directly able to inhibit *N. gonorrhoeae* adherence to epithelial cells, with clinical vaginal strains providing the most inhibition (181–184), while acidification due to lactic acid, particularly by *L. crispatus*, was able to inhibit *N. gonorrhoeae* growth in culture (185).

Women with BV were 3.5 times more likely to subsequently acquire *M. genitalium* than women with a normal vaginal microbiota in a dose-dependent manner, where the risk increased by 16% with each point increase in the Nugent score (186). The mechanisms for this have not been well explored.

T. vaginalis and BV commonly cooccur, and data suggest that BV precedes *T. vaginalis* infection (187, 188). This is possibly related to a low abundance of *Lactobacillus* spp. and a higher vaginal pH associated with BV, which favors the growth of *T. vaginalis* organisms (187).

Women with Nugent-defined BV are at a higher risk of HIV acquisition (189). Furthermore, in multiple African cohorts, HIV acquisition was associated with high-diversity microbiota and/or high relative abundances or absolute concentrations of specific microbes such as *Prevotella*, *Sneathia*, and other anaerobes (21, 190). Cellular immune responses and inflammation triggered in response to BV could provide more accessible target cells at the vaginal mucosa for HIV to infect, through their recruitment, activation, or barrier disruption (57). Alternatively, it is possible that the lower numbers of lactobacilli resulting from BV would lead to an increase in vaginal pH (191) associated with lower lactic acid levels (particularly L-lactic acid), which could in turn enable HIV infection (192, 193).

The absence of vaginal lactobacilli has also been associated with herpes simplex virus 2 (HSV-2) shedding (194, 195), while on the other hand, incident HSV-2 infections are associated with a 30% increased odds of having a subsequent BV episode (196), suggesting a reciprocal relationship between BV and HSV-2. The protective effect could be due to the adhesion of lactobacilli to epithelial cells (197) or the production of L-lactic acid, which has virucidal activity at low pH (164, 198).

Women with high-risk HPV are more likely to have a vaginal microbiota dominated by bacteria other than *Lactobacillus* spp., especially *L. gasseri*, which has been implicated in HPV clearance (199, 200). Having a high-diversity or an *L. iners*-dominated CST puts women at a higher risk of HPV acquisition or persistence, suggesting that a low-diversity, healthier CST could protect against HPV via the production of several antimicrobial factors, including lactic acid (7, 199).

Vulvovaginal candidiasis. Vulvovaginal candidiasis (VVC), usually caused by *Candida albicans*, occurs more readily in a *Lactobacillus*-dominated microbiota, particularly with *L. iners* (201). Lower abundances of *Megasphaera* spp. and *Mageeibacillus indolicus* and higher abundances of *Bifidobacterium bifidum*, *Aerococcus christensenii*, *L. mucosae*, *L. crispatus*/*L. helveticus*, *Streptococcus equinus*/*S. infantarius*/*S. lutetiensis*, *P. bivia*, and *Dialister propionicifaciens* have been found in women with candidiasis (202). However, other studies point to a positive association between microbial dysbiosis and VVC instead. In a U.S. study, the *C. albicans* concentration was inversely associated with the *Lactobacillus* abundance and positively associated with the IL-8 concentrations (203). All of these studies, however, were associative, and we are not able to infer the directionality of the relationship between vaginal bacteria and candida. *In vitro*, however, lactobacilli can inhibit *C. albicans* growth through competition for adhesion sites and nutrients or the secretion of fungicidal compounds (204–206). Both cells and supernatants from *L. crispatus* and *L. gasseri* strains reduced the adhesion of *Candida* to HeLa cells, suggesting that *Lactobacillus* spp. produce compounds with fungistatic and fungicidal activities (207). *Lactobacillus* biosurfactants, lipopeptides that reduce pathogen growth and adhesion, showed antimicrobial activity

against *C. albicans*, including via disrupting preformed biofilms (208) or competitive exclusion (209). Pretreating HeLa cells with *L. crispatus* induced the downregulation of TLR2/4 expression; increased IL-8, HBD-2, and HBD-3 concentrations; and decreased the adhesion and growth of *C. albicans* (205). The production of lactic acid by probiotic *Lactobacillus* spp. was also associated with reduced fungal growth (210).

CONCLUSION

The female genital microbiota and the consequent genital immune milieu are highly dynamic. While cytokines are an important part of the FGT immune system, the multiple physiological and behavioral factors associated with a diverse microbiota can lead to a cascade effect of overwhelming chronic inflammation, genital epithelial barrier damage, and increased risk of other infections. Research around this topic has increased significantly in recent years, and the role of certain key bacteria as well as community dynamics in modulating genital health is now well established. However, data describing the means through which particular taxa influence immunity in the FGT, and the ways in which specific cytokines interact with the genital milieu, are lacking. Understanding the mechanisms through which the host inflammatory response, triggered by shifts in the microbiota, can influence FGT health is essential for prevention and therapeutic strategies.

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