HIV & Inflammation: Clues to Prevention Paradox

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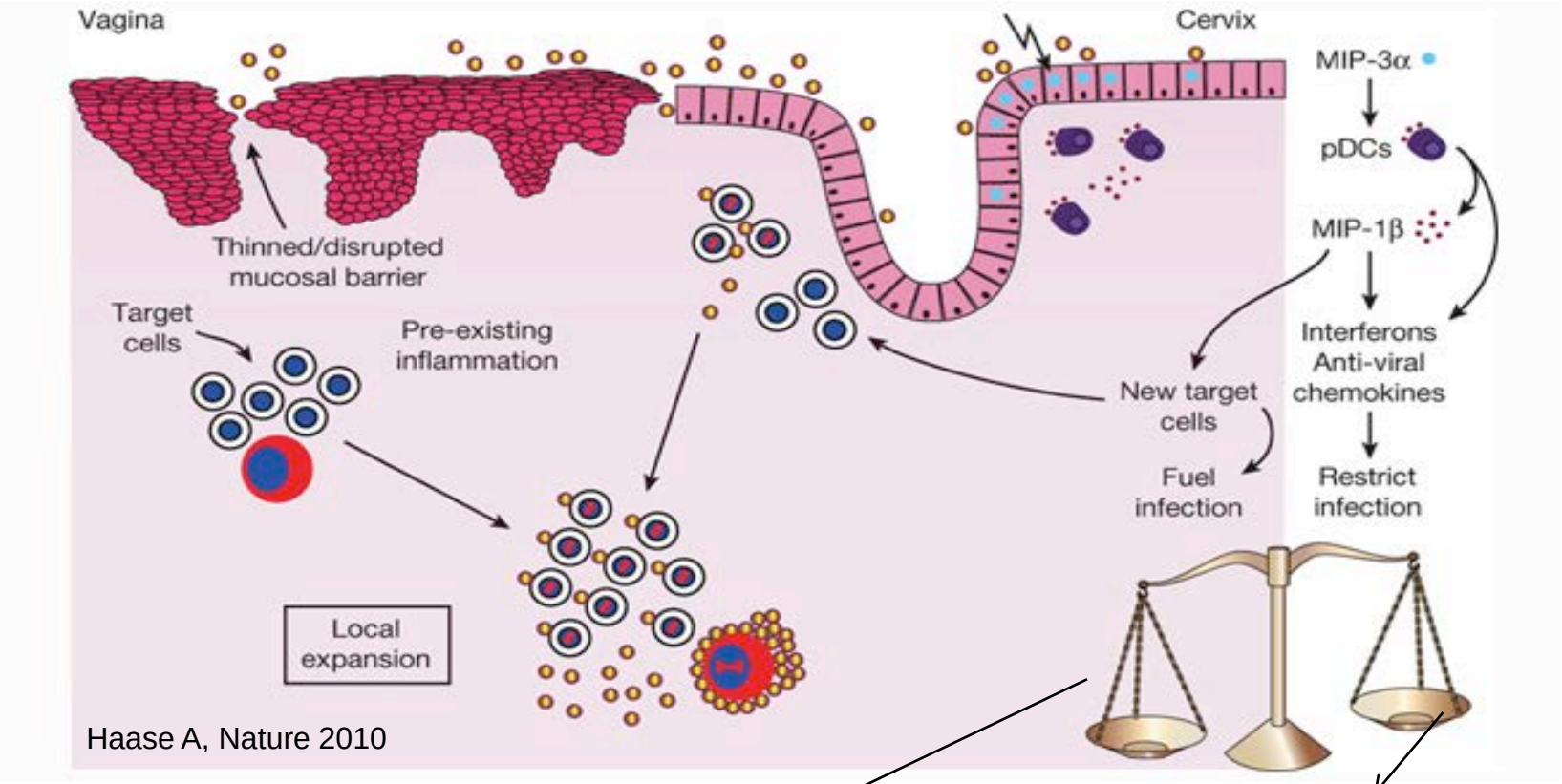
Inflammation: Modulator of Mucosal Defense

- What is inflammation?
- What role does it play in HIV prevention?
- Is it friend or foe?
- Can it be exploited to promote protection?

What is inflammation?

