

# **Development of Biomarkers Predictive of Microbicide Safety**

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# Limitations of Pre-Clinical & Clinical Trials

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- Pre-clinical studies focus on cytotoxicity in cell lines or explants
  - What is an acceptable selectivity index for vaginally applied drugs?
- Rabbit vaginal irritation model
- Clinical trials rely on colposcopy & adverse events
- Experience with N-9 suggests this may not be sufficient
- Measurement of cytokines & other inflammatory markers may provide insight into potential toxicity of surfactant-type agents
  - Precisely how or whether the inflammatory response to N-9 was responsible for the ↑susceptibility to HIV unknown
  - Biological/functional significance of ↑ cytokine levels in vaginal secretions not defined

# Safety Biomarkers

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- Goal is to identify/develop assays that predict **safety** of products that will be used repeatedly and intermittently, both vaginally and rectally
  - No cytotoxicity; high selectivity index
  - Non-inflammatory
  - No deleterious effect on normal vaginal flora
  - **Preserve or enhance mucosal immunity**
  - Little or no systemic absorption
  - Little or no selection for resistant variants

**In vitro**

**RVI**

**Phase I/II**  
(colposcopy)  
(cytokines)

**Phase III**

**Comprehensive  
Murine Model**

**Comprehensive  
Phase I Trial  
Focusing on Mucosal  
Immunity and Intrinsic  
Antimicrobial activity  
of Genital Tract  
Secretions**



# **Goal: Develop Comprehensive Murine Model to Assess Safety**

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- Inflammatory responses
- Determine effect on mucosal immunity
- Impact of frequent & intermittent application
- Biologic significance
  - Do observed changes in immune mediators enhance sexually transmitted infection?

# Experimental Design

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- Balb/c mice treated w/ Depo-Provera 5 days prior to intravaginal gel application
- 40  $\mu$ l of formulated gel applied daily for 14 days
- Vaginal washes collected on Days 0, 3, 7, 14 & 21
- Groups of mice (n=5) sacrificed D 7, 14, and 21  
Vaginal tissue excised & analyzed by H/E staining, FACS, or RT-PCR

# Results Summarized:

- Significant  $\uparrow$  in MCP-1, MIP-2, and IL-1 $\beta$  in vaginal washes at days 3 and 7 ( $p < .01$ ) for N-9 treated mice compared to baseline.
- PRO 2000 treated animals demonstrated only a significant  $\uparrow$  in MIP-2 & only on day 3 ( $p < .001$ ).
- There was a significant increase in the total number of leukocytes in vaginal tissue by FACS after 7 days of N-9, but not PRO 2000, treatment ( $p < 0.05$ ),
- Histology demonstrated N-9, but not PRO 2000, induced epithelial cell disruption & inflammation
- N-9, but not PRO 2000, triggered NF $\kappa$ B and AP-1

# Biological Significance of Inflammatory Response to N-9

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- Mice pretreated with Depo-Provera and then received 40  $\mu$ l N-9, PRO 2000 or HEC intravaginally for 7 days.
- 12 hours after last dose, mice challenged with low dose of HSV-2 (G) ( $\log_{10}4$  pfu)
- N-9  $\uparrow$  susceptibility to HSV compared with mice treated with PRO 2000 ( $p = 0.002$ ) or HEC ( $p = 0.03$ ).
- PRO 2000 treated mice showed no increase in susceptibility

