Lactic acid dampens inflammatory responses elicited by microbial TLR agonists from vaginal and cervical epithelial cells

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Background

- The female reproductive tract (FRT) is a primary route of transmission of sexually transmitted infections (STI) including HIV.
- *Lactobacillus* spp. dominate the microbiota of the healthy FRT.
  - Produce lactic acid (LA, both L and D isomers) to >1%.
  - Associated with positive reproductive and sexual health outcomes.
- The FRT is lined with epithelial cells which are a physical and immunological barrier to infection (Fig.1).
- Inflammation in the FRT increases the risk of STI and HIV acquisition1.
- Inflammatory FRT imbalances such as bacterial vaginosis increases susceptibility to STI/HIV by 2-3 fold2.
- Lactobacilli impair pathogen mediated inflammation from FRT cells3.
- We have shown LA is virucidal against HIV4, but the impact of LA on pathogen-induced inflammation from FRT epithelial cells is unknown.

Methods

The effect of LA (pH 3.9) on the viability and inflammatory response of epithelial FRT cells was assessed.

- Vaginal (VK2), endocervical (End) and ectocervical (Ect) epithelial cell lines and cervicovaginal primary cells were used.
- Cells were treated in transwells (physiologically relevant format).
- Cell viability (MTS assay) and monolayer integrity (diffusion of fluorescent dextrins, Fig. 2) were determined following treatment.
- Cytokine release from FRT epithelial cells stimulated with toll-like receptor (TLR) agonists ± LA was determined using a Luminex-based multiplex assay (ProCartaplex, eBioscences).
- The effect of D-LA and L-LA at neutral pH was also determined and compared to media pH adjusted to low pH with HCl.

Results

**Virucidal concentrations of LA are relatively non-toxic and do not disrupt FRT epithelial cell monolayers.**

- L-LA and D-LA up to 0.3% (pH3.9) have minimal effect on FRT epithelial cell line viability (Fig. 3A).
- Low pH alone (pH 3.9, adjusted with HCl) was non-toxic.
- Treatment of epithelial cell lines with 0.3% L- or D-LA, or low pH alone does not significantly alter dextran diffusion (B&C).

**LA induces an anti-inflammatory response from primary FRT epithelial cells and cell lines**

- 0.3% L-LA (pH 3.9) induces production of the anti-inflammatory cytokine IL-1RA from primary and FRT epithelial cell lines (Fig 4A).
- Similar effect seen with D-LA, but not L-LA at neutral pH (pH 7.4) or low pH alone (HCI, Fig. 4B).

LA induces an anti-inflammatory state in FRT epithelial cells which inhibits TLR-induced inflammation.

- 0.3% L-LA inhibits the pro-inflammatory response to TLR agonists.
  - Observed in FRT epithelial cell lines (Fig. 5A-F) and primary cells (G&H).
  - Similar effect seen with D-LA but not neutralised L-LA or low pH (not shown).
- Pre-treatment of cells with L-LA protects cells from subsequent TLR-induced inflammation (Fig. 5I&J).

Conclusions and Significance

- Virucidal, relatively non-toxic concentrations of LA (0.3%) elicit an anti-inflammatory response from cervicovaginal epithelial cells of the FRT and reduce the TLR-induced production of pro-inflammatory cytokines and chemokines known to activate/recruit HIV target cells.
- D-LA had a similar anti-inflammatory effect, but L-LA at neutral pH or low pH (HCl adjusted) media alone did not.
- Pre-treatment of cells with LA was able to induce an anti-inflammatory state that protected from later TLR challenge. These results suggest the potential for LA to be used in topical microbicides to maintain an anti-inflammatory state in the FRT, and help reduce inflammation, cell activation and subsequent HIV and STI susceptibility.

References