The Global HIV Vaccine Enterprise (Enterprise) is a unique collaboration of the world’s leading HIV vaccine research funding, policymaking, advocacy and stakeholder organizations.

**Timely Topics in HIV Vaccines**

- Topic important for the field as a whole
- Multiple stakeholders involved
- Required a neutral convener
- Clear objectives
Capturing Participant Information for Mucosal Sampling:

An Investigator’s Guide

Satellite Session - Oct 7th, 2013
AIDS Vaccine Conference, Barcelona, Spain

Chaired by Georgia Tomaras, PhD and David Masopust, PhD
11:00 - 11:10 - Georgia Tomaras, PhD (Duke University, Durham, NC, USA) and David Masopust, PhD (University of Minnesota, Minneapolis, MN, USA) – Introduction to the session

11:10 - 11:25 - Thomas Hope, PhD (Northwestern University School of Medicine, Chicago, IL, USA) - The increasing focus on mucosal immunity and mucosal sampling in HIV vaccine studies.

11:25 - 11:35 – Helene Zinszner, PhD/Patricia D’Souza, PhD (Division of AIDS, NIH-NIAID, Bethesda, MD, USA) - Purpose, scope, and format of the guide.

11:35 - 12:15 - Short case studies and Q&A

Panelists:
- Catherine Cosgrove, PhD (MRC Clinical Trials Unit, London, UK)
- John Hural, PhD, (HIV vaccine Trial Network, FHCRC, Seattle, CA, USA)
- Sandhya Vasan, MD /Nicos Karasavva, PhD (Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand)
- Arthur Sekiziyivu, MD, MSc (Makerere University-Walter Reed Project, Kampala, Uganda)
Stopping HIV-1 at Portal of Entry

Mucosal sampling includes measurement of antibodies (IgG, SIgA, mIgA) and determination of effector cells (NK, PMNs, monocytes, CD8⁺ T cells) and target cells (CD4⁺ T cells) with the overall goal to understand if there is a sufficient quality/quantity to impact HIV-1 acquisition.

Tomaras
Effective Vaccine-Induced Mucosal Immunity - Possible?

Steps for Vaccine – Elicited Immune Response Intervention

Step 1: Inhibit initial infection at mucosal barrier.

Step 2: Inhibit local replication at portal of entry.

Step 3: Limit Spread and Replication of Virus

Ab Virion Capture?

Ab FcR function?

**Abstract**

Number: P13.71 LB

Human HIV-1 Vaccine Induced Antibody Durability and Env IgG3 Responses

Kelly E Seaton\(^1\), Nicole L. Yates\(^1\), William T. Williams\(^1\), Aaron Deal\(^2\), Vicki Ashley\(^3\), Judith Lucas\(^1\), Nathan Vandergrift\(^1\), Wes Rountree, \(^1\) Larry Liao, \(^1\) Allan de Camp, \(^2\) Youyi Fong, \(^2\) David Montefiori, \(^2\) Paul Spearman, \(^4\) Marnie Elizaga, \(^5\) Susan Barnett, \(^6\) Marguerite Koutsovko, \(^6\) Patricia Bourgugnon, \(^6\) GSK PROHIV-002 Protocol Team, \(^7\) HVTN 088 Protocol Team, \(^8\) RV144 Protocol Team, \(^9\) VAX003 Protocol Team, \(^10\) Julie McElrath, \(^10\) Larry Corey, \(^10\) Nelson Michael, \(^10\) Punneet Pitsuttithum, \(^10\) Supachai Rooks-Ngarm, \(^10\) Jerome Kim, \(^10\) Gerald Voss, \(^10\) Peter Gilbert, \(^10\) Barton F. Haynes, \(^10\) Georgia D. Tomaras

\(^1\) Duke Human Vaccine Institute, Durham, NC; \(^2\) Fred Hutchinson Cancer Research Center, Seattle, WA; \(^3\) Duke University, Durham, NC; \(^4\) University of Alabama, Birmingham, AL; \(^5\) Novartis Vaccines and Diagnostics, Cambridge, MA; \(^6\) GSK Vaccines, Belgium; \(^7\) MHRP, Bethesda, MD; \(^8\) Mahidol University, Thailand; \(^9\) Ministry of Public Health, Thailand

