Can We Use Biomarkers in Microbicide Trials to Predict Efficacy & Safety?

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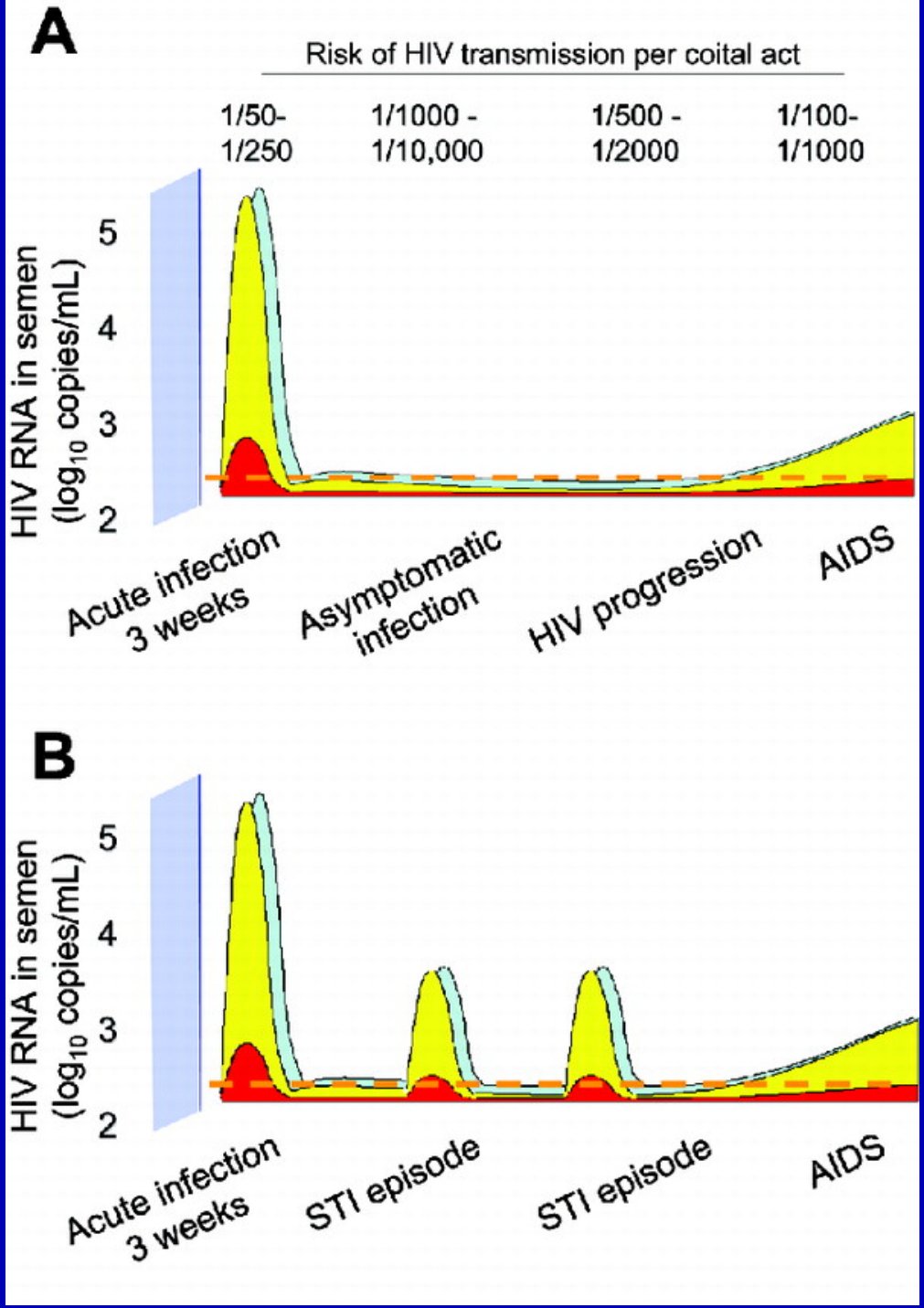
Attributes of Effective Microbicide

- Active against R5 and X4 isolates
- Active against multiple clades
- Block cell-free & cell-associated infection
  - Mϕ, T cells, DCs
- Rapid onset of action; prolonged activity
  - Coitally independent
- Active in genital tract environment
  - pH, cervical secretions, semen
- Active against other STI

Biomarker(s) Predictive of Efficacy:

- No gold standard
- No animal model recapitulates HIV transmission
- Optimal assays not defined
  - Cell lines & lab isolates
  - Explant models may provide additional insights
  - Extent of anti-viral activity predictive of in vivo protection *unknown*
    - IC50/IC90 probably NOT sufficient
    - Which clades and how many different isolates need to be tested in vitro?
  - Formulation impacts efficacy



Prediction of the efficiency of HIV transmission according to HIV burden in the genital tract.

A: **Probability of male-to-female HIV transmission per coital act**

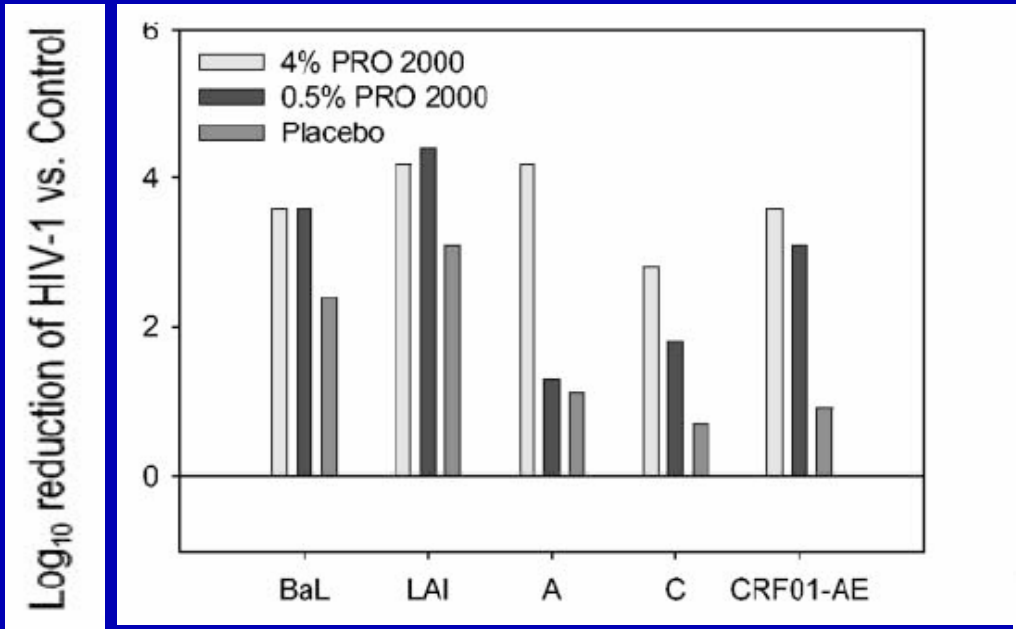
. *Yellow,* Expected distribution of viral burden in semen among men over time;