- **Protective** tissue response marked by recruitment of WBCs, release of cytokines, chemokines, and antimicrobial proteins
- Serves to eliminate offending agent and damaged tissue
- Chronic inflammation associated with HIV progression

Inflammation and HIV Acquisition



Transmitting viral load
Stage of HIV infection
Virulence
Tropism (R5>X4)
Target cells (#; activation)

Epithelial barrier
Protective mediators
Microbiota
Host genetics (CR5Δ32)

Mucosal Mediators of "Inflammation" Protective or Facilitators of HIV

- Protective:
  - Directly inhibit HIV infection
  - Maintain epithelial barrier
  - Promote healthy vaginal flora
  - Promote innate immune responses
- Facilitators:
  - Recruit and activate immune target cells
  - Activate NFkB pathways to promote HIV replication
  - Disrupt epithelial barrier
  - Interfere with innate responses

Cytokines/Chemokines

- Activators: Enhance HIV infection
  - TNFα, IL-1, IL-6, IL-12, chemokines
    - Activate NF-κB, which binds to HIV LTR to initiate or increase viral transcription.
    - Recruit and activate immune target cells
- Suppressors: Inhibit HIV infection
  - IFNα: Antiviral activity, suppresses RT
  - RANTES, MIP1α, MIP1β: inhibit co-receptor binding
  - IL-10: Inhibits HIV replication
  - IL-13: Down modulates CCR5 expression

Antimicrobial proteins

- Defensins:
  - Inhibit HIV in vitro (HBDs and HNP1-3)
  - BUT also recruit immune cells and induce inflammatory responses
- SLPI
  - Anti-inflammatory
  - Direct antiviral activity (?)
  - Higher levels associated with reduced HIV acquisition/transmission
- Lactoferrin
  - Direct inhibitory activity in vitro
  - BUT “alarmin”: recruits and activates immune cells

Mucosal inflammation and HIV

- Increased risk of transmission
  - IL-1β and IL-8 associated with higher cervicovaginal HIV-1 RNA concentrations, even after controlling for plasma viral load and vaginal microbial cofactors
- Increased risk of acquisition
  - Higher viral set point
  - Lower CD4 count

Mitchell, C et al AIDS Res Hum Retro 2011 27: 35
Roberts, L et al JID 2012 205(2): 194

Factors associated with ↑ HIV Risk

- Sex
- STI
  - HSV, HPV, Bacterial STD, Trichomonas
- BV
  - Decrease SLPI
  - Increase IL-1β
- Pregnancy
- Adolescents
- Depot medroxyprogesterone

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Sex/Semen and Mucosal Inflammation

- Buffers protective acidic pH
- Enriched in cytokines/chemokines
- Triggers MIP-3α, GM-CSF, MCP-1, IL-6, and IL-8 from genital tract epithelial cells
- Induces TNFalpha

Sharkey DJ et al Mol Hum Reprod. 2007;13(7):491-501.
Berlier W et al Hum Reprod. 2006;21(5):1135-42.
Lisco, A et al JID 2012; 1:97-105

Sex Increases Immune Targets

- Women who had sex within 3 days had higher cervical CD3+ (76 ± 4%) and CD4+ T lymphocytes (58 ± 6%) compared to women who last had sex >3 days prior to evaluation (CD3+ 54 ± 6%, CD4+ 39 ± 4%).

Prakash M et al. Am J Obstet Gynecol. 2003;188(2):376-81

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HPV

- HPV associated with increased HIV risk*
- ? Mechanisms
- Compared concentrations of immune mediators in CVL from HIV-negative women with high risk HPV positive (HRHPV+) CIN-3 (n=37), HRHPV+ CIN-1 (n=12), or PAP negative controls (n=57).

*Smith-McCune KK et al PLoS One **2010**; 5:e10094

Cervical Dysplasia Associated with Lower “Protective” and “Higher” Inflammatory Mediators

Mhatre et al, under review

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Bacterial vaginosis and HIV acquisition: a meta-analysis of published studies

Julius Atashili[a,b], Charles Poole[a], Peter M. Ndumbe[b], Adaora A. Adimora[a] and Jennifer S. Smith[a]

