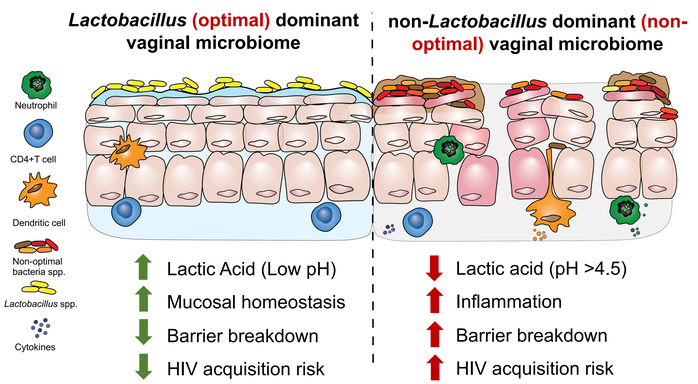
Neutrophil extracellular traps impair vaginal barrier function and increase HIV acquisition

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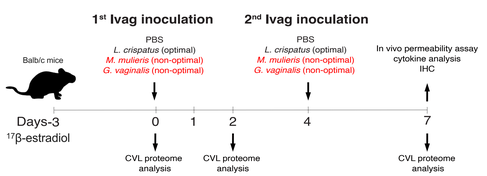
BACKGROUND



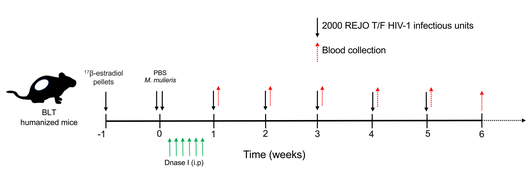
- Understanding the molecular mechanisms underlying the role of the vaginal microbiome in HIV acquisition is an essential step towards safer and more effective HIV prevention.
- Unpublished data from >700 women from the CAPRISA004 cohort show that women with a **non-optimal microbiome** were at significantly higher risk of sexual HIV acquisition (HR=2.0), and that this strongly correlated with vaginal **epithelial barrier disruption**, **inflammation** and **neutrophil accumulation;**
- **Gap in knowledge:** Despite the strong association of neutrophils, barrier disruption and inflammation with HIV acquisition, the cellular and molecular mechanisms that mediate this risk remain largely unknown.

METHODOLOGY

(A) Bacterial challenges studies



(B) HIV transmission studies



Experimental readout: # challenges required to reach systemic viremia.
- Weekly plasma HIV titer (RT-PCR analysis)

RESULTS

Neutrophil infiltration in the FGT is increased in the presence of non-optimal bacteria species

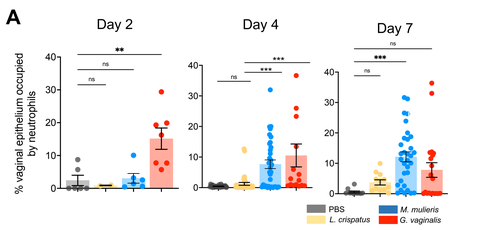


Figure 1. (A) Percentage of vaginal epithelium occupied by Ly6G⁺ neutrophils cells on day 2, 4 and 7. Statistical analysis: One-way ANOVA, Mean +/- SEM. *p<0.05; ****p<0.0001, ns=not significant.

Non-optimal bacteria challenge impacts vaginal epithelial barrier integrity

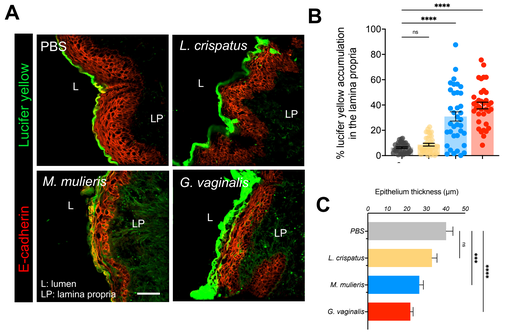


Figure 2. (A) Representative images of barrier integrity after bacterial challenge **(B)** Percentage of lamina propria occupied by lucifer yellow. **(C)** Vaginal epithelial thickness measurements. Bar indicates mean values +/- SEM. *p<0.05; ****p<0.0001, ns=not significant. scale bar = 50 μm

Non-optimal bacteria species induce neutrophil extracellular traps (NETs) formation

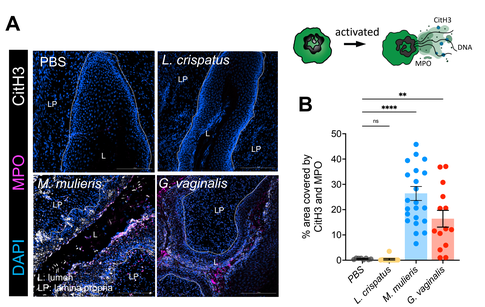


Figure 3. (A) Representative images of the vaginal tissue at day 7 post-challenge to visualize neutrophil extracellular traps; Lu = vaginal lumen; E=vaginal epithelium; LP=lamina propria. Dotted line represents border between epithelium and lamina propria. **(B)** Percentage of vaginal mucosa covered by myeloperoxidase (MPO) and citrullinated histone 3 (CitH3) after bacterial challenges. ****p<0.0001. Bar indicates mean values +/- SEM. scale bar = 50 μm

NET removal from non-optimal bacteria induced inflammation rescues vaginal barrier integrity

