Human IL-36 gamma as an Indicator of Vaginal Infection and Promoter of Mucosal Inflammation

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Abstract

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: Utilizing 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 analyzed by real-time qPCR analysis. Cell pellets and culture supernatants were also collected and analyzed 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.

Disclosure of Interest Statement:
No pharmaceutical grants were received in the development of this study.

Results - qPCR

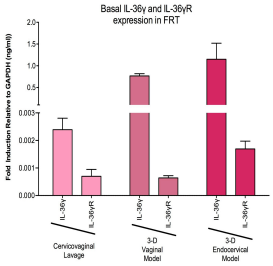

Figure 2. Expression of IL-36γ and IL-36R in the human lower female reproductive tract.
Constitutive expression of IL-36γ and IL-36R in 3-D human vaginal and endocervical cell cultures and cervicovaginal lavage. mRNA levels were normalized against GAPDH and expression of IL-36γ was reported as fold change compared to PBS treated samples.

Results - qPCR

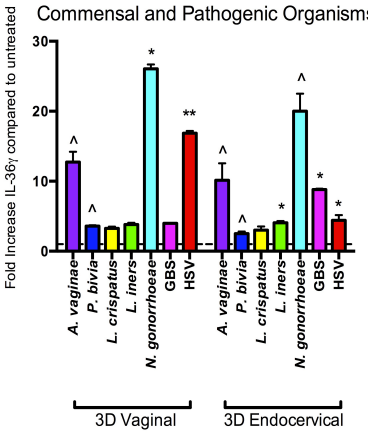

Figure 3. Independently developed IL-36γ qRT-PCR assay indicates increased expression of IL-36γ following exposure to pathogens, not commensals.
qRT-PCR targeting IL-36γ performed on vaginal epithelial cells exposed to TLR agonists, commensal bacteria, or pathogenic bacteria resulted in the differential upregulation of the gene. 3D vaginal and endocervical EC infected for 24 hours with *A. vaginae, P. bivia, Lactobacillus crispatus, Lactobacillus iners, Neisseria gonorrheae,* group B streptococcus (GBS), and Herpes simplex virus (HSV). cDNA of infected 3D vaginal EC were assayed by qRT-PCR to quantify IL-36γ gene expression. Expression of IL-36γ was determined and reported as fold change relative to untreated samples. Horizontal dashed line indicates IL-36γ expression in untreated cells. ^$P<0.05$; *$P<0.01$; **$P<0.001$.

Introduction

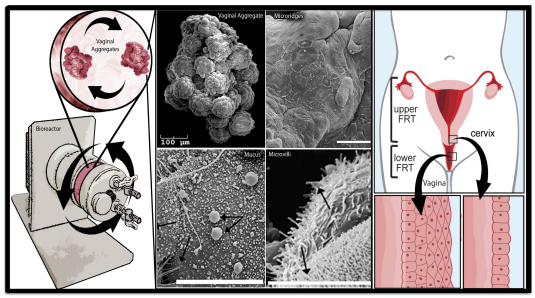

Figure 1. Schematic for the culturing 3-D human vaginal and cervical EC in rotating wall vessels (RWV).
EC cultures are started as monolayers (ML) in a tissue culture flask. When cells reach confluency, they are removed from flask, incubated with Cytodex carrier beads, and applied to RWV bioreactor. The low fluid shear and simulated microgravity environment of the STLV keeps the cells in free fall, allowing the cells to affix to the beads and form cellular aggregates. Media is changed daily to sustain cellular metabolism using syringe plungers to eliminate unwanted gases. The aggregates are periodically sampled to monitor development and cell viability. E) Complete aggregation formation and cellular differentiation (as evidenced by electron microscopy images (middle panels)) occurs after 28 days, at which time the aggregates are harvested from the bioreactor. Aggregates are seeded into 24 well plates to conduct experiments.[2, 3, 4]