# Balance Between Protection & Infection

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## Infection

*High Inoculum*

↑Target cells

Loss of protective barrier

Inflammation or disruption

STI

Microbicides

Trauma

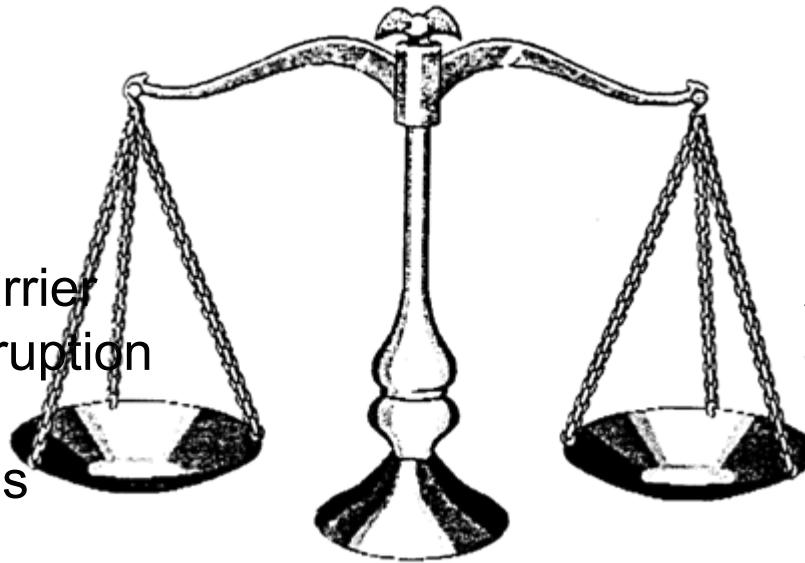
Loss of protective mediators

Defensins

SLPI

Calprotectin

Lactoferrin



## Protection

*Low inoculum*

Intact mucosa

