P06.10 LACTIC ACID DAMPENS INFLAMMATORY RESPONSES ELICITED BY MICROBIAL TLR AGONISTS FROM VAGINAL AND CERVICAL EPITHELIAL CELLS

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Introduction Vaginal lactobacilli are associated with favourable sexual health outcomes and acidify the vagina to pH <4.0 by producing 0.3–1% D and L isomers of lactic acid (LA). Epithelial cells that line the vagina and cervix have barrier and immune functions in the lower female reproductive tract (FRT). Here we investigate the immune modulatory effects of L-LA on lower FRT epithelial cells that might influence HIV susceptibility.
Methods The effect of apically applied L-LA (0.3% w/w, pH3.9) was assessed on vaginal (VK2), endocervical (End), ectocervical (Ect) epithelial cell lines and primary ectocervical cells grown in transwells. Elicited immune mediators were quantified following apical stimulation with toll-like receptor (TLR) agonists ± L-LA by flow cytometry and luminex-based assays.
Results L-LA had little impact on FRT epithelial cell viability. Stimulation of FRT epithelial cell lines with the TLR3-agonist poly (I:C) (PIC) induced high-levels of pro-inflammatory cytokines (IL-6/IL-8), and their variable induction with TLR agonists Pam (3) CSK(4) (TLR1/2) and lipopolysaccharide (TLR4). Conversely, L-LA treatment significantly reduced PIC-induced IL-6 (~30-fold) and IL-8 (3–4.5–fold, p£0.03) secretion, compared to PIC-only treated FRT epithelial cell lines. Irrespective of TLR stimulation, L-LA elicited a 4–11-fold ($p < 0.01$) increase in the anti-inflammatory cytokine IL-1RA in FRT epithelial cell lines. Neither 0.3% L-LA at neutral pH nor acidity alone (HCl, pH 3.9) elicited the abovementioned effects indicating that immune modulation is mediated by the protonated form of L-LA and is not due to low pH. L-LA also reduced PIC-induced secretion of RANTES and MIP3a in all cells, associated with recruitment of HIV target cells to the mucosa. Similar anti-inflammatory effects of L-LA were observed in primary ectocervical cells.
Conclusion L-LA found in *lactobacillus*-dominated vaginal microbiota elicits an anti-inflammatory effect on lower FRT epithelial cells and dampens inflammation induced by microbial TLR agonists suggesting a role in mitigating inflammation-induced HIV susceptibility at the vaginal mucosa.
Disclosure of interest statement This study was funded by NHMRC Project APP1088564. No pharmaceutical grants were received in the development of this study.

P06.11 THE IMPACT OF PERIODIC PRESUMPTIVE TREATMENT FOR VAGINAL INFECTIONS ON THE VAGINAL MICROBIOME AMONG WOMEN PARTICIPATING IN THE PREVENTING VAGINAL INFECTIONS TRIAL

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Background A randomised trial of monthly periodic presumptive treatment (PPT) with intravaginal metronidazole 750 mg plus miconazole 200 mg reduced bacterial vaginosis (BV) by 35% compared to placebo. We further assessed the effect of the intervention on detection of select bacterial species in the vaginal microbiome.
Methods HIV-uninfected, non-pregnant women aged 18–45 years from the US and Kenya were randomised to receive PPT or matching placebo for 5 consecutive nights each month for 12 months. Vaginal fluid specimens were collected every other month using Dacron swabs and tested using species-specific quantitative PCR assays that target the 16S rRNA gene. Relative risks [RR] were generated using generalised estimating equations with a log link and exchangeable correlation structure to separately assess the effect of the intervention on species detection.
Results Of 234 women enrolled, 221 (94%) had specimens for analysis (PPT n = 110; placebo n = 111). The proportion of follow-up visits with individual species detected was lower in the PPT arm versus placebo for: BVAB1 (13.8% vs. 23.7%; RR = 0.60, 95% CI 0.39–0.93), BVAB2 (30.7% vs. 42.5%; RR = 0.72; 95% CI 0.55–0.95), BVAB3 (22.9% vs. 31.0%; RR = 0.75, 95% CI 0.54–1.03), Atopobium vaginae (59.7% vs. 72.7%; RR = 0.82, 95% CI 0.71–0.94), Leptotrichia/Sneathia (49.4% vs. 60.6%; RR = 0.81, 95% CI 0.68–0.97), and Megasphaera species (26.8% vs. 43.8%; RR = 0.61, 95% CI 0.46–0.82). Lactobactillus crispatus and L. jensenii were more frequently detected in the PPT arm (L. crispatus: 31.8% vs. 26.7%, p = 0.19; L. jensenii: 31.8% vs. 25.1%; p = 0.07). However, these increases were not statistically significant. The prevalence of *Gardnerella vaginalis* and *L. iners* during follow-up was high (90% and 91%, respectively) and did not differ by arm.
Conclusions Use of monthly PPT for one year significantly reduced BV prevalence as well as colonisation with a number of bacterial species strongly associated with BV. The role of PPT to improve vaginal health should be considered.
Disclosure of interest statement R. S. M. has received honoraria for invited lectures and consulting as well as donated study product for this trial from Embil Pharmaceutical Company. R. S. M. currently receives research funding from Hologic/Gen-Probe. J. E. B. received honoraria from Symbiomix, Inc for consulting and donated reagents from Hologic/Gen-Probe. J. S. has received consultancy payments from Akesis, Hologic, Symbiomix, and Starpharma, and has grants/pending grants from Akesis, BD Diagnostic, Hologic, Cepheid, Quidel, Symbiomix, Starpharma, and Viamet. All other authors declare that they do not have a commercial or other association that might pose a conflict of interest.

