

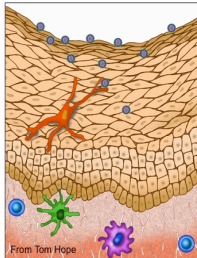
Prevention of HIV-1 Infections by Mucosal Delivery of Anti-HIV Antibodies using Human Vaginal *Lactobacillus* (LB)

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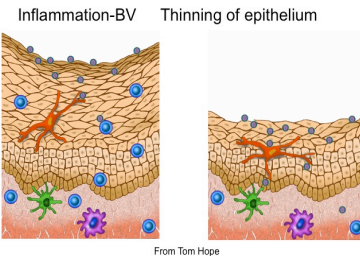
Background

HIV Transmission to Females

- HIV penetrates intact genital epithelium
- Gradient of virus entry
- HIV moves interstitially and can enter tissue where target cells (LC) are abundant
- Rare event 1-5 founder viruses (Keele et al. PNAS 2008)



HIV Transmission to Females

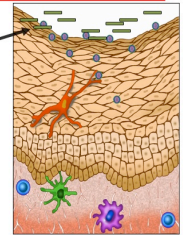


HIV Transmission to Females

- Abundant bacterial biofilm coats epithelium, *Lactobacillus* sp.
- Osel's approach: Use live commensal bacteria
 - promote vaginal health
 - deliver proteins that prevent HIV

Potential advantages:

- Coitally independent
- Discreet
- Long-term persistence
- oSEL Inexpensive
- Safe



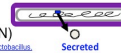
Human Vaginal Biology and *Lactobacillus*

- Lactobacilli* are important to vaginal health
- Estrogen, glycogen-LB produce lactic acid, pH <5
- LB may decrease risk of HIV and STDs
- Bacterial vaginosis (BV) –no lacto, high pH, inflammation
- CAPRISA-004 found that inflammation was a key factor in women who became infected



Live Topical Microbicide

- Lactobacillus* are part of the normal vaginal microbiome
- We have used a recombinant human vaginal *Lactobacillus jensenii* to deliver a topical microbicide
 - Modified CV-N - HIV entry inhibitor
 - Single domain antibodies* - deliver passive immunity mucosally
- Advantages of a live microbicide
 - Colonizing, replicating, durable, stable
 - Discreet
 - Not coital dependant
 - Protein delivery at site of infection
 - Cheap to manufacture
- Proof of concept (using modified CV-N)
 - Prevention of vaginal SHIV transmission to macaques by a live recombinant *Lactobacillus*
 - Lagenaur et al. Mucosal Immunol 2011 Jul 6 2011

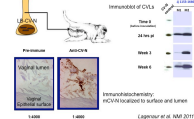


Proof of Concept:

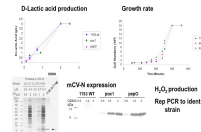
Cyanovirin-N (CV-N), A Candidate Protein-Based Topical Microbicide

Small, acid-stable (pH 4-5.2), identified at NCJ
Monomeric CV-N (PS1G), high thermodynamic stability
Binds to oligomannose-S9
Activity against multiple tested clades of HIV
Made recombinant LB producing modified CV-N

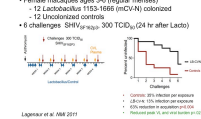
Intravaginal Inoculation of *Lactobacillus* Expressing *oCV-N* in Rhesus Macaques



Phenotypic Characteristics of *L. jensenii* 1153 with Integrated *oCV-N*

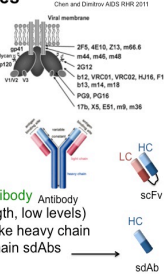


SHIV Challenge Protocol and Results



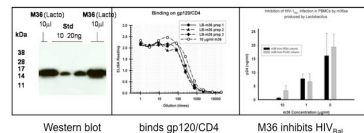
Recently Identified Broadly Neutralizing Antibodies

- Few broadly neutralizing Ab identified B12 (CD4bs), 2F5 and 4E10(gp41/MPEP) ZG12 (glycan)
- New techniques for isolating neutralizing Ab VRC 01, VRC 02 (CD4bs) PG9/PG16 (V2/V3), m9/m36 (CD4i)
- Vaccine- Generate an immune response
- Use *Lactobacillus* to deliver an antibody
 - Tried single chain scFv (full length, low levels)
 - Camels, llamas and sharks make heavy chain only (HC) antibodies, single domain sdAbs
 - full length, high expression



Expressed Single Domain Antibody M36

- sdAb M36 expressed in *L. jensenii* 1153
- M36 binds gp120/CD4
- M36 inhibits HIV_{Bal} p24 assay in PBMC



Improvements in Single Domain Antibodies

- Single amino acid change improved HIV IC₅₀ Chen et al. Antiviral Research 2010
- Several new single domain antibodies described with breadth and potency
- Make best single domain antibody, make a recombinant LB and test efficacy

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