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Topical Microbicides Program

Update 1997

**National Institute of Allergy and Infectious Diseases
National Institutes of Health
Bethesda, Maryland**

Revised December 1997



Topical Microbicides Program

Update 1997

***National Institute of Allergy and Infectious Diseases
National Institutes of Health
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Revised December 1997

TOPICAL MICROBICIDES UPDATE 1997

Preface

Program staff have prepared this document in order to facilitate collaborative efforts in development and evaluation of safe, effective topical microbicides. It summarizes the Topical Microbicides Research Program of the National Institute of Allergy and Infectious Diseases (NIAID). The program includes efforts of the Division of Acquired Immunodeficiency Syndrome (DAIDS) and the Division of Microbiology and Infectious Diseases (DMID). NIAID has established an internal topical microbicides working group which is comprised of program staff responsible for research portfolios (basic, applied, and clinical/behavioral science) involved in the development and evaluation of topical microbicides. The NIAID Topical Microbicides Working Group coordinates research efforts, information exchange and growth and development of the Institute's topical microbicides program. The group also assists investigators and sponsors who are interested in research and development and/or evaluation of products.

Inquiries should be directed to:

Dr. Penelope J. Hitchcock
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Sexually Transmitted Diseases Branch
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NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

UPDATE ON TOPICAL MICROBICIDES

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Update 1997

Section 1

- Chronology of the Initiative
- Research Strategy
- Funding Overview

Update 1997

Chronology of the Initiative:

Although the public interest in developing safe, effective, female-controlled methods that will prevent STDs and HIV infection has been apparent for a number of years, the NIH's topical microbicide initiative grew out of four international conferences held in 1992 and 1993. From those conferences a consensus emerged that prevention of AIDS as well as prevention of STDs in women and their infants will depend upon the development of safe, effective, female-controlled barrier methods that will block transmission of these pathogens.

The barrier methods that are currently available have limitations. Most require consent, if not active cooperation of the male partner, and therefore cannot be independently implemented at the discretion of the female partner. Although epidemiological studies have demonstrated that the use of condoms can reduce the risk of acquiring HIV infection, gonorrhea and pregnancy, there are many situations in which personal, social or cultural barriers interfere with a women's ability to successfully negotiate and implement condom use. Specifically, the need for a method which can be implemented by women is grounded in the high prevalence of non-consensual sex; sex without condom use; and risky behaviors that occur without partner knowledge.

The following list accounts for the administrative activities associated with the topical microbicides research initiative:

Summer 1992	Topical Microbicides Meeting, Sponsored by Population Council New York, NY
Winter 1993	Contraceptive Development Meeting, Sponsored by CONRAD and USAID, Dominican Republic
Spring 1993	Topical Microbicides Workshop, Sponsored by NIH and FDA, Bethesda, MD
Summer 1993	Topical Microbicides Round Table, Sponsored by Population Council, International AIDS Conference, Amsterdam
Summer 1993	PHS Advisory Group on Topical Microbicides, Coordinated by Patsy Fleming, Washington, DC
Fall 1993	Secretary Shalala identifies \$10 million for NIH Topical Microbicide Initiative
Fall 1993	Topical Microbicides Meeting, Sponsored by World Health Organization, Geneva
Fall 1993	International Working Group on Vaginal Microbicides (IWGVM) Established
Winter 1993	FDA convenes PHS Working Group to evaluate existing literature on the microbicidal activities of OTC spermicides

	1994	House and Senate include topical microbicides in report language
Spring	1994	Contraceptive Efficacy and STD Prevention Meeting, Sponsored by NICHD, Washington, DC
Spring	1994	Semi-annual meetings of IWGVM; begin work on Guidelines for Microbicide Preclinical Development and Clinical Evaluation
Fall	1994	CDC briefing on Topical Microbicides
Winter	1994	Topical Microbicides Initiative described in NIH response to UN's Cairo document
	1995	House and Senate include topical microbicides in report language
Winter	1995	NIH Director briefs Ms. Fleming
Winter	1995	NIAID staff briefs Senators Kassenbaum, Mikulski, Frist and Representative Morella
Summer	1995	NIAID staff briefs Ms. Fleming
Summer	1995	NIAID established Topical Microbicides Working Group
Fall	1995	NIAID's Preclinical Guidelines for Topical Microbicides completed
Fall	1995	White House Summit on AIDS; recommendations include research on Topical Microbicides
Winter	1995	Presidential Advisory Council on AIDS recommends research on Topical Microbicides
	1996	House and Senate include topical microbicides in report language
Winter	1996	IWGVM Guidelines for Microbicide PreClinical Development and Clinical Evaluation Published
Winter	1996	Vice President Gore convenes task force to discuss ways to enhance collaborations between government and industry - vaccines and microbicides are given top priority
Spring	1996	CDC Topical Microbicides Meeting
Spring	1996	Vaginal Physiology Meeting, Sponsored by NIAID, Bethesda, MD
Summer	1996	Symposium on Topical Microbicides, Sponsored by NIAID and UNAIDS, International AIDS Meeting, Vancouver, BC
Fall	1996	Contraceptive Development Meeting, Sponsored by Rockefeller Foundation and CONRAD, Raleigh, NC

Winter	1997	Topical Microbicides Symposium, Retrovirus Meeting, Sponsored by IDSA, Washington, DC
Winter	1997	HIV and Heterosexual Transmission Meeting, Sponsored by NICHD and NIAID, Bethesda, MD
Spring	1997	Topical Microbicides Symposium and keynote lecture, Women and AIDS Meeting, Pascedena CA

Update 1997

Research Strategy:

The research strategy is comprised of three critical, complementary components: basic research, product development research and clinical evaluation, including behavioral research on acceptance and compliance.

The research goals include:

1. Define the molecular basis and chronology of the early steps in the infectious process.
2. Define vaginal and cervical ecology and the natural defense mechanisms of the female reproductive tract.
3. Facilitate preclinical development of topical microbicides.
4. Facilitate clinical evaluation of topical microbicides.
5. Define product characteristics and use-patterns that facilitate acceptance and compliance of topical microbicides.

**Department of Health and Human Services
Investment in Microbicide Research
FY 1997 (actuals)**

NIH	Grants	Contracts	Total \$
NIAID	45	15	13,124,804
NICHD	20	11	6,344,099
OAR	NIAID grant*	NIAID contract*	845,329
OAR	1 - NIMH grant	0	112,670
ORWH	NIAID grant*	0	100,000
FIC	3	0	100,000
NIDA	2	0	106,821
Total NIH	71*	26	20,733,723
Total CDC	7	3	3,969,283
Total DHHS	78*	29	24,703,006

*The supplements to NIAID grants and contracts have not been counted in the totals.

Key to abbreviations:

NIH = National Institutes of Health
 NIAID = National Institute of Allergy and Infectious Diseases
 NICHD = National Institute of Child Health and Human Development
 OAR = Office of AIDS Research
 NIMH = National Institute of Mental Health
 ORWH = Office of Research on Women's Health
 FIC = Fogarty International Center
 NIDA = National Institute of Dental Research
 CDC = Centers for Disease Control
 DHHS = Department of Health and Human Services

Basic and Applied Research

Section 2

- Topical Microbicides Program Projects
- Studies in HIV Pathogenesis
- Preclinical Development

Section 2

Basic and Applied Research

TOPICAL MICROBICIDES PROGRAM PROJECTS:

The NIAID has four program projects for basic and applied research in topical microbicides. These multi-disciplinary research efforts in microbiology, immunology, reproductive biology, reproductive toxicology and cell biology are aimed at identifying effective strategies for intravaginal protection against HIV infection and other STDs.

Request for Applications

Primary Investigators

Abstracts

Accomplishments

RESEARCH ON TOPICAL MICROBICIDES FOR PREVENTION OF STDs/HIV INFECTION

NIH Guide.

RFA AVAILABLE: AI-94-13

P.T.,

National Institute of Allergy and Infectious Diseases

Letter of Intent Receipt Date: May 13, 1994
Application Receipt Date: July 13, 1994

PURPOSE

The Sexually Transmitted Diseases Branch of the Division of Microbiology and Infectious Diseases (DMID), National Institute of Allergy and Infectious Diseases (NIAID), invites grant applications for program projects to conduct research necessary for the development of topical microbicides for intravaginal use to prevent sexually transmitted diseases (STDs), including Human Immunodeficiency Virus (HIV) infection. The NIAID wishes to expand research in this area through the conduct of multi-disciplinary research in microbiology, immunology, reproductive biology, reproductive toxicology, and cell biology. Basic and applied research that will lead to effective strategies for intravaginal protection against STDs, including HIV infection, are encouraged.

HEALTHY PEOPLE 2000

The Public Health Service (PHS) is committed to achieving the health promotion and disease prevention objectives of "Healthy People 2000," a PHS-led national activity for setting priority areas. This Request For Applications (RFA), "Research on Topical Microbicides for Prevention of STDs/HIV", is related to the priority areas of "Sexually Transmitted Diseases" and "HIV Infection". Potential applicants may obtain a copy of "Healthy People 2000" (Full Report: Stock No. 017-001-00474-0) or "Healthy People 2000" (Summary Report: Stock No. 017-001-10473-1) through the Superintendent of Documents, Government Printing Office, Washington, DC 20402-9325 (telephone 202-783-3238).

ELIGIBILITY REQUIREMENTS

Research grant applications may be submitted by domestic, for-profit and non-profit organizations, public and private institutions, such as universities, colleges, hospitals, laboratories, units of State and local governments, and eligible agencies of the Federal government. Foreign organizations are not eligible to apply. Applications from minority individuals and women are encouraged.

MECHANISM OF SUPPORT

The mechanism of support will be the Program Project (P01) grant. Multidisciplinary approaches that involve collaborative efforts among investigators in microbiology, immunology, reproductive biology, reproductive toxicology, and cell biology specialties are strongly encouraged. The total project period for applications submitted in response to this RFA may not exceed five years. At this time, the NIAID is administratively limiting the duration of P01 grants to four years; this administrative limitation may change in the future.

FUNDS AVAILABLE

The estimated total funds (direct and indirect costs) available for the first year of support for awards under this RFA will be \$1,500,000. In Fiscal Year 1995, the NIAID plans to fund approximately two program projects.

Applications may not request budgets in excess of \$750,000 total costs in the first year or more than 4 percent annual inflationary increases for future years. An application with a first year requested amount in excess of \$750,000 total direct cost will require written approval by senior NIAID or NIAMS officials via the program officer for acceptance of the application for processing. The usual PHS policies governing grants administration and management will apply. This level of support is dependent on the receipt of a sufficient number of applications of high scientific merit. Although this program is provided for in the financial plans of the NIAID awards pursuant to this RFA are contingent upon the availability of funds for this purpose. Funding beyond the first and subsequent years of the grant will be contingent upon satisfactory progress during the preceding years and availability of funds.

RESEARCH OBJECTIVES

Background

The HIV pandemic has focused attention on sexually transmitted diseases (STDs), both because HIV infection is a fatal STD and because other STDs are implicated as risk factors for sexual transmission of HIV. Current global estimates indicate that 14 million people are infected with HIV, the cause of Acquired Immunodeficiency Syndrome (AIDS). The majority of these infections were acquired through sexual intercourse. Unless effective prevention measures to stop sexual transmission of HIV are implemented, the number of AIDS cases will continue to grow.

Separate from the HIV epidemic, STDs cause significant morbidity and mortality and contribute greatly to increasing health care costs. In the United States in 1993, an estimated 12 million new cases of STDs occurred, 64 percent of which were in people less than 24 years old, including three million in teenagers. In 1993, cost estimates associated with these infections exceeded six billion dollars.

Furthermore, STDs disproportionately affect the female, the fetus, and the newborn. Gonococcal and chlamydial infections cause pelvic inflammatory disease, infertility, and ectopic pregnancy. Several common STDs adversely affect pregnancy and result in spontaneous abortion, stillbirth, chorioamnionitis, premature rupture of membranes, preterm delivery, and postpartum endometritis. Neonatal infections include gonococcal conjunctivitis, which may lead to blindness; chlamydial pneumonia, which may lead to chronic respiratory disease; congenital syphilis and herpes encephalitis. Moreover, genital infections due to human papillomavirus are causally associated with cervical cancer, one of the most common cancers in women throughout the world.

It is now clear that the risk of becoming infected or infecting others with HIV is substantially increased if one has an STD such as chancroid, genital herpes, syphilis, trichomoniasis, gonorrhea, or chlamydial infection. Over 75 studies on the role of STDs in HIV transmission have been conducted. In 15, STD effects could be assessed independently of sexual behavior effects; both ulcerative and non-ulcerative STDs increased risk of HIV transmission. Although the individual risk of HIV transmission associated with genital ulcer diseases appears to be higher than the discharge diseases (up to ten-fold compared to three to five fold), the high prevalence of discharge diseases results in a much higher population attributable risk.

Furthermore, data from over 80 reports on the natural history of STDs in HIV infected people suggest that, at a community level, HIV infection may increase the prevalence of some STDs (e.g. genital ulcers). If co-infection with HIV prolongs or augments the infectiousness of individuals with STDs, and if the same STDs increase risk of HIV acquisition, these infections may greatly amplify one another. This "epidemiological synergy" may be fueling the explosive growth of the HIV pandemic in some populations (reviewed by

Wasserheit, 1991).

Based on the recommendations from four international conferences, a consensus has emerged that safe, effective, female-controlled chemical barriers that will block transmission are needed to prevent sexually transmitted HIV infection as well as other STDs. Currently available mechanical and chemical barriers have many limitations. Although the male condom, if used consistently and correctly, is a very effective barrier against transmission of HIV and the discharge diseases, it requires the active cooperation of the male partner and, therefore, cannot be independently implemented at the discretion of the female partner. Although the female condom only requires partner consent, virtually nothing is known about its efficacy in preventing bacterial and viral STDs. Spermicides have in vitro activity against most sexually transmitted pathogens including HIV; however, well designed clinical studies demonstrating, unequivocally, that spermicides prevent STDs/HIV infection have not been done. Furthermore, several studies have revealed that spermicides can cause mucosal erosions and ulcers; whether these lesions might increase risk of transmission of HIV infection is presently unknown.

In addition to the inherent limitations of these existing methods, there are many situations in which personal, social, or cultural barriers interfere with a woman's ability to successfully negotiate and implement the use of barriers that could decrease risk of infection. Specifically, the need for a method that can be implemented by women is grounded in the high prevalence of: 1) non-consensual sex; 2) sex without condom use; and 3) risky behaviors that occur without partner knowledge. Just as oral contraceptives dramatically enhanced the ability of women to avoid unwanted pregnancy, effective female-controlled topical microbicides are urgently needed to enhance the ability of women to avoid sexually transmitted infections. Furthermore, chemical barriers that inactivate pathogens in vaginal/cervical secretions, as well as in the ejaculate could reduce female-to-male as well as male-to-female transmission.

Topical microbicides are defined herein as preparations for intravaginal use that are microbicidal (virucidal and/or bactericidal); these products will prevent sexually transmitted infections. The ideal microbicide should have the following characteristics: colorless, odorless, tasteless (physically "invisible"), stable, easy to store, fast acting for appropriate duration, effective pre- and post-coitus, inexpensive, available without a prescription, and safe for use at least one to two times daily. Candidate classes of microbicidal compounds include, but are not limited to, detergents, chemicals such as iodophores, carbohydrates, antibodies, defensins, and pyocins.

Ideally topical microbicides would not be inherently spermicidal but could be formulated with or without spermicidal activity. Non-contraceptive microbicides would be extremely useful for women who wish to become pregnant, or for those women who use one of the many safe, effective methods for contraception that either have no protective effect against infection or, arguably, exacerbate risk of infection. Indeed, a person's contraceptive choices may change over a lifetime. However, no matter what an individual's current contraceptive preference, if they are sexually active, they will desire/require protection from sexually transmitted infections.

Scope of Research

A fundamental objective of the NIAID's STD/HIV research programs is to develop topical microbicides effective in preventing and controlling sexually transmitted infections. Whereas it is recognized that safe, spermicidal-microbicides are important outcomes in the search for female-controlled barrier methods to prevent HIV infection and STDs, the development of spermicides which are not microbicidal is not an objective of NIAID.

Arguably the most productive approach to identifying safe, effective molecular strategies for blocking the early steps in the infectious process is based on

multi-disciplinary efforts including microbiology, immunology, reproductive biology, reproductive toxicology, and cell biology. Applicants are strongly encouraged to include microbiology and two additional disciplines in the program project.

A wide range of basic and applied research questions must be answered in order to meet this programmatic objective. Research issues and areas of high priority to the NIAID and to this RFA include, but are not limited to, the following:

Diseases of interest

Applicants are encouraged to address the following sexually transmitted diseases of high scientific priority to the NIAID:

- HIV infection;
- Chlamydial infection;
- Gonorrhea;
- Trichomoniasis;
- Genital Ulcer Diseases, including syphilis, genital herpes (herpes simplex virus 1 and 2) and chancroid; and
- Human papillomavirus infection.

The goal, as envisioned, is to develop topical microbicides with activity against a combination of pathogens including viral, bacterial and protozoan. With respect to HIV prevention, it is theoretically possible that microbicides may be used to prevent HIV infection primarily or secondarily. For example, a microbicide might be virucidal and prevent HIV infection through direct viral neutralization. Another plausible outcome is development of a safe microbicide that was bactericidal but did not inactivate enveloped viruses such as HIV. Given the role of the discharge STDs in increasing risk of HIV transmission, a microbicide with this specificity would prevent gonorrhea and chlamydial infection directly and HIV infection secondarily. For these reasons, research on two sexually transmitted diseases, preferably one viral and one bacterial, is highly recommended.

Early steps in infectious processes

Studies delineating the chronology and biology of the early steps in the host/pathogen interaction including, but not limited to, the role of inflammation in altering kinetics and infectious dose and the role of cell-free versus cell-associated pathogens in the transmission of disease are encouraged.

Microbicide evaluation

Of interest are in vitro (using established tissue culture systems), ex vivo (using primary human cells eg. vaginal and cervical epithelium or sperm) and pre-clinical characterization of bactericidal and virucidal activity of currently available spermicidal products, new topical microbicides and inactive ingredients (carriers) in existing or new products.

Biology of the reproductive tract

It is critical to identify and characterize the "endogenous" anatomical, physiological, hormonal, immunological and microbiological factors of the female reproductive tract that play a role in resistance and susceptibility to infection including, but not limited to, vaginal pH, mucus, estrogen and other hormones, cervical ectopy and normal flora including lactobacilli.

Reproductive toxicology

Using materials from human subjects (ie. cell or organ cultures) or

established model systems, studies on the effects of topical microbicides on the normal vaginal environment, including but not limited to, alteration of vaginal pH, mucus, surface receptors, normal flora (e.g. lactobacilli and yeast), and induction of inflammatory processes are encouraged. Studies on characterization of spermicidal, teratogenic, mutagenic or carcinogenic properties of topical microbicides are also of interest. Appropriate models for studying the reproductive toxicology of topical microbicides might include, but are not limited to, those that measure vaginal/cervical irritation/ inflammation, or effects on sperm, or on embryogenesis. If animal models are included, these should be well-established models, eg. those developed for characterizing adverse effects of spermicidal contraceptives.

Projects may involve collaboration among investigators at several institutions. Consortium arrangements should follow "Guidelines for Establishing and Operating Consortium Grants, January 1989", available from the individuals listed under INQUIRIES, below.

Project Leaders and Program Directors should budget for an annual one day progress review meeting at a site to be designated (either in Bethesda or in association with a relevant national meeting).

STUDY POPULATIONS

NIH policy is that applicants for NIH clinical research grants and cooperative agreements are required to include minorities and women in study populations so that research findings can be of benefit to all persons at risk of the disease, disorder or condition under study; special emphasis must be placed on the need for inclusion of minorities and women in studies of diseases, disorders and conditions which disproportionately affect them. This policy is intended to apply to males and females of all ages. If women or minorities are excluded or inadequately represented in clinical research, particularly in proposed population-based studies, a clear compelling rationale must be provided.

The composition of the proposed study population must be described in terms of gender and racial/ethnic group. In addition, gender and racial/ethnic issues must be addressed in developing a research design and sample size appropriate for the scientific objectives of the study. This information must be included in the form PHS 398 (rev. 9/91) in Sections 1-4 of the Research Plan AND summarized in Section 5, Human Subjects. Applicants are urged to assess carefully the feasibility of including the broadest possible representation of minority groups. However, NIH recognizes that it may not be feasible or appropriate in all research projects to include representation of the full array of United States racial/ethnic minority populations (i.e., Native Americans [including American Indians or Alaskan Natives], Asian/Pacific Islanders, Blacks, Hispanics).

The rationale for studies on single minority population groups should be provided.

For the purpose of this policy, clinical research is defined as human biomedical and behavioral studies of etiology, epidemiology, prevention (and preventive strategies), diagnosis, or treatment of diseases, disorders or conditions, including but not limited to clinical trials.

The usual NIH policies concerning research on human subjects also apply. Basic research or clinical studies in which human tissues cannot be identified or linked to individuals are excluded. However, every effort should be made to include human tissues from women and racial/ethnic minorities when it is important to apply the results of the study broadly, and this should be addressed by applicants.

If the required information is not contained within the application, the application will be returned. Peer reviewers will address specifically

whether the research plan in the application conforms to these policies. If the representation of women or minorities in a study design is inadequate to answer the scientific question(s) addressed AND the justification for the selected study population is inadequate, it will be considered a scientific weakness or deficiency in the study design and will be reflected in assigning the priority score to the application.

All applications for clinical research submitted to NIH are required to address these policies. NIH funding components will not support applications that do not comply.

LETTER OF INTENT

Prospective applicants are asked to submit, by May 13, 1994, a letter of intent that includes a descriptive title of the overall proposed research, the name, address and telephone number of the Program Director, and the number and title of this RFA. Prospective applicants are also asked to submit a list of the project leaders and other key investigators and their institution(s). Although the letter of intent is not required, is not binding, does not commit the sender to submit an application, and does not enter into the review of subsequent applications, the information that it contains allows NIAID staff to estimate the potential review workload and to avoid conflict of interest in the review. The letter of intent is to be sent to Dr. Olivia Preble at the address listed under INQUIRIES.

APPLICATION REQUIREMENTS

In preparing the application, the applicant should bear in mind the research objectives of this RFA. P01 applications should be prepared using the guidance and instructions provided NIAID PROGRAM PROJECT GRANTS AND MULTIPROJECT COOPERATIVE AGREEMENTS (Revised February 1994), which may be requested from Dr. Olivia Preble at the address listed under INQUIRIES. Failure to follow these instructions may result in delays in the review or in an incomplete application.

Applications are to be submitted on form PHS 398 (rev. 9/91), the standard application form for research grants. Application kits are available at most institutional offices of sponsored research and may be obtained from the Office of Grants Information, Division of Research Grants, National Institutes of Health, Westwood Building, Room 449, Bethesda, MD 20892, telephone 301/594-7248. Applicants must adhere to the format and requirements specified in the PHS 398 application kit.

For purposes of identification and processing, mark "yes" in item 2a on the face page of the application and type in the RFA number AI-94-13 and the title "RESEARCH ON TOPICAL MICROBICIDES FOR PREVENTION OF STDs/HIV." The RFA label available in the form PHS 398 must be affixed to the bottom of the face page of the original application. Failure to use this label could result in delayed processing of the application such that it may not reach the review committee in time for review.

The signed, typewritten original of the application, including the Checklist, and three exact single-sided copies must be sent to:

Division of Research Grants
National Institutes of Health
Westwood Building, Room 240
Bethesda, MD 20892

At the time of submission, two additional copies and all five sets of appendices must also be sent to Dr. Olivia Preble at the address listed under INQUIRIES.

To ensure their review, applications must be received by both the Division of Research Grants and Dr. Olivia Preble by July 13, 1994. Applications not

received by this special date will be considered non-responsive and will be returned to the applicant without review.

If the application submitted in response to this RFA is substantially similar to a grant application already submitted to the NIH for review, but has not yet been reviewed, the applicant will be asked to withdraw either the pending application or the new one. Simultaneous submission of essentially identical applications will not be allowed, nor will essentially identical applications be reviewed by different review committees. Therefore, an application cannot be submitted in response to this RFA that is essentially identical to one that has already been reviewed. This does not preclude the submission of substantial revisions of applications already reviewed, but such applications must include an introduction addressing the previous critique.

REVIEW CONSIDERATIONS

Review Procedures

Applications will be reviewed by DRG staff for completeness and by NIAID staff to determine administrative and programmatic responsiveness to this RFA. Those judged to be incomplete or non-responsive will be returned to the applicant without review. Those considered complete and responsive may be subjected to a triage review by an NIAID peer review group, to determine their scientific merit relative to the other applications submitted in response to this RFA. The NIAID will withdraw from competition those applications judged by the triage peer review group to be noncompetitive for award and will so notify the applicant investigator and the institutional business official. Those applications judged to be competitive for award will be further reviewed for scientific and technical merit by a Review Committee convened by the Division of Extramural Activities, NIAID. The second level of review will be provided by the National Advisory Allergy and Infectious Diseases Council.

Review Criteria

The review criteria for P01 grant applications are the review criteria for large, multi-component, interdisciplinary program projects as outlined in the document entitled NIAID PROGRAM PROJECT GRANTS AND MULTIPROJECT COOPERATIVE AGREEMENTS (Revised February 1994). The application should include a justification for the appropriateness of the proposed studies for the P01 grant mechanism. The distinguishing features of a program project grant include:

A unifying well-defined goal or problem area of research to which each project relates and contributes, thereby producing a research environment that allows each research effort to share the creative strengths of others.

A program director who possesses recognized scientific and administrative competence; she/he should show a substantial commitment of time and effort to the program and exercise leadership in its quality control.

Each research project should, as assessed by peer review, stand on its own independent scientific merit, as well as complement other projects whenever feasible.

These multiple projects require the participation of established investigators in several disciplines, or investigators with special expertise in several areas of one discipline. All investigators should contribute to and share in the responsibilities of fulfilling the program objective.

Applicants from institutions that have a General Clinical Research Center (GCRC) funded by the NIH National Center for Research Resources may wish to identify the GCRC as a resource for conducting the proposed research. If so, a letter of agreement from either the GCRC Program Director or Principal Investigator could be included with the applications.

AWARD CRITERIA

Awarding decisions will be made on the basis of scientific and technical merit as determined by peer review, program priorities and balance, and the availability of funds. The totality of the awarded projects will reflect the programmatic objectives described above.

INQUIRIES

Written and telephone inquiries concerning this RFA are encouraged. The opportunity to clarify any issues or questions from potential applicants is welcome.

Requests for the RFA and the document entitled "NIAID PROGRAM PROJECT GRANTS AND MULTIPROJECT COOPERATIVE AGREEMENTS", (Revised February 1994) as well as inquiries regarding programmatic issues may be directed to:

Dr. Penelope J. Hitchcock
Division of Microbiology and Infectious Diseases
National Institute of Allergy and Infectious Diseases
Solar Building, Room 3A-21
Bethesda, MD 20892
Telephone: (301) 402-0443
Fax: (301) 402-1456
Email: penny@exec.niaid.pc.niaid.nih.gov

Direct inquiries regarding review issues, address the letter of intent to, and mail two copies of the application and all five sets of appendices to:

Dr. Olivia Preble
Division of Extramural Activities
National Institute of Allergy and Infectious Diseases
Solar Building, Room 4C20
Bethesda, MD 20892
Telephone: (301) 496-8208
FAX: (301) 402-2638

Direct inquiries regarding fiscal matters to:

Ms. Sharie Bernard
Division of Extramural Activities
National Institute of Allergy and Infectious Diseases
Solar Building, Room 4B32
Bethesda, MD 20892**
Telephone: (301) 496-7075

SCHEDULE

Letter of Intent Receipt Date:	May 13, 1994
Application Receipt Date:	July 13, 1994
Scientific Review Date:	October, 1994
Advisory Council Date:	February, 1995
Earliest Date of Award:	April, 1995

AUTHORITY AND REGULATIONS

This program is described in the Catalog of Federal Domestic Assistance, No. 93.856 - Microbiology and Infectious Diseases Research. Awards will be made under the authority of the Public Health Service Act, Title III, Section 301 (Public Law 78-410, as amended; 42 USC 241) and administered under PHS grants policies and Federal Regulations 42 CFR Part 74. This program is not subject to the intergovernmental review requirements of Executive Order 12372 or Health Systems Agency review.

**PRIMARY INVESTIGATORS
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Hillier, Sharon, P01 AI39061-01
University of Pittsburgh
Vaginal Microbicides for Prevention of STDs/HIV

The long term goals of these studies are 1. to develop clinically relevant models for evaluation of intravaginal microbicides using currently available microbicidal compounds; and 2. to assess the utility of naturally-occurring microbicidal systems as vaginal microbicides. The intent is to provide new information which could lead to the development of female-controlled methods for prevention of sexual transmission of STDs/HIV.

The studies proposed in this application will evaluate known microbicides (nonoxynol-9, benzalkonium chloride, chlorhexidine) and novel naturally-occurring microbicides in four model systems: 1. in vitro; 2. in raft (organotypic cultures); 3. a primate model; and 4. HIV+ and HIV- women. The spectrum of STD agents to be evaluated include HIV (human model); HPV and HSV 2 (organotypic model); *C. trachomatis* (in vitro, organotypic model and monkey model); *N. gonorrhoeae* (in vitro); and *T. vaginalis* (in vitro). In addition, the effect of these microbicidal agents on the normal vaginal flora including lactobacilli will be evaluated in vitro, in the primate model and in humans. Similarly, tissue toxicity of microbicidal compounds will be evaluated in both primate and Phase I human studies. This multidisciplinary approach will yield new information on currently available microbicidal compounds, lead to development of model systems for evaluation of new microbicides and will assess the potential use of naturally-occurring antimicrobial systems as vaginal microbicides.

In project 1, a primate model will be used to evaluate the early stages of infection of *C. trachomatis*, the effect of available microbicides on the vaginal and cervical epithelium and the effect of these microbicides on chlamydial infection. In project 2, organotypic cultures will be used to evaluate the effect of microbicides on cell phenotype, viability, growth and differentiation, as well as the effects of these compounds on viral gene expression using HPV and HSV-2 gene constructs. In project 3, the lactobacillus-peroxidase-halide antimicrobial system will be evaluated in the vagina of HIV+ and HIV- women, and the effects of exogenous suppositories (containing lactobacillus, recombinant myeloperoxidase, or a combination of both) on *C. trachomatis* infection and recovery of HIV will be evaluated. In project 4, antimicrobial lipid mixtures which are known to inactivate enveloped viruses (HIV and HSV) will be evaluated for their potential effectiveness as vaginal microbicides. These projects will be supported by an administrative/epidemiology core, and three scientific cores. A bacteriology/parasitology core will provide vaginal cultures for projects 1 and 3, and will perform in vitro testing of known microbicides. The chlamydia core will provide purified *C. trachomatis* for project 1 infection studies, cultures and DNA amplification methods for use in project 1 and 3, as well as comparing MIC results using standard methods and organotypic model systems. The clinical core will provide tissue for project 2, human subjects for projects 3 and 4, and will investigate the effects of microbicides on HIV recovery from the vagina and cervix.

The development and use of effective vaginal microbicides should decrease the sexual transmission of HIV, as well as other STDs that not only facilitate the spread of HIV, but also cause long term health care problems in millions of women each year. The proposed studies will yield new information and tools that have immediate applicability to the development of vaginal microbicides.

**UNIVERSITY OF PITTSBURGH-MAGEE WOMEN'S HOSPITAL
TOPICAL MICROBICIDE PROJECT**

PI: Dr. Sharon Hillier

Grant #: AI-39061

Funded: May 1995

Accomplishments to Date:

- Development of reproducible, objective testing systems for the *in vitro* evaluation of microbicides against *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Trichomonas vaginalis* and members of the normal vaginal microflora. A wide range of microbicides, including surfactants, (N-9, chlorhexidine) and peptides, (magainins, Peptidyl-MIMs). Specific inhibitors of viral entry (PRO-2000) have also been screened for activity against other STDs.
- Expansion of the nonhuman primate model for preclinical evaluation of topical microbicides, including microbiologic cultures of the vagina, effects on the vaginal epithelium assessed by colposcopy and biopsy, and the capacity of microbicides to prevent cervical chlamydial infection. Studies evaluating N-9 and chlorhexidine have been completed.
- Development and evaluation of synthetic milk lipids, important endogenous microbicides in the infant gut, for use as vaginal microbicides. *In vitro* screening of four compounds has been completed, and one compound, octyl-glycerol, has emerged as a potential microbicide. Evaluation of toxicity and activity in animal models are ongoing.
- Preparation and evaluation of a vaginal suppository containing *Lactobacillus crispatus* and purified human myeloperoxidase as a vaginal microbicide. The *L. crispatus* acts as the source of H₂O₂, which reacts with the myeloperoxidase in the presence of chloride to form reactive hydroxyl compounds which have potent microbicidal activities. *In vitro* and animal model studies are ongoing.
- Assessment of microbicides on the vaginal ecology of HIV-infected and noninfected women. The effects of three commercially available preparations of nonoxynol-9 and one chlorhexidine gluconate product have been evaluated. Simultaneously, evaluation of the effects of microbicides on the vaginal microflora, vaginal/cervical epithelium and HIV shedding detected by RNA PCR are evaluated.

Kreider, John, P01 AI37829-01
Pennsylvania State University
Analysis of Microbicides in Model Systems

The long-term objective of this Program Project is to develop a multiphased, preclinical laboratory system which can predict the effectiveness and safety of microbicides in clinical trials. A broad spectrum of sexually transmitted diseases (STDs) will be used including papillomavirus, HIV-1, HSV-2, *T. pallidum*, and *Chlamydia*. Microbicides will first be tested *in vitro* for mammalian cell toxicity and effectiveness against the STDs. However, the ultimate question is whether potential clinically useful microbicides can inhibit/destroy STD pathogens in the human vaginal environment with little or no toxicity to the vaginal wall. To this end, we have developed a unique model system in which xenografts of human vaginal tissue in immune deficient mice provide an environment similar to that which prevails in the human vagina. The microbicides which we shall initially evaluate include C31G, a surface active, two component agent. We shall evaluate structure/activity relationships, formulations, and delivery systems. Nonoxynol-9 will be included as a positive control. The laboratory systems which we shall refine in this project, should provide guidelines for the rapid, future development of more effective and safer microbicides.

**PENNSYLVANIA STATE UNIVERSITY
MICROBICIDES IN MODEL SYSTEMS**

PI: Dr. John Kreider

Grant #: AI-37829

Accomplishments to Date:

- Human vaginal xenograft model has been developed.
- Infection of human vaginal xenografts with HSV-2; demonstration of virus macromolecules by PCR, RT/PCR and by *in-situ* hybridization.
- Infection of human vaginal xenografts with HPV 11; production of transformed lesions; demonstration of HPV 11 macromolecules by *in-situ* hybridization.
- Demonstration that C31G is more potent than N-9 in the inactivation of enveloped viruses.
- Microbicidal intervention to prevent HSV-2 infection in an *in vivo* model of vaginal infection.
- Development of an effective microbicide that inactivates papillomaviruses.
- Developed and evaluated a new formulation of C31G (designated high molecular weight, or C14/C16), in which the alkyl chains of the compound have been increased from 12 carbons to 14 carbons (for the amine oxide) and 16 carbons (for the betaine). This formulation has increased efficacy against all of the viruses and bacteria tested (HIV, HSV, Chlamydia). This increased efficacy appears to be correlated with a lower critical micellar concentration (CMC).
- Prepared gel formulations of C31G and placebo for antimicrobial testing. This material will now be tested in model systems.
- Pre-exposure of isolated, purified EB to C31G, at pH 7.2 and 5.7, for 1 hour at 4°C results in reduced attachment to and infectivity for HEC-1B cells - due to a C31G concentration-dependent and pH-dependent destabilization of the chlamydial envelope (release of chlamydial LPS and proteins).
- When HEC-1B cells are infected with Chlamydiae and then exposed to dilute concentrations of C31G (which do not alter HEC-1B cell viability), chlamydial infectivity is also reduced. C31G gains access to the developing chlamydial inclusion causing damage/destruction to metabolically active RB as well as apparent alteration of the inclusion membrane.
- Our preliminary studies show that the broad spectrum antiviral and antibacterial microbicide C31G also has anti-chlamydial activity.

The long term objective of this Program Project is to develop protegrins as a safe and effective chemoprotective barrier to protect women from the acquisition HIV, gonorrhea, chlamydia, syphilis, herpes or trichomonas infection during sexual intercourse. Protegrins are recently discovered natural peptide antibiotics that were originally isolated from porcine leukocytes. They contain 16-18 amino acids, including 4 cysteine residues that form 2 intramolecular disulfide bonds. Unlike defensins, to which they show primary sequence homology, and many other antibiotic peptides of animal origin, the antimicrobial activity of protegrins is enhanced by the presence of physiological salt concentrations and serum. The relatively simple structure of protegrins makes them highly amenable to solid phase chemical synthesis, and synthetic and native protegrins show identical antimicrobial activity in vitro. Protegrins rapidly inactivated HIV, *C. trachomatis*, *N. gonorrhoeae* and *T. pallidum* in vitro when added in micromolar concentrations, typically 5 µg/ml or below. Somewhat higher concentrations of protegrins also inactivated herpes simplex type II and *Trichomonas vaginalis*. Synthetic protegrin congeners with a single disulfide bond, and an *enantiopro*tegrin molecule composed exclusively of D-amino-acids have been synthesized. Initial tests of these congeners against *N. gonorrhoeae* and *T. vaginalis* revealed that they also possessed strong inactivating activity. Despite their unusually broad antimicrobial spectrum, protegrins were not cytotoxic towards mammalian fibroblasts, lymphocytes or macrophages, even when tested at concentrations of 50 µg/ml. The specific aims of the overall program project include designing protegrin congeners with enhanced efficacy against STD organisms (which will also allow us to examine the structure-function relationships of protegrins), testing the protegrin congeners against laboratory and clinical isolates of these organisms, and ascertaining the ability of topical protegrins to protect the lower genital tracts of female mice from infection by *C. trachomatis*. This Program Project Grant combines the talents and experiences of a consortium of leading STD scientists in an integrated and interactive program to develop a novel, female-controlled modality to prevent STD.

UNIVERSITY OF CALIFORNIA
TOPICAL PROTEGRINS TO PREVENT AIDS AND HIV INFECTION
PI: Dr. Robert Lehrer
Grant #: AI-37945

Accomplishments to Date:

- Protegrins are a family of small (2 kDa) antimicrobial peptides that were originally isolated from porcine white blood cells. Their broad antimicrobial spectrum includes Gram positive and Gram negative bacteria, mycobacteria, selected fungi and certain enveloped viruses, including HIV-1. Five native porcine protegrins have been identified and sequenced at the peptide and nucleotide level, and all of these have been prepared by solid phase synthesis.
- For the purposes of this grant, we have synthesized over 100 protegrin-like peptides and tested their activity against HIV-1, *N. gonorrhoeae* and *C. trachomatis*. Optimal candidates for future study and development have been identified from these studies.
- These studies allowed us to establish the minimal essential structure required for potent activity against HIV-1, *N. gonorrhoeae* and *C. trachomatis*. In addition, we were able to define the optimal cystine disulfide array needed to achieve optimal function in physiological media.
- We determined the solution structure of protegrin PG-1 by 2-D NMR. X-ray crystallographic studies are in progress.
- We have begun to examine the activity of protegrins against herpes simplex, type I and II.

Certain other events relevant to topical protegrin prophylaxis deserve mention here, although they are not funded by this award. Protegrins have been awarded broad patent protection (U.S. 5,464,823, et. seq.) and were licensed to a company (IntraBiotics Pharmaceuticals, Sunnyvale, CA) which has performed extensive preclinical safety testing of topically applied protegrins. With FDA approval, a Phase I clinical trial of a topical protegrin (code name IB-367) that is designed to prevent oral mucositis in cancer patients was announced on April 8, 1997. Pending the satisfactory completion of the ongoing Phase Ia studies in normal volunteers, the Phase I study will take place at the Fred Hutchinson Cancer Center in Seattle, Washington and the Brigham and Woman's Hospital in Boston, Mass.

Sexually transmitted diseases (STD) are a major public health problem worldwide. Control of this epidemic will require multiple approaches including vaccine development, increased use of barrier methods, improved patient education and development of new antimicrobial drugs. An important strategy that warrants exploration is the development of topical microbicides designed for intravaginal use and intended to prevent infection at the portal of entry. In this application we propose three projects designed to evaluate the in vitro and in vivo efficacy and toxicity of candidate microbicidal compounds and to further explore the pathogenesis of sexually transmitted viral (HSV and HIV) and bacterial (chlamydia) pathogens.

We have selected a series of polysulfated carbohydrates and surface-active agents expected to have in vitro microbicidal activity. In Project 1 (Microbicidal agents for HSV and HIV-1: In vitro studies), Dr. Patricia Spear and her colleagues will determine the in vitro antiviral and cytotoxic activities of these candidate microbicides. Additionally they will construct HSV mutants and explore the role of envelope glycoproteins in initiating viral infection. They will also investigate HSV and HIV infection of primary human cells derived from the female reproductive tract and determine the ability of lead compounds to protect these cells. In Project 2 (Effects of microbicides on chlamydial infections) Dr. Morris Cooper will evaluate the antichlamydial activity and cellular cytotoxicity of the compounds in cell and human fallopian tube organ cultures. As a possible target for microbicidal action he will also examine the role of chlamydial glycosaminoglycan ligands in initiating infection of human genital epithelial cells. Microbicides found to have potent antimicrobial activity and low cellular toxicity in Projects 1 and 2 will be evaluated in Project 3 (Studies with animal models of STD) by Dr. Lawrence Stanberry to determine whether they can prevent genital HSV-2 or chlamydial infection in guinea pigs. Additional studies in project 3 are intended to increase our understanding of the pathogenesis of genital herpes and facilitate the development of chemoprophylactic approaches to the control of HSV infections. Compounds with in vivo activity (and in vitro activity against HIV) will be referred to the Toxicology Core, directed by Dr. Shirley Reising, for evaluation of their spermicidal activity, genital tract irritant effects, mucosal immunotoxicological properties and effects on fertility and vaginal flora.

We expect this cooperative effort will identify broad spectrum intravaginal microbicides that will be safe and effective in women. In addition, these projects will yield new information regarding the pathobiology of HSV, HIV and chlamydia that will be useful in designing strategies to prevent sexually transmitted diseases.

**CHILDREN'S HOSPITAL RESEARCH FOUNDATION
PROGRESS IN MICROBICIDE RESEARCH
PI: DR. LAWRENCE STANBERRY
GRANT #: AI-37940**

Accomplishments to Date:

- Nonionic detergents have broad antimicrobial activity but there has been no rational strategy for selecting specific agents for *in vitro* and *in vivo* evaluation as possible intravaginal microbicides. Recently we determined that the critical micellar concentration (CMC) of nonionic detergents correlated with the concentration of detergent required to inhibit herpes simplex virus (HSV) infection in cell culture. The CMC is the concentration at which a monolayer of a nonionic surfactant in water begins to form micelles. This finding should facilitate development of nonionic detergents as intravaginal microbicides by allowing rational prioritization of agents for evaluation.
- Nonoxynol 9 (N-9) is a nonionic detergent contained in intravaginal spermicide preparations. N-9 has antimicrobial activity against a variety of sexually transmitted pathogens including HIV and herpes simplex virus. Using an experimental model of genital herpes we showed that N-9 can protect animals against intravaginal HSV-2 challenge but protection was only seen with high concentrations of N-9. Spermicides containing low concentrations of N-9 were ineffective in this model. Our results suggest that while N-9 can afford some protection *in vivo* against herpes simplex virus, that commercially available N-9 containing spermicides are unlikely to be useful as intravaginal microbicides.
- Cholic acid is a naturally occurring bile acid that has *in vitro* activity against HIV. We have shown that *in vitro* cholic acid is active against herpes simplex virus at low concentrations but high concentrations are required to inhibit *Chlamydia trachomatis*. Using an experimental model of genital herpes we have shown that cholic acid affords protection against intravaginal HSV-2 challenge. The *in vivo* effectiveness of cholic acid was greatly influenced by the vehicle used for intravaginal delivery, illustrating the importance of formulation in developing effective intravaginal microbicides. Our results suggest that cholic acid containing intravaginal microbicides may be useful in controlling the spread of viral pathogens such as HIV and HSV but may be ineffective in controlling bacterial infections such as Chlamydia.
- Through the efforts of personnel at the National Institute of Allergy and Infectious Diseases, the Children's Hospital Research Foundation - Cincinnati, the University of Chicago, and Southern Illinois University, collaborations have been established with several companies in the pharmaceutical and biotech industries to evaluate a wide range of novel compounds as potential microbicides. Classes of compounds include stannous salts, organometallic compounds, synthetic peptides, modified polysaccharides, biflavonoids, arylalkenyl polymers, quinonyl aldehyde polymers and synthetic polyanionic compounds. These collaborations represents an unprecedented effort by industry and academia to identify new agents that may be useful in the development of safe and effective microbicides.

