HIV Prevention Trials Network

PROTOCOL CLARIFICATION MEMORANDUM #1

HPTN 056: Characterization of Baseline Mucosal Indices of Injury and Inflammation in Men For Use in Rectal Microbicide Trials

Version 1.0 Dated 02 December 2003

28 March 2005

Summary of Clarification

The purpose of this memo is to clarify a discrepancy in the protocol regarding when CD4 testing should be performed.

CD4 Testing

The Inclusion Criteria erroneously states that CD4 is to be performed on all participants.
Sections 3.0 (Study Population) and 5.2.2 (Clinical Procedures) correctly state that CD4 testing is only required for participants enrolled into Groups 3 or 4.

DATE: **4 March 2005**

RE: **LETTER OF AMENDMENT #2 FOR HPTN 056: Characterization of Baseline Mucosal Indices of Injury and Inflammation in Men For Use in Rectal Microbicide Trials, Version 1.0 Dated 02 December 2003**

TO: **Dr. Ian McGowan, HPTN 056 Investigator of Record**

CC: **HPTN 056 Protocol Team**

FROM: **Scott Mitchell Rose, HPTN CORE Protocol Specialist**

THE FOLLOWING INFORMATION IMPACTS THE HPTN 056 STUDY AND MUST BE FORWARDED TO YOUR INSTITUTIONAL REVIEW BOARDS (IRBS) AND/OR ETHICS COMMITTEES (ECS) AS SOON AS POSSIBLE FOR THEIR INFORMATION AND REVIEW. IT MUST BE APPROVED BEFORE IMPLEMENTATION.

THE FOLLOWING INFORMATION MAY ALSO IMPACT THE SAMPLE INFORMED CONSENT. YOUR IRB/EC WILL BE RESPONSIBLE FOR DETERMINING THE PROCESS OF INFORMING SUBJECTS OF THE CONTENTS OF THIS LETTER OF AMENDMENT.

PLEASE FILE THIS LETTER AND ANY IRB CORRESPONDENCE IN YOUR REGULATORY FILE AND OTHER PERTINENT FILES. YOU ARE <u>NOT</u> REQUIRED TO SUBMIT THESE DOCUMENTS TO THE PROTOCOL REGISTRATION OFFICE UNLESS THE CHANGES RESULT IN A CHANGE TO THE INFORMED CONSENT FOR YOUR SITE.

Summary of Revisions

- The Inclusion Criterion requiring that the participants be between 18 and 50 years of age has been modified to at least 18 years of age.

- Clarification of inclusion criteria regarding CD4 count >200 cells/mm^3 at screening, which contradicts pages vi and 9.
  Should read CD4 >200 cells/mm^3 to be confirmed at screening (Groups 3 and 4).

If the HPTN 056 protocol is amended in the future, this Letter of Amendment will be incorporated into the next version.

Upon receipt of IRB/EC approval, the following protocol modifications, indicated by ~~strikethrough~~ and **<u>underscored</u>** text, will be implemented:

1. Study Schema:

   Study Population: HIV-1 infected and uninfected men aged 18~~ 50 years~~ **<u>and over</u>**.

2. Section 3.1, first bullet:

   Age 18~~ to 50 years~~ **and over**

3. Section 3.1, last bullet:

   CD4 >200 cells/mm^3 to be confirmed at screening **(Groups 3 and 4)**

DATE: **18 February 2004**

RE: **LETTER OF AMENDMENT FOR HPTN 056: Characterization of Baseline Mucosal Indices of Injury and Inflammation in Men For Use in Rectal Microbicide Trials, Version 1.0 Dated 02 December 2003**

TO: **HPTN 056 Investigator of Record**

CC: **HPTN 056 Protocol Team**

FROM: **Leigh Peterson, HPTN CORE Protocol Specialist**

THE FOLLOWING INFORMATION IMPACTS THE HPTN 056 STUDY AND MUST BE FORWARDED TO YOUR INSTITUTIONAL REVIEW BOARDS (IRBS) AND/OR ETHICS COMMITTEES (ECS) AS SOON AS POSSIBLE FOR THEIR INFORMATION AND REVIEW. IT MUST BE APPROVED BEFORE IMPLEMENTATION.

THE FOLLOWING INFORMATION MAY ALSO IMPACT THE HPTN 056 INFORMED CONSENT FORMS. YOUR IRB/EC WILL BE RESPONSIBLE FOR DETERMINING THE PROCESS OF INFORMING SUBJECTS OF THE CONTENTS OF THIS LETTER OF AMENDMENT.

PLEASE FILE THIS LETTER AND ANY IRB CORRESPONDENCE IN YOUR REGULATORY FILE AND OTHER PERTINENT FILES. YOU ARE NOT REQUIRED TO SUBMIT THESE DOCUMENTS TO THE PROTOCOL REGISTRATION OFFICE UNLESS THE CHANGES RESULT IN A CHANGE TO THE INFORMED CONSENT FOR YOUR SITE.

Summary of Revisions

- The Inclusion Criterion requiring that the participants be able and willing to communicate in English has been deleted.
- Two Exclusion Criteria have been modified to exclude both HIV-1 seropositive and HIV-1 seronegative participants who have had recent HSV-2 outbreaks.
- The protocol has been revised to exclude use of non-steroidal anti-inflammatory drugs including ibuprofen and naprosyn throughout the study.

If the HPTN 056 protocol is amended in the future, this Letter of Amendment will be incorporated into the next version.

Upon receipt of IRB/EC approval, the following protocol modifications, indicated by ~~strikethrough~~ and underscored text, will be implemented:

1. Section 3.1, third bullet:

   - ~~Able and willing to communicate in English~~

2. Section 3.2, seventh bullet:

   - Three or more HSV-2 outbreaks ~~(HIV-1 seropositive subjects only)~~ in the past 12 months prior to screening

3. Section 3.2, eighth bullet:

   - One or more HSV-2 outbreaks ~~(HIV-1 seropositive subjects only)~~ in the past 6 months prior to screening

4. Section 3.2, twelfth bullet:

   - Impaired coagulation or current use of anticoagulants or medications such as warfarin or aspirin, or non-steroidal anti-inflammatory drugs including ibuprofen and naprosyn that, in the opinion of the Investigator, would make participation in the study unsafe or complicate interpretation of study outcome data

5. Section 5.2, third bullet:

   - Subjects must agree to refrain from using anticoagulants or other medications such as warfarin or aspirin, or non-steroidal anti-inflammatory drugs including ibuprofen and naprosyn throughout the study.

6. Section 5.5:

   Enrolled study participants may not use anticoagulants or medications such as warfarin or aspirin, including non-steroidal anti-inflammatory drugs including ibuprofen and naprosyn.

HPTN 056

Characterization of Baseline Mucosal Indices of Injury and Inflammation in Men For Use in Rectal Microbicide Trials

A Study of the HIV Prevention Trials Network

Sponsored by:

Division of AIDS, US National Institute of Allergy and Infectious Diseases
US National Institute of Child Health and Human Development
US National Institute on Drug Abuse
US National Institute of Mental Health
US National Institutes of Health

Protocol Chair(s):

Ian McGowan, MD, PhD
University of California, Los Angeles
Los Angeles, CA USA

and

Peter Anton, MD
University of California, Los Angeles
Los Angeles, CA USA

Version 1.0
Final Version
02 December 2003

HPTN 056

Characterization of Baseline Mucosal Indices of Injury and Inflammation in Men For Use in Rectal Microbicide Trials

TABLE OF CONTENTS

HPTN 056

LIST OF ABBREVIATIONS AND ACRONYMS

AE	Adverse event
AIDS	Acquired Immunodeficiency Syndrome
CARE	Center for AIDS Research and Education
CCR5	Chemokine receptor 5
CDC	Centers for Disease Control and Prevention
CHADD	Center for HIV & Digestive Diseases at UCLA
CL	(HPTN) Central Laboratory
CLIA	Clinical Laboratory Improvement Amendments
CORE	(HPTN) Coordinating and Operations Center
CRF	Case report form
CXCR4	CXC chemokine receptor 4
DAIDS	Division of AIDS
EC	Ethics Committee
ELISA	Enzyme-Linked Immunosorbent Assay
FDA	(United States) Food and Drug Administration
GALT	Gut-associated lymphoid tissue
GCP	Good Clinical Practices
HIV	Human Immunodeficiency Virus
HPTN	HIV Prevention Trials Network
HPTU	HIV Prevention Trials Unit
HRA	Health Research Associates
HSV-2	Herpes simplex virus-2
IRB	Institutional Review Board
LDMS	Laboratory Data Management System
LL	local laboratory
MIP	macrophage inflammatory protein
MMC	mucosal mononuclear cells
MSM	men who have sex with men
N-9	Nonoxynol-9
NIAID	(United States) National Institute of Allergy and Infectious Diseases
NIH	(United States) National Institutes of Health
PBMC	Peripheral blood mononuclear cells
PCR	polymerase chain reaction
PID	Participant identification number
RANTES	Regulated on activation normal T cell expressed and secreted
RCC	Regulatory Compliance Center
RT	Reverse transcriptase
SAE	Serious adverse event
SDF	Stromal-derived factor
SDMC	(HPTN) Statistical and Data Management Center
SIV	Simian Immunodeficiency Virus
SSP	Study-specific procedures
STD	Sexually transmitted disease
UCLA	University of California, Los Angles

HPTN 056

PROTOCOL TEAM ROSTER

Ian McGowan, MD, PhD, Principal Investigator HPTN 056
UCLA Medical Center,
MRL 2734, 675 Charles E. Young Dr. South
Los Angeles, CA 90095
Tel: 310-206-3580
Fax:310-206-8824
Email: *imcgowan@mednet.ucla.edu*

Peter Anton, MD, Protocol Co-Chair
UCLA Medical Center,
MRL 2734, 675 Charles E. Young Dr. South
Los Angeles, CA 90095
Tel: 310-206-5797
Fax: 310-206-8824
Email: *panton@mednet.ucla.edu*

Lydia Soto-Torres, MD, MPH, NIAID Medical Officer
Division of AIDS/NIAID/NIH
6700B Rockledge Drive, Room 4112
Bethesda, MD 20892
Tel: 301-594-9705
Fax: 301-402-3684
Email: *Lsoto-torres@niaid.nih.gov*

Leigh Peterson, PhD, CORE Protocol Specialist
Family Health International
2224 East NC Hwy 54
Durham, NC 27713
Tel: 919-544-7040 ext. 349
Fax: 919-544-0207
Email: *lpeterson@fhi.org*

Beverly Bell, CORE Protocol Specialist-Back-up
Family Health International
2224 East NC Hwy 54
Durham, NC 27713
Tel: 919-544-7040 ext. 310
Fax: 919-544-0207
Email: *bbell@fhi.org*

John Boscardin, Statistician
School of Public Health
UCLA
21-254B CHS
Los Angeles CA 90095
Tel: 310-825-6402
Fax: 310-267-2113

Marie Fuerst, Study Nurse
UCLA Medical Center,
MRL 2734, 675 Charles E. Young Dr. South
Los Angeles, CA 90095
Tel: 310-825-9254
Fax: 310-206-8824
Email: *mfuerst@mednet.ucla.edu*

Peter Kerndt, MD, Principal Investigator, Los Angeles HPTU
3850 Wilshire Blvd, Suite 1640
Los Angeles, CA 90010
Tel: 213-744-3066
Fax: 213-749-9606
Email: *Prk40@aol.com*

Audrey Williams, CORE Senior Project Assistant
Family Health International
2224 East NC Hwy 54
Durham, NC 27713
Tel: 919-544-7040 ext. 257
Fax: 919-544-0207
Email: *awilliams@fhi.org*

HPTN 056: Characterization of Baseline Mucosal Indices of Injury and Inflammation in Men For Use in Rectal Microbicide Trials

SCHEMA

Purpose: The purpose of this study is to obtain preliminary data on a range of mucosal parameters that might be valuable in the safety evaluation of rectal microbicides and to determine the magnitude and biological variability of those indices in a range of male subjects who are likely to participate in future Phase I rectal microbicide studies.