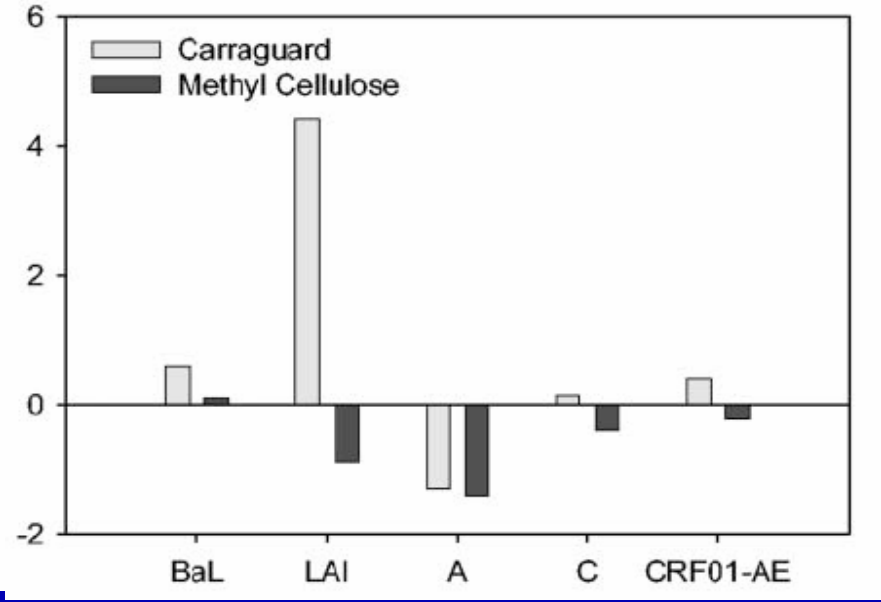
***Red,* theoretical effect of intervention**

D*ashed line,* a potential threshold for HIV transmission.

Journal of Infectious Diseases 2005;191:1391-1393

Prevention of PBMC Infection by Different Clades





Primary isolates: A (Central Africa & Asia), C (Central & South Africa), and CRF01-AE (Asia)
Virus cultured with PBMCs in the presence or absence of product and placbeo;
Infection was measured by the release of p24gag in the supernatants
from day 4 or 7 of culture.

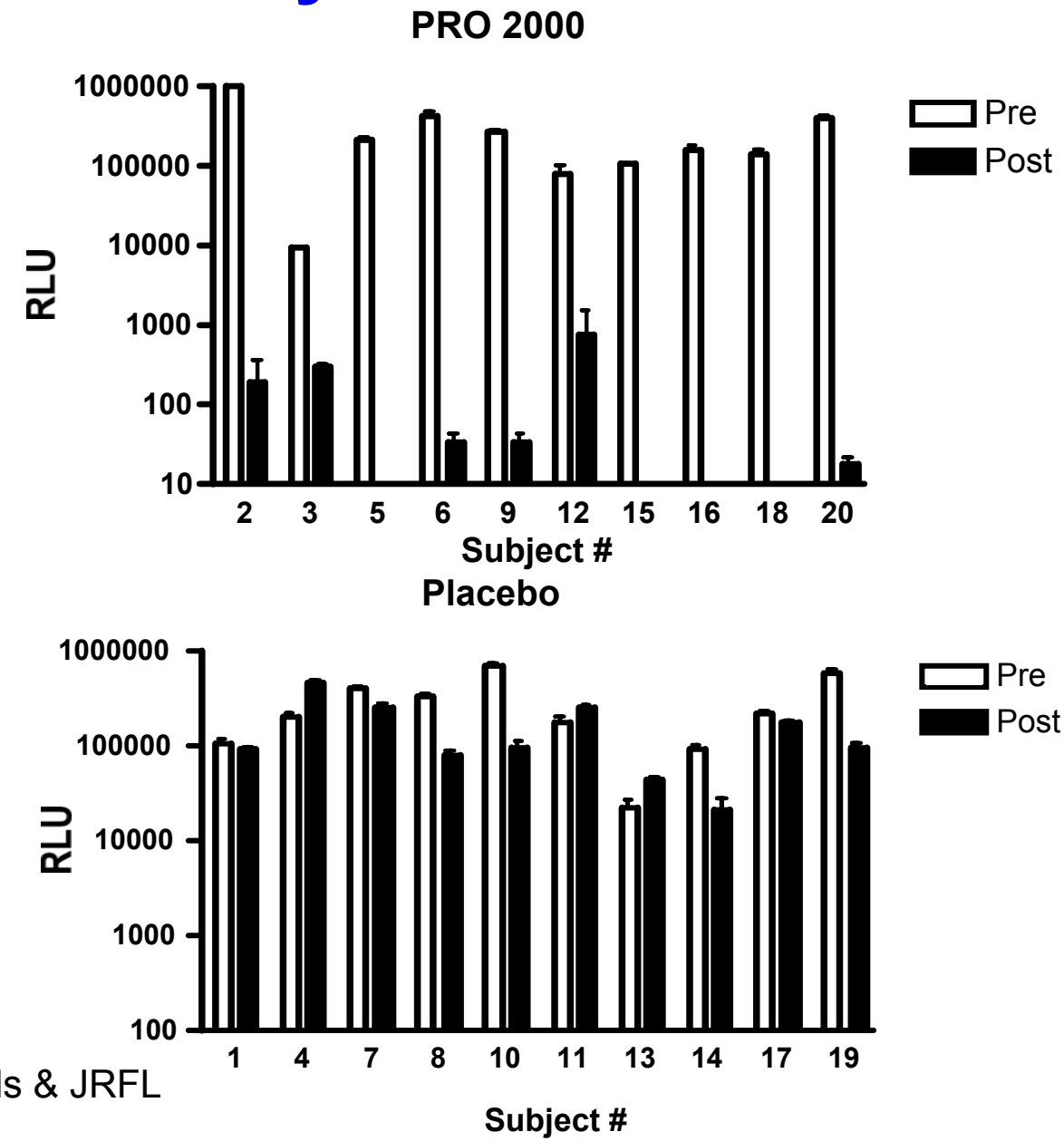
Dezzutti, CS et al, AAC:2004; 48, 3834

Development of Biomarker of Efficacy

- To determine the extent of anti-HIV & anti-HSV activity in cervical fluid obtained 1 h after gel application using a spiking strategy
  - 0.5% PRO 2000 vs. matched Placebo gel
- Enrolled 20 women

