Cervicovaginal Microbiota: Simple Is Better

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http://dx.doi.org/10.1016/j.immuni.2015.05.006

Vaginal microbiota differs within individuals and between human populations. Anahtar et al. (2015) identify a specific vaginal cervicotype commonly found in healthy South African women that causes localized inflammation including activation of antigen-presenting cells and vaginal recruitment of HIV target cells.

Healthy vaginal flora has long been characterized as lactobacillus dominant with low bacterial diversity and an accompanying low pH. Conversely, "unhealthy" flora is often characterized by high diversity bacterial populations with increased anaerobic bacteria and lactobacilli present at reduced levels (Brotman, 2011). In the last decade, data from diverse human populations combined with culture-independent methods of microbiota analysis have suggested that this division is too simplistic. Thus, while lactobacillus dominance is often associated with health, the converse might not be necessarily true. What defines a "healthy" vaginal microbiome? Does it depend on factors such as ethnicities, contraceptive use, sexual behavior, or all of the above? Now, a new study by Anahtar et al. (2015) surveyed a cohort of young healthy South African women and found that only a minority (37%) had a lactobacillus-dominant vaginal microbiota. The women surveyed fell into four distinct microbiome clusters or "cervicotypes;" two were lactobacillus dominant, but the remaining two were characterized by high bacterial diversity (Figure 1). Cervicotype 1 (CT1) is dominated by *Lactobacillus crispatus*; CT2 dominated by *L. iners*; CT3, *Gardnerella*; and CT4 consisting of a complex mixture of bacteria including *Prevotella*. The study found that such cervicotypes can change over time in a given individual, but do not depend on sexual behavior or contraceptive use. Moreover, the cervicotype was a better predictor of local inflammatory signature than sexually transmitted infections (STIs) including chlamydia and Trichomoniasis, indicating that localized vaginal inflammation is controlled at the level of the endogenous microbiota.

The extent of vaginal microbial diversity was demonstrated in an North American study analyzing the vaginal microbiota of women of reproductive age across multiple ethnicities that showed the presence of five distinct "community states." One of these states was represented by high alpha diversity and low lactobacillus presence, suggesting that our concept of the "normal" vaginal microbiome needs to be reassessed to include these populations (Ravel et al., 2011). The high diversity and non-lactobacillus-dominant vaginal community states are highly represented in African American and Hispanic populations (Ravel et al., 2011). Because Anahtar et al. found a similar high diversity microbiome within the same ethnic population, this raises questions about what determines the diverse nature of the vaginal microbiota. In the case of gastrointestinal microbiota, non-Western diets (Cox and Blaser, 2013) have been linked to specific microbial profiles, but it is unclear whether similar links exist for the vaginal microbiota.

Bacterial vaginosis (BV) is a relatively undefined polymicrobial disorder characterized by mix of aerobic and anaerobic vaginal microbiota—one of the commonly accepted ways of scoring BV, the Nugent score, involves analyzing a gram stain for mixed diversity population (reviewed in Ma et al., 2012). However a high diversity vaginal microbiome accompanied by a high Nugent score might not always be linked to disease. The study by Anahtar et al. highlight that of the two cervicotypes that have high diversity, CT3 and CT4, only CT4 is strongly associated with the presence of inflammatory vaginal cytokines (Figure 1). Although none of the women presented with BV symptoms, authors found CT4 to be strongly associated with localized genital inflammation including increased inflammatory cytokine production. Therefore, CT4 might serve as a better and more reliable indicator of genital inflammation than the Nugent score.

This inflammatory cervicotype, CT4, is not characterized by dominance of a single genus but is rather composed of multiple genera including *Gardnerella*, *Prevotella*, *Mobiluncus*, and *Sneathia*. The other high diversity cervicotype, CT3, is characterized by *Gardnerella* dominance but is not significantly associated with inflammatory cytokines indicating that a single genus, even *Gardnerella*, is not a consistent marker of vaginal inflammation. The mechanism behind this has been hard to unravel and is likely due to multiple microbial and host interactions. Anahtar et al. shed some much-needed light on this subject. In vitro stimulation of immortalized human vaginal epithelial cells with the bacterial isolates representing CT4 induce higher levels of IL-1α, IL-1β, and IL-8 than *L. crispatus* (dominant in CT1) or *L. iners* (dominant in CT2), indicating a direct inflammatory consequence of CT4 bacteria on epithelial cells. The bacterial epithelial co-culture data support previous findings indicating that vaginal microbes can modulate epithelial cell responses in a strain-specific manner (Doerflinger et al., 2014). Further, while the number of vaginal antigen-presenting cells (APCs) does not vary with vaginal microbial populations, the transcriptional state of these cells is drastically altered in women with CT4. Gene-expression patterns of vaginal APCs in women with CT4 overlap with those of LPS-treated macrophages and DCs, suggesting that these cells might be directly activated