Hypothesis 1: The practice of anoreceptive sexual intercourse by male subjects, the presence or absence as well as the stage of HIV-1 infection (defined by plasma viral load), and the site of mucosal biopsy (10 cm versus 30 cm) will influence immunological, virological and histopathological parameters in rectal mucosa.

Hypothesis 2: The immunological, virological and histopathological parameters measured in this study will remain stable, within individuals, over a six-week period.

Design: This study will be an observational study at a single site (UCLA Medical Center) in which subjects will undergo endoscopic collection of distal colonic and rectal tissue biopsies at defined time points over a six-week period.

Study Population: HIV-1 infected and uninfected men aged 18-50 years.

Study Size: A total of 16 participants will be enrolled into four groups:

- Group 1: HIV-1-seronegative, practicing anal-receptive sex* (n = 4)
- Group 2: HIV-1-seronegative, not practicing anal-receptive sex ** (n = 4)
- Group 3[#]: HIV-1-seropositive, anal-receptive active* subjects with high plasma viral burden (>10,000 copies RNA / ml plasma) (n = 4)
- Group 4[#]: HIV-1-seropositive, anal-receptive active* subjects with low plasma viral burden (<50 copies RNA / ml plasma) (n = 4)

* Average of 1 episode of anal receptive intercourse per week for the past two months
** No history of anal receptive intercourse for the past two months
[#] CD4>200 cells/mm^3

Treatment Regimen: There is no treatment regimen in this observational study.

Study Duration: Participant accrual will take approximately four months and each participant will complete six weeks of follow-up. The total duration of the study will be approximately seven months. Data analysis will require an additional two months.

Primary Objective: To determine the variability and contrast the differences between study groups of a range of immunological, virological and histopathological parameters in multiple rectal tissue biopsies from two sites in the recto-sigmoid colon (10 cm and 30 cm) measured three times over six weeks. Parameters to be studied include:

- Histopathology (all subjects)
- Mucosal mononuclear cell phenotype by flow cytometry (all subjects)
- Mucosal immunoglobulin production by ELISA (all subjects)
- Mucosal cytokine mRNA by reverse transcriptase (RT) PCR (all subjects)
- Tissue and rectal secretion viral load (in HIV-1 seropositive subjects)

Secondary Objective: To determine the variability and stability within an individual of a range of immunological, virological and histopathological parameters (listed above) in multiple rectal tissue biopsies by assaying samples from two sites in the recto-sigmoid colon (10 cm and 30 cm) from participants in relevant study groups measured three times over six weeks.

1.0 INTRODUCTION

HPTN 056, "Characterization of Baseline Mucosal Indices of Injury and Inflammation in Men For Use in Rectal Microbicide Trials," will be conducted under the auspices of the HIV Prevention Trials Network (HPTN) since the investigators are closely affiliated with the Los Angeles HIV Prevention Trials Unit (HPTU) and anticipate using the LA HPTN community outreach program to facilitate enrollment into the study. In addition, the HPTN clinical trials support staff will provide critical guidance to ensure conduct that the study complies with the Division of AIDS (DAIDS) Good Clinical Practices (GCP) guidelines for studies involving human subject participation.

1.1 Background and Prior Research

The gastrointestinal tract is the body's largest lymphoid organ, containing significant numbers of activated, memory T lymphocytes making it a prime site for HIV infection and amplification. Understanding the impact of HIV on the gastrointestinal-associated lymphoid tissue (GALT) is essential because of (1) the profound drop in mucosal CD4-positive lymphocytes during early Simian Immunodeficiency Virus (SIV) infection[4] with similar mucosal lymphopenia seen in patients with Human Immunodeficiency Virus (HIV) infection[5], (2) the persistence of tissue viral replication in the setting of undetectable plasma viral activity[6], and (3) the growing appreciation of the compartmentalization of HIV infection[7,8]. The implications of this area of research are protean for developing novel rectal microbicides as well as successful vaccination strategies.

The rectal and vaginal epithelia differ significantly in their capacity to resist the traumatic friction accompanying sexual intercourse and in the immune parameters activated to provide protection against invasive pathogens. The rectum is lined by a single cell columnar epithelium scattered with activated intra-epithelial lymphocytes. The lamina propria and, as such, systemic exposure, is immediately subjacent and separated from the lumen by a single cell layer attached to the basement membrane by reticular fibers. The vaginal epithelium, in contrast, is a stratified epithelium with a low density of intraepithelial lymphocytes. The endo-cervical epithelium is more akin to that of the rectum with significant numbers of activated lymphocytes whose density fluctuates with hormonal cycles[9].

A recent study has demonstrated that up to 41% of men who have sex with men (MSM) use vaginal microbicides for lubrication during rectal sex[10]. Many of these contain Nonoxynol-9 (N-9), a product that has been demonstrated to cause rapid exfoliation of rectal epithelia in mice[11] and variable abnormality in humans[1,12]. Evidence of this injury is apparent within 15 minutes of exposure to N-9 with resolution of injury within 8 hours[1]. Assessment of mucosal parameters characterizing this insult, which might guide the development of future rectal microbicides, is currently not available.

Co-receptor expression (e.g., CCR5, CXCR4, etc.) on exposed mucosal immunocytes is important for HIV-1 entry. In healthy HIV-1 seronegative individuals, the expression level of CCR5 is increased seven-fold in mucosal mononuclear cells

(MMC) compared to peripheral blood mononuclear cells (PBMC)[13]. CXCR4, however, is expressed on $CD45RO^+$ T cells in similar levels in MMC and PBMC. It was recently shown that MMC are more easily infected with HIV-1 than PBMC[13,14]. Explanations for the high susceptibility to HIV-1 of MMC may include the increased expression of HIV-1 co-receptors, especially CCR5, as well as the activation status of the MMC. The expression of CCR5 has been shown to be up-regulated by pro-inflammatory and T helper (Th)-1 cytokines, while Th-2 cytokines up-regulate CXCR4[15,16]. This suggests that expression of CCR5 and CXCR4 is partly controlled by Th1/Th2 type of cytokines, which have been shown to be up-regulated in rectal mucosa from HIV-infected patients[17]. It will be important to ascertain whether microbicidal agents trigger similar responses and associated increased vulnerability to infection.

RANTES, macrophage inflammatory protein (MIP)-1α and MIP-1β[18] are the natural ligands for CCR5 while stromal-derived factor (SDF)-1 is the ligand for CXCR4. The physiological function of β-chemokines and their receptors is to direct migration of recruited lymphocyte subsets to sites of inflammation and immune activation[19,20], furthering the inflammatory cascade. Blocking chemokine activity has proved to be effective for inhibiting the migration of certain leukocytes[21] while up-regulation of chemokine receptors and their ligands are characteristic correlates of mucosal inflammation[22,23]. Immune activation of resting $CD4^+$ T cells has been shown to trigger viral replication and spread[24,25].

It remains unclear which factors may augment or reduce the risk of HIV transmission via the rectal route. The increased cellular and soluble inflammation associated with HIV infection[26] might well increase the transmission of HIV infection. The vulnerability of the rectal epithelium to the trauma of sexual intercourse suggests that the sub-epithelial mucosa may frequently be exposed to infected lumenal ejaculate and therefore to the risk of systemic infection. The implications are that one cannot rely simply on epithelial integrity and focus on strategies associated with lumenal absorption of infectious agents. Efforts must also be directed towards containment of potentially inflammatory sequelae that may favor HIV transmission. Similarly, one cannot rely on gross or microscopic morphological changes to identify adverse consequences of trial therapies as many safety risks may be subtle and require alternate modes of detection (PCR, flow cytometry, immunohistochemistry, permeability studies etc).

1.2 Rationale

The development of new rectal microbicides will involve defining the safety and efficacy profile of each agent. The safety profile for a rectal microbicide may differ from that needed for a vaginal microbicide as the two sites differ anatomically and physiologically. It is known that microbicides may cause local mucosal irritation[1] and the rationale for the aims of this study is to define baseline values for a broad range of immunological, virological and histopathological parameters that might be perturbed following application of a rectal microbicide.

Several assays are available for assessing injurious responses to microbicidal therapy. We have selected those procedures with which we have demonstrated expertise and which we believe will best demonstrate potential anatomic and immunologic changes to an intact mucosal surface. We have included indices that will reflect changes in soluble inflammation (such as changes in chemokine and cytokine mRNA levels) as well as alterations in cellular activation markers and co-receptor expression that might otherwise be under appreciated using routine histopathology.

Much of our previous data have been obtained from samples acquired from 30 cm in the recto-sigmoid colon. However, it is known that there are regional differences in the concentration of lymphoid follicles and lymphoid aggregates throughout the colon[2,3]. These differences include increased numbers of lymphoid aggregates in the rectum as well as the cecum. Data generated from samples collected at 30 cm are useful if one is interested in mucosal responses at the upper range of ejaculate contact and lubricant migration during sex. In contrast, data from samples collected at 10 cm (which would include a region with a high density of lymphoid tissue compared to 30 cm) are clearly pertinent because of the local exposure and traumatic friction associated with ano-receptive intercourse at this level. For these reasons, immunologic, activation, inflammatory, and virologic parameters will be characterized in samples from the recto-sigmoid at both 10 cm and 30 cm.

Although the sample size in this study is limited (four subjects per arm with a total population of 16 patients) the Center for HIV & Digestive Diseases at UCLA (CHADD) has conducted previous studies of mucosal viral load and cytokine gene expression with sample sizes of seven subjects per arm and have been able to demonstrate statistically significant differences between different study subgroups. In addition, this study will provide critical data to power future studies in which these types of assays are employed.

