Vaginal lactic acid inhibits production of pro-inflammatory mediators from human cervicovaginal epithelial cells associated with HIV acquisition

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3rd International Workshop on Microbiome in HIV Pathogenesis, Prevention and Treatment
Vaginal microbiome affects HIV risk in young women in Sub-Saharan Africa

IN DEPTH

Young women in South Africa are up to 8 times more likely to be infected with HIV compared to young men.

INFECTION DISEASE

Vaginal microbiome affects HIV risk
Unusual bacteria in vagina help explain high infection rates in South African women

Subclinical Genital Inflammation – Vaginal Microbiome

- high load and diversity of anaerobic bacteria (Prevotella) is associated with genital inflammation and an increased risk of HIV acquisition in contrast to

- vaginal microbiota dominated by Lactobacillus spp. (L. crispatus) associated with lack of genital inflammation and lower risk of HIV acquisition

Passmore et al 2016 Curr Opin HIV AIDS 11:156
Arnold et al Mucosal Immunology
Cohen 2016 Science 353:6297
Anahtar et al 2015 Immunity 42: 965
Gossmann et al 2017 Immunity 46:1
How do lactobacilli protect against HIV?
Protective Vaginal Microbiota Produces more Lactic Acid

<table>
<thead>
<tr>
<th>Acid</th>
<th>Lactobacillus (mM)</th>
<th>High diversity (BV) (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactic</td>
<td>~120(^1,2)</td>
<td>≤20(^4)</td>
</tr>
<tr>
<td>Acetic</td>
<td>2-4(^3)</td>
<td>≤120(^1,3)</td>
</tr>
<tr>
<td>Propionic</td>
<td>&lt;1</td>
<td>2-4(^3)</td>
</tr>
<tr>
<td>Butyric</td>
<td>&lt;1</td>
<td>2-4(^3)</td>
</tr>
<tr>
<td>Succinic</td>
<td>&lt;1(^4)</td>
<td>≤20(^4)</td>
</tr>
</tbody>
</table>

*L. crispatus acidifies vagina to lower pH\(^5\)*

Does lactic acid have a protective role in the FRT?

Lactic Acid (LA): Protonated form has Antimicrobial, Antiviral and Immunomodulatory Activities

- In the vagina 1.0 ± 0.2%
  DL-LA  pH 3.5 ± 0.3\(^1\)

- Uncharged LA (but not H\(_2\)O\(_2\))
  Bactericidal BV-associated bacteria but not vaginal lactobacilli\(^2\)
  HIV virucidal\(^3\) ex vivo tissues\(^4\)

- Inactivates N. gonorrhoeae\(^5\), Chlamydia trachomatis\(^6\), HSV\(^7\)

- Uncharged LA mediates anti-inflammatory effects cervicovaginal epithelial cells that could protect against HIV

Cervicovaginal Epithelial Cells Provide both Physical and Immunological Barriers in the FRT

**Physical Barriers**
- Mucous
- Ciliary clearance
- Epithelial cells

**Immune Defense**
- Epithelial cells respond to MAMPs/PAMPs: immune mediators: antiviral and pro-inflammatory (paradoxically promote HIV infection in women)

**Microbiota**
- Competition, bacteriocins, organic acid metabolites (i.e. lactic acid – a major protective factor produced by vaginal lactobacilli)

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**Endocervix**

**Ectocervix and Vagina**
(apparent preference)

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[References]
- Shattock and Moore 2003 Nat Micro Reviews
- Carias et al 2013 J Virol 87: 11388
- Wira et al 2011 Am J Rep Immunol
- Stieh et al 2014 Plos Path 10:e1004440
Vaginal Bacteria Regulate Epithelial Innate Immunity in a Strain/Isolate Specific Manner

- **Lactobacilli** sp (e.g. *L. crispatus*) – largely non-inflammatory while BV-associated bacteria (e.g. *Atopobium vaginae*, *Prevotella amnii*) trigger a proinflammatory cytokine response from FRT epithelial cells\(^1,2,3,4\)

- Lactobacilli but not BV-associated bacteria dampen TLR agonist response by reducing pro-inflammatory cytokines elicited by FRT epithelial cells\(^5,6\)

- Lactobacilli: anti-inflammatory effect

Does lactic acid contribute to this anti-inflammatory effect?