**Acidic pH**/vaginal flora

**Innate immunity**

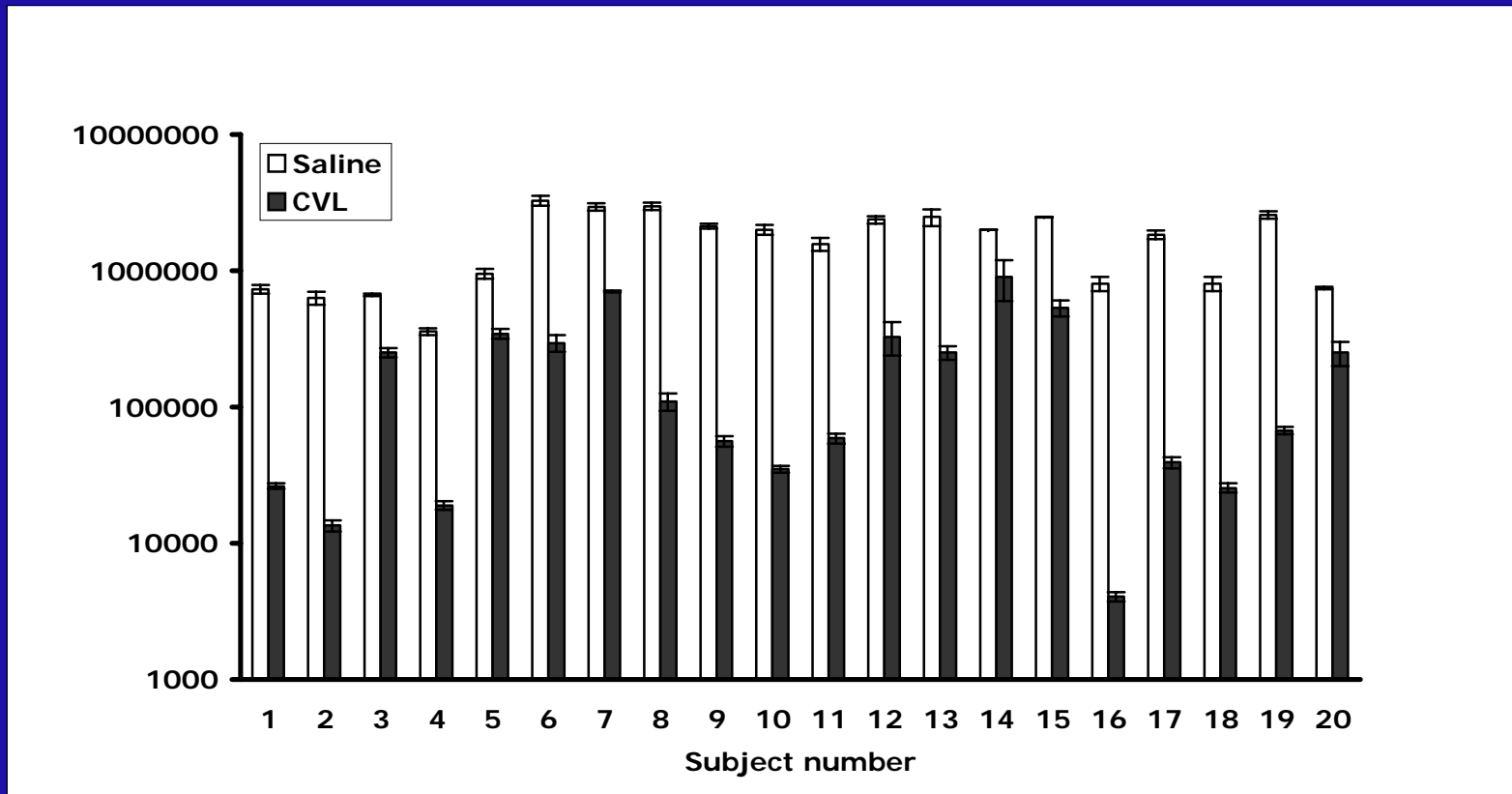
**Defensins**

**SLPI**

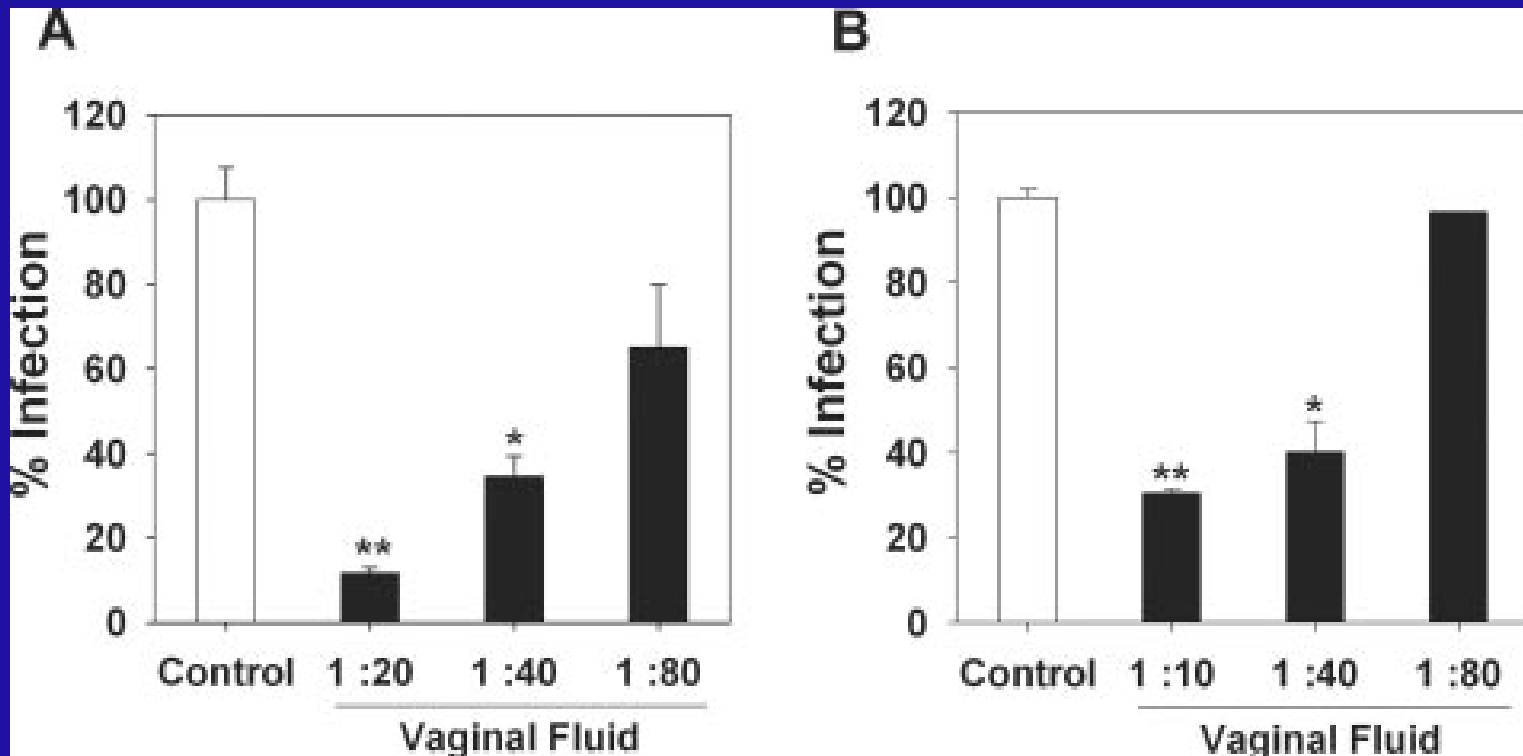
Calprotectin

Lactoferrin

# Cervical Secretions Protect Against HSV, Independent of pH



# Vaginal Secretions Provide Innate Protection Against HIV



Cells rx'd with PBS or pooled vaginal fluid diluted in DMEM & then challenged with BaL (A) or IIB (B)