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Figure 4: Env IgG responses remain detectable 6-17 years post last vaccination (Clade C gp140/MF59)

IgG binding antibody response magnitude to SF162deltaV2 gp140 (Panel A) and to Con6 gp120 (Panel B) was measured in up to 36 HVTN 088 Vaccinees. Binding antibody responses are presented as MFI.
Vaccine induced mucosal Env IgG responses can be detected 6-17 years post-last vaccination. The presence of vaccine induced Env IgG responses 6-17 years post-vaccination is proof-of-concept that maintenance of vaccine induced mucosal responses is possible.
The first step to an in-depth understanding of potentially protective humoral and cellular immune responses present at the mucosal site is to ensure high quality and reliable mucosal sampling.

Many factors can influence the quality of the mucosal sample. Thus, collective guidance from experienced investigators can save time and money and move the field forward faster.
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The Increasing Focus on Mucosal Immunity and Mucosal Sampling in HIV Vaccine Studies

Thomas J. Hope

Northwestern University
Feinberg School of Medicine
Why do we need to understand mucosal immunity and mucosal immune responses?

• This is the portal of entry for the majority of HIV transmissions.
• Antibodies at these sites can be produced locally (mix of systemic and local antibodies).
• T cells in at mucosal sites are different from cell in blood. (effector cells, $\alpha 4\beta 7$)
• Mucosal barriers are different in the different compartments.
The mucosal sites of transmission are very different.
Differences in mucosal immunity at rectal and FRT compartments

- Different Epithelial barriers
- Distinct tissue structure.
- Different Antibodies (FRT IgG>IgA, Gut IgA>IgG)
- Mucus (FRT MUC5AC/B, FRT MUC2)
Collecting mucosal samples

A swab

Biopsy

www.vaccineenterprise.org
Virus interaction with the FRT over menstrual cycle

Vishwanathan et. al. 2011

A

Vishwanathan et. al. 2011

folicular phase
(95% of infections detected)

luteal phase
(5% of infections detected)

number of macaques showing initial viremia

day of menstrual cycle

progesterone
estradiol
window of highest susceptibility to SHIV

mean occurrence of initial viremia
More virus is seen in the endocervix in the luteal phase of macaques.

- Virus in Ecto
- Virus in Endo
In luteal phase pigtail macaques there is an increase in the density of ectocervical and vaginal suprabasal CD4+ cells. However, there is no change in CD4+ density during cycle times within the endocervix.
Mucus: The first barrier
Mucus distribution/structure is distinct between FRT and gut

**MUC5AC**

**MUC2**

www.vaccineenterprise.org
Identification of the first cell infected Dual Reporter System

VSVG pseudotype

Transduce (infect) most living cells

HIV envelope

Transduce (infect) HIV target cells
Luciferase can reveal the needle-in-a-haystack, spectral imaging can identify infected cells.
Measuring Luminescence in Whole Tissue Using IVIS - Luciferin (JRFL)

Legend:
1 Vagina, Lower
2 Vagina, Upper
3 Cervix
4 Uterus
5 Ovary

Rotation of the tissue and reimaging using IVIS
Measuring Luminescence in Whole Tissue Using IVIS - Tissue in Luciferin (JRFL)

Legend:

1. Vagina, Lower
2. Vagina, Upper
3. Cervix
4. Uterus
5. Ovary
Surprise #1 - Ovaries

Virus is able to reach and infect susceptible cells in all aspects of the Upper reproductive tract. Only a subset of ovaries are positive. A mechanism beyond simple diffusion seems to play a role. Target cell infiltration associated with cysts (common).
Infected CD4+ T cells in the ovary
Take home messages

• To understand mucosal transmission and protection, we need study mucosal sites and samples
• Big differences between mucosal portals of entry
• Menstrual cycle can influence barrier and protection
• Mucus plays a role (can enhance?) barrier function.
• Sites of infection can be widely distributed throughout mucosal sites.
• CD4 T cells are the primary target of initial infection
ACKNOWLEDGEMENTS

Collaborators

Ron Veazey
Audrey French
Kerrie McCotter
Brianne Condron
Ellen Kersh
Michael Hendry
Jim Smith
Karla Satchell
Igal Szleifer
Francois Villinger
Ruth Ruprecht