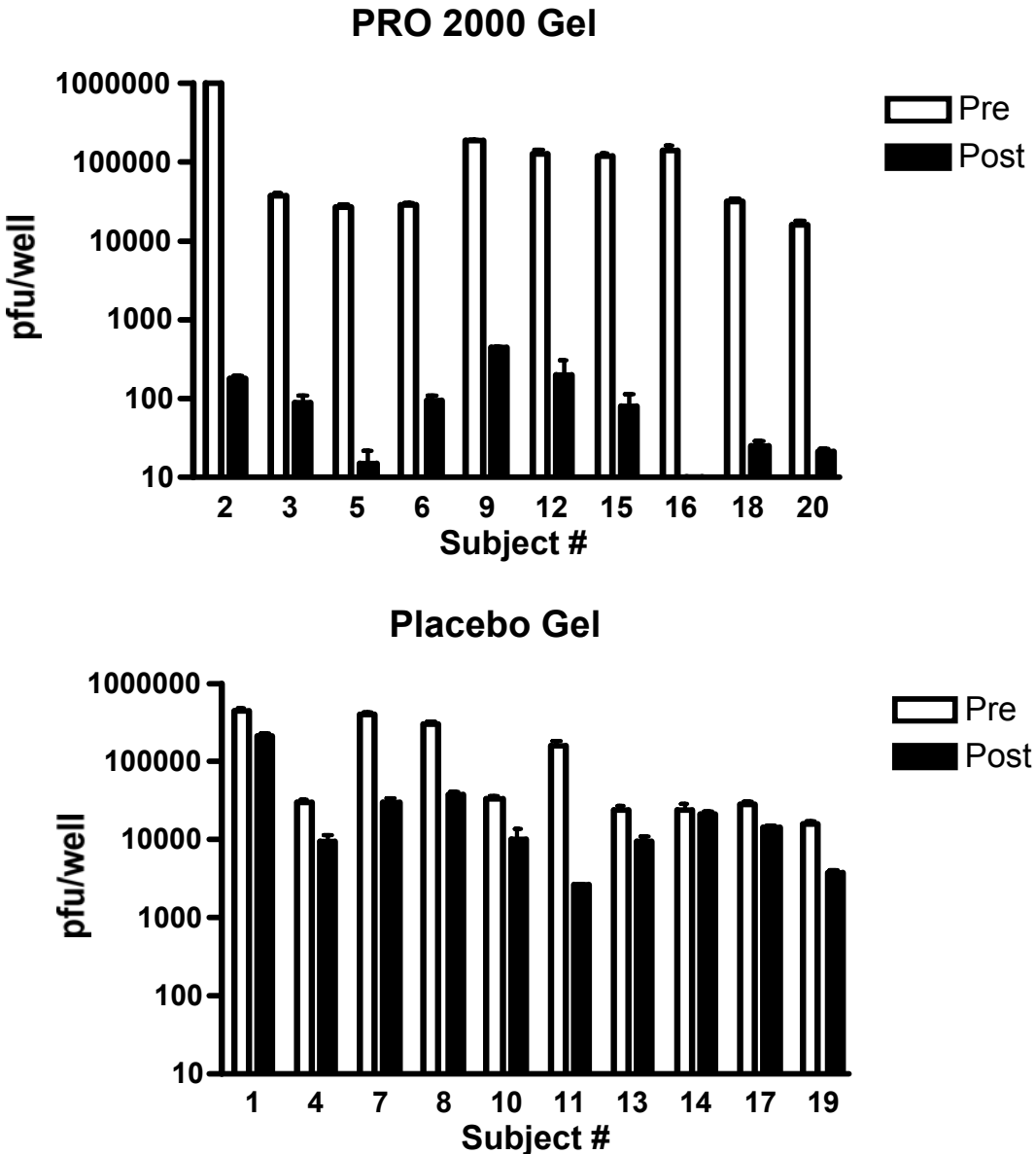
Keller, MJ et al JID 2006 Jan 1;193(1):27-35.

Anti-HIV Activity of CVL Pre & Post Gel



HeLaCD4-CCR5 cells & JRFL

CVL Obtained Post PRO 2000 Inhibits HSV Infection



Conclusions: Single Dose Trial

- CVL obtained 1 h post-application PRO 2000 significantly inhibits HIV infection
  - Extent of activity correlated with in vitro activity of similar drug concentration: 100-300 μg/ml
- CVL inhibits HSV-2 infection by > 4-logs
- Activity is ↓↓ if spike with virus in seminal plasma (work in progress)
- Post-coital pilot clinical study planned
- This inexpensive clinical assay may provide biomarker predictive of efficacy & should be conducted early in clinical development
  - Limitation: Not applicable to drugs that act intracellularly

Keller, et al. JID 193: Jan., 2006

Safety: Limitations of Pre-Clinical & Clinical Trials

- Clinical experiences with N-9 & Cellulose Sulfate demonstrate that current assays to predict safety are inadequate
  - Pre-clinical studies focus on cytotoxicity in cell lines or explants; acceptable selectivity index not known
  - Rabbit vaginal irritation model current FDA standard
  - Clinical trials rely on colposcopy & adverse events
  - Modification of these assays by including measurement of select/limited # cytokines & chemokines also not predictive
  - Need for functional correlates

Safety Biomarkers

- 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

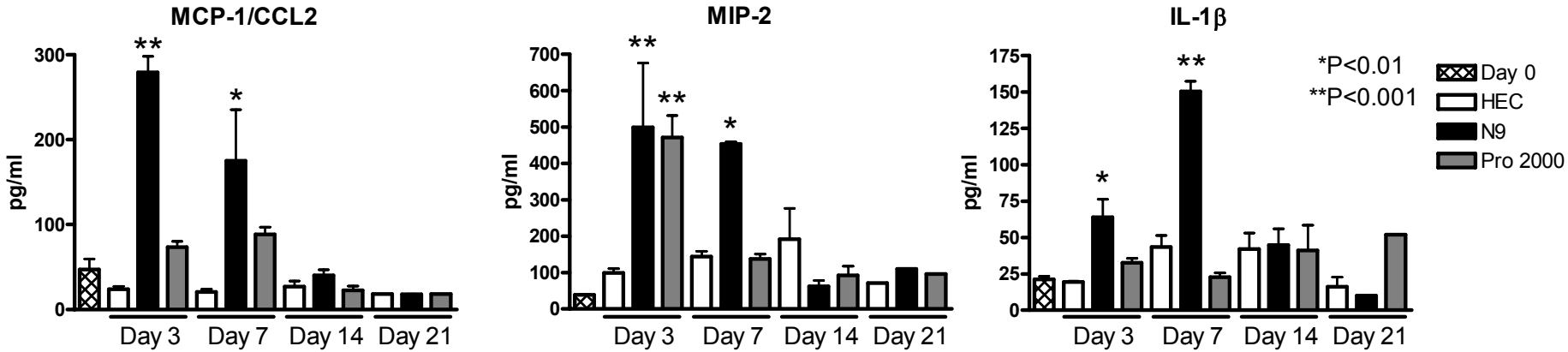
Goal: Develop Comprehensive Murine Model to Assess Safety