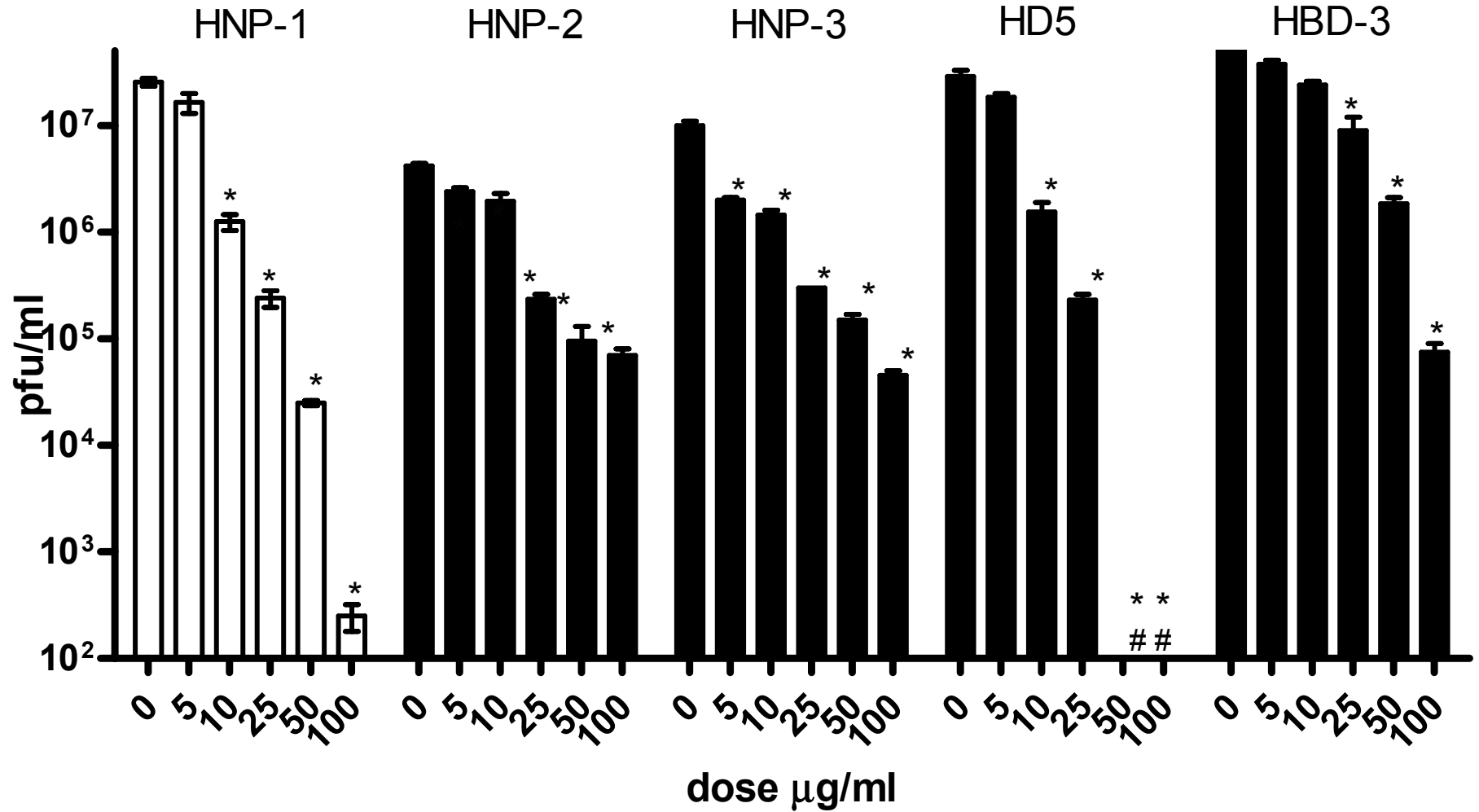
**Venkataraman JI 2005, 175:7560**

# Cationic Peptides Contribute to Intrinsic Anti-Viral Activity

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- HSV:
  - Activity correlates with HNP concentration
  - Anti-viral activity lost in 10% serum, which inactivates defensins
  - Anti-HSV activity ↓ in subjects with LOW conc of defensins (HIV-infected); restored by adding back CVL from pooled healthy controls
  - Other Defensins & SLPI inhibit HSV in vitro
- HIV:
  - Majority of activity in cationic fraction; restored if add back cationic fraction to cationic depleted fluid
  - Physiological conc of individual peptides alone not sufficient to inhibit HIV, but added together, partially restored anti-HIV activity

# Cumulative Anti-HSV Effects of Human Defensins



\*  $P < 0.05$ ; Hazrati, et al, J Immunol 2006 177:8658-66

# **Pilot Trial to Evaluate the Mucosal Response to PRO 2000 vs Placebo Gel**

- Objectives:
  - Investigate impact of 14 daily applications of 0.5% PRO 2000 or matched Placebo gel on cytokines, chemokines, and mediators of mucosal immunity
  - Evaluate functional significance of any observed changes in specific mediators
    - Anti-viral activity
    - Anti-bacterial activity
  - 24 healthy women enrolled (12 placebo, 12 Drug)
    - CVL obtained on Days 0, 7, 14, 21
    - Colposcopy done at Day 0 and Day 14

# Results

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- **No significant colposcopic findings in either group**
- **No increase in inflammatory cytokines**
- **Decline in select mediators observed on Days 7 & 14 between PRO 2000 & Placebo which returned towards baseline on Day 21**
  - Significant differences were observed for IL-1RA ( $p < 0.05$ )
  - Trend towards significance for IL-6, IL-8, HBD-2, SLPI, IgG and IgA ( $p < 0.1$ )
- **Subgroup analysis indicates**
  - Cycle effect: concentration of select factors is ↓ in women who are cycling compared to OCP users
  - Drug effect: Among cyclers, further ↓ in PRO 2000 compared to Placebo group, statistically significant
- **No loss in intrinsic anti-viral or anti-bacterial activity in CVL**

# Conclusions

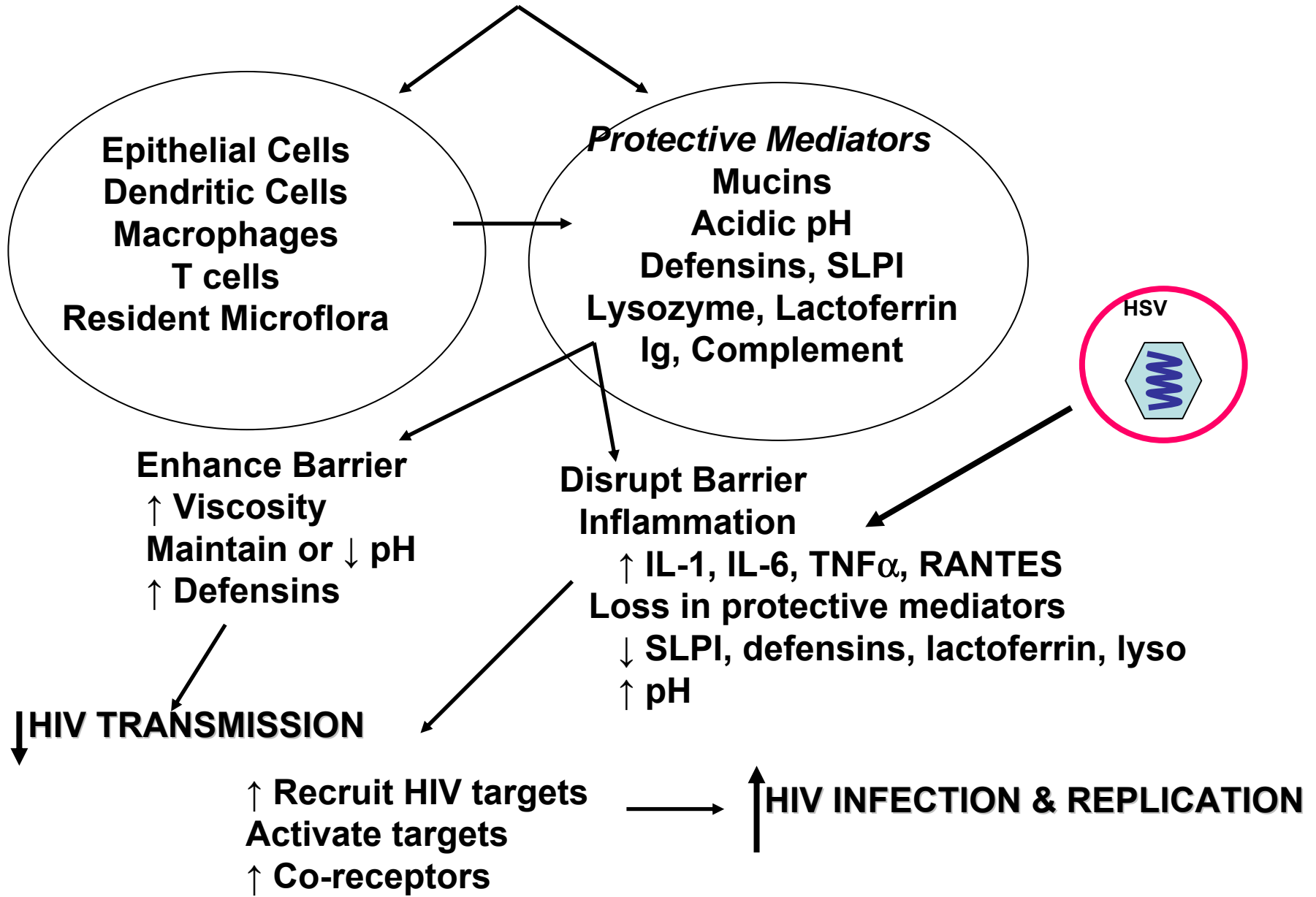
- PRO 2000 does not trigger an inflammatory response
- PRO 2000 triggered ↓ in selective protective mediators
  - No significant change in TGF- $\beta$  or lactoferrin
  - Cyclers had lower levels of immune mediators compared to women on OCPs
- Trend towards ↓ in mediators requires further study
  - Carbomer effects?
  - Signaling pathways? (TLR, NOD2/CARD15)
- No significant loss in anti-viral or anti-bacterial activity

# Future Directions

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- Additional long-term studies warranted to determine whether a sustained loss in mediators could lead to increased susceptibility to infection.
- Testing additional compounds could provide an assessment as to whether these assays prove predictive of safety.
- If validated, this strategy should be included in the algorithm to assess future-generation microbicide safety and help identify which candidate drugs to prioritize in development.

# Microbicides



# Proposed New Safety Algorithm

## **In vitro:**

Cell lines  
Primary cells  
Explants

**Cell viability; growth**

**Cytokines**

**Innate immune mediators**

**Functional assays**

## **Animal Models:**

Rabbit  
Mouse  
Macaque

**Histology**

**Recruitment of cells**

**Innate immune mediators**

**Functional assays**

## **Clinical Trials:**

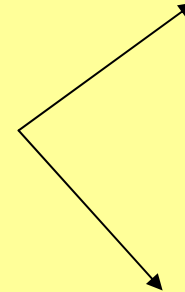
**Clinical symptoms**

**Colposcopy**

**Cytokines**

**Innate immune mediators**

**Functional assays**



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