Basic and Applied Research

STUDIES IN HIV PATHOGENESIS:

Research on early steps in HIV infection was solicited in a request for applications in 1994. Studies focused on development of primate models, on mechanisms of mucosal transmission and on identification of host factors that determine resistance and susceptibility were funded. Nine awards were made in areas related to topical microbicides development.

Request for Applications
Primary Investigators
Abstracts

MECHANISMS OF AIDS PATHOGENESIS

NIH GUIDE, Volume 23, Number 17, May 6, 1994

RFA: AI-94-018

P.T. 34; K.W. 071508, 0765033, 0705048

National Institute of Allergy and Infectious Diseases (NIAID)

Letter of Intent Receipt Date: September 16, 1994

Application Receipt Date: November 16, 1994

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I. PURPOSE

The National Institute of Allergy and Infectious Diseases (NIAID) invites applications from single institutions or consortia of institutions for research project grants (R01s) each focusing on an hypothesis for AIDS-related pathogenesis. This Request For Applications (RFA) specifically solicits applications for *in vivo* research of AIDS-related pathogenesis utilizing state-of-the-art methods and approaches. *In vivo* research includes studies of human clinical or epidemiologic cohorts, of animal models, or of appropriate specimens from humans or animals. Research supported by this RFA is limited to one or more of three scientific areas:

1. non-human primate models of HIV immunopathogenesis,
2. sexual/mucosal transmission of HIV or SIV, and
3. host factors that modulate HIV or SIV infection or disease.

Where scientifically justified, applicants are encouraged to include both human and animal studies. While the research necessary to test a proposed pathogenesis hypothesis may be possible within a single laboratory, NIAID anticipates that highly competitive applications may require separate components at the same or different institutions specializing in different scientific disciplines (e.g., molecular biology, biochemistry, cellular biology, cellular immunology, genetics and biophysics).

II. HEALTHY PEOPLE 2000

The Public Health Service (PHS) is committed to achieving the health promotion and disease prevention objectives of "Healthy People 2000," a PHS-led national activity for setting priority areas. This Request for Applications (RFA), MECHANISMS OF AIDS PATHOGENESIS, is related to the Priority area of "HIV infection." Potential applicants may obtain a copy of "Healthy People 2000" (Full Report: Stock No. 017-001-00474-0) or "Healthy People 2000" (Summary Report: Stock No. 017-001-00473-1) through the Superintendent of Documents, Government Printing Office, Washington, DC 20402-9325 (telephone 202-783-3238)."

III. ELIGIBILITY REQUIREMENTS

Applications may be submitted by domestic and foreign for-profit and non-profit organizations, public and private, such as universities, colleges, hospitals, laboratories, units of State or Local Government, and eligible agencies of the Federal Government. Successful applications from foreign institutions are limited to three years of support with no support for indirect costs. Domestic applications may include international components but these components will receive no support for indirect costs. Applications from minority individuals and women are encouraged.

IV. MECHANISM OF SUPPORT

Awards made under this RFA will use the National Institutes of Health (NIH) individual research grant (R01) award mechanism. Responsibility for the planning, direction and execution of the proposed project will be solely that of the applicant. The total project period for applications submitted by domestic institutions may not exceed four years; the total project period for applications submitted by foreign institutions may not exceed three years.

Applicants are strongly encouraged to coordinate, through the use of consortium arrangements or subcontracts, integrated approaches with individuals or institutions having relevant reagents and expertise

in their use, demonstrated ability in a particular area of relevant research, or access to relevant animal or patient populations.

Applicants from institutions which have a General Clinical Research Center (GCRC) funded by the NIH National Center for Research Resources may wish to identify the GCRC as a resource for conducting the proposed research. In such case, a letter of agreement from either the GCRC Program Director or Principal Investigator should be included with the application.

V. FUNDS AVAILABLE

The estimated total funds (direct and indirect costs) available for the first year of support for awards under this RFA will be \$6 million. Because the nature and scope of the research proposed in response to this RFA may vary, it is anticipated that the size of the awards will vary also. The NIAID anticipates 8 - 16 awards under this RFA. It is the intent of NIAID to fund applications in each of the three scientific areas. Awards and level of support depend upon receipt of a sufficient number of applications of high scientific merit. Although this program is provided for in the financial plans of the Institute, awards pursuant to this RFA are contingent upon the availability of funds for this purpose. Funding beyond the first and subsequent years of the grant will be contingent upon satisfactory progress during the preceding years and availability of funds.

Applicants are encouraged to discuss budget requests with Division of AIDS (DAIDS) Program Staff (listed in INQUIRIES) prior to submission. Requests for expensive equipment are not encouraged. Investigators are encouraged to use animals efficiently and to minimize expenses for animal purchase and/or support. The purchase of animals should be strongly justified.

This RFA is a one-time solicitation. Future competing continuation applications will compete with all unsolicited investigator-initiated applications and be reviewed by an appropriate Study Section according to the customary NIH referral and peer review procedures.

VI. RESEARCH OBJECTIVES

A. BACKGROUND

NON-HUMAN PRIMATE MODELS OF HIV IMMUNOPATHOGENESIS

Non-human primate models of HIV disease are vital tools for pathogenesis research. Where no single animal model satisfies all investigative requirements, comparison of data from multiple models may contribute to the understanding of the pathogenic aspects of HIV disease. The simian immunodeficiency virus (SIV) and chimeric SIV-HIV infections of macaques are well described. Clues to HIV pathogenesis also may be discovered from analyses of lentivirus-infected non-human primates in which there is little or no apparent disease, *e.g.*, HIV-infected chimpanzees and African green monkeys infected with African Green Monkey (AGM) lentiviruses.

SEXUAL/MUCOSAL TRANSMISSION

The overwhelmingly predominant mode of transmission of HIV is through exposure of mucosal surfaces to infected sexual fluids (semen, cervical/vaginal, rectal) and during birth. However, epidemiological data suggest that sexual transmission, in general, is relatively inefficient, in that exposure does not always produce infection. Some individuals who are exposed to HIV via mucosal surfaces over prolonged periods remain seronegative. The likelihood of transmission is increased by factors that decrease the

integrity of the mucosal epithelium. Epidemiologic studies have implicated sexually transmitted diseases that produce genital ulcerations or inflammation, use of intrauterine devices, and lack of male circumcision as cofactors facilitating HIV transmission.

Studies in the SIV monkey model indicate that 100- to 1,000-fold more infectious virus is required to produce infection following application to intact genital mucosal surfaces than by intravenous injection. Studies of mucosal infection in the SIV model suggest that mucosal dendritic cells may be the first cells infected. Subsequently, infected dendritic cells may migrate to lymph nodes where they infect other cells.

HOST RESISTANCE TO INFECTION OR DISEASE

Genes that confer host resistance to retroviral infection in animals have been described and include both immune response genes and non-immune response genes. For example, the product of the *Fv-1* allele prevents the development of leukemia in inbred mice infected with specific MuLV strains. Recent experimental evidence suggests that host genes may also modulate infection or disease caused by lentiviruses including HIV. *In vitro* evidence for the relative resistance of human T lymphocytes to HIV infection has been described. Resistance of PBMCs from individual monkeys to infection by SIV has also been observed and has been reported to be predictive of disease course. Investigation of these and other resistance mechanisms may reveal novel events in infection or replication of lentiviruses and could lead to novel prevention or therapeutic strategies.

The NIAID Multicenter AIDS Cohort Study (MACS) has shown that the median time from infection with HIV to development of AIDS is approximately 10 years, although this time period is highly variable among different individuals. A small proportion of the cohort participants who seroconverted in the study developed AIDS and died within two or three years of HIV infection, while others have maintained stable, high CD4+ cell counts ten or more years following HIV infection. To date, no independent epidemiologic factor or set of host or viral factors has been identified which reliably predicts disease progression or a state of relative "immunologic non-progression" (as judged by CD4+ cell stability). Studies of host factors and other determinants of HIV infection and disease are needed to gain a better understanding of the pathogenesis of HIV.

B. GOALS

The NIAID HIV/AIDS Research Agenda (October 1993) identified eight critical areas of research in HIV pathogenesis:

- molecular biology of viral and cellular processes;
- cellular biology and immunology related to course and dynamics of HIV infection;
- viral genetic and phenotypic correlation with disease;
- host factors affecting infection and/or progression;
- external factors affecting infection and/or progression;
- mechanisms of HIV-related immunodeficiency;
- animal models of HIV infection and/or pathogenesis; and
- mechanisms of sexual transmission and mucosal immunity.

Reviews of the DAIDS pathogenesis programs by an ad hoc committee and by the AIDS Research Advisory Committee (ARAC) concurred with these eight critical areas of research and recommended that three of these scientific areas receive particular emphasis, specifically: non-human primate models of HIV immunopathogenesis, sexual/mucosal transmission of HIV or SIV, and host factors that modulate

HIV or SIV infection or disease. The reviews strongly encouraged NIAID to promote studies of *in vivo* pathogenesis in these areas utilizing state-of-the-art *in vitro* approaches.

This RFA is intended to foster applications for integrated, multi-disciplinary pathogenesis research linking *in vitro* studies to *in vivo* disease and focusing specifically on these three scientific areas. The most relevant studies are expected to examine molecular and cellular biology, virology, and immunology within the context of animal models and/or well-defined human cohorts or patient samples.

C. RESEARCH SCOPE

Research proposals responsive to this RFA should provide innovative focused approaches to test an hypothesis of HIV pathogenesis in non-human primate models, HIV or SIV sexual/mucosal transmission, or host factors that modulate HIV or SIV infection or disease. NIAID anticipates that investigators will propose studies testing hypotheses using state-of-the-art methods on specimens from human and/or animal models. While the research necessary to test a proposed pathogenesis hypothesis may be possible within a single laboratory, NIAID anticipates that highly competitive applications may require separate components at the same or different institutions specializing in different scientific disciplines (*e.g.*, molecular biology, biochemistry, cellular biology, cellular immunology, genetics and biophysics). If applicable, applicants are encouraged to select component leaders based on scientific excellence rather than on proximity. If multiple components are proposed, the interactive role of each component in investigating the hypothesis should be described.

The following are examples of major issues or gaps in knowledge in the three RFA scientific areas that were identified in the NIAID HIV/AIDS Research Agenda:

PATHOGENESIS IN HUMANS AND NON-HUMAN PRIMATES

- Course of infection (infected cells, non-productively infected cells, virus production, altered biology of infected and uninfected cells) in different organ systems (blood, thymus, lymph nodes, gastrointestinal tract, bone marrow, spleen, nervous system, etc.) from early to systemic disease.
- Clinical course and mechanisms of disease following infection with viruses of differing tropisms (macrophage, T-cell, neurotropic), high/low replication properties, or syncytium /non-syncytium inducing abilities.
- Role of accessory viral genes (*vif*, *nef*, *vpu*, *vpr*, etc.) in infection, cellular tropism, and disease progression *in vivo*.
- Steps in T-cell development and/or regulation that are affected by lentivirus infection and the lentivirus genes or gene products that are responsible.
- Mechanisms of defects in T-cell signalling in uninfected and SIV (SHIV or HIV) infected cells.
- Differences in pathogenesis between control and treated infected non-human primates in ongoing (already funded) therapeutic and vaccine SIV or SHIV studies.

HIV OR SIV SEXUAL/MUCOSAL TRANSMISSION

- Mechanisms of mucosal transmission by free virus, cell-associated virus, or both.
- Genetic composition of HIV in the donor compared to HIV phenotype/genotype that is sexually transmitted to the recipient.
- Types of cells initially infected (i.e., lymphocytes, macrophages, and dendritic cells).
- Mechanisms, time course, and pathways of viral spread from site of initial infection.
- Differences, if any, in the mechanisms of establishment of HIV infection by rectal, vaginal, oral, or other mucosal routes.
- Host mechanisms operative at mucosal surfaces that protect exposed individuals from HIV infection.
- Role of co-factors other than STDs in facilitating or hindering mucosal transmission *in vivo*.

HOST FACTORS THAT MODULATE HIV OR SIV INFECTION OR DISEASE

- Mechanisms for differences in susceptibility of cells from different hosts to HIV or SIV infection *in vitro*.
- Identification of host genes (alleles) and/or factors that prevent or enhance infection, resistance to infection, and/or disease progression. Investigations of variations in normal immune response are not included under this topic.
- Mechanisms by which host factors prevent or enhance infection, provide resistance to infection, and/or disease progression *in vitro* and *in vivo*.
- Mechanisms by which primates are resistant to lentivirus disease; i.e., Chimpanzees infected with HIV and African Green Monkeys infected with AGM virus.
- Mechanisms responsible for observed variations in susceptibility to HIV or SIV infection and/or rates of progression in different individuals.
- Comparison of known mechanisms of resistance to retroviruses in non-primates (mice, chickens, etc.) with observations in primates.

D. RESTRICTIONS AND EXCLUSIONS

Descriptive, non-hypothesis driven, research is not within the scope of the this RFA. For example, natural history epidemiologic studies in many DAIDS-supported cohorts are critically important for collecting information on cause and course of disease. The information provided by such studies may provide a foundation for hypotheses that may be tested in research supported by this RFA. This RFA is intended to support the next step in research, the testing of these hypotheses.

This initiative will not support drug or vaccine trials in animals or humans.

Proposed studies should minimize expense for animal support and maximize use of animals in ongoing supported non-human primate research. For example, the applicant may be able to coordinate SIV pathogenesis projects with DAIDS supported vaccine or drug development studies in macaques. The applicant is responsible for establishing components and/or collaborative arrangements. A list of Principal Investigators who have access to non-human primates in NIAID-sponsored research will be available from DAIDS Program Staff (see "INQUIRIES") upon request but these investigators are under no obligation to collaborate with RFA applicants. When requested by investigators, DAIDS Program Staff will help identify potential participants and aid in the establishment of collaborations.

Clinical trials and recruitment or retention of cohorts will not be supported under this RFA. However, identified costs for patient visits, sample storage and handling specific to the applicant's proposed research are appropriate. Proposed analyses of samples acquired from epidemiologic or clinical trials are also appropriate. Proposed studies should maximize use of epidemiologic or clinical cohorts in ongoing research to minimize expenses. The Applicant is responsible for establishing components and/or collaborative arrangements. A list of Principal Investigators who have access to epidemiologic or clinical cohorts in NIAID-sponsored research will be available from DAIDS Program Staff (see "INQUIRIES") upon request but these investigators are under no obligation to collaborate with RFA applicants.

VII. SPECIAL REQUIREMENTS

A. TRAVEL – NIAID AIDS PATHOGENESIS MEETING

Principal Investigators and other key members (as designated by the Principal Investigators) of the projects will be requested to attend an annual NIAID AIDS Pathogenesis Meeting, including grantees of the Mechanisms of AIDS Pathogenesis RFA, to be held each year at a site designated by NIAID (Bethesda, Maryland is anticipated). Principal Investigators and component leaders will be requested to present significant findings in symposium format. Data presented at this meeting are selected by the individual presenters in consultation with their Principal Investigator thus affording appropriate protection of proprietary or commercially sensitive information. At the discretion of the DAIDS Program Staff, other investigators may also be invited to attend. Funds for the Principal Investigator and key scientific personnel to travel to these meetings should be included in the application budget. For budgetary purposes, applicants should anticipate lodging for three nights in the Bethesda, Maryland area (estimated food and lodging costs for 1994 = \$158/night) and travel at economy airfares. To best utilize limited funds, applicants are encouraged NOT to include funds for travel to other scientific meetings in their budgets.

B. NO INITIAL REVIEW SITE VISIT

One important factor that should influence the care and detail with which applications in response to this RFA are prepared is a long-standing NIAID policy of no site visits in the initial review of applications. Peer reviewers will base their comments and recommendations solely on the written application, which must be complete and prepared according to the RFA guidelines. Applicants are strongly encouraged to discuss research plans and organizational structure with DAIDS Program Staff in the early stages of preparation of the application. (See program contact list in "INQUIRIES".)

VIII. STUDY POPULATIONS

INCLUSION OF WOMEN AND MINORITIES IN RESEARCH INVOLVING HUMAN SUBJECTS

It is the policy of the NIH that women and members of minority groups and their subpopulations must be included in all NIH supported biomedical and behavioral research projects involving human subjects, unless a clear and compelling rationale and justification is provided that inclusion is inappropriate with respect to the health of the subjects or the purpose of the research. This new policy results from the NIH Revitalization Act of 1993 (Section 492B of Public Law 103-43) and supersedes and strengthens the previous policies (Concerning the Inclusion of Women in Study Populations, and Concerning the Inclusion of Minorities in Study Populations) which have been in effect since 1990. The new policy contains some new provisions that are substantially different from the 1990 policies. All investigators proposing research involving human subjects should read the "NIH Guidelines For Inclusion of Women and Minorities as Subjects in Clinical Research", which have been published in the Federal Register of March 9, 1994 (FR 59 11146-11151), and reprinted in the NIH GUIDE FOR GRANTS AND CONTRACTS of March 18, 1994, Volume 23, Number 11.]

Investigators may obtain copies from these sources or from program staff (listed in INQUIRIES) who may also provide additional relevant information concerning the policy.

IX. LETTER OF INTENT

Prospective applicants are asked to submit, by the date indicated in "SCHEDULE", a letter of intent that includes:

- number and title of this RFA;
- descriptive title of the R01 application
- names, addresses, telephone numbers, and EMAIL addresses (if available) of the Principal Investigator, component leaders and other key personnel;
- primary institution and component institutions (if different);

Although a letter of intent is not required, is not binding, and does not enter into the review of the application, the information that it contains is helpful in planning for the review of expected applications. It allows NIAID to estimate the potential workload for reviewers and to avoid possible conflict of interest in the review process.

The letter of intent should be sent to the staff person in the NIAID Division of Extramural Activities listed in "INQUIRIES."

X. APPLICATION PROCEDURES

Applications are to be submitted on form PHS 398 (rev. 9/91), the standard application form for research grants. Application kits are available at most institutional offices of sponsored research and may be obtained from the Office of Grants Information, Division of Research Grants (DRG), National Institutes of Health, Westwood Building, Room 449, Bethesda, MD 20892, telephone 301/594-7248. Applicants must adhere to the format and requirements specified in the PHS 398 application kit.

For purposes of identification and processing, mark "YES" in item 2a on the face page of the application and type in the RFA number AI-94-018 and the title "MECHANISMS OF AIDS PATHOGENESIS." The RFA label available in the form PHS 398 must be affixed to the bottom of the face page of the original application. Failure to use this label could result in delayed processing of the application such that it may not reach the review committee in time for review.

Any questions regarding the format for submission of a R01 package may be directed to the staff person in the Division of Extramural Activities listed in "INQUIRIES."

SEND:

- The signed, typewritten original of the application,
- The checklist,
- Three exact single-sided copies, and
- One set each of the appendices and reprints

TO: By U.S. Mail

Division of Research Grants
National Institutes of Health
Westwood Building, Room 240
Bethesda, MD 20892

Or by express mail or a courier service

Division of Research Grants
National Institutes of Health
Westwood Building, Room 240
5333 Westbard Avenue
Bethesda, MD 20816

ALSO SEND:

- Two additional copies of the application,
- Five sets of appendices and reprints

TO: Dr. Dianne Tingley at address listed in "INQUIRIES"

To ensure their review, applications must be received by both the Division of Research Grants and Division of Extramural Activities by the time listed under SCHEDULE. Applications not received by the receipt date may be considered non-responsive and may be returned to the applicant without review. If the application submitted in response to this RFA is substantially similar to a grant application already submitted to the NIH for review, but has not yet been reviewed, the applicant will be asked to withdraw either the pending application or the new one. Simultaneous submission of essentially identical applications will not be allowed, nor will essentially identical applications be reviewed by different review committees. Therefore, an application cannot be submitted in response to this RFA that is essentially identical to one that has already been reviewed. This does not preclude the submission of substantial revisions of applications already reviewed, but such applications must include an introduction addressing the previous critique.

XI. REVIEW CONSIDERATIONS

A. REVIEW PROCEDURES

Applications will be reviewed by DRG staff for completeness and by NIAID staff to determine administrative and programmatic responsiveness to this RFA. To be considered responsive, the application must be directed towards the attainment of the stated programmatic goals (see "RESEARCH OBJECTIVES"). Therefore, applicants are encouraged to discuss their research plans with program staff before completing their applications.

Those applications judged to be incomplete or nonresponsive will be returned to the applicant without review. Those applications that are complete and responsive may be subjected to a triage before or during the initial scientific peer review by a peer review group convened by the Division of Extramural Activities, NIAID, to determine their scientific competitiveness relative to the other applications received in response to this RFA. The NIAID will remove from further competition those applications judged to be noncompetitive for award and will notify the applicant and institutional business official.

Those applications judged to be competitive for award will be further reviewed for scientific and technical merit by a review committee convened by the Scientific Review Branch, Division of Extramural Activities, NIAID. A second level of review will be provided by the National Advisory Allergy and Infectious Diseases Council (see "SCHEDULE"). In the event of multiple highly qualified applications, final funding recommendations will be based on Program priorities.

B. REVIEW CRITERIA

The factors, when applicable, considered in evaluating the scientific merit of each application will be:

- Preliminary data leading to the hypothesis for the proposal including data for component studies.
- Clarity and significance of hypothesis to be tested by the applicant.
- Relationship of Specific Aims to the hypothesis, *e.g.*,
 - o Will accomplishment of Specific Aims provide significant proof or refutation of hypothesis?
 - o Does the application justify additional components to fulfill the Specific Aims?
 - o Are necessary components included in the application?
- Integration of *in vitro* and *in vivo* approaches and of components.
- Innovative nature and technical merit of experimental approach.
- Availability and acquisition of clinical specimens (tissues/biological fluids), and cost savings by utilization of resources not funded through this R01 application. Documentation of availability of specimens.
- Efficient use of animals and utilization of animal resources not funded through this R01 application. Documentation of availability of animals or animal specimens.

- Adequacy of resources and environment including biohazard containment facilities if relevant.
- Documented research experience, accomplishments, and other qualifications of Principal Investigators and other technical personnel in the proposed research areas.
- Time commitment of the Principal Investigators (and Component Investigators) to conduct the proposed research;

XII. AWARD CRITERIA

The final number and specific amounts of awards to be made will depend upon the consideration of the following:

- Results of the initial scientific and technical merit review;
- Potential contribution of the proposed research to the goals and objectives of the RFA;
- Program balance within the three areas of research; and
- Availability of funds.

XIII. INQUIRIES

Prospective applicants should carefully review the RFA. The contacts listed below welcome the opportunity to clarify issues or questions from potential applicants.

A. DAIDS PROGRAM STAFF

Direct written, telephone, or electronic mail (EMAIL) inquiries regarding scientific and responsiveness issues to:

Dr. Gregory Milman	(scientific organization)
Dr. Opendra K. Sharma	(sexual/mucosal transmission of HIV or SIV)
Dr. Patricia D'Souza	(non-human primate models of HIV immunopathogenesis)
Dr. Janet M. Young	(host factors that modulate HIV or SIV infection or disease)

Basic Research and Development Program
Division of AIDS
National Institute of Allergy and Infectious Diseases
Solar Building, Room 2B35
Bethesda, MD 20892
Telephone: (301) 496-8378
EMAIL: MAPS@EXEC.NIAID.PC.NIAID.NIH.GOV

B. STAFF OF DIVISION OF EXTRAMURAL ACTIVITIES

Direct letters of intent, inquiries regarding the scientific review process and format of applications, and two copies of the application including five sets of appendices and reprints, to:

Dr. Dianne Tingley
Chief, AIDS Preclinical Research Review Branch
Scientific Review Program
Division of Extramural Activities
National Institute of Allergy and Infectious Diseases
Solar Building, Room 4C16
Bethesda, MD 20892
Telephone: (301) 496-0818
EMAIL: MAPS@EXEC.NIAID.PC.NIAID.NIH.GOV

Applicants who wish to use express mail or a courier service are advised to follow the carrier's requirements for showing a street address. The address for the Solar Building is: 6003 Executive Boulevard, Rockville, MD 20852

Direct inquiries regarding fiscal matters and budget format to:

Ms. Jane Unsworth
AIDS Grants Management Section
Grants Management Branch
Division of Extramural Activities
National Institute of Allergy and Infectious Diseases
Solar Building, Room 4B22
Bethesda, MD 20892
Telephone: (301) 496-7075
EMAIL: MAPS@EXEC.NIAID.PC.NIAID.NIH.GOV

XIV. SCHEDULE

Letter of intent receipt date	: September 16, 1994
Application receipt date	: November 16, 1994
Scientific review date	: March 1995
Advisory Council date	: June 1995
Earliest Award date	: September 1995 and/or November - December 1995

Applications should be received by the application receipt date listed above. If an application is received after that date, an application may be returned to the applicant without review. The Division of Research Grants will not accept any application in response to this announcement that is essentially the same as one currently pending initial review, unless the applicant withdraws the pending application. The Division of Research Grants will not accept any application that is essentially the same as one already reviewed. This does not preclude the submission of a substantial revision of an application already reviewed, but such an application must follow the guidance in the PHS Form 398 application instructions for the preparation of revised applications, including an introduction addressing the previous critique.

XV. AUTHORITY AND REGULATIONS

This program is described in the Catalog of Federal Domestic Assistance, 93.856 - Microbiology and Infectious Diseases Research and 93.855 - Immunology, Allergy and Transplantation Research. Awards are made under authorization of the Public Health Service Act, Title IV, Part A (Public Law 78-410, as amended by Public Law 99-158, 42 USC 241 and 285) and administered under PHS grant policies and Federal Regulations 42 CFR Part 52 and 45 CFR Part 74. This program is not subject to the intergovernmental review requirements of Executive Order 12372 or Health Systems Agency review.

The Public Health Service strongly encourages all grant recipients to provide a smoke-free workplace and promote the non-use of all tobacco products. This is consistent with the PHS mission to protect and advance the physical and mental health of the American people.

Grants funded by RFA on Mechanisms of AIDS Pathogenesis *In Vivo*

Braun, Jonathan, 1R01 AI38545-01
UCLA School of Medicine
Role of the gp120 VH3 Superantigen in HIV-1 Pathogenesis

Compans, Richard W., 1R01 AI38501-01
Emory University School of Medicine
Determinants of HIV/SIV Mucosal Transmission

Desrosiers, Ronald C., 1R01 AI38559-01
New England Primate Center
Early Events in SIV Infection

Fultz, Patricia, 1R01 AI38580-01
University of Alabama, Birmingham
Mucosal Infection of Macaques with an Acutely Lethal SIV

Haase, Ashley T., 1R01 AI38565-01
University of Minnesota
Pathogenesis of Sexual Transmission of Primate Lentivirus

Marx, Preston A., 1R01 AI 38573-01
Aaron Diamond AIDS Research Center
Early Events in Mucosal Transmission of SIV and SHIV

Narayan, Opendra, 1R01 AI38492-01
University of Kansas Medical Center
Pathogenesis and Prevention of SHIV Disease

Overbaugh, Julie M., 1R01 AI38518-01
University of Washington
Early Infection in Women Exposed Mucosally to HIV-1

Ruprecht, Ruth M., 2R01 AI32330-05
Dana-Farber Cancer Institute
SIV and HIV Pathogenesis during Ontogeny

Unsolicited grants on sexual transmission

Pauza, David, R01 AI36643
University of Wisconsin
Virus variation and sexual transmission of SIV

Phillips, David, R01 AI 37793
Population Council
Mechanisms of transmission of HIV

Pomerantz, Roger, R01 AI33810
Thomas Jefferson University
HIV transmission in vivo

Braun, Jonathan, 1R01 AI38545-01

UCLA School of Medicine

Role of the gp120 VH3 Superantigen in HIV-1 Pathogenesis

HIV gp120 has recently been identified as an immunoglobulin superantigen (SAg), binding most members of the large VH3 gene family (~10 % of total serum IgM) in non-infected individuals. Our concept is that virus has evolved this superantigen to enhance the efficiency of infection during mucosal transmission. Our preliminary studies have also shown that the level of SAg-binding antibodies is polymorphic host trait, and that individuals highly susceptible to HIV transmission correspond to those with the high-phenotype SAg-binding Ig trait. This preliminary finding supports the prediction that the high antibody phenotype is a host susceptibility factor in HIV transmission. To test this idea, we will determine whether these antibodies enhance *in vitro* infection via an Fc α receptor-dependent mechanism, and the types of cells targeted by this mechanism. In parallel, we will measure the levels of superantigen-binding IgA in gateway mucosal sites, and determine whether individuals highly susceptible to mucosal transmission are high-expressors for these antibodies. Family and genotypic studies are also developed to determine whether genetic factors contribute to the high-expressor phenotype.

Paradoxically, a striking predominance of anti-SAg antibodies is also associated with long-term survivors of HIV infection. To understand this correlation, we considered that anti-gp120 antibodies might also promote viral uptake by cell types bearing Fc γ and/or C3R. Since such cells are mostly non-T cells, this may serve to redirect virus away from T cells and hence reduce viral pathogenesis. Our initial *in vitro* studies demonstrate such immune sequestration of gp120 by anti-SAg IgM. The final two aims further characterizes and validates this protective mechanism in two ways. First, studies are designed to whether immune sequestration by anti-SAg inhibits HIV infection *in vitro*, through identifying the receptors and cell types involved in immune sequestration of gp120 in PBMC, and the role of this mechanism on HIV infection of T cells *in vitro*. Second, reagents already developed to manipulate anti-Sag activity will be used to test whether anti-SAg Ig inhibits HIV pathogenesis in SCID-hu mice. If these ideas are validated, then this project will have defined a novel and important immunogenetic host factor in HIV infection, and an new class of target for design of vaccine or other protective therapies.

Compans, Richard W., 1R01 AI38501-01
Emory University School of Medicine
Determinants of HIV/SIV Mucosal Transmission

The objective of this proposal is to determine the detailed mechanism by which HIV can initiate infection at the mucosal surfaces of the female genital tract, with the ultimate goal of preventing mucosal transmission. Specifically, we will be seeking to answer the following questions using the SIV model: To what extent does the virus strain influence the efficiency of infection at mucosal surfaces? What cell types are involved in virus attachment and subsequent infection? What host factors may influence the susceptibility to HIV/SIV infection at mucosal surfaces? To determine if specific genotypes of SIV are preferentially transmissible by the mucosal route, we will determine if specific sequence changes can be detected in SIV isolates following mucosal inoculation of rhesus macaques with SIV virus stocks obtained from infectious molecular clones. We will also determine if SIV variants with altered cell tropism are selected after passage in primary genital tract cell cultures. We will develop in vitro culture systems to investigate the molecular and cellular determinants of virus binding; the susceptibility of such cell cultures to infection by free vs cell associated virus; identify the receptors which mediate virus infection; study mechanism(s) by which SIV can traverse epithelial cell layers; and determine the effects of specific hormones or growth factors on the susceptibility to infection. In addition, we will determine the role of distinct subsets of epithelial T cells a) as sites for virus replication and b) as effector cells in virus-specific immune responses. To specifically address these issues, we will: 1) characterize T cell subsets and cytokine production in the vaginal mucosa of uninfected and infected macaques, and 2) identify T cell subsets representing cellular targets following infection in vivo with either T cell tropic or macrophage tropic SIV strains. Immunohistochemistry and in situ hybridization techniques will be utilized to accomplish these objectives. The combined expertise of the participating investigators in studies of SIV envelope gene variation and the cell biology of virus-epithelial cell interactions will lead to a highly interactive collaborative group with the central focus of determining the mechanisms of SIV and HIV transmission across mucosal surfaces of the genital tract.

Desrosiers, Ronald C., 1R01 AI38559-01
New England Primate Center
Early Events in SIV Infection

Although HIV infection is generally associated with prolonged clinical latency, an expanding body of evidence points to the importance of early events for determining the outcome of HIV infection. Primary infection of humans with HIV-1 is often associated with an acute mononucleosis-like clinical syndrome characterized by fever, malaise, pharyngitis, lymphadenopathy, headache, diarrhea, rash and meningoencephalitis and is accompanied by a burst in virus replication detectable in the blood approximately 1-3 weeks following exposure. The appearance of specific antiviral immune responses is associated with dramatic reductions in the level of viremia, but there is no clearance of the virus. Limitations inherent in studying human subjects have precluded detailed investigation of many of the early events critical for virus transmission, dissemination and eventual disease progression. The SIV/macaque model will be used to address these critical issues. Whether there is selective transmission of particular SIV genotypes across the mucosal surface will be determined. The initial cellular targets of primary infection by the mucosal and intravenous routes will be identified and the sequential targets of the spreading viral infection will be characterized. Analyses will include subsets of CD4+ lymphocytes and progenitor cells in thymus and bone marrow as possible preferential targets. The influence of varying viral genotype on transmission, dissemination to tissues and tissue-specific disease manifestations will be investigated. Finally, the importance of cytotoxic T lymphocytes for controlling the early burst in virus replication will be investigated in this manipulable experimental setting. These studies will be achieved through the cooperation of three principal investigators at the New England Regional Primate Research Center with extensive experience in the disciplines of Virology, Molecular Biology, Pathology and Immunology.

Fultz, Patricia, 1R01 AI38580-01
University of Alabama, Birmingham
Mucosal Infection of Macaques with an Acutely Lethal SIV

The major route of transmission of HIV-1 is across mucosal surfaces between sexual partners or infected mothers and their fetuses or neonates. However, essentially nothing is known about the cells that facilitate passage of virus or virus-infected cells across the mucosa or that are initially infected after passage of virus to primary sites of replication. To devise effective treatments and vaccines, it is important to understand the pathogenesis of HIV following mucosal transmission and to determine whether there are differences between mucosal and parenteral infection in primary and secondary sites of virus replication and in initial immune responses to the virus. Such insight into infectious diseases can be gained most efficiently through the use of animal models. Infection of pig-tailed macaques (*Macaca nemestrina*) with a virulent simian immunodeficiency virus, designated SIVsmmPBj14, provides several advantages over other SIV models. Two important advantages are: first, rapid onset of disease, which reproduces the multisystemic acute HIV-1 infection in humans and results in death in less than 2 weeks; and second, the virus preferentially replicates in gut-associated lymphoid tissue. Based on preliminary experiments indicating that the disease course following mucosal infection is altered relative to that observed after intravenous infection with SIVsmmPBj14, the following hypothesis will be tested: Mucosal infection of pig-tailed macaques with SIVsmmPBj14 results in less severe disease sequelae because viral replication occurs in mucosal lymphocytes and macrophages. As a result, virus dissemination is inhibited more effectively by cytotoxic cells in mucosal tissues compared with similar effector cells induced in peripheral lymphoid organs after parenteral infection. Macaques will be infected with SIVsmmPBj14 by atraumatic exposure of genital or rectal mucosal surfaces. At regular intervals during the first few hours and days after inoculation, the animals will be euthanatized, and the kinetics of viral replication and dissemination, and tissue sites and cell types that support viral replication will be determined using immunohistochemistry, in situ hybridization, and PCR-based assays. In addition, leukocytes that infiltrate areas of active SIV replication will be characterized phenotypically and functionally by assessing cell surface antigens, cytolytic activity, and use of specific T-cell receptor V β and V δ gene segments. Finally, biologic and molecular properties of viruses isolated from peripheral and mucosal tissues and fluids will be determined. These studies should provide important insight into the earliest events that occur following mucosal infection by a primate lentivirus.

Haase, Ashley T., 1R01 AI38565-01
University of Minnesota
Pathogenesis of Sexual Transmission of Primate Lentivirus

The objective of the proposal is to test the hypothesis that the determinants of sexual/mucosal transmission of primate lentiviruses are viral replication at the portal of entry and the local virus-specific cellular immune response. If the host mounts an effective immune response before there is sufficient replication to generate ineradicable latently infected cells or cytotoxic T lymphocyte (CTL) escape mutants, infection will be aborted or transient. If not, infection will be persistent and progress to immune depletion and disease. Identifying the critical determinants of outcome will lay the foundations for design and evaluation of lentiviral vaccines.

Experiments to identify these determinants and test their hypothesized role in sexual transmission are proposed that utilize a non-human primate model, rhesus monkeys infected intravaginally with simian immunodeficiency virus (SIV) or SIV-HIV chimeras (SHIV) with different replicative capabilities. The extent of viral replication will be assessed locally, in the draining lymph nodes, and systemically, by double-label *in situ* PCR and hybridization methods that identify the number and types of infected cells in tissue specimens and the state of viral gene expression (latent or productive infection) by the abundance and class of viral transcripts. The timing and breadth of the virus-specific cellular immune response in specific anatomic compartments and evidence of CTL escape mutants will be sought by characterizing specific CTL epitopes with defined MHC class I contexts, the clonality of the CD8⁺ T lymphocyte response, and changes in the sequences of the epitopes in the evolving viral quasi-species. These analyses will provide insight into the immune correlates of protection at the portal of entry in sexual transmission of lentiviruses.

Marx, Preston A., 1R01 AI 38573-01
Aaron Diamond AIDS Research Center
Early Events in Mucosal Transmission of SIV and SHIV

Better delineation of the viral targets and pathways involved in sexual transmission of HIV-1 is crucial to the global effort to develop a protective vaccine, and to other preventive strategies. Studies to date suggest that viruses found in newly infected individuals are commonly macrophage-tropic and non-syncytium inducing, irrespective of the viral phenotypes found in their corresponding sexual partners. Moreover, genetic studies in this setting have found evidence of a stronger pressure to conserve sequences in gp120 than in gp41, p17, or *nef*, suggesting that a selective mechanism may be involved in sexual transmission. This possibility is further supported by our recent finding that minor variants of the HIV-1 populations present in seminal fluids of chronically infected men are preferentially transmitted to their male or female sexual partners. These preliminary but intriguing results emphasize the need to define the initial cellular targets for HIV-1 in the genital mucosa and the subsequent pathway of viral spread. However, these unresolved questions are extremely difficult to pursue experimentally in humans. We therefore propose to examine several of the issues concerning sexual transmission in a rhesus macaque model using both SIV and SHIV chimeric viruses. Specifically, we will attempt to define the identity of the first cell(s) infected by SIV in the vaginal and cervical mucosa of macaques using in situ PCR coupled with immunohistochemistry. Employing culture and PCR methods, we will also track the kinetics and the pathway of SIV spread from the genital mucosa to the draining (internal iliac) lymph nodes before systemic dissemination. In addition, we will perform a series of experiments to examine directly the issue of selective transmission. Mixtures of SIV variants with known differences in their biological phenotypes will be inoculated both mucosally and intravenously into macaques to determine whether virus with a particular biological property (e.g., macrophage-tropism) will be preferentially selected in animals inoculated mucosally. Similar experiments will also be performed using mixtures of SHIV chimeric viruses with divergent biological phenotypes. Lastly, we will determine the impact of early mucosal immune responses, particularly cytotoxic T lymphocytes, in preventing the establishment of mucosal infection or in aborting the subsequent spread of the virus. We believe that the proposed studies will contribute considerably to our understanding of sexual transmission of HIV-1.

Narayan, Opendra, 1R01 AI38492-01
University of Kansas Medical Center
Pathogenesis and Prevention of SHIV Disease

This application addresses the great need for an animal model that reflects the pathogenic potential of HIV-1. Building upon our experience with the SIVmac and ruminant lentiviruses, we passaged SHIV through macaques and in pigtail macaques, and derived a virulent SHIV swarm that caused persistent, productive infection with a severe precipitous loss of CD4⁺ T cells.

In this proposal, we will examine the pathogenesis of infection in pigtail macaques with this SHIV swarm, addressing the hypothesis that loss of CD4⁺ T cells is caused by viral lysis of the cells, a phenomenon evident by apoptotic changes in the lymphoid tissues. We hypothesize two periods of lysis of the T cells which are matched by a decline in CD4⁺ T cell count in peripheral blood. The first period of decline occurs early after infection and the second after the immune responses to the virus break down.

We will determine whether particular sequences in the HIV-1 env gene are associated with the pathogenic effects of the virus and whether such genotypes are selected in particular tissues. Cloning and sequencing of env genes obtained by PCR from tissues, and examination of biological properties of viruses isolated from tissues, and examination of chimeric viruses constructed with the env genes from tissues will be used to answer these questions. We will derive pathogenic molecular clones of SHIV directly from affected tissues. This will provide information on the molecular basis of SHIV pathogenicity and supersede use of limited tissue material for reproducing disease. Finally, we will infect pigtail macaques with SHIV Δ nef vaccine and challenge them with virulent SHIV. Prevention of disease, rather than infection by the virulent virus, is the goal of this experiment.

We expect that pigtail macaques infected with the virulent SHIV swarm will develop AIDS, with loss of CD4⁺ T cells (<200/ μ l) and opportunistic infections; that apoptosis will be demonstrable in lymphoid tissues during the periods of CD4⁺ loss, and that some macaques will also develop disease in non-lymphoid tissues, enabling us to link specific genotypes with particular tissues. We also expect that macaques infected with the SHIV Δ nef will develop persistent non-pathogenic infection and will not develop disease when challenged with the virulent SHIV virus swarm.

The overall goal of this study is to define the virological and immunological properties of HIV-1 variants that are transmitted to and persist in women following mucosal infection. Worldwide, HIV-1 is predominantly a heterosexually acquired virus, but very little is known about HIV-1 variants that are transmitted to the mucosa, particularly in women. Yet, for a HIV vaccine to be effective, it will be essential that it protect against the virus variants that are most frequently transmitted.

The PAVE cohorts, which have been established to identify and monitor high risk HIV-1 negative individuals in preparation for HIV vaccine efficacy trials, provide an excellent population for studying early infection because a change in serostatus is detected within a one-two month period. In this study, we propose to analyze the virological and immunological responses in a PAVE cohort of female commercial sex workers in Mombasa, Kenya, where transmission occurs through mucosal exposure. This cohort also provides an opportunity for temporal studies of newly infected individuals, allowing analyses of the interplay of the specific virus, viral tropism and the immune response in defining the viruses that persist in the host.

The specific AIMS include: 1: To define the envelope genotype of virus variants that are transmitted. To compare the envelope sequences of viruses in the mucosa to those in the blood at early times post-infection. To analyze the dynamics of the virus population over time in blood and the mucosa; 2: To determine the phenotype of viruses that are present in the cervix and blood at early times post-infection; 3: To define early immune responses to autologous virus and to determine the potential role of cellular and humoral immune response in affecting selection for certain variants in blood and mucosa. To determine the potential role of cellular and humoral immune response in affecting selection for certain variants in blood and mucosa; 4: To define the early target cells for infection in the mucosa and determine if the types of cells infected in the host correlate with the cell culture-defined properties of the viruses.

Maternal transmission of HIV-1 is a growing problem worldwide. We will test two key issues in congenital HIV-1 infection in primate models: the mechanism of neonatal infection across intact mucosa, and the fate of mucosal Langerhans cells (LC) during disease progression. Our group is uniquely qualified to conduct this work. First, by inoculating simian immunodeficiency virus (SIV) into amniotic fluid, we created a primate model for *in utero* infection. Second, by exposing neonatal macaques orally to SIV following C-section, we created a primate model which mimics intrapartum mucosal transmission. For both models, high infection rates were achieved. Using oral infection at birth with SIV Δ 3, which is deleted in *nef*, *vpr* and *NRE*, and which is attenuated in adult macaques after intravenous (i.v.) infection, we made a startling finding: SIV Δ 3 replicated to high levels and caused AIDS in infants. This led us to hypothesize that a) mucosal LC play a pivotal role in the early dissemination of SIV across neonatal mucosal surfaces; b) that these steps require neither *Nef* nor *Vpr*; and c) that infection of LC may play a key role in the pathogenesis of primate immunodeficiency virus infection in neonates.

The Specific Aims are to:

1. Compare the susceptibility of cultured neonatal LC to lentiviral infection with that of adult cells and investigate the mechanism(s) of virus dissemination from acutely infected LC; compare the rate of virus production by LC exposed to isogenic HIV-1 and SIV differing only in *nef* or *vpr*.
2. Determine the portal of virus entry (using wild-type SIV_{mac251}), initial target cells and pattern of virus spread following oral or i.v. infection of neonates. Are LC the earliest cells infected after oral infection? Do LC, and dendritic cells in general, undergo apoptosis in infected neonates?
3. Determine the infection rate and fate of LC during the clinically asymptomatic period after seroconversion following oral or i.v. neonatal infection, and after full-blown AIDS has developed.
4. Determine infection rate and fate of LC after *in utero* infection; test whether viral load, number or phenotype of apoptotic cells, or the rate of disease progression differ in neonates infected before and after acquiring the capability to mount immune responses.
5. Test whether immune activation precipitates wide-spread apoptosis in SIV-infected infant macaques. Are LC, and dendritic cells in general, lost as a consequence?

The proposed work, which is based on our prior accomplishments, is highly significant because it will yield new insights into lentiviral pathogenesis during ontogeny.

Pauza, David, R01 AI36643
University of Wisconsin
Virus variation and sexual transmission of SIV

Sexual transmission is the predominant mode for epidemic spread of human immunodeficiency virus (HIV). In man, sexual transmission is correlated with a change in the virus population. The newly infected recipient manifests only a portion of the complex virus population found in the donor. Similar data were obtained in animal infection experiments wherein juvenile male rhesus monkeys were inoculated intrarectally with simian immunodeficiency virus (SIV). Again, the population of viruses in the newly infected recipient was much different than the complex population in the inoculum. Current data from animal and human studies favor selection among individual viruses as the model for mucosal infection and implicate virus structural elements as determinants of transmission efficiency. The objectives of this proposal are to identify virus sequences associated with efficient mucosal transmission and to establish quantitative differences in mucosal infectivity for molecularly cloned viruses in the rhesus monkey.