2.0 STUDY OBJECTIVES AND DESIGN

2.1 Primary Objective

The primary objective of this study is to determine the variability and contrast the differences between study groups of a range of immunological, virological and histopathological parameters in multiple rectal tissue biopsies from two sites in the recto-sigmoid colon (10 cm and 30 cm) measured three times over six weeks. Parameters to be studied include:

- Histopathology (all subjects)
- Mucosal mononuclear cell phenotype by flow cytometry (all subjects)
- Mucosal immunoglobulin production by ELISA (all subjects)
- Mucosal cytokine mRNA by RT-PCR (all subjects)
- Tissue and rectal secretion viral load (in HIV-1 seropositive subjects)

2.2 Secondary Objective

The secondary objective of this study is to determine the variability and stability within an individual of a range of immunological, virological and histopathological parameters (listed above) in multiple rectal tissue biopsies by assaying samples from two sites in the recto-sigmoid colon (10 cm and 30 cm) from participants in relevant study groups measured three times over six weeks.

2.3 Study Design

This is an observational study to be conducted among 16 MSM residing in the Los Angles, California area. Half of the 16 participants will be HIV-1-infected. The study design is summarized in the Schema above and the Schedule of Events in Appendix I. Participants will undergo endoscopic collection of intestinal tissue biopsies at two sites in the recto-sigmoid colon (10 cm and 30 cm) at defined time points over a six-week period.

After providing written informed consent, potential study participants will undergo eligibility screening, including medical history and a directed physical exam. Eligible participants will undergo endoscopic collection of rectal biopsies at baseline (primary objective) and on two subsequent occasions at two-weekly intervals for six weeks (secondary objective). Details of the assays performed are listed in Section 5: Study Procedures.

3.0 STUDY POPULATION

Sixteen MSM, half of whom will be HIV-1-infected, will be included in this study. Participants will be selected for the study according to the criteria in Sections 3.1 and 3.2. Participants will be recruited, screened, and enrolled concurrently as described in Section 3.3 and assigned to one of the four study groups listed below:

- Group 1: HIV-1-seronegative, practicing anal-receptive sex* (n = 4)
- Group 2: HIV-1-seronegative, not practicing anal-receptive sex ** (n = 4)
- Group 3[#]: HIV-1-seropositive, anal-receptive active* subjects with high plasma viral burden (>10,000 copies RNA / ml plasma) (n = 4)
- Group 4[#]: HIV-1-seropositive, anal-receptive active* subjects with low plasma viral burden (<50 copies RNA / ml plasma) (n = 4)

* Average of 1 episode of anal receptive intercourse per week for the past two months
** No history of anal receptive intercourse for the past two months
[#] CD4>200 cells/mm^3

Issues related to participant retention and withdrawal from the study are described in Sections 3.4 and 3.5, respectively.

3.1 Inclusion Criteria

Men who meet all of the following criteria are eligible for inclusion in the study:

- Age 18 to 50 years
- HIV-1-status as documented by licensed ELISA/Western blot confirmed at screening
- Able and willing to communicate in English
- Able and willing to provide written informed consent to take part in the study
- Able and willing to provide adequate information for locator purposes
- For subjects practicing anal-receptive sex (Groups 1, 3, and 4), report average of one episode per week for the past two months
- For HIV-1-infected participants (Groups 3 and 4), documented plasma viral load of either >10,000 copies/RNA/ml plasma or <50 copies RNA/ml plasma for at least the last two months
- HIV-1-infected participants should not have changed their antiretroviral therapy within the previous six weeks
- For subjects not practicing anal-receptive sex (Group 2), no history of anal receptive intercourse for the past two months
- The anal-receptive groups (Groups 1, 3, and 4) will be asked to refrain from anal intercourse for 24 hours prior to and for one week after sigmoidoscopy
- CD4>200 cells/mm^3 to be confirmed at screening

3.2 Exclusion Criteria

Men who meet any of the following criteria will be excluded from the study:

- Active, serious infections (other than HIV-1 infection) requiring parenteral antibiotic therapy within 15 days prior to screening
- Any other clinical condition or prior therapy that, in the opinion of the investigator, would make the patient unsuitable for the study or unable to comply with the study requirements. Such conditions would include, but not be limited to, current or recent history of severe, progressive, or uncontrolled renal, hepatic, hematological, gastrointestinal, endocrine, pulmonary, neurological, or cerebral disease
- Diagnosed inflammatory bowel disease (ulcerative colitis or Crohn's disease) or rectal malignancy
- Rectal surgery including fistulectomy
- Diagnosed bleeding disorder including hemophilia and thrombocytopenia

- Prosthetic heart valve or diagnosed with a valvular abnormality
- Three or more HSV-2 outbreaks (HIV-1 seropositive subjects only) in the past 12 months prior to screening
- One or more HSV-2 outbreaks (HIV-1 seropositive subjects only) in the past 6 months prior to screening
- Hemorrhoidectomy in the past six months prior to screening
- Active diarrheal disease (> 3 times /day) or bleeding disorder
- Bleeding hemorrhoids (at the time of screening or within the last six weeks) or the presence of anal fistulae within the last six weeks
- Impaired coagulation or current use of anticoagulants or medications such as warfarin or aspirin that, in the opinion of the Investigator, would make participation in the study unsafe or complicate interpretation of study outcome data
- Positive rectal cultures for chlamydia or gonorrhea at screening or within the previous month
- A history of unprotected anal intercourse in the previous three months
- Enrolled in any other clinical trial for the duration of their participation in HPTN 056

3.3 Recruitment Process

Subject recruitment will draw on support from UCLA CARE Clinic and Health Research Associates (HRA) for direct outreach. Subjects recruited from the UCLA CARE clinics are predominately white and male, although more women and minorities are now being treated there. Recruitment of subjects through HRA reflects increased ethnic/minority diversity (2% African American, 12% Hispanic; over 40% economically disadvantaged).

If needed, participants may be recruited for this study through local health care providers who treat large numbers of HIV-infected patients. Participants also may be sought through advertising (posters, brochures, and postings on the site's HPTU website), announcements in newsletters distributed to participants in previous studies, and outreach at venues frequented by MSM.

Site staff will meet at least weekly to discuss current recruitment status, targets, and strategies. Staff also will follow-up with all persons who express an interest in the study to ensure that screening appointments are scheduled and carried out in a timely manner.

3.4 Participant Retention

Once a participant enrolls in this study, the study site will make every effort to retain him for six weeks of follow-up in order to minimize possible bias associated with loss-to-follow-up. Study site staff is responsible for developing and implementing local standard operating procedures to target this goal. Components of such procedures include:

- Thorough explanation of the study visit schedule and procedural requirements during the informed consent process, and re-emphasis at each study visit;
- Thorough explanation of the importance of all four study treatment groups to the overall success of the study;
- Use of appropriate and timely visit reminder mechanisms (via email and/or telephone); and
- Immediate and multifaceted follow-up on missed visits.

3.5 Participant Withdrawal

Regardless of the participant retention methods just described, participants may voluntarily withdraw from the study for any reason at any time. The Investigator also may withdraw participants from the study in order to protect their safety and/or if they are unwilling or unable to comply with required study procedures. Participants also may be withdrawn if the study sponsor or government or regulatory authorities terminate the study prior to its planned end date.

Every reasonable effort will be made to complete a final evaluation (as described in Section 5) of participants who terminate from the study prior to week six, and study staff will record the reason(s) for all withdrawals from the study in participants' study records. Participants who withdraw from the study will not be replaced.

4.0 STUDY TREATMENT/PRODUCT/INTERVENTION

This is an observational study and has no treatment, product, or therapeutic intervention associated with it. A number of diagnostic procedures will be performed and these are discussed in Section 5.0.

5.0 STUDY PROCEDURES

An overview of the study visit and procedures schedule is presented in Appendix I. Participant accrual will take approximately four months and each participant will complete six weeks of follow-up. The total duration of the study will be approximately seven months. Presented below is additional information on visit-specific study procedures. Detailed instructions to guide and standardize all study procedures across sites will be provided in the study-specific procedures manual.

All routine laboratory tests (HIV-1 antibody test, HIV-1 viral load, CD4 count, and rectal cultures for chlamydia and gonorrhea) will be conducted by the UCLA Medical Center Clinical Laboratory which is CLIA certified. The plasma viral load will be measured at the UCLA Clinical Laboratory using the Roche Amplicor HIV-1 Monitor Test, Version 1.5. All laboratory results will be reported to the trial participants once the results are available. Participants who receive unexpected results such as HIV seropositivity and/or rectal infection with chlamydia or gonorrhea will be referred to available sources of medical and psychosocial care and support for appropriate counseling, clinical assessment, and treatment.

Appendix I presents the study visits and procedures' schedule. Detailed instructions to guide and standardize all study procedures will be provided in the study-specific procedures (SSP) manual. Unless otherwise specified, the laboratory procedures listed below are performed at the local study site laboratories.

5.1 Pre-screening

If desired, study staff may pre-screen potential study participants. During these interactions, study staff may explain the study to participants and ascertain presumptive eligibility, to be confirmed at an on-site Screening Visit (see Section 5.2). Pre-screening data may be recorded and stored at the study site in the absence of written informed consent from potential participants, provided the information is collected in such a manner that it cannot be linked to participant identifiers. A local IRB approved script will be used to conduct any telephone interviews.

5.2 Screening Visit (up to –7 days prior to enrollment)

Written informed consent will be obtained before any screening procedures are initiated. For potential participants who do not meet the study eligibility criteria, the screening process will be discontinued when ineligibility is determined. If a participant is not enrolled within seven days after signing the informed consent form, the screening process must be repeated.

The outline of the study will be described to the potential study participants including the following points:

- All anal-receptive participants should refrain from anal-receptive activity including penile, digital or sex toy insertion 24 hours prior to the Enrollment visit, Visit 2 and Visit 3.

- Anal-receptive activity should be avoided for one week after the Enrollment visit, Visit 2 and Visit 3.

- Subjects must agree to refrain from using anticoagulants or other medications such as warfarin or aspirin throughout the study. Should a subject need to take such medications after joining the study, the subject will be withdrawn.

- Subjects who either have received or do receive positive rectal cultures for chlamydia or gonorrhea at screening or within the previous month will not be allowed to participate in the study.