Results - IL-36γ ELISA

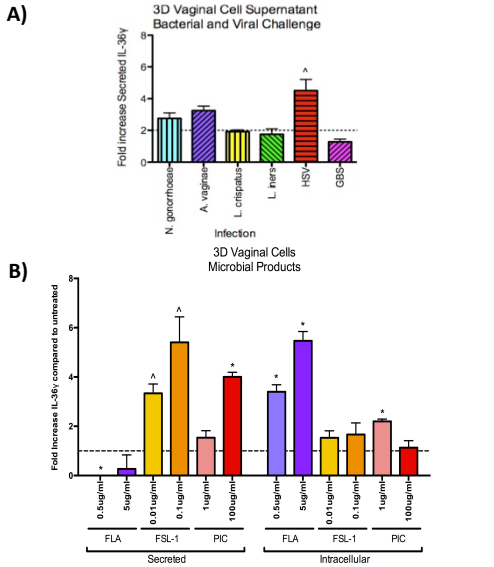

Figure 4. Pathogenic bacteria induce IL-36γ secretion by 3D vaginal EC. IL-36γ is secreted in a dose-dependent manner following TLR agonist exposure in 3D vaginal EC.
A and B) Pathogenic bacteria increased secretion of IL-36γ. Colonization with commensal bacteria does not significantly alter IL-36γ expression. 3D vaginal aggregates were plated into 24-well plates and infected with pathogenic bacteria (*N. gonorrhoeae* and *A. vaginae)* and commensal bacteria (*L. crispatus and iners*) for a 24 hour time period. Cell supernatants and cell pellets were assayed by ELISA for IL-36γ levels. All wells challenged with bacteria were infected at a MOI of 10. **A)** Fold increase of IL-36γ in infected 3D vaginal cell supernatants compared to untreated levels. **B)** Exposure to Poly(I:C) and FSL-1 induces secretion of IL-36γ. Exposure to flagellin upregulates intracellular production of IL-36γ but does not result in extracellular secretion of the cytokine. Horizontal dashed line indicates IL-36γ expression in untreated cells. ^$P<0.05$; *$P<0.01$; **$P<0.001$.

Results – qPCR

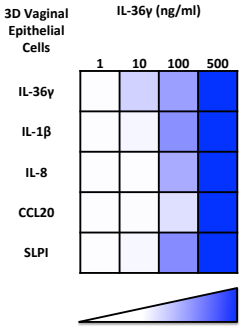

Figure 5. 3D Exposure to IL-36γ increases expression of IL-36γ, IL-1β, IL-8, CCL20, and in 3-D vaginal epithelial cells.
qRT-PCR targeting IL-36γ performed on vaginal and epithelial cells exposed to recombinant IL-36γ resulted in the upregulation of the gene in a dose dependent fashion. 3D vaginal cells exposed to recombinant IL-36γ for 24 hours. Expression of IL-36γ increased in a dose dependent fashion and suggests that IL-36γ has autocrine activity in vaginal cells. Recombinant IL-36γ also induced increased levels of the cytokine IL-1β, IL-8, the chemokine CCL20, and antimicrobial peptide SLPI in a dose-dependent manner. For all figures, gene expression was determined and reported as increasing color saturation correlating to increased expression.

Results – Bioplex

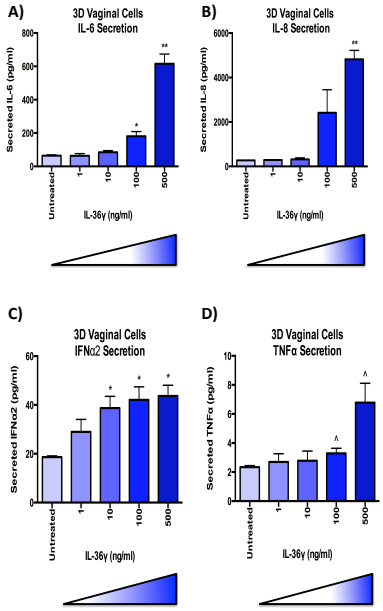

Figure 6. Exposure to recombinant IL-36γ results in the increased secretion of IL-6, IL-8, IFNα2 and TNFα.
3D vaginal EC were exposed to increasing doses of IL-36γ (1ng/ml, 10ng/ml, 100 ng/ml, and 500ng/ml) for 24 hours. The supernatants were separated from the cells and a Bioplex assay targeting cytokines IL-6, IL-8, IFNα2 and TNFα was performed on these supernatants. Cytokine secretion was determined and reported as picograms per milliliter. ^$P<0.05$; *$P<0.01$; **$P<0.001$.

Conclusions

- Specific vaginotropic microorganisms and microbial products induce the expression of IL-36γ in epithelial cells in the female reproductive tract.
- Commensal bacteria commonly found in a healthy vagina does not significantly induce IL-36γ, however, pathogenic bacteria induces IL-36γ expression and secretion.
- Treatment with specific microbial products triggered secretion of IL-36γ and an inverse relationship was observed between secreted levels of IL-36γ and intracellular levels of IL-36γ .
- Recombinant IL-36γ stimulated autocrine activity in 3-D vaginal epithelial cells.
- IL-36γ induced expression and secretion of proinflammatory cytokines, chemokines and AMP in a dose dependent fashion.
- IL-36γ is a potential biomarker that signals pathogenic insult and inflammation in the vagina.

Acknowledgements

Herbst-Kralovetz Lab Members

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Funding
NIH 1R15AI113457-01A1 (MMH-K)