P06.12 HUMAN IL-36 GAMMA AS AN INDICATOR OF VAGINAL INFECTION AND PROMOTER OF MUCOSAL INFLAMMATION

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Introduction IL-36γ (also designated as IL-1F9) has been recently identified and belongs to the IL-1 family of cytokines. Despite expression of IL-36γ at other mucosal sites, it has not previously been reported in the vaginal or cervical epithelium. Overall, there is a paucity of information regarding the induction and physiological function of IL-36γ.

Methods Utilising our human 3-D vaginal EC model, that more accurately recapitulates *in vivo* human vaginal tissue, we tested the hypothesis that IL-36γ induction in the vaginal epithelium is microbe-dependent by testing a panel of STI microbes and microbial products. To further investigate the induction and regulation of IL-36γ, 3-D vaginal EC were treated with poly (I:C), flagellin or FSL-1 for 24 h. Human 3-D cells were analysed by real-time qPCR analysis. Cell pellets and culture supernatants were also collected and analysed by IL-36γ ELISA, Western blot and cytometric bead array.

Results Following exposure to STI pathogens (herpes simplex virus and bacterial vaginosis (BV)-associated bacteria) and specific microbial products, IL-36γ expression was significantly increased relative to untreated and Lactobacilli spp. bacteria in the vaginal EC model. All microbial products tested significantly ($p < 0.05$) induced expression of IL-36γ in a dose- and TLR-dependent manner. Treatment with IL-36γ significantly ($p < 0.05$) induced proinflammatory cytokines and antimicrobial peptides (AMP). Recombinant IL-36γ treatment resulted in cytokine and AMP production, thereby promoting inflammation in the local microenvironment.

Conclusion We show that human 3-D vaginal EC express IL-36γ and this cytokine is elicited in a microbe-dependent manner at this mucosal site. Furthermore, we demonstrate that IL-36γ is an important driver for epithelial activation and inflammation following infection with STI-related pathogens and BV-associated bacteria, as such this novel cytokine may play an important role in host defense in the vaginal epithelium.

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P06.13 INFLAMMATORY CYTOKINE BIOMARKERS IDENTIFY WOMEN WITH ASYMPTOMATIC GENITAL INFECTIONS THAT INCREASE THE RISK OF HIV INFECTION

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Introduction Untreated sexually transmitted infections (STIs) and bacterial vaginosis (BV) cause genital inflammation and increased risk of HIV infection. WHO-recommended syndromic STI and BV management is limited as large numbers of women with asymptomatic infections go untreated. The purpose of this study was to evaluate genital cytokine profiles as a biomarker to identify women with asymptomatic, treatable infections.

Methods Luminex was used to measure the concentrations of 42 cytokines in cervicovaginal lavages (CVL) from 227 HIV-uninfected women from Durban, South Africa, and nine cytokines in endocervical swabs from 264 women from Bondo, Kenya and Pretoria, South Africa. Women were screened for BV and treatable STIs (*Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, *Mycoplasma genitalium*) using microscopy and molecular assays. Nonparametric receiver operating characteristic curves and logistic regression were used to identify cytokine profiles associated with STIs/BV.

Results In women from Durban, concomitant increased IL-1α and IL-1β and decreased IP-10 concentrations in CVLs predicted the presence of a treatable genital condition, correctly classifying 76% of women (sensitivity 72%, specificity 81%, PPV 86% and NPV 64%). In a separate validation cohort of women from Bondo and Pretoria, IL-1α, IL-1β and IP-10 concentrations in endocervical swabs correctly classified 72% of the participants according to STI/BV status. This approach performed substantially better than clinical signs in both cohorts from Durban (sensitivity 19%, specificity 92%, PPV 79% and NPV 40%) and Pretoria and Bondo (sensitivity 29%, specificity 78%, PPV 68%, NPV 39%).

Conclusion Across two cohorts of women residing in different regions in sub-Saharan Africa, genital IL-1α, IL-1β and IP-10 together was the best immunological predictor of the presence of an STI or BV. Supplementing syndromic management with point-of-care assessment of biomarkers of genital inflammation may improve STI/BV management for women, enabling more effective treatment of asymptomatic infections and potentially reducing their risk of HIV infection.

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P06.14 THE EFFECT OF SEXUAL INTERCOURSE ON VAGINAL COLONISATION WITH CANDIDA

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