5.2.1 Administrative, Behavioral, and Regulatory Procedures

- Obtain informed consent
- Assess eligibility for study based on inclusion/exclusion criteria
- Assign participant ID number
- Collect locator and demographic information
- Provide HIV pre-test counseling, when indicated
- Provide contact information for study clinicians

5.2.2 Clinical Procedures

- Medical history, physical examination and ascertainment of current medications
- Collect blood for HIV-1 serology, HIV-1 plasma viral load (Groups 3 & 4) and CD4 count (Groups 3 & 4)
- Perform anoscopy with visualization of the rectal mucosa
- Collect 2 rectal swabs for *Chlamydia trachomatis* and *Neisseria gonorrhea* cultures

5.2.3 Laboratory Procedures

- HIV-1 serology
- HIV-1 plasma viral load (Groups 3 & 4)
- T cell count (Groups 3 & 4)
- Rectal *Chlamydia trachomatis* and *Neisseria gonorrhea* cultures

5.3 Enrollment

5.3.1 Administrative, Behavioral, and Regulatory Procedures

- Update locator information and remind participant that he will receive visit reminders via email and/or telephone
- Remind participant of increased risks associated with anal-receptive activity within one (1) week of biopsies
- Subjects will be telephoned by the study nurse the day after the sigmoidoscopy to check whether the subject has experienced any adverse events related to the procedure

5.3.2 Clinical Procedures

- Provide test results from screening visit and post-test counseling
- Provide treatment of sexually transmitted diseases (STDs) if clinically indicated; offer of STD testing and treatment of partners
- Perform anoscopy with application of Sno-Strip© (x 2) and Weck-cel© sponge to rectal mucosa for approximately 5 minutes to collect rectal secretions
- Perform flexible sigmoidoscopy with collection of 10 rectal biopsies at 10 cm and 10 rectal biopsies at 30 cm from the anal margin
- Perform adverse event assessment

5.3.3 Laboratory Procedures

- Routine histopathological assessment of rectal biopsies
- Tissue HIV-1 viral load (for HIV-1 seropositive subjects)
- Luminal HIV-1 viral load (for HIV-1 seropositive subjects)
- Mucosal cell isolation and flow cytometry
- Mucosal cytokine PCR profile
- Luminal immunoglobulin analysis

5.4 Follow-up Visits 2 and 3

Visit 2 will occur two weeks (± 2 days) after the enrollment visit and Visit 3 will occur two weeks (± 2 days) after Visit 2.

5.4.1 Administrative, Behavioral, and Regulatory Procedures

- Update locator information
- Review sexual behavior diary
- Remind participant of increased risks associated with anal-receptive activity within one (1) week of biopsies
- Subjects will be telephoned by the study nurse the day after the sigmoidoscopy to check whether the subject has experienced any adverse events related to the procedure

5.4.2 Clinical Procedures

- Perform anoscopy with application of Sno-Strip© (x 2) and Weck-cel© sponge to rectal mucosa for approximately 5 minutes to collect rectal secretions
- Perform flexible sigmoidoscopy with collection of 10 rectal biopsies at 10 cm and 10 rectal biopsies at 30 cm from the anal margin
- Perform adverse event assessment

5.4.3 Laboratory Procedures

- Routine histopathological assessment of rectal biopsies
- Tissue HIV-1 viral load (for HIV-1 seropositive subjects)
- Luminal HIV-1 viral load (for HIV-1 seropositive subjects)
- Mucosal cell isolation and flow cytometry
- Mucosal cytokine PCR profile
- Luminal immunoglobulin analysis

5.5 Concomitant Medications

Enrolled study participants may not use anticoagulants or medications such as warfarin or aspirin.

6.0 SAFETY MONITORING AND ADVERSE EVENT REPORTING

The study site Investigators are responsible for continuous close safety monitoring of all study participants, and for alerting the protocol team if unexpected concerns arise. The protocol team will meet via conference call every two to four weeks during the period of study implementation, and additional ad hoc calls will be convened, if required.

Study participants will be provided with a 24-hour telephone number and instructed to contact the study clinician to report any events they may experience, except for life-threatening events, for which they will be instructed to seek immediate emergency care. Depending on the severity of the event, the clinician will instruct the participant to present to the study site (for more mild events) or to an emergency room (for more serious events) for immediate evaluation. Where feasible and medically appropriate, participants will be encouraged to seek medical care where the study clinician is based, and to request that the clinician be paged or otherwise contacted upon their arrival. With appropriate permission of the participant, records from all non-study medical providers related to such events will be obtained and required data elements will be recorded on study case report forms (CRF).

Since this is an observational study in which participants will not receive any investigational agents, no adverse event reporting will be undertaken per se. However, because events may occur that are related to the study procedures, specific events will be recorded and reported to the Safety Desk of the DAIDS Regulatory Compliance Center and the Institutional Review Board (IRB) in accordance with all applicable local IRB requirements.

Specific events to be recorded in the CRF are as follows:

- Bleeding;
- Pain;
- Perforation;
- Other, specify (ulceration, abrasion, erythema, edema).

Study site staff will document on study case report forms all listed events reported by or observed in enrolled study participants regardless of severity and presumed relationship to

study procedures. All participants reporting such events will be followed clinically, until the event resolves (returns to baseline) or stabilizes. All participants will be reminded at study visits that there may be an increased risk of perforation, bleeding, infection or acquiring HIV if he engages in anal-receptive activity within one (1) week after the biopsies have been taken.

7.0 STATISTICAL CONSIDERATIONS

7.1 Review of Study Design

This study will be an observational study in which subjects will undergo endoscopic collection of distal colonic and rectal tissue biopsies at Enrollment (Week 0), Week 2 and Week 4 over a six-week period. The study is not formally powered to demonstrate statistical significance between the two biopsy sites or the four study groups as the total study population is only 16.

7.2 Data Analysis

7.2.1 Primary Analyses

The primary objective is to acquire pilot data on the distributional characteristics of various immunological, virological and histopathological parameters at the two different biopsy locations. Summary statistics and graphical displays will be used to examine the distributions of these parameters. Analysis of variance techniques will be used to estimate systematic differences between the two biopsy sites.

7.2.2 Secondary Analyses

The secondary aims of the study involve examination of differences between study groups and variability over time of the various parameters. Exploratory graphics and descriptive statistics will be used to examine and summarize the data in an informal manner. Where appropriate, more formal statistical analysis will be conducted: analysis of variance techniques will be used to examine group differences, and variance component analysis will be employed to assess stability across time points.

8.0 HUMAN SUBJECTS CONSIDERATIONS

8.1 Ethical Review

This protocol and the template informed consent form contained in Appendix II (and any subsequent modifications) will be reviewed and approved by the HPTN Protocol Review Committee and DAIDS Prevention Science Review Committee with respect to scientific content and compliance with applicable research and human subjects regulations.

The protocol, site-specific informed consent form, participant education and recruitment materials, and other requested documents (and any subsequent modifications) also will be reviewed and approved by the ethical review bodies responsible for oversight of research conducted at the study site.

Subsequent to initial review and approval, the responsible local Institutional Review Board (IRB) will review the protocol at least annually. The Investigator will make safety and progress reports to the IRB at least annually, and within three months of study termination or completion. These reports will include the total number of participants enrolled in the study, the number of participants who completed the study, all changes in the research activity, and all unanticipated problems involving risks to human subjects or others. The study site will submit documentation of continuing review to the DAIDS Protocol Registration Office, via the HPTN CORE, in accordance with the current DAIDS Protocol Registration Policy and Procedures Manual.

8.2 Informed Consent

Written informed consent will be obtained from each study participant prior to screening. Participants will be provided with a copy of their signed informed consent form if they are willing to receive it.

The study site is responsible for developing the study informed consent form for local use that describes the purpose of the study, the procedures to be followed, and the risks and benefits of participation, in accordance with all applicable regulations.

Study staff will document the informed consent process as instructed in the study-specific procedures (SSP) manual.

Written informed consent also will be obtained for long-term specimen storage and possible future testing, however consent for specimen storage is not required for study participation. Study staff will document the informed consent process in accordance with the DAIDS Standard Operating Procedure for Source Documentation.

8.3 Risks

Study participants may experience discomfort when undergoing phlebotomy. During phlebotomy, participants may feel dizzy or faint, or develop a bruise, swelling or infection where the needle is inserted. The risks of the blood draws include pain, bruising, and light headedness and on rare occasions, infection.

Flexible sigmoidoscopy is a commonly practiced medical procedure and the endoscopic procedures done in this trial will not involve any unusual risks or discomforts. The risks associated with these procedures include mild discomfort and the feeling of having a “bloated stomach”. Endoscopic biopsies are painless and heal quickly within 3-5 days. On extremely rare occasions, the endoscopic procedure or biopsies may lead to pain, infection, bleeding or perforation (a hole or tear in the lining of the gut) of the gastrointestinal tract. Perforation occurs approximately once

out of every 100,000 procedures. If this extremely rare complication occurs, surgery to repair the tear may be necessary.

Participants may become embarrassed, worried, or anxious when completing their HIV-related interviews and/or receiving HIV/STD counseling. They also may become worried or anxious while waiting for their HIV test results or after receiving HIV-positive test results. Trained counselors will be available to help participants deal with these feelings. Although the study site will make every effort to protect participant privacy and confidentiality, it is possible that participants' involvement in the study could become known to others, and that social harms may result (i.e., because participants could become known as HIV-infected or at "high risk" for HIV infection). For example, participants could be treated unfairly or discriminated against, or could have problems being accepted by their families and/or communities.

8.4 Benefits

There may be no direct benefits to participants in this study. However, participants and others may benefit in the future from information learned from this study. Specifically, information learned in this study may lead to the development of a safe and effective microbicide that reduces the risk of HIV transmission.

In addition, participants will receive HIV counseling and testing as part of the study screening process, as well as rectal exams. Participants also will be screened for a number of STDs, and provided STD treatment if applicable.

8.5 Incentives

Pending IRB approval, participants will be compensated for their time and effort in this study, and/or be reimbursed for travel to study visits and time away from work. Site-specific reimbursement amounts will be specified in the study informed consent forms.

8.6 Confidentiality

All study-related information will be stored securely at the study site. All participant information will be stored in locked file cabinets in areas with access limited to study staff. All laboratory specimens, reports, study data collection, process, and administrative forms will be identified by a coded number only to maintain participant confidentiality. All records that contain names or other personal identifiers, such as locator forms and informed consent forms, will be stored separately from study records identified by code number. All local databases will be secured with password-protected access systems. Forms, lists, logbooks, appointment books, and any other listings that link participant ID numbers to other identifying information will be stored in a separate, locked file in an area with limited access.

Participant's study information will not be released without the written permission of the participant, except as necessary for monitoring by the NIAID and/or its contractors and representatives of the HPTN CORE, Statistical and Data Management Center, and Central Laboratory, the site IRB, and US government and regulatory agencies.

A Certificate of Confidentiality will be obtained for this study from the US Department of Health and Human Sciences. This certificate protects study staff from being compelled to disclose study-related information by any US Federal, State or local civil, criminal, administrative, legislative or other proceedings. It thus serves to protect the identity and privacy of study participants.

8.7 Communicable Disease Reporting Requirements

Study staff will comply with all applicable local requirements to report communicable diseases identified among study participants to local health authorities. More specifically, confidential morbidity reports for chlamydia and gonorrhea, including the subject's name, will be filed with the Department of Health Services, Los Angeles County. Positive HIV serology will be reported to the same agency using an anonymous number created by the UCLA Medical Laboratory and the Investigator. Participants will be made aware of all reporting requirements during the study informed consent process.

8.8 Study Discontinuation

The study also may be discontinued at any time by NIAID or the HPTN, the IRB(s) or US government and regulatory agencies.