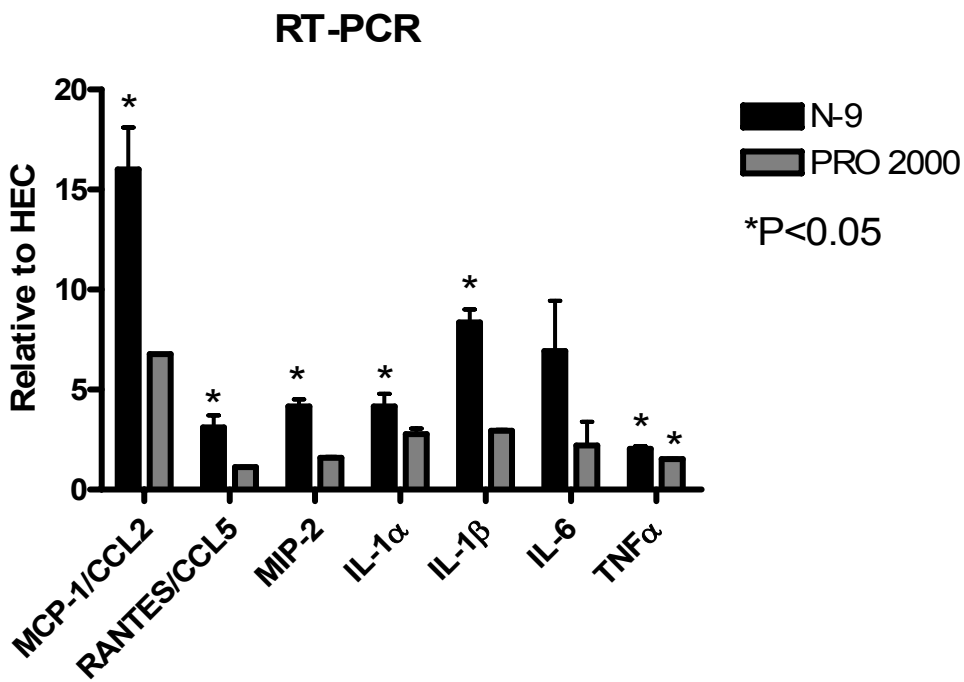
- Inflammatory responses
- Determine effect on mucosal immunity
- Impact of frequent & intermittent application
- Biologic significance
  - Do observed changes in immune mediators enhance sexually transmitted infection?

Experimental Design

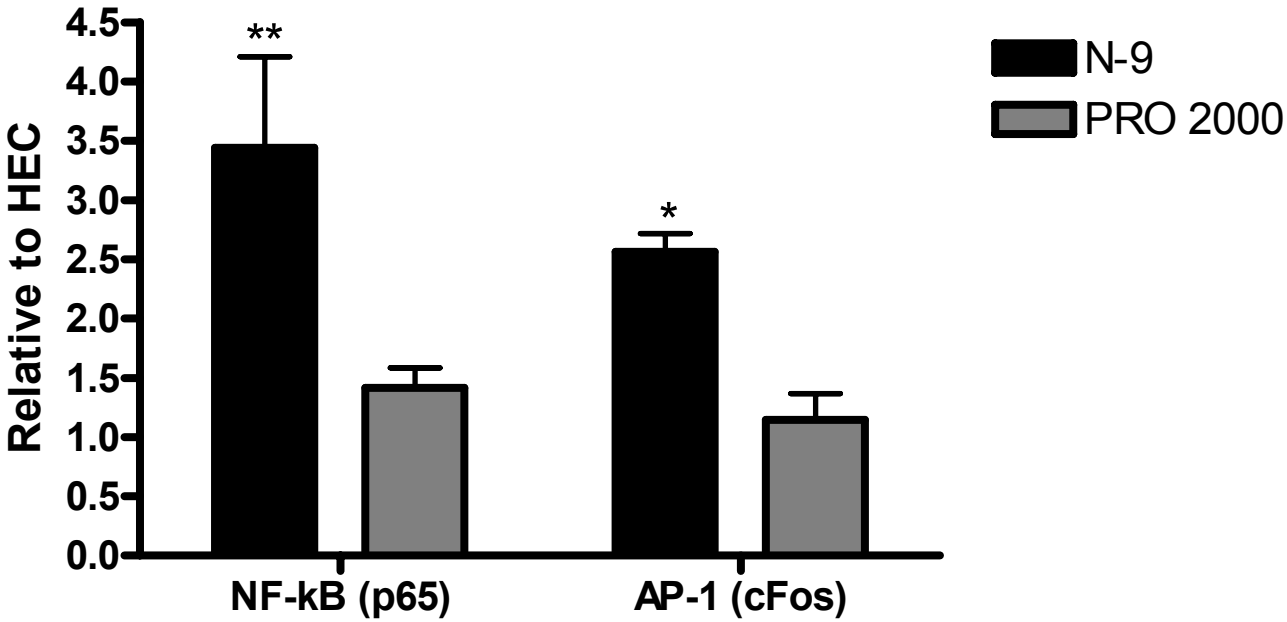
- Balb/c mice treated w/ Depo-Provera 5 days prior to intravaginal gel application
- 40 µl of formulated gel applied daily for 14 days
- Vaginal washes collected on Days 0, 3, 7, 14 & 21
- Groups of mice (n=5) sacrificed D 7, 14, and 21 Vaginal tissue excised & analyzed by H/E staining, FACS, or RT-PCR

Cytokines & Chemokines ↑ in Response to Microbicides





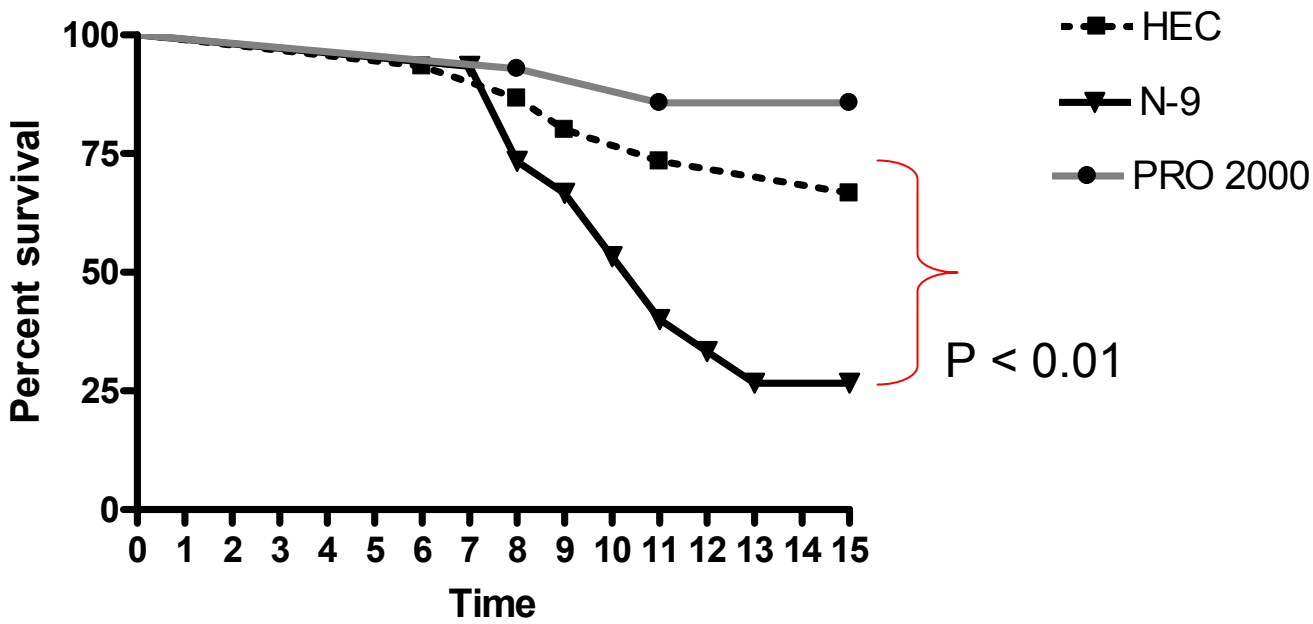
↑in Nuclear NF-κB (p65) and AP-1 (cFos) Following N-9 (Day 7)



