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 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 epithelium. Overall, there is paucity of information regarding the induction and biological function of IL-36γ.

Method: Using 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.

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 control. Upon exposure to BV and L. crispatus, the IL-36γ transcript was upregulated at the transcriptional and translational level. In vitro, exposure to flagellin upregulated intracellular production of IL-36γ but did not result in extracellular secretion. IL-36γ expression was significantly increased relative to untreated samples.

Conclusions: We show that human 3-D vaginal EC express IL-36γ and this cytokine is induced 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. As such, this novel cytokine may play an important role in host defense in the vaginal epithelium.

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Results - qPCR

Figure 2. Expression of IL-36γ and IL-36β in the human lower female reproductive tract.

Figure 3. Independently developed 3-D IL-36γ qRT-PCR assay indicates increased expression of IL-36γ following exposure to pathogens, not commensals.

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.

Figure 5. 3D Exposure to IL-36γ increases expression of IL-36γ, IL-1β, IL-6, IL-8, CCL20, and SLPI in 3-D vaginal epithelial cells.

Figure 6. Exposure to recombinant IL-36γ results in the increased secretion of IL-6, IL-8, IFNα2 and TNFs.

Results - Bioplex

Figure A. 3D Vaginal Cell Supernatant Bacterial and Viral Challenge

Figure B. 3D Vaginal Cells Microbial Products

Figure C. 3D Vaginal Cells TNFα Challenge

Figure D. 3D Vaginal Cells IFNα2 Challenge

References

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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 induce 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 stimulates 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.