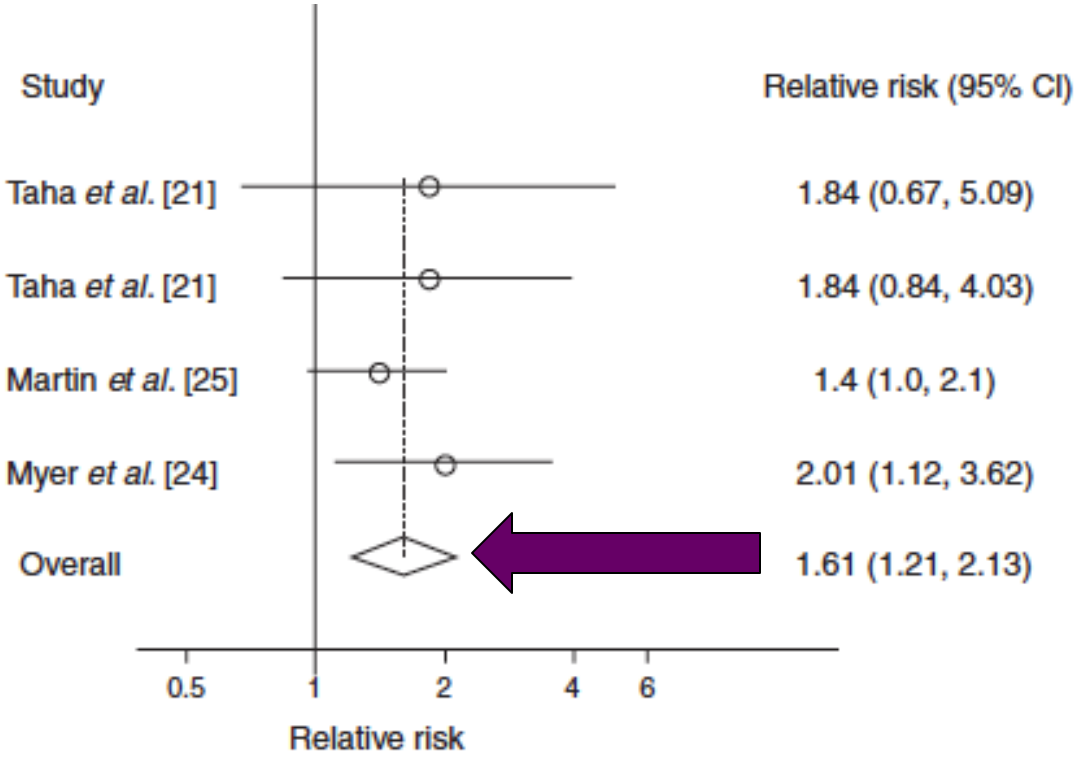

Fig. 1. Forest plot of relative risk estimates of incident HIV infection by bacterial vaginosis status, stratified by HIV-risk group. Studies are identified by the references. The horizontal lines represent the 95% confidence intervals (CI). Overall heterogeneity $P = 0.7$.

- Possible mediators
  - Loss of H2O2 (directly virucidal)
  - Activation of CD4 by alkaline pH
  - **Upregulation of cytokines that promote local HIV replication (TNF-alpha, IL-1 beta)**
  - Direct stimulation of HIV expression from T cells/monocytes by BV-associated bacteria

Atashili 2008

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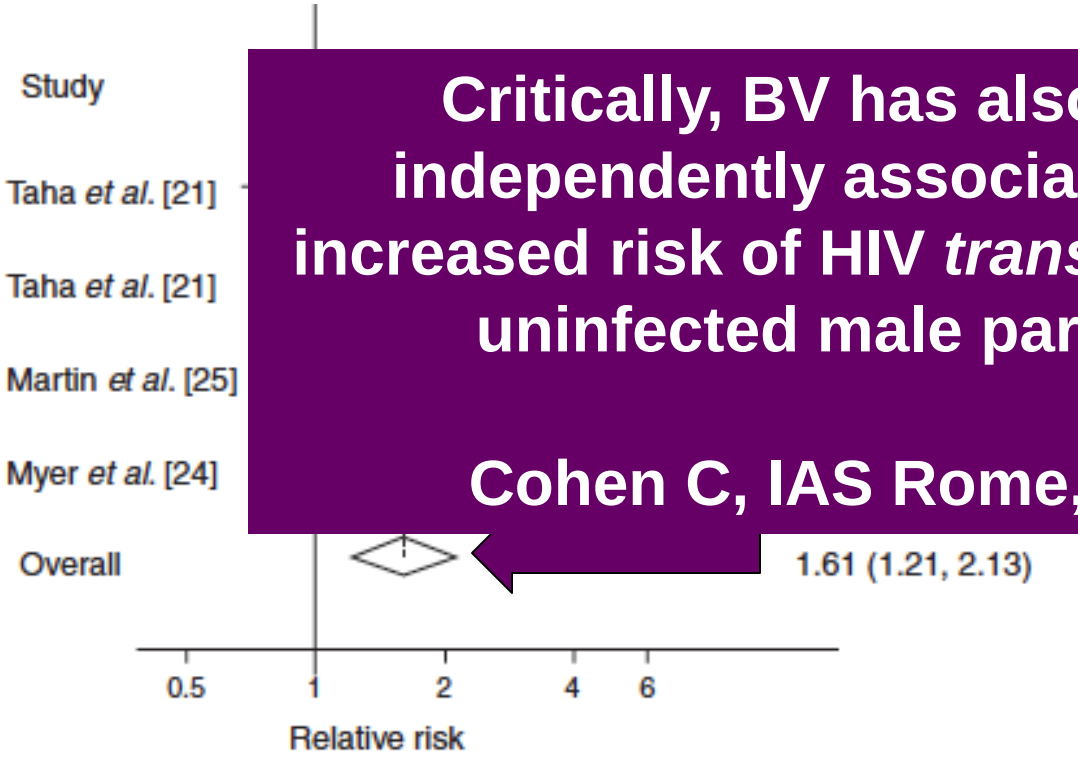