Biological Significance of Inflammatory Response to N-9

- Mice pretreated with Depo-Provera and then received 40 μl 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 ↑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

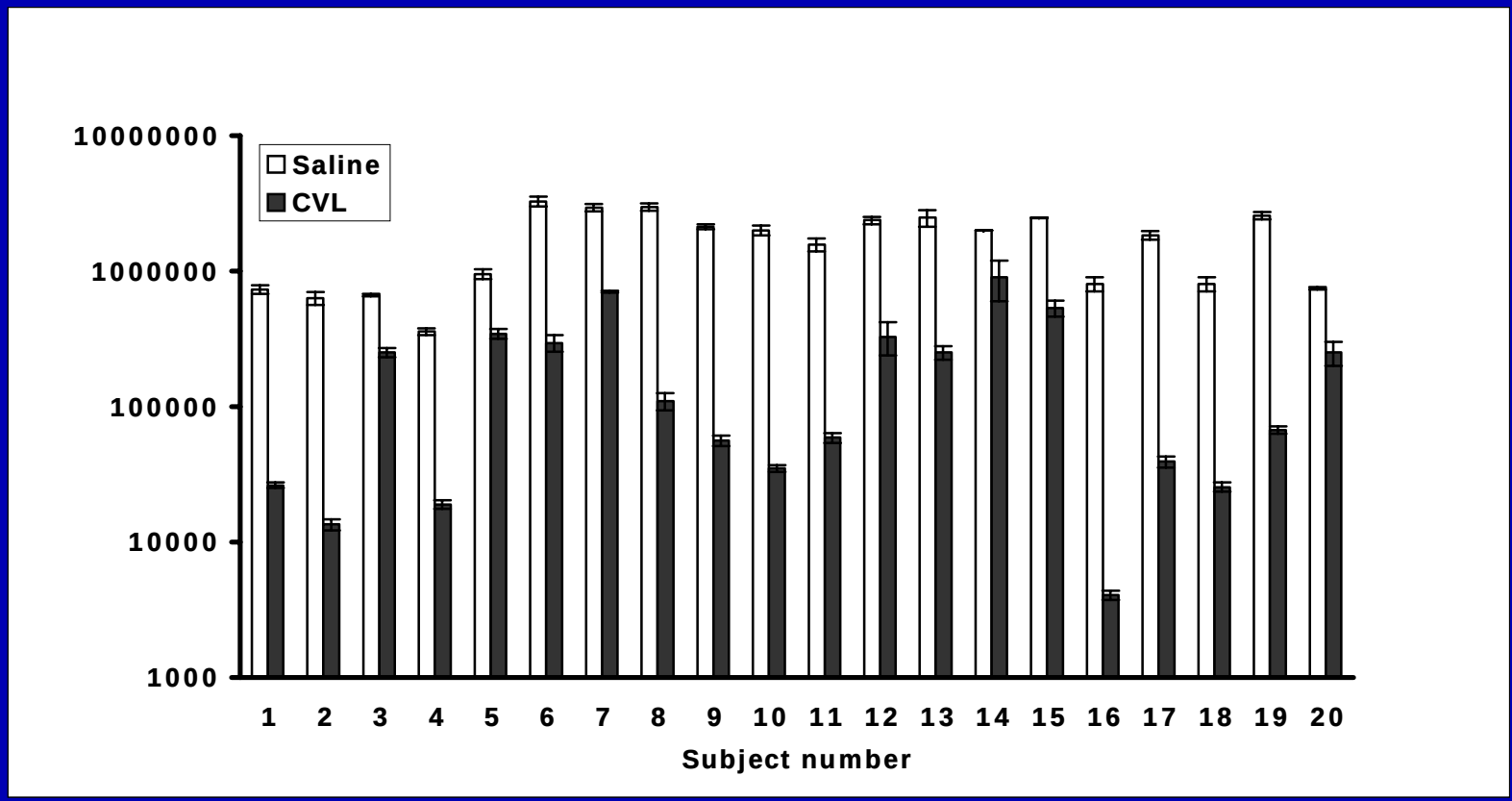
Chronic N9 Exposure in Mice Increases Susceptibility to Herpes



