Lactic acid produced by an optimal vaginal microbiota promotes cervicovaginal epithelial barrier integrity: implications for HIV transmission

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Background:
Women with a *Lactobacillus spp*.-dominated vaginal microbiota have a decreased risk of HIV acquisition compared to women colonized with 'non-optimal' vaginal microbiota, the latter being associated with decreased cervicovaginal epithelial barrier integrity. Lactic acid (LA) is a key metabolite of *Lactobacillus spp*. with antimicrobial and anti-inflammatory properties that is differentially produced by *Lactobacillus* species as L- and D- isoforms. However, the impact of LA in promoting epithelial barrier integrity through modulation of junctional molecules is unknown.

Methods:
Cervicovaginal epithelial (Ect) cells were cultured in a transwell system and treated apically for 1 h with 0.3% L-LA or D-LA (pH 3.9), or acidity alone (pH 3.9, HCl adjusted). Transepithelial electrical resistance (TEER) across the cell monolayer was determined prior to and 24 h post-treatment to measure epithelial barrier integrity. Expression of junctional molecule mRNA after L or D-LA treatment was determined by RNASeq and qRT-PCR, and protein levels were determined by Western blot.

Results:
Treatment of Ect cells with L- or D-LA significantly increased TEER by 1.5-fold (n= 4; $p<0.05$), in contrast to pH control treatment. RNASeq and gene ontology enrichment analysis were consistent with the TEER functional data demonstrating that L- and D-LA caused significant differential expression (FDR<0.05) of at least 11 genes associated with intracellular junctions and barrier function, including claudin-1 (CLDN1, Log_2 fold change L-LA 1.12/ D-LA 1.17), claudin-4 (CLDN4, Log_2FC 1.47/1.63) and occludin (OCLN, Log_2FC 0.49/0.55), with no differential gene expression between isoforms. These findings were confirmed by qRT-PCR. In addition, tight junction protein levels were significantly increased by L-LA treatment (CLDN1 FC = 1.56, TJP2 FC = 1.42) but not with the pH control (n= 5; $p<0.05$).

Conclusion:
LA significantly increases cervicovaginal epithelial barrier integrity by increasing the expression of junctional molecules, which has implications for the paracellular penetration of HIV through cervicovaginal tissue and subsequent HIV acquisition.

Disclosure of Interest Statement:
G.T. and A.H. are co-inventors on patent applications on the anti-inflammatory effects of LA on cervicovaginal epithelium (Patent Application AU201501042 and US Patent Application 20150306053). The remaining authors declare no conflict of interest.