We identified numerous envelope gene and long terminal repeat (LTR) SIV sequences in rhesus monkeys that were infected following atraumatic intrarectal inoculation. We will use cloned envelope and LTR sequences that were correlated specifically with intrarectal transmission, to construct recombinant chimeric viruses. Individual infectious molecular clones of SIV will be characterized for *in vitro* properties and cellular tropism. Based on these criteria, we will select viruses with unique properties and study their ability to cross the mucosal barrier in rhesus monkeys.

One recombinant virus that is identified as efficiently transmitted across the mucosa and another that is inefficiently transmitted by this route will be compared in a dose ranging study to provide a quantitative definition of mucosal infectivity. Quantitative infectivity studies are needed to understand the phenomenology of sexual transmission in man. It is possible that individual virus strains present at very low frequency in the natural inoculum, might still mediate infection because of their enhanced efficiency for mucosal transmission. In this circumstance, the efficient viruses must be high priority targets for vaccine development and, because these viruses are present in small numbers, transmission may be preventable by prior vaccination. Identification of common genotypes or phenotypes of efficiently transmitted viruses are of immediate interest for understanding the rates of sexual transmission in man. Current studies examine the complexity of HIV-1 sequence present in genital fluids and organs of reproduction and it is crucial to focus these studies on the fraction of viruses that present the greatest risk. Basic knowledge of virus structural elements involved in sexual transmission will also facilitate studies of the cellular and molecular mechanisms that regulate infection efficiency in the living host.

Phillips, David, R01 AI37793
Population Council
Mechanisms of transmission of HIV

The proposed research is based on our recent observation that when HIV-infected transformed T-cells or monocytes are added to monolayers of epithelial cells derived from the human intestine or cervix they attached tightly to the epithelium. Adherence triggers directional release of HIV onto the epithelium. Virus is subsequently taken up by the epithelia which becomes productively infected. We theorize that this in vitro model could mimic the manner which HIV is sexually transmitted in vivo. We propose to develop systems that more accurately reflect the natural conditions which exist during sexual transmission of HIV and to employ these models to clarify the mechanisms involved in mononuclear cell-to-epithelia adhesion, directional secretion of HIV, and virus uptake. Infection by cell-free virus will also be studied.

To date, we have used cell lines of mononuclear cells derived from human cancers. We propose to use HIV-infected primary mononuclear cells because they are more representative of the HIV-infected cells found in blood and genital tract secretions. In preliminary experiments we have been able to infect epithelia with HIV-infected primary mononuclear cells. As infection may occur by both cell-free and cell-to-cell mechanisms, work will also involve infection via cell-free virus. These studies will include the development of a quantitative blocking assay of cell-free virus infection of ME180 cells - a cervix-derived cell line which is similar to the human cervix and which we have found in preliminary experiments can be infected by cell-free virus. Research on both cell-free and cell-associated epithelial infection systems will involve virus strains believed to be involved in natural infection.

Our preliminary studies suggest that rearrangement of microtubules and microfilaments may be involved in directional secretion of virus. We will use EM localization techniques to further investigate this phenomenon. Since cell adhesion molecules (CAM) are likely to be involved in adherence of mononuclear cells to epithelia we will carry out blocking experiments with antibodies to CAM, sugars, enzymes, and lectins. The role of cytokines in virus uptake and phagocytosis by cervix-derived epithelial cells will be elucidated with blocking experiments of cell-free virus and by phagocytosis of microspheres by cervix-derived epithelia. Lastly, blocking experiments will be carried out to determine whether antibodies to gp120 can block cell-mediated infection of epithelia.

We believe that the proposed studies will lead to a better understanding of the basic mechanisms of sexual transmission of HIV and to new AIDS prevention strategies.

Pomerantz, Roger, R01 AI33810
Thomas Jefferson University
HIV transmission in vivo

The understanding of human immunodeficiency virus type 1 (HIV-1) pathogenesis, in vivo, requires the ability to precisely quantitate HIV-1 proviral DNA and RNA in a variety of tissues and secretions. Although HIV-1 has long been known to infect semen and cervical/vaginal secretions of HIV-1-seropositive individuals, the molecular mechanisms affecting the sexual transmission of HIV-1, in vivo, are poorly defined. Understanding the factors which affect the infectivity of HIV-1-seropositive individuals is of critical importance.

Utilizing two novel molecular techniques, developed in our laboratory, the precise HIV-1-proviral load in the cellular components of semen and cervical/vaginal secretions, and the HIV-1 RNA levels in the genital secretions shall be determined. An in situ polymerase chain reaction (PCR) for HIV-1 DNA, which allows amplification of a segment of the HIV-1 genome in intact cells, and a quantitative HIV-1 reverse transcriptase PCR technique will be utilized. Total HIV-1 RNA, in both cell-associated and cell-free fractions, of these genital secretions shall be evaluated. As well, an aberrant pattern of HIV-1 RNA, previously correlated with proviral latency, will be investigated in the cellular components of these genital secretions.

HIV-1 proviral and HIV-1 RNA total levels and patterns, in genital secretions, will be correlated with other clinical markers of HIV-1 infection and initial mode of HIV-1 infection. As well, the levels of HIV-1 proviral-harboring cells and HIV-1 RNA in genital secretions shall be followed, longitudinally, through disease progression and correlated with clinical parameters of genital inflammation and various treatment modalities using anti-retroviral agents. In addition, levels of HIV-1 proviral-containing cells in the peripheral blood will be assessed, utilizing in situ PCR, and correlated with HIV-1 RNA and DNA levels in genital secretions.

The cellular tropism of HIV-1 strains and the presence of defective viral genomes, in genital secretions, will be assessed via cloning and sequencing techniques. The hypotheses, that macrophage-tropic strains correlate with increased sexual transmission while partially defective viruses lead to aberrant viral RNA patterns and possibly decreased transmissibility, will be tested.

To further assess correlation of molecular parameters, described above, with clinical infectivity of HIV-1-seropositive individuals, couples both discordant and concordant for HIV-1 infection will be evaluated. In these ways, it is suggested that a more precise understanding of the mechanisms involved in controlling the sexual transmissibility of HIV-1 will be gained.

Basic and Applied Research

PRECLINICAL DEVELOPMENT

NIAID's support contracts provide key capacities for *in vitro*, and *in vivo* evaluation of new products. The NIAID also has the capacity for assessment of local and systemic toxicity, formulation research and development as well as formulation manufacturing. Included is an outline of the steps to be considered in preclinical development of topical microbicides for the prevention of STDs, including HIV infection.

Preclinical contract support
NIAID Guidelines for Preclinical Development

Preclinical Antiviral Evaluation for Genital Herpes

In vitro antiviral screen for herpesviruses

Contract #: N01-AI-35177

PI: **Earl Kern**

University of Alabama at Birmingham

The Collaborative Antiviral Testing Group (CATG) is responsible for the DMID's preclinical antiviral evaluation activities. The CATG began in 1971 with the goal of performing antiviral therapies in animal models to expedite the clinical development of effective therapies for viral diseases. In 1988, the *in vitro* antiviral screening facilities were also established. Currently, two of the eight CATG are evaluating topical microbicide products in the herpes model systems. Dr. Christopher Tseng is the Project Officer of the *in vitro* screens and animal models for herpes viruses.

Animal models of human herpes viral infections

Contract #: N01-AI-65289

PI: **David Bernstein**

Children's Hospital Medical Center
Cincinnati, Ohio

Animal models of human herpes viral infections

Contract #: N01-AI-65290

PI: **Earl Kern**

University of Alabama at Birmingham

Both mice and guinea pigs inoculated intravaginally with HSV-2 are used as models for genital herpes. The murine model is used primarily for primary infection. The guinea pig model not only results a primary genital infection, but also gives spontaneous recurrent vesicular lesions appear on the external genital skin. Treatment with topical or oral acyclovir was effective in both models. Recently, the murine model was used in a preliminary prophylactic study. The results demonstrated that quinobene derivatives could protect animals from genital herpes infection.

Animal models of human papillomavirus infections

Contract #: N01-AI-35158

PI: **John Kreider**

Pennsylvania State University

Rabbits inoculated on the back with cottontail rabbit papillomavirus are used as a model for evaluating experimental antiviral agents with potential for the treatment of human papillomavirus infections. Dr. Kreider is developing this model to be useful for the evaluation of a compound's anti-papillomavirus microbicide potential. In addition, he is developing a model using rabbit oral papillomavirus and infection of mucosal surfaces.

DAIDS Preclinical Contract Support
Project Officer: **Nancy Miller, Ph.D.**

Contract #: NO1-AI-65303
PI: **Dr. Michael Wyand**
GTC Mason Laboratories
Worcester, MA

Contract #: NO1-AI-65314
PI: **Dr. Phillip Markham**
Advanced BioScience Laboratories
Kensington, MD

Both of these contracts cover the purchase and housing of monkeys, administration of vaccines or virus, evaluation of immune responses (when relevant), and determination of whether or not monkeys were infected after exposure to virus.

Non-Human Primate Models For Evaluation of AIDS Therapies
Project Officer: Roberta Black, Ph.D.

Contract number: N01-AI-65310

PI: Louis N. Martin, Ph.D.

Tulane University
Tulane Regional Primate Research Center
18703 Three Rivers Road
Covington, LA 70433

Contract number: N01-AI-65311

PI: Che-Chung Tsai, DVM, Ph.D.

University of Washington
Grant and Contract Services
3935 University Way NE
Seattle, WA 98105

Contract number: N01-AI-65312

PI: Mark Lewis, Ph.D.

Henry M. Jackson Foundation
1401 Rockville Pike, Suite 600
Rockville, MD 20852

Contract accomplishments pertaining to development of topical microbicides to prevent infection by HIV and other STDs:

- Screened potential topical microbicides for activity against multiple SIV strains in vitro
- Development of in vivo macaque models of vaginal or rectal transmission of SIV and SHIV strains is in progress

***In Vitro* Test Systems for Evaluating Chemotherapies against HIV**

Project Officer: Dr. Steven Turk

Contract #s: N01-AI-55264 and N01-AI-55265

P.I.: Robert Buckheit, Jr., Ph.D.

P.I.: Owen Weislow, Ph.D.

Southern Research Institute
Frederick Research Center
431 Aviation Way
Frederick, MD 21701

SRA Technologies, Inc.
8110 Gatehouse Rd., Suite 600
Falls Church, VA 22042

Contract accomplishments pertaining to development of topical microbicides to prevent infection by HIV:

- Utilized cytotoxicity and HIV-1 acute-infection assays to evaluate compounds *in vitro* in CEM cells, PBMCs, and/or macrophages;
- Developing an *in vitro* virucidal assay to evaluate compounds for direct inactivation of cell-free HIV-1. This assay will be used to evaluate the pH sensitivity of a panel of different HIV-1 subtype clinical isolates.

Special *In Vitro* Evaluations of Strategies to Combat HIV/AIDS

Part A: Specialized *In Vitro* Virological Evaluations of Strategies to Combat HIV/AIDS.

Project Officer: Dr. Roger Miller

Contract #: N01-AI-55281

P.I.: Robert Buckheit, Jr., Ph.D.

Southern Research Institute

Frederick Research Center

431 Aviation Way

Frederick, MD 21701

Contract accomplishments pertaining to development of topical microbicides to prevent infection by HIV and other STDs:

- Established an *in vitro* assay using human cervical carcinoma (ME180) cells to screen compounds for effectiveness in blocking transmission of cell-associated HIV-1;
- Obtained over 750 compounds from governmental, academic and private sector sources and successfully tested the compounds in the primary screening assay;
- Identified approximately 20 colorless compounds possessing significant anti-HIV blocking activity;
- Screened the above compounds in secondary assays measuring toxicity against hydrogen peroxide secreting (beneficial) lactobacillus, non-specific inhibition of blocking activity by interaction with mucin, and determination of effect on HIV binding and attachment to a CD4 positive cell line.

Feline Immunodeficiency Virus (FIV) Model Studies

Project Officer: **Roger Miller, Ph.D.**

Immune Based Therapies for AIDS

Contract#: N01-AI-35155

PI: **Wayne Tompkins, D.V.M.**

Department of Microbiology, Pathology & Parasitology
College of Veterinary Medicine
North Carolina State University
4700 Hillsborough Street
Raleigh, NC 27695-8401

Contract accomplishments pertaining to development of topical microbicides to prevent infection by HIV and other STDs:

- Development of a feline/FIV mucosal challenge model for evaluation of topical microbicides;
- Determination of the *in vitro* macrophage tropism of various FIV isolates;
- Localization of virus and immune cells in the reproductive tract of cats after FIV vaginal infection.

DAIDS Pre-Clinical Contract Support
Project Officer: Chuck Litterst

Contract #: N01-AI-65307

PI: **Jon Mirsalis, Ph.D.**

SRI International

Menlo Park, CA

Safety assessment contract. Can determine vaginal irritation, and bioavailability and distribution of compounds administered intravaginally in animals.

Contract #: N01-AI-55268

PI: **Valentino Stella, Ph.D.**

University of Kansas

Lawrence, KS

Formulation research and development. Can develop formulations for compounds to be administered intravaginally.

Contract #: N01-AI-55268

PI: **Larry Augsburger, Ph.D.**

University of Maryland

Baltimore, MD

Formulation manufacturing. Can manufacture clinical dosage forms for compounds to be administered intravaginally.

DAIDS Pre-Clinical Contract Support
Project Officer: Mohamed Nasr

Contract #: N01-AI-55285
PI: **Larry Callahan, Ph.D.**
Herner & Co.
Arlington, VA

Management of information resources on therapeutic agents for HIV and opportunistic infections. Perform literature searches, maintain, update HIV and OI's computerized data bases that currently contain 42,000 chemical structures and their biological activities.

National Institute of Allergy and Infectious Diseases'
CONSIDERATIONS FOR PRE-CLINICAL DEVELOPMENT OF TOPICAL MICROBICIDES:

In 1993, the National Institute of Allergy and Infectious Diseases, launched a research initiative to develop topical microbicides. Topical microbicides are products for intravaginal and/or rectal use that protect against sexually transmitted diseases (STDs), including Human Immunodeficiency Virus (HIV) infection. The research initiative consists of three parallel tracks including basic science, product development and clinical research.

This document presents an outline of the steps to be considered in preclinical development of topical microbicides for the prevention of STDs, including HIV infection. It is intended to be a comprehensive list of studies which could be performed with a potential topical microbicide prior to, or concurrent with, clinical studies in humans. Since candidate products will have different active agents with unique mechanisms of action and formulations, preclinical approaches will have to be tailored for the specific product. The precise studies that will need to be performed will also depend on several factors including: 1) the indication for which the product will be marketed; and 2) the regulatory requirements of the host/sponsoring country. Again, this document is meant to serve as a general guideline for preclinical product development. A product sponsor should contact their regulatory agency about specific requirements for licensure as soon as a product's potential has been delineated.

I. Recommendations for Tests of Active Agents and Clinical Formulations

The tests described in this section are designed to obtain information regarding the products potential for prevention of HIV and STDs. If the product indication is for the prevention of HIV infection, in vitro assays will include a variety of HIV strains (both lab-adapted and clinical isolates). Since the precise mechanism of mucosal transmission of HIV is unknown, tests for evaluating prevention of cell-associated HIV transmission are also needed. It is desirable that an agent be evaluated for activity against HIV and other STDs regardless of its intended HIV indication since a clinical outcome of HIV prevention may be achieved by the prevention of other STDs. Similarly, data on the effect of the agent on lactobacilli as a surrogate for normal vaginal flora is desirable.

1. In vitro Activity against HIV*:

- A) Laboratory-adapted virus in T-cell lines;
- B) Laboratory-adapted virus in peripheral blood mononuclear cells (PBMC);
- C) Clinical HIV isolates (depending upon the microbicide and the mechanism of action, it may be appropriate to include drug resistant isolates);
- D) Activity against cell-associated virus; and
- E) Anti-viral activity in semen and, if possible, vaginal fluids or in an in vitro system that is physiologically appropriate.

* If the microbicide contains more than one active agent, each must be tested separately.

2. In vitro Activity against other STD pathogens:

- A) *Neisseria gonorrhoeae*;
- B) *Chlamydia trachomatis*;
- C) *Haemophilus ducreyi*;
- D) *Trichomonas vaginalis*; and
- E) Herpes simplex virus (HSV).

3. Spermicidal activity (e.g. mucous penetration test; sperm motility).

4. In vitro Activity against *Lactobacillus crispatus* and *Candida albicans*.
5. Interactions with latex/polyurethane or other physical barrier materials as appropriate.
6. Screening for vaginal irritation in a rabbit model (standard 10 day application of active agent and/or preclinical formulation).

Due to the potential for increased HIV transmission in the presence of significant cervical/vaginal inflammation and ulceration, it is recommended that the active agent be tested in a rabbit vaginal irritation model early in the development process. A 4% nonoxynol-9-containing spermicidal gel (e.g. Conceptrol) should be used as a positive control. If significant irritation occurs, termination of product development may be warranted.

7. Animal model efficacy testing of clinical formulation:

This section describes animal model systems to be considered for assessing product efficacy via topical administration for prevention of HIV and STDs. This list is not intended to be comprehensive. For example, newer models which are presently under development, such as the mouse HSV skin or genital infection models, may be considered. Depending upon the indication, tests for animal model efficacy of the clinical formulation may include:

HIV	STDs
SIV in Macaques	Genital herpes in guinea pigs
FIV in cats	<i>C. trachomatis</i> in mouse or primate
	<i>H. ducreyi</i> in rabbits
	<i>T. pallidum</i> in rabbits
	Human papillomavirus (HPV) in nude mice
	Rabbit papillomavirus model

II. Requirements for Tests of Clinical Formulations [Clinical formulations must be produced under good manufacturing practices, (GMP)].

This section is based on U.S. Food and Drug Administration (FDA) published documents (see references).

1. Physical-chemical properties of active agent*:
 - A) Solubility;
 - B) Stability (pH rate profile; accelerated degradation);
 - C) Standard Chemistry Requirements (Chemistry, Manufacturing; and Control (CMC) section of Investigational New Drug (IND) application.

* Well-characterized analytical methods to detect and measure the physical-chemical properties of the active agent are essential for product development. For example, quantitative analytical methods will be needed to determine drug diffusion and potential for systemic absorption of the active agent.

2. Define and assess characteristics of clinical formulation:

The combination of the active agent and a delivery vehicle may result in alterations in physical-chemical properties and microbicidal activity. Thus, the following studies of the clinical formulation are recommended.

- A) Stability, real and accelerated time of both clinical formulation and vehicle alone;
 - B) Formulation release studies (i.e. the kinetics of release of active agent from solid and semi-solid formulations); and
 - C) In vitro activity against pathogen, if possible, in comparison with active agent alone.
3. In vitro mutagenesis (can be done in parallel with Phase I clinical studies):
- A) Gene mutation tests;
 - B) Tests for chromosomal abnormalities; and
 - C) Primary DNA damage assays.
4. Good laboratory practices (GLP) safety assessment of clinical formulation produced in compliance with GMP:
- A) Rabbit vaginal/cervical irritation model;
 - B) Tests for systemic absorption;
 - C) Studies of exposure to rectal mucosa; pubic and other skin areas in animal models;
 - D) Oral toxicity studies;*
 - E) Gross necropsy or more specialized studies if warranted including penile irritation and absorption in male rabbits;*
 - F) Systemic toxicity studies in one species with single dose administration;
 - G) Hypersensitivity and photosensitivity studies;
 - H) Segment I reproductive toxicology as defined by the U.S. FDA (spermicidal activity)^{ref 1}
- * may be done concurrent with or after Phase I clinical studies depending upon the topical microbicide.
5. Segment II reproductive toxicology studies as defined by the FDA^{ref 1} (standardized tests in two animal species performed subsequent to Phase I studies in humans).
6. Toxicology studies in a rodent and non-rodent species (possibly concurrent with clinical trials):
- A) Duration equal to intended duration of clinical studies (up to twelve months);
 - B) Pharmacokinetics study; serum drug levels, C_{max} , "Area Under the Curve", tissue distribution and metabolite profiles should be presented and compared to human data; and
 - C) If Phase I trials establish that the drug is absorbed in humans and the mucosal route of delivery in animals cannot achieve much higher blood levels than those seen in Phase I, 1-3 month toxicity study during which the clinical formulation is given parenterally or orally may be required to define all potential toxicities.
7. Carcinogenicity testing (concurrent with Phase III clinical trials):
- A) Two animal species (rats and mice); and
 - B) Intravaginal administration for 2 years at maximum tolerated dose.

8. Segment III reproduction studies as defined by the FDA^{ref} (perinatal and post-natal studies in rats) can be done concurrent with Phase III clinical trials.

SOURCES:

1. U.S. Food and Drug Administration Guidance for the Development of Vaginal Contraceptive Drugs (January 27, 1995; clarifications February 8, 1995)
2. U.S. Food and Drug Administration Points to Consider in the Preclinical Pharmacology/Toxicology Development of Topical Microbicides Intended for Viral Sexually Transmitted Diseases (STD) Including HIV (DRAFT)
3. Federal Register Notice : International Conference on Harmonization - Guideline on detection of Toxicity to Reproduction for Medicinal Products (September 22, 1994)

Date: 8/20/95

Prepared by: Z. Rosenberg and P. Hitchcock and the
NIAID Topical Microbicides Working Group

Clinical and Behavioral Research

Section 3

- Capacity and Infrastructure
- Clinical Trials of Existing Products

Vaginal Contraceptive Film Study

Phase I Advantage 24 Study

Phase II/III Advantage 24 Study

- Clinical Trials of New Products

BufferGel Study

Lactin-vaginal Suppository Studies

- Behavioral Research

Clinical and Behavioral Research

CAPACITY AND INFRASTRUCTURE

The NIAID has the capacity to conduct single and multi-site trials for Phase I (safety studies in low risk populations); Phase II (safety studies in high risk populations); Phase III (efficacy studies); and Phase IV (post-market evaluations).

Contract and cooperative agreement mechanisms are utilized. In addition, investigator initiated research, using the R01 mechanism, can be utilized for the conduct of clinical trials. Contracts include the HIV Network for Prevention Trials, HIVNET; the Vaccine and Treatment Evaluation Unit, VTEU; and the STD Clinical Trials Unit, STD CTU. Cooperative Agreements include the STD Cooperative Research Centers.

- HIV Network for Prevention Trials (HIVNET)
- Vaccine and Treatment Evaluation Unit (VTEU)
- STD Clinical Trials Unit (STD CTU)
- STD Cooperative Research Centers (STD CRCs)

Capacity and Infrastructure

HIV Network for Prevention Trials (HIVNET)

- Description
- Primary Contractors
- Domestic Sites
- International Sites

HIV Network For Prevention Trials (HIVNET)

The HIV Network For Prevention Trials (HIVNET) is a cooperative network of eight domestic and nine international field sites, a data management and statistical center, a central support laboratory, and a central specimen repository, a master contractor for the domestic sites, and a master contractor for international sites. The primary mission of the HIVNET is to evaluate interventions for the prevention of the spread of HIV infection and other sexually transmitted diseases (STDs). To accomplish this mission, the HIVNET will conduct intermediate and phase III randomized controlled field trials with HIV seroincidence as the primary endpoint. HIVNET will also conduct pilot, phase I, and phase II intervention and feasibility studies which lead to or support phase III studies. To promote coordination and collaboration among HIVNET investigators conducting prevention research, four Science Direction Working Groups have been formed within the HIVNET: vaccines; behavioral research; microbicides, barriers, and STDs; and perinatal.

Currently, the domestic sites are involved in a multi center Vaccine Preparedness Study (VPS) of 4800 HIV-negative individuals at high risk of HIV infection. The goals of VPS are to determine the seroincidence and subtypes of HIV transmitted, the virology and immunology of the incident infections, the most effective means of recruiting and retaining different high risk groups for prevention trials, valid instruments for determining and monitoring high risk behavior of study participants, and factors that determine individual participation and community acceptance of prevention trials. Similar research is also ongoing at the nine international field sites although each protocol is independent. In addition, three of the international sites are involved in prevention efficacy trials: vaginal topical microbicides for the prevention of HIV sexual transmission in Kenya; HIV immune globulin for prevention of perinatal transmission in Haiti; and behavioral risk reduction through peer counseling in Zimbabwe.

HIV Network for Prevention Trials - HIVNET
National Institutes of Health
National Institute of Allergy & Infectious Diseases, Division of AIDS

Primary Contractors		
Organization	Contract	Principal Investigator
Abt Associates Inc.	Domestic Master	George Seage, DSc
Family Health International	International Master	Ward Cates, PhD
Fred Hutchinson Cancer Research Center/ University of Washington	Statistical Center	Steve Self, PhD
Viral & Rickettsial Disease Laboratory, California Department of Health Services	Central Laboratory	Haynes Sheppard, PhD
Biomedical Research, Inc.	Repository Contractor	James L. Leef, PhD

Domestic Sites	
Site	Principal Investigator(s)
Bronx-Lebanon Hospital Center, New York City	Jerome Ernst, MD
Denver Department of Public Health	Frank Judson, MD
Fenway Community Health Center, Boston	Kenneth Mayer, MD
Health Research Association, Los Angeles	Peter R. Kerndt, MD, MPH
Howard Brown Health Center, Chicago	David McKirnan, PhD
Johns Hopkins University, Baltimore	Alice Tang, PhD
New York Blood Center, New York City	Cladd E. Stevens, MD Beryl A. Koblin, PhD
New York University Medical Center, NYC	Michael Marmor, PhD
Public Health Foundation Enterprises, Inc. San Francisco	Susan Buchbinder, MD
University of Pennsylvania, Philadelphia	George Woody, MD David Metzger, PhD
University of Washington, Seattle	Connie Celum, MD, MPH

HIV Network for Prevention Trials - HIVNET
National Institutes of Health
National Institute of Allergy & Infectious Diseases, Division of AIDS

International Sites			
Site	Country	Principal Investigator(s)	
		U.S.	In-Country
University of Pittsburgh Universidade Federal do Rio de Janeiro	Brazil	Lee H. Harrison, MD	Mauro Schechter, MD, MPH
Cornell University Institute Nationale de Laboratoire et de Recherches	Haiti	Jean Pape, MD	Jean Pape, MD
Johns Hopkins University Malawi College of Medicine	Malawi	Taha E. Taha, MD, PhD	George Liomba, MD, FRCPath
Centre for Epidemiological Research in South Africa	South Africa	Salim S. Abdool Karim	Salim S. Abdool Karim
Perinatal Research Unit Barangwanath Hospital	South Africa	James McIntyre, MD	James McIntyre, MD
Johns Hopkins University Chiang Mai University	Thailand	Kenrad E. Nelson, MD	Chirassak Khamboonruag, MD, PhD Chawalit Natpratan, MD, MPH
University of Maryland at Baltimore Medical Research Foundation of Trinidad and Tobago	Trinidad	Farley Cleghorn, MD, MPH	Courtenay Bartholomew, MD
Case Western Reserve Makerere University	Uganda	Jerrold Ellner, MD	Roy Mugerwa, MBChB, MMed
Johns Hopkins University Makerere University	Uganda	Brooks Jackson, MD	Francis Mmimo, MBChB, FRCOG
University of Alabama University Teaching Hospital	Zambia	Susan Allen, MD, MPH	Yusuf Ahmed, MD Chewe Luo, MD Rosemary Musonda, PhD
Stanford University Zimbabwe AIDS Prevention Project	Zimbabwe	Julie Parsonnet, MD	Jacob Mufunda, MD

International Sites Phasing Out in 1998			
Johns Hopkins University National AIDS Research Institute	India	Robert Bollinger, MD	D.A. Gadkari, MD
University of Washington University of Nairobi	Kenya	Joan Kreiss, MD, MSPH	J.O. Ndinya-Achola

HIVNET RESEARCH PLAN

EXECUTIVE SUMMARY

The HIV epidemic continues to grow worldwide despite major advances in understanding the pathogenesis of HIV infection and in our ability to treat the disease. Control of the epidemic requires improved methods and strategies for preventing HIV infection. Development and testing of an HIV vaccine that is simple to administer, inexpensive and that induces long-lasting immunity to all known HIV strains would be the optimal preventive measure and is the highest priority of the HIVNET. Although promising HIV vaccine products are in various stages of development, an effective vaccine will not be available for several years. Therefore, other promising HIV prevention approaches need to be developed and evaluated for interrupting HIV transmission. Such approaches include topical microbicides, treatment of sexually transmitted diseases that are cofactors for HIV transmission, prophylaxis to prevent mother to child transmission, and behavioral risk reduction strategies. Even in the presence of an efficacious vaccine, control of the epidemic will probably require combinations of methods and strategies to protect individuals and the public against HIV infection.

Mission and Resources of the HIVNET

The HIV Network for Prevention Trials (HIVNET) was established in 1993 by the Division of AIDS (DAIDS) of the National Institute of Allergy and Infectious Diseases (NIAID). Its goal is to conduct domestic and international multi-center trials to evaluate the safety and efficacy of promising interventions to prevent sexual, perinatal, and parenteral transmission using HIV seroincidence as the primary trial endpoint. The interventions to be evaluated encompass HIV vaccines, topical microbicides, sexually transmitted disease (STD) treatment, prophylaxis to prevent mother to child transmission, and behavioral risk reduction strategies. HIVNET studies are carried out in close coordination and collaboration with other agencies that are involved in prevention research including the National Institute of Mental Health (NIMH), the National Institute on Drug Abuse (NIDA), the National Institute of Child Health and Human Development (NICHD), the National Institute on Alcohol Abuse and Alcoholism (NIAAA), the Fogarty International Center (FIC), the Centers for Disease Control and Prevention (CDC), the Walter Reed Army Institute of Research (WRAIR), the U.S. Agency for International Development (USAID), the Population Council, the Contraceptive Research and Development Agency (CONRAD), UNAIDS, and the World Health Organization (WHO). Experts from these agencies have provided advice on the design and conduct of HIVNET studies.

The HIVNET was designed to rapidly and systematically test promising HIV prevention interventions in response to new scientific developments. The HIVNET is funded through a series of peer reviewed contracts and subcontracts consisting of the Domestic Master Contractor (DMC), the International Master Contractor (IMC), the Statistical and Clinical Coordinating Center (SC), the Central Laboratory (LC), and eight domestic and nine international HIVNET field sites that are supported through subcontracts from the DMC and IMC (Appendix 1).

Through the peer reviewed proposals for contracts and subcontracts, the HIVNET has assembled an experienced, multi-disciplinary team of investigators and clinical sites to undertake Phase III prevention trials. The HIVNET mission also is to conduct the necessary clinical, epidemiologic and laboratory research and Phase I and Phase II clinical trials which are antecedents to large-scale prevention efficacy trials.

HIVNET Governance

The HIVNET has developed governance procedures to promote the efficient conduct of prevention studies and to ensure that the design, implementation and analysis of HIVNET studies meet the highest possible standards. Scientific direction of the HIVNET resides with the Scientific Steering Group (SSG). The four Science Direction Working Groups (SDWGs) (Vaccines, Perinatal, Microbicides/Barriers/STDs, and Behavioral), which report to the HIVNET SSG, have the responsibility to analyze and identify research needs in their relevant fields. HIVNET investigators can propose research ideas for HIVNET studies and outside investigators can submit proposals for collaborative studies. These proposals are further discussed and developed by the relevant SDWG. Research proposals are peer-reviewed by HIVNET's independent Prevention Research Group (PRG), which critiques the proposals based on their importance for HIV intervention research, scientific merit, and the advantage of the HIVNET to undertake the research. Proposals with the highest scores are then considered by the HIVNET SSG, which determines its priority for protocol development and implementation. The final decision to allocate funds for the study is made by NIAID.

Research Accomplishments and Future Directions

Since November 1994, when the domestic and international field sites were funded, the HIVNET has initiated studies in each of targeted prevention research areas. Summaries of the research objectives, study design and current status of these studies are provided in Appendix 2.

The initial research priority of the eight domestic and nine international HIVNET sites was to determine the feasibility of conducting HIV vaccine and other prevention intervention trials in various populations at high risk of HIV infection and to prepare these communities for anticipated vaccine trials. In addition, the HIVNET has initiated a series of randomized controlled prevention

trials of behavioral, microbicide and perinatal transmission interventions. Although still in progress, the research accomplishments of these studies, represented by numerous publications and abstracts, have been extensive, with major contributions to understanding the critical biologic, epidemiologic, and behavioral determinants that affect sexual, perinatal, and parenteral transmission and willingness to participate in prevention trials among the diverse risk groups participating in HIVNET cohorts.

Research in Preparation for Prevention Trials

The domestic HIVNET sites have implemented a multi-center Vaccine Preparedness Study (VPS) to determine feasibility of conducting an HIV vaccine efficacy trial. The study, which will be completed in April 1997, has enrolled 4892 participants at high risk of HIV infection (gay men, injection drug users and women at heterosexual risk) in a prospective cohort study which simulates the size and study procedures of a large-scale vaccine trial (Table 1). The research goals of the VPS are to: 1) determine seroincidence and cofactors for transmission in the context of behavioral interventions that will be available to vaccine trial participants, 2) assess knowledge, attitudes and willingness to participate in vaccine and non-vaccine prevention trials, 3) identify effective and efficient methods to rapidly recruit and retain individuals at risk for HIV infection, 4) develop reliable, valid, consistent, and cost-effective measures to quantify risk behaviors, 5) develop reliable and efficient procedures for obtaining truly informed consent for participation in vaccine trials, 6) develop and evaluate risk reduction measures that will be provided to vaccine trial participants, 7) characterize clinical, epidemiological, virological, and immunological outcomes of participants who become infected with HIV, 8) determine if highly exposed but uninfected cohort participants have immunologic responses to HIV, and 9) determine the feasibility of home collection of oral fluid and blood for diagnosis of early HIV infection. Seroincidence in the cohort has been stable at 2% per year with a 95% retention rate after 12 months of follow-up. Willingness to participate in vaccine trials has been high and is being monitored in response to new information about trials and vaccine products.

With similar scientific goals, the nine international HIVNET sites have recruited and followed more than 8000 participants in cohorts of female commercial sex workers, gay men, STD clinic patients, long distance truck drivers, factory workers, and pregnant women. The annual HIV seroincidence at these sites ranges from <1% to greater than 12%, with one year follow-up rates ranging from 70-98% (Table 2). HIV strains isolated from participants in the diverse cohorts have been characterized and represent all the major subtypes or clades of HIV.

Prevention Trials--Studies in progress and future directions

HIV Vaccines

An effective HIV vaccine has the greatest potential for helping to control the global epidemic of HIV, and HIVNET will be ready to evaluate promising vaccine concepts as they are identified for

efficacy testing. One promising vaccine concept, the ALVAC vcp205 prime/gp120 boost combination will soon be ready for expanded testing, and the HIVNET will focus on evaluation of this concept over the next few years. A series of trials of this vaccine candidate are in various stages of planning, although the eventual design of these trials will be dependent on data from early clinical trials currently underway in the AIDS Vaccine Evaluation Group (AVEG). A Phase II trial in the U.S. (in collaboration with AVEG) to assess the safety and immunogenicity of the ALVAC vcp205 prime/gp120 boost combination is slated to begin in early 1997. If the Phase II study satisfies safety and immunogenicity criteria, the HIVNET will proceed with an intermediate size trial of this HIV vaccine concept in the US in mid-1998. In addition to U.S. studies, the HIVNET will conduct a Phase I study to assess the safety and immunogenicity of the ALVAC vcp205 vaccine in African populations scheduled to begin in late 1996 in the Uganda HIVNET site. Immunogenicity data from this Uganda study will be used to develop an appropriate ALVAC HIV vaccine for East African countries.

Prevention of Perinatal Transmission

Perinatal transmission is a principal source of HIV-related morbidity and mortality in developing countries. Antiretroviral therapy administered prior to and at delivery to reduce the rate of vertical transmission has been shown to result in a substantial reduction in HIV transmission in the U.S. and other industrialized countries. Implementation of this approach in developing countries is precluded by cost and by limited access to prenatal care. A priority of the HIVNET is to identify antiretroviral therapies that can be administered over a shorter course (e.g., at delivery) and at reduced cost for reducing vertical transmission in the developing country setting. A Phase III study in the Malawi site, which was jointly funded by the National Cancer Institute (NCI), evaluated the effect of chlorhexidine wash of the birth canal to prevent perinatal transmission; this study did not show an effect on HIV transmission, but did show a significant drop in the rate of neonatal sepsis. A Phase III study of effect of HIV Immunoglobulin (HIVIG) to prevent perinatal transmission in Haiti will begin in September 1996. A Phase I study of the safety of nevirapine in seropositive pregnant women and their newborns in Uganda will begin in June 1996. If the latter trial shows no unexpected safety concerns, HIVNET will proceed with a Phase III trial in Uganda to evaluate peripartum use of nevirapine and also zidovudine compared to placebo in preventing perinatal transmission. The HIVNET will consider evaluating other promising antiretroviral drugs (e.g., PMPA, protease inhibitors) as soon as initial safety studies are completed by the U.S.-based Pediatric AIDS Clinical Trials Group (ACTG).

Topical Microbicides and STD Treatment

The development of female controlled methods to protect against sexual transmission has been a high priority of HIVNET. The Kenya HIVNET site completed a Phase II safety study of a nonoxynol-9 (N9) vaginal microbicide gel in January 1996 and will begin a Phase III efficacy trial of this product for preventing sexual transmission among commercial sex workers in June 1996.

To further clinical development of new topical microbicide products for vaginal use, the HIVNET will undertake a series of Phase I/II trials to establish the safety and acceptability of new products in different populations. An initial trial at the Rhode Island HIVNET site starting in August 1996 will examine the safety and acceptability of an acid buffering gel. If there are no safety concerns in U.S. participants, the trial will be extended to the HIVNET sites in Thailand, India, Malawi and Zimbabwe. The goal of HIVNET will be to complete early clinical testing and move one or more new products into efficacy trials in the next few years.

Microbicides also may be a promising strategy for preventing rectal transmission of HIV. Microbicide products, such N9, have been used extensively by gay men, but the safety of these products for rectal use has not been established. Therefore the HIVNET is conducting a Phase I study of the safety of N9 containing gel for rectal use. These studies also will conduct research to identify more acceptable and effective delivery systems for rectal use products. Depending on safety and acceptability data from the Phase I studies, an intermediate sized trial could be proposed for the U.S. gay cohorts within a few years.

Behavioral Interventions

Behavioral interventions to promote condom use, STD treatment and partner reduction may reduce HIV transmission. There now appears to be considerable agreement within the prevention research community regarding key principles that should be incorporated into such interventions to produce convincing and sustained behavioral change. However, to date, these interventions have not been evaluated systematically in randomized controlled trials with HIV infection as the primary endpoint. Therefore, a priority of the HIVNET has been to evaluate the efficacy of these promising behavioral interventions for reducing HIV transmission. The HIVNET is currently conducting a Phase III study of peer counseling to prevent sexual transmission in factory workers at the Zimbabwe site. The Thailand, India, and Uganda sites are developing a joint Phase I study of condom promotion for prevention of heterosexual transmission among stable, sero-discordant couples. The domestic HIVNET sites, in conjunction with NIMH investigators, are planning an intermediate-size trial (Phase IIb) to evaluate an individualized behavioral counseling intervention for gay men to reduce HIV and/or STD transmission.

Among injection drug users (IDUs), broad agreement exists about the efficacy of methadone treatment and use of clean needles in reducing HIV transmission. Therefore, a priority of domestic HIVNET IDU sites has been to promote the use of these interventions in their cohorts as part of routine counseling. The NYU HIVNET site is currently planning a Phase IIb study of using social networks to enhance syringe exchange use among IDUs in the New York City cohort. An additional strategy would be to simultaneously address multiple factors associated with HIV-related risk behaviors including depression, alcoholism, and sexual exposure to HIV, among IDUs. However, such studies do not appear feasible in the current HIVNET IDU cohorts in which needle sharing and HIV seroincidence have dropped significantly. Therefore, additional IDU cohort development will be needed for both vaccine and prevention trials.

The seroincidence in non-drug using women enrolled in HIVNET cohorts has been unexpectedly high. Further analysis of the risk factors that are predictive of seroconversion will be undertaken to strengthen the counseling for risk behavior reduction that is provided during followup visits.

Addressing Multiple Priorities Efficiently

Efficacy trials represent a significant investment of resources, particularly because evaluations of HIV prevention interventions involve large numbers of participants followed over several years. The HIVNET will be limited in its ability to carry out multiple prevention trials by financial constraints and participant availability. These limitations -- as well as the need to rapidly respond to the availability of candidate vaccines suitable for efficacy trials -- are best addressed by careful allocation of resources. When more than one large-scale intervention is possible in a given site or population and when resources are limited, interventions will be chosen according to the following priorities: the likelihood that the intervention will significantly slow the spread of HIV, the ability of the trials to provide unique scientific insights, and the ability of the HIVNET to make a unique contribution to science and public health.

The use of flexible study designs in the evaluation of prevention strategies also may enable the HIVNET to address priorities more efficiently. The HIVNET Statistical and Clinical Coordinating Center has been developing statistical design strategies that improve efficiency and conserve resources. These strategies include the use of: 1) intermediate size (Phase IIb) trials, which provide a rapid and efficient strategy for preliminary evaluation of vaccines and other prevention interventions prior to an investment in larger scale efficacy trials, and 2) factorial study designs, which simultaneously evaluate multiple interventions. Factorial studies are more economical and efficient than trials that are conducted simultaneously with separate cohorts.

Community Education

Prevention efficacy studies require an informed and prepared community working in collaboration with researchers. The HIVNET is building credibility and trust among communities regarding the array of prevention strategies that could be studied in the HIVNET. To foster trust and mutual understanding of trial issues and to ensure that the studies respect cultural and ethnic differences among participants, the HIVNET has involved community members in all phases of the research process. The HIVNET is currently developing a systematic, multi-faceted community education plan that will include activities at the national and local site levels and a needs assessment for community education materials and strategies.

Conclusions

The HIVNET is a flexible and coordinated multi-center, collaborative research infrastructure ideally suited to implement and carry out HIV prevention intervention efficacy trials. A variety of promising preventive intervention strategies have been described that could potentially contribute to the control of the HIV epidemic; however, few of these have been critically evaluated with HIV seroincidence as a primary endpoint. The HIVNET has the capacity to launch a broad range of Phase I through Phase III HIV prevention trials to evaluate these interventions. While doing research critical to the design of future efficacy trials, the HIVNET also has contributed valuable information and specimens that are critical to our understanding of pathogenesis of the early phases of HIV infection.

Table 1

HIVNET Domestic Cohorts

<u>Institution</u>	<u>Cohort</u>	<u>Estimated Recruitment</u>
Johns Hopkins University BALTIMORE	IDU	490
Fenway Community Health Center, BOSTON	MSM WAHR	650 250
Howard Brown Health Center CHICAGO	MSM WAHR	650 300
Denver Dept. of Public Health DENVER	MSM	650
Health Research Association LOS ANGELES	MSM WAHR	600 200
Bronx-Lebanon Hospital Center NEW YORK CITY	IDU WAHR	250 150
New York Blood Center NEW YORK CITY	MSM WAHR	650 150
NYU Medical Center NEW YORK CITY	IDU WAHR	500 200
University of Pennsylvania PHILADELPHIA	IDU WAHR	500 200
Public Health Foundation SAN FRANCISCO	MSM	650
University of Washington SEATTLE	MSM	650

MSM = Men who have sex with men

WAHR = Women at heterosexual risk

IDU = Injection drug user (male or female)

Table 2

HIVNET International Cohorts

INSTITUTION(S)	Cohort(s) (Existing or to be established)	Recruitment #
U. Maryland/ Medical Research Foundation of Trinidad and Tobago	STD Clinic Patients Female CSWs MSM	952 21
South Africa - Bargwanath Hospital/PHRU & RHRU	Female Adolescents HIV+ Pregnant women and their infants Women w/history of > 2 STDs Injection/oral contraceptive users Female CSWs	1200 720/900 — 3600 —
JHU/Uganda - Makerere Univ.	HIV+ Pregnant women and their infants	852
CWRU/Uganda - Makerere Univ.	Military (community based population) STD Clinic Family Planning Clinic	1200 1600 974
U.Pitt/Brazil - Univesidade Federal do Rio de Janeiro	MSM Female CSW	1500 —
South Africa - CERSA	Female Partners of Migrant Workers (community based population) HIV+ Pregnant women and their infants	2000 1124
JHU/Malawi - Malawi College of Medicine	Sugar Corporation Workers (male) HIV+ Pregnant women and their infants High Risk Women	7000 990 3660
Cornell/Haiti - GHESKIO	Discordant Couples STD Patients	60 1000
Stanford/Zimbabwe - University of Zimbabwe Medical School	Male Factory Workers (and their female partners) Family Planning Clinic HIV+ Pregnant women and their infants	1950 2400 1000
UAB/Zambia - Univ. Teaching Hospital	STD Clinic Discordant Couples HIV+ Pregnant women and their infants	189 700 1280
JHU/Thailand - RIHES	Ex-Military Army Recruits Male CSW Factory Workers Infants of HIV+ women Discordant couples STD Clinic & HIV Testing Site Attendees	380 821 1245 499 92 562 1380

Capacity and Infrastructure

Vaccine and Treatment Evaluation Unit (VTEU)

- Description

Phase I studies of topical microbicides are being planned for the Vaccine and Treatment Evaluation Program (VTEU) at the Children's Hospital in Cincinnati Ohio. All sites in the Network are potential sites for Phase I/II studies. In addition, the network can and has been used for multi-center trials of vaccines and therapies, this potential could be tapped for domestic Phase III topical microbicide trials.