Implications

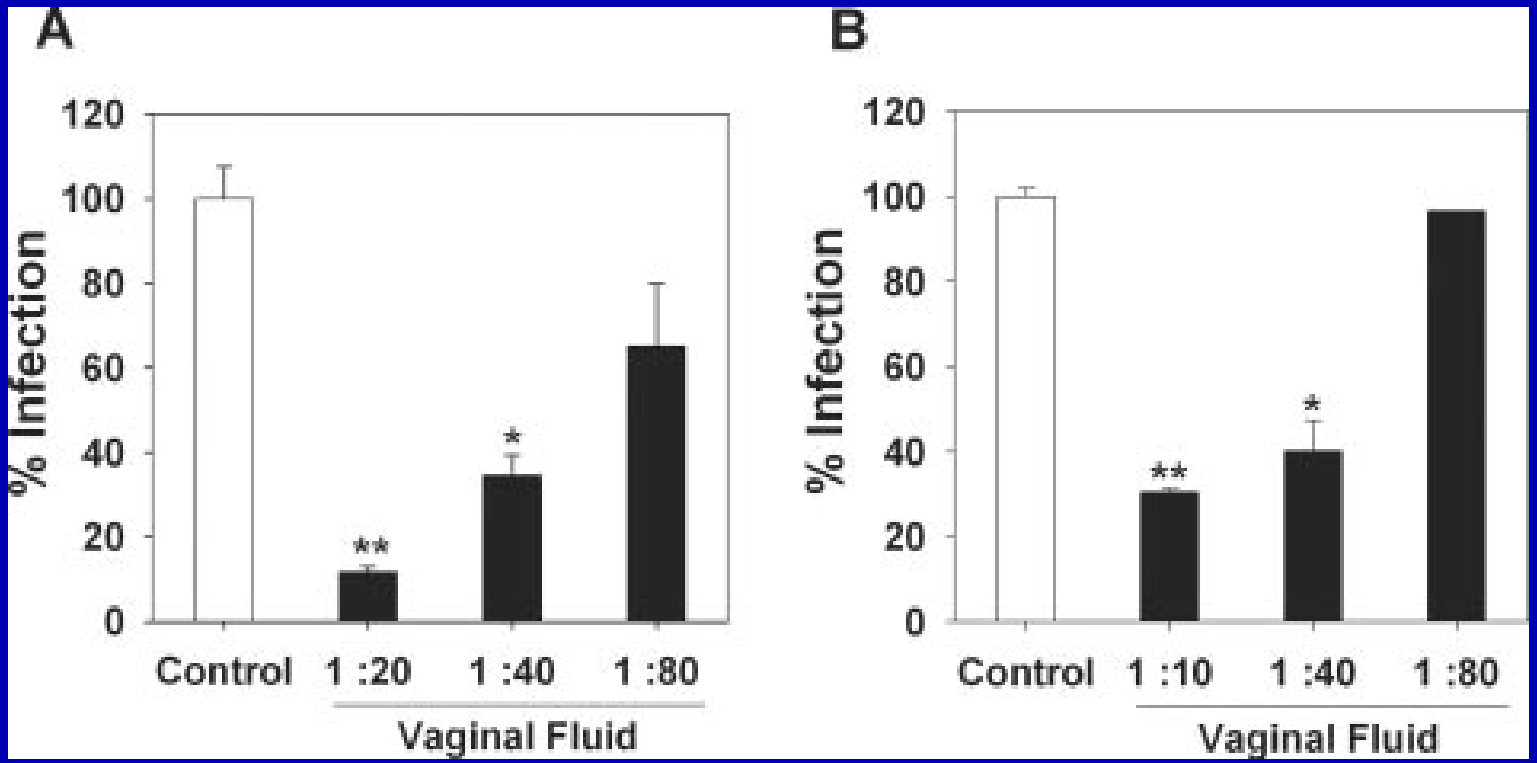
- This simple surrogate murine model may provide inexpensive biomarker predictive of microbicide inflammation
- Validation will require testing other drugs in this model and correlating results with clinical studies
  - 1% Tenofovir: No inflammation; no ↑ HSV susceptibility
  - Others in progress

Cervical Secretions Protect Against HSV, Independent of pH



John, Keller, et al. J. Infect Dis. 2005;192(10):1731-40

Vaginal Secretions Provide Innate Protection Against HIV



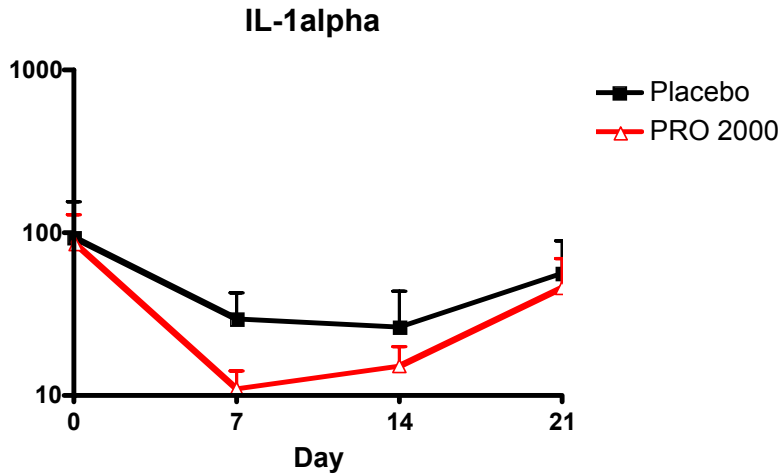
Cells rx'd with PBS or pooled vaginal fluid diluted in DMEM & then challenged with BaL (A) or IIIB (B)

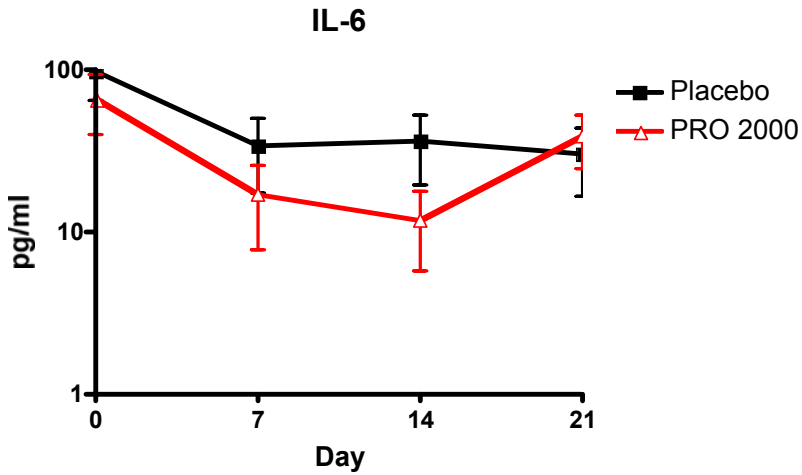
Venkataraman JI 2005, 175:7560

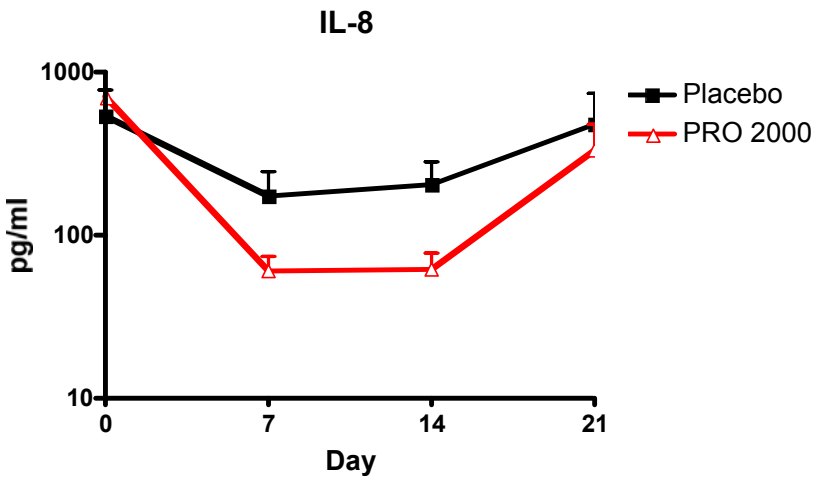
Pilot Trial to Evaluate the Mucosal Response to PRO 2000 vs Placebo Gel