Critically, BV has also been independently associated with increased risk of HIV *transmission* to uninfected male partners

Cohen C, IAS Rome, 2011

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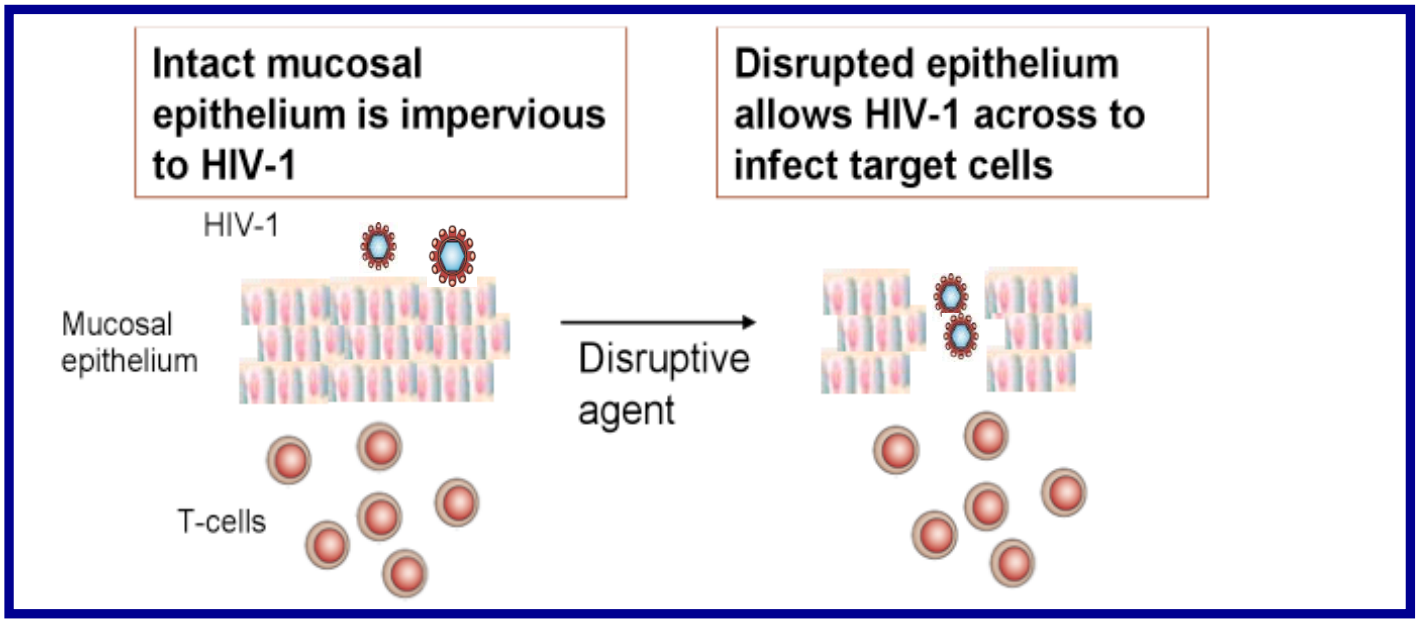
Pregnancy Associated with ↓ HBDs

