Abstract

Background: Little is known about how female reproductive hormones estradiol-17β (E) and progesterone (P) influence vaginal barrier and immune function. Furthermore the synthetic progestin contraceptive Depo-Provera (DMPA) promotes vaginal SIV acquisition in macaques and may enhance HIV acquisition in women. We have studied the effects of endogenous and exogenous hormones on vaginal epithelial barrier function and molecular mechanisms of immune defense.

Methods: We conducted an Affymetrix 1.0 ST microarray study to examine gene expression in MatTek vaginal (VEC) and endocervical (VEN) tissues after differentiation in media containing physiologic E (75nM) or E+P (75nM and 700nM, respectively) or DMPA (130μM). These hormone levels represent the 10X physiologic menstrual cycle serum peak since a 7-fold local amplification has been reported (Huhlinen, K et al. 2012). RNA was isolated from triplicate wells and subjected to microarray analysis by the BU microarray core using Affymetrix GeneChip® Gene 1.0 ST Array.

Results: We identified gene expression patterns associated with each treatment group. Many of the unique genes of interest were involved in immune function, cell death, and cell differentiation. Notably, many of the genes identified were upregulated in response to E+P treatment, indicating a potential role for E+P in promoting immune function.

Conclusions: Female reproductive hormones and DMPA have distinct effects on molecular pathways underlying immune defense in vaginal and endocervical epithelium. E appears to fortify vaginal epithelial barrier function.

The EpiVaginal™ Tissue Model

The EpiVaginal™ Tissue Model is a unique in vitro platform that allows for the study of the human vagina, fallopian tube, and endocervix in a 3D, multi-cellular environment. This model is useful for studying the effects of various conditions and treatments on the vaginal epithelium.

Macrophage transmigration into VEC-FT TNF-treated (inflamed) vaginal model:

E decreases adherence and increases barrier function. VEC-FT and E+P+DMPA-treated for 24h after differentiating in hormones as detailed. Tissues were then seeded apically with 5×10⁴ Monkey-derived macrophages (MDMs) stained with CFDA and incubated for 2h. Representative 3-D reconstructions shown in profile taken with Zxis 170 confocal microscope to a depth of 250 microns from the tissue surface. TEER was not affected (data not shown).

Conclusions:

• Hormones influence innate immunity and chemotactic properties in FGT epithelium.

• Hormones may influence migration of MDMs into Vaginal Epithelium.

• Vaginal ectocervix and endocervix appear to have distinct responses to hormones.

Future Directions:

• RNAseq experiments using tissue models for more precise quantification of differences in gene expression and identification of miRNAs.

• Data to be further validated by RT-qPCR and histology. Pathways to be confirmed using additional dopa.

• Additional macrophage migration studies to be performed, as well as with the addition of DMPA treatment prior to transmigration.

Key Points:

- Endogenous and exogenous hormones influence vaginal barrier and immune function.
- Macrophage transmigration into VEC-FT TNF-treated (inflamed) vaginal model: E decreases adherence and increases barrier function.

Table 2: E-treated VEN vs untreated E+P vs U Gene Fold Change p-value Gene Fold Change p-value Gene Fold Change p-value

<table>
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<th>Gene</th>
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<th>p-value</th>
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Expression of Innate Immune Genes and Migration of PBMCs in Female Genital Epithelium is influenced by Endogenous and Exogenous Reproductive Hormones

Ayesh Islam¹, Jai G Marathe², Jeff Pudney¹, Joseph Politch¹, Seyoum Ayehunie³, Robin R. Ingalls³ and Deborah J. Anderson¹,²,³

¹Department of Obstetrics and Gynecology, ²Microbiology and ³Section of Infectious Diseases, Boston University School of Medicine and Boston Medical Center; ⁴MatTek Corporation