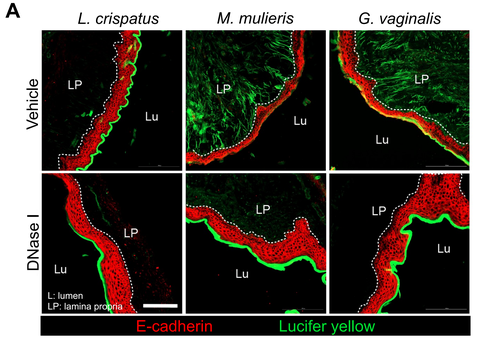
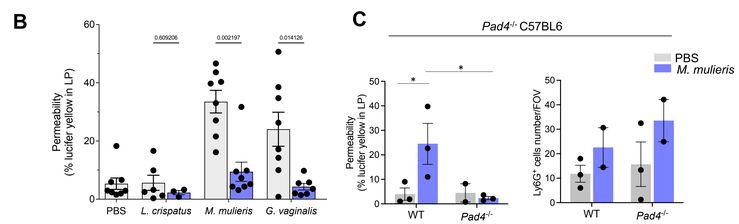


Figure 4. (A) Representative images of vaginal barrier integrity at day 7 post-challenge in control and DNase I administered animals **(B)** Percent area of vaginal mucosa covered by lucifer yellow. **(C)** Percent area of vaginal mucosa covered by lucifer yellow in *Pad4*$^{-/-}$ and control animals. Absolute number of Ly6G positive cells in *Pad4*$^{-/-}$ and control animals. Lu = vaginal lumen; E=vaginal epithelium; LP=lamina propria. Dotted line represents border between epithelium and lamina propria. scale bar = 50 μm



M. mulieris inoculation enhances vaginal HIV acquisition in humanized BLT mice

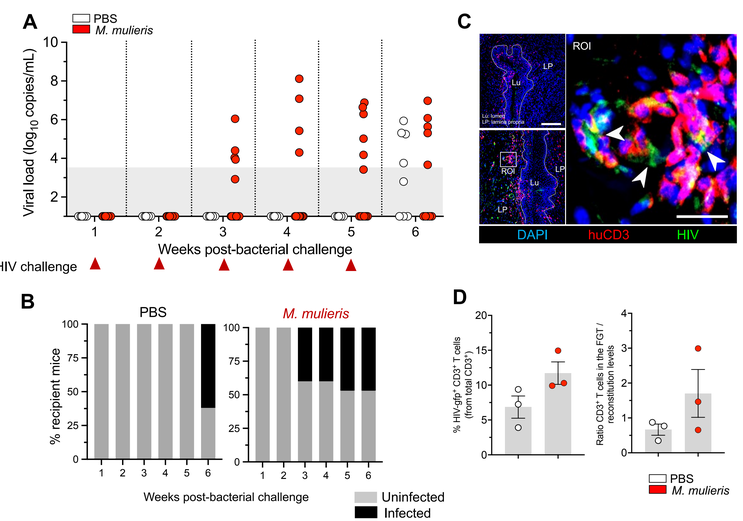


Figure 5. (A) Presence of HIV RNA in plasma after bacterial challenge of control (open circle n=8) *M. mulieris* inoculated mice (red circle, n=9) overtime. Grey box represents the limit of detection of the assay. **(B)** Proportion of animals that remained uninfected and in the PBS control and *M. mulieris* overtime. **(C)** Representative image of HIV-GFP infected huCD3⁺ T cells in the vaginal mucosa of PBS and *M. mulieris* inoculated BLT humanized mice. **(D)** Proportion of double positive HIV-GFP⁺huCD3+ T cells from the total CD3⁺ T cell per field of view **(E)** Proportion of double positive HIV-GFP⁺huCD3⁺ T cells from the total CD3⁺ T cell per field of view normalized to their corresponding CD4⁺ T cell count in peripheral blood at 18 weeks post-tissue implantation.

NET impairment restores the vaginal mucosa barrier and prevents HIV acquisition

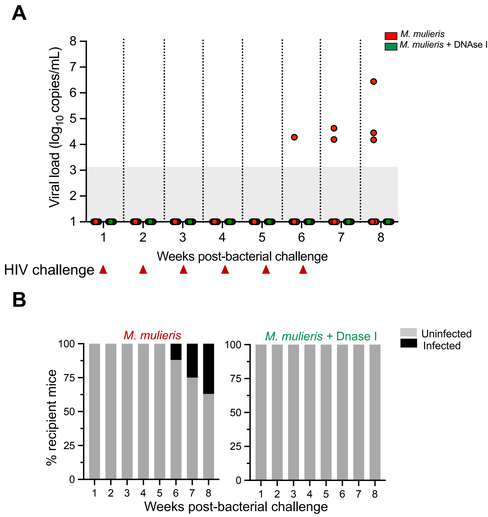
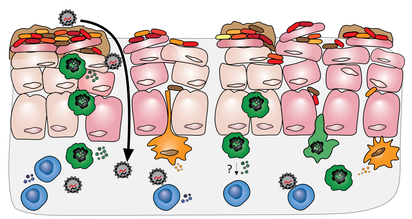


Figure 6. (A) Presence of HIV RNA in plasma after bacterial challenge *M. mulieris* (red circle n=8) and *M. mulieris* + DNase I treated huBLT mice (green circle, n=9) overtime. Grey box represents the limit of detection of the assay. **(B)** Proportion of animals that remained uninfected in the *M. mulieris* and *M. mulieris* + DNase I treatment groups overtime.

PLAIN LANGUAGE SUMMARY



Our research shows that key bacteria species present in women with a non-*Lactobacillus* dominant vaginal microbiota activate local immune cells that damages the vagina's protective lining, making it easier for the HIV virus enter the body. Importantly, restoring this barrier helps the tissue become protective again, pointing to new strategies to reduce HIV risk.

ACKNOLEDGEMENTS

CIHR IRSC Canadian Institutes of Health Research / Instituts de recherche en santé du Canada
Health Sciences Centre FOUNDATION
Research Manitoba
NIH