Non-optimal bacteria species induce neutrophil-driven inflammation and epithelial barrier disruption in the female genital tract

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The mucosal surface of a healthy female genital tract (FGT) is comprised of physicochemical, immunological and microbial components that serve as a rapid, first line of defense against infections. Alterations of these components have been associated with higher HIV acquisition risk. Analysis from >700 women from the CAPRISA004 cohort show that women with a non-*Lactobacillus* dominant vaginal microbiome were at significantly higher risk of sexual HIV acquisition, and that this strongly correlated with loss of barrier integrity, inflammation and neutrophil accumulation. However, how FGT barrier function is impacted by changes in the vaginal microbiota, and a mechanistic understanding of mucosal neutrophils in this process, remain unclear. Here, we utilized microscopy and proteomic approaches to better define the interplay between vaginal microbial species, epithelial barrier function and neutrophil activation *in vivo*. Balb/c mice intravaginally inoculated with *Lactobacillus crispatus* (optimal bacteria species) had little impact on FGT biology, whereas inoculation with *Mobiluncus mulieris* or *Gardnerella vaginalis* (non-optimal bacteria species) induced inflammation, increased cytokine release and upregulation of neutrophil-related signatures in vaginal secretions. We found that the presence of non-optimal bacterial species causes substantial damage to the vaginal epithelium and results in high neutrophil recruitment along with an increase in the release of extracellular matrix-modifying enzymes shortly after challenge. Excitingly, we also show that neutrophils response to these non-optimal bacteria species directly impacts FGT barrier function *in vivo*. Non-optimal bacteria also caused substantial damage to the vaginal epithelial barrier in humanized BLT mice, also accompanied by high neutrophil influx. We are currently addressing whether changes in vaginal barrier integrity directly impact HIV acquisition *in vivo* using humanized BLT mice. Together, our work provides a mechanistic understanding of how composition of the vaginal microbiome can alter epithelial barrier function and innate immune responses to modulate HIV risk.