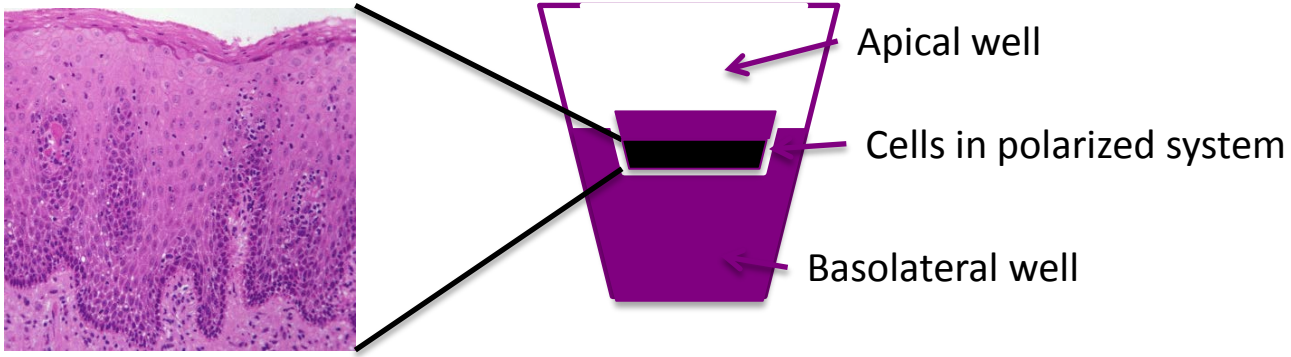
and Higher “Inflammatory” Cytokines

Measurement individual mediators may not capture complex interactions

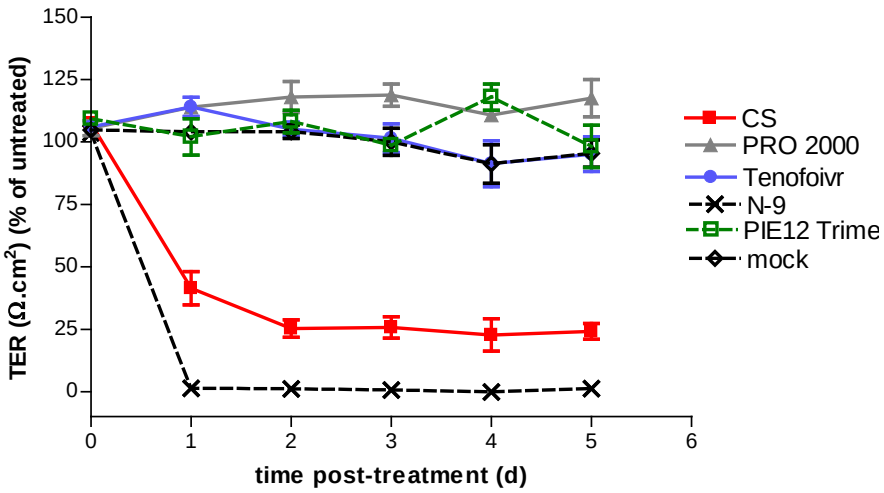
- "Functional assays"
  - Measure antimicrobial activity of secretions collected by swab or lavage
    - HIV
      - ??
    - HSV-2
      - Correlates with concentrations of HNP1-3, IL-8, Lf
    - E.coli
      - Proteomic studies suggest that this activity is mediated by host proteins and proteins secreted by Lactobacillus

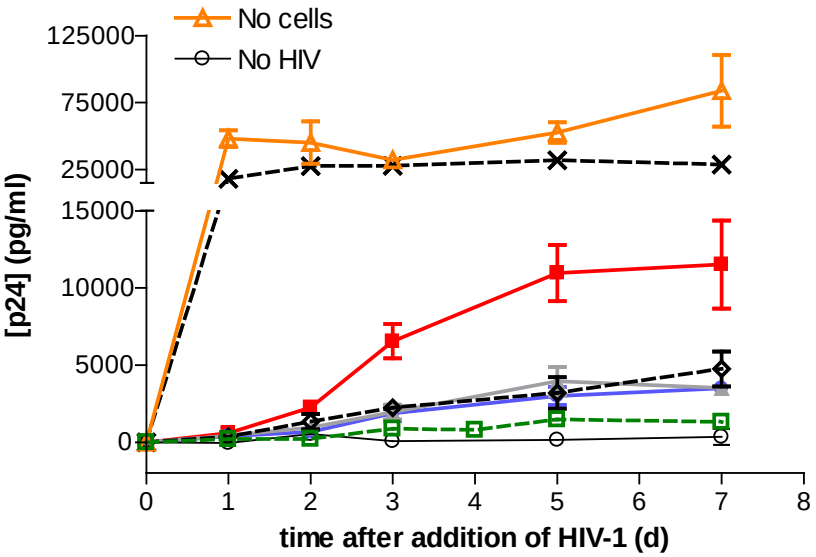
Inflammatory Cytokines May Disrupt the Epithelial Barrier