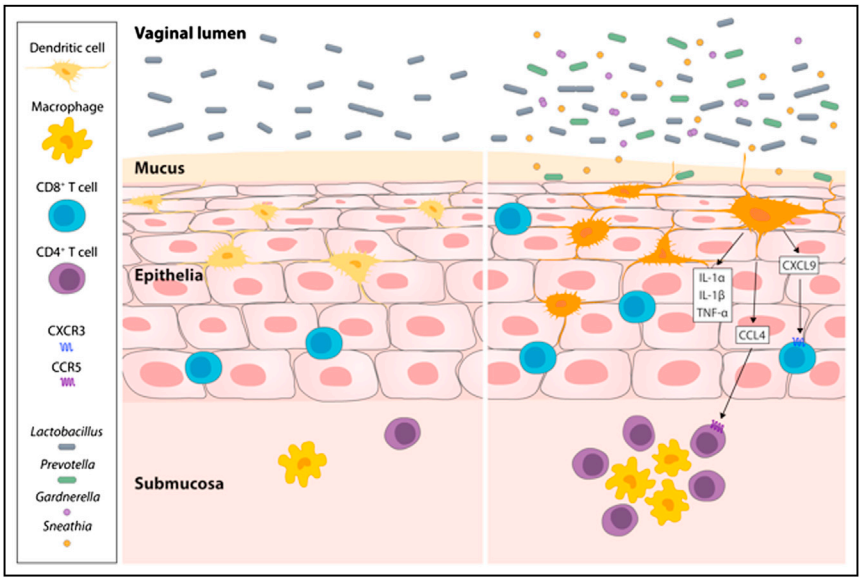

Figure 1. Vaginal Microbial Composition Influences Inflammation and T Cell Abundance
(Left) Low complexity vaginal flora represented by cervicotype CT1 and CT2 are *Lactobacillus* dominant and are not associated with inflammatory cytokine production. (Right) High-diversity vaginal flora, (specifically a cervicotype CT4 enriched for multiple bacterial genera including *Prevotella*, *Sneathia*, and *Gardnerella*) is strongly correlated with increased inflammatory cytokine production by both epithelial cells and activated vaginal APCs. These APCs are potentially directly activated by the vaginal microbiota and secrete both inflammatory cytokines, as well as chemokines resulting in T cell recruitment. Increased numbers of $CCR5^+$ CD4 T cells in hosts with this cervicotype might explain the previously reported link between high-diversity vaginal flora and increased HIV susceptibility. Memory CD4 T cells in the female reproductive tract are often found in close proximity with macrophages in "memory lymphocyte clusters" (Iijima and Iwasaki, 2014). The induction of a localized inflammatory milieu by high-diversity microbiota will likely influence the formation of memory lymphocyte clusters and will be an important consideration in the design of vaccines against STIs, as well as acquisition of HIV-1.

by contact with vaginal bacteria. Importantly, this effect is localized, as the systemic APCs do not display this inflammatory signature.

Women with traditional signs of BV accompanied by a high Nugent score have been shown to be at a higher risk of contracting sexually transmitted diseases, including HIV-1 and herpes simplex virus type 2 (HSV-2) (Petrova et al., 2013). Additionally, increased inflammatory cytokines in the cervicovaginal lavage has also been linked with increased HIV-1 shedding and transmission (Mitchell et al., 2011). Inflammation-induced breach in the vaginal epithelial barrier, recruitment of target cells, and reduction in antimicrobial peptide secretion have been proposed as mechanisms for increased susceptibility to STIs. Here, Anahtar et al. demonstrate that inflammatory cervicotype is also associated with an increased number of vaginal T cells. They trace this T cell recruitment to upregulation of chemokine gene expression by vaginal APCs (Figure 1) thereby providing a potential mechanism linking high-diversity vaginal microbiota and HIV-1 susceptibility. This study suggests that vaginal APCs are stimulated by contact with the vaginal resident flora to upregulate both inflammatory cytokines and chemokine expression, resulting in a significant increase in vaginal $CCR5^+$ CD4 T cells. The vaginal mucosa is a restricted space for lymphocyte migration including T cells (Shin and Iwasaki, 2013), and a small increase in the number of accessible target CD4 T cells could conceivably make a large difference in HIV-1 infectivity. Conversely, the influence of microbiota on T cell trafficking could be important for the formation of effective mucosal memory in the primary infections, as well as efficacy of vaccines against STIs.

Although the microbiota field has made significant mechanistic advances into host-commensal dynamics in the gastrointestinal tract and the skin, much less is understood about the vaginal microbiota. These connections are required before advances in microbiota-based treatment and therapy can be made in women's health. Anahtar et al. have given us mechanistic insight into the ways in which specific bacterial species might activate vaginal APCs and recruit T cells into the vaginal mucosa. Key candidate bacteria identified in this study can be further explored in gnotobiotic mice to probe their causative role in inflammation and disease susceptibility to STIs. Further, this study, together with others, now bolsters the view that a diverse microbial community in the vaginal mucosa is associated with an inflammatory phenotype and might serve as a risk factor for HIV-1 acquisition.

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