Vaccine and Treatment Evaluation Program

NIAID has a major responsibility within the U.S. Public Health Service for vaccine research, development, and evaluation of methods to control and prevent infectious diseases. Within this mission, the Division of Microbiology and Infectious Diseases (DMID) is charged with the evaluation of control measures for infectious diseases other than AIDS. This work encompasses clinical trials in humans of candidate vaccines, other biologicals, and drugs as preventive and therapeutic measures.

To facilitate testing of vaccines for safety and effectiveness under controlled conditions, the Institute established Vaccine Evaluation Units in the early 1960s and subsequently Vaccine and Treatment Evaluation Units (VTEU) in 1992. Currently, DMID and the Division of Intramural Research within NIAID support seven VTEUs and four special emphasis units at nine university-based and independent medical research institutions around the country. The VTEUs have broad capabilities to conduct prophylactic and therapeutic studies. The Special

Emphasis Sites for Clinical Research have specific capacity in the areas of maternal immunization and viral respiratory diseases, group B streptococcus, and tuberculosis. Together the VTEUs and the Special Emphasis Sites for Clinical Research comprise the Vaccine and Treatment Evaluation Program (VTEP).

VTEP scientists accelerate the development of new vaccines and therapies by evaluating candidate vaccines in early human trials for safety, immunogenicity, and protective efficacy. The vast experience of researchers with vaccines, combined with the units' unique facilities and researchers' access to various population groups for studies, makes the VTEP a national resource for early evaluation of vaccines in carefully controlled phase I and phase II clinical trials. Some of the VTEUs also have the ability to evaluate candidate anti-infective therapies.

After a vaccine has been tested in phase I and II trials, it is evaluated in phase III studies. These studies can involve large numbers of volunteers if disease

incidence is low. To enlist the large numbers of subjects needed, phase III studies often are conducted simultaneously in several medical centers and usually are designed as randomized, placebo-controlled trials. Phase III trials provide the statistically significant data on vaccine efficacy that are necessary before the vaccine can be made available for general use. Post-licensure studies also can be performed to answer important questions.

In addition, the Institute maintains a series of support contracts for its vaccine development efforts, including a facility for preparing pilot lots of vaccines and formulating vaccines for human trials. A separate contract provides research support for the vaccines and drugs filed under investigational new drug applications by DMID. Another contract provides NIAID with a resource center to aid in conducting multicenter phase II and III clinical trials. This contract also provides NIAID with form design; data coordination, reporting, and analysis; and various other technical services.

Current Active Institutions

Vaccine and Treatment Evaluation Units

1. Baylor College of Medicine, Houston, Texas
2. Harbor-UCLA Medical Center, Torrance, California
3. James N. Gamble Institute, Cincinnati, Ohio
- *4. Johns Hopkins University, Baltimore, Maryland
5. St. Louis University, St. Louis, Missouri
6. University of Maryland, Baltimore, Maryland
7. University of Rochester, Rochester, New York

Special Emphasis Sites for Clinical Research

8. Baylor College of Medicine, Houston, Texas
9. Baylor College of Medicine, Houston, Texas
10. Brigham and Women's Hospital, Boston, Massachusetts
11. Case Western Reserve University, Cleveland, Ohio

Support Contracts

12. Ogden Bioservices Corporation, Gaithersburg, Maryland
13. Program Resources, Incorporated, Reston, Virginia
14. Technical Resources International, Incorporated, Rockville, Maryland

Contract Numbers

AI-25135
AI-45249
AI-45252
AI-15095
AI-45250
AI-45251
AI-45248

AI-15103
AI-15126
AI-25152
AI-45244

AI-25137
AI-15129
AI-15131

* Supported by the Division of Intramural Research.

Capacity and Infrastructure

STD Clinical Trials Unit (STD CTU)

- Description
- Statement of Work
- Abstract

STD Clinical Trials Unit

The STD Clinical Trials Unit will support clinical trials to test safety and efficacy of biomedical and behavioral interventions aimed at the prevention and control of sexually transmitted diseases. Diseases or pathogens of interest include, but are not limited to: chlamydial infection; gonorrhea; syphilis; chancroid; human papillomavirus (HPV) infection; genital herpes (herpes simplex virus 1 & 2 infections), human herpes virus 8 (Kaposi's sarcoma virus) and human immunodeficiency virus (HIV) infection as it is associated with other STDs (both in terms of increased transmission rates of HIV and alteration of the natural history of STDs). The syndromes of interest include, but are not limited to: pelvic inflammatory disease (and sequelae - infertility, ectopic pregnancy and chronic pelvic pain); adverse outcomes of pregnancy; and cervical cancer as well as other genital cancers associated with HPV infection..

These prevention and control strategies encompass primary, secondary and tertiary prevention. The interventions of interest include but are not limited to: topical microbicides, vaccines, diagnostics tests, screening tests, therapeutics and behavior change. Although the scope of interventions is broad, the number of microbicides, vaccines, diagnostic tests, behavioral interventions, that are appropriate for clinical trials is finite. That said, a prioritization process with respect to category of intervention and specific intervention will be implemented and those interventions of highest priority will be selected for study in the STD CTU.

Given currently available funds, it is anticipated that the trials will be Phase I and Phase II in scope. Infrequently, but when appropriate, Phase III (efficacy) and Phase IV (post-marketing application) trials may be conducted in the STD CTU.

ATTACHMENT A

**SEXUALLY TRANSMITTED DISEASES CLINICAL TRIALS UNIT (STD CTU)
WORK STATEMENT**

Introduction:

The objective of the acquisition is to support clinical trials to test safety and efficacy of biomedical and behavioral interventions aimed at prevention and control of sexually transmitted diseases (STDs). Diseases or pathogens of interest include, but are not limited to: chlamydial infection; gonorrhea; syphilis; chancroid; human papillomavirus (HPV) infection; genital herpes (herpes simplex virus 1 & 2 infections), human herpes virus 8 (Kaposi's sarcoma virus) and human immunodeficiency virus (HIV) infection as it is associated with other STDs (both in terms of increased transmission rates of HIV and alteration of the natural history of STDs). The syndromes of interest include, but are not limited to: pelvic inflammatory disease (and sequelae - infertility, ectopic pregnancy and chronic pelvic pain); adverse outcomes of pregnancy; and cervical cancer as well as other genital cancers associated with HPV infection. Viral hepatitis and cytomegalovirus infection are addressed in other programs of the National Institute of Allergy and Infectious Diseases (NIAID) and are not of interest in this solicitation.

These prevention and control strategies encompass primary, secondary and tertiary prevention. The interventions of interest include but are not limited to: topical microbicides, vaccines, diagnostics tests, screening tests, therapeutics and behavior change. Although the scope of interventions is broad, the number of microbicides, vaccines, diagnostic tests, behavioral interventions, that are appropriate for clinical trials is finite. That said, a prioritization process with respect to category of intervention and specific intervention will be implemented and those interventions of highest priority will be selected for study in the STD CTU.

Given currently available funds, it is anticipated that the trials will be Phase I and Phase II in scope. Infrequently, but when appropriate, Phase III (efficacy) and Phase IV (post-marketing application) trials may be conducted in the STD CTU; however no protocol or budget for Phase III or IV trials should be submitted in the proposal.

It is anticipated that one, completion, contract will be awarded.

Background:

The mission of the NIAID's STD program is to foster, develop, and administer a research program that will contribute to the reproductive health of people and specifically lead to prevention and control of STDs. The broad, public health goals of the research program are:

- prevention of STDs;
- prevention of infertility;
- prevention of adverse outcomes of pregnancy;
- prevention of reproductive tract neoplasia;

- prevention of human immunodeficiency virus (HIV) infection; and
- prevention of other chronic STD sequelae.

In order to accomplish our mission, strategies for primary, secondary and tertiary prevention of STDs must be evaluated. Strategies for primary prevention (prevent infection) would include, but are not limited to, topical microbicides, vaccines or behavior change, e.g. increased use of male condoms; secondary prevention strategies (prevent transmission of the infection to others) might include diagnostic screening and treatment; diagnosis and use of single-dose therapies; or behavior change, e.g. self-screening based on mild symptoms or risk behaviors; tertiary prevention strategies (interventions to prevent disease progression and chronic sequelae) might focus on pelvic inflammatory disease and infertility; adverse outcomes of pregnancy; HPV and cervical cancer; or HIV infection.

Statement of Work

The Contractor, independently and not as an agent of the government, shall furnish all services, qualified professional and technical personnel, volunteer populations, materials, equipment and facilities not otherwise provided by the government under the terms of this contract, as needed to evaluate biomedical and behavioral interventions to prevent and control sexually transmitted diseases (STDs).

Specifically the contractor shall:

- I. Provide facilities and staff to conduct Phase I and Phase II clinical trials to evaluate interventions to prevent and control STDs, including human challenge studies as appropriate. Contract efforts may include but are not limited to clinical trials to evaluate topical microbicides, vaccines, diagnostic tests, therapeutics and behavioral interventions.**

{Notes to Offeror:

- 1. The offeror should describe how an existing clinical and laboratory facility can support the various studies, or describe the establishment of such facilities or collaborations to support the various studies.*
- 2. A well-trained, experienced, and balanced staff to conduct clinical trials is required. The primary investigator (PI) should have an M.D. and/or Ph.D. degree and should have demonstrated expertise in clinical trials related to STDs involving appropriate populations. The team of experts in the diseases of interest should include a nursing staff, experts in adolescent medicine; behavioral science; infectious diseases including STDs, pelvic inflammatory disease and adverse outcomes of pregnancy; obstetrics/gynecology, including colposcopy; clinical trials evaluating vaccines, diagnostic tests, therapeutics, behavioral interventions; epidemiology and biometry. Effective nursing staff are critical to the success of intervention trials and a Research Nurse Coordinator should be identified. Technical personnel, trained and experienced in collecting specimens, and performing assays and laboratory procedures should be included. The offeror should describe coverage of administrative responsibilities.*
- 3. A two page biosketch of each proposed professional should be included in the proposal.}*

II. Provide and retain patients as required for all clinical trials to ensure timely completion of protocols.

{Note to Offeror:

4. The availability of appropriate populations is critical to the effective testing of candidate interventions. Offeror's technical proposal should include a detailed description of the types of populations available; consideration should be given to incidence and prevalence of STDs. Estimates of the target populations for the clinical trials efforts are as follows: adolescents, 14-17 years old, (50%); young adults, 18-24 years (30%); adults 25 years and older (20%). Studies may require testing in healthy volunteers at low risk for STDs as well as populations at high risk for STDs. Depending upon the study, monogamous, contracepting women may be required. In other studies sero-positive and/or sero-negative volunteers may be required.

5. For contracts dealing with clinical research, it is NIH policy that women and members of minority groups and their sub-populations must be included in all NIH supported biomedical and behavioral research projects involving human subjects, unless a clear and compelling rationale and justification is provided that inclusion is inappropriate with respect to the health of the subjects or the purpose of the research. All investigators proposing research involving human subjects should read the "NIH Guidelines For Inclusion of Women and Minorities as Subjects in Clinical Research". A copy of this publication is contained in this RFP Gopher under the RFP REFERENCES - FORMS, FORMATS and INSTRUCTIONS directory.}

III. Conduct rigorously designed Phase I and Phase II clinical trials to evaluate safety and effectiveness of topical microbicides, diagnostic tests, therapeutics and behavioral interventions for the prevention of STDs. ALL STUDIES ARE SUBJECT TO PROJECT OFFICER APPROVAL.

{NOTES TO OFFEROR:

6. Two sample, detailed, protocols and consent forms should be submitted with the proposal and should demonstrate a thorough understanding of clinical evaluation of the interventions described.

- i. One Phase I protocol should focus on an approach to the diagnosis of subclinical pelvic inflammatory disease; the offeror should first describe the characteristics of the diagnostic approach followed by an appropriate protocol. The protocol need not describe the use of a currently available product. If a theoretical product or approach is described, the offeror should include sufficient detail to permit determination of the appropriateness of the protocol. The Offeror should consider the Meeting Summary of the NIAID Workshop on Pelvic Inflammatory Disease, December 1994, Attachment 1.*
- ii. One Phase II protocol should address the safety/efficacy of a vaginal topical microbicide to prevent chlamydial infection in an adolescent population. The microbicide protocol need not involve the use of a currently available product. The offeror should describe the "theoretical product" in sufficient detail so as to enable determination of the protocol's appropriateness. The Offeror should consider the International Working Group on Vaginal Microbicides' Recommendations for the Development of Vaginal Microbicides, Attachment 2 and the WHO*

Manual for Colposcopy, copies of which are available through the WHO, Geneva Switzerland.

- iii. *The protocols should neither be underway presently nor have been sponsored or reviewed by the NIAID previously. The protocols should document how the Offeror can best address the intent of this RFP.*

7. It is anticipated that a representative Phase I study might involve 12-20 patients for a two week time period or a 2-4 month time period depending upon the nature of the intervention being tested; a representative Phase II study would involve 100 patients for a 2-6 month time period. However, the Offeror should justify the number of participants and the study duration proposed in the sample protocols.}

- IV. **Conduct rigorously designed Phase I and Phase II clinical trials to determine vaccine safety, immunogenicity, reactogenicity, optimal dose and schedule; where appropriate, conduct challenge studies in human volunteers immunized with candidate vaccines for gonorrhea or chancroid. Both vaccines and controls shall be challenged with virulent pathogens to assess protection. ALL STUDIES ARE SUBJECT TO PROJECT OFFICER APPROVAL.**

{NOTE TO OFFEROR:

8. Two sample, detailed, protocols and consent forms should be submitted with the proposal and should demonstrate a thorough understanding of clinical evaluation of the interventions described.

- i. *One Phase I protocol should address the safety of a vaccine for human papillomavirus infection designed to ameliorate disease by inducing regression of genital warts. This protocol need not involve the use of a currently available or "under-development" vaccine. The offeror should describe the "theoretical vaccine" in sufficient detail so as to enable determination of the protocol's appropriateness. If the protocol submitted has been conducted previously it must be so identified. The protocol should document how the Offeror can best address the intent of this RFP.*
- ii. *One Phase II protocol should describe a study to assess the safety/effectiveness of a prophylactic vaccine for gonorrhea or chancroid, using the human challenge model of experimental urethritis or skin infection, respectively. This protocol need not involve the use of a currently available or "under-development" vaccine. The offeror should describe the "theoretical vaccine" in sufficient detail so as to enable determination of the protocol's appropriateness. If the protocol submitted has been conducted previously it must be so identified. The protocol should document how the Offeror can best address the intent of this RFP.*

9. It is anticipated that a representative Phase I study would involve 12-20 patients for a two week time period or a 2-4 month time period depending upon the nature of the intervention being tested; a representative Phase II study would involve 100 patients for a 2-6 month time period. A representative human challenge experiment would involve 25-50 people for a two week period. However, the Offeror should justify the number of participants and the study duration proposed in the sample protocols.}

- V. Collect, appropriately store and conduct assays on sera and other clinical specimens to determine volunteer eligibility and from study participants, as required in the protocol.

{Note to Offeror:

10. The Offeror should include a complete description of the clinical and laboratory facilities and capabilities required to run assays, in order to document the 1) appropriateness of the population; 2) the safety of the intervention; and 3) the effect of the intervention. A plan for long term follow-up (up to, but not to exceed, monthly evaluations for one year) should be included. Should non-routine or "special" assays be necessary, testing may be arranged by the government, and the offeror will be asked to ship specimens to the appropriate site for assay.}

- VI. Organize and administer the clinical and associated laboratory research activities of the contract:

- a. Receive and process concepts for proposed studies.
- b. Develop and implement a protocol review process that ensures scientific needs are met and that individual protocols are prioritized across the spectrum of existing protocols.
- c. Write protocols and case report forms for review and approval by the NIAID project officer. The NIAID project officer will review and approve all protocols and case report forms.
- d. Track the current status of concepts, protocols, active trials, abstracts and publications.
- e. Prepare clinical trials agreement which includes a delineation of responsibilities of industry, the STD clinical trials unit and the NIAID in the implementation of each trial. The list of responsibilities should be submitted for review to the Project Officer prior to the initiation of each trial.

{NOTE TO OFFEROR:

11. With respect to VI.e., the list of responsibilities is to be included, regardless of who has primary responsibility. A sample list is given in Attachment 3.}

- VII. Coordinate submission of protocols, consent forms (including parental assent forms, where applicable), site registration information, local Institutional Review Board (IRB) approval documents, and other Investigative New Drug (IND) or New Drug Application (NDA) information to the Clinical and Regulatory Affairs Branch, Division of Microbiology and Infectious Diseases, NIAID, for those protocols in which the NIAID holds the IND. Ensure that documents are revised and finalized as necessary after review by the Project Officer, the NIAID Clinical and Regulatory Affairs Branch, the local IRB and the regulatory authorities.

- a. Serve as coordinator for the conduct of clinical trials in the case of multi-site trials.

- b. Receive and ship reagents and samples, code patients, reagents, and samples, and coordinate all of the subcontractors' activities (if applicable).
- c. Develop and follow a detailed plan for on-site monitoring; offeror shall monitor according to the plan to ensure completeness and accuracy of study data and adherence with established research standards and protocol requirements and regulatory guidelines.

VIII. Develop policies and standard operating procedures for the conduct of the clinical trials, site evaluation criteria, publication criteria, and manuals of operation. All policies and standard operating procedures are subject to approval of project officer.

IX. Attend and participate (the Principle Investigator shall) in the semi-annual meeting of the Directors of the STD Cooperative Research Centers (STD CRCs) in Bethesda, MD to discuss ongoing protocols and future clinical trials agenda with the STD CRC Directors.

{NOTE TO OFFEROR:

12. The Contractor should budget for travel to the meetings.

13. The STD CRC Directors will act as consultants to the project officer and the Principle Investigator of the STD CTU. Additional consultants and advisory activities may be sought and arranged on an as needed basis, by the project officer.}

X. Organize and conduct an annual, 1-2 day investigators meeting on site. It is possible that the project officer would request the meeting be held in Bethesda, MD, in that case, the Offeror no more than six STD CTU staff shall attend, including the Principle Investigator and the Nurse Coordinator. This meeting shall include presentation and review of data, progress updates on all studies, discussion of proposed protocols and establishment of the group's standard operating procedures and research priorities to update the proposed scientific agenda for subsequent submission to the Project Officer. If held on site, all of the doctoral level staff and the Nurse Coordinator shall attend this meeting. It is estimated that three to six NIH staff would attend on site meetings.

{NOTE TO OFFEROR:

14. The Contractor should budget for the cost of the meetings.}

XI. Collect, store, manage and analyze data from the conduct of the clinical trials. Prepare and publish all manuscripts and presentations involving data from the clinical trials, including those sponsored by pharmaceutical trials. The project officer shall have access to all data generated with the support of this contract and shall review and approve all resulting manuscripts and abstracts before publication.

{NOTE TO OFFEROR:

15. *The current guidelines for publications resulting from work sponsored by this contract are provided in Attachment 4.*

{GENERAL NOTES TO THE OFFEROR:

16. In general, direct patient costs will not be reimbursed as allowable costs under this contract, except for the cost to house participants in the human challenge studies. Exception for other direct patient costs, on a case by case basis, may be made by the project officer. The Offeror should present a plan to indicate how the patient costs associated with the submitted protocols can be covered. Typically this has involved industrial support or co-sponsorship by a General Clinical Research Center.

17. Award of the contract does not commit the government to approve the studies outlined or the proposed protocols. The project officer will determine which studies are actually undertaken.

18. For the purposes of planning and budgeting, assume that a total of ten to fifteen clinical trials may be initiated over the life of the contract with approximately 50% being Phase I and 50% being Phase II.

19. A detailed organizational chart should be provided that shows the administrative structure, reporting structure, supervisory roles, the interactions between the groups and projects, etc.

20. The proposal length should be approximately 50 pages, excluding the protocols.}

Reporting Requirements

The Contractor shall submit to the Contracting Officer and to the Project Officer technical progress reports covering the work accomplished during each reporting period; in addition to the number of hard copies requested in each case, an electronic copy shall be submitted on diskette or by electronic mail (Email). These reports are subject to technical inspection and requests for clarification by the Project Officer. These shall be brief and factual and prepared in accordance with the following format:

A. Technical Reports

In addition to those reports required by SECTION I and other terms of this contract, the Contractor shall prepare and submit the following reports in the manner stated below:

(1) Quarterly Technical Progress Reports - by the fifteenth working day of the month following the end of each three month period, the Contractor shall submit four (4) copies of the quarterly Technical Progress Report, comprising three (3) copies to the Project Officer and one (1) copy to the Contracting Officer. Such reports shall include the following specific information:

- a. A cover page that lists the contract number and title, the period of performance being reported, the Contractor's name and address, the author(s), and the date of submission;
- b. SECTION I - An introduction covering the purpose and scope of the contract effort;
- c. SECTION II - A description of overall progress plus a separate description for each task or other logical segment of work on which effort was expended during the report period. The description shall include pertinent data and/or graphs in sufficient detail to explain any significant results achieved and preliminary conclusions resulting from analysis and scientific evaluation of data accumulated to date under the project;
- d. SECTION III - Substantive performance; a description of current technical or substantive performance and any problems encountered and/or which may exist along with proposed corrective action. An explanation of any difference between planned progress and actual progress, why the differences have occurred and if behind planned progress what corrective steps are planned;
- e. An anticipated work plan for the next three months; and
- f. Pre-prints and reprints shall be submitted along with the report.

Quarterly Technical Progress Reports are not due for periods in which an annual or final report is due.

(2) Annual Reports - On the anniversary date of the contract, the Contractor shall submit four (4) copies of an annual Technical Progress Report, as above, comprising three (3) copies to the Project Officer and one (1) copy to the Contracting Officer. Such reports shall detail, document, and summarize the results of the entire contract work for the period covered and shall include information regarding numbers of women and minority subjects enrolled in trials. These reports shall be in sufficient detail to explain comprehensively the results achieved. Also to be included in the report is a summary of work proposed for the next reporting period. An annual report will not be required for the period when the final report is due. Pre-prints and reprints not submitted in the semi-annual report shall be submitted. The annual report will also append all relevant manufacturing documents available for the project officer's confidential review, these include, but are not limited to: quality control (QC) documentation; formal accelerated and real-time stability protocols; component specifications; manufacturing procedures.

(3) Final Report - By the expiration date of the contract, the Contractor shall submit ten (10) copies of a comprehensive Final Report, as above, comprising nine (9) copies to the Project Officer and one (1) copy to the Contracting Officer. These final reports shall detail, document and summarize the results of the entire contract period of performance. These reports shall be in sufficient detail to explain comprehensively the results achieved. Pre-prints and reprints not included previously shall be submitted.

(4) Summary of Salient Results - With the annual and final reports the Contractor shall submit a summary (not to exceed 200 words) of salient results achieved during performance of the contract.

(5) Other Reports - To facilitate compliance with regulatory requirements, the Contractor shall submit to the Project Officer (10) copies of a brief summary of each ongoing and completed protocol one year and thirty days after FDA approvals go into effect.

- B. If the Contractor becomes unable to deliver the reports specified hereunder within the period of performance because of unforeseen difficulties, notwithstanding the exercise of good faith and diligent efforts in performance of the work, the Contractor shall give the Contracting Officer immediate written notice of any anticipated delays with reasons.

Concept Review and Approval

TITLE: Sexually Transmitted Diseases Clinical Study Group (STD CSG)

BRANCH: Sexually Transmitted Diseases Branch, DMID

MECHANISM: RFP

DURATION: 5 Years

PROJECT DESCRIPTION: The STD Clinical Study Group will provide the expertise and environment for conducting clinical studies in STD patient populations, including, but not limited to, evaluation of: 1) diagnostic tests for silent Pelvic Inflammatory Disease (PID); 2) therapeutic interventions for PID that prevent tubal scarring; 3) immunotherapies to prevent PID; and 4) other tools and interventions such as topical microbicides, diagnostic tests, treatment regimens, behavioral interventions, and vaccines aimed at the prevention and control of STDs.

BACKGROUND: Significant progress has been made towards the development of tools and interventions to prevent STDs and long term sequelae, including vaccines, diagnostic tests, topical microbicides, and behavioral interventions. However, advances such as these require infrastructure for clinical studies in STD populations in order to evaluate and ultimately implement these prevention tools successfully. Importantly, the development of diagnostic procedures for complex clinical problems such as PID requires access to at-risk patients and an evaluation capability. And finally, establishment of clinical infrastructure to conduct human challenge studies for STDs (under the contract mechanism) would facilitate both vaccine and microbicide development and evaluation efforts.

The STD Clinical Study Group would provide the clinical component to establish the relevance of laboratory and preclinical findings in humans. Such clinical studies are time-consuming and expensive, and require dedicated clinical units staffed by experts in STDs. The need for a dedicated STD Clinical Study Group also reflects the special requirements for populations with high and low prevalence of infection and disease, for characterization of these populations with respect to pre-existing infections, co-infections and incident infections, and for measurement of risk-associated behaviors during the course of clinical studies which could confound results of the clinical studies.

OBJECTIVE: To provide dedicated clinical facilities and laboratories staffed by multidisciplinary teams of STD experts in clinical, behavioral, and basic biomedical research to conduct clinical studies in STDs, with special emphasis on PID.

RELATIONSHIP TO ONGOING PROGRAMS: This initiative would interface with and complement virtually all ongoing or anticipated programs in the STD Branch, including the STD Cooperative Research Centers, the Research on Molecular Immunology of STDs P01s, the Topical Microbicides P01s and the STD Diagnostic Development Cooperative Agreements. In addition, the STD Clinical Study Group would provide opportunities for collaboration with the Division's VTEUs on Phase I/II studies of STD vaccines and the Collaborative Antiviral Study Group.

University of North Carolina at Chapel Hill

Contract #: N01-AI-75329

PI: **Myron S. Cohen, M.D.**

Abstract

The overall objective of this Sexually Transmitted Diseases Clinical Trial Unit (STD CTU) contract is to conduct clinical trials to test safety and efficacy of biomedical and behavioral interventions aimed at prevention and control of sexually transmitted diseases (STDs). Diseases or pathogens which may be studied under this contract include, but are not limited to: chlamydial infection; gonorrhea; syphilis; chancroid; human papillomavirus (HPV) infection; genital herpes (herpes simplex virus 1 & 2 infections), human herpes virus 8 (Kaposi's sarcoma virus) and human immunodeficiency virus (HIV) infection as it is associated with other STDs (both in terms of increased transmission rates of HIV and alteration of the natural history of STDs). The syndromes to be studied under this contract may include, but are not limited to: pelvic inflammatory disease (and sequelae - infertility, ectopic pregnancy and chronic pelvic pain); adverse outcomes of pregnancy; and cervical cancer as well as other genital cancers associated with HPV infection. The prevention and control strategies to be studied may encompass primary, secondary and tertiary prevention. The interventions to be studied may include, but are not limited to: topical microbicides, vaccines, diagnostics tests, screening tests, therapeutics and behavior change.

STD Cooperative Research Centers (STD CRCs)

- RFA
- List of STD CRCs and Directors

The Sexually Transmitted Diseases Cooperative Research Centers (STD CRCs)

The Sexually Transmitted Diseases Cooperative Research Centers (STD CRCs) are cooperative agreements designed to provide multidisciplinary approaches to STD research. The STD CRCs bridge basic biomedical, clinical, behavioral and epidemiological research, promote productive collaborations among academic researchers and facilitate the development of intervention-oriented research.

Seven STD CRCs are currently funded; these include Boston University, Johns Hopkins University, Indiana University, University of Alabama at Birmingham, University of North Carolina, University of Pittsburgh/McGee's Women's Hospital, and University of Washington. More than thirty research projects are included in the CRCs; the studies conducted are directly linked to the five strategic aims. In collaboration with the Centers for Disease Control and Prevention (CDC), NIAID established a fellowship program in the CRCs designed to strengthen the link between the public health departments and the universities; we hope to renew this program in the currently funded CRCs. In summer of 1996 NIAID launched a summer research program for Howard University Medical Students within the STD CRCs.

SEXUALLY TRANSMITTED DISEASES COOPERATIVE RESEARCH CENTERS (STD CRCs)

NIH GUIDE,

RFA: AI-94-16

P.T.

National Institute of Allergy and Infectious Diseases

Letter of Intent Receipt Date: August 5, 1994

Application Receipt Date: November 15, 1994

PURPOSE

The purpose of this RFA is to stimulate multidisciplinary, collaborative research to further understanding of sexually transmitted diseases (STDs) and effective approaches to their prevention and control. The Sexually Transmitted Diseases Branch of the Division of Microbiology and Infectious Diseases (DMID) of the National Institute of Allergy and Infectious Diseases (NIAID) invites grant applications for recompetition of the Sexually Transmitted Diseases Cooperative Research Centers (STD CRCs). The Institute recognizes that although each STD presents unique diagnostic, therapeutic, and prevention challenges, all STDs share a common mode of transmission, populations at risk for one STD are at risk for others, comorbidity is common, and the presence of one infection may influence the acquisition and natural history of another. Therefore, a research program that addresses these diseases as a group is likely to be highly productive. The CRCs (1) provide a multi-disciplinary approach to STD research by bridging biomedical, clinical, behavioral, and epidemiological research; (2) foster interaction among STD investigators; and (3) facilitate intervention-oriented research.

HEALTHY PEOPLE 2000

The Public Health Service (PHS) is committed to achieving the health promotion and disease prevention objectives of "Healthy People 2000," a PHS-led national activity for setting priority areas. This Request For Applications (RFA), *Sexually Transmitted Diseases Cooperative Research Centers (STD CRCs)*, is related to the priority areas of STDs and acquired immunodeficiency syndrome (AIDS). Potential applicants may obtain a copy of "Healthy People 2000" (Full Report: Stock No. 017-001-00474-0) or "Healthy People 2000" (Summary Report: Stock No. 017-001-00473-1) through the Superintendent of Documents, Government Printing Office, Washington, DC 20402-9325 (telephone 202-783-3238).

ELIGIBILITY REQUIREMENTS

Applications will be limited to domestic institutions but may include an international component. Applications may be submitted by domestic for-profit and non-profit research institutions; public and private organizations, such as universities, colleges, hospitals, laboratories, units of State or local governments; and eligible agencies of the Federal government. Applications from minority individuals and women are encouraged.

MECHANISM OF SUPPORT

The administrative and funding mechanism to be used to undertake this program will be the Cooperative Agreement (U19), an "assistance" mechanism, rather than an "acquisition" mechanism, in which substantial NIH scientific and/or programmatic involvement with the awardee is anticipated during performance of the activity. Under the cooperative agreement, the NIH purpose is to support

and/or stimulate the recipient's activity by involvement in and otherwise working jointly with the award recipient in a partner role, but it is not to assume direction, prime responsibility, or a dominant role in the activity. Details of the responsibilities, relationships, and governance of the study funded under cooperative agreement(s) are discussed later in this document under the section Terms and Conditions of Award.

The total project period for applications submitted in response to this RFA may not exceed 5 years. At present, the NIAID is administratively limiting the duration of U01/U19 cooperative agreements to four years; this administrative limitation may change in the future. At this time, the NIAID has not determined whether and how this solicitation will be continued beyond the present RFA.

The anticipated award date is July 1, 1995.

FUNDS AVAILABLE

The estimated total funds (direct and indirect costs) available for the first year of support for awards under this RFA will be \$6.2 million. In Fiscal Year 1995, the NIAID plans to fund five STD CRCs or more if additional money becomes available. The final number of awards to be made is dependent upon the availability of funds. The initial year's total costs, including direct and indirect costs, should not exceed \$1.2 million for each award. The usual PHS policies governing grants administration and management will apply. This level of support is dependent on the receipt of a sufficient number of applications of high scientific merit. Although this program is provided for in the financial plans of the NIAID, awards pursuant to this RFA are contingent upon the availability of funds for this purpose. Funding beyond the first and subsequent years of the grant will be contingent upon satisfactory progress during the preceding years and availability of funds.

RESEARCH OBJECTIVES

Background

In 1993, an estimated 12 million cases of STDs occurred in the United States. Approximately 65% occurred in people under 24 years of age; three million occurred in teenagers. Associated health care costs exceeded \$6 billion. Women and infants bear a disproportionate share of disease burden and sequelae of STDs, including infertility, ectopic pregnancy, cervical cancer, fetal wastage, low birth weight, and congenital/perinatal infection. Additionally, STDs disproportionately impact the health of some minority populations. Both the incidence of STDs and their long-term and potentially fatal sequelae are consistently higher among African and Hispanic Americans than among white Americans.

- o Chlamydial infection is the most prevalent bacterial STD in the U.S. and a major cause of pelvic inflammatory disease (PID); over four million cases are diagnosed annually. Due to delays in diagnosis or inadequate therapy, 10 to 40 percent of women with chlamydial cervicitis develop PID.
- o Gonorrhea, the other major cause of PID, occurs at an annual rate of approximately 1.4 million new cases, 25 percent of which occur in teenagers. Antibiotic resistance has increased within the last decade; up to 10 percent of isolates may be resistant to one or more antibiotics.
- o In the U.S., as many as 40 million people are believed to be infected with human papillomavirus (HPV). High risk HPV types are causally associated with cervical cancer. Globally, cervical cancer is the most common cause of cancer-related death in women.

- o In the 1990's, infectious syphilis reached the highest level in 40 years. Syphilis rates among African-Americans are reported to be 56 times higher than in whites. Increases in women were echoed by five-fold increases in congenital/perinatal cases.
- o Approximately 65 million Americans are afflicted with genital herpes (HSV). HSV is a painful, recurrent, incurable disease with annual costs estimated to exceed \$96 million.
- o Both ulcerative and non-ulcerative STDs increase the risk of human immunodeficiency virus (HIV) transmission approximately 3-5 fold, independent of the effect of sexual behavior; and HIV infection, which decreases immune function, may alter the natural history and increase the prevalence of some STDs.

Objectives and Scope

In spite of decades of STD prevention and control programs and STD research, rates of infection continue to grow. This is now complicated by the emergence of a new fatal STD, HIV infection. The alarming dimensions of STDs and related health problems described above point to the need for more effective research approaches that will lead to essential tools for preventing and controlling STDs. The 1986 report of the NIAID Study Group on STDs and Congress have recommended a multidisciplinary approach to research programs seeking to prevent and control STDs. By contrast, STD research historically has not been conducted by multidisciplinary, collaborative teams of scientists. Each scientific area tended to build its own empirical base and report findings to peers. In response to both the dimension of the problem and advice provided to the Institute, the STD CRCs were created to achieve the following goals: integrate clinical observations into basic biomedical research; apply the findings of basic research to the development of improved diagnostics, therapeutics, vaccines and topical microbicides; and integrate behavioral and epidemiologic research needed to ensure the optimal utilization of these tools.

The purpose of this RFA is to stimulate multidisciplinary, collaborative research to further understanding of STDs and effective approaches to their prevention and control. The scientific complexity of the STD problems is such that coordinated, multidisciplinary research is required to solve these problems. This will be accomplished through collaborations among scientists from various disciplines working in the scientific areas of biomedical, clinical, behavioral, and epidemiological research. In this RFA, the term scientific area refers to the four broad categories of investigation: (1) biomedical, (2) clinical, (3) behavioral, and (4) epidemiologic research. The term discipline refers to investigators' specialized areas of expertise or training. Some examples of disciplines associated with the four scientific areas include but are not limited to (1) immunology, virology, and molecular biology; (2) gynecology, infectious diseases, and adolescent medicine; (3) psychology, sociology, and anthropology; (4) epidemiology, biostatistics, and computer modeling.

In order to attain these goals, applications should include:

- o at least three projects, one of which should be in behavioral or epidemiologic research (behavioral/epidemiologic research must include both behavioral or epidemiologic outcomes and microbiologic/disease outcomes as described in the next section);
- o two projects that link disciplines within a single scientific area and at least one project that links disciplines in two different areas (disciplines and scientific areas are defined above);
- o research on two or more STD pathogens or syndromes selected from Diseases, Syndromes, and Areas of Interest listed below;

- o a strong clinical capability (described below) with accessible patient populations to participate the clinical and behavioral/epidemiologic research projects; and
- o provisions for the Principal Investigator of each CRC (also known as the CRC Director) to attend meetings with NIAID staff twice each year and for all CRC Project Leaders to attend CRC workshops twice during the program period.

Diseases, Syndromes, and Areas of Interest

In designing specific projects, applicants are encouraged to consider research proposals in the following areas of programmatic interest. Biomedical research projects are encouraged to use human material to address research questions.

- o Pathogens: *C. trachomatis*, *N. gonorrhoeae*, *H. ducreyi*, *T. pallidum*, *T. vaginalis*, herpes simplex virus 1 and 2, and human papillomavirus.
- o Adverse Outcomes of Pregnancy: Research is needed to define the epidemiology, pathogenesis, and immunology of STD-related adverse outcomes of pregnancy.
- o Human Papillomavirus Infection: The NIAID's priorities in HPV include research on the epidemiology, natural history, pathogenesis, and immunology of infection as well as the development of a culture system and improved diagnosis and treatment.
- o Pelvic Inflammatory Disease (PID): Additional research is needed in a number of areas, including diagnosis, epidemiology, pathogenesis, treatment, and long term sequelae.
- o Inter-Relatedness of Sexually Transmitted Infections: Because of the HIV epidemic, it is now recognized that infection with one sexually transmitted infection can alter susceptibility to and natural history of other STDs. Understanding the molecular bases of these interactions is needed. For example, research is needed to examine the role of classical STDs in the acquisition and progression of HIV infection and on the role of HIV in alterations of the natural history, diagnosis, or response to treatment of STDs. If this area is selected, the application should include only one project on STDs and HIV infection.
- o Immunology: Basic immunological research related to vaccine development for any of the pathogens or syndromes listed above is of programmatic interest. In order to make critical advances in this area, functional collaborations between immunologists and microbiologists focused on pathogenesis will be extremely important.
- o Behavioral/Epidemiologic Research: Much STD research to date has focused on diagnosis, treatment, and vaccine development without integrating approaches to modify the sexual and other behaviors associated with acquisition and transmission STDs or development of their sequelae. Behavioral research is needed to decrease risk-associated behaviors and to increase health behaviors, specifically those related to seeking early diagnosis, treatment, and immunization. STD CRCs, because of their multi-disciplinary approach, are in a unique position to assess behavioral measures and evaluate interventions because, as stated earlier, behavioral and epidemiologic research projects in the STD CRCs must include both behavioral and microbiologic and/or disease outcomes. (For more information on areas of programmatic interest, see Program Announcement Number PA-93-108, NIH Guide, Volume 22, Number 32, September 3, 1993.)
- o Topical Microbicides: Basic biomedical and clinical research leading to topical microbicide development to prevent sexually transmitted infections

is needed. Topical microbicides are products for intravaginal use that are microbicidal (virucidal and/or bactericidal) but not necessarily spermicidal; they are used by women to prevent sexually transmitted infections (HIV and other STDs). Examples of such research include, but are not limited to, identifying early events in the infectious process, characterizing vaginal physiology and normal flora, developing methods to measure and assess clinical significance of vaginal/cervical inflammation. (See also RFA AI-94-13.)

Clinical Capability

As stated earlier, applications must have a strong clinical capability and access to patient populations to serve the clinical and behavioral/epidemiologic research projects. In describing the clinical and laboratory facilities, the application should include specific information on the institution's present patient load, projections for patient involvement in future clinical investigations, history of recruitment of subjects, and disease prevalence as well as on the availability of appropriate biohazard facilities and safety procedures.

Optional Developmental Component

Applications may include a Developmental Fund Core to provide support for new investigators or pilot projects. Eligible investigators are individuals in the early or mid stages of their career who have NOT held an NIH grant including an R29, RO1, PO1, UO1, any research career or training grant (K or T awards), or any other type of grant or contract with annual direct costs in excess of \$37,500 for research in STDs including HIV.

Potential awardees and specific research projects to be pursued need not be identified in the CRC application. However, the application should include a one page description of the kind of project that might be funded under this mechanism and how it interdigitates with CRC research projects. Approval of the developmental funds portion of the application does not in any way commit the investigators to the execution of the sample project. In addition, the application must provide a description of review process and selection criteria for proposed projects.

Budget Issues

Budget requests within each project may include research-related costs for supplies, patient involvement and medical care, funds for limited investigator travel, and costs of publication. Proposals for studies that do not receive the majority of funding through the CRC will not be counted as a project. There must be concordance between the science proposed and the budget requested. Furthermore, if additional sources of funding have been identified for a project, then letters documenting a funding commitment must be included in the application.

STD CRC award funds may be utilized to support the following research-related activities:

- o PI Level of Effort: The applicant Principal Investigators must allot 15% of time to the administration of the CRC. If less effort is indicated, justification must be provided, including a written plan explaining how they will be able to successfully and fully meet the responsibilities demanded by these endeavors.
- o Shared Research Resources (Cores): The STD CRCs may include funds for equipment, supplies, and services to expand and/or maintain clinical, laboratory, biostatistical, or behavioral facilities shared by research staff from at least two research projects.
- o Developmental Funds Core for New Investigators: This optional core sets aside and restricts funds solely to cover salaries and research costs for new investigator or pilot projects. There is no ceiling on the total dollar amount of the developmental funds pool, but once identified as developmental funds these monies constitute a restricted portion of the total CRC budget and will not be available for other CRC activities.

The annual total amount for each developmental award may not exceed \$40,000 and may be used for salary, technical support, laboratory supplies, and equipment. Supplies and equipment expenditures for each award may not exceed \$20,000 annually. Projects and investigators funded under the developmental core may not receive subsequent awards from this pool. The duration of support is limited to three years. If the investigator achieves independent funding through a traditional research grant (R01) or a FIRST (R29) award prior to the end of the developmental award, the award must be terminated, and unexpended funds must be returned to the developmental funds pool.

In general, the CRCs should try to advance learning experiences in STD research and to make medical students, house staff and postdoctoral candidates more aware of STD research opportunities in the clinical, biomedical, and behavioral sciences. It is permissible for projects to include post-residency personnel who spend a maximum of one third of their time in clinical activities related to the research focus of the project. Formal training programs in clinical or basic sciences that include stipends for these students are supported by other funding mechanisms at the NIH, such as the National Research Service Awards (T-32s) and do not, therefore, fall within the purview of this request.

SPECIAL REQUIREMENTS

Collaborative Organization

The application should include a plan to maintain close collaboration and communication among members of the STD CRC and an organizational chart showing the name, the organization and the scientific discipline of the Principal Investigator, the Project Leaders, and the key personnel for the projects and cores. The application must also include a signed letter of agreement from each collaborator and/or consultant to the program indicating willingness to participate in the program and a description of the exact nature of the participation.

Terms and Condition of Award

The following terms and conditions will be incorporated into the award statement and provided to the Principal Investigator as well as the institutional official at the time of award.

These special Terms of Award are in addition to, and not in lieu of, otherwise applicable OMB administrative guidelines, HHS Grant Administration Regulations at 45 CFR Parts 74 and 92, and other HHS, PHS, and NIH Grant Administration policy statements.

The administrative and funding instrument used for this program is the cooperative agreement (U19), an "assistance" mechanism (rather than an "acquisition" mechanism), in which substantial NIH scientific and/or programmatic involvement with the awardee is anticipated during the performance of the activity. Under the cooperative agreement, the NIH purpose is to support and/or stimulate the recipient's activity by involvement in and otherwise working jointly with the award recipient in a partner role, but it is not to assume direction, prime responsibility, or a dominant role in the activity.

Awards will be made to an institution on behalf of a Principal Investigator who will be responsible for the coordination of STD CRC scientific and administrative activities. Support of all CRC activities will be coordinated through a Central Operations Office located within the applicant organization.

1. Awardee Rights and Responsibilities

Awardees will have primary responsibility for defining the details for the project within the guidelines of the RFA and for performing the scientific activity, and agree to accept close coordination, cooperation, and participation of NIAID staff in all aspects of scientific and technical management of the project. Specifically, awardees have primary responsibilities as described below.

Under the Cooperative Agreement, a partnership relationship exists between the recipient of the award and NIAID in which successful applicants are responsive to the guidelines and conditions set forth in the RFA. At the same time, investigators are expected to define research objectives and approaches in accord with their own interests and perceptions of novel and exploitable approaches to the research which ultimately is likely to result in improved prevention and control of STDs.

It is the primary responsibility of the Principal Investigator to clearly state the objectives and approaches of the research, to plan and conduct the research stipulated in the proposal, and to ensure that the results obtained are analyzed and published in a timely manner. NIAID may periodically review and generate internal reports from data and progress reports developed under this cooperative agreement. The data obtained will, however, be the property of the awardee.

The multi-disciplinary and collaborative nature of the STD CRCs creates an extraordinary opportunity for information exchange and scientific advancement in STD research. Principal Investigators are expected to take advantage of this opportunity by participation in both formal events established expressly for this purpose and informal investigator-initiated dialogues.