9.0 LABORATORY SPECIMENS AND BIOHAZARD CONTAINMENT

9.1 Local Laboratory Specimens

As described in Section 5, the following types of specimens will be collected for testing at the local laboratory (LL):

- Blood for HIV-1 serology, CD4+ and viral load
- Weck-Cel© to measure luminal immunoglobulins
- Sno-strip© to measure luminal HIV-1 RNA
- Cultures for *Chlamydia trachomatis* and *Neisseria gonorrhea*
- Rectal biopsy to measure cytokine mRNA profiles, histopathology and flow cytometry to measure immune activation

The study site will adhere to standards of good laboratory practice and local standard operating procedures for proper collection, processing, labeling, transport, and storage of specimens to the local laboratory, and the HPTN Central Laboratory Manual.

9.2 Specimen Storage and Possible Future Research Testing

Study site staff will store all specimens collected in this study at least through the end of the study. In addition, study participants will be asked to provide written informed consent for their intestinal tissue to be stored after the end of the study for possible future testing. Storage of all tissue samples will follow local standard operating procedure to ensure the anonymity and confidentiality of the trial subjects. The specimens of participants who do not consent to long-term storage and additional

testing will be destroyed at the end of the study, after all protocol-required and quality assurance testing has been completed.

9.3 Biohazard Containment

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood and shipping and handling of all specimens for this study, as currently recommended by the United States Centers for Disease Control and Prevention. All infectious specimens will be transported in accordance with US regulations (42 CFR 72).

10.0 ADMINISTRATIVE PROCEDURES

10.1 Study Activation

Following ethical review and approval, the study site will submit required administrative documentation (as listed in the study-specific procedures manual) to the HPTN CORE. CORE staff will work with study site staff and complete "protocol registration" in accordance with DAIDS procedures. Included in this step will be CORE and DAIDS review of the study informed consent form.

Pending successful protocol registration and submission of all required documents, CORE staff will "activate" the site to begin study operations. Study implementation may not be initiated until a study activation notice is provided to the site.

10.2 Study Coordination

Study implementation will be directed by this protocol as well as the SSP manual. The SSP manual will outline procedures for conducting study visits; data and forms processing; safety assessment, management and reporting; and other study operations. The Protocol Team will develop study case report forms and as part of the study activation process, the Investigator will identify all case report forms to be used as source documents.

Close coordination between protocol team members will be necessary to track study progress, respond to queries about proper study implementation, and address other issues in a timely manner. Rates of accrual, adherence, and follow-up will be monitored closely by the team as well as the HPTN Study Monitoring Committee. The Protocol Chair, DAIDS Medical Officer, Protocol Biostatistician, and CORE Protocol Specialist will address issues related to study eligibility as needed to assure consistent case management and documentation.

10.3 Study Monitoring

On-site study monitoring will be performed in accordance with DAIDS policies. Study monitors will visit the site to

- verify compliance with human subjects and other research regulations and guidelines;

- assess adherence to the study protocol, study-specific procedures manual, and local counseling practices; and

- confirm the quality and accuracy of information collected at the study site and entered into the study database.

Site investigators will allow study monitors to inspect study facilities and documentation (e.g., informed consent forms, clinic and laboratory records, other source documents, and case report forms), as well as observe the performance of study procedures. Investigators also will allow inspection of all study-related documentation by authorized representatives of the HPTN CORE, NIAID, and US government and regulatory authorities. A site visit log will be maintained at the study site to document all visits.

10.4 Protocol Compliance

The study will be conducted in full compliance with the protocol. The protocol will not be amended without prior written approval by the Protocol Chair and NIAID Medical Officer. All protocol amendments must be submitted to and approved by the relevant local IRB and the DAIDS Regulatory Compliance Center (RCC) prior to implementing the amendment.

10.5 Investigator's Records

The study site investigator will maintain, and store in a secure manner, complete, accurate, and current study records throughout the study. “In accordance with US regulations, the investigator will retain all study records for at least three years after submission of the HPTU’s final Financial Status Report to DAIDS, which is due within 90 days after the end of the HPTU’s cooperative agreement with DAIDS, unless otherwise specified by DAIDS or the HPTN CORE.” Study records include administrative documentation — including site registration documents and all reports and correspondence relating to the study — as well as documentation related to each participant screened for and/or enrolled in the study — including informed consent forms, locator forms, case report forms, notations of all contacts with the participant, and all other source documents.

10.6 Use of Information and Publications

Publication of the results of this study will be governed by HPTN policies. Any presentation, abstract, or manuscript will be submitted by the Investigator to the HPTN Manuscript Review Committee and DAIDS for review prior to submission.

11.0 REFERENCES

1. Phillips DM, Taylor CL, Zacharopoulos VR, Maguire RA. Nonoxynol-9 causes rapid exfoliation of sheets of rectal epithelium. *Contraception* 2000;**62**(3)**:**149-54.

2. O'Leary AD, Sweeney EC. Lymphoglandular complexes of the colon: structure and distribution. *Histopathology* 1986;**10**(3)**:**267-83.

3. Langman JM, Rowland R. The number and distribution of lymphoid follicles in the human large intestine. *J Anat* 1986;**149:**189-94.

4. Veazey RS, DeMaria M, Chalifoux LV, et al. Gastrointestinal tract as a major site of CD4+ T cell depletion and viral replication in SIV infection. *Science* 1998;**280**(5362)**:**427-31.

5. Schneider T, Jahn HU, Schmidt W, Riecken EO, Zeitz M, Ullrich R. Loss of CD4 T lymphocytes in patients infected with human immunodeficiency virus type 1 is more pronounced in the duodenal mucosa than in the peripheral blood. Berlin Diarrhea/Wasting Syndrome Study Group. *Gut* 1995;**37**(4)**:**524-9.

6. Hockett RD, Kilby JM, Derdeyn CA, et al. Constant mean viral copy number per infected cell in tissues regardless of high, low, or undetectable plasma HIV RNA. *J Exp Med* 1999;**189**(10)**:**1545-54.

7. Poles MA, Elliott J, Vingerhoets J, et al. Despite high concordance, distinct mutational and phenotypic drug resistance profiles in human immunodeficiency virus type 1 RNA are observed in gastrointestinal mucosal biopsy specimens and peripheral blood mononuclear cells compared with plasma. *J Infect Dis* 2001;**183**(1)**:**143-8.

8. Clayton F, Snow G, Reka S, Kotler DP. Selective depletion of rectal lamina propria rather than lymphoid aggregate CD4 lymphocytes in HIV infection. *Clin Exp Immunol* 1997;**107**(2)**:**288-92.

9. Patterson BK, Landay A, Andersson J, et al. Repertoire of chemokine receptor expression in the female genital tract: implications for human immunodeficiency virus transmission. *Am J Pathol* 1998;**153**(2)**:**481-90.

10. Gross M, Buchbinder SP, Celum C, Heagerty P, Seage GR, 3rd. Rectal microbicides for U.S. gay men. Are clinical trials needed? Are they feasible? HIVNET Vaccine Preparedness Study Protocol Team. *Sex Transm Dis* 1998;**25**(6)**:**296-302.

11. Phillips DM, Zacharopoulos VR. Nonoxynol-9 enhances rectal infection by herpes simplex virus in mice. *Contraception* 1998;**57**(5)**:**341-8.

12. Tabet SR, Surawicz C, Horton S, et al. Safety and toxicity of nonoxynol-9 gel as a rectal microbicide. *Sex Transm Dis* 1999;**26**(10)**:**564-71.

13. Anton PA, Elliott J, Poles MA, et al. Enhanced levels of functional HIV-1 co-receptors on human mucosal T cells demonstrated using intestinal biopsy tissue. *AIDS* 2000;**14**(12)**:**1761-5.

14. Lapenta C, Boirivant M, Marini M, et al. Human intestinal lamina propria lymphocytes are naturally permissive to HIV-1 infection. *Eur J Immunol* 1999;**29**(4)**:**1202-8.

15. Weissman D, Dybul M, Daucher MB, Davey RT, Jr., Walker RE, Kovacs JA. Interleukin-2 up-regulates expression of the human immunodeficiency virus fusion coreceptor CCR5 by CD4+ lymphocytes in vivo. *J Infect Dis* 2000;**181**(3)**:**933-8.

16. Patterson BK, Czerniewski M, Andersson J, et al. Regulation of CCR5 and CXCR4 expression by type 1 and type 2 cytokines: CCR5 expression is downregulated by IL-10 in CD4-positive lymphocytes. *Clin Immunol* 1999;**91**(3)**:**254-62.

17. Kotler DP, Reka S, Clayton F. Intestinal mucosal inflammation associated with human immunodeficiency virus infection. *Dig Dis Sci* 1993;**38**(6)**:**1119-27.

18. Raport CJ, Gosling J, Schweickart VL, Gray PW, Charo IF. Molecular cloning and functional characterization of a novel human CC chemokine receptor (CCR5) for RANTES, MIP-1beta, and MIP-1alpha. *J Biol Chem* 1996;**271**(29)**:**17161-6.

19. Furie MB, Randolph GJ. Chemokines and tissue injury. *Am J Pathol* 1995;**146**(6)**:**1287-301.

20. Springer TA. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell* 1994;**76**(2)**:**301-14.

21. Gonzalo JA, Lloyd CM, Wen D, et al. The coordinated action of CC chemokines in the lung orchestrates allergic inflammation and airway hyperresponsiveness. *J Exp Med* 1998;**188**(1)**:**157-67.

22. Luster AD. Chemokines--chemotactic cytokines that mediate inflammation. *N Engl J Med* 1998;**338**(7)**:**436-45.

23. MacDermott RP, Sanderson IR, Reinecker HC. The central role of chemokines (chemotactic cytokines) in the immunopathogenesis of ulcerative colitis and Crohn's disease. *Inflamm Bowel Dis* 1998;**4**(1)**:**54-67.

24. Nabel G, Baltimore D. An inducible transcription factor activates expression of human immunodeficiency virus in T cells. *Nature* 1987;**326**(6114)**:**711-3.

25. Pantaleo G, Fauci AS. Immunopathogenesis of HIV infection. *Annu Rev Microbiol* 1996;**50:**825-54.

26. Olsson J, Poles M, Spetz AL, et al. Human immunodeficiency virus type 1 infection is associated with significant mucosal inflammation characterized by increased expression of CCR5, CXCR4, and beta-chemokines. *J Infect Dis* 2000;**182**(6)**:**1625-35.

27. Farzadegan et al. Sex differences in HIV-1 viral load and progression to AIDS. *Lancet* 1998;**352:**1510-14.

28. Katzenstein et al. The relation of virologic and immunologic markers to clinical outcomes after nucleoside therapy in HIV infected adults with 200-500 CD4+ cells per cubic millimeter. *N Engl J Med* 1996;**335:**1091-8

29. Anton PA, Poles MA, Elliott J, et al. Sensitive and reproducible quantitation of mucosal HIV-1 RNA and DNA viral burden in patients with detectable and undetectable plasma viral HIV-1 RNA using endoscopic biopsies. *J Virol Methods* 2001;**95**(1-2)**:**65-79.

30. Elliott J, Boscardin J, Poles M, McGowan I, Fuerst M, Taing P, Anton P. Mucosal cytokines/chemokines are increased by up to 75-fold in HAART-treated subjects with detectable plasma viral loads compared to those with fully suppressed viremia. *9th Conference on Retroviruses and Opportunistic Infections* 2002. (Abstract D56e).