Microbicides may disrupt this barrier directly or by increasing inflammatory cytokines





Putting it all together..

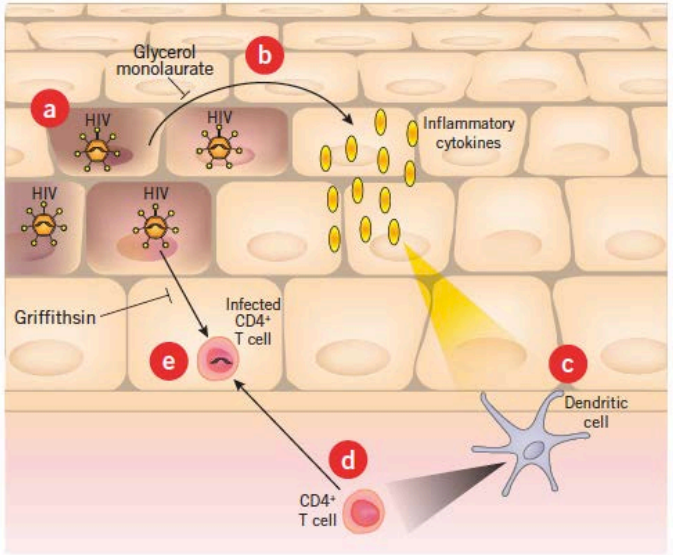
- Factors associated with increased HIV risk characterized by increases in inflammatory cytokines, increase in activated immune target cells, and lower levels of protective mediators
- Similar mucosal environment observed prior to HIV seroconversion; higher inflammatory mediators associated with higher viral set point
  - CAPRISA 002
  - CAPRISA 004

But this is only a snap-shot

- Association is not causality
- Inflammatory signaling cascades are complex
- Some inflammation is protective
  - Primes innate immune responses
- Too much may increase HIV risk
  - MTN 001 data
    - Higher levels in U.S. vs. Durban participants
    - Lower levels after 6 wks vaginal TFV
    - Is this protective or facilitating HIV infection??

Interventions?

- Directly block inflammation
  - Must be fine-tuned
  - Not disrupt ability of mucosa to respond appropriately to other pathogens
    - Ex. Glycerol monolaurate blocks DC/T cell recruitment by blocking MIP3a responses (Haase et al)



Interventions

- Treat/prevent underlying causes
  - STD rx efforts have failed to reduce risk of HIV transmission or acquisition
  - May reflect persistent inflammation
    - Ex. Activated T cells persist after resolution of genital herpes lesions
  - Vaccines may hold greater promise
- Augment natural host defenses
  - INFs, TLR agonists, recombinant defensins
  - Double-edged swords

Future Directions: Knowledge Gaps

- Assessment of inflammatory status complex
- Measurements of individual mediators may not tell the whole story
  - Need to consider complex interactions between mediators/signaling cascades/downstream events
  - Functional assays may provide more comprehensive measure but biological significance of measures unclear
- Inflammation & Microbicide Trials
  - Inflammation increases HIV risk in both placebo and rx arms
  - BUT may interfere with drug activity

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Gaps in Knowledge that Impact Prevention Efforts: PrEP and Vaccines

- What are the driving forces that enable virus to establish infection?
- How much virus is needed to transmit?
- What accounts for R5 viruses predominating?
- What are the first cells infected & what allows that infection to be amplified and disseminated?
- How do site specific differences in mucosal immunity impact HIV risk and prevention?
  - Vagina, ectocervix: (Type II mucosa)
    - Stratified squamous epithelia
    - Sparse submucosal immune cells
    - IgG predominant immunoglobulin
  - Endocervix and gut: (Type 1 mucosa)
    - Simple columnar
    - pIgA receptor; IgA predominates
    - MALT