HIV VACCINE
TRIALS NETWORK

Mucosal Immunology Group Scientific Agenda

1 Introduction / Background

In order to advance rational HIV vaccine designs that can efficiently generate long-lived mucosal immunity, better tools for measuring mucosal immune responses in humans are needed. Standardization of specimen collections has focused primarily on mucosal secretions to evaluate antibody responses. By contrast, less effort has been devoted to optimize and standardize the collection of mucosal secretions and tissues to identify both innate and adaptive cellular responses. Thus, precise and comprehensive characterization of mucosal cellular immunity using well-standardized specimen collection methods and detection assays are critically needed. This Scientific Agenda will guide vaccine and study designs to ultimately determine which regimens efficiently induce mucosal HIV-specific responses, and furthermore, which strategies may potentially enhance HIV-1 infection through mucosal immune activation.

1.1 Mission of the Mucosal Immunology Group

The mission of the Mucosal Immunology Group is to identify critical areas of need for a Scientific Agenda that will guide future mucosal studies. Addressing the questions within the Scientific Agenda will lead to improvement and standardization of mucosal specimen collection for use in clinical trials and assay development for an enhanced understanding of HIV-specific cellular immunity in the mucosa.

1.2 Formation of Scientific Review Committee

A Mucosal Scientific Review Committee (mSRC) comprised of scientific experts and leaders will be formed and have the following responsibilities: 1) establish a Scientific Agenda, 2) advise the mucosal immunology group on the state of the field, 3) review scientific proposals submitted by investigators, and 4) make recommendations for prioritization and funding.

1.3 Top Priorities

The DAIDS-sponsored Mucosal Immunity Workshop held in June 2009 presented an opportunity to identify areas of focus in mucosal immunology that will advance the field. The recommendations brought forward from that meeting form the basis for the Mucosal Immunology Group Scientific Agenda. The Scientific Agenda sets the course for the Mucosal Immunology Group in two major areas:

a) **Develop standardized protocols for mucosal sample collection, storage, and transportation for use in clinical trials.**

b) **Develop standardized assays to measure and characterize the major effector and memory mucosal immune responses in the GI and GU tracts.**

2 Components of Top Priorities

The following sections provide specific focus areas within each top priority.

2.1 Develop standardized protocols for mucosal sample collection, storage, and transportation for use in clinical trials

Scientists with relevant experience will be asked to join one of three working groups: Gastrointestinal (GI), Genitourinary (GU), and Systems Biology. The Working Groups, in addition to proposals submitted by others in the field, will focus on conducting studies that address the questions and priorities set forth in the Mucosal Immunology Group Scientific Agenda. The critical areas that need scientific focus have been identified as the following:

GI – Specific Sample Collection

- Flexible sigmoidoscopy is the least invasive and preferred method for maximum cell yields. Confirm and develop standardized protocols as needed for this type of tissue collection.
- Address the type and maximum number of biopsies that can be collected from the different mucosal sites (i.e., Standardize use of Jumbo Biopsy Forceps to improve tissue acquisition.)
- Survey of sampling from multiple mucosal regions within the tissue (e.g., upper vs. lower GI tract) to compare immune response in the different locations. The goal being to facilitate the standardization of sampling location.
- Address and score the efficiency of isolation from biopsies; Determine what fraction of cells are being extracted.
- Determine baseline phenotypic and functional ranges for cells across population demographics.
- Standardize time lapse between mucosal sample collections.

GU – Specific Sample Collection

- Determine the best sampling method strategy to address the limitations in sampling within the female reproductive tract.
- Address the challenges of very limited cell numbers when GU sampling: biopsies vs. use of cytobrush vs. cervico-vaginal lavage to sample GU cells. Can we improve cell yield and viability?
- Improve and standardize methods to collect secretions; Determine baseline ranges of cytokines, antibodies, etc. from Weck-Cel sponge-collected secretions.
- Investigate origin of T cells in menstrual blood by characterizing the cellular phenotype of menstrual blood.

- Understand how other physiologic events such as recent sex, effects of semen, hormonal contraception, and common co-infections affect cell yields.