31. Kozlowski PA, Lynch RM, Patterson RR, Cu-Uvin S, Flanigan TP, Neutra MR. Modified wick method using Weck-Cel sponges for collection of human rectal secretions and analysis of mucosal HIV antibody. *J Acquir Immune Defic Syndr* 2000;**24**(4)**:**297-309.

32. John GC, Sheppard H, Mbori-Ngacha D, et al. Comparison of techniques for HIV-1 RNA detection and quantitation in cervicovaginal secretions. *J Acquir Immune Defic Syndr* 2001;**26**(2)**:**170-5.

APPENDICES

Appendix I: Schedule of Study Visits and Procedures

	Screening (up to –7 days)	Enrollment (Week 0)	Follow-up (Week 2 ±2 days Week 4 ±2 days)
Obtain Informed Consent	X		
Assess Eligibility	X		
Assign PID	X		
Collect/update locator information	X	X	X
Collect demographic information	X		
HIV pre-test counseling	X		
Provide contact information for study clinicians	X		
Medical History and Physical examination	X		
Medication assessment	X		
Provide STD/HIV test results		X	
Anoscopy	X	X	X
Collect rectal secretions (Sno-Strip© and Weck-cel© sponge)		X	X
Rectal swabs for chlamydia and gonorrhea	X		
Provide STD treatment (if applicable)		X	
Offer STD testing/treatment to partners		X	
Flexible Sigmoidoscopy/collection of biopsies		X	X
Adverse Event Assessment		X	X
Perform laboratory evaluations:			
HIV-1 serology	X		
HIV-1 plasma viral load*	X		
CD4+ T-cell count*	X		
Chlamydia and gonorrhea cultures	X		
Histopathology assessment of biopsies		X	X
Tissue HIV-1 viral load*		X	X
Luminal HIV-1 viral load*		X	X
Mucosal cell isolation and flow cytometry		X	X
Mucosal cytokine PCR profile		X	X
Luminal immunoglobin analysis		X	X

* HIV-1 seropositive subjects only

Appendix II: Sample Informed Consent Forms

DIVISION OF AIDS
HIV Prevention Trials Network (HPTN)
INFORMED CONSENT FORM
SCREENING AND ENROLLMENT

HPTN 056:
Characterization of baseline mucosal indices of injury and inflammation in men for use in rectal microbicide trials

Final Version 1.0
02 December 2003

Short Title for the Study

A study of the rectal tissue lining in males to determine those factors that impact on the function of this tissue to act as an immune barrier.

Introduction

You are being asked to participate in this research study either because:

- you are male and have had at least one experience per week for the past two months of receptive anal sex;
- or you are male and have had at least one experience per week for the past two months of receptive anal sex and are HIV positive with a viral load above 10,000;
- or you are male and have had at least one experience per week for the past two months of receptive anal sex and are HIV positive with a viral load below 50;
- or you are male, HIV negative, and do not practice anal sex.

This study is sponsored by the U.S. National Institutes of Health (NIH). The person in charge of this study at this site is Ian McGowan, M.D., Ph.D. and Peter Anton, M.D. from the Department of Medicine, Division of Digestive Diseases at the University of California, Los Angeles. Before you decide if you want to be a part of this study, we want you to know about the study.

This is a consent form. It gives you information about this study. The study staff will talk with you about this information. You should read the information below, and ask questions about anything you do not understand before deciding whether or not to participate. Please take the consent form home to discuss this with family or friends, if desired. If you agree to take part in this study, you will be asked to sign this consent form or make your mark in front of a witness. Drs. McGowan or Anton will meet with you before you sign to answer any additional questions you may have. You will be offered a copy to keep.

Disclosure statement

Your health care provider may be an investigator of this research protocol, and as an investigator, is interested in both your clinical welfare and in conduct of this study. Before entering this study or at any time during the research, you may ask for a second opinion about your care from another doctor who is in no way associated with this project. You are not under any obligation to participate in any research project offered by your physician.

Why Is This Study Being Done? (Purpose of Study)

One purpose of this study is to begin investigations to determine the immune and viral profiles, and tissue changes caused by injury during anal sex in the rectal lining (mucosa) of male subjects. We will attempt to identify differences in these profiles between men who are HIV positive or negative and practicing anal sex, and those men who are HIV negative and do not practice anal sex. This is an observational study that is not associated with any treatment.

A second purpose of this study is to determine if specific immune, viral, and tissue measurements taken from two sites in the recto-sigmoid colon remain the same within an individual over a six-week period.

What Do I Have To Do If I Am In This Study? (Procedures)

Screening Visit
If you agree to participate in this study, you will have one screening visit to determine if you are eligible to enroll in the study based on your HIV status and blood viral load. The screening visit will take approximately 2 hours. There is a possibility you may be found ineligible for the study after this initial screening visit and will not be able to proceed.

After you read, discuss, and sign this informed consent form, you will be asked to begin the screening process. The study staff will ask you where you live and other questions about you, your health, current medications you are taking, and your sexual practices. You will also have counseling about HIV and the HIV test.

If your answers to these questions indicate that you may be eligible for the study, you will be given a physical exam, give approximately 3 tablespoons of blood from your arm for an HIV test to determine/confirm your HIV status, and if you are infected with HIV, your plasma viral load and CD4 count will be determined.

An anoscope (a small plastic tube) will be inserted into your rectum (approximately two inches) to view the lining surface. In addition, two samples (secretions) will be taken from your rectum to test for gonorrhea and chlamydia.

All screening tests must be completed within seven days. If all tests are not done within seven days, and you are still interested in participating in this study, you will have to start the screening tests over from the beginning.

Enrollment Visit
If you are eligible and decide to take part in the study, the study staff will ask you to confirm/update information on where you live and how you can be contacted. You will be given the results of the tests performed during the screening visit (HIV, CD4 count, gonorrhea and chlamydia). If you are found to have gonorrhea and/or chlamydia, you will be treated and you will be informed that you can bring your partner for testing and treatment if necessary. If any chlamydia or gonorrhea is identified, this will be reported, including your name, in a confidential fashion to the Los Angeles County Department of Public Health. If your HIV antibody test is positive this will be anonymously reported to the Los Angeles County Department of Public Health by mail using a non-name code (i.e., the report will not include personal-identifying information).

Also, during the enrollment visit, you will be asked to undergo the following procedures:

Sno-Strip© collection for mucosal viral load and Weck-cel© sponge collection for immunoglobulins (a type of antibody): Prior to the flexible sigmoidoscopy (examination of anus, rectum, and colon), samples of secretions will be obtained from your rectal lining. An anoscope (a small plastic tube) will be inserted into your rectum (approximately two inches) to view the lining surface. Through this scope, two strips of absorbent paper (Sno-Strips©) will be placed on the rectal lining for approximately five minutes. This will be followed by placement of two Weck-cel© sponges (similar to a Q-tip©) which will remain on the rectal lining for an additional five minutes.

Sigmoidoscopy: Prior to the procedure, you will report to the endoscopy suite where the procedure will again be explained to you and all of your questions will be answered. You may not take aspirin or any aspirin containing medications for at least five days prior to the procedure. Tylenol is allowed. You will not need to take any preparation for the procedure at home. A Fleets® enema will be given to you in the endoscopy suite after the Sno-Strip© and Weck-cel© are collected and prior to the flexible sigmoidoscopy. A Fleets® enema is a small amount of fluid (approximately one cup) that is inserted into your rectum, retained for approximately five minutes and then expelled in the toilet.

There are no sedative medications routinely given for flexible sigmoidoscopies. If requested, special provisions will be made to provide you with sedative medication to control for discomfort. If used, you will need to arrange for someone to drive you home. The flexible sigmoidoscopy requires about 15 minutes to perform. During the procedure, your doctor places a tube within your rectum through which he will be able to examine its lining and through which the small biopsies will be taken. You will be consented separately for the flexible sigmoidoscopy each time you are seen.

Biopsy: The doctor performing the sigmoidoscopy will take up to 20 research biopsies (painless snipping of small pieces of tissue) from the colon/rectum. These pieces of tissue that are removed are about the size of a pinhead. The sites from which the biopsies are taken will typically heal completely within a week. Some biopsy samples will be stored for potentially important future research questions.

Blood draw: In the endoscopy suite, we ask that you allow us to collect approximately three tablespoons of blood for research purposes. This blood will be used to measure the number

of immune cells in your blood and some of the specific tags on the cells surface that are indicators for inflammation and compare them to the same markers on the immune cells from your colon biopsies. In those individuals who are HIV positive blood will also be used to measure HIV RNA in blood and to compare with levels of HIV RNA and DNA in tissue from your colon/rectum.

The enrollment visit will take approximately two hours.

Follow-up visits
You will be asked to return to the clinic for two follow-up visits. The first follow-up visit will occur two weeks (±2 days) after the enrollment visit and the second follow-up visit will occur two weeks (±2 days) after the first follow-up visit.

At each follow-up visit, the study staff will ask you to confirm/update information on where you live and how you can be contacted. Also at each follow-up visit, you will be asked to undergo the following procedures (see above descriptions):

- Sno-Strip© collection for mucosal viral load and Weck-cel© sponge collection for immunoglobulins
- Sigmoidoscopy
- Biopsy
- Blood draw

Lab results (HIV viral load and rectal biopsy) will be provided to subjects at the visit following collection of these samples. Each follow-up visit will take approximately two hours.

How Many People Will Take Part In This Study? (Number of Subjects)
Sixteen people will take part in this study.

How Long Will I Be In This Study? (Study Duration)

You will be in this study about eight weeks.

Why Would The Doctor Take Me Off This Study Early? (Involuntary Withdrawal)

The study doctor may need to take you off the study early without your permission if:

- The study is cancelled by the U.S. National Institutes of Health (NIH) or the Institutional Review Board (IRB). (An IRB is a committee that watches over the safety and rights of research subjects.)
- You are not able to attend the study visits or follow the procedures required by the study.
- If you have to take aspirin or any other drug likely to reduce your blood clotting (anticoagulants) such as warfarin.
- If your samples prove to be unsuitable, your participation might be terminated before completion of the study. The investigator may withdraw you from participating in this research if circumstances arise which warrant doing so. The investigators, Drs. McGowan and/or Anton will make the decision and let you know if it is not possible for you to continue. The decision may be made either to protect your health and safety, or because it

is part of the research plan that people who develop certain conditions may not continue to participate.

What Are The Risks Of The Study? (Risks and Discomforts)

Risks of Sno-Strip© and Weck-Cell© Collections
There are minimal risks of the Sno-Strip© and Weck-Cell© collections. You may have minor discomfort from the insertion of the speculum and the lining of your rectum may become irritated. This irritation may last for the remainder of the day. The absorbent paper strip and sponges do not have any associated risks.

Risks of the Endoscopic Procedures: Flexible sigmoidoscopy is a commonly practiced medical procedure and the endoscopic procedures done in this trial will not involve any unusual risks or discomforts. The risks associated with these procedures include mild discomfort and the feeling of having a "bloated stomach".