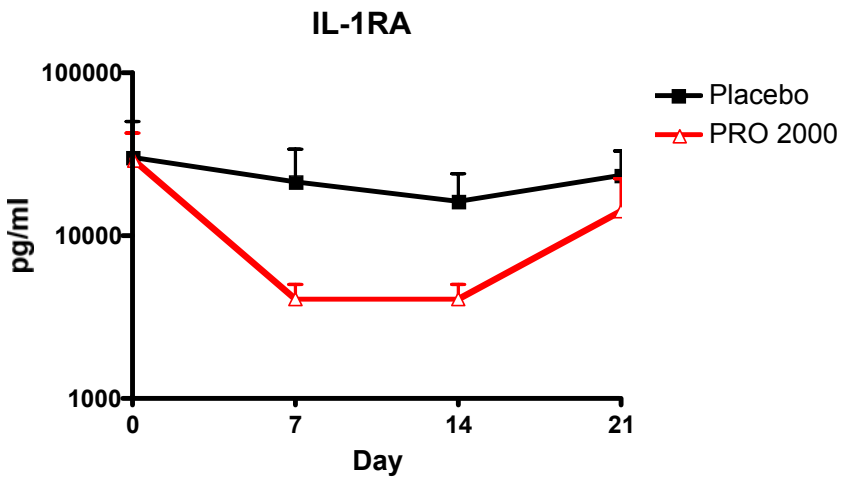
- Objectives:
  - Investigate impact of 14 daily applications of 0.5% PRO 2000 or matched Placebo gel on cytokines, chemokines, and mediators of mucosal immunity
  - Evaluate functional significance of any observed changes in specific mediators
    - Anti-viral activity
    - Anti-bacterial activity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

PRO 2000 Triggers Modest Loss in Mediators



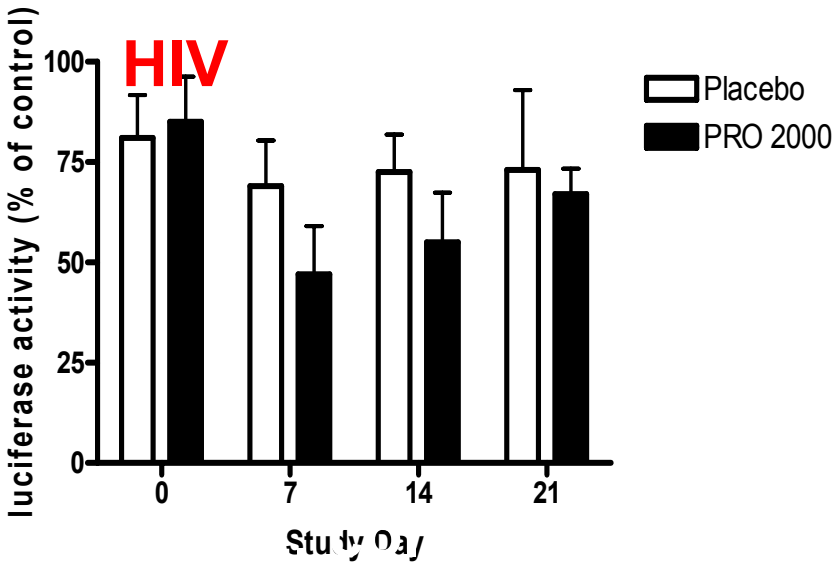


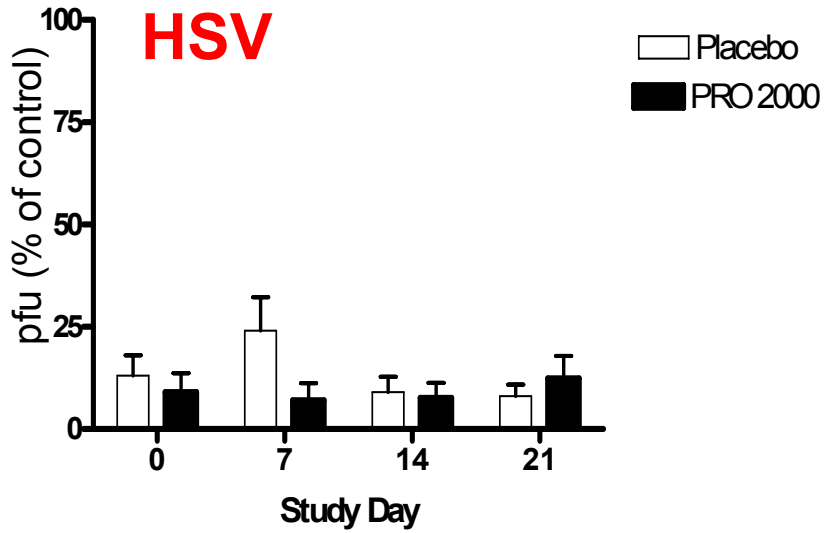


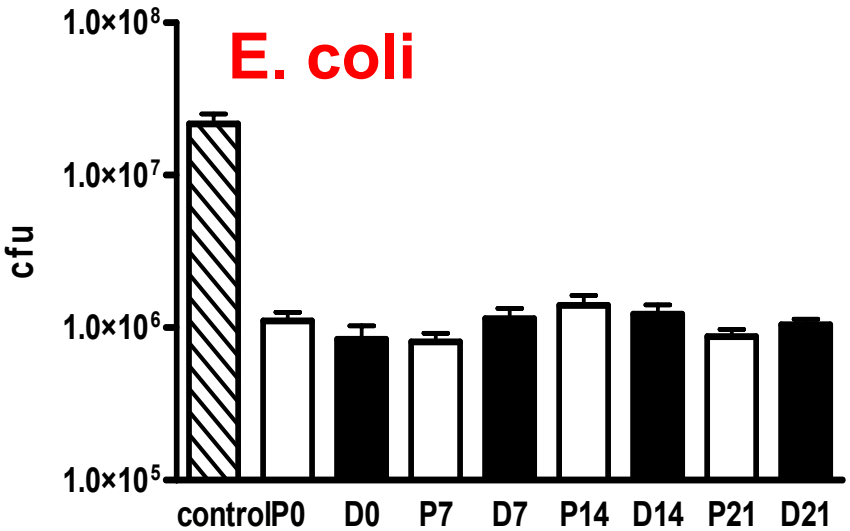


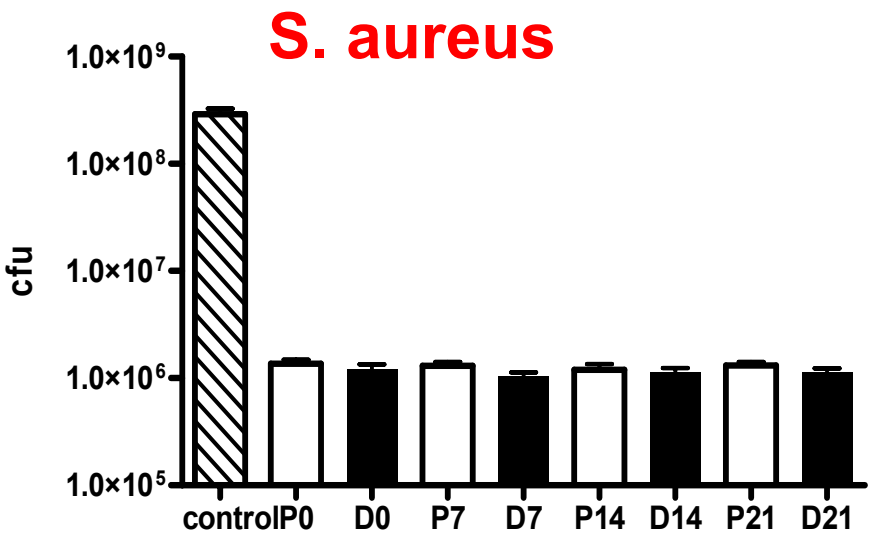
No inflammatory response
↓Cytokines on Days 7 & 14; $p < 0.05$ for IL-1RA (D7 & 14),
Drug effect persisted independent of cycle effect (IL1RA, IL-1, IL-8)