Thank You to mucus and tissue donors and gynecologists who collect our samples.
A Mucosal Sampling Guide

Helene Zinszner for Patricia D’Souza, PhD
NIAID/NIH
October 7, 2013
Difficulties in mucosal sampling

- Based on assays established for the evaluation of systemic responses
  - not rate limiting vs low yield, unique collection
  - frozen vs fresh samples
- Lack of standardization
  - different labs use different methods for collection, different sample preparations, and different assays
  - difficult to compare results across labs
- Participant Information
  - role of STIs, menstrual cycle
  - hormonal contraception
  - inter-current infections
  - alcohol use
  - ...
Evolution of the Mucosal Guide

**Concept**

S. Cu-Uvin Mucosal Immunology Group 2012 talk – Clinical factors that can impact mucosal immunity

J. McElrath suggests ‘Mucosal Sampling Questionnaire’

D’Souza proposes as Enterprise Timely Topic

**Foundation**

Enterprise compiles current Network practices (ACTG; HVTN; IAVI; MHRP; MTN; European network)

S. Cu-Uvin & K. Broliden create consensus framework

Guide structure (e.g., subsections) developed

**Development**

Cross-Network SMEs contribute primary content

In-depth review by additional Mucosal Immunologists
Pat D’Souza, NIAID-DAIDS- MIG
Mary Gross, FHCRC- HVTN- MIG
Amapola Manrique, the Enterprise
Helene Zinszner, the Enterprise

Expert contributors

Michelle Andrasik (University of Washington, HVTN); Chuka Anude (National Institute of Allergy and Infectious Diseases); Rahul Bakshi (Johns-Hopkins University, MTN); Kristina Broliden (Karolinska Instituta); Susan Cu-Uvin (Brown University, ACTG), Shelly Karuna (HIV Vaccine Trial Network); Julie McElrath (Fred Hutchinson Cancer Research Center , HVTN); Ian McGowan (University Pittsburgh, MTN); Devika Singh (University of Washington, HPTN, MTN). Finally, the guide was critically reviewed and revised by an extensive group of additional experts including: Peter Anton (University of California LA); Stephen De Rosa (University of Washington); Florian Hladik (Fred Hutchinson Cancer Research Center); Rupert Kaul (University of Toronto); ; Douglas Kwon (Ragon Institute); Alan Landay (Rush University Medical Center); Kenneth Mayer (The Fenway Institute); Richard Novak (University of Illinois); Hariett Park (International AIDS Vaccine Initiative); Jo-Ann Passmore (University of Cape Town); Steven Safren (Massachusetts General Hospital)
Purpose of the Mucosal Sampling Guide

➢ key participant characteristics that can affect mucosal immunity
  o Factors to consider for conclusive interpretation and potential cross-trial comparison of mucosal immunology data.

➢ Intended as a resource, not a requirement
  o Point investigators to participant factors that can impact interpretation of mucosal immunity data
  o Minimize chance that important data element will be missed
Format - The Guide is organized in six categories

1. DEMOGRAPHIC
   - Age
   - Race/ethnicity/tribe
   - Sex at birth/self-identified gender
   - Relationship status
   - Education
   - Employment/Income

2. REPRODUCTIVE HISTORY
   - Menstrual data
   - Menopausal status
   - Pregnancy report
   - Contraception
   - Vaginal Practices
   - Gyn surgery
   - STIs
   - Dysplasia

3. MEDICAL HISTORY
   - Medical conditions
   - HIV status
   - Surgeries
   - Vaccinations
   - Medications
   - Allergies
   - BMI

4. SEXUAL HISTORY
   - Sexual activity
   - Sexual preference
   - HIV status of partner

5. RISK BEHAVIORS
   - Drug use
   - Smoking
   - Drink alcohol
   - Sexual behavior
   - Sexual behavior of partner

6. SYMPTOMS
   - Vaginal discharge
   - Rectal discharge
   - Enlarged/painful lymph nodes
   - Pelvic pain
   - Genital Ulcers
The guide will be reviewed and revised on an as-needed basis and at least annually after launch.
3. MEDICAL HISTORY
3.7 Body Mass Index (BMI)

Rationale
BMI may have a significant impact on vaccine-elicited immunogenicity. Obesity, defined as BMI ≥ 30 kg/m², has been associated with impaired adaptive and innate immune system responses to infections or vaccination and increased susceptibility to infectious agents and cancer.