Endoscopic biopsies: These biopsies are painless and heal quickly within 3-5 days. On extremely rare occasions, the endoscopic procedure or biopsies may lead to pain, bleeding or perforation (a hole or tear in the lining of the gut) of your gastrointestinal tract. Perforation occurs approximately once out of every 100,000 procedures. If this extremely rare complication occurs, surgery to repair the tear may be necessary. There may be an increased risk of perforation, bleeding, infection or acquiring HIV if you engage in anal-receptive activity within one (1) week after the biopsies have been taken.

Risks of the Blood Draws: The risks of the blood draws include pain, bruising, and lightheadedness and on rare occasions, infection. You may feel dizzy or faint.

The procedures listed above may involve risks that are currently unforeseeable.

You may become embarrassed, worried, or anxious when completing your HIV-related interviews and/or receiving HIV/STD counseling. You also may become worried or anxious while waiting for your HIV test results or after receiving HIV-positive test results. Trained counselors will be available to help you deal with these feelings. Although study site will make every effort to protect your privacy and confidentiality, it is possible that your involvement in the study could become known to others, and that social harms may result (i.e., because you could become known as HIV-infected or at "high risk" for HIV infection). For example, you could be treated unfairly or discriminated against, or could have problems being accepted by your families and/or communities.

Are There Benefits To Taking Part In This Study? (Benefits)

This is a descriptive, pilot trial. You will not receive any direct benefit from participating in this trial and you should not expect your condition to improve as a result of participating in this research.

This study will add to the current understanding of the immune defenses of the gastrointestinal tract and will provide the basis to develop new treatments for HIV. This knowledge may help to manage these diseases in the future.

What About Confidentiality? (Confidentiality)

Efforts will be made to keep your personal information confidential. We cannot guarantee absolute confidentiality. Your personal information may be disclosed if required by law or if necessary to protect your rights or welfare. Any publication of this study will not use your name or identify you personally.

This research is covered by a Certificate of Confidentiality issued by the Department of Health and Human Services (DHHS). This Certificate will protect the investigators from being forced to release any research data in which you are identified, even under a court order or subpoena. Disclosure will be necessary, however, upon request of DHHS for audit or program evaluation purposes. There are other situations in which confidential information could be released. These include:

- If you are involved in child abuse
- If you are diagnosed with a communicable disease
- If you are a threat to your own health or to others

This protection, however, is not absolute. A Certificate of Confidentiality does not prevent you or a member of your family from voluntarily releasing information about yourself or your involvement in this research. Note however, that if an insurer or employer, learns about your participation, and obtains your consent to receive research information, then the investigator may not use the Certificate of Confidentiality to withhold this information. This means that you and your family must also actively protect your own privacy.

Upon receipt of your blood and biopsies, we will record your name and patient ID number once and assign a number to the samples. The protocol book containing your name will be stored, and locked in the laboratory. Depending on the research results, we might have to consult your medical records. In this case, we would also keep the records locked in the laboratory.

Authorization To Use And Disclose Protected Health Information For Research Purposes

Federal law requires that researchers, healthcare providers, and health plans protect the privacy of information that identifies you. The privacy law, Health Insurance Portability & Accountability Act (HIPAA) requires that researchers get your permission to be able to use or disclose your protected health information for research purposes in this study. By signing this consent form, you give that permission.

By signing this consent form, you authorize Drs. Ian McGowan and Peter Anton from the Department of Medicine, Division of Digestive Diseases at the University of California, Los Angeles and their research staff and business associates (together referred to as the "researchers") to use and disclose your protected health information for the purposes of this research study.

Your protected health information that may be used and disclosed includes:

- Contact Information
- Demographic Information

- Medical History
- Physical examination
- Current medications
- Blood storage
- STD/HIV testing
- Anoscopy
- Rectal secretions
- Rectal swabs
- Sigmoidoscopy/biopsies
- Adverse events
- Sexual Behavior
- Laboratory evaluations, including the following:
  - HIV serology, HIV plasma load, tissue HIV viral load, luminal HIV viral load
  - CD4+ T-cell count
  - Gonorrhea and Chlamydia testing
  - Histopathology of biopsies
  - Mucosal cell isolation and flow cytometry
  - Mucosal cytokine PCR profile
  - Luminal immunoglobin analysis

The Researchers may use and share your health information with:

- Family Health International (FHI)
- UCLA Office of Protection of Research Subjects
- U.S. National Institutes of Health (NIH) and the Division of AIDS (DAIDS)
- Government representatives, when required by law
- Data Safety Monitoring Board
- The study monitors supporting this study

The researchers will protect your health information by using and disclosing it only as permitted by you in this consent form, and as directed by state and federal law. Once your health information has been disclosed as permitted by this consent form, the information can no longer be considered protected.

After signing this consent form, you can change your mind at any time and not let the researchers disclose or use your protected health information (revoke this authorization).

- If you revoke this authorization, you must send a written letter to the Principal Investigator, Dr. Ian McGowan at the UCLA Center for Health Sciences, Division of Digestive Diseases, 675 Charles E. Young Drive South, MRL 2736, Los Angeles, CA 90095 to inform him of your decision.

- If you revoke this authorization, researchers may only use and disclose the protected health information **already** collected about you in this research study. Once you revoke this authorization, no further protected health information will be collected from you for this research study and you may not be allowed to continue to participate in the study.

- If you revoke this authorization, your protected health information may still be used and disclosed should you have an adverse event (a bad effect).

This Authorization does not have an expiration date.

What Are The Costs To Me? (Costs to You)

There will be no cost to you or your insurance carrier for study-related visits, study products, physical examinations, laboratory tests or other procedures.

Will I Receive Any Payment?

You will be paid $75 for each flexible sigmoidoscopy (including the concurrent blood draw). You will be paid in cash at the end of each scheduled visit. The total received for those subjects undergoing 3 sigmoidoscopies/blood draws will be $225.00.

What Happens If I Am Injured? (Research-Related Injury)

If you are injured as a result of being in this study, you will be given immediate treatment for your injuries. However, you may have to pay for this care. There is no program for compensation either through this institution or the U.S. National Institutes of Health (NIH).

What Are My Rights As A Research Subject? (Research Subject's Rights)

Your participation in this research is VOLUNTARY. If you choose not to participate, it will not affect your relationship with UCLA (or UCLA Medical Center) or your right to health care, or other services to which you are otherwise entitled. If you decide to participate, you are free to withdraw your consent and discontinue participation at any time without prejudice to your future medical care. You are not waiving any legal claims, rights, or remedies because of your participation in this research study. You will not be giving up any of your legal rights by signing this consent form.

During the course of the study, you will be informed of any significant new findings (either good or bad) that might cause you to change your mind about continuing in the study. If significant new information is provided to you, your consent to continue participating in the study will be re-obtained.

You will not be allowed to review the information collected about you on this research study until after the study is completed. When the study is over, you will have the right to access the information. If you want the results of the study, let the study staff know.

What Do I Do If I Have Problems Or Questions? (Problems or Questions)

For questions about this study or a research-related injury, contact the following site personnel:

- The Principal Investigator, Dr. Ian McGowan at the UCLA Center for Health Sciences, Division of Digestive Diseases, 675 Charles E. Young Drive South, MRL 2736, Los Angeles, CA 90095 or you may phone him at (310) 206-3580

- The Co-Investigator, Dr. Peter Anton at the UCLA Center for Health Sciences, Division of Digestive Diseases, 675 Charles E. Young Drive South, MRL 2734, Los Angeles, CA 90095 or you may phone him at (310) 206-5797

- The research nurse coordinator, Marie Fuerst, MS, RN can be reached through our clinical trial office at (310) 825-9254

- Alternatively, you may call the UCLA page operator at (310) 825-6301 and have Drs. McGowan or Anton paged at any time, 24 hours a day

If you have any further questions, comments or concerns about the study, the informed consent process, or your rights as a research subject, you may write or call:

- The Office of Protection of Research Subjects, UCLA, Box 951694, Los Angeles, CA 90095-1694, (310) 825-8714

What if a commercial product is developed as a result of this research study?

All tissue and/or fluid samples are important to this research study. Your sample will be owned by the University of California or by a third party designated by the University (such as another university or a private company). If a commercial product is developed from this research project, the commercial product will be owned by the University of California or its designee. You will not profit financially from such a product.

SIGNATURE OF RESEARCH SUBJECT

I have read (or had someone read to me) and understand the information provided above. I have been given an opportunity to ask questions and all of my questions have been answered to my satisfaction. I have been given a copy of this form, as well as a copy of the Subject's Bill of Rights.

BY SIGNING THIS FORM, I WILLINGLY AGREE TO PARTICIPATE IN THE RESEARCH IT DESCRIBES.

Name of Subject: _____

Signature of Subject:_____ Date:_____

SIGNATURE OF INVESTIGATOR OR CO-INVESTIGATOR

I have explained the research to the subject and answered all of his/her questions. I believe that he/she understands the information described in this document and freely consents to participate.

Name of Investigator or Co-Investigator:_____

Signature of Investigator or Co-Investigator:_____

Date (must be same as subject's):_____

UNIVERSITY OF CALIFORNIA, LOS ANGELES **UCLA**

BERKELEY * DAVIS * IRVINE * LOS ANGELES * RIVERSIDE * SAN DIEGO * SAN FRANCISCO SANTA BARBARA * SANTA CRUZ

OFFICE FOR PROTECTION OF RESEARCH SUBJECTS
405 HILGARD AVENUE
LOS ANGELES, CALIFORNIA 90024-1694

RIGHTS OF HUMAN SUBJECTS IN MEDICAL EXPERIMENTS

Any person who is requested to consent to participate as a subject in a research study involving a medical experiment or who is requested to consent on behalf of another has the right to:

1. Be informed of the nature and purpose of the experiment.

2. Be given an explanation of the procedures to be followed in the medical experiment, and any drug or device to be utilized.

3. Be given a description of any attendant discomforts and risks reasonably to be expected from the experiment.

4. Be given an explanation of any benefits to the subject reasonably to be expected from the experiment, if applicable.

5. Be given a disclosure of any appropriate alternative procedures, drugs or devices that might be advantageous to the subject, and their relative risks and benefits.

6. Be informed of the avenues of medical treatment, if any available to the subject after the experiment if complications should arise.

7. Be given an opportunity to ask any questions concerning the experiment or the procedure involved.

8. Be instructed that consent to participate in the medical experiment may be withdrawn at any time and the subject may discontinue participation in the medical experiment without prejudice.

9. Be given a copy of any signed and dated written consent form used in relation to the experiment.

10. Be given the opportunity to decide to consent or not to consent to a medical experiment with out the intervention of any element of force, fraud, deceit, duress, coercion or undue influence on the subject's decision.

