Mucosal Assays in HIV Prevention Trials: Vaginal Microbicide Trials

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Use of Mucosal Assays in Microbicide Trials
August 25, 2015
Today’s discussion

• Mucosal assays that have been used in microbicide trials to collect female genital tract specimen
• Mucosal assays for future trials
Specimen collected

- Dependent on molecule tested
  - e.g. hydrophobic vs. hydrophilic or luminal vs. intracellular
- Mucosal (cervical, vaginal) swabs / sponges, tearflo strips, and cytobrushes
  - Cervicovaginal fluid collected by InStead Cup
- Cervicovaginal lavage (typically 5 or 10 ml)
- Cervical, vaginal tissue
- Blood/PBMCs
Collection devices

- Dacron Swab
- FLOQ Swabs
- Ophthalmic sponges
Mucosal fluid processing

- **Swabs/sponges**
  - Low volume of PBS (or saline) added to swab, soaked for ~10 min,
  - Vortexed
  - Placed in Spin-X insert (without filter), centrifuged
  - Elute collected (can retain pellet)
  - Repeat process if desired

- **CVL**
  - Centrifuged to remove cellular debris (can retain pellet)
  - Whole CVL has been used for functional assays
Mucosal Fluid collection

Comparison between cervicovaginal lavage (CVL) and swabs (Dacron or flocked)

Mucosal fluid assays

- Defining soluble cytokines/chemokines
- Functional assays:
  - Anti-HIV activity
    - Typically assayed using in vitro cell lines (e.g. TZM-bl assay or Jurkat-Tat-CCR5 assay)
  - Anti-HSV activity
    - HSV plaque reduction using Vero cell line
  - Anti-\textit{E. coli} activity
    - Colony forming unit reduction based on plate counts
Soluble cytokines / chemokines / innate factors from baseline CVL

Murphy, K., et al, Am J Reprod Immunol 2015 Jun 21 Epub
Anti-HIV activity in CVL from FAME-02: comparison between DPV film and gel users

Bunge, K., et al. in submission J AIDS 2015
FGT mucosal tissue assays

• Define cell populations (cytobrush or biopsy)
• Ex vivo challenge assay
  – Eliminated cleansing and numbing of area prior to biopsy collection
    • Consistency between FGT and GI biopsy collection
  – Limited in the number and frequency of biopsy collection
  – Requirement for fresh tissue
Cell population recovery

Cytobrush vs. CVL

- While possibly representative, tissue cell populations are different from luminal cell populations
- Cells have migrated out of the tissue for a reason, which should be taken into consideration

Ex vivo challenge assay: Inter-person variability in HIV replication

- Placebo users in FAME-02 clinical trial, n = 29
- Paired cervical and vaginal tissue

Ex vivo challenge assay: Intra-person variability in HIV replication

- 31 evaluable women enrolled
- 4 cervical and 4 vaginal biopsies were collected
- 2 of each were challenged with BaL or JR-CSF (data not shown)
- Significant intra-person variability for cervical and vaginal tissue

Lack of HIV replication in cryopreserved cervical tissue

• Placebo users from MTN-013
• Fresh cervical tissue was collected from local clinical site and used immediately
• Frozen cervical tissue was cryopreserved and shipped to the lab at end of study

Dezzutti, C.S., et al, 2015 in submission
Future mucosal assays

• Incorporation of omics into trials: collection of additional specimens, addition of preservatives, different processing?
• Upper genital tract sample collection (uterus / fallopian tube)?
• Biomarkers of HIV risk:
  – Inflammation (soluble and cellular)
  – Y chromosome (PSA)
  – HIV nucleic acid (exposed uninfected)
• Biomarkers of product efficacy:
  – Adherence (PK?)
  – Ex vivo challenge assay
  – Pharmacogenomics
• Development of a specimen repository – what specimens to collect?
Key points

• Establishing baseline (normative) values for the population(s) in the trial so product effects can be defined

• Inter-person is similar to intra-person variability for HIV replication in cervical and vaginal tissue; placebo groups equivalent to baseline specimen

• Close relationship between clinic and laboratory for specimen management and testing

• Focused working groups to provide best practices on specimen collection, processing, and assay development
Acknowledgements

- Pam Kunjara
- Kevin Uranker
- Julie Russo
- Cory Shetler
- Sarah Yandura
- Sidney Lawlor
- Dana Tirabassi

- Lisa Rohan lab
- Sharon Hillier lab
- Ian McGowan lab
- UPMC Clinical Staff
- Participants

MTN: UM1 AI106707
     UM1 AI068653
     UM1 AI 068615
FAME: U19 AI082639

OPP1084465

National Institutes of Health
Bill & Melinda Gates Foundation