Effect of the vaginal environment on infected leukocytes

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“Trojan Horse” workshop, Boston, 10/19/2013
Effect of the multiple vaginal environments on infected leukocytes

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Only a minority of women have a ‘normal’ lactobacillus dominated vaginal microbiota.

- pH 3.5
- 1% Lactic acid

- pH 5-6
- 0.3% Acetic acid
- Succinic acid
- Butyric acid
- Putrescine
- Cadaverine
- Tyramine (fishy odor)

- ‘Normal’ ~ 38%
- Intermediate ~ 33%
- BV ~ 29%

% of U.S. women on any given day

Allsworth and Peipert, 2007
BV is strongly associated with increased risk of infections and poor birth outcomes:

Populations with high prevalence of BV are at high risk of HIV:

BV increases several factors that may increase susceptibility to infections, e.g., inflammatory cytokines, and it also eliminates protection by Lactic Acid!
Most prevalent vaginal microbiota communities:

Pre-menopause, *L. crispatus, L. iners*, and IV (‘BV’)

Peri-menopause, IV-A (Prevotella) and *L. gasseri*

Post-menopause: IV-A

  Vulvovaginal atrophy post-menopause: IV-A

  Risk of VVA 25-fold higher with IV-A vs *L. crispatus*

Low pH immobilizes and kills human leukocytes and prevents transmission of cell-associated HIV in a mouse model

Olmsted, Khanna, et al, BMC Infect Dis 2005
VAGINAL ACIDITY INACTIVATES CELLS AND PATHOGENS WITHOUT INJURING MUCOSA

Lactobacilli acidify the vagina with lactic acid
Rapid fluctuation of vaginal microbiota (over 4 months)

Brotman, Ravel Cone, et al STI 2010
Temporal Dynamics of the Human Vaginal Microbiota

Communities

IV-A

L. crispatus

IV-B

L. iners

Nugent Scores

Vaginal pH and microbicidal lactic acid concentration when *L. crispatus* dominates the microbiota (Nugent Scores 0-3)

Our observations are based on Johns Hopkins students: *L. crispatus* and *L. iners*

\[ \Delta \text{pH due to loss of CO}_2 \]

O’Hanlon, Moench, Cone *in press* PLoS One 2013
*L. crispatus* acidifies vaginal pH to \( \sim 3.5 \pm 0.3 \)  

and produces a \( \sim \)racemic mixture of D- and L lactic acid.

When vaginal isolates of *L. crispatus* are cultured, they acidify the medium to essentially the same pH and D-/L- ratio as the vagina from which they were obtained.  
Boskey Cone Whaley Moench, Human Reproduction 2001

Essentially all our samples from **Hopkins students** are *L. crispatus* and *L. iners*.

We have collected over 250 Hopkins student samples with Nugent scores 0-3.

At least 70 of these students were black,

We had only **one** BV sample, and the donor was white.   
O’hanlon *in prep.*

Most of our low Nugent score samples from the **Baltimore STD clinic** are *L. iners*.

*L. iners* produces only the L-isomer.  
Witkin et al, mBio, 2013

*L. iners* in vitro is less susceptible to the diamines produced by BV-bacteria than *L. crispatus*. So *L. iners* “survives” BV better?  
Li Han Lai JHU Masters Thesis 2010
Acetic acid concentrations during BV

pH 7.0: [acetic acid] mM at pH 4.5

Lactic acid

Healthy flora: 1.2%

pH 7.0: [lactic acid] mM at pH 4.5
L-Lactic acid is more potent than D- and DL-LA in inactivating HIV$_{\text{Ba-L}}$.

Vaginal concentrations of lactic acid potently inactivate HIV
Aldunate, Tysssen, Johnson, Zakir, Sonza, Moench, Cone, Tachedjian, J. Antimicrob Chemother 2013
Acid inactivation of PBMC at 37°C in RPMI media acidified with HCL or 0.5% Lactic Acid

$\text{pKa of Lactic acid } 3.8$

$\text{LA}^- + \text{H}^+$

Fraction live PBMC

Exposure (minutes)

pH 3.5
pH 4.5
pH 5.5
Human vaginal epithelium and shed cells stained for glycogen

Rakoff et al, AJOG, 1944
Mucus penetrating particles 100 nm in diameter delivered acyclovir more effectively than free drug for preventing HSV infections in the mouse vagina. “Advective” transport caused by osmotically driven absorption of water from the lumen.
Literature values for vaginal pH and %LA

O'Hanlon et al, PLoS One 2013

Relative infectivity compared to untreated control

[L-Lactic acid] in % (w/w)
L-LA inactivates different HIV-1 subtypes, X4 and R5 strains, patient isolates and HIV-2

Inactivation by LA is pH dependent and appears irreversible

Vaginal concentrations of lactic acid potently inactivate HIV

Aldunate, Tysssen, Johnson, Zakir, Sonza, Moench, Cone, Tachedjian, J. Antimicrob Chemother 2013
BV increases risks/rates of many different genital tract infections. These include:

- HIV-1
- HSV-2
- CMV
- Trichomonas vaginalis
- Chlamydia trachomatis
- Neisseria gonorrhoeae
- Treponema pallidum
- Viridans streptococci

Other infections and conditions affected include:

- Urinary tract infections
- Genital warts
- Cervical inflammation
- Cervical intraepithelial neoplasia
- Pelvic inflammatory disease
- Endometritis
- Tubal infertility
- Anovulation
- Preterm delivery
- Cervical intraepithelial neoplasia
- Tubal infertility
- Endometritis
- Pelvic inflammatory disease
- Cervical intraepithelial neoplasia
- Cervical inflammation
- Genital warts
- Urinary tract infections
- Viridans streptococci

The protective effect if 'healthy' or 'intermediate flora' present at day of trial entry:

- Urethritis in male sexual partners
- Infection after hysterectomy
- Delivery of low birthweight infant
- Preterm delivery
- Anovulation

The odds ratio compared to women without BV on day 1 is as follows:

- 50%
- 70%
- 80%
- 90%
- 94%

Protective effect if no BV on day 1:

- 50%
- 70%
- 80%
- 90%
- 94%
Microbicide production by vaginal lactobacilli: vaginal acidity (pH) and lactic acid are more potent than previously reported.

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Microbicides 2008, New Delhi, Poster TA-23
Only a minority of women have a ‘normal’ vaginal microbiota

‘Normal’:
- Viscoelastic Mucus
- Light gram positive monoculture
  - pH 3.5
  - 1% Lactic acid

BV:
- Variable discharge
- Heavy polyculture
  - pH ≥4.5
  - Succinic acid
  - Acetic acid
  - Butyric acid
  - Putrescine
  - Cadaverine
  - Tyramine
  - (fishy odor)

% of U.S. women on any given day

- ‘Normal’ ~ 38%
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The rate at which human sperm are immobilized and killed by mild acidity

Olmsted, Dubin, Cone and Moench

Fertil Steril 2000
Probability per intercourse: ~1.4%
Cervicovaginal fluid (CVF) and semen block the microbicidal activity of (the virtually undetectable) hydrogen peroxide produced by vaginal lactobacilli

O'Hanlon, Lanier, Moench and Cone  
BMC Infect Dis 2010

µM concentration of H₂O₂ detected by Amplex Red® assay in CVF (n = 8) and semen (n = 6) samples, versus the mM concentration of exogenous H₂O₂ added to the samples.
Microbicide production by vaginal lactobacilli: vaginal acidity (pH) and lactic acid are more potent than previously reported.

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Microbicides 2008, New Delhi, Poster TA-237