(10/89)

DIVISION OF AIDS
HIV Prevention Trials Network (HPTN)

INFORMED CONSENT FORM
SPECIMEN STORAGE

HPTN 056:
Characterization of baseline mucosal indices of injury and inflammation in men for use in rectal microbicide trials

Final Version 1.0
02 December 2003

INTRODUCTION

You have decided to take part in a National Institute of Health (NIH) Division of AIDS research study. While you are in this research study there may be some samples (blood, fluid, and tissue) taken from you that might be useful for future research. You are being asked to agree to the storage of these samples. This consent form gives you information about the collection, storage and use of your samples. The study staff will talk with you about this information. Please ask if you have any questions. If you agree to the storage of your samples, you will be asked to sign this consent form. You will get a copy to keep. You may decide not to have any samples stored other than what is needed to complete this study and still be in this research study or any future study.

On the checklist at the end of this consent form, you will be asked to indicate if you would permit part of these samples to be shared with other researchers. If you agree to have your sample shared with other researchers and later decide to withdraw, we may not be able to retrieve any or all of your samples from other researchers. The researcher is not required to store your sample(s) indefinitely.

HOW WILL YOU GET THE SAMPLES FROM ME?

There will be NO ADDITIONAL samples taken from you for storage. After all the tests are done for this research study, there may be some left over samples of blood, fluid, and tissue. If you agree, left over samples will be kept and used for future research.

HOW WILL YOU USE MY SAMPLES?

Your samples will only be used to look for immune or viral responses that may play an important role in understanding HIV/AIDS infection. Tests may also include examining your genes (DNA), since they might affect your response to disease in important ways. Your genes might make you more or less susceptible to becoming infected, your responses to infection or to treatment stronger or weaker, or make HIV progress more rapidly or slowly. No other kinds of genetic test will be done by anyone on your stored specimens without first explaining the test to you and obtaining your permission.

The researchers do not plan to contact you or your regular doctor with any results from tests done on your stored samples. This is because research tests are often done with experimental procedures, so the results from one research study are generally not useful for making decisions on managing your health. Should a rare situation come up where the researchers decide that a specific test result would provide important information for your health, the researchers will notify your study doctor and your study doctor will try to contact you. If you wish to be contacted with this type of test result, you must give the study doctor or nurse any change to your address and/or phone number. If you want your regular doctor to be told about this type of test result, you must provide the study doctor or nurse with your regular doctor's name, address and phone number.

Your samples will not be sold or used directly to produce commercial products. Research studies using your samples will be reviewed by the National Institutes of Health and a special committee at the researcher's institution (an Institutional Review Board).

HOW LONG WILL YOU KEEP MY SAMPLES?
There is no time limit on how long your samples will be stored.

HOW WILL MY SAMPLES BE STORED?
Your samples will be stored at special facilities that are designed to store samples safely and securely. The storage facilities are designed so that only approved researchers will have access to the samples. Some employees of the storage facilities will need to have some access to your samples in order to store them and to keep track of where they are, but these people will not have information that directly identifies you. An Institutional Review Board will oversee the storage facilities to protect you and other research volunteers from harm.

DOES STORAGE OF MY SAMPLES BENEFIT ME?
There are no direct benefits to you. The benefit of doing research on stored samples includes learning more about HIV/AIDS infection.

WHAT ARE THE RISKS?
There are few risks related to storing your samples. When tests are done on the stored samples there is a small but possible risk to your privacy. It is possible that if others found out information about you that is learned from tests (such as information about your genes) it could cause you problems with your family (having a family member learn about a disease that may be passed on in families or learning who is the true parent of a child) or problems getting a job or insurance.

WHAT ABOUT CONFIDENTIALITY?
In order to keep your information private, your samples will be labeled with a code that can only be traced back to your research clinic. Your personal information (name, address, phone number) will be protected by the research clinic. When researchers are given your stored samples to study they will not be given your personal information. The results of future tests will not be included in your health records.

We will do everything we can to protect your privacy. In addition to the efforts of the study staff to help keep your personal information private, we have gotten a Certificate of Confidentiality from the U.S. Federal Government. This certificate means that researchers cannot be forced to tell people who are not connected with the research, such as the court system, about your

participation. Also, any publication of the research will not use your name or identify you personally.

People who may review your records include: The UCLA Office of Protection of Research Subjects, National Institutes of Health (NIH), study staff, study monitors, and their designees. Having a Certificate of Confidentiality does not prevent you from releasing information about yourself and your participation in the study.

Even with the Certificate of Confidentiality, if the study staff learns of possible child abuse and/or neglect or a risk of harm to yourself or others, we will be required to tell the proper authorities.

Every tissue or fluid sample contains genetic information. Recent studies have found normal and disease producing genetic variations among individuals. Such variations may permit identification of individual participants. Despite this possible limitation, every precaution will be taken to maintain your confidentiality now and in the future.

We have learned from past research that we will not always be able to predict future research findings and new technologies. You should be aware that unforeseeable problems may arise from new developments. Possible problems include insurance or employment discrimination based on genetic information. Within the limits imposed by technology and the law, every effort will be made to maintain the privacy of your genetic information.

WHAT ARE MY RIGHTS?

Allowing your samples to be stored is completely voluntary. You may decide not to have any samples stored other than what is needed to complete this study and still be in this research study or any future study.

If you decide now that your samples can be stored for future research, you may change your mind at any time. You must contact your study doctor or nurse and let them know that you do not want your samples used for future research. Your samples will then not be used.

WHAT DO I DO IF I HAVE QUESTIONS?

For questions about the storage of your samples, contact:

- Dr. Ian McGowan at the UCLA Center for Health Sciences, Division of Digestive Diseases, Suite #510, 100 Medical Plaza, Los Angeles, CA 90095 at (310) 794-1700, or

- Dr. Peter Anton at the UCLA Center for Health Sciences, Division of Digestive Diseases, Suite #510, 100 Medical Plaza, Los Angeles, CA 90095 or you may phone him at (310) 206-5797

For questions about your rights related to the storage of your samples for research, contact:

- The Office of Protection of Research Subjects, UCLA, Box 951694, Los Angeles, CA 90095-1694, (310) 825-8714

INFORMATION ABOUT YOUR SAMPLE

Please indicate by checking and initialing the category below if you want to receive specific information about what the study found about your samples. It is your responsibility to let the investigator know if your address and/or telephone number changes. The contact information is in this informed consent form under "Identification of Investigators".

☐ _____Specific Information about what the study found about me

☐ _____I do not want any information about my sample

Research is a long and complicated process. Obtaining general information from a project may take years. Even if there is general information from a project, there may not be personal information for every participant.

Please carefully read the statements below and think about your choice. No matter what you decide, it will not affect your care.

I agree to have my left over samples (blood, fluid, and tissue) stored and tested for future research related to HIV infection.

_____Yes

_____No

BY SIGNING THIS FORM, I WILLINGLY AGREE TO PARTICIPATE IN THE RESEARCH IT DESCRIBES.

Name of Subject: _____

Signature of Subject:_____ Date:_____

SIGNATURE OF INVESTIGATOR OR CO-INVESTIGATOR

I have explained the research to the subject and answered all of his/her questions. I believe that he/she understands the information described in this document and freely consents to participate.

Name of Investigator or Co-Investigator:_____

Signature of Investigator or Co-Investigator:_____

Date (must be same as subject's):_____

Appendix III: Laboratory procedures

1. RNA and DNA extraction

Three samples will be combined for joint RNA and DNA extraction as we have already demonstrated low inter-biopsy variability and low assay variability (0.14 $\log_{10}$)[29]. Viral RNA will only be measured in the seropositive subjects and results will be reported as number of copies of HIV RNA/g total RNA; the extracted DNA will be retained for future investigations if needed. Tissue viral load will be determined at both 30 cm and 10cm allowing a comparison between the two sites.

2. Cytokine profiles

Cytokine mRNA profiles will be generated for each of three biopsies in each subject group, at the same two levels. The profile will include RANTES as a soluble mediator of inflammation, pro-inflammatory/Th-1 cytokine, -interferon; and Th-2 cytokine, IL-10. In addition, an aliquot of pooled mRNA from all these biopsies will be combined to quantify a 3-item cytokine/chemokine profile As there are no means at present to determine absolute quantitative results, findings will be reported as fold difference in detection compared to healthy, seronegative controls. We have already demonstrated that significant fold-differences occur between healthy recruits and HIV-infected subjects which give us confidence in being able to observe injury-related inflammation[30] . Comparisons can then be made between the two levels of sampling and whether pooled RNA can provide a less labor-intensive means of characterizing mucosal cytokine mRNA profiles.

3. Histopathology

Routine histopathology will be recorded in a blinded fashion by an experienced gastrointestinal histopathologist. Initially, only routine diagnostic criteria will be applied to determine if the sample shows epithelial disruption and separation from the basement membrane (yes/no) in 3 sections and whether the sample shows evidence of mild, moderate or severe inflammation. A second, more detailed review will be conducted quantifying the number of intra-epithelial apoptotic bodies, lamina propria lymphocytes, eosinophils, plasma cells and neutrophils in 10 crypt sections. These will determine the baseline rate of epithelial disruption detected by histopathologic examination as well as the number of inflammatory cells in each study group pre-intervention. If significant differences between the 4 groups are not appreciated, this will provide assurance that routine histopathology will be adequate for future trials. This is important as detailed examination is time intensive and requires expert histopathologists.

4. Flow cytometry

Samples for flow cytometry will only be obtained from the 30 cm level due to time and cost restraints. Six biopsies will be pooled to provide adequate mucosal mononuclear cells for 3 tubes using 4-color staining (Tube 1: CD20-CD4-CD45-CD8, Tube 2: HLA-DR-CD38-CD4-CD8, Tube 3: RO-CCR5-CD4-CD8). In previous studies, we have been able to appreciate several fold differences in CCR5 expression of CD4+ memory T cells from blood and gut compartments as well as between HIV+, active inflammatory bowel disease and healthy controls in mucosal samples[13]. As an additional indicator of immune activation reflecting microbicidal irritation, activation markers on mucosal mononuclear cells will also be assessed using flow cytometry. The two markers selected for monitoring would be HLA-DR on both CD4+ and CD8+ T cells and CD38 on CD8+ T cells. Both markers have been demonstrated by us to occur in an elevated range in HIV-positive subjects with inflammation as compared to healthy controls in the mucosa, demonstrating there is adequate range to appreciate an increase in seronegative subjects.

5. Weck-Cel© application

Application of Weck-Cel© sponges will be used to assess levels of total secreted immunoglobulins prior to microbicide use as an index of non-specific humoral response to injury.

6. Measurement of luminal HIV RNA

Luminal HIV-1 RNA will be measured using two Sno-Strips© applied to the rectal mucosa. HIV-1 RNA will be extracted and amplified using the Roche Amplicor Ultrasensitive kit. Results will be normalized and reported as number of copies per Sno-Strips© as previously described[32]. Assays and results will be performed with input from Dr. Cellum's laboratory to enable result comparison.

The specialized mucosal assays (viral load, flow cytometry, Weck-cel©) will be measured in the UCLA AIDS Institute Core Mucosal laboratory co-directed by Dr. Ian McGowan.