Expression of Innate Immune Genes and Migration of PBMCs in Female Genital Epithelium is influenced by Endogenous and Exogenous Reproductive Hormones

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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.

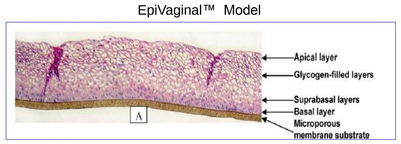
Methods: We conducted an Affymetrix 1.0 ST microarray study to examine gene expression in MatTek vaginal (VEC) and endocervical (VEN) tissues after differentiation in media containing physiologic E (75nM) or E+P (75 and 700nM, respectively) or 130 nM DMPA for 10 days. To assess barrier function, tissues were seeded apically with CMFDA-stained macrophages and infiltration was assessed by confocal microscopy.

Results: Our study confirms the hormonal responsiveness of these tissues, and identifies several genes that are significantly up and down-regulated following exposure to hormones. Pathways identified by DAVID and Ingenuity Pathway Analysis (IPA) reflect classical hormone responses and epithelial differentiation, as well as a number of others that potentially affect the acquisition of HIV and other sexually transmitted infection acquisition including mediators of innate immunity, cell death, and tight junction molecules. VEC-DMPA showed increased membrane lipid storage but decreased steroid (E) responses and retinol metabolism. VEC-E showed increased lysozyme expression (3x) and decreased Caspse14 (-8x) expression versus hormone untreated VEC tissue ($p < 0.05$). Notably, gene expression profiles of VEN were distinct from VEC: VEN increased MMP expression and appeared more immunologically responsive. E treatment of VEC prevented infiltration of macrophages by >50%, providing further evidence of its barrier enhancing effects.

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

EpiVaginal™ Model

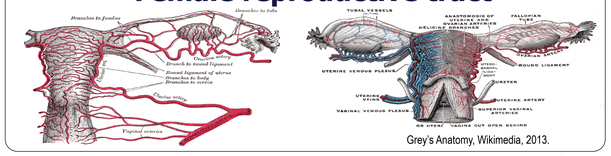


Native Human Explant Vaginal Tissue

S. Ayehunie et al. / Toxicology 279 (2011) 130–138

MatTek EpiVaginal™ tissues are produced from primary, human-derived vaginal and ectocervical epithelial cells (VEC) and grown as a stratified squamous epithelial tissue (A) that resembles vaginal explant tissue (C). Partial thickness (PT) tissue contains only VEC, while full thickness (FT) tissue consists of VEC with a fibroblast-containing lamina propria. MatTek is developing an endocervical tissue model (VEN-PT) from endocervical epithelial cells that is grown as a polarized columnar epithelial layer.

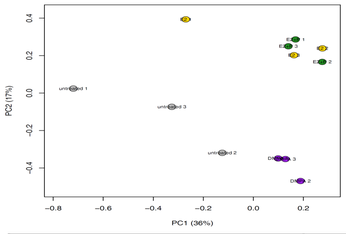
Female reproductive tract



Grey's Anatomy, Wikimedia, 2013.

VEC-PT hormone array

MatTek EpiVaginal partial thickness tissues were treated with E (75nM) or E+P (75nM and 700nM, respectively) or DMPA (130nM). These hormone levels represent the 10X physiologic menstrual cycle serum peak since a 7-fold **local amplification** has been reported (Huhtinen, 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.



The principal component analysis indicated that E and E+P treated samples were similar to one another and distinct from untreated (U) tissues. DMPA tissues were also distinct from the E and E+P.