2. NIAID Staff Responsibilities

The NIAID will have substantial scientific/programmatic involvement during the conduct of this activity, through technical assistance, advice and coordination above and beyond normal program stewardship for grants, as described below.

The NIAID will work closely with the Principal Investigators and shall be represented by a Scientific Coordinator (Program Officer). The Scientific Coordinator will be a program officer in the STD Branch of NIAID. During the award period, the NIAID Scientific Coordinator may provide appropriate assistance, advice, and guidance in: design of research activities; coordination and facilitation of information, technology, and reagent exchange between STD CRCs; data collection and analysis; assistance in review and selection of developmental fund applicants; and technical and administrative activities of CRCs. However, it is again emphasized that the role of NIAID will be to facilitate and not to direct the activities of the STD CRC. It is anticipated that decisions in all activities outlined within this RFA will be reached by consensus of the investigators and that the NIAID Scientific Coordinator will be given the opportunity to offer input to this process.

3. Collaborative Responsibilities

The CRC Principal Investigators and NIAID Scientific Coordinator will meet twice a year to review progress of the CRCs at the NIH in Bethesda, Maryland (or at a site designated by NIAID). The first such meeting will be a Post Award Meeting. In addition, two workshops for the Principal Investigators and CRC Project Leaders will be convened during the project period to share STD research advances, and to discuss STD research needs and opportunities, to develop collaborations. It is likely that workshops will be convened in Year 1 and Year 3 of the project period at the NIH in Bethesda, Maryland (or at a site designated by NIAID). Applicants should be aware that there are no additional travel monies available. Funds for travel to all meetings must be included in applicant's budget.

A critical element of the STD CRCs' success is the degree of communication among its members. Therefore, additional informal meetings among participants from different CRCs as well as regular telephone and written communication will be encouraged.

4. Arbitration

Any disagreement that may arise on scientific/programmatic matters (within the scope of the award) between award recipients and the NIAID may be brought to arbitration. An arbitration panel will be composed of three members -- one selected by the Principal Investigator, a second member selected by the NIAID, and the third member selected by the two prior members. This special arbitration procedure in no way affects the awardee's right to appeal an adverse action that is otherwise appealable in accordance with PHS regulations at 42 CFR part 50, subpart D, and HHS regulation at 45 CFR part 16.

In the event that research supported by the Cooperative Agreement results in development of a therapeutic or other medical intervention, NIAID will retain the option to cross-file or independently file an application for investigational clinical trial (i.e., an Investigational New Drug Application [IND]) to the United States Food and Drug Administration. Reports of data generated by the CRC or any of its members which are required for inclusion in INDs and Clinical Brochures and for cross-filing purposes will be submitted by the Principal Investigator to the Scientific Coordinator upon request. Such reports will be in final draft form and include background information, methods, results, and conclusion. They will be subject to approval and revision by NIAID and may be

augmented with test results from other Government sponsored projects prior to submission to the appropriate regulatory agency.

STUDY POPULATIONS

A strong emphasis is placed on studying STDs in populations that are disproportionately affected. These populations include women, adolescents, and minorities. Subjects may be recruited or specimens obtained from domestic sites or through collaborations with foreign institutions in developing countries if the collaboration is beneficial to the foreign country and offers the potential for collection of STD data that are pertinent to U.S. populations and could not be generated as effectively in the United States.

SPECIAL INSTRUCTIONS TO APPLICANTS REGARDING IMPLEMENTATION OF NIH POLICIES CONCERNING INCLUSION OF WOMEN AND MINORITIES IN CLINICAL RESEARCH STUDY POPULATIONS

It is the policy of the NIH that women and members of minority groups and their subpopulations must be included in all NIH supported biomedical and behavioral research projects involving human subjects, unless a clear and compelling rationale and justification is provided that inclusion is inappropriate with respect to the health of the subjects or the purpose of the research. This new policy results from the NIH Revitalization Act of 1993 (Section 492B of Public Law 103-43) and supersedes and strengthens the previous policies (Concerning the Inclusion of Women in Study Populations, and Concerning the Inclusion of Minorities in Study Populations) which have been in effect since 1990. The new policy contains some new provisions that are substantially different from the 1990 policies.

All investigators proposing research involving human subjects should read the "NIH Guidelines For Inclusion of Women and Minorities as Subjects in Clinical Research", which have been published in the Federal Register of March 9, 1994 (FR 59 11146-11151), and reprinted in the NIH GUIDE FOR GRANTS AND CONTRACTS of March 18, 1994, Volume 23, Number 11. Investigators may obtain copies from these sources or from the program staff or contact person listed in the NIH Guide. Program staff may also provide additional relevant information concerning the policy.

(NOTE: When the proposed study or studies in the RFA or PA involves a gender specific study or a single or limited number of minority population groups, this should also be stated to inform potential applicants and reviewers.)

For the purpose of this policy, clinical research is defined as human biomedical and behavioral studies of etiology, epidemiology, prevention (and preventive strategies), diagnosis, or treatment of diseases, disorders or conditions, including but not limited to, clinical trials.

If the required information is not contained within the application, the application will be returned.

Peer reviewers will address specifically whether the research plan in the application conforms to these policies. If the representation of women or minorities in a study design is inadequate to answer the scientific question(s) addressed AND the justification for the selected study population is inadequate, it will be considered a scientific weakness or deficiency in the study design and will be reflected in assigning the priority score to the application.

All applications for clinical research submitted to NIH are required to address these policies. NIH funding components will not award grants or cooperative agreements that do not comply with these policies.

NOTE: Peer review groups need adequate information about the composition of proposed study populations in all applications involving human subjects. To avoid delays in review of such applications, the NIAID therefore requires that, as a minimum, the application must contain demographic data about the clinic and/or in-patient population from which study subjects will be drawn: average hospital admissions per year; percentage distribution of black/hispanic/other minority/non-minority populations; gender; etc. Studies using non-hospital populations, such as community-based studies, should provide similar data about populations in the area or region from which the study subjects will be drawn. In the absence of current data, historical demographic information and/or previous recruitment data for similar studies from the proposed sites should be provided.

LETTER OF INTENT

Prospective applicants are asked to submit, by August 7, 1994, a letter of intent that includes a descriptive title of the overall proposed research; the name, address, and telephone number of the Principal Investigator; the number and title of this RFA; and a list of the key investigators and their institution(s). Although the letter of intent is not required, is not binding, does not commit the sender to submit an application, and does not enter into the review of subsequent applications, the information that it contains allows NIAID staff to estimate the potential review workload and to avoid conflict of interest in the review. The letter of intent is to be sent to Dr. Olivia Preble at the address listed below.

APPLICATION PROCEDURES

Before preparing an application, the applicant should carefully read the new information brochure accompanying each RFA, NIAID Program Project Grants and Multiproject Cooperative Agreements. Instructions for formatting the application as outlined in the brochure should be followed carefully. Failure to follow the instructions may result in unnecessary delays in the review process.

Applications are to be submitted on the standard research grant application form PHS 398 (rev. 9/91). For purposes of identification and processing, item 2 on the face page of the application must be marked "YES" and the RFA number and the words "SEXUALLY TRANSMITTED DISEASES COOPERATIVE RESEARCH CENTERS (STD CRCs)" must be typed in.

Applications from multi-component consortia must contain a single face page and additional face pages for each institution involved, an overall budget page, and separate budget pages for each institution involved. Each consortium institution is allowed 25 pages for the research plan if different plans are proposed by the different member institutions. For additional information, refer to page 8 of the PHS 398 application form.

These application forms may be obtained from the institution's office of sponsored research or its equivalent and from the Office of Grants Information, Division of Research Grants, National Institutes of Health, 5333 Westbard Avenue, Room 449, Bethesda, Maryland, 20892, telephone (301) 594-7248.

The RFA label available in the PHS 398 (rev. 9/91) application form must be affixed to the bottom of the face page of the application. Failure to use this label could result in delayed processing of the application such that it may not reach the review committee in time for review. In addition, the RFA title and number must be typed on line 2a of the face page of the application form and the YES box must be marked.

Submit a signed, typewritten original of the application, including the checklist, and three (3) signed, exact, single-sided photocopies, in one package, to:

DIVISION OF RESEARCH GRANTS
National Institutes of Health
Westwood Building - Room 240
5333 Westbard Avenue
Bethesda, Maryland 20892

PLEASE NOTE: Applicants who wish to use express mail or courier service should change the zip code to 20816.

At the time of submission, two additional exact copies of the grant application and five sets of appendix material must also be sent to:

Dr. Olivia Preble
Division of Extramural Activities
National Institute of Allergy and Infectious Diseases
National Institutes of Health
9000 Rockville Pike, Solar Building, Room 4C-20
Bethesda, Maryland 20892

PLEASE NOTE: If sending the application by express mail or courier service to Dr. Preble, use the following address: 6003 Executive Blvd., Room 4C-20, Rockville, Maryland, 20852.

Applications must be received by the official receipt date as indicated in the RFA heading. All components, subparts and sections of the application must be collated into the application, and the packages sent to the DRG and to the NIAID must each be complete in themselves. Applications which do not conform to the instructions contained in PHS 398 (rev. 9/91) application kit will be judged nonresponsive and will be returned to the applicant.

Current NIH policy permits a component research project of a multiproject grant application to be concurrently submitted as a traditional individual research project (R01) application. If, following review, both the multiproject application and the R01 application are found to be in the fundable range, the investigator must relinquish the R01 and will not have the option to withdraw from the multiproject grant. This is an NIH policy intended to preserve the scientific integrity of a multiproject grant, which may be seriously compromised if a strong component project(s) is removed from the program. Investigators wishing to participate in a multiproject grant must be aware of this policy before making a commitment to the Principal Investigator and awarding institution.

THE FOUR COPIES OF THE APPLICATIONS WHICH ARE SENT TO DRG MUST BE RECEIVED AS A SINGLE PACKAGE FROM THE PRINCIPAL INVESTIGATOR AND CONFORM TO THE INSTRUCTIONS CONTAINED IN PHS 398 (rev. 9/91) APPLICATION KIT OTHERWISE THE APPLICATION WILL BE JUDGED NON-RESPONSIVE AND WILL BE RETURNED TO THE APPLICANT.

REVIEW CONSIDERATIONS

Review Method

Upon receipt, applications will be reviewed for completeness by the Division of Research Grants (DRG) for completeness and for responsiveness by NIAID staff. Incomplete and non-responsive applications will be returned to the applicant without further consideration or review.

Those applications that are complete and responsive may be subjected to a triage by a peer review group to determine their scientific merit relative to other applications received in response to this RFA. The NIAID will remove from competition those applications judged to be non-competitive for award and will notify the Principal Investigators and institutional business officials. For applications found non-competitive, summary statements will be very brief and will generally contain a one paragraph resume, based on reviewers' comments, indicating the major reason(s) for the findings.

Those applications judged by the reviewers to be competitive for award will be further reviewed for scientific and technical merit by a review committee convened by the Division of Extramural Activities, NIAID. The second level of review will be provided by the National Advisory Allergy and Infectious Diseases Council.

REVIEW CRITERIA

The review criteria are stated in Draft NIAID Program Project Grants and Multiproject Cooperative Agreements which accompanies each RFA. In addition, applicants are expected to address research priorities, objectives, and other requirements stated in this RFA as well as the following:

- o the scientific and technical significance, merit, and originality of the research projects and anticipated contributions to the prevention and control of STDs;
- o the scientific expertise and experience of the Principal Investigator, the Project Leaders and key project and core personnel;
- o documentation of a strong clinical capability, adequate and appropriate patient populations, disease prevalence, and historical success of recruitment and retention of subjects;
- o documentation of the sponsoring institution's commitment to the cooperative program and willingness to accept the participation and assistance of NIAID staff;
- o adequacy of proposed plan for coordination and communication within the applicant STD CRC and with NIAID and other STD CRCs; and
- o adequacy of review plan and selection criteria for new investigators making application to the optional Developmental Funds.

In addition, applications from existing STD CRCs must include a comprehensive progress report (as stated above under Objectives and Scope) and demonstrate successful collaborative activities supported through their CRC. Applicants who have not had an STD CRC are should explain how they will establish successful collaborative efforts.

AWARD CRITERIA

Funding decisions will be made on the basis of scientific and technical merit as determined by peer review, program needs and balance, and (c) availability of funds. Program needs and balance take into account pathogen(s) proposed for study, the potential impact on health of women, minorities and adolescents, as well as geographic distribution of the CRCs.

INQUIRIES

Written and telephone inquiries concerning this RFA are encouraged. The opportunity to clarify any issues or questions from potential applicants is

welcome. Investigators seeking information about programmatic issues should contact:

Dr. Heather Miller
Sexually Transmitted Diseases Branch
DMID, NIAID, NIH
9000 Rockville Pike, Solar Building, Room 3A-26
Bethesda, Maryland 20892
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Direct inquiries regarding review issues, address the letter of intent to, and mail two copies of the application and all five sets of appendices to:

Dr. Olivia Preble
Division of Extramural Activities
National Institute of Allergy and Infectious Diseases
National Institutes of Health
9000 Rockville Pike, Solar Building, Room 4C-20
Bethesda, Maryland 20892
Telephone: 301/496-8208

If the package for NIAID will be sent via overnight, express, or courier service, use the actual street address and local zip code for the Solar Building: 6003 Executive Boulevard, Bethesda, MD 20852.

Direct inquiries regarding fiscal matters to:

Ms. Katherine Phillips
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9000 Rockville Pike, Solar Building, Room 4B-33
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Schedule

Letter of Intent Receipt Date:	August 5, 1994
Application Receipt date:	November 15, 1994
Scientific Review Date:	January, 1995
Advisory Council Date:	June, 1995
Earliest Award Date:	July 1, 1995

AUTHORITY AND REGULATIONS

This program is described in the Catalog of Federal Domestic Assistance No. 93.855 Immunology, Allergic and Immunological Diseases Research and 93.856 Microbiology and Infectious Diseases Research. Grants are awarded under the authority of the Public Health Service Act, Section 301 (42 USC 241) and administered under PHS grants policies and Federal Regulations, most specifically at 42 CFR Part 52 and 45 CFR Part 74. This program is not subject to the intergovernmental review requirements of the Executive Order 12372 or Health Systems Agency review.

ADDENDUM

STD CRCs can serve as important resources to increase awareness of the prevention, diagnosis, and treatment of STDs in the public health, the lay and the local medical communities. The NIAID has been involved with the Centers for Disease Control's (CDC) Accelerated Research Program in STDs. Several proposed projects for this CDC program involve collaborations between the existing STD CRCs and state and local health departments. Applicants with interest in this area should contact the Division of STDs/HIV, National Center for Prevention Services, CDC for information on support of outreach projects.

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Clinical and Behavioral Research

8

CLINICAL TRIALS OF EXISTING PRODUCTS

Clinical trials of over-the-counter spermicides and disinfectants to assess the safety and effectiveness for prevention of STDs and HIV infection are a top priority. Currently available products are in use (and in some cases are being recommended) as barrier methods to reduce the risk of STDs and HIV infection in the absence of definitive clinical evidence that supports claims of disease prevention.

- N-9 vaginal contraceptive film
- Advantage 24
 - Phase I
 - Phase II/III

Clinical and Behavioral Research

CLINICAL TRIALS OF EXISTING PRODUCTS

Title: N-9 Film Clinical Trial in Cameroon

Dates: 1993 - 1997

Principle Investigator: Ron Roddy

Institution: Family Health International

Mechanism: R01

- Abstract
- Protocol
- Results
- NIAID press release
- Overview: Results of study

R01 34714-02
Roddy, Ronald
Family Health International
HIV and Nonoxynol 9

The long-term goals of the study are to reduce the sexual transmission of HIV and to reduce the prevalence of sexually transmitted infections (STI). The specific aims are 1) to measure the effect of nonoxynol-9 (N-9) film use on the rate of HIV infection by comparing the rate of infection of women using condoms and N-9 film with that of women using condoms and placebo film, 2) to measure the effect of N-9 film on the rate of genital ulcers and symptoms of irritation by comparing the rate of genital ulcers and irritation of women using N-9 with that of women using placebo, and 3) to measure the effects of N-9 film use on the vaginal ecology by comparing the vaginal flora patterns before initiation of N-9 use with the flora patterns after N-9 use.

A randomized, double-blind, multicenter, placebo-controlled trial conducted in San Pedro Sula and Tegucigalpa, Honduras will be conducted. An N-9 film containing about 70 mg of N-9 will be compared with a placebo film with identical non-active ingredients, packaging, and labeling as the N-9 film. This product was chosen because it has been used in previous studies with women who had approximately the same frequency of sexual contact as the women for the proposed study, and was not associated with an increase in yeast infections or genital ulcers. About 1,500 women at high-risk for HIV infection will be randomized to receive either condoms and N-9 or condoms and placebo. The recruitment period will be for 1 year and the follow-up period will be for 1 year.

Survival analysis techniques will be used for intent-to-treat analysis of the N-9 data. Data on demographic characteristics, potential confounders and effect modifiers will be collected at enrollment and during the follow-up period. Colposcopy will be used to assess genital epithelial disruption. Interim analyses will be reviewed by an oversight committee at 4 month intervals.

This study will provide valuable information to women and those attempting to slow the rate of HIV infection among women.

**PROTOCOL FOR PHASE III RANDOMIZED CONTROLLED TRIAL
OF VAGINAL MICROBICIDES**

A sample Protocol prepared by Dr R. Roddy, FHI

FHI

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INTRODUCTION

The introduction should briefly cover the rationale for the study, a description of the experimental product, including its mechanism of action, and data from preclinical, Phase I, and II studies. The relevant published literature should be critically reviewed.

[Example]

The vast majority of HIV infections in the world are sexually transmitted among heterosexual partners. The current prevention strategies include abstinence, mutually exclusive sexual relationships with an HIV-negative partner, and the use of condoms. All of these strategies require male consent and cooperation. In many female-male relationships, the gender-power differences are such that the woman does not have control over if and when she will have sexual intercourse. An example of this is when a husband is the partner who decides when the couple will have sex. Another example is when a woman's male relative (father, brother, etc.) decides to have sexual intercourse with her despite her own wishes. A woman may adhere to the practice of intercourse with only one man, but have no control over the male partner's sexual activity with other women. The use of the male condom and, perhaps, to a lesser extent, the female condom, requires male cooperation. There is no strategy or method currently available which is within the personal control of women.

Nonoxynol-9 (N-9) is a nonionic detergent that has been used as a spermicide for over 30 years. N-9 acts by disrupting the cell membrane of the sperm but is not specific in its activity. N-9 will disrupt the membrane of epithelial cells, bacteria, and viruses. This mode of action allows N-9 to inactivate many sexually transmitted pathogens in vitro, including *N. gonorrhoeae*, *C. trachomatis*, *H. ducreyi*, *T. pallidum*, *T. vaginalis*, and herpes simplex virus.¹⁻⁸ N-9 has also been shown in vivo to be effective as a prophylaxis against cervical infection by *N. gonorrhoeae* and *C. trachomatis*, and vaginal infection by *T. vaginalis* and the bacteria associated with bacterial vaginosis (9-11).

The evidence for N-9 activity against HIV includes in vitro studies, animal model studies, and three human studies. Several in vitro studies demonstrate that N-9 inactivates free and cell-associated HIV; one indicates that inactivation occurs in less than 60 seconds (12-15). A study of vaginal transmission of simian immunodeficiency virus (SIV) in macaque monkeys demonstrated a protective effect of N-9 (16). N-9 foam was inserted into the vagina of six monkeys immediately before introduction of cell-free SIV suspension to the vagina (104 intravenous animal infectious doses). This dose of SIV is usually 100% infectious. The N-9/SIV inoculation was repeated four times over 2 weeks. Three of the six monkeys did not become infected. N-9 gel was inserted into an additional six monkeys followed by intravaginal SIV inoculation four times over 2 weeks. Two of these monkeys did not become infected. Five of 12 monkeys were protected by N-9 from a dose of SIV about 1,000 times that usually found in infected monkeys.

Kreiss et al. conducted a randomized placebo-controlled trial with sex workers in Nairobi, Kenya (17). The N-9 sponge with 1 gm of N-9 was compared with a glycerin vaginal suppository containing mineral oil or a water-based vaginal cream for HIV prophylaxis. HIV seronegative women (138) were enrolled; 74 were assigned to N-9 and 64 to placebo. The group assigned to N-9 had a higher prevalence of genital ulcers at the time of enrolment; 16%

for N-9 vs. 3% for placebo. The sites of the genital lesions were generally external; 88% for N-9 and 100% for placebo. During follow-up for the study, the N-9 group had a higher incidence of genital ulcers than the placebo group (15% vs. 8%) and the majority of the lesions were external (67% for the N-9 group and 63% for the placebo group). Twenty-seven (life-table cumulative = 45%) of the N-9 group and 20 (life-table cumulative = 36%) of the placebo group seroconverted during the study. Using proportional hazards modeling to control for the presence of a genital ulcer at enrolment, a rate ratio of 1.6 (95% CI: 0.8, 2.8) was estimated and indicates no protection from HIV infection. The authors also concluded that the N-9 sponge was associated with an increase in genital ulcers (RR=3.3). The authors did acknowledge many limitations of their study and suggested that other studies be conducted to assess different formulations of N-9.

An editorial by Stone and Peterson 18 has pointed out some of the weaknesses of the Kreiss et al. study. They include the high probability of selection bias even though a randomization process was followed; this is reflected in the difference in prevalence of genital ulcers at enrolment. Risk of seroconversion by level of compliance was not reported. The distribution of condom use among the study participants was not reported, and no adjustment was attempted for condom compliance. A concern raised by the Kreiss et al. study is whether N-9 may actually increase susceptibility to HIV infection. A major unanswered question is why the women had so much external irritation and so little internal irritation where the highest N-9 concentration was located. One possible explanation could be that N-9 was not responsible for an increase in genital ulcers or vulvitis, but that the placebo used (a glycerin suppository with mineral oil or a vaginal cream) was responsible for a decrease in ulcers and vulvitis. The sponge may have produced a drying effect and the placebo may have had a lubricating effect. Either explanation is compatible with the data collected, and is dependent only upon which product is selected to be the referent for the estimations.

A prospective cohort study conducted by Feldblum and Zekeng among female sex workers in Cameroon has reported a decrease in the rate of HIV infection among women who consistently used a 100 mg N-9 suppository (19). Condoms and N-9 were distributed to 273 women. During a mean of 8 months follow-up, 19 seroconversions occurred. Comparing consistent N-9 users (>67% of intercourse protected) with those who used N-9 less consistently, and using proportional hazards modeling to adjust for condom use produced a rate ratio of 0.1 (95% CI: 0.1, 0.6). There was also a decrease in gonorrhea and genital ulcers among consistent users of N-9.

The major weaknesses of the study were the lack of randomization, the high potential for selection bias, and no unexposed reference group. Women who decide to use N-9 consistently may have different behavioral and exposure risks than women who decide to use N-9 infrequently. Consistent users may be more health conscious, which may lead to a bias that may overestimate the effect of N-9 by an unknown quantity. No women declined to use N-9; therefore, no unexposed group existed. The lack of an unexposed group requires the study to make comparisons with women exposed to N-9 as the reference group and this underestimates the effect of N-9 by an unknown amount. However, while the magnitude of the estimate is difficult to ascertain, the direction of the effect is clearly toward protection. A preliminary report from a prospective cohort study conducted by Feldblum and Hira among 114 HIV discordant couples in Zambia indicated a decrease in the HIV rate among consistent users of N-9 film (100% use reported) when compared with less consistent users (<100%) (20). The couples were

followed for a mean of 16 months. About three quarters (83) of the women were seronegative. Only six seroconversions occurred among women in the study, limiting the to crude estimates. A rate ratio of 0.5 (95% CI: 0.1, 3.9) was estimated. There was no increase in genital ulcers among the consistent N-9 users. The major weaknesses of this study are the same as in the Cameroon study discussed above. In brief, there is no unexposed reference group and selection bias may distort the estimate. The small number of seroconversions also limits the of the study data. N-9 does cause genital irritation. Two human studies have demonstrated that frequent, high, cumulative doses of N-9 produce epithelial disruption. In a phase I study, using 150 mg N-9 suppositories four times a day cumulatively, 43% of women developed epithelial disruption (21).

A dosing study compared cumulative placebo use four times a day with cumulative 150 mg N-9 suppository use once every other day, once a day, twice a day and four times a day. An increase in N-9 use produced an increase in epithelial disruption (22). In vitro studies have shown that N-9 has an effect on bacteria known to be usual vaginal flora (23). Lactobacilli (especially the H202 producing strains) are sensitive to N-9 and some believe this bacteria is important for maintaining a healthy vaginal ecosystem (24). Human studies have raised questions about the possibility of N-9 produced changes in the vaginal flora which might lead to urinary tract infections (25). One hypothesis Kreiss and coworkers give for a possible increase in vulvitis in their study is that N-9 affects the usual microflora allowing overgrowth of fungal species. The work done on vaginal ecology to date has not been with sex workers. The vaginal flora of women who have sexual intercourse multiple times a day with different men is not clearly understood. If we are to know whether the effect of N-9 on the vaginal flora is important, we first need to understand the vaginal flora of these women.

In summary, the evidence concerning the prophylactic effect of N-9 on HIV transmission is mixed and unclear. In vitro evidence, animal data, and two human use studies indicate that N-9 may be protective. However, a methodologically stronger human study (Kreiss et al.) and data on genital irritation caused by N-9 provide reason for concern and caution. The question remains: is N-9 protective from HIV infection despite the potential risk of increased genital irritation?

OBJECTIVES

The major objectives of the study should be presented in a concise manor such that the reader can understand what is to be measured and what comparisons will be made.

[Example]

1. To measure the effect of N-9 use on the rate of HIV infection by comparing the rate of infection of women using condoms and N-9 with that of women using condoms and placebo.
2. To measure the effect of N-9 on the rate of genital ulcers and symptoms of irritation by comparing the rate of genital irritation of women using N-9 with that of women using placebo.

3. To measure the effects of N-9 use on the vaginal ecology by comparing the vaginal flora patterns before initiation of N-9 use with the flora patterns after N-9 use.

Secondary study questions can be examined with the collected data and include:

STUDY DESIGN

A randomized, double-blind study is highly recommended. Observational studies will not be likely to control for the myriad of possible confounding variables associated with sexual behavior. Masking or blinding is important to prevent bias being introduced by the study staff and participants.

[Example]

The study is a randomized, double-blind, multicentre, placebo-controlled trial. Approximately 1,000 women will be recruited by outreach workers and followed for a minimum of 1 year.

Each study clinic will recruit and follow 334 women.

All sexually transmitted infections (STI) diagnosed during the study will be treated at no cost to the study participants.

Women will be randomized to use either condoms and N-9 or condoms and placebo. This is a design similar to multiple-treatment clinical trials.

A computer generated randomization list will be drawn up for each clinic because we plan to stratify by clinic during the ; to have the most efficient design, each clinic (strata) will need roughly equal numbers of N-9 and placebo users. Assignments to the study groups will be done using random blocks of size eight. Blocking is done to assure equal numbers are assigned to each group throughout the study so that the preliminary analyses will be efficient.

The group assignments will be placed in opaque envelopes and will not be known by the field study staff until the actual time for assignment. The assignment to a group will not take place until after the woman has signed the consent form and is ready to enrol in the study.

PARTICIPANT SELECTION

Number of Participants

Sufficient information should be presented to indicate the current estimate of seroprevalence and HIV incidence at the study site. The assumptions such as type I and II error, rate of infection, the amount of difference expected, and the expected loss to follow-up. A brief discussion of the effect of changes in the assumptions on the study size should address such issues as changes in the rate of infection, loss to follow-up, recruitment problems, and length of follow-up.

The effectiveness may be altered by the success of other concurrent interactions such as condom use, diagnosis and treatment of STD in the trial population, counselling or by the existence of other routes of HIV transmission (IVDU and anal intercourse).

- [Example]

The study will enrol a total of 1,000 women. The HIV seroprevalence will be approximately 25% and 1334 women will be screened in order to enrol the 1,000. Approximately 958 HIV-negative women are necessary for the study if the assumptions used for study size calculations are two-sided $\alpha=0.05$ testing, $\alpha=0.1$ (power=0.90), a 20% loss to follow-up per year, a 10% incidence per year, a rate ratio of 0.5, a recruitment period of 1 year, and a minimum of 1 year of follow-up (26). If recruitment is extended to 1.5 years, 30% loss to follow-up per year occurs, minimum follow-up is only 6 months, and power is reduced to 0.80, we need to recruit 897 HIV-negative women. If we assume a worst case scenario with 1.5 years for recruitment, 30% loss to follow-up, 6 months of follow-up time, and only 5% incidence per year, the study will have a power of 0.71 if the 1000 HIV-negative women are enrolled. We will enrol the larger number because we cannot be sure of the incidence of seroconversion, loss to follow-up, or the rapidity of enrolment. Follow-up times will vary by time of entry into the study. A woman enrolled the first month of the study and followed until the last month of the study would have a follow-up time of 2 years (1 year of recruitment time plus 1 year of follow-up time) while a woman enrolled in the last month of the recruitment period would have 1 year of follow-up.

Inclusion Criteria

Women at high-risk of HIV infection through heterosexual contacts should be the major population studied. Wherever appropriate, the involvement of community based organizations should be sought:

- o to establish and maintain the appropriate infrastructure for the trial;
- o for the design of the study, especially with regard to the feasibility of the project and acceptability issues; and
- o for implementation of the project.

However, women not affiliated with an "organization/community" should not be excluded.

Criteria used to judge whether a participant should be enrolled should be listed. When possible HIV+ women should be included in the study to determine the safety of the product in women who are HIV infected. Promotional campaigns for successful products will not be able to single out HIV- women for product use because most women will not know their infection status.

Inclusion criteria:

Volunteers to participate in the study willing to comply with the study design;

Women of reproductive age (e.g. 18-45);
 HIV negative women (measure rate of seroconversion);
 HIV positive women (measure incidence of gonococcal and chlamydial infections;
 Women with genital lesions detected at entry should not be excluded.

[Example]

- * Sex worker (greater than three partners per month)
- * Age 18 to 50
- * Resident of study city
- * Willing to keep a coital log and use the study products as instructed
- * Willing to sign a consent form and learn the results of her HIV test

Exclusion criteria:

Criteria used to judge whether a participant should be excluded from enrolment should be listed.

Women currently using intravenous drugs, crack or cocaine.
 Current and regular practice of anal intercourse (This efficacy study has been designed specifically to prevent the transmission of HIV to women via vaginal intercourse.)

[Example]

- * Pregnant or less than 42 days postpartum
- * Allergic to N-9 or latex

STUDY PRODUCT ADMINISTRATION AND COMPLIANCE

Description of Study Products

Instructions for Administration

Blinding and Unblinding Procedures

A description of the blinding procedures should be described and under what conditions the blinding should be broken.

[Example]

The group assignments will be placed in opaque envelopes and will not be known by the field study staff until the actual time for assignment. The assignment to a group will not take place until after the woman has signed the consent form and is ready to enrol in the study.

The assignment will be to a group, but neither the study staff nor the participants will know if that group is placebo or N-9. The study staff in the field and the statisticians will not know which products are placebo and which are N-9.

The N-9 products are identically packaged. The casing for the N-9 products will be labelled A or B so that the study staff will know which product to provide to the participants.

Unblinding of individual participants for any reason is discouraged. In the case of a medical emergency, use of the vaginal product should be discontinued and appropriate treatment given.

EXPERIMENTAL PROCEDURES

The procedures of the study should be sufficiently described. This should include the method for data collection.

[Example]

The data will be gathered from monthly interviews, pelvic examinations, and clinic and laboratory tests. The interview will be structured, use a standardized questionnaire, and be recorded on an Initial or Follow-up Questionnaire Forms. The information from the Coital Log and the interview about product use will be included on the Follow-up Questionnaire Form. An Initial Physical Examination Form will be used to collect the information gathered during the enrolment examination and a Follow-up Physical Examination Form will be used for subsequent examinations. Clinical lab tests will be recorded on the Physical examination forms. Two forms will be used to record the laboratory tests (Central Lab Form and HIV Report Form). The HIV Report Form will be separate to maintain control of confidentiality of the study participants' serostatus.

A coital log will be used to assess compliance with the vaginal products and condom use (proportion of intercourses used). The coital log will be pictorial with a condom and vaginal product represented and a place to indicate whether the man was a client or non-client. Each time sexual intercourse takes place the participant will place an X over the box that represents the type of partner and over the condom and/or vaginal product representation if used. The coital logs will be maintained by each participant and brought to the clinic at each visit. The interviewer will review the coital log with the women.

Demographic variables will be collected: age, education, marital status, number of living children, and amount of money earned in an average week. Variables to assess the randomization process and potential confounders will be collected at enrolment and during the study: duration of sex work, number of sexual partners per week, history and acquisition of STI, types of sexual acts conducted, condom use, antibiotic use, sex during menses, contraceptive method, blood transfusions, receipt of injections, douching, and use of vaginal agents.

Behavioural variables that may also confound the effect measure will be measured before and during the study include: injecting drug use (ever, never, last month), non-injecting drug use (ever, never, last month), alcohol use (amount last month), anal intercourse (ever, never, number of times last month), and oral intercourse (ever, never, number of times last month).

Questions about sexual networks will seek information about types of sexual partners (new partners, regular customers, non-customers, coerced sexual relationships, etc.) and condom129

and N-9 use with these different types of partners. The interviews will be conducted monthly so women will not have a long time period to recall.

Study Time and Events Schedule

[Example]

Times are from the date of the start of the field portion of the study:

Month 1	Pretest questionnaires, forms, the consent process, lab procedures.
Months 2-4	Recruitment of study participants will begin at one clinic each of the first three months (the study will be phased in one clinic at a time) to allow the supervisory staff time to assure that study procedures are incorporated properly.
Months 2-16	Recruitment of study participants (if recruitment does not proceed as planned, it will be extended to 18 months), interim at months 6, 10 and 14.
Months 17-28	Continued follow-up for each woman (if this is not possible, follow-up will be reduced to 6 months), interim at months 18 and 22.

Event	Timing
Initial Screening	Initial clinic contact
HIV pretest counseling	During initial screening
HIV testing consent	During initial screening
Start Coital log	During initial screening
Study enrolment	Two weeks after initial HIV screening
N-9 study consent	At study enrolment
Randomization	At study enrolment
Initial questionnaire	At study enrolment
Initial physical examination	At study enrolment
Check coital log	At study enrolment
Study Follow-up	Every 1 month after enrolment
Follow-up HIV testing	Every 1 month
Follow-up questionnaire	Every 1 month
Follow-up physical examination	Every 1 month
Check coital log	Every 1 month
Measurements/Evaluations	

[Example]

Screening Phase

Potential study participants will be briefly informed about the study and invited to attend the nearest study clinic by the study recruiters (the recruiters will use the screening questionnaire). The women will have the study further explained to them at the clinic by the study staff. If a woman thinks she would like to participate in the study, she will have the HIV Testing Consent Form explained to her, be given pretest counseling, be HIV tested, instructed in keeping the coital log, asked to keep the coital log for one month and return to the clinic in one month to possibly be enrolled in the study. If the woman returns to the clinic at one month, she will be given the results of her HIV test and receive post-test counseling.

HIV serostatus will be determined by sequential method. The HIV testing scheme is as follows: a sample of blood will be collected for HIV 1+2 ELISA testing. If an ELISA is positive (by optical density determination) or indeterminate, a Western blot will be performed.

This sequence of testing assures a high specificity and reduces misclassification.

Baseline Phase

[Example]

The baseline phase will include the initial questionnaire, physical examination, and laboratory tests.

Genital ulcers will be noted upon physical examination. Genital ulcers will be defined as any break in the epithelium of the vulva, vagina, or cervix. A clinical diagnosis will be made for most genital ulcers. Syphilis serologic testing will be available, but no cultures for chancroid or antigenic tests for herpes virus will be used. The syphilis testing will be done by the study laboratory technicians. The participants will be treated for genital ulcers dependant upon the presumptive diagnosis. A woman with a genital ulcer will be advised to not have sex until the ulcer has healed. She will be asked to return to the clinic at weekly intervals for repeat examinations to determine the healing of the ulcer.

Vaginal ecology of 300 women from one the clinics will be monitored with the Gram stain. A swab will be rolled over the lateral walls of the vagina and then rolled on a 1 cm² area of a slide. The specimen will be heat fixed and Gram stained using the fast method (34). The slide will be interpreted by the study laboratory technician using the scale developed by Hillier and colleagues (35,36).

All women will be monitored with colposcopic examinations. The examination will specifically search for signs of genital irritation; erythema and epithelial disruption. The description for lesions found with the colposcope will follow that found in the draft WHO Manual for the standardization of colposcopy for the evaluation of vaginally administered products.

Endocervical specimens will be taken with the supplied swab for the Pace-2 DNA probe for gonorrhea and chlamydia infection. The swab will be transported to the central laboratory in the preservative provided and processed according to manufacturer's instructions.

Vaginal pH will be measured using ColorpHast indicator sticks with a pH range of 4.0 to 7.0. The pH stick will be pressed against the lateral vaginal wall and read while still moist by comparing the color change to the color chart provided with the sticks. The pH will be reported as the specific pH number and not just < or > a value.

A saline and 10% KOH wet-mount will be conducted in the clinic. Motile trichomonads and clue cells will be identified by microscopy in the saline preparation. Budding yeast and pseudohyphae in the KOH preparation will indicate the presence of *Candida* species.

The enrolment examination and specimen collection sequence will be as follows:

- * The inguinal lymph nodes palpated
- * The vulva and perineum inspected for lesions (colposcope used as needed to assist with evaluation)
- * The introitus examined for discharge
- * A speculum lubricated only with water is placed in the vagina
- * Visual inspection of the vagina and cervix with the colposcope
- * A pH strip is placed against the lateral vaginal wall and compared to the pH chart provided with the strips
- * A swab from the vaginal pool placed in a test tube with a few drops of normal saline for preparation of the wet-mount (saline and KOH)
- * A second swab rolled against the vaginal wall and then rolled onto a glass slide for a Gram stain
- * The ectocervix cleaned, any discharge from the endocervix noted, and a swab inserted into the endocervix for a specimen for the gonorrhea and chlamydia DNA probe test; this swab will also be used to judge the color of the cervical mucus (swab test)
- * The vagina inspected again as the speculum is removed
- * A bimanual pelvic examination performed

Follow-up Phase

[Example]

The follow-up phase will include the follow-up questionnaire, physical examination, and laboratory tests.

HIV testing will occur at initiation and then monthly. All women will receive pretest and post-test counseling from the counsellors. The data will be analyzed with and without women who seroconvert within 3 months of enrolment to determine if accounting for a lag period between infection and seroconversion is necessary.

Examination for genital ulcers and signs of genital irritation will be conducted at initiation and at each follow-up visit. Colposcopes will be used to examine the vulva, vagina, and cervix at each visit to assist the examiner in locating micro-ulcerations. Special attention will be given to intravaginal areas that are exposed to the highest doses of N-9. Women will be advised to return to the clinic immediately if they become aware of a genital ulcer. An interview conducted during the monthly visit will elicit symptoms of irritation and problems encountered with vaginal product use. Syphilis will be the only diagnosis for genital ulcers that will have supporting laboratory evidence (RPR and TPHA). The diagnosis of herpes and chancroid will be made clinically; although such diagnosis is recognized to be inaccurate.

Women with genital ulcers will be continued in the study, treated according to the presumptive diagnosis, and asked to return to the clinic for weekly examinations until the genital ulcer is healed. She will be asked to not have sexual intercourse during the time she has a genital ulcer.

Vaginal specimens for Gram stain will be taken from the vaginas of 300 women at one of clinics at the initial examination and at follow-up visits 1 and 2. The Gram stains will be interpreted as normal, intermediate, or abnormal. Women with abnormal Gram stains will be treated for bacterial vaginosis.

The following will be the sequence for the follow-up physical examination:

- * The inguinal lymph nodes palpated
- * The vulva and perineum inspected for lesions (colposcope used as needed to assist with evaluation)
- * The introitus examined for discharge
- * A speculum lubricated only with water is placed in the vagina
- * Visual inspection of the vagina and cervix using the colposcope
- * A swab from the vaginal pool placed in a test tube with a few drops of normal saline for preparation of the wet-mount (saline and KOH)
- * A specimen taken from the endocervix for the gonorrhea and chlamydia DNA probe test
- * The vagina inspected again as the speculum is removed
- * A bimanual pelvic examination performed

STATISTICAL ANALYSIS

Analytical Methods

- [Example]

We will conduct an intention-to-treat by comparing the rates of HIV infection by assigned group. We will also explore dose response by comparing the two experimental groups at various frequencies of vaginal product use. The dependent variables for include HIV serostatus (HIV+, HIV-), genital ulcer (present, absent), and vaginal flora (normal, intermediate, abnormal). The independent variables include vaginal product use (N-9, placebo) and condom use (proportion of sexual acts used). A coital log will be used to assess compliance with vaginal product and condom use (proportion of intercourses used). Effect modification of one independent variable on the other will be assessed.

will begin with the estimation of the prevalence of the demographic variables and potential confounders at enrolment stratified by experimental group. Prevalence ratios will be estimated for the variables with the placebo group as the referent.

To measure the effect of N-9 use on the rate of HIV infection during the trial, group sequential will be performed. The accumulating data will be examined six times, the first five for interim analyses and the sixth for the final . Interim analyses will be performed approximately every 4 months. The two-sided log rank test will be used to compare the rate of infection in women using condoms and N-9 with women using condoms and placebo. With the planned study size of 500 HIV-negative participants per group, there will be 90% power to detect a relative difference of 50% in the HIV infection rate between the two groups at the end of the study (H0: There is no difference in the rate of HIV infection for the two treatment groups).

The Lans-DeMets error spending function approach will be used to spread the Type I error rate among the interim analyses and final so that the overall significance level is 0.05 (27). Specifically, the use function $*(t) = 2\{1 - (z^*/2/*t)\}$ (where there is the proportion of failures that have accrued at some interim assessment and $*$ is the standard normal distribution function) will be used. This function will give sequential boundaries similar to O'Brien and Fleming (28). EaSt software will be used to obtain the value of the log rank needed to assess statistical significance at both interim and final analyses. A blinded will be performed.

Multicentre studies are designed with the assumption that results will be pooled unless there is clear evidence that pooling would be inappropriate (29). Thus, if there is no evidence of interaction, clinic site will be treated as a stratification factor in the . If there is evidence of interaction, the effect of N-9 will be estimated for each clinic separately. We will assess whether the rate ratio is constant over time. SAS software will be used to calculate the value of the rate ratio for the data observed at each and to carry out the hazards .

Adjustment of estimates for covariable effects will be conducted if necessary. A 10% change in the rate ratio estimate with the addition of a covariable to a model will be considered evidence of confounding. Statistical significance testing will not be used to judge confounding (30).

FAST*PRO software (31) will be used to obtain a set of marginal probability distributions or maximum likelihood estimates and confidence intervals for rate differences and ratios. The probability distributions will provide a graphic representation of the uncertainty about the estimates. The software will calculate the probability that the true value lies within a specified range by estimating the area under the probability curve for the range. For instance, the probability that the rate ratio is less than one (a protective effect) will be calculated.

The total number of genital ulcer events in each experimental group will be divided by the number of person-months of follow-up for the corresponding group. Each separate episode of genital ulcer will be counted and each woman may have multiple events. The rate difference and ratio will be calculated and FAST*PRO used to estimate the probabilities of the values lying within specified ranges. A dose response will be assessed for genital ulcers and N-9 use.

The incidence difference $[(\text{\#seroconversions/person-months, N-9 users}) - (\text{\#seroconversions/person-months, placebo})]$ will be used as the measure of direct incremental effect. The reciprocal of the incidence difference $(1/\text{incidence difference})$ will be used to denote the number of people who would have to use N-9 to produce a theoretical benefit (or harmful effect) for one person (32). The rate ratio will be used to demonstrate the relative effect. Finally, if appropriate, the proportionate increment will be used to express the effect in the form of a percentage increase or decrease.

Data and Safety Monitoring Board

A Data and Safety Monitoring Board (DSMB) will be set up to monitor the study every 4-6 months. The DSMB will be given the following data in summary form by randomized group: number of participants enrolled, amount of person-time accumulated, number of HIV seroconversions, number of severe genital ulcers (defined as ulcers that bleed), number of participants withdrawn, and number of participants lost to follow-up. Rate ratios for HIV seroconversion and severe genital ulcers will be calculated. The DSMB will be given the data used for the summary so that they can repeat the if they desire. EaSt software will be used to graphically represent the stopping boundaries and determine statistical significance.

STUDY PRODUCT INVENTORY, STORAGE AND END-OF-STUDY DISPOSITION

The clinics will receive sufficient clinical supplies of investigational products to study the number of participants to be enrolled at the center. All supplies must be stored in a limited access area that is securely locked (e.g., substantially constructed cabinet, or other substantially constructed enclosure).

Dr. ***** is required to maintain records of the disposition of all clinical supplies received to perform this study, including dates, quantity, and use by participants.

Under no circumstances is Dr. ***** allowed to release these clinical supplies for use by a person not named as a subinvestigator for the study or to administer these supplies to persons who are not enrolled in this study.