Storage and Transportation Issues

- Develop standardized protocols for freezing and thawing cells and/or biopsy samples keeping in mind practicality for collection in a clinical trial setting.
- Determine optimal transportation conditions; compare the effects of transporting fresh vs. frozen samples.
- Develop standardized protocols for the transportation of sensitive samples, as storage and transportation may differ depending on the questions being asked (e.g., Cytobrush-derived T cells maintain function and viability if stored at 37°C, but exhibit 75% loss of T cells if frozen).

2.2 Develop standardized assays to measure and characterize the major effector and memory mucosal immune responses in the GI and GU tracts

Standardized assays

- Develop novel high-throughput, clinically relevant functional assays for a small number of cells.
- Develop and improve miniaturized cellular and soluble functional assays to overcome the limitations of low cell yields collected from mucosal samples.
- Establish normative values of the different cell types and responses in healthy populations.
- Establish mucosal positive control antigens for assays.
- Develop and standardize flow cytometry with gating strategies; develop an IQA-like program to address issues with gating for both recently disaggregated cells and cells utilized for flow assays, such as ICS.
- Develop statistical methods to account for the high backgrounds, sampling variability, and low cell numbers found in mucosal samples.
- Identification of appropriate surrogate markers to permit the use of PBMCs for GU and GI assays; this would alleviate challenges associated with very low cell numbers from mucosal tissue.

- **Systems Biology:**
  - Develop validated assays and technology platforms for incorporating systems biology approaches into mucosal immune response studies that drive our understanding of correlates of protection against HIV infection and disease progression.
  - Development and implementation of data analysis protocols.

Sample Processing

- Develop standardized tissue processing protocols to obtain functional cells and maximize cell yields.
- Develop standardized protocols for handling cells before extracting nucleic acid for genomic analysis.
- Need for methodology to control for trauma-dependent sample phenotype; e.g. assess blood contamination of mucosal tissues to ensure subsequent analyses are relevant to mucosa.
- Determine if cells and/or tissue can be frozen and function optimally in functional assays upon thawing; survey a broad panel of procedures to determine which is most representative for evaluation of cell functionality.
- Characterization of expanded cells: need to explore optimized cell expansion methods to improve cell yields (especially GU tissue) as well as shorten the time in culture for expansion.

3 Mucosal Working Groups

Name	Working Group	Institution
Peter Anton	**GI**	UCLA
Jason Brenchley	**GI**	NIAID
Steve De Rosa	**GI**	FHCRC
Mark de Souza	**GI**	AFRIMS
Paul Johnson	**GI**	Harvard
Julie McElrath	**GI**	FHCRC
Ian McGowan	**GI**	Univ. of Pittsburgh
Alexandra Schuetz	**GI**	AFRIMS
Barbara Shacklett	**GI**	UC Davis
Elizabeth Sinclair	**GI**	UCSF
Ron Veazey	**GI**	Tulane
Susan Cu-Uvin	**GU**	Miriam Hospital; Brown
Charlene Dezzutti	**GU**	Univ. of Pittsburgh
Craig Hendrix	**GU**	Johns Hopkins
Florian Hladik	**GU**	FHCRC
Rupert Kaul	**GU**	Univ. of Toronto
Alan Landay	**GU**	Rush Univ.
Maria Lemos	**GU**	FHCRC
Rick Novak	**GU**	UIC
Jo-Ann Passmore	**GU**	Univ. of Cape Town
Robin Shattock	**GU**	Univ. of London
Charles Wira	**GU**	Dartmouth

Blake Ball	**Systems**	Univ. of Manitoba
Mark Cameron	**Systems**	VGTI
Chris Love	**Systems**	MIT
Leonid Margolis	**Systems**	NIH
Rafick Sékaly	**Systems**	OHSU/VGTI
Phil Bergin	**Ad Hoc**	IAVI
Patricia D'Souza	**Ex Officio**	NIH/DAIDS
Jean-Louis Excler	**Ad Hoc**	US-MHRP
David Masopust	**SRC**	Univ. of Minnesota
Bali Pulendran	**SRC**	Emory
Marco Schito	**Ex Officio**	NIH/DAIDS
Georgia Tomaras	**Ad Hoc**	Duke
Otto Yang	**SRC**	UCLA