Considerations
Some human studies have shown that systemic inflammation varied at different levels of adiposity and was observed even among individuals with normal weights. It has been proposed that adipose tissue inflammation, either in excess (in individuals with elevated BMI) or in normal weight individuals, may modulate the immune responses to antigen. Assessment of adiposity and the pro-inflammatory biomarkers secreted by adipose tissue can thus provide additional insight into immune regulation and may be important to explore in responses to vaccination.

Measurements of BMI and adiposity can be both straightforward and relatively inexpensive (waist circumference measurements, for example) or more complex and expensive (i.e. fasting leptin and adiponectin levels) and the more complex and expensive methods should be justified as they raise significant operational concerns.
I is important to have a starting weight and height for later use.

Caveats
The influence of BMI and of sex on vaccine-elicited immunogenicity may be related, which should be considered in analyses.

References (with links for online version)
How to use the Guide

- View in context of trial-specific objectives, study intervention, and operational feasibility
  - Not all categories will apply to all studies
  - Rationale, considerations, caveats and literature references should help end-user decide whether to collect a specific data element or not

- There are multiple ways in which guide recommendations could be implemented
  - CRF; questionnaire; participant interview; protocol development

- The guide is a living document
When and Where Can I Find the Mucosal Guide?

- Launch targeted for Q4 2013
  - Additional reviews of technical content in progress
  - Web-based version under development
  - Options for print release being evaluated

- Initial release on the Global HIV Vaccine Enterprise website
  - www.vaccineenterprise.org
  - www.vaccineenterprise.org/content/timely-topics-hiv-vaccines

SIGN UP

timelytopics@vaccineenterprise.org
Case Studies & Discussion

Catherine Cosgrove, PhD (MRC Clinical Trials Unit, London, UK)

John Hural, PhD, (HIV vaccine Trial Network, FHCRC, Seattle, CA, USA)

Sandhya Vasan, MD / Nicos Karasavva, PhD (Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand)

Arthur Sekiziyivu, MD, MSc (Makerere University-Walter Reed Project, Kampala, Uganda)
• Clear menstrual cycle history
  • Mid-cycle - often more secretions/ mucus
  • Erratic or irregular cycles make mid-cycle collection more difficult
  • Bleeding may contaminate sample

• Sexual history

• Contraception history
  – Intra-uterine devices may make Instead cup collection hazardous
  – Progesterone contraception may make menses very light, difficulty timing collections
## Mucosal Sampling Experiences in HVTN/FHCRC Studies

<table>
<thead>
<tr>
<th>Data Category (Mucosal Guide)</th>
<th>Mucosal Sample Collected</th>
<th>Mucosal Immune Endpoint Affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reproductive History</td>
<td>Semen</td>
<td>Laboratory recovered no immune cells for cellular assays in samples from participant with prior vasectomy</td>
</tr>
<tr>
<td>Genital Surgery (male)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reproductive History</td>
<td>Cervical Cytobrush</td>
<td>DMPA use decreases the number of vaginal T cells over time (Hladik et al., manuscript in preparation)</td>
</tr>
<tr>
<td>Contraception (female)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reproductive History</td>
<td>Cervical Cytobrush</td>
<td>Laboratory can observe decreased immune cell yields in samples from participants with history of vaginal births, which may be due to inconsistent contact of cytobrush with cervix when cervical Os is wider.</td>
</tr>
<tr>
<td>Pregnancy History (female)</td>
<td></td>
<td>Similarly, a stenotic cervical Os may prevent proper insertion of cytobrush for optimal immune cell recovery.</td>
</tr>
</tbody>
</table>
IgG and IgA Quantification via Rectal Secretion Collection: Optimization of Sponge Type and Placement

Sponge 2 cm Above Pectinate line

IgA standard curve
IgG standard curve

IgA Volunteer#1 spear sponge (shallow)

- Shallow 1-1, 15.3 ug/ml
- Shallow 2-1, 0 ug/ml

IgA Volunteer #1 spear sponge (deeper)

- Deep 1-1, 46.7 ug/ml
- Deep 1-2, 9.0 ug/ml

IgA Volunteer#2 Cylindrical sponge (shallow)

- Shallow 4-1, 12.8 ug/ml
- Shallow 4-2, 0 ug/ml
- Shallow 4-3, 0 ug/ml

IgA Volunteer#2 Cylindrical sponge (deeper)

- Deeper 3-1, 213.3 ug/ml
- Deeper 3-2, 97.5 ug/ml
- Deeper 3-3, 33.4 ug/ml

IgG Volunteer#1 spear sponge (shallow)

- Shallow 2-1, 58.1 ug/ml
- Shallow 2-2, 0 ug/ml

IgG Volunteer#1 spear sponge (deeper)

- Deep 1-1, 0 ug/ml Yield 0 ug
- Deep 1-2, 0 ug/ml Yield 0 ug

IgG Volunteer#2 cylindrical sponge (shallow)

- Shallow 4-1, 14.9 ug/ml
- Shallow 4-2, 0 ug/ml
- Shallow 4-3, 0 ug/ml

IgG Volunteer#2 cylindrical sponge (deeper)

- Deeper 3-1, 43.5 ug/ml
- Deeper 3-2, 27.7 ug/ml
- Deeper 3-3, 11.0 ug/ml

Tested positive using hemoccult test.

Disclaimer: The opinions herein are those of the author and should not be construed as official or representing the views of the Department of Defense or the Department of the Army.
## Demographic information – RV217 and RV262 mucosal sampling

<table>
<thead>
<tr>
<th>Category</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong> (date of birth or best estimate)</td>
<td>Age-associated changes in cervical epithelium could influence cellular and humoral factors in cervico-vaginal secretions</td>
</tr>
<tr>
<td><strong>Sex</strong> (at birth and self identified)</td>
<td>There may be sex-specific differences in induction of mucosal immunity</td>
</tr>
<tr>
<td><strong>Race/ethnicity</strong></td>
<td>Race variations in HIV prevalence could represent genetic variation that potentially influences mucosal immunity. May relate to socio-behavioural risk for HIV infection.</td>
</tr>
<tr>
<td><strong>Education</strong> (highest level attained)</td>
<td>Analytical variable – adherence to study, retention, socio-behavioural risk for HIV infection.</td>
</tr>
<tr>
<td><strong>Employment/occupation</strong></td>
<td>Analytical variable – adherence to study, retention, socio-behavioural risk for HIV infection.</td>
</tr>
<tr>
<td><strong>Marital status</strong></td>
<td>May relate to socio-behavioral risk for HIV infection</td>
</tr>
</tbody>
</table>
## Other participant information

<table>
<thead>
<tr>
<th>Medical history</th>
<th>Chronic conditions, non-study vaccinations (eligibility), allergies, concomitant meds, HIV test, mucosal sampling in acutely infected cases – stage of HIV disease versus mucosal immunity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reproductive history</td>
<td>LMP, LH &amp; FSH – phase of menstrual cycle could affect immune factors in cervico-vaginal secretions, contraception use, pre/post coital vaginal and anal washing practices. <em>Excluded</em> – <em>positive Urine HCG, current IUD use, h/o Gyn surgery (hysterectomy, D &amp; C, cervical biopsy etc), childbirth, abortion in last 6 weeks, h/o TSS, abnormal PAP smear, h/o of STI.</em></td>
</tr>
<tr>
<td>Sexual history</td>
<td>Type of sex – receptive, unprotected, vaginal, anal, male/female, regular or casual partner, number of different partners, HIV status of partner (infected, unknown), partner STI and behavior (drugs, alcohol, multiple partners). <em>Deferred sample collection – h/o intercourse, douching, cervical cap/diaphragm, ejaculation, menses within last 48-72 hours.</em></td>
</tr>
<tr>
<td>Risk behaviour</td>
<td>Drug use, alcohol consumption, sex and drugs/alcohol, smoking, sex in exchange for money, goods, drugs, services, multiple concurrent partners,</td>
</tr>
<tr>
<td>Symptoms</td>
<td>Genital ulceration, vaginal or rectal discharge, cervicitis, proctitis, pelvic pain, leukocyte on urinalysis, STI diagnosis. <em>Defer collection until after treatment and symptom resolution.</em></td>
</tr>
</tbody>
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