PROCEDURES FOR PARTICIPANT OR STUDY DISCONTINUATION

[Example]

Responses to the investigational product will be carefully followed by the responsible clinic personnel. Any participant withdrawn from the study for any reason after receiving the investigational product may not be re-enrolled in the study.

Loss to Follow-Up Considerations

[Example]

For each participant, clinic staff must obtain the names, addresses, and telephone numbers of contacts who would be expected to know the whereabouts of the participant enrolled in this study at the time of study enrolment. In the event that a participant misses a scheduled appointment, the clinic staff must try to establish communication with the participant through all possible means (e.g., telephoning and writing to the participant and contacts). The need to attend all scheduled follow-up visits must be emphasized to all study participants at every visit. If these attempts at contact have been unsuccessful over a period of three months and the clinic staff cannot contact the study participant, the participant will be considered lost to follow-up.

When a participant is declared lost to follow-up by the Investigator, the participant will be withdrawn from the study and a Study Termination Form will be completed.

Premature Discontinuation of a Participant

[Example]

Participants must be withdrawn from the study at any time that:

1. The participant feels it is in her best interests.
2. The Investigator believes it is in the participant's best interests.
3. The participant becomes pregnant.

Withdrawal for any other reason is discouraged. All reasons for study withdrawal must be documented on the Study Termination Form in the participant's files.

Discontinuation of the Study

[Example]

The sponsor reserves the right to terminate this study for any reason at any time during its conduct. All investigators will be notified by the sponsor if this is considered necessary and will be reimbursed for work completed. The sponsor will also instruct the investigator if any additional procedures are necessary to close-out the study.

ADVERSE EXPERIENCES

Surveillance

Surveillance for adverse experiences

[Example]

Medical problems, abnormal clinical findings, and diagnoses noted at scheduled study visits and at all nonscheduled visits will be recorded. All such conditions will be followed until resolution or until prognosis is clear (maximum: 3 months).

Continued study participation in the case of an AE

[Example]

As noted previously, participants who develop a sexually transmitted disease will be treated and continued in the study. The decision whether or not participants who develop other adverse experiences should discontinue assigned study product use temporarily or permanently will be made based on the clinical judgment of the investigator. Temporary cessation of assigned product use is not an indication for discontinuation from the study.

Unresolved adverse experiences at the last visit

[Example]

Participants with unresolved AEs possibly or probably related to product use at the final visit will be followed-up until resolution of the adverse experience or until the prognosis of the adverse experience is clear (a maximum of three months after discontinuation).

Reporting

[Example]

All Adverse Experiences

Each participant will be advised to contact the investigator (or study coordinator) immediately if she has any medical problems during the course of this study. All participants reporting adverse experiences during this study will be followed clinically, until the adverse experience resolves or stabilizes, including examination if necessary.

All adverse experiences occurring during this study will be recorded on the Adverse Experience Log. Each adverse experience reported will be graded on a 3-point scale of severity. Severity describes the intensity of the adverse experience based on the investigator's opinion (mild, moderate, or severe). The resolution (and date resolved) or continuation of each adverse experience will also be noted on the Adverse Experience Log.

For information on the classification of serious/non-serious adverse experiences, unexpected adverse experiences, and the relationship of the study product to the adverse experience, please refer to Appendix.

- Serious Adverse Experiences

All serious adverse experiences, even though they may not appear to be related to the product under investigation, will be reported promptly by telephone or fax to:

Preliminary telephone or Fax reports of all serious and unexpected adverse experiences will be followed by detailed descriptions on the IRB Preliminary Notification Form. Adverse Experiences that are fatal, life-threatening, or serious and unexpected will include copies of hospitalization summaries, pathology reports, operative reports, laboratory reports, autopsy reports and/or other documents as appropriate.

PROTECTION OF HUMAN PARTICIPANTS

Institutional Review Board Review and Approval

[Example]

This study has been reviewed by three IRB committees. The study proposal, consent process and forms were approved by the sponsor's IRB. The study has been reviewed by the Ministry of Public Health's National Ethical Committee. The Faculty of Medicine Ethical Review Board has also reviewed the proposal.

Serving as the sponsor's Institutional Review Board is a Protection of Human Subjects Committee (PHSC), which convenes several times during the year to review research proposals that involve human participants. All continuing research projects are reviewed at least annually or at more frequent intervals relative to the degree of risk as determined by the PHSC. The committee is composed of eight voting scientific and non-scientific men and women of diverse cultures and disciplines and three ex-officio/non-voting committee members, sufficiently qualified to execute the committee's charge. The committee consists of legal, clergy, consumer, public health, social science and medical representatives. The PHSC functions as an Institutional Review Board for the sponsor in accordance with 45 CFR 46 and all other applicable U.S. Federal regulations. The IRB has been dealing with HIV and AIDS related studies since 1987.

Participants - 1,000 female sex workers in generally good health will be recruited. Sex workers are sought as participants for the study because they have the highest risk for acquiring HIV infection, currently have the highest prevalence and incidence of HIV infection, and have the highest prevalence and incidence of STI. This subgroup will also benefit the most from the health care provided by the study.

The participants will be asked to use a condom and a vaginal product (N-9 or placebo) during each sexual intercourse. They will return to the clinic monthly for a follow-up pelvic examination and a structured interview. The participants will receive a much more extensive STI work-up than they usually receive. If an STI is diagnosed, they will receive treatment at no personal cost.

Research Material - Standard clinical procedures will be conducted to collect specimens for standard laboratory and clinic tests. A standardized interview will be conducted by trained personnel. All specimens and interview data are collected specifically for the study.

Participant Recruitment - Outreach workers familiar with the communities in which study participants live and work will be recruiters for the study. The outreach workers will give an explanation of the study to the potential participants. Women attending the clinics in the study cities will also be recruited. Each potential participant will have the study fact sheet and consent form explained in the appropriate language, and consent will be obtained by the formally trained health educators. The requirements for remaining in the study will be emphasized, the need for condom use thoroughly explained, and the lack of knowledge about protection by the vaginal product acknowledged. The fact that half the participants will be using a placebo will be stressed. The participants will be trained to complete coital logs and the importance of accuracy stressed. They will be told that they will not be judged by these data.

Risks - There is little or no risk from the diagnostic tests, pelvic examination, or interview. There is a possibility of increased risk for HIV infection from use of the N-9, but there also may be the benefit of a decrease in risk of HIV infection from N-9 use. We do not know the risk/benefit ratio of N-9 use for HIV transmission; that is the purpose of the study. N-9 has been shown to increase epithelial disruption with frequent use. This knowledge has prompted us to choose the smallest dose of N-9 available and a formulation that has demonstrated no association with genital ulcers or yeast infections in actual use in two studies. Our concern for the safety of the study participants also is the reason we will have an oversight committee to review the data every 4 months.

Women who seroconvert will continue in the study and, if they so choose, will continue to receive health care through the study doctors. Women who have a genital ulcer with a known etiology that is treatable, such as syphilis, will receive therapeutic treatment or palliative treatment (in the case of genital herpes). It is expected that a large proportion of women will develop a genital ulcer at one time or another during the study. About 36% of the low-risk women we attempted to enrol into a study in the Dominican Republic had epithelial disruption or signs of inflammation and could not enter the study. The women for this proposed study could have genital ulcers caused by yeast infections, trichomoniasis, trauma, bacterial or viral infections, condom use, or N-9 use. We will not drop women from the study because they have a genital ulcer. Women will be advised not to have sex while they have a genital ulcer.

Protection Against Risks - Because N-9 carries some chance of not reducing the risk of HIV, it is necessary that this study have an impartial DSMB. Group sequential monitoring for this study means that every 4-6 months we will conduct an interim of the main outcome of the study, HIV seroconversion. The information to be given to the DSMB includes, but is not limited to, the number of women enrolled in the study, the number of person-months, the number of women lost to follow-up, the number of seroconversions by experimental group, and the of the data.

All data and participant records will be confidential. Study files will be kept in locked filed cabinets. Participants' names will not be placed on the forms. Each clinic will keep a master log with the participant's study ID number, name, and contact information, and access to the master log will be limited. Those participants who choose to use the study physicians for

health care not associated with the study will have medical records kept separately from the study records in secure cabinets. Non-study health information will not be kept on study forms or be sent out of the clinic.

-Every effort will be made to minimize risk due to medical procedures and treatments undertaken during the study according to the best medical practices available.

Risk/Benefit Ratio - Having STI diagnosed and treated during the study is clearly beneficial to the participants and a small risk may be associated with the side effects of the medicines used for STI treatment. The risk of HIV infection in a group of sex workers similar to the one we have chosen was about 10% per year in 1990. This indicates that the women are already at high risk for HIV infection. We will be giving condom education, distributing condoms, providing counseling, and treating STI (which may reduce risk). The benefit to women as a group and to sex workers as a high-risk subgroup will be great if the N-9 is found to reduce the rate of HIV infection. We will monitor the study closely for indications that the risks of HIV infection are increased from N-9 use and stop the study if necessary. The risk/benefit ratio for this study is appropriate and the likelihood is great that new and useful information will be obtained.

Informed Consent

Before participants are enrolled in this study, the purpose and nature of the study as well as possible adverse experiences must be explained to them. Each participant must sign a statement of informed consent which complies with U.S. Federal Regulations. The participant must agree that she understands the investigational nature of the study, its inherent risks and benefits, other treatment alternatives, her rights to terminate participation in the study without affecting her health care at the center, whom to contact with questions regarding the study, and that she freely has given informed consent to participate in the study.

The signed informed consent form will become a permanent part of the participant's clinic records. While the informed consent will be audited in the same manner as other records, a copy of it will not be transferred to the sponsor.

Participant Confidentiality

The confidentiality of all participants enrolled in this study will be protected to the full extent possible. Participant's clinic records may be audited by the sponsor staff, regulatory personnel, or other individuals authorized to audit the study. However, study participants should not be identified by name on any study form or on any other documentation sent to sponsor and will not be reported by name in any report or publication resulting from data collected in this study.

RECORDKEEPING AND DATA COLLECTION

Data management is an important consideration. Interviewers will check the questionnaires before the participants leave the clinic to insure that answers to questions missed or left blank can be ascertained. The physician and nurse will be responsible for completion of

the physical examination form. They will be instructed to check the form for completeness before the participant leaves the clinic. The clinic supervisor will be responsible for assuring that the forms are properly completed and retraining will be undertaken if necessary. The laboratory technician will complete the lab forms and keep duplicate records in the lab. All forms will have three copies. One copy will be kept at the site, one with the data entry personnel, and the original will be sent to sponsor. The forms will be given to data entry personnel who will be responsible for checking the forms for completeness, initiating queries when necessary, and entering the data. Double entry of data will be conducted to provide a check on accuracy of the entry process. The 3.5" disk with the keyed data will be sent to the sponsor the first of each month. The data will be checked by a project assistant at the sponsor's headquarters and queries initiated if necessary. During the first 8 months, 50% of the data on the disk will be checked against the forms during the monitoring visit and, if no difficulties are detected, 20% of the data on the disk will be checked thereafter. The original forms will be transported by the data monitor after the site visit.

Study Forms and Data Entry

Accurate source documents and study forms must be documented and maintained at the designated times for all participants enrolled in the study.

All data required to be collected according to this protocol will be documented in Study Forms supplied by sponsor. Copies of the individual forms and instructions for their use are included in the Study Procedure Manual.

The original of each Study Form will be submitted to the sponsor. A copy will be retained in the participant's clinic study file. A third copy will be retained by the data entry team. It is imperative that the original and all copies match in every detail. Therefore, no changes may be made on one that does not appear on the other. All changes on the forms should be made on all copies, dated and initialed by the Investigator.

All sponsor data queries will be answered by the Investigator in a timely fashion.

Record Retention

Copies of Study Forms and other pertinent documentation (e.g., drug inventory forms, protocol, patient clinic records and original laboratory reports, etc.) must be retained by the Investigator until the sponsor advises the Investigator that it is no longer necessary.

SITE VISITS

Various authorized individuals may visit the study site to audit the progress of this study (e.g., sponsor personnel, regulatory personnel). A site visit log will be maintained at the study site in which all site visits made by authorized individuals are recorded. All clinical records and study forms for the participants enrolled in this study, will be made available for review by these authorized individuals.

Data Monitoring

During the course of this study, a sponsor data monitor will maintain personal contact with each participating clinic center on a periodic basis. At each site visit, the monitor will ascertain that: 1) the clinic facilities continue to be acceptable for the purposes of the study; 2) the study protocol is properly followed; 3) any changes in the protocol have been approved by the reviewing IRB, reported to sponsor and approved by the sponsor's IRB; 4) participants' records are complete and accurate; 5) a log is kept with the participants' names, addresses, telephone numbers, clinic file numbers and study participant numbers; 6) the study product inventory log is current and shows the study products received and used; 7) clinic staff are carrying out their activities as agreed upon at study initiation.

Quality Assurance

In the interests of the sponsor and the investigator, quality assurance procedures may be conducted by the sponsor. The study site may be visited by a quality assurance (QA) monitor. The QA monitor will compare participant's case records with the data on file at the sponsor's headquarters to assure the quality and accuracy of the data in this study.

PUBLICATION AND PRESENTATION OF STUDY RESULTS

SCIENTIFIC INTEGRITY

ADDITIONAL INVESTIGATOR RESPONSIBILITIES

INVESTIGATOR AGREEMENT

[Example]

I, the Key Investigator, agree to conduct this study in full accordance with the provisions of this protocol and will comply with all requirements regarding the obligations of clinical investigators as fully outlined in the Statement of Investigator, which I have also signed.

I have read and understand the information in the protocol, including the potential risks and side effects of the product under investigation and will ensure that all associates, colleagues, and employees assisting in the conduct of the study are informed about the obligations incurred by their contribution to the study.

Signature of Key Investigator

Date

**NIAID NEWS**Office of
Communications

National Institute of Allergy and Infectious Diseases

National Institutes of Health

FOR RELEASE
Thursday, April 3, 1997

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NIAID Evaluates N-9 Film as Microbicide

A large two-year study supported by the National Institute of Allergy and Infectious Diseases (NIAID) showed that vaginal contraceptive film containing a commonly used spermicide had no effect on transmission of HIV/AIDS, gonorrhea or chlamydia infections when provided as part of an overall HIV/STD prevention program.

Investigators at Family Health International (FHI), a nonprofit health research organization based in the United States, collaborated with those at the Cameroon Ministry of Public Health to conduct this study with female sex workers in two cities in Cameroon. Of the 1,292 women who enrolled, 941 completed 12 months of follow-up. Volunteers were given contraceptive films to be used before sexual intercourse. The films contained either the spermicide nonoxynol-9 (N-9) or a placebo. The women were supplied with male latex condoms and counseled monthly about reducing their number of partners and other safe sex practices. They also were examined and treated monthly for any STDs.

Preliminary analysis of the results from this recently completed study showed the overall rate of HIV transmission to be 6.7 percent, half the transmission rate that was previously estimated in this population. This rate reduction was the same in both the N-9 film users and the placebo group.

"We are encouraged by the apparent effectiveness of the overall intervention program that included counseling, STD treatment and encouragement of condom use," says Rodney Hoff, Ph.D., chief of the Efficacy Trials Branch in the AIDS Vaccine Research and Prevention Program. "Correct and consistent condom use is highly effective, but women must depend on the willingness of their partners to use male condoms. We and other public health officials are committed to developing an STD/HIV prevention method that can be controlled by a woman. This study is one part of that ongoing effort."

The search for woman-controlled methods has focused on the development of virus- and bacteria-killing products that women can apply intravaginally before having sex. Known collectively as topical microbicides, these products could give women the means to protect themselves from STDs.

"We had hoped that the N-9 film might increase a woman's available options for HIV and STD protection," says Willard Cates, Jr., M.D., M.P.H., FHI's senior vice

president for biomedical affairs. "These results show that we must accelerate our research programs dedicated to finding new products and techniques for women to use."

Until safe and effective vaginal microbicides are developed, the Centers for Disease Control and Prevention (CDC) recommends consistent and correct use of male latex condoms, with or without the use of a spermicide, to prevent sexual transmission of HIV and other STDs in high-risk populations. The CDC does not currently recommend the use of spermicides alone. The agency will continue to monitor data from this and other studies of vaginal products with N-9 to determine if their use results in prevention benefits or adverse effects.

N-9 is a detergent-like chemical that has been widely used for more than 30 years in over-the-counter gels, foams, creams and films designed to kill sperm. Researchers have shown that N-9 can kill HIV and other STD microbes in laboratory experiments. Previous studies in small numbers of women suggested that N-9 had some benefit as a topical microbicide, but also prompted some concern that frequent use or use of high doses of N-9 could disrupt the cells that line the genital tract, thereby increasing the chances of HIV infection.

The primary investigators for this study were Ronald E. Roddy, M.P.H., of FHI and Leopold Zekeng, Ph.D., of the Cameroon Ministry of Health. The sex workers volunteered at clinics in Yaounde and Douala between March 1995 and December 1996. To be eligible, participants could not be HIV-positive, pregnant, or allergic to latex or to N-9. Of the eligible volunteers, 941 women were randomized into two groups and completed the study. One group of 478 women were given condoms and contraceptive film containing 70 mg of N-9, and a second group of 463 women were given condoms and film containing placebo (an inert substance). Neither the women nor the study investigators knew which product a woman received.

The rates of HIV and other STD transmission were essentially the same for both groups and were measured in woman-years. For every 100 women using N-9 film and condoms for one year, 6.7 became infected with HIV, 33.3 became infected with gonorrhea and 20.6 with chlamydia. Infection rates for those provided placebo film and condoms were 6.6 for HIV, 31.1 for gonorrhea and 22.2 for chlamydia per 100 woman-years. Women using N-9 film and condoms had 42.2 genital sores per 100 woman-years compared with 33.5 sores in the placebo group. Women in the N-9 film group reported 147,996 acts of sexual intercourse, and those in the placebo film reported 146,942 acts.

Additional funding for this study was provided by the U.S. Agency for International Development and the Mellon Foundation. The N-9 film was provided by Apothecus Pharmaceutical Corp. of Oyster Bay, N.Y.

NIAID is a component of the National Institutes of Health (NIH). NIAID conducts and supports research to prevent, diagnose and treat illnesses such as HIV disease and other sexually transmitted diseases, tuberculosis, asthma and allergies. NIH is an agency of the U.S. Department of Health and Human Services.

NIAID press releases, fact sheets and other materials are available on the Internet via the NIAID home page at <http://www.niaid.nih.gov>.

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**A Randomized Controlled Trial
of the Effect of N-9 film on
Male-to-Female Transmission of HIV-1**

Family Health International

Funded By

- ◆ **Efficacy Trials Branch
Division of AIDS
National Institute of Allergy and
Infectious Diseases**
- ◆ **US Agency for International
Development**
- ◆ **Mellon Foundation**

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◆ Sylvie Rano

◆ Pierre Tina

Family Health International Staff

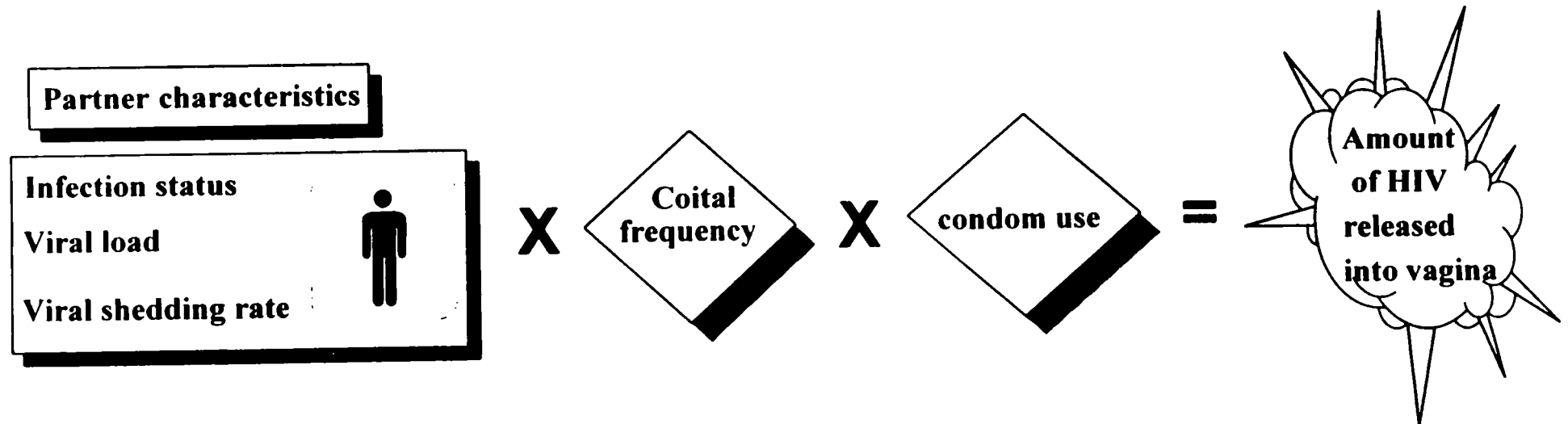
- ◆ Kelley A. Ryan
- ◆ Sharon S. Weir
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- ◆ Rosalie C. Dominik
- ◆ Douglas J. Taylor
- ◆ Angie J. Wheelless

Primary Question

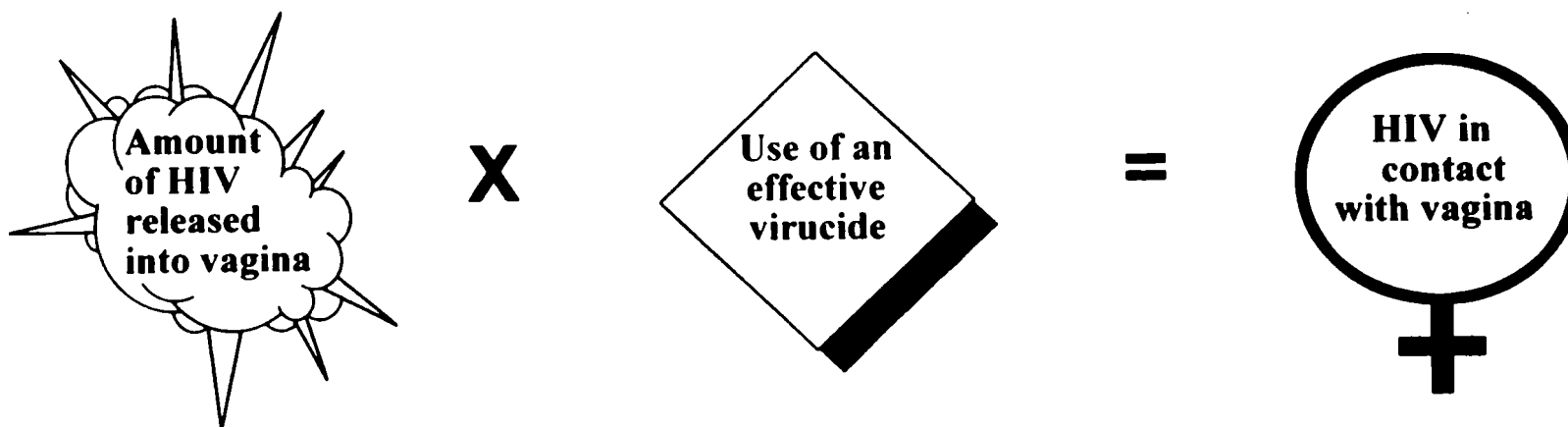
What is the effect of N-9 film use on male-to-female transmission of HIV-1?

- ◆ **Condom use encouraged**
- ◆ **STD treated**

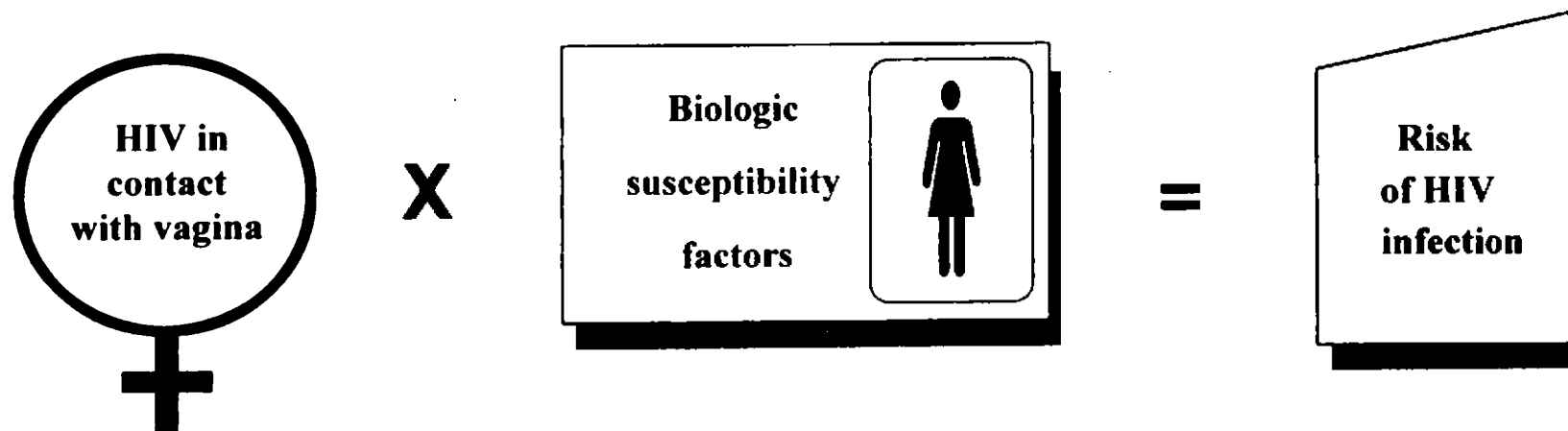
Exposure to HIV Adjusted for Condom Use



II. Exposure to HIV Adjusted for Condom and Virucide Use

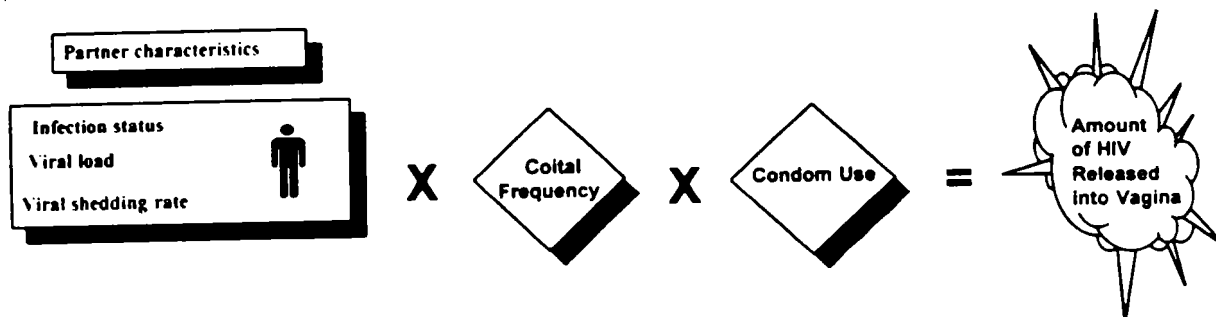


III. Susceptibility

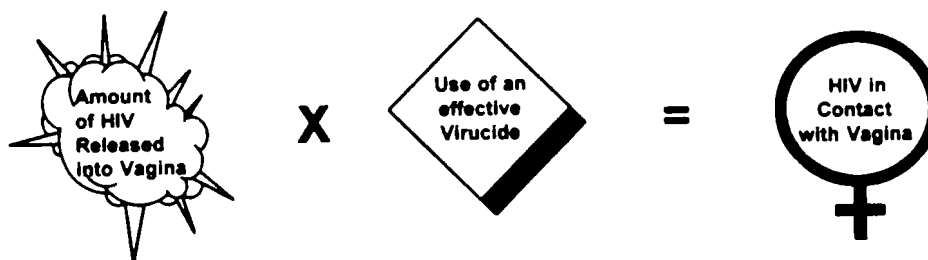


HIV Transmission Model

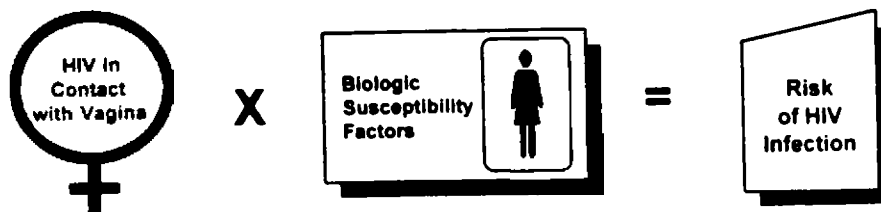
I. Exposure to HIV Adjusted for Condom Use



II. Exposure to HIV Adjusted for Condom and Virucide Use



III. Susceptibility

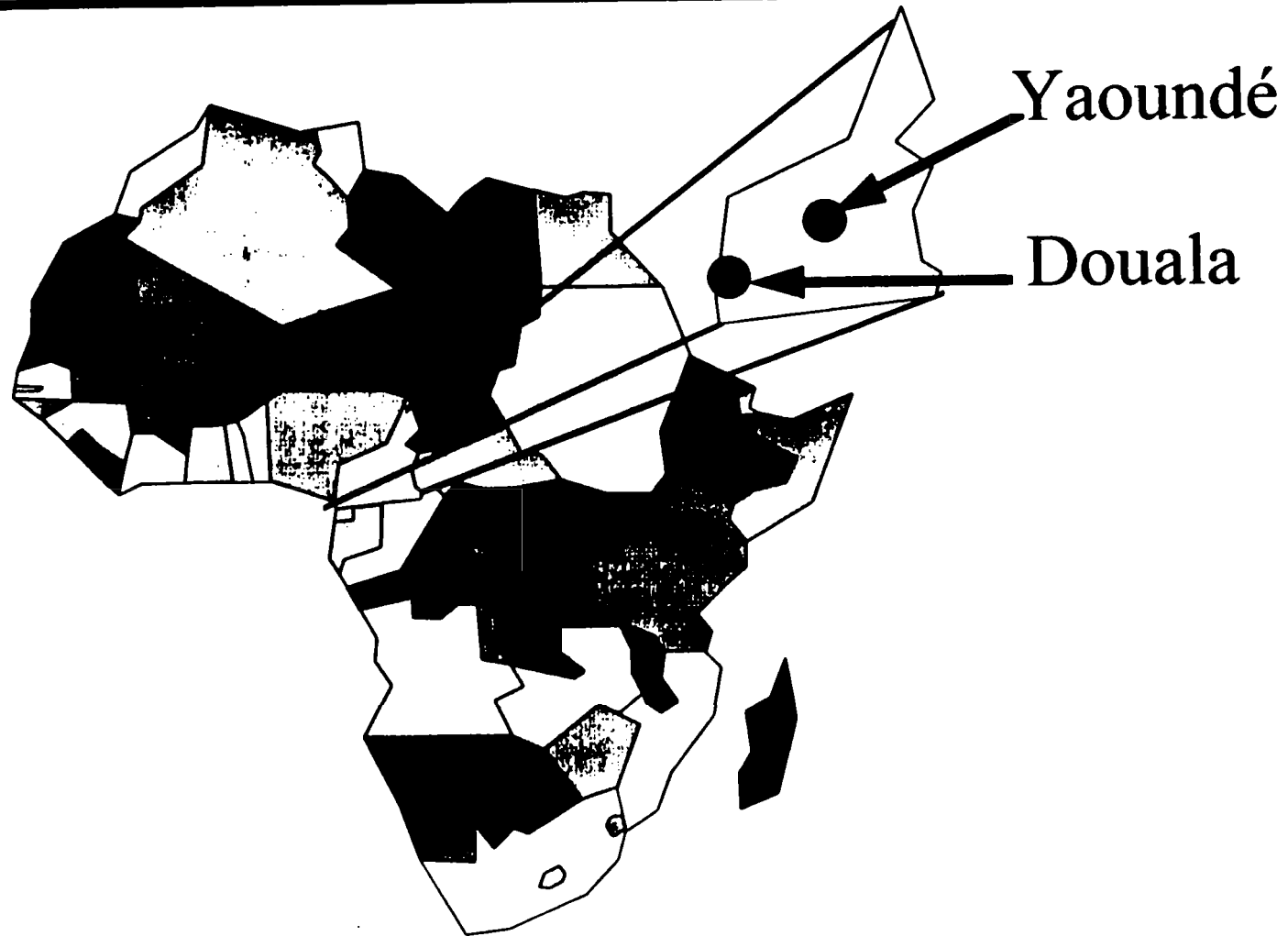


Secondary Questions

Effect of N-9 use on:

- ◆ **Genital lesions**
- ◆ **Cervical gonorrhea**
- ◆ **Cervical chlamydial infection**

Cameroon Sites



Study Population

Sex workers from the cities of:

- ◆ **Yaoundé (Pop. 686,423)**
 - **Family Planning clinic**
 - **Community clinic**

- ◆ **Douala (Pop. 1,503,770)**
 - **STD clinic**

Inclusion Criteria

- ◆ **> 3 partners per month**
- ◆ **Age 18 to 45**
- ◆ **Resident of study city**
- ◆ **Willing to:**
 - **sign a consent form**
 - **learn the results of HIV tests**
 - **use the study products as instructed**
 - **keep a coital log**

Exclusion Criteria

- ◆ **Pregnant, or less than 42 days postpartum**
- ◆ **Allergic to N-9 or latex**
- ◆ **HIV seropositive**

Study Size Estimate

◆ Assumptions

- $\alpha = 0.05$, two-sided; power = 90%
- HIV incidence = 10/100 woman-years
- rate ratio = 0.5
- at least 1 year FU, 20% per year loss to FU

◆ Enroll 1,000 women

◆ Expected 88 seroconversions

Clinical Objectives

◆ HIV

- enzyme immunoassay
- Western blot

◆ Genital lesions

- colposcope

◆ Gonorrhea and chlamydial infection

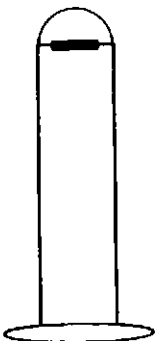
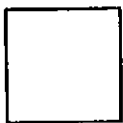
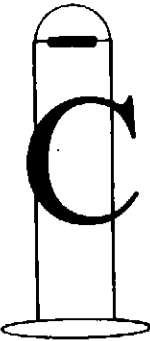
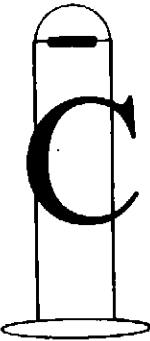
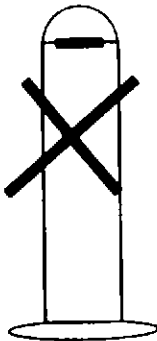
- Gen-Probe

Subgroup Analysis,

30-day time period before HIV test

- ◆ **Film use analysis**
 - percent of sex in which film was used
- ◆ **Film frequency analysis**
 - categories by absolute number
- ◆ **Condom use analysis**
 - percent of sex in which condoms used

Coital Log

C			~~C~~		
~~NC~~			NC		
~~C~~			C		
NC			~~NC~~		

Consent Process

- ◆ **Counseling**
 - **HIV and STD**
 - **explanation of HIV testing**
 - **condom education**
- ◆ **Study explanation**
- ◆ **Consent form**
- ◆ **Q&A; then sign**

Screening Visit

- ◆ **Consent process for screening**
- ◆ **Randomization for questionnaire study**
- ◆ **Coital log training**
- ◆ **Interview**
 - **condoms distributed**
- ◆ **HIV test**
- ◆ **Reimbursement for transportation**

Enrollment Visit - slide 1

- ◆ **Consent process**
 - placebo explanation
- ◆ **Randomization for film**
- ◆ **Interview**
 - coital log review
 - condom and film distribution

Enrollment Visit - slide 2

- ◆ **Pelvic exam**
 - **GC and CT tests**
 - **treatment**
- ◆ **HIV test**
- ◆ **Reimbursement for transportation**

Monthly Follow-up Visit - slide 1

◆ Counseling

- voluntary participation
- condom and film use
- interview, exam, and HIV testing
- fill in coital log

◆ Interview

- coital log review
- condom and film distribution

Monthly Follow-up Visit - slide 2

- ◆ **Pelvic exam**

- **colposcope for genital lesions**
- **GC and CT testing**
- **treatment**

- ◆ **HIV test**

- ◆ **Reimbursement for transportation**

Films and Condoms

- ◆ **N-9 film**

- 70 mg N-9, polyvinyl alcohol, glycerin

- ◆ **Placebo film**

- polyvinyl alcohol, glycerin, polysorbate-60

- ◆ **In vitro testing of films**

- N-9 inactivated HIV at 0.035%
 - placebo had no effect on HIV or cells

- ◆ **Condoms**

- latex, plain-lubricated condoms

Statistical Analysis - Main Comparison

- ◆ **Log-rank test for survival curves stratified by center**
- ◆ **Kaplan-Meier estimates of survival distributions**
- ◆ **Crude rate ratios and 95% CI**
- ◆ **Cox regression rate ratios and 95% CI stratified by center**

One Interim Analysis

- ◆ Two-sided log-rank test
- ◆ Lan-Demets spending function, 0.004
- ◆ 84 comparisons with p-values
- ◆ critical value = 1.76, $p = 0.54$

Group Assignment

- ◆ **Individual randomization**
- ◆ **Computer generated allocation**
 - **block sizes of 8**
 - **list for each clinic**
- ◆ **Opaque envelope concealment**
 - **distributed by clerk between counseling and interview**

Masking

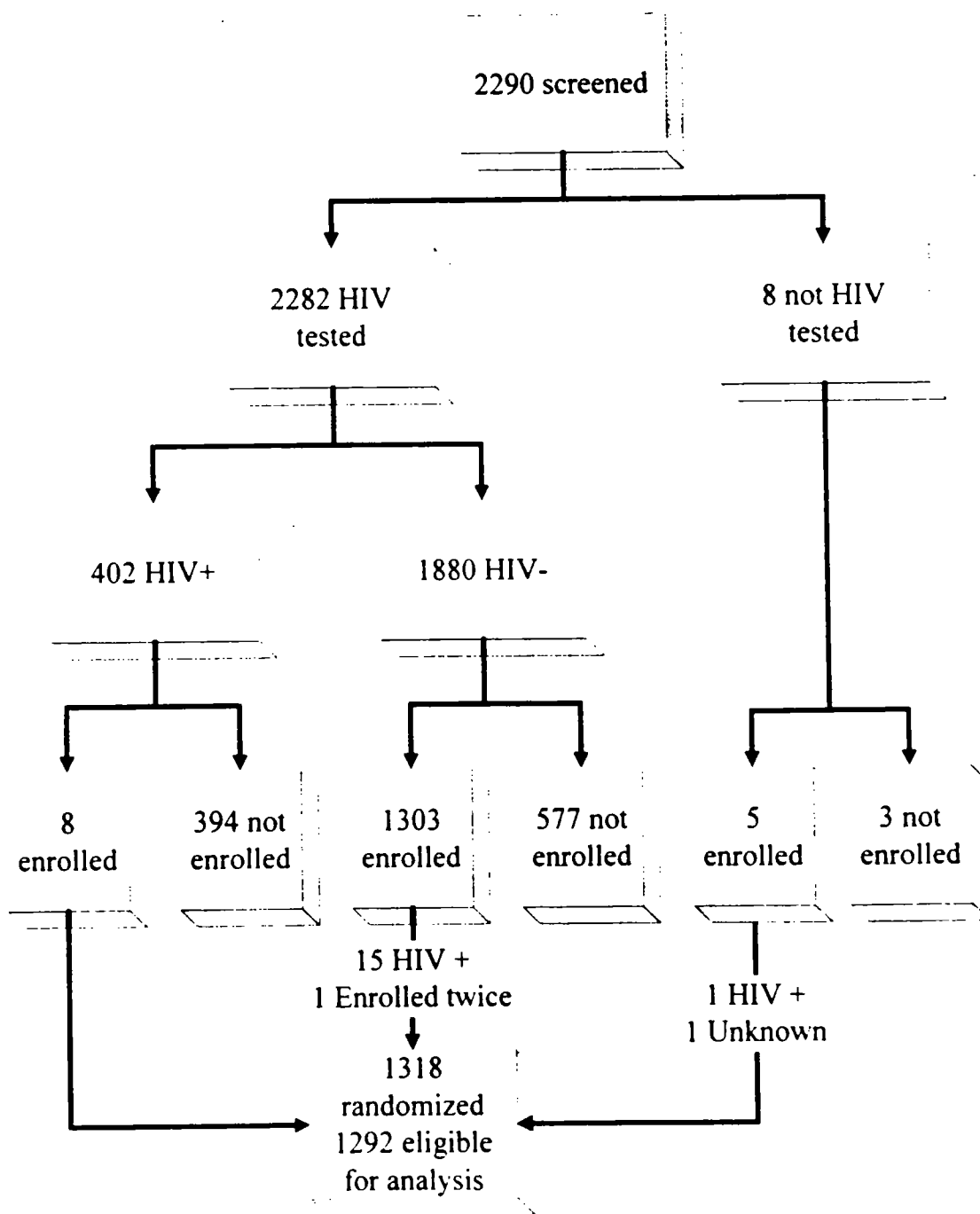
- ◆ **Triple-masking**
 - participants
 - Cameroonian study staff
 - biostatisticians
- ◆ **Similarities of products**
- ◆ **Evidence of successful masking**

Break for 15 minutes

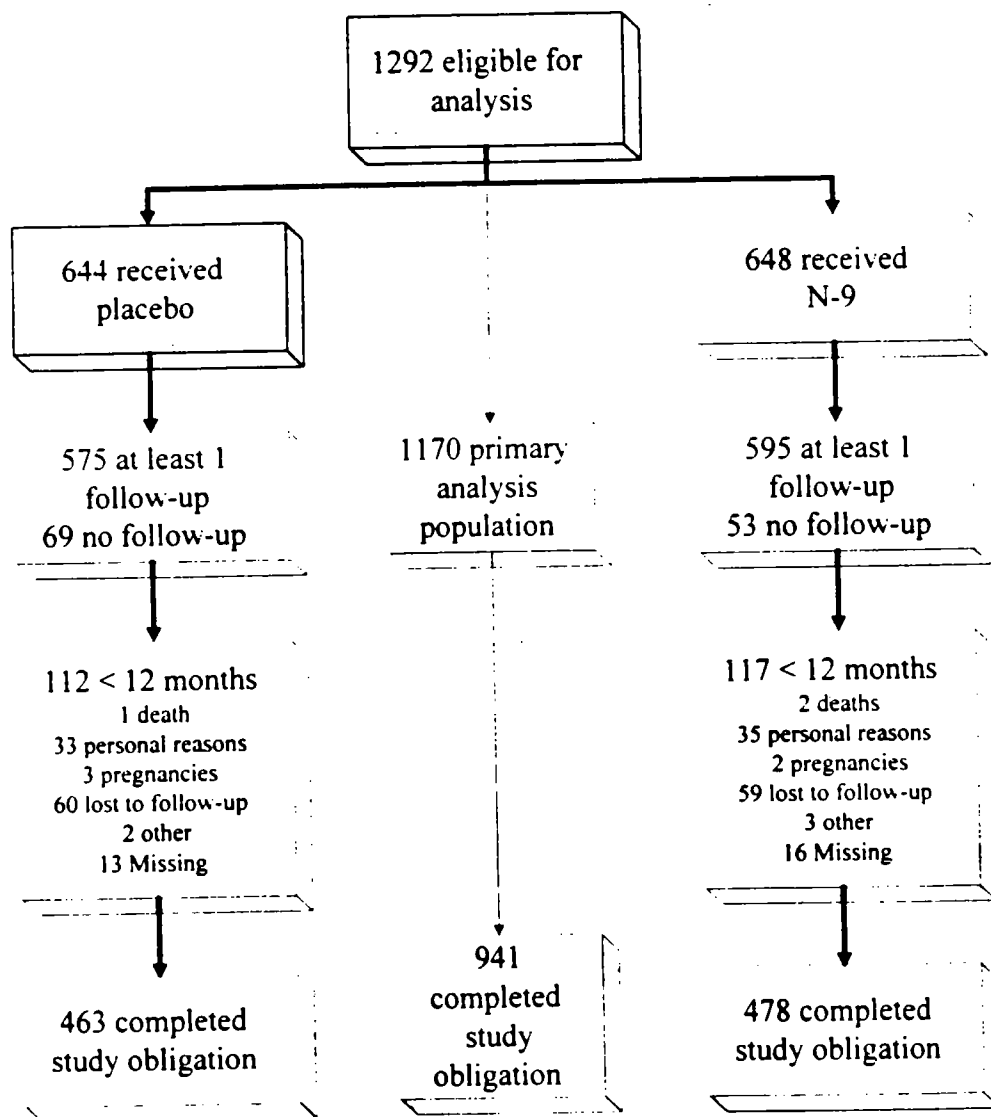
Masking Evidence

Personnel	Number	A %	B %	No opinion %
Counselor	8	13	38	49
Interviewer	9	22	44	34
Outreach workers	68	16	13	71
Data analyst	6	33	0	67
Medical staff	11	18	0	82
Admin staff	15	27	0	73
Lab staff	9	11	0	89
Total	126	18	13	69