Intrinsic Antimicrobial Activity is Retained Following Gel Application









Conclusions: 14 d Safety Trial

- **No significant colposcopic findings in either group**
- **No increase in inflammatory cytokines**
- **↓ in mediators D7 & 14; returned to baseline D21**
  - Significant for IL-1RA ($p < 0.05$)
  - Trend towards significance IL-6, IL-8, HBD-2, SLPI, IgG & 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**

Keller, et al AIDS, 2007

Future Directions

- 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.

Proposed New Safety Algorithm

In vitro:
- Cell lines
- Primary cells
- Explants

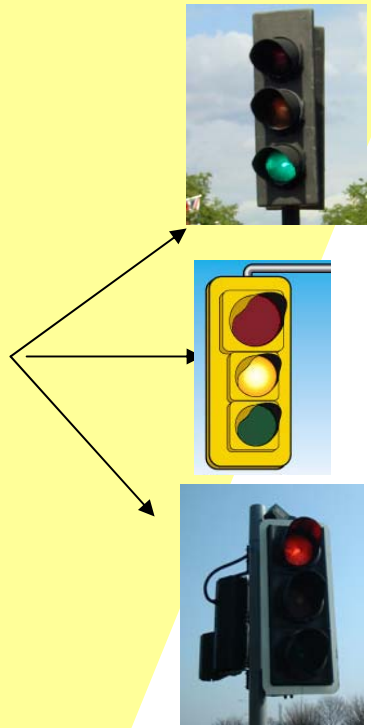
Cell viability; growth
Cytokines
Innate immune mediators
Functional assays

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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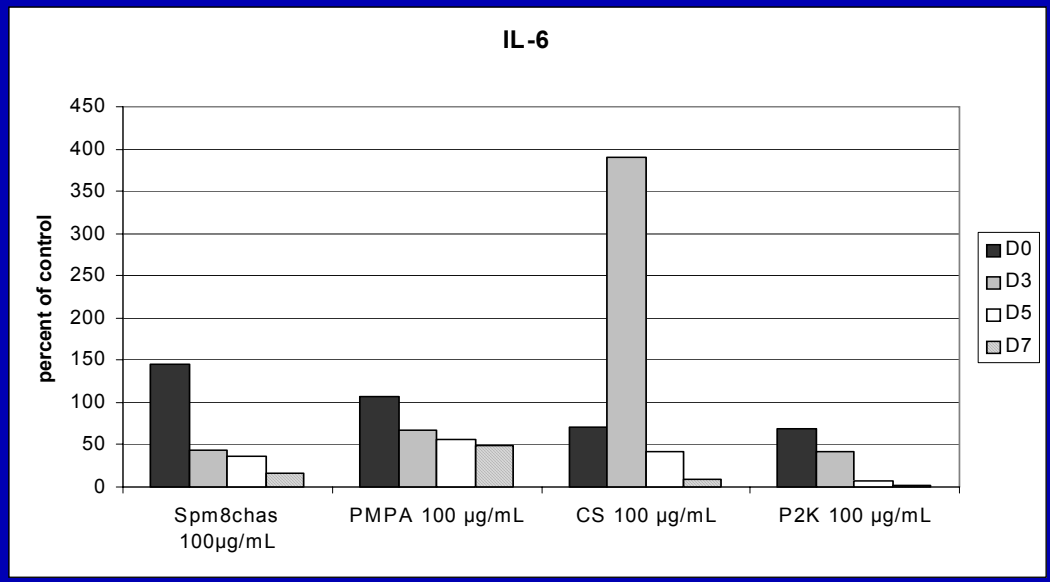
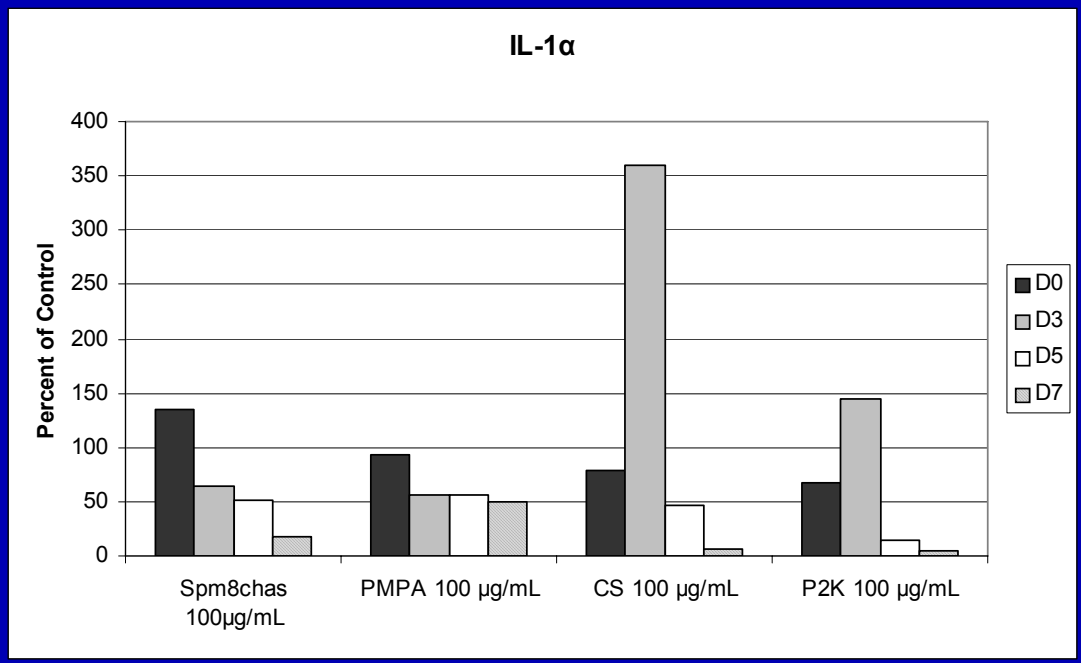
Cytokine Response to Microbicides





Impact of Microbicides on SLPI Anti-Inflammatory Anti-HIV Protein