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Experimental System for Evaluating Immune Modulatory Effects of Lactic acid

Human Epithelial Cells:
Vaginal: VK2/E6E7
Ectocervix: Ect1/E6E7
Endocervix: End1/E6E7

Primary ectocervical cells
Organotypic cervicovaginal tissue model

Add LA ± TLR agonist apically SFKM:
TLR1/2 [Pam(3)CSK(4)] (HIV gp120, BV)
TLR3 (polyIC - PIC)
TLR4 (LPS) (HIV gp120, BV)

Soluble immune mediators relevant to HIV infection: cytokine bead array/luminex

Hearps et al Mucosal Immunol 2017 10: 1480
Cervicovaginal Epithelial Cells Viable in 0.3% Lactic Acid pH 3.9

Cell Viability

- VK2 Cells
- End Cells
- Ect cells

Monolayer integrity

Transepithelial electrical resistance (TEER) - Ect cells

Cells pre-exposed to CVF

Hearps et al. Mucosal Immunol 2017 10: 1480
0.3% L-Lactic Acid pH 3.9 Elicits an Anti-inflammatory Effect on Cervicovaginal Epithelial Cells

Lactic acid elicits the production of IL-1RA from FRT epithelial cells in the absence or presence of TLR agonists

Similar increase in IL-1RA with if add LA in presence of TLR agonists: polyIC, LPS, Pam3C
0.3% L-Lactic Acid pH 3.9 Protects Against TLR-agonist Mediated Inflammation

n≥ 4

Similar effects IL-8, TNF, RANTES

Hearps et al Mucosal Immunol 2017 10: 1480
Low pH Alone does not Reproduce Lactic Acid’s Anti-inflammatory Effects

**Anti-inflammatory Cytokine**

**Pro-inflammatory Chemokine**

Pretreated with 0.3% LA pH 3.9 or HCl pH 3.9 for 1 h
Washed cells, add PIC, cytokines 18 h

\[ n \geq 5 \]
L-LA and D-LA Inhibit TLR-Agonist Inflammatory Responses from Epithelial Cells: Role of Protonated LA

**Anti-inflammatory Cytokine**

**Pro-inflammatory Chemokine**

**Ect cells - IL-1RA**

**Ect cells - MIP3**

IL-6, TNF, RANTES, IL-8

n ≥ 4

Hearps et al Mucosal Immunol 2017 10: 1480
L-LA elicits anti-inflammatory effect on Ectocervical Epithelial cells pre-treated with CVF and in presence of SP

Cervicovaginal Fluid

CVF-exposed Ect cells - IL-6

![Graph showing IL-6 levels with different treatments](image)

**CVF - 4 h, then add LA+/- PIC**

Seminal Plasma

SP-exposed Ect Cells - IL-8

![Graph showing IL-8 fold change with different treatments](image)

10% SP +/- LA 12h, wash, 18-24 h

Hearps et al Mucosal Immunol 2017 10: 1480
Lactic acid is Anti-inflammatory in a Cervicovaginal Tissue Model

Anti-inflammatory Cytokine

Little change in IL-1β

No toxicity MTT, TEER

Pro-inflammatory Chemokine

3 h LA (pH 3.9) apically, Wash, incubate for 18 h

Hearps et al Mucosal Immunol 2017 10: 1480
Mechanism?
L-LA inhibits pro-inflammatory immune mediator production at a stage prior to gene transcription

1 h treatment, media removed, additional 4 h incubation, qRT-PCR - mRNA

- Investigating NF-KB pathways
RNA-Seq – Distinct Gene Expression Profile for Lactic acid vs HCl relative to PolyIC (DEGUST)

Relative to PIC

Parallel coordinates plot

FDR <0.05
Log FC 0.5

Studying lactic acid specific effects on FRT epithelial cells that could potentially provide protection against HIV and other STIs
Summary – Lactic acid dampens inflammation

- Anti-inflammatory Cytokines: IL-1RA ↑
- Pro-inflammatory Cytokines: IL-6, TNF-α ↓
- Chemokines: IL-8, MIP-3α, RANTES ↓
- LA’s immune modulatory effect mediated by protonated form and by both the L and D stereoisomers
- LA’s effect not simply a low pH effect on cells
- LA pretreatment inhibits pro-inflammatory mediators elicited by TLR agonists
- LA’s anti-inflammatory effect observed in the presence of genital secretions
Conclusion

Lactic acid elicits the production of a proinflammatory cytokine and inhibits production of inflammatory cytokines and chemokines associated with HIV transmission.

These data might explain in part the HIV protective properties of LA producing lactobacilli, along with LA’s bactericidal activity against BV-associated bacteria and potent HIV virucidal activity.

Lactic acid and/or lactic acid producing *Lactobacillus* spp. could potentially be used to reduce the risk of HIV infection as an adjunct to antiretroviral-based PrEP.

1. LA treatment as little as 0.5 – 1 h elicits anti-inflammatory response.
2. LA pretreatment able to protect subsequent inflammatory challenge.
3. LA protects against a range of inflammatory stimuli: TLR agonists, TNF, SP.
Vaginal Environment and Impact on HIV Susceptibility

Lactobacillus spp.

Viscoelastic mucus

Suppression of other endogenous bacteria

Selection for acid tolerant bacteria

LACTIC ACID (pH < 4.5)

Glycogen breakdown

Non-inflammatory environment

HIV

Aldunate et al 2015 Frontiers in Physiology 6:164
Acknowledgments

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