Table1: Selected genes E vs Untreated				Selected genes DMPA vs E			
		fold change	p			fold change	p
IL36A	interleukin 36, alpha	3.59	0.04	KRT20	Keratin 20	7.10	<0.0001
LYZ	lysozyme	3.13	0.05	CD36	CD36 molecule (thrombospondin receptor)	3.57	0.0002
CD14	CD14 molecule	2.87	>0.001	TRIM31	Tripartite motif containing 31	2.92	0.0006
IL2RG	interleukin 2 receptor, gamma	2.16	>0.0001	CD53		2.72	0.0005
IFRD1	interferon-related developmental regulator 1	2.08	0.04	CYP2B6	cytochrome P450, family 2, subfamily B, polypeptide 6	-4.75	0.0001
CXCR7	chemokine (C-X-C motif) receptor 7	-2.03	0.01	TFF1	Trefoli factor 1	-4.88	<0.0001
DAPK1	death-associated protein kinase 1	-2.11	0.01	IL36A	interleukin 36, alpha	-5.11	0.0191
C3	complement component 3	-2.12	0.001	CYP2B7P1		-6.50	<0.0001
CD53	CD53 molecule	-2.63	0.003	CYP2A7		-12.9	<0.0001
LRMP	lymphoid-restricted membrane protein	-2.96	0.007	GREB1	growth regulated by estrogen in breast cancer	-13.8	<0.0001
CD36	CD36 molecule (thrombospondin receptor)	-3.13	0.0004	CYP2A6	cytochrome P450, family 2, subfamily A, polypeptide 6	-21.7	0.0005
IFITM2	interferon induced transmembrane protein 2	-3.74	0.003	OLFML3	olfactomedin-like 3	-25.7	<0.0001
CASP14	caspase 14, apoptosis-related cysteine peptidase	-8.25	0.023	PAPSS2	3'-phosphoadenosine 5'-phosphosulfate synthase 2	-53.1	<0.0001

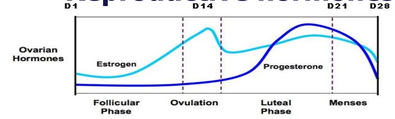
E vs U: **115 genes Up** 2-fold with p<0.05 and FDR under 0.25 versus untreated. Pathways (DAVID: Huang da et al 2009) included: response to wounding (p<0.0005), inflammatory response (p<0.005), and many involved in steroid metabolism.

41 genes Down: differentiation and lipid binding (p<0.01) were decreased. Ingenuity pathways analysis (IPA) suggested a decrease in cell death.

DMPA vs E: **48 genes Up** CD36 (fatty acid/glucose metabolism and sterile inflammation) CD53 (signal transduction, regulation of cell growth/activation). DMPA increases positive regulation of lipid storage at the membrane and the O-glucan biosynthesis pathway.

132 genes down: several cytochrome p450s, growth and immune related genes. DAVID pathway analysis: DMPA decreases differentiation, response to steroids, extracellular matrix organization and retinol metabolism.

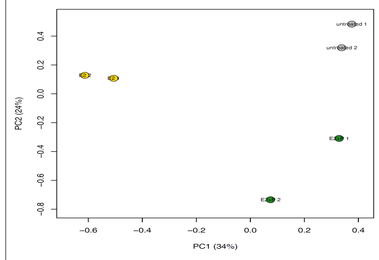
Reproductive hormones



Peak serum hormone levels (95th CI)	Estradiol	Progesterone
Molarity/Volume	2.2 nmol/L	70 nmol/L
Weight/Volume	600pg/mL	22ng/mL

VEN-PT hormone array

MatTek endocervical partial thickness tissues were treated with E (75nM) or E+P (75nM and 700nM, respectively). RNA was isolated in duplicates (3 pooled wells per sample) and subjected to microarray analysis by the BU microarray core using Affymetrix GeneChip® Gene 1.0 ST Array.



The principal component analysis indicated that E and E+P treated samples were different from one another and distinct from untreated (U) tissues.

Furthermore, expression profiles of VEN tissues did not globally resemble those of the VEC tissues.

Table 2: E-treated VEN vs untreated				E+P vs U			
Gene		Fold Change	p-value	Gene		Fold Change	p-value
MMP9	matrix metallopeptidase 9	6.39	<0.0001	OLFM4	olfactomedin 4	3.87	3.2E-07
CD200	CD200 molecule	3.58	<0.0001	CD200	CD200 molecule	3.06	6.5E-07
CCL5	chemokine (C-C motif) ligand 5	2.5	0.0086	DUOX2	dual oxidase 2	2.43	1.5E-05
CXCR7	chemokine (C-X-C motif) receptor 7	2.29	<0.0001	LIPN	lipase, family member N	2.07	2.7E-05
MMP10	matrix metallopeptidase 9	2.16	<0.0001	CXCL10	chemokine (C-X-C motif) ligand 10	-2.01	0.00011
MUC13	mucin13	-2.13	0.0172	IFIT1	interferon-induced protein with tetratricopeptide repeats 1	-2.03	0.00028
CXCL14	chemokine (C-X-C motif) ligand 14	-2.51	<0.0001	AMTN	amelotin	-2.04	0.02018
CXCL10	chemokine (C-X-C motif) ligand 10	-2.54	0.0003	PTGS2	prostaglandin-endoperoxide synthase 2	-2.09	7.2E-05
HLA-DRA	major histocompatibility complex (MHC), class II, DR alpha	-3.29	<0.0001	IFI44L	interferon-induced protein 44-like	-2.17	2.9E-05
HLA-DRB6	MHC, class II, DR beta 6 (pseudogene)	-3.64	<0.0001	TMEM27	transmembrane protein 27	-2.27	2.7E-05

E vs U: **59 genes Up** 2-fold with p<0.05 and FDR under 0.25 versus untreated. E-treated tissue involved metal metabolism and immune related genes (eg: CCL5, CXCR7). DAVID Pathways included: several carbohydrate biosynthesis functions as well as retinol metabolic process (p<0.05), involved in epithelial barrier function.

33 genes Down: DAVID Pathways: reduction immune response (p<0.00005) and in cytokine activity (p<0.001).

E+P vs U: **10 genes Up** including CD200 (inhibitory to macrophages in various tissues).

6 genes Down: CXCL10 was down regulated and IPA identified a decrease in interferon signaling (p<0.0005) in genes changed with p<0.05.

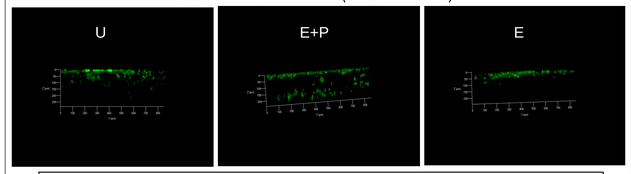
E+P vs E: **12 Up** including 2 HLA molecules and 4 small RNAs.

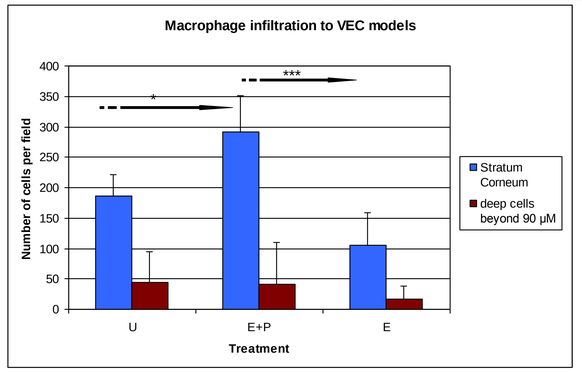
49 Down: various carbohydrate biosynthetic processes, negative regulation of innate immunity (SERPING1, IGF2), and regulation of the inflammatory response (CCL5).

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

E decreases adherence and increases barrier function

VEC-FT tissues were TNF-α treated for 24h after differentiating in hormones as detailed. Tissues were then seeded apically with $5x10^5$ Monocyte-derived macrophages (MDMs) stained with CMFDA and incubated for 2h. Representative 3-D reconstructions shown in profile taken with Zeiss 710 confocal microscope to a depth of 250 microns from the tissue surface. TEER was not affected (data not shown).





ANOVA (Fisher PLSD test) shows **Stratum Corneum** penetration of MDMs in Untreated vs E+P (p=0.04) and E+P vs E (p=0.0006) are significantly different. Thus, E appears to **decrease adherence** of macrophages and **increase resistance of tissues** to MDM infiltration in the context of TNF-α induced inflammation.

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

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