Vaginal Stratum Corneum: Demilitarized Zone or Battlefront?

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Vaginal Epithelium

- Stratum corneum
- Suprabasal layer
- Basal layer
Epithelial Cells in Vaginal Stratum Corneum (Corneocytes)

- lose nuclei and organelles
- glycogen-filled
- no tight junctions
- continuous turnover of apical cells
Vagina and Endocervix: Comparison of Apical Surfaces

Vagina

Endocervix
Stratum Corneum: Demilitarized Zone?
Few immune cells are present in the healthy SC

Pudney J, Quayle AJ, and Anderson DJ
Does the vaginal stratum corneum prevent stimulation of basal epithelial cells by products of luminal bacteria?
MatTek VEC culture

- lumen
- lamina propria
- medium
- Stratum corneum
MatTek VEC culture

Basal epithelial cells express TLRs 1/2, 3, 5
(notably they do not express TLR4, the LPS receptor)
Primary and immortalized cultures of vaginal basal epithelial cells produce proinflammatory cytokines and chemokines when stimulated with TLR agonists.

\[ \text{II}-6 \]
\[ \text{II}-8 \]
\[ \text{TNF-alpha} \]
\[ \text{RANTES} \]
\[ \text{MIP-1alpha} \]

These products recruit and activate immune cells.
MatTek VEC culture

TLR agonists added to **apical** surface
- FSL
- Poly (I:C)
- Flagelin

Basal epithelial cells express TLRs 1/2, 3, 5

No cytokines detected in apical or basal supernatants
Basal epithelial cells express TLRs 1/2, 3, 5.

TLR agonists added to scarified surface:
- FSL
- Poly (I:C)
- Flagelin

Cytokines detected in apical and basolateral supernatant.
MatTek VEC culture

Basal epithelial cells express TLRs 1/2, 3, 5

TLR agonists added to basolateral surface

- FSL
- Poly (I:C)
- Flagelin

Cytokines detected in basolateral supernatant
Evidence that the healthy intact vaginal stratum corneum is a passive protective barrier:

- Does not contain many immune cells
- Protects basal epithelial cells from stimulation with products from endogenous luminal bacteria
Stratum Corneum: Battlefront
Can pathogens infiltrate the stratum corneum?
Infiltration of Stratum Corneum by Inert Beads

0.1um (virus)

1um (bacterium)
HIV enters the vaginal stratum corneum where it may encounter Langerhans cells and transmit infection.

Thomas Hope 2009
Question:

How long does HIV remain viable (infectious) in the stratum corneum?
Assessment of HIV infectivity in the Stratum Corneum

TZM-bl (HIV indicator) cells were added to the apical surface of VEC cultures to assess infectious HIV.
HIV Infection of TZM-bl cells on Vaginal Surface

The graph shows relative O.D. (405nm) for different conditions:

- **HIV + TZ 1 hr before addition to culture**: The highest value, indicating a significant infection.
- **HIV + TZ**: Moderate infection.
- **HIV 1 hr before addition of TZ**: The lowest value, suggesting minimal infection.
Can HIV-infected cells infiltrate the stratum corneum from the apical side?
Cell-Associated HIV Transmission
PBMCs Adhere to Vaginal Stratum Corneum
Apical to basal leukocytic infiltration of the vaginal stratum corneum

Macrophages

Seminal leukocytes
Does the vaginal stratum corneum retain soluble immunological mediators?
Igs in the Vaginal Stratum Corneum

• Does the SC absorb Igs from the lumen?
• Do antibodies retain their activity in the SC?
• Time course of Ig retention in the SC?
IgG Uptake by Apical Vaginal Epithelial Cells

IgG-Cy3

Negative Control
Evidence that Ig Uptake is not receptor mediated

- Absorption and retention occurs at 4°C
- Absorption and retention of IgY
- Ig receptors not detected in stratum corneum
- The stratum corneum absorbs and retains other types of immune mediators
  - lysozyme
  - type-1 defensins
Do antibodies retain activity in the stratum corneum?
Retention of HSV-Cy3 mab by vaginal stratum corneum

After 1 hour

After 12 hours
Suppression of HSV-2 Infection in vitro with Anti-HSV Mab

Treatment Groups:
- No treatment
- Anti-HSV Mab
- Anti-HSV Mab Preincubation/Rinse

Time After HSV Infection:
- 2 hrs
- 24 hrs
- 48 hrs

p<0.01
p<0.05
p<0.05
VRCO1 HIV neutralizing mab blocks HIV infection of TZM-bl cells in vaginal stratum corneum
Cell-free and cell-associated pathogens (eg. HIV) can enter the SC.

Soluble immunological mediators and leukocytes provide immune defense in the stratum corneum.
Implications for microbicide research

- Assess concentrations, activity and half-life of microbicides in SC layer

- Design microbicides to fortify the SC - monoclonal antibodies
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IPCP-HTM: Monoclonal Antibody Multipurpose Microbicides
ICAM antibody blocked leukocyte infiltration into vaginal epithelium
Ig deposits in human vaginal epithelium

Intracellular IgA in superbasal layer

Intracellular IgG in superbasal layer

IgG in stratum corneum

Intercellular IgG