Progress through Screening



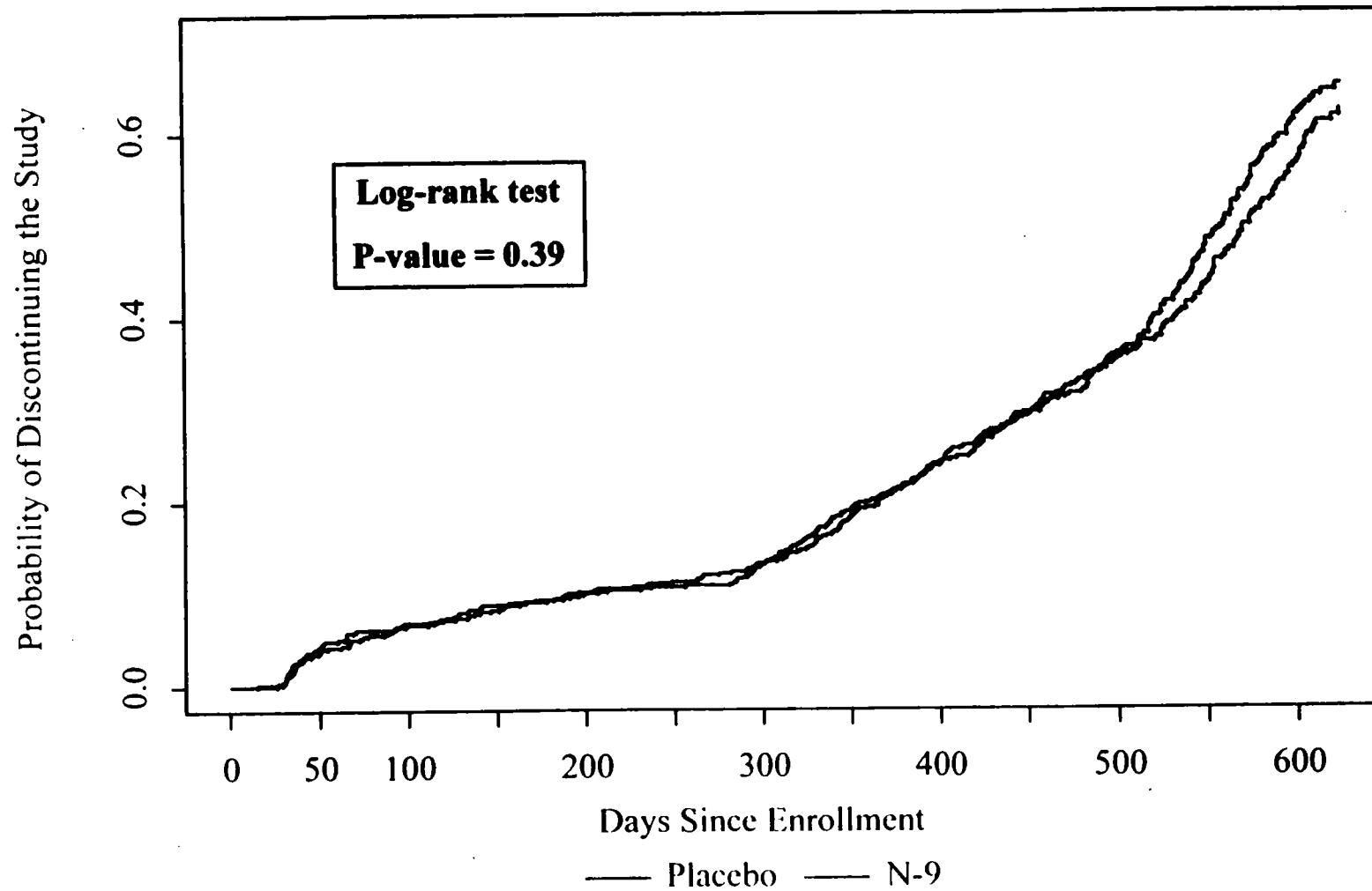
Progress through the Study



Rate of Early Discontinuation

	Number of women	Woman- years	Number of events	Event Rate
Placebo	575	697	268	38.4
N-9	595	720	278	41.0
	Rate Ratio 1.1		95% CI 0.96-1.26	

Study Discontinuation Curves



Demographics

Characteristic	Placebo n = 575 %	N-9 n = 595 %
Age, mean (SD)	25.5 (5.7)	26.0 (5.6)
Current marital status		
not married-NLWM	97	95
not married-LWM	1	2
married - NLWM	2	3
Ability to read		
yes	99	99
Highest education		
none	1	1
primary - middle	73	70
high school	24	27
university	1	1
home economics	1	1
Number of living children, mean (SD)	1.4 (1.5)	1.4 (1.4)

Medical History

Characteristic	Placebo n = 575 %	N-9 n = 595 %
Contraceptive method		
none	87	86
hormonal	5	6
other	8	8
Douching		
yes	25	27
Use vaginal substance		
yes	43	47
STD in last 3 months		
yes	8	10
Ever had STD		
yes	35	32
Medicine in last 30 days		
yes	11	12

Sexual Behavior

Characteristic	Placebo n = 575 %	N-9 n = 595 %
Time as sex worker, mean (SD)	3.7 (3.5)	4.5 (4.05)
Ever had oral sex yes	27	24
Ever had anal sex yes	17	16
Condom used with last client yes	47	49
Condom used with last non- client yes	24	26

Sexual Behavior

Characteristic	Placebo n = 575 %	N-9 n = 595 %
Work from bar yes	79	75
Work from street yes	80	81
Work from hotel yes	43	42
Work from home yes	56	56
Number of non- clients in last 30 days, mean (SD)	1.3 (1.1)	1.4 (1.4)
Number of new clients in last 30 days, mean (SD)	10.4 (14.5)	12.4 (21.5)
Number of repeat clients in last 30 days, mean (SD)	4.3 (4.8)	4.8 (6.5)

Baseline Clinical Examination

Finding	Placebo n = 575 %	N-9 n = 595 %
Vulvar ulcers		
yes	15	15
Vaginal ulcers		
yes	1	1
Cervical ulcers		
yes	5	3
Gonorrhea		
yes	10	13
Chlamydia		
yes	12	11
Vaginal inflammation		
none	94.9	95.1
mild/moderate	4.8	4.7
severe	0.4	0.2

Excluded and Included, Primary Analysis Population

Characteristic	Excluded		Included	
	Placebo	N-9	Placebo	N-9
	n=83 %	n=64 %	n = 575 %	n=595 %
Age, mean (SD)	24.9 (4.9)	25.2 (5.5)	25.5 (5.7)	26.0 (5.6)
Condom used with last client yes	46	49	48	49
Douching yes	24	16	25	27
Use vaginal substance yes	40	43	43	47
STD in last 3 months yes	15	10	8	10
Time as sex worker, mean (SD)	3.8 (3.2)	4.2 (4.8)	3.7 (3.5)	4.5 (4.1)
Ever had anal sex yes	12	16	17	16

Discontinued and Continued, Primary Analysis Population

Characteristic	Discontinued		Continued	
	Placebo	N-9	Placebo	N-9
	n=265 %	n=294 %	n = 306 %	n=298 %
Age, mean (SD)	25.1 (5.4)	25.9 (5.4)	25.8 (5.9)	26.1 (5.8)
Condom used with last client				
yes	48	49	47	48
Douching				
yes	23	28	26	26
Use vaginal substance				
yes	44	46	43	48
STD in last 3 months				
yes	7	11	9	10
Time as sex worker, mean (SD)	3.6 (3.6)	4.4 (3.9)	3.8 (3.4)	4.5 (4.2)
Ever had anal sex				
yes	16	15	19	16

Number of Reported Partners During the Study, Last 30 Days

Type of Sex Partner	Placebo n = 335 Mean (SD)	N-9 n = 330 Mean (SD)
New client	6.5 (8.1)	7.1 (9.0)
Repeat client	4.4 (6.0)	5.0 (6.2)
Non-client	1.5 (1.4)	1.6 (2.2)
Total sex partners	12.4 (11.8)	13.7 (13.6)

Condom and Film Use

	Placebo n = 575	N-9 n = 595
No. of sex acts reported	146,942	147,996
Condom use		
Proportion	88%	91%
Median	90%	91%
25th %	77%	80%
Film use		
Proportion	84%	82%
Median	88%	89%
25th%	74%	75%

Reported Problems and Discomfort

	Placebo n = 575 %	N-9 n = 595 %
Problem with		
Condoms	18	19
Films	20	21
Discomfort from		
Condoms	12	10
Films	13	12

Condom Breakage and Slippage

	Placebo n = 575 %	N-9 n = 595 %
At least one condom broke	64	69
At least one condom slipped off during sex	17	20

Proportions of Condom Breakage

	Number of condoms used	Number of condoms broken	Percent breakage
Clients			
Placebo	89,360	3,527	4.0
N-9	93,113	3,783	4.1
Non-clients			
Placebo	40,333	1,965	4.9
N-9	41,113	1,988	4.8

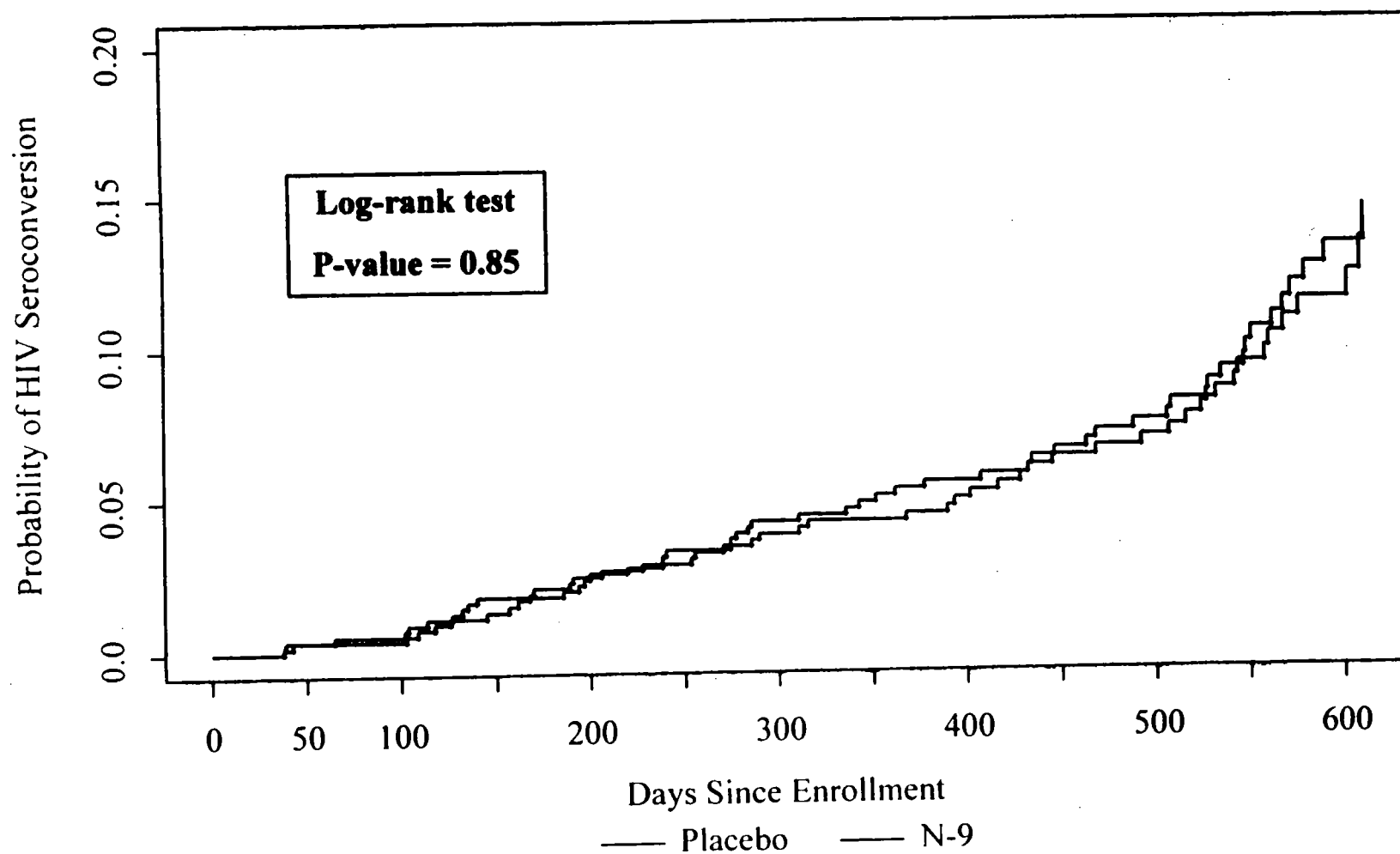
Non-vaginal Sex During the Study

	Placebo n = 575 %	N-9 n = 595 %
Oral sex yes	11	10
Anal sex yes	6	5

Measure of N-9 Effect on HIV Transmission

	No. of women	Woman- years	No. of events	Event rate
Placebo	575	697	46	6.6
N-9	595	720	48	6.7
	Rate ratio 1.01		95% CI 0.68-1.52	

HIV Seroconversion Curves



Primary and Coital Log Populations

Characteristic	Primary		Coital log	
	Placebo	N-9	Placebo	N-9
	n= 575 %	n=595 %	n =407 %	n=407 %
Age, mean (SD)	25.5 (5.7)	26.0 (5.6)	25.5 (5.7)	26.1 (5.7)
Condom used with last client yes	48	49	48	50
Douching yes	25	26	23	26
Use vaginal substance yes	43	47	42	49
STD in last 3 months yes	8	10	9	10
Time as sex worker, mean (SD)	3.7 (3.5)	4.5 (4.1)	3.8 (3.4)	4.6 (4.1)
Ever had anal sex yes	17	15	16	14

Excluded and Included, Coital Log Population

Characteristic	Excluded		Included	
	Placebo	N-9	Placebo	N-9
	n= 164 %	n=185 %	n = 407 %	n= 407 %
Age, mean (SD)	25.5 (5.5)	25.6 (5.3)	25.5 (5.7)	26.1 (5.7)
Condom used with last client yes	46	45	48	50
Douching yes	30	29	23	26
Use vaginal substance yes	46	43	42	49
STD in last 3 months yes	7	12	9	10
Time as sex worker, mean (SD)	3.5 (3.5)	4.2 (3.9)	3.8 (3.4)	4.6 (4.1)
Ever had anal sex yes	21	20	16	14

Film Use Analysis - HIV

Strata	No. Women	Woman- years	No. events	Event rate
100%				
Placebo	162	198	9	4.5
N-9	196	241	12	5.0
75-99%				
Placebo	123	137	11	8.1
N-9	101	112	7	6.2
51-74%				
Placebo	54	61	5	3.4
N-9	37	37	3	8.0
0-50%				
Placebo	51	59	5	8.5
N-9	58	64	10	15.6
No sex				
Placebo	21	25	1	4.0
N-9	17	20	3	15.2
No log				
Placebo	164	218	15	4.2
N-9	186	245	13	5.3

Film Frequency Analysis - HIV

Strata Acts/month	No.Women	Woman- years	No. events	Event rate
0-10				
Placebo	62	72	8	11.1
N-9	63	72	11	15.3
>10-31				
Placebo	182	213	13	6.1
N-9	188	212	14	6.6
>31-53				
Placebo	122	140	8	5.7
N-9	100	112	6	5.4
>53				
Placebo	24	30	1	3.3
N-9	41	59	1	1.7
No sex				
Placebo	21	25	1	4.0
N-9	17	20	3	15.2
No log				
Placebo	164	218	15	4.2
N-9	186	245	13	5.3

Condom Use Analysis - HIV

Strata	No. Women	Woman- years	No. events	Event rate
100%				
Placebo	181	220	12	5.5
N-9	212	270	10	3.7
75-99%				
Placebo	129	151	9	6.0
N-9	111	118	14	11.9
51-74%				
Placebo	47	46	3	6.5
N-9	39	33	4	12.1
0-50%				
Placebo	33	38	6	15.8
N-9	30	34	4	11.7
No sex				
Placebo	21	25	1	4.0
N-9	17	20	3	15.2
No log				
Placebo	164	218	15	4.2
N-9	186	245	13	5.3

Number of Women Using Films and Condoms

Condom Use	0-50%	Film Use 51-74%	75-100%
0-50%			
Placebo	20	4	9
N-9	19	4	7
51-74%			
Placebo	6	18	23
N-9	10	10	19
75-100%			
Placebo	25	32	253
N-9	29	23	271

Measure of N-9 Effect on the Occurrence of Genital Lesions

	No. of women	Woman- years	No. of events	Event rate
Placebo	393	319	107	33.5
N-9	410	306	129	42.2
	Rate ratio 1.26		95% CI 0.97-1.63	

Measure of N-9 Effect on Gonorrhea Transmission

	No. of women	Woman- years	No. of events	Event rate
Placebo	435	357	111	31.1
N-9	441	342	114	33.3
	Rate ratio 1.07		95% CI 0.83-1.39	

Measure of N-9 Effect on Chlamydia Transmission

	No. of women	Woman-years	No. of events	Event rate
Placebo	420	365	81	22.2
N-9	451	384	79	20.6
	Rate ratio 0.93		95% CI 0.68-1.26	

Study Limitations and Strengths

◆ Limitations

- loss to follow-up
- high proportion of condom use

◆ Strengths

- balanced randomization
- effective masking
- large study size

Conclusions

- ◆ **The 70mg N-9 vaginal film**
 - **No effect on the rate of HIV infection**
 - **Increase in rate of genital lesions**
 - **No effect on the rate of gonorrhea**
 - **No effect on the rate of chlamydia infection**
- ◆ **Safe to use but will not prevent STD/HIV**

Clinical and Behavioral Research

CLINICAL TRIALS OF EXISTING PRODUCTS

Title: Phase I Rectal Microbicide Study
Dates: 1996 - 1997
Study Chairs: Connie Celum, Susan Buchbinder
Institution: University of Washington
Mechanism: HIVNET

- Study Summary

HIVNET 008

PHASE I RECTAL MICROBICIDE STUDY

Study Purpose	To assess the safety/toxicity of a nonoxynol-9 (N9) gel for use during anal intercourse among men who have sex with men (MSM), as well as to assess the acceptability of, and compliance with, a regimen of N9 gel use in this population.
Study Rationale	N9 is currently available over-the-counter in a range of concentrations in products for spermicidal or lubricating purposes, although there is a paucity of data on toxicity associated with its use vaginally, and no data on the safety of its use rectally. This study will provide data on the safety of N-9 in a commercially-available gel formulation for use by HIV-negative and HIV-positive MSM during anal intercourse.
Study Chairs	Connie Celum M.D., M.P.H. and Susan Buchbinder M.D.
Participants	Up to 70 men who have sex with men (MSM)
Time Line/ Current Status	Target enrollment of 35 couples was achieved and participant dosing was completed in May 1997. Remaining laboratory assays should be completed by October 1997.
Study Site	University of Washington
Study Design	This is a prospective, frequency escalation study of the safety of Advantage 24, a commercially available contraceptive gel containing nonoxynol-9, for use by MSM during anal intercourse. Successive waves of HIV-seroconcordant MSM couples will use Advantage 24 at increasing frequencies over the course of eight weeks. Participants will apply Advantage 24 daily, and will use it in the context of anal intercourse at least three times per week during the study period. Participants will be counseled to use non-N-9 condoms for all episodes of anal intercourse. Study participants will record their use of Advantage 24, sexual activity, and any health symptoms in daily diaries. Men who apply the product rectally will undergo anoscopy with colposcopy and rectal biopsy to assess the effect of Advantage 24 on the ano-rectal tissue and the rectal mucosa. Men who apply the product to the penis will undergo genital exams and urine leukocyte esterase testing to assess the effect of Advantage 24 on the penile epithelium and urethral mucosa. Each participant will serve as his own control for clinical comparisons of exposed tissue before and after use of Advantage 24. Study participants also will complete interviews and questionnaires on the acceptability of Advantage 24 for use during anal intercourse.

HIVNET 008

Primary Objectives

- ◆ Assess the safety/toxicity of Advantage 24 in escalating frequencies on the rectal tissue of low-risk (monogamous, HIV-seroconcordant) HIV-negative MSM and HIV-positive MSM.
- ◆ Assess the acceptability of, and compliance with, a regimen of Advantage 24 for rectal use in low-risk (monogamous, HIV-seroconcordant) HIV-negative MSM and HIV-positive MSM.

SAFETY, ACCEPTABILITY, AND BIOLOGIC EFFECTS OF NONOXYNOL-9 AS A RECTAL MICROBICIDE.

Celum CL, Tabet SR, Paradise M, Surawicz C, Chesney M, Gross M, Coletti AS, Brar H, Elliott T, Fleming T, Sheppard HW, Rosenberg Z, Levine H, Holt R, and Buchbinder SP from the HIV Network for Prevention Trials (HIVNET).

Objectives: To assess safety, acceptability, and effect of nonoxynol-9 (N-9) on rectal viral STDs when used as a topical rectal microbicide.

Methods: 25 HIV-negative and 10 HIV-positive seroconcordant monogamous male couples were enrolled in a phase 1 frequency use escalation study of N-9 (52 mg/ml) gel applied daily for 6 weeks. 10 HIV-negative couples used 1 applicator/day for 5 weeks escalating to 2 applicators/day for the 6th week, and 25 couples used 2 applicators/day then 4 applicators/day. Men were provided and counseled to use condoms. Safety visits of insertive partners included genital exam and urine LE, and for receptive partners, anoscopy with colposcopy, rectal biopsies and mucosal samples for semi-quantitative assays of HPV DNA, and in HIV-positive men, quantitative HIV RNA PCR and HSV DNA PCR, before and after product use. Daily diaries were kept documenting N-9 use, sexual activity, and symptoms.

Results: 34 of the 35 couples have completed the study as of April 1997. Compliance with product use was high and macroscopic inflammation has been minimal. The most frequent complaint has been rectal fullness after product use among receptive partners. Data from anoscopy, rectal biopsies, and semi-quantitative assays of HPV, HSV, and HIV to assess biologic plausibility will be presented.

Conclusions: The first phase 1 study of a rectal microbicide, nonoxynol-9 gel, provides useful data on safety and effects on rectal viral STDs. The rectal fullness data have implications for acceptability and possible delivery systems for rectal microbicides.

HIVNET 003

PHASE I/II TRIAL OF NONOXYNOL-9 AND HIV INFECTION AMONG PROSTITUTES IN MOMBASA, KENYA

Study Purpose	To determine the safety and toxicity of a vaginal gel containing nonoxynol-9.
Study Rationale	There is a need to identify safe, acceptable, affordable, female-controlled barrier methods for prevention of HIV transmission.
Study Chair	Joan Kreiss, M.D., M.S.P.H., University of Washington
Participants	60 HIV negative commercial sex workers (CSWs) in Mombasa, Kenya
Product Description	Advantage-24 is a vaginal gel containing 3.5% N-9 with approximately 52.5 mg of N-9 delivered each application.
Time Line/Current Status	Accrual and follow-up are complete. First volunteer entered September 1995 and last enrolled in November 1995.
Study Site	Ganjoni Clinic, Mombasa, Kenya
Study Design	

Time and Events Schedule

N-9 Dose <i>n</i> =60	Accrual Target	Days 1-14	Washout Period	Days 1-14
52.5 mg	A		B	
Placebo	B		A	2 weeks

Volunteers are randomized to placebo or N-9 in this cross over design clinical trial.

- Primary Objectives** ♦ To evaluate the safety and toxicity of a vaginal gel containing 52.5 mg of N-9
- ♦ To determine if once-daily dosing of the N-9 preparation is safe for use by women in this population

HIVNET 003

Results

- ◆ 60 subjects were randomized to receive N-9 and placebo; 30 were randomized to receive N-9 followed by placebo and 30 to receive placebo first followed by N-9.
- ◆ 52/60 subjects had 100% follow-up
- ◆ Compliance with gel application was 97% for placebo and 98% for N-9
- ◆ Incidence of genital symptoms was similar during placebo and N-9 periods
- ◆ Four (4) instances of genital epithelial disruption:
 - 3 occurred while on placebo
 - 1 occurred during N-9 treatment
- ◆ No differences in lesions by visual or colposcopic detection

HIVNET 004

EFFICACY OF NONOXYNOL-9 FOR THE PREVENTION OF HIV INFECTION AMONG COMMERCIAL SEX WORKERS IN MOMBASA, KENYA

Study Purpose	To evaluate the effect of intravaginal N-9 gel use on acquisition of HIV and other STDs.
Study Rationale	There is a need to identify safe, acceptable, affordable, female-controlled barrier methods for prevention of HIV transmission.
Study Chair	Joan Kreiss, M.D., M.S.P.H., University of Washington
Participants	638 HIV-negative commercial sex workers (CSWs)
Product Description	Advantage 24 is a vaginal gel containing 3.5% N-9 with approximately 52.5 mg of N-9 delivered with each application.
Time Line/Current Status	Recruitment will begin June 1996 for a period of 9 months. Participants will be followed monthly for 2 years.
Study Site	Ganjoni Clinic, Mombasa, Kenya
Study Design	Randomized double-blind placebo-controlled clinical trial
Primary Objectives ♦	- To determine the effect of intravaginal N-9 gel use on HIV acquisition ♦ To assess the effect of N-9 gel use on the acquisition of other STDs ♦ To monitor safety and toxicity of N-9 gel over a prolonged period of use
	<u>Secondary Objective</u>
	♦ To evaluate factors influencing the acceptability and utilization of spermicides for disease prevention

Clinical and Behavioral Research

CLINICAL TRIALS OF NEW PRODUCTS

Clinical trials of new products are in progress. Ideally the products are/will be non-irritating, safe, and effective in prevention of STDs and/or HIV infection. The availability of products without contraceptive efficacy is a high priority.

- BufferGel Study
- Lactin-Vaginal Suppository Studies

Clinical and Behavioral Research

CLINICAL TRIALS OF NEW PRODUCTS

Title:	BufferGel Study
Dates:	1997
Protocol Team Co-chairs:	Kenneth Mayer, Kenrad Nelson
Institution:	Brown University International HIVNET Sites
Mechanism:	HIVNET

- Study Summary

HIVNET 009

HIVNET Phase I Vaginal Microbicide Study

Study Purpose	To test the ability of a non-detergent microbicide to block transmission of HIV and other acid sensitive STD pathogens.
Study Rationale	There is a need to identify safe, acceptable, affordable, female-controlled barrier methods for prevention of HIV transmission.
Protocol Team Co-Chairs	Kenneth Mayer, MD, Memorial Hospital of Rhode Island, Brown University Kenrad Nelson, MD, Johns Hopkins University
Participants	20 HIV-negative women, 10 sexually abstinent and 10 sexually active, will be enrolled in Rhode Island, U.S. A total of 20 HIV-negative women, 5 sexually abstinent and 15 sexually active, will be enrolled at each of four international sites: Blantyre, Malawi; Chiang Mai, Thailand; Harare, Zimbabwe; and Pune, India.
Product Description	BufferGel is a microbicide based on acidic buffering. BufferGel is an aqueous gel formulated at pH 3.9-4.0 with sufficient buffer capacity to acidify (to pH<5) approximately twice its own volume in human semen. Two additional microbicidal products (to be named) will be tested at the international sites.
Time Line/ Current Status	The domestic phase of the study is complete. Screening and enrollment in the international phase is expected to begin in November 1997, after determination of Highest Practical Dose Frequency, as indicated by the domestic phase of the study.
Sites	U.S. site - Providence, Rhode Island International sites -Blantyre, Malawi Chiang Mai, Thailand Harare, Zimbabwe Pune, India
Study Design	This will be a multi-center Phase I, dose frequency escalation and tolerance study among sexually abstinent and sexually active women.
Primary Objectives	◆ To compare differences in vulvar and cervicovaginal mucosal health at baseline and after 14 to 28 days of study product use. ◆ To assess the local toxicity effects of BufferGel for vaginal use on vulvar and cervicovaginal mucosa in HIV-negative women who are at low risk for HIV infection.

HIVNET 009

Secondary Objectives

- ◆ To collect qualitative data on participants' attitudes and behaviors regarding product use.
- ◆ To assess the acceptability of, and compliance with a short-term regimen of BufferGel for vaginal use in low-risk, HIV-negative women.
- ◆ To examine qualitative aspects in acceptability and compliance as a pilot for substantive qualitative and quantitative assessments in future studies.

Clinical and Behavioral Research

CLINICAL TRIALS OF NEW PRODUCTS

Title: Lactin-Vaginal Suppository Studies

Dates: 1997 - 1999

Principle Investigators: Sharon Hillier, Pamela Murray

Institution: University of Pittsburgh - Magee Women's Hospital

Mechanism: STD Cooperative Research Center
Richard Sweet, Primary Investigator

- Background
- Update - Status of IND

1. Background Overview of Investigational Plan

Not all women who are exposed to a male sexual partner who has a sexually transmitted disease are infected during intercourse. The efficiency of transmission is influenced by a number of factors including the relative virulence of the microorganism, the infectious dose, the humoral and cellular immunity at the mucosal surface and nonspecific defense mechanisms. Nonspecific defense mechanisms include mucins, lysozyme, lactoferrin, zinc, polyamines and components of the indigenous microflora of the vagina.

Lactobacilli function as endogenous microbicides in the human vagina by producing H_2O_2 , organic acids and other factors which inhibit the growth and/or survival of some genital pathogens.(1-6) The broad, long term objective of this study is to evaluate a vaginal suppository containing hydrogen peroxide (H_2O_2) producing lactobacilli for maintenance of an optimal vaginal ecosystem which is resistant to infection. Both *in vitro* (7) and human studies support a role for H_2O_2 -producing lactobacilli in protecting against genital infections.(2,3,8-10) The proposed study is a randomized, double-blinded, placebo-controlled trial of a suppository containing viable *Lactobacillus* as prophylaxis against genital tract infection. Over four years, 924 young women between the ages of 14-21 who attend an adolescent health clinic will be recruited. At enrollment, 462 girls will be randomly allocated to receive *Lactobacillus* and 462 girls will receive identical placebo. Active or placebo suppositories will be self-administered at monthly intervals, and the girls will be followed prospectively for 1 year at 4 month intervals. The working hypothesis is that use of a suppository containing H_2O_2 -producing lactobacilli which colonize the vagina will positively effect the vaginal ecosystem and decrease acquisition of bacterial vaginosis and gonorrhea.

Specifically, we propose to:

- 1) Assess the relationship between genital tract infection and lack of vaginal lactobacilli at enrollment in 924 young women recruited into the treatment trial. We hypothesize that vaginal lactobacilli will be negatively associated with gonorrhea and bacterial vaginosis at baseline.
- 2) Determine the frequency of behaviors associated with lack of vaginal lactobacilli. Specific demographic, medical and behavioral factors to be evaluated include age, race, gravidity, parity, contraceptive method, number of recent sexual partners, douching practices, use of vaginal products, and antibiotic use.
- 3) Assess the effect of monthly prophylactic use of the *Lactobacillus* suppository on the vaginal ecosystem. Characteristics which will be evaluated at enrollment and each follow-up visit include vaginal pH, vaginal lactobacilli (LB^+ and LB^-), anaerobic microorganisms (including *Prevotella*, *Porphyromonas*, and *Bacteroides*), *Gardnerella vaginalis* and genital mycoplasmas.
- 4) Determine whether women randomly allocated to receive the *Lactobacillus* suppository have decreased acquisition of bacterial vaginosis or gonorrhea compared to women receiving placebo, after accounting for other potentially confounding factors such as race, douching and multiple sexual partners.

- 5) Evaluate the effect of the *Lactobacillus* on acquisition of other infections including chlamydia, trichomoniasis, vulvovaginal candidiasis, urinary tract infection and pelvic inflammatory disease. Preliminary data does not support a protective role of endogenous lactobacilli against these infections, but it is hypothesized that monthly use of high concentrations of exogenous lactobacilli may exert a biological effect.
- 6) Determine the demographic, behavioral and microbiologic factors associated with loss or acquisition of H₂O₂-producing lactobacilli. The behavioral and microbiological factors which predict successful colonization by exogenous lactobacilli, or acquisition of lactobacilli from endogenous sources will be evaluated. In addition, the factors related to loss of lactobacilli in the active suppository group and acquisition of lactobacilli in the placebo group will be determined.

Update - Status of IND

Protocol No. 96-014 entitled "A Trial of Microbicidal *Lactobacilli* (Lactin-Vaginal) for Prevention of Genital Tract Infections" is on clinical hold. In order to address concerns, two studies: 1) potency study and 2) dose-ranging study were suggested to be done before initiating this protocol.

Protocol No. 97-022, entitled "A Study of Microbicidal *Lactobacilli* (Lactin-Vaginal) at Two Potencies," a Phase 2 potency study, has been approved; enrollment is underway. This Phase 2 study will:

- address questions regarding the suppository potency and dosage required for vaginal colonization;
- evaluate *Lactobacillus* suppository safety as assessed by colposcopy (to determine if suppository use causes vaginal, vulvar, or cervical irritation) and clinical signs pre- and post-treatment; and
- provide data for comparison of this test article to other vaginal products.

Safety data will be used to support the larger study in young women (96-014).

The second Phase 2 (dose-ranging) study entitled "Dose Ranging Study of Microbicidal *Lactobacilli*" is planned after the potency study. Following the completion of these two studies and contingent upon suppository safety, the large randomized study entitled "A Trial of Microbicidal *Lactobacilli* for Prevention of Genital Tract Infections" will then be conducted.

Clinical and Behavioral Research

BEHAVIORAL RESEARCH

Research on acceptance and compliance as well as other behavioral science questions should be conducted prior to and during all phases of clinical research. In January 1996 The Johns Hopkins University, CDC, and NIAID co-sponsored a workshop to identify key research issues in these areas.

The Office of AIDS Research has provided supplemental funding to examine the acceptability of vaginal products to prevent STDs and HIV infection.

Agenda for Meeting on Behavioral Research
Participant List
Abstract

Social Science Research Priorities for Topical Microbicides
January 15-16, 1997
Cross Keys Inn, 5100 Falls Road, Baltimore, MD

AGENDA

Wednesday, January 15

A.M. Chair: Margaret Scarlett

- 8:30-9:00 Coffee and continental breakfast
9:00-9:15 Introductions and objectives of meeting
9:15-10:15 Roundtable introductions
10:15-10:45 Overview of social science approaches and methods for microbicide research
(P Bentley)
- 10:45-11:00 Break
- 11:00-11:30 Acceptability research: perspectives from the women's health movement
(L. Heise)
11:30-12:00 Contraceptive decision-making models (S. Baker)
12:00-12:30 Ethical issues in safety and efficacy trials for microbicide research (A. Novick)
12:30-1:00 Inventory of social and behavioral research related to microbicides
(L. Sussman and M. Scarlett)
- 1:00-2:00 Lunch

P.M. Chair: Lori Heise

- 2:00-2:15 Introduction to working group objectives (Penny Hitchcock and Lori Heise)
2:15-5:30 Working groups
- Group 1 - Formative research/clinical trials research: social and behavioral questions, methods, and instruments (J. Auerbach, facilitator)
- Group 2 - Formative research/post-marketing research: social and behavioral questions, methods, and instruments (H. Miller, facilitator)
- Group 3 - Formative/clinical/post-marketing research for rectal use of products: social and behavioral questions, methods, and instruments (M. Chesney, facilitator)

Thursday, January 16

A.M. Chair: Linda Sussman

8:30-9:00 Continental breakfast
9:00-10:00 Group 1 feedback and discussion
10:00-11:00 Group 2 feedback and discussion

11:00-11:15 Break

11:15-12:15 Group 3 Feedback and discussion
12:15-1:00 General discussion

1:00-2:00 Lunch

P.M. Co-Chairs: Peggy Bentley and Penny Hitchcock

2:00-5:00 Setting research priorities and mobilizing the research

Adjourn

"Social Science Research Priorities of Topical Microbicides"

January 15-16, 1996

Cross Keys Inn, 5100 Falls Road, Baltimore, MD

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Children's Research Foundation
Adolescent Development and STD Acquisition
Office of AIDS Research Supplement for behavioral research on topical microbicides

This proposal seeks to broaden the original grant entitled, Adolescent Development and STD Acquisition in Females (#AI35087). The current proposal extends the original application with the following specific aims: 1) Among girls who have used a vaginal product (e.g., spermicides and/or fungicides), determine how use and accessibility of the product is related to psychosexual development; 2) Determine the relationship of perception of control over general health and STD acquisition to psychosexual development; 3) Determine the relationship of vaginal product and condom use to perception of control over general health and STD acquisition.

This study will build upon a three year longitudinal study of 174 adolescent girls. The girls are seen every six months, during which time they are interviewed regarding sexual behaviors and attitudes, and if sexually experienced, they receive evaluation for STD. All girls are evaluated for seroconversion for relevant viruses (e.g., herpes simplex virus). The girls will receive reimbursement for transportation. At two visits (six months apart), the core interview from the parent grant will be conducted as well as additional questions regarding vaginal product use, and perception of control over general health and STD acquisition.

At enrollment, the girls had a mean age of 14.5 years; 76% were African-American and 24% were Caucasian. At the time of enrollment, 41% were sexually experienced and 35 of these had a history of a pregnancy, STD, or abnormal Pap smear. By the third study visit, 54% were sexually experienced. Ninety percent of the sample had been seen at least once during the second or third visit for follow-up; 73% had made all three visits.

The results can be used in efforts to enhance an adolescent girl's ability to make reproductive health decisions, including the use of a topical microbicide.¹⁷ The ability to evaluate whether there are subgroup differences based on developmental level or sexual experience will aid the establishment of targeted interventions.

Product Development through SBIR Grants

Section 4

Section 4

PRODUCT DEVELOPMENT THROUGH THE SBIR GRANT

Small Business Innovative Research Grants are awarded to small businesses for product development. The mechanism, either grant or contract, provides for a Phase I award, \$90,000 over a six month period and a Phase II award, \$750,000 over a two year period. With the new FAST TRACK system, an applicant can submit a proposal for Phase I and Phase II simultaneously. If the application is scientifically meritorious, consecutive awards are made. Research and development, manufacturing development and clinical evaluation can be proposed. NIAID has made several SBIR awards for topical microbicide development.

Reprotech
Chrisope Technologies
Demeter

ReProtect, L.C.
225 W. Lafayette Avenue
Baltimore, MD 21217
(410) 523-4267

We propose to develop an improved barrier contraceptive, a spermicidal and microbicidal lubricant to be used with a diaphragm or cervical cap for protection against pregnancy and sexually transmitted diseases including AIDS. This contraceptive consists of a potent acidic buffer that will reinforce the natural mild acidity of the vagina (pH 4), and can block the powerful neutralizing action of semen. Mild acidity kills sperm and inactivates HIV: our pilot experiments also demonstrate that $\text{pH} < 5$ inactivates most major STD pathogens, including leukocytes that can be infected with HIV. Using a mouse/HSV2 model we have demonstrated that this acid-buffering lubricant gives highly significant protection against vaginal and genital skin transmission.

We propose to maximize the acid-buffering capacity of this lubricant by varying its formulation within limits set by rheological and other characteristics. We will test the optimized formulation *in vitro* against STD pathogens, leukocytes, and sperm, use the mouse HSV-2 model to determine its duration of protection against vaginal and genital skin viral challenge, and the rabbit to test its contraceptive efficacy and vaginal toxicity. An IND application will be drafted in preparation for initial safety trials in humans.

Chrisope Technologies, Inc.
3941 Ryan Street
Lake Charles, LA 70605
318-479-1000

The goal of this project is to develop a commercially available vaginal suppository containing a H₂O₂-producing strain of *Lactobacillus* for use in colonization of the vagina. Vaginal lactobacilli are the primary microbial defense against infection. *Lactobacillus* predominant vaginal flora is associated with decreased frequencies of gonorrhea and chlamydial infections. Women with H₂O₂-producing lactobacilli have fewer vaginal infections including bacterial vaginosis and trichomoniasis. While only half of women of child-bearing age are colonized by H₂O₂-producing lactobacilli, there are no commercially available products in the U.S. suitable as vaginal suppositories. During Phase I studies, a strain of *Lactobacillus* was selected based on adherence to vaginal epithelial cells, H₂O₂ production, growth characteristics and viability stability during storage. Pilot production methods to preserve these characteristics in a suppository format were developed. In this study, commercial scale cGMP production and packaging methods for the suppository will be optimized. Animal toxicity studies and a dose-ranging efficacy and safety study in women will be performed. An efficacious and safe *Lactobacillus* suppository for colonization of the vagina could lead to maintenance of an intact vaginal ecosystem resulting in decreased prevalence of genital tract infections among women of childbearing age.

Demeter BioTechnologies, Ltd.
Brightleaf Square, Suite 19-D
905 West Main Street
Durham, NC 27701
919 682-7181

The increasing incidence of sexually transmitted diseases (STD) and women's need to control their own reproductive health are the impetus for development of novel methods of STD prophylaxis and contraception. Peptidyl Membrane Interactive Molecules (***Peptidyl MIMsTM***) from Demeter BioTechnologies Ltd. are unique peptide antibiotics that kill STD-pathogens and sperm *in vitro* at low concentrations while sparing mammalian cells. Phase I experiments will evaluate twenty ***Peptidyl MIMsTM*** for activity against STD pathogens/sperm and vaginal flora. We will perform LD₅₀ on parenteral ***Peptidyl MIMsTM*** to prove the concept and establish initial values for Phase II dosing and toxicity. Phase II pharmacokinetic studies will focus on the bioavailability of compounds administered intravaginally to rats in a new chemically-stable gel formulation. This will include histopathology and other effects of the gel on the vaginal environment. The goal is to develop an effective microbicide and/or microbicide/spermicide with low systemic and local toxicity, minimal absorption and minimal disruption of the vaginal environment and to evaluate the gels' persistence and effectiveness in animal models of trichomoniasis and gonorrhea. Clinical studies would follow.

International Working Group on Microbicides

Section 5

Section 5

THE INTERNATIONAL WORKING GROUP ON MICROBICIDES

The International Working Group on Microbicides (originally International Working Group on Vaginal Microbicides) was established in 1994. The group meets semi-annually to further the global microbicides initiative.

Mission Statement

Current Members

Guidelines for Microbicide Development

INTERNATIONAL WORKING GROUP ON MICROBICIDES

Mission statement:

The international working group on microbicides (iwgm) is committed to the development, production and distribution of safe and effective, acceptable and affordable, vaginal microbicides to prevent human immunodeficiency virus infection and other sexually transmitted diseases. The working group members, experts in relevant scientific areas from governmental and non-governmental organizations, meet regularly to communicate, consult and facilitate collaboration on: (1) scientific advances; (2) technical problems; and (3) needs and opportunities in microbicide product development.

**INTERNATIONAL WORKING GROUP
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April 1997

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Recommendations for the development of vaginal microbicides

The International Working Group on Vaginal Microbicides*

Vaginal microbicides are products for vaginal administration that can be used to prevent HIV infection and other sexually transmitted diseases (STD). We recognize two potential sources of vaginal microbicides: existing spermicides and new products (new products may or may not be spermicidal). This document is meant to serve as a general guide for development and evaluation of existing and new products. For new products preclinical studies will be required. Depending upon indication, *in vitro* activity against HIV, target STD, and sperm should also be assessed. Compatibility with barrier method materials should also be evaluated. The physical-chemical properties of the active agent and the clinical formulation should be assessed. Animals studies should be conducted to assess its safety and predict dosing; use of various models to assess local toxicity is indicated and microbicidal activity of the product may be evaluated if appropriate models are available. Carcinogenicity testing and segment III reproduction studies (perinatal and post-natal studies in rats) may be performed concurrently with Phase III clinical trials. All vaginal microbicides, including existing spermicides and new products, should be clinically evaluated for safety and efficacy. Safety studies are necessary because irritation of vaginal and cervical mucosae has been recently associated with spermicide use and those lesions might increase HIV transmission. Efficacy studies to assess prevention of HIV infection and/or STD, depending upon the product indication, should then be conducted with products that have been evaluated for safety and appear to be non-toxic to tissue. For spermicidal microbicides contraceptive efficacy studies will be needed.

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Keywords: HIV prevention, sexually transmitted disease prevention, vaginal microbicides

Introduction

Heterosexual transmission of HIV is a serious public health concern, as it accounts for about three-quarters of all HIV-1 infections worldwide. Globally, there are currently three men infected for every two women, but by the year 2000 it is projected that number of new infections among women will be close to that among men [1]. While condoms, when used consistently and correctly, are effective in preventing the sexual spread of HIV, there is an urgent need for methods women can use for HIV prophylaxis, such as vaginal microbicides [2].

Vaginal microbicides are products for vaginal administration that can be used to prevent HIV infection and/or other sexually transmitted diseases (STD). An ideal vaginal microbicide would be safe and effective, and also tasteless, colourless, odourless, nontoxic, stable in most climates, and affordable. Currently available spermicides are being clinically evaluated for prevention of infection because their active ingredients — all surfactants such as nonoxynol-9, octoxynol-9, menfegol, benzalkonium chloride or chlorhexidine — have been shown to have antiviral and antibacterial activity *in vitro*. Among the new classes of vaginal microbicides, some products are

From the International Working Group on Vaginal Microbicides, c/o UNAIDS, Geneva, Switzerland. *See Appendix for contributors.

Note: The proposals in this document were endorsed by consultants to and members of the International Working Group on Vaginal Microbicides, but do not necessarily reflect the policies of their respective agencies and/or organizations.

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not spermicidal and may not interfere with procreation: for example, inhibitors of virus absorption, defensins and inhibitors of HIV reverse transcriptase [3-6].

The International Working Group on Vaginal Microbicides (IWGVM) was formed in November 1993 at a meeting held at the World Health Organization (WHO), Geneva, Switzerland. The goal of the IWGVM is to facilitate the development, production and distribution of safe, acceptable, effective, and affordable vaginal microbicides to prevent HIV infection and other STD. The working group members, experts in relevant scientific areas from governmental and non-governmental agencies, meet regularly to facilitate communication, consultation, and collaboration on scientific advances and technical problems in microbicide development, and to identify needs and opportunities to promote product development.

The present document outlines the guidance the IWGVM has developed on the evaluation of vaginal microbicides.

Preclinical considerations

This section presents steps to be considered in preclinical development of new vaginal microbicides for the prevention of HIV and STD. It is less relevant to the evaluation of existing spermicides as vaginal microbicides. For example, in the United States, the Food and Drug Administration (FDA) does not require pharmacology/toxicology studies for evaluation of nonoxynol-9 and octoxynol-9 as spermicides, and perhaps as vaginal microbicides [7]. In Europe and Japan regulatory authorities might rule similarly for approved spermicides. However, it might be wise to do some of the tests proposed in this section with spermicidal products to avoid toxicity problems in human studies and to improve prospects for developing a safe, effective microbicide. Indeed, several currently marketed spermicidal products have been associated with genital mucosal lesions [8-11].

The preclinical guidance provided here is a list of studies which may be valuable and performed prior to, or concurrently with, clinical studies. Since candidate products will have different active agents with unique mechanisms of action and formulations, preclinical approaches will have to be tailored for the specific product. The precise studies that will need to be performed will also depend on several factors including (1) the indication for which the product will be tested and marketed, and (2) the regulatory requirements of the host/sponsoring country. Therefore, a product sponsor should contact regulatory agencies about specific requirements for approval of its product as soon as its potential has been delineated.

In vitro activity and safety

The tests described in Table 1 are designed to obtain information regarding the products' potential for prevention of HIV and STD. If the product indication is for the prevention of HIV infection, *in vitro* assays will include a variety of HIV strains (both laboratory-adapted and clinical isolates). Since the precise mechanism of mucosal transmission of HIV is unknown, tests for evaluating prevention of cell-associated HIV transmission are also recommended. It is desirable that an agent be evaluated for activity against HIV and other STD regardless of its intended HIV indication since a clinical outcome of HIV prevention may be achieved by the prevention of other STD [12,13]. Similarly, data on the effect of the agent on lactobacilli as a surrogate for normal vaginal flora is desirable.

Table 1. Recommended tests for *in vitro* activity*.

Activity against HIV
• Laboratory-adapted HIV virus in T-cell lines
• Laboratory-adapted HIV virus in peripheral blood mononuclear cells
• Clinical HIV isolates (depending upon the microbicide and the mechanism of action, it may be appropriate to include drug-resistant isolates)
• Activity against cell-associated virus
• Antiviral activity in semen and, if possible, vaginal fluids or in an <i>in vitro</i> system that is physiologically appropriate
Activity against other sexually transmitted pathogens
• <i>Neisseria gonorrhoeae</i>
• <i>Chlamydia trachomatis</i>
• <i>Haemophilus ducreyi</i>
• <i>Trichomonas vaginalis</i>
• Herpes simplex virus
Activity against other vaginal organisms
• <i>Lactobacillus crispatus</i>
• <i>Candida albicans</i>

*Tests should be performed on active agent and clinical formulation; if the microbicide contains more than one active agent, each must be tested separately.

As vaginal microbicides should be compatible with other methods, such as the male or female condom used to prevent HIV and STD transmission, it is suggested that the both active ingredient and the final formulation be evaluated for compatibility with latex, polyurethane or other physical barrier materials be assessed early on during preclinical development.

In vitro mutagenesis studies can be done in parallel with Phase I clinical studies, and should include gene mutation tests, tests for chromosomal abnormalities and primary DNA damage assays.

Physical-chemical considerations

Clinical formulations must be produced under good manufacturing practices. The properties of the active agent should be known. Well-characterized analytical methods to detect and measure the physical-chemical properties of the active agent are essential for product

Recommendations for the development of vaginal microbicides

development. For example, quantitative analytical methods will be needed to determine drug diffusion and potential for systemic absorption of the active agent, and to meet the Standard Chemistry Requirements (Chemistry, Manufacturing, and Control section of the Investigational New Drug application to the FDA in the USA and similar requirements of regulatory bodies elsewhere).

As the combination of the active agent and a delivery vehicle may result in alterations in physical-chemical properties and microbicidal activity, it is recommended to assess the stability (real and accelerated time) of both clinical formulation and vehicle alone, to do formulation release studies (i.e., the kinetics of release of active agent from solid and semi-solid formulations), and to assess *in vitro* activity against the target pathogens, if possible, in comparison with active agent alone.

Animal studies

Due to the potential for increased HIV transmission in the presence of significant cervical/vaginal inflammation and ulceration, it is recommended that the active agent and the clinical formulation of the product be tested in a rabbit vaginal irritation model (standard 10-day application of active agent and/or preclinical formulation) early in the development process. A 4% nonoxynol-9-containing spermicidal gel should be used as a positive control. If significant irritation occurs, termination of product development may be warranted.

In addition, systemic absorption and the toxicity of the formulation to the rectal mucosa, pubic and other skin areas should be assessed in animal models. Oral toxicity studies, and gross necropsy or more specialized studies, if warranted (including penile irritation and absorption in male rabbits), may be performed concurrently with or after Phase I clinical studies depending upon the topical microbicide. Systemic toxicity studies in one species with single dose administration, hypersensitivity and photosensitivity studies, and segment I reproductive toxicology (spermicidal activity) are required prior to Phase I, and segment II reproductive toxicology studies (standardized tests in two animal species) may be performed subsequent to Phase I studies in humans [14].

Toxicology studies in a rodent and non-rodent species (possibly concurrent with clinical trials) with duration equal to intended duration of clinical studies (up to 12 months) should be performed. Pharmacokinetics data (serum drug levels, maximum concentration, area under the curve, tissue distribution and metabolite profiles) should be presented and compared to human data. If Phase I trials establish that the drug is absorbed in humans and the mucosal route of delivery in animals cannot achieve much higher blood levels than those seen

in Phase I, a 1–3 month toxicity study during which the clinical formulation is given parenterally or orally may be required to identify all potential toxicities.

If possible, animal model data on the product's potential efficacy should be obtained. Examples of animal models to be considered are listed in Table 2.

Table 2. Examples of animal models for testing the efficacy of clinical formulations against HIV and sexually transmitted diseases (STD).

HIV
• Simian immunodeficiency virus in macaques
• Feline immunodeficiency virus in cats
STD
• Genital herpes in guinea pigs
• <i>Chlamydia trachomatis</i> in mouse or primate
• <i>Haemophilus ducreyi</i> in rabbits
• <i>Treponema pallidum</i> in rabbits
• Human papillomavirus in nude mice
• Rabbit papillomavirus model

Newer models, such as the mouse herpes simplex virus genital infection models, are presently under development and may be considered.

Carcinogenicity testing is necessary, but may be performed concurrently with Phase III clinical trials. Such testing should involve two animal species (rats and mice), and intra-vaginal administration for up to 2 years at maximum tolerated dose. Segment III reproduction studies (perinatal and post-natal studies in rats) can be performed concurrently with Phase III clinical trials [14].

Clinical considerations

Like all clinical trials, trials on vaginal microbicides should be conducted in accordance with the current version of the Declaration of Helsinki and the Good Clinical Practice guidelines applicable in the country where the trial will take place [15]. In particular, the studies must be ethically sound and be reviewed in advance by an institutional (ethical) review board or other appropriate group, employ informed consent, assure confidentiality, and be monitored for the completeness and accuracy of study data. A standardized approach to collecting all clinical data, administering subject interviews and analysing data should be used. Laboratory tests should be conducted in laboratories with proper quality assurance procedures. A Data and Safety Monitoring Board (DSMB) composed of a group of physicians, epidemiologists, statisticians and ethicists should oversee the conduct of all long-term clinical trials. The DSMB should review the study at intervals specified in the protocol and advise the sponsors on modification or termination of the trial according to rules for termination of the study defined beforehand.

Use of currently available spermicides or new vaginal microbicides may cause changes and even lesions of the genital mucosa [8–10]. Not knowing what role, if any,

these play in the transmission of HIV or STD, the IWGVM believes that information on the status of the genital mucosa must be collected both during safety and efficacy studies on vaginal microbicides. Because changes in genital mucosa may occur independently of symptoms, assessment of the genital mucosa should be by objective methods, such as vaginal speculum examination, while irritation/inflammation should be assessed in Phase I and II studies using colposcopy.

Community-based organizations should be involved as much as possible in the design and implementation of the studies, especially with regard to its feasibility and acceptability, including establishment and maintenance of the appropriate clinical and social infrastructure for the trial.

Design and implementation of Phase I, II and III clinical trials

Phase I trials

The main objectives of Phase I trials are usually (1) to gather initial information on the incidence and extent of toxicity for a product that has never been used in humans, (2) to obtain data on the pharmacokinetics of the product, and (3) to aid in the selection of a dose for subsequent studies. Open-label studies, some probably with dose escalation, in a small number of healthy, non-pregnant female volunteers not at risk of pregnancy or STD, who would use the study drug for one to a few days (per dose-escalation level) should be adequate. If the main objective of the study is to gather safety information, colposcopy should be used to assess the status of the genital mucosa. Systemic toxicity of the product should be assessed by appropriate laboratory tests. Volunteers in early Phase I studies of a new product should abstain from vaginal intercourse thereby avoiding its potential confounding effect on the incidence of genital lesions.

Phase II trials: further safety studies and pilot studies

After Phase I studies are completed, the product's safety should be confirmed in larger studies among healthy, non-pregnant female volunteers not at risk of pregnancy or STD. Ideally the study should be a randomized, double-blind comparison of the new product to placebo (i.e., the product formulation without its active ingredient) or to a reference product on one hand, and to an untreated group on the other. Use of a placebo group would enable assessment of the safety characteristics of the vehicle; this information is critical to future studies. A cross-over design, in which the product, placebo and non-use would be given sequentially, would also be acceptable. However, subject dropouts and the need to include a wash-out period for products with persistent effects limit the utility of this design and should be taken into consideration. Finally, the utility of open-label stud-

ies is limited because findings cannot be compared to the placebo. When participants in Phase II studies have sexual intercourse and lubricated condoms are used, the lubricant should contain no spermicidal products.

Study should be designed in collaboration of biostatisticians. The appropriate sample size, duration of the study, and virtually all other aspects of the study design will be effected by many factors, for example, product characteristics, outcome measures, and will require biostatistical expertise.

When the safety of the study drug has been documented in low-risk, healthy women, it is recommended that additional safety studies be conducted in other populations prior to or at the beginning of Phase III studies; safety should be monitored during the Phase III study as well. Examples of other populations of women include commercial sex workers, pregnant women, HIV-infected women, women using reproductive hormones, intrauterine device users, intravenous drugs or crack cocaine users, peri-menopausal or post-menopausal women, and breastfeeding women. Consideration should also be given to an assessment of the product's administration via rectal route. Finally, it is suggested that all clinical studies should be regarded as opportunities to collect data about product acceptability and its ease of use.

Phase III studies

Phase III studies aim to assess the balance between efficacy and toxicity of a new product. For vaginal microbicides, Phase III studies should determine whether the product will prevent heterosexual transmission of HIV and/or other STD depending upon the product among women at high risk of acquiring those infections. The incidence of adverse events (i.e., potential toxicity) should be assessed to obtain information on the long-term safety of the product. Phase III studies should be randomized, double-blind, controlled trials. Again, the study should be designed with biostatisticians. Phase III studies will need to be sufficiently large to assess the risks and benefits of a product.

Furthermore, inconsistent use of the product, and use of condoms or other interventions such as treatment of incident STD will complicate analyses. As male condoms are the only currently available effective method to prevent heterosexual transmission of HIV, condom use must be recommended and condoms made available to all study participants. In other words, the study design will test whether the microbicide will improve the protection afforded by condom use. If in the future, a vaginal microbicide were shown to be effective in preventing HIV infection, placebo-controlled trials would become inappropriate and new microbicides should be compared to the vaginal microbicide with known efficacy. Phase III studies will also enable an assessment of product acceptability for women and men, compliance with product use, effects on the male partner, and pregnancy rates, if appropriate.

Recommendations for the development of vaginal microbicides

Study populations

Study participants for Phase I and initial Phase II studies should be volunteers at low risk of HIV infection and STD, ideally of reproductive age (i.e., 18–45 years of age). Participants in Phase III studies should be HIV-uninfected women at high risk of acquiring HIV infection and other STD through vaginal intercourse.

Depending on the microbicide being evaluated and study type, women with systemic diseases, women who use other drugs that could modify the pharmacokinetics or the effects of the study product, or women who are pregnant or nursing (unless the product has been demonstrated to be safe for administration in these situations) may need to be excluded from the study. Women presenting with clinically apparent genital infections and/or lesions at enrollment should be treated prior to inclusion. For initial safety studies, the exclusion criteria may need to be more rigorous, and it may be necessary to exclude women with (1) abnormal liver or renal function; (2) a history of genital problems; (3) colposcopic abnormalities; (4) other STD; and (5) continued use of other vaginal products such as douches, tampons, or spermicides (during the study period).

Excluding HIV-infected subjects from participation in Phase III studies may not be possible, mainly for confidentiality considerations. However, these participants will contribute safety information and may contribute to assessment of the products' efficacy against other pathogens. Because of the ease of intravenous or rectal HIV transmission, it is also preferable to exclude women who are intravenous drug users or who frequently and regularly practice anal intercourse. As it is not possible to ensure that the participants will not engage in these high-risk behaviours, it is recommended to quantify the incidence of high-risk behaviours in Phase III studies.

Study outcomes and their detection

It is recommended that histories on the incidence of vaginal discharge, genital discomfort (including vaginal pain and burning sensations in the vagina and/or the vulva), vaginal dryness, painful urination or intercourse, lower abdominal pain and genital infection be collected in all studies on vaginal microbicides. Reasons for discontinuation should be recorded and adverse events documented.

Toxicity to the genital mucosa should be assessed by objective methods. Colposcopy should be used in initial safety studies. In later studies, vaginal speculum examination may be sufficient, but it is probably wise to monitor local safety with colposcopy in a subset of participants, as recommended for the development of new spermicides [4]. Colposcopic examination should follow the WHO manual for the standardization of colposcopy (available

on request from UNAIDS). This manual describes the lesions as ulcers, abrasions, ecchymotic, and petechial haemorrhages, sub-epithelial haemorrhages plus oedema, erythema, oedema, or abnormal vaginal and cervical discharge. Signs of cervical/vaginal dysplasia, neoplasia or metaplasia are not described in the WHO manual but would be recognized by a skilled colposcopist and should also be recorded. In Phase I/II safety studies, the toxicity to the genital mucosa should be evaluated between menses, to avoid difficulties assessing the genital mucosa when menses are present.

In studies focusing on the assessment of safety of the product to the genital mucosa the frequency of clinical, gynaecological, and colposcopic examinations should be frequent, not less than once every 7–10 days. When genital lesions occur, more frequent examinations may be necessary. Systemic toxicity should be assessed using appropriate laboratory tests, at least at enrollment and at the end of each observation period. Assessment of STD and other genital infections is recommended to exclude them as confounders in the analysis of the data.

In Phase III studies, these evaluations should take place at study enrollment, and at regular intervals (possibly monthly) thereafter, for as long a follow-up period as possible. Currently the populations that would be appropriate for Phase III studies (those with high sero-incidence rates) may be difficult to follow. It is anticipated that follow-up will not, in most cases, exceed 1 year; but every effort should be made to improve it by establishment of appropriate infrastructure. Evidence of local toxicity should be sought by pelvic examination (and colposcopy in a subset of participants if possible). Screening for incident HIV infection should be with state-of-the-art laboratory methods, such as third-generation HIV serologic tests, HIV culture, HIV antigen detection and polymerase chain reaction. The diagnosis of STD should be by using state-of-the-art laboratory techniques. Organisms to be considered to measure the incidence of STD include (1) *Trichomonas vaginalis*, (2) *Neisseria gonorrhoeae*, (3) *Chlamydia trachomatis*, (4) *Treponema pallidum*, (5) *Haemophilus ducreyi*, and (6) herpes simplex virus. Compliance with both condom and microbicide use could be assessed with coital log charts and subject interviews. Incidence of anal intercourse and intravenous drug use during the study should be obtained and recorded. Pregnancies should be determined by testing. If the male partner can be evaluated, genital irritation and infections that are reported should be confirmed if possible.

Handling of participants who develop adverse reactions

Participants should discontinue use of study products immediately when a serious adverse reaction occurs [16].

In Phase I and Phase II studies, study products should be discontinued when genital ulceration occurs. When genital abrasion, petechial haemorrhage, ecchymosis, or subepithelial haemorrhage and swelling occurs, study products should be discontinued if after 24–72 h of continued study product use the condition worsens.

When in a Phase III study, a genital ulceration occurs, study products may continue but treatment for bacterial causes of genital ulceration (chancroid and/or syphilis) should be given immediately. If the ulcer worsens after 7 days, use of study products should be discontinued, at least until the lesion is cured. When other genital lesions occur, study products should be discontinued if, after 7 days of continued study product use, the condition worsens. Product use may be resumed after the lesion resolves.

Women who discontinue therapy for any reason should be encouraged to continue follow-up to assess genital irritation, to detect potential toxicity that might arise after cessation of study drug use, and to assess their STD and HIV outcomes for intention-to-treat analyses.

References

- Mertens TE, Belsey E, Stonebuner R, et al.: Global estimates and epidemiology of HIV-1 infections and AIDS: further heterogeneity in spread and impact. *AIDS* 1995, 9 (suppl A):S259–S272.
- Stein ZA: HIV prevention: the needs for methods women can use. *Am J Public Health* 1990, 80:460–462.
- Elias C, Heise L: *The Development of Microbicides: A New Method of HIV Prevention for Women. Program Division Working Papers [No. 6]*. New York: The Population Council; 1993.
- Global Programme on AIDS, World Health Organization: *Report on a Meeting on the Development of Vaginal Microbicides for the Prevention of Heterosexual Transmission of HIV*. Geneva: GPA/WHO; 1993. [Document WHO/GPA/RID/CRD/94.1; 11–13 November 1993].
- Hitchcock PJ, Claypool L: HIV and other STD: Working Group Report. In *Barrier Contraceptives*. Edited by Mauck C, Cordero M, Gabelnick H, Spieler JM, Rivera R. New York: Wiley-Liss; 1994:353–362.
- Stone AB, Hitchcock PJ: Vaginal microbicides for preventing the sexual transmission of HIV. *AIDS* 1994, 8 (suppl 1):S285–S293.
- US Food and Drug Administration: *Guidance for the Development of Vaginal Contraceptive Drugs (27 January 1995)*. [Clarifications: 8 February 1995]. Washington, DC: US Government; 1995.
- Niruthisard S, Roddy RE, Chutivongse S: The effects of frequent nonoxynol-9 use on the vaginal and cervical mucosa. *Sex Transm Dis* 1991, 18:176–179.
- Roddy RE, Cordero M, Cordero C, Fortney JA: A dosing study of nonoxynol-9 genital irritation. *Int J STD AIDS* 1993; 4:165–170.
- Kreiss J, Ngugi E, Holmes K, et al.: Efficacy of nonoxynol-9 contraceptive sponge use in preventing heterosexual transmission of HIV in Nairobi prostitutes. *JAMA* 1992, 268:477–482.
- Goeman J, Ndoye I, Sakho LM, et al.: Frequent use of menfegol spermicidal vaginal foaming tablets associated with a high incidence of genital lesions. *J Infect Dis* 1995, 171:16–24.
- Laga M, Manoka A, Kivuvu M, et al.: Non-ulcerative sexually transmitted diseases as risk factors for HIV-1 transmission in women: results from a cohort study. *AIDS* 1993, 7:95–102.
- Grosskurth H, Mosha F, Todd J, et al.: Impact of improved treatment of sexually transmitted diseases on HIV infection in rural Tanzania: randomised controlled trial. *Lancet* 1995, 346:530–536.
- Federal Register Notice: *International Conference on Harmonization — Guideline on Detection of Toxicity to Reproduction for Medicinal Products (22 September 1994)*. Washington, DC: US Government; 1994.
- World Medical Association Declaration of Helsinki. In *International Ethical Guidelines for Biomedical Research Involving Human Subjects*. Geneva: Council for International Organization of Medical Sciences; 1993:47–50.
- World Health Organization: *World Health Organization Guidelines on Good Clinical Practice*. Geneva: WHO; 1995. [WHO Technical Report Series No. 850, Annex 3].

Appendix

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Scientific Considerations

Section 6

Challenges for the development of female-controlled vaginal microbicides

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Introduction

Several authors have presented a strong case for developing an HIV prevention technology within the personal control of women [1-4]. The proponents of such technology acknowledge the significant and growing risk of HIV infection faced by women throughout the world and, simultaneously, recognize the serious limitations of current AIDS prevention strategy. Attempts to limit the spread of HIV infection primarily through encouraging a reduced number of sexual partners, widespread condom promotion, and the control of other sexually transmitted diseases (STD) have proven inadequate for many women worldwide [1,5,6]. Underlying gender power inequalities severely limit the ability of many women to protect themselves from HIV infection, especially in the absence of a prevention technology they can use, when necessary, without their partner's consent. The development of new prevention methods controllable by women would fill an important gap in the global response to the AIDS pandemic. This review outlines the challenges to be faced in developing microbicides — pharmaceutical products that would reduce a woman's risk of acquiring HIV and, potentially, other STD when applied intravaginally.

The principal challenge

Globally, most women are at greatest risk of acquiring

HIV infection through heterosexual vaginal intercourse with an infected man. To avoid infection via this route, a prevention method must establish an effective barrier between the infectious elements in the male genital secretions/ejaculate and those cells of the female reproductive tract susceptible to infection. Such a barrier might be physical (such as that provided by condoms), chemical (such as that provided by an intravaginal microbicide), or immunological (such as the mucosal immunity that could result from an effective vaccine). Ideally, such a barrier would be selective, providing effective protection against HIV and other STD without impairing a woman's ability to conceive. Such an ideal barrier could be combined with a spermicide (or another method of birth control) for use when contraception was explicitly desired. Given our limited knowledge regarding several critical questions of reproductive biology, the feasibility of developing non-contraceptive microbicides is unresolved. Compared with contraceptive microbicides, the pursuit of non-contraceptive products presents a number of additional challenges and will most certainly take a longer time to be realized, but is an essential goal when viewed from the perspective of women who desire conception but still require protection against HIV. Ultimately, vaginal microbicides could be made available in a range of product formulations — including gels, foams, suppositories, films — to match women's preferences for acceptable and optimal use.

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Biological issues

Developing a new microbicide compound for preventing the sexual transmission of HIV depends on resolving a number of undetermined scientific issues. These range from defining more precisely the chemical and physical properties of potentially microbicide compounds and product formulations, to elucidating the exact biological mechanism of HIV transmission. Despite a considerable amount of research effort, much remains to be learned about the basic biology of the sexual transmission of HIV [7,8].

Evidence suggests HIV-infected lymphocytes and macrophages are primary infectious elements in semen [7,9]. These immune cells are abundant in normal semen and have been shown to increase in the presence of genital tract inflammation (such as that resulting from common STD) and HIV infection [10]. Significant amounts of cell-free virus have also been found in semen [11–13]. Discerning whether HIV transmission involves cell-associated virus, cell-free virus, or both, is a critical question for microbicide development. For example, if it were convincingly demonstrated that virus associated with immunologically competent cells, such as lymphocytes and macrophages, was the predominant mechanism of sexual transmission, this would have significant implications for the types of microbicide compounds one would pursue. In such a scenario, the virucidal action could target primarily the infected host cells instead of the virus itself.

Some recent evidence suggests that cell-associated virus is a principal means of HIV transmission. At The Population Council, Phillips *et al.* have developed an *in vitro* model of viral transmission using a confluent epithelial cell culture [14–16]. When HIV-infected CD4 lymphocytes were added to this culture system, lymphocytes were soon observed in close association with the epithelial surface. Subsequent observation by electron microscopy demonstrated the directional release of mature viral particles into the space between the lymphocyte and epithelium, as well as the rapid uptake of virus by the epithelial cells. In contrast, when cell-free virus was added to the system, infection of epithelial cells was found to be a relatively rare event.

It is also well established that Langerhans cells are present in the vagina and cervix and could be recipients of cell-free or cell-associated HIV [17,18]. One possible scenario is that cell-associated virus is primarily responsible for transmission across intact epithelial surfaces, but that cell-free virus can infect mucosa when present in high concentration or when epithelial surfaces are disrupted. Studies of discordant couples, for example, have demonstrated that older/post-menopausal women are at increased risk of HIV seroconversion [19,20], a finding attributed to their increased risk of epithelial disruption dur-

ing sex because of low estrogen levels and atrophic vaginal mucosa. This may also partially explain why HIV transmission is augmented in the presence of genital ulcerative diseases that result in mucosal disruption.

This scenario might also help to explain the conflicting data regarding the safety and efficacy of currently available vaginal spermicides in preventing HIV infection [21,22]. Two recent observational studies in sub-Saharan Africa (one among sex workers and another among discordant couples) have suggested that consistent use of vaginal spermicides containing nonoxynol-9 may provide partial protection against HIV infection [23,24]. These studies contradict a randomized controlled trial among female sex workers in Nairobi which found that daily use of the nonoxynol-9-impregnated 'Today' sponge appeared to be associated with increased risk of HIV seroconversion [25]. These conflicting data have prompted a variety of interpretations and recommendations regarding the relative safety and efficacy of existing vaginal spermicides as methods of HIV prevention [21,22,26–28].

More recent studies have suggested that the toxicity of nonoxynol-9 may be related to the dose and/or frequency of application [23,29–31]. These results suggest that, to the extent that nonoxynol-9 does cause mucosal inflammation, micro-ulcerations, or sores, the effects may be limited to situations of high-dose and/or high-frequency use. It remains to be established, however, whether nonoxynol-9 offers women measurable protection from HIV in typical use and at what point the negative consequences of potentially disrupting the epithelium outweigh the positive benefits of killing the virus. Given the non-selective detergent properties of compounds such as nonoxynol-9 and other available spermicides, there may be a narrow range between 'enough' and 'too much' microbicide spermicide.

This line of reasoning suggests the need to develop more specific intravaginal compounds that prevent the attachment of HIV-infected lymphocytes to epithelial surfaces, the secretion of virus from these cells, or the uptake of virus by the mucosal epithelium, without indiscriminantly disrupting cell membranes. One such class of compounds may be polyanionic polysaccharides, such as dextran sulfate. Dextran sulfate has been demonstrated to inhibit HIV replication *in vitro* [32,33]. A broad range of polyanionic polysaccharides have also been shown to inhibit the cell-associated transmission of HIV using the *in vitro* epithelial cell culture model described above [34]. This activity has been demonstrated at concentrations 100–1000 times lower than those associated with significant cytotoxicity. The hypothesized mechanism of action of these polyanionic compounds is that, by coating the epithelial surface with a highly charged macro-

molecular film, they may physically repel HIV and HIV-infected cells from the epithelium and thereby prevent the directional release of HIV that occurs when these infected lymphocytes attach to mucosal cells. Many polyanionic polysaccharides are currently used as food additives and, as non-detergent compounds, they could have considerably less local toxicity than existing vaginal spermicides. It should be emphasized, however, that although this is certainly a promising class of compounds to begin evaluating, there are currently no clinical data regarding their safety or effectiveness for intravaginal use in preventing HIV transmission.

Another basic issue related to the biology of HIV transmission is our incomplete understanding of the precise cells and tissues within the female reproductive tract that are infected in the course of transmission. Disruptions of epithelial surfaces probably augment susceptibility to infection, but data from animal models, as well as case reports of infections acquired during artificial insemination, indicate that such disruption is not required for transmission to occur [35,36]. Phillips *et al.* [15] suggest that cell-associated virus may infect typical mucosal cells. But does this happen throughout the vagina? Is the cervix a more or less susceptible site? What is the role of the upper genital tract structures, if any? A recent study of HIV transmission in hysterectomized macaque monkeys suggests that the presence of a cervix is not required for transmission [37]. However, this finding may not easily generalize to humans, and even if applicable does not exclude an additional (and potentially more important) route of infection via the cervix or upper genital tract. Answers to such questions of basic biology are essential to understand fully the interactions of various contraceptive methods with HIV infection, as well as the possibility of identifying a non-contraceptive microbicide [7,4].

Another important issue that bears directly on the feasibility of developing a non-contraceptive microbicide is the question of whether HIV infects or attaches to sperm. Several authors have suggested that HIV may become associated with sperm, either by attaching to the surface or crossing the cell membrane [33,38]. Others have refuted these findings after failing to find a physical association between HIV and sperm in assays used to separate motile sperm from other elements of semen, as well as failure to identify HIV DNA using genetic amplification techniques on the sperm fractions of semen from more than 200 seropositive men [9]. Current opinion favors the lack of a significant relationship between HIV and viable sperm, but the data are by no means complete, leaving open the possibility of an uncommon but perhaps biologically relevant association [39]. Obviously, a significant association of virus with sperm would make the development of a non-contraceptive microbicide extremely problematic.

Emerging data further suggest that the complex chemical, microbiological, and immunological environment of the male and female reproductive tracts may play a major role in determining the likelihood of HIV transmission. Understanding the ecology of the vaginal environment is essential for future microbicide development, because any compound introduced intravaginally has the potential to disrupt the balance of vaginal flora, pH, or immune function. For example, recent evidence has shown that the normally low pH of the vagina may be virucidal in its own right [40,41]. Voeller and Anderson have shown that at pH levels below 5.5 both HIV and HIV-infected host cells are rapidly inactivated. Semen typically neutralizes the normally acidic pH of the vagina. This finding suggests that a microbicidal preparation should be formulated to include an acidic buffer if feasible. It also raises the possibility of developing a prevention strategy based on keeping the pH of the vagina low.

The disturbance of vaginal pH may also partially explain why non-ulcerative genital tract infections have been shown to augment the transmission of HIV to women [42]. Vaginal infections, such as trichomoniasis and bacterial vaginosis, are very common and often remain unrecognized or untreated in many women [43]. These infections are commonly associated with an alkaline change in pH [44,45]. This suggests that the routine screening and treatment of women for these common reproductive tract infections may help reduce HIV transmission, especially in areas where heterosexual transmission predominates.

Another mechanism by which reproductive tract infections augment women's susceptibility to HIV infection is by attracting large numbers of potential 'host cells' into the genital tract mucosa through the inflammatory process. Data indicate that, unlike semen, healthy female vaginal secretions contain relatively few CD4 lymphocytes and macrophages, except during menstruation [46]. Future preparations of vaginal compounds for use as microbicides must take into account effects on the vaginal flora. Disruption of the vaginal flora might result in subclinical inflammation or pH alteration that could potentially impair the natural defenses of the reproductive tract. The potential for such effects, for example, has recently been reported for nonoxynol-9 [47,48].

A final set of issues crucial to microbicide development relates to the mucosal immunity of the male and female reproductive tracts. In addition to the systemic immune defenses of the body, most mucosal surfaces exhibit immune protection against potential pathogens through the local activity of immunologically competent cells and the secretion of antibody that prevents primary infection of epithelial surfaces. Currently, very little is known about the local immunity of the reproductive tract [49,50].

Existing data on women suggest that the mucosal secretory immune system of the endocervix provides a primary immune defense against lower reproductive tract pathogens, primarily through the local production of immunoglobulin A (IgA) [46,51,52].

The majority of people acquiring HIV today are infected by mucosal exposure to cell-free or cell-associated virus through heterosexual contact. It is not clear that the current candidate HIV vaccines, many of which are primarily targeted to stimulate systemic immunity, will provide an adequate immunological barrier at the mucosal surfaces of the reproductive tract and thereby prevent primary mucosal infection. This uncertainty provides an important reason to pursue microbicide development even in the face of possible advances in vaccine research. Women may still need a microbicide even after the first generation of vaccines becomes available.

Clinical testing

Many concerns have been expressed regarding the feasibility of designing clinical trials of microbicides that are both scientifically valid and ethically sound. Although clearly a complex and costly procedure, the task of testing a candidate microbicide is both feasible and considerably less problematic than testing a vaccine. Since a microbicide would be applied with each sexual contact, it is not necessary to determine how many days, months, or years protection lasts, as in the case of vaccines. Below we explore some of the more salient issues regarding the design of clinical trials for a female-controlled microbicide.

Before entering human testing, a candidate compound would go through standard testing for toxicity in at least two species of laboratory animals. With minor modification, the typical product-development sequence for a new vaginal contraceptive could be applied to the pursuit of new microbicidal compounds [53]. The sequence of preclinical, animal testing required for evaluation of new intravaginal compounds, as well as the simian models for retrovirus transmission, are discussed elsewhere and will not be reviewed here [54,55]. The preclinical evaluation of potential compounds will obviously require significant attention to the possible mutagenicity of vaginal compounds, especially those that are potentially non-contraceptive. Following successful testing *in vitro* and in animal models, a carefully selected group of promising microbicidal compounds would be brought to human trials. For a typical compound, completion of all three phases of human clinical trials can take at least 5 years, but may take longer for microbicide evaluation, given the sample size requirements and special requirements of study populations described below.

One important question concerns the appropriate study populations for Phase I safety testing. Since studies on safety, tolerance, and absorption can be performed in populations not at significant risk of HIV infection, there is little need to conduct initial Phase I research among vulnerable populations. In particular, special caution should be applied in the recruitment of sex workers for Phase I trials. Given the economic, political, and violent realities of many of these women's lives, it is impossible to ensure (even through cash payments) that they will not 'work' during the trial, thereby exposing themselves to HIV while using an intravaginal compound whose safety in humans has yet to be demonstrated. There are, however, compelling reasons to repeat Phase I safety and toxicity testing in developing countries and among high-risk women following initial testing in the country of origin, and the accumulation of adequate safety data in low-risk populations. As with nonoxynol-9, product formulations demonstrated to be safe when used infrequently may exhibit considerable toxicity under conditions of higher frequency application.

The major challenge with respect to microbicide development is to design a Phase III effectiveness trial that is both scientifically rigorous and ethically defensible. From a scientific standpoint, the most rigorous and therefore the most desirable design is a randomized controlled trial (RCT), in which trial participants are randomly assigned to receive either the compound under investigation or a placebo (or an 'active control' if a compound with previously demonstrated effectiveness exists). Since it would be unethical to withhold a form of HIV prevention known to be protective, participants in a microbicide trial would be counseled to use both the vaginal product and a condom during each act of intercourse. Provision of active, ongoing counseling, assertiveness training, and free condom supplies would decrease the risk of HIV infection in all trial participants. Statistical techniques would be used to determine any incremental benefit offered by the experimental compound over condom use alone. Many of the practical issues of clinical trial design for evaluating the effectiveness of spermicides in preventing more common STD (including a discussion of study population, site selection, and follow-up procedures) have been reviewed recently [56].

This type of trial design raises important ethical issues [57-59]. Researchers have an obligation to minimize harm and to maximize the benefits to all trial participants. In the optimal design, therefore, all participants, including those in the control group, would benefit from receiving condoms, reproductive health-care, and intensive HIV counseling. Therefore, under optimal conditions, the women in the control group should have a lower HIV seroconversion rate than women not participat-

ing in the study. Historic controls could be used to document improved use of condoms by all participants as a direct measure of the benefit of participating in the trial.

The hypothetical results presented in Table 1 illustrate this point. In this example, researchers randomly assign a group of women with a 4% seroincidence rate per year to receive either the test compound or a placebo. In addition, both groups receive extensive counseling and free condoms, which raise their condom use from 10% before the trial to 50% after. In the control group, the women's seroincidence declines from 4 to 3%, a 25% drop, whereas in the experimental group, the rate declines to 1.5%, a 62.5% drop. This design would confirm a 50% protective effect of the test compound while at the same time reducing the rate of seroconversion in the control group.

Researchers would have to undertake a variety of other safeguards to guarantee the ethics of microbicide trials. Among them is the need to ensure informed consent. This requires both the absence of direct or structural coercion and full disclosure of trial procedures, including the randomization process and the use of a placebo if applicable. With respect to microbicide testing, this would require that counseling, condoms, and reproductive health-care be made available to women who choose not to be in the trials (as well as to participants), so that women would not feel obliged to enroll in the trial only to gain access to these often scarce resources. Also, researchers would have to take special care to insure that women understood the contraceptive activity of both the test substance and the control. Some women may wish to protect themselves further from pregnancy during the trial, while others may choose not to participate if doing so might prevent them from conceiving. Discussing the issue of contraceptive activity is especially important in settings where access to abortion is restricted by law or by lack of services.

Perhaps the most vexing ethical issue, however, derives from the principle of justice, which mandates that the burdens and benefits of research be distributed equitably [57]. A particularly extreme example of injustice would be where a new prevention technology is tested extensively in sub-Saharan Africa, but once proven, is unavailable to Africans due to its high cost [60,61]. This precise example

has been the focus of much debate with regard to HIV vaccines, given the high cost of vaccine development, the prominence of private sector interest, and the long-term investment required to make vaccines available in many developing countries.

There are a variety of scientific exigencies that make it almost certain that Phase III testing of a microbicide (as opposed to Phase I safety testing) would take place primarily in the Third World. Two conditions are required to measure a significant decrease in HIV seroconversion attributable to an effective microbicide: a study population of women with a high seroincidence rate attributable primarily, if not exclusively, to the sexual transmission of HIV; and a sufficiently large study population to achieve statistical significance. It is important to avoid any confounding that may result from 'contamination' with other routes of transmission, such as injection drug use. Compared to sexual transmission, it is relatively easy to become infected through parenteral exposure, hence, such contamination would obscure the effects of the product under study and greatly increase the required sample size. As Table 2 illustrates, the number of trial participants required to achieve statistical significance quickly becomes unmanageable in populations with annual HIV seroincidence rates <2–4%. For example, the number of participants required in each arm of a clinical trial seeking to establish 50% efficacy of a test compound in 3 years drops from 3039 to 743 women as the seroincidence of the study population increases from 1 to 4% (Table 2). Note that even at a high annual seroincidence rate of 4%, however, it is likely that data from multiple sites would have to be pooled for analysis. As with vaccine evaluations, the greatest chance for success lies in the design of multicenter, multicountry studies that include both developed and developing country populations of women.

Study populations that fit the criteria outlined above are found more commonly in developing countries and often involve vulnerable women, such as sex workers, who are subject to economic and physical exploitation. Although there are populations of women with annual seroincidence rates in the range of 2–3% in the United States (for example, in the major urban centers of the states of New York and New Jersey), a large proportion of these women are injecting drug users, making them less appropriate for

Table 1. Hypothetical results for a study population comprising commercial sex workers with 4% annual HIV seroincidence.

Random assignment	Initial		Post-intervention	
	Seroincidence	Condom use	Condom use	Seroincidence
Experimental group	4%	10%	50%	1.5%
Control group	4%	10%	50%	3%

Table 2. Number of participants required for microbicide efficacy trials.

Effectiveness (%)	Annual HIV seroincidence			
	1%	2%	3%	4%
25	29008	14376	9499	7061
30	19521	9677	6396	4755
50	6078	3016	1996	1486
70	2552	1268	840	626
90	1118	556	369	276
99	646	322	214	160

Assumptions: Alpha = 0.05, 1-Beta = 0.90, two-tailed test, table entry = total sample size, two-armed study, 3-year trial (participants enrolled for 2 years and followed for 1 year each). No loss to follow-up. (For formula see [74].) Note: sample size calculations should be based on estimates of the seroincidence that can be expected in a given study population after the anticipated effect of alternative intervention strategies (i.e., condom promotion) has been taken into account.

microbicide testing because of their risk of acquiring infection through non-sexual means. Clearly, the political and ethical questions raised by this must be addressed prior to initiating Phase III testing. If the developing world is to bear the major burden of clinical research related to microbicides, women in developing countries must realize the benefits of such compounds once proven.

Programmatic issues

A number of important programmatic issues must also be considered in relation to microbicide development. Perhaps most important is a commitment to seeking women's input at each stage of the research and development effort. Soliciting women's needs and preferences at the outset will help ensure that the product, once developed, will be used and accepted. The importance of this type of consultation for contraceptive development has recently been summarized by the International Women's Health Coalition and the World Health Organization's Special Programme of Research, Development, and Research Training in Human Reproduction [62].

Women's input is especially critical with respect to the initial formulation criteria for a new microbicide product. Table 3 lists some of the dimensions along which a new product might vary. In all likelihood, a broad range of product formulations will ultimately be necessary. The ideal formulation for an adolescent in the United States, for example, might be quite different from that required by a married woman in rural India. An important step in product development will be to conduct field research to establish women's needs and preferences in different geographic and cultural settings.

Table 3. Some dimensions of microbicide product specification.

Contraceptive activity
Interaction with existing contraceptive use
Capacity for covert use ('detectability')
Method of application (e.g., jelly, film, etc.)
Coital dependence
Postcoital effectiveness
Onset and duration of action
Spectrum of microbicidal activity
Disposability
Affordability
Smell/taste
Messiness
Shelf life
Stability at room temperature
Ease of insertion
Appropriateness for anal/oral sex

Experience from testing the acceptability of various contraceptives suggests that one of the more critical design features for microbicide development will be its method of application [63,64]. A microbicidal compound could be manufactured in a number of ways, each with unique implications for its acceptability among users. Cultural factors, for example, might affect whether women would prefer a vaginal cream applied with an applicator, or a vaginal sponge inserted by hand. In parts of Africa, for example, women use a variety of astringent substances to dry and tighten the walls of the vagina before sex [65]. Where this form of 'dry sex' is common and preferred, a moist vaginal creme would probably receive little use.

Contraceptive research also suggests that the timing of insertion will be another factor critical to the acceptability of new microbicidal products. Couples tend to prefer methods that do not interrupt the spontaneity of intercourse, a property determined in part by the product's onset and duration of action.

Microbicidal products that become effective quickly and/or that can be inserted long before intercourse are likely to be preferred. There is also a great need for a product that could be used postcoitally to protect women subject to non-consensual sex. The need for such a product is evidenced by emerging data concerning the widespread prevalence of non-consensual and coercive sex in women's lives, even within married and consensual unions [66]. A postcoital method might also have some utility for women, especially adolescents, in communities where 'planning' to have sex is unacceptable [67].

Another set of programmatic issues revolves around the introduction, distribution, and postmarketing surveillance of any new microbicidal product. The development of appropriate information, public education, and counseling materials must complement the development of any new biotechnology to ensure that women realize the maximum benefit of such new products and that providers sup-

ply proper instructions for correct usage. In contrast to vaccines, which often require delicate handling, constant refrigeration and clinical personnel, the programmatic challenge of microbicides will be to adapt and apply the lessons learned from condom promotion to a new product targeted at women. In many cases, microbicides could potentially be incorporated into the same procurement, storage, and distribution systems presently used for condoms. Moreover, many of the same social-marketing techniques used to create demand for condoms could also be used to promote microbicides. Even in very poor countries like Zaïre, social-marketing campaigns have been able to increase greatly the sale of condoms using a skillful mix of media, packaging, pretested slogans and advertisements, and aggressive, widespread distribution through multiple outlets [68]. Also promising are opportunities to distribute and promote microbicides through family planning programs, mothers' clubs, and other women's organizations. Indeed, for this type of product, word-of-mouth and 'woman-to-woman' promotion may be the most effective.

Although microbicide development will most probably depend on a public sector-supported research and development effort, efficient manufacture and distribution will require some degree of profitability. Therefore, the major challenge (as with condom supplies today) will be identifying resources to finance the recurrent costs of commodity procurement and distribution. In many places, this will require substantial infrastructure development, as well as the political will to provide public subsidy for the purchase and distribution of microbicial products for individual use in the public good.

Public sector leadership and partnership with the private sector

There is an urgent need for public sector organizations to provide leadership in co-ordinating research and development of HIV prevention technologies — including both microbicides and vaccines. This will require not only support for basic and clinical research needed to answer the outstanding questions of reproductive biology and provide efficacy testing infrastructure, but also, more essentially, the organization of this research into a product development path that will result in safe and effective vaginal products. These products would be eligible for registration with drug regulatory agencies and available for global distribution. A constellation of concerns regarding inadequate liability insurance, cumbersome regulatory requirements, high costs of clinical testing infrastructure, political caution, and anticipated low returns on investment have prevented the private sector from taking the lead in

this area. This has long been the case in the area of contraceptive development [69–71].

While there is need for leadership, the public sector should avoid, if possible, taking on the entire responsibility for product research and development. The most efficient motors of invention, product design and technology advancement are in the private sector. The relatively small scale of public sector laboratories, erratic patterns of donor support and budget allocations, and the inefficiencies of multiple contracted research agreements lead to slow product development processes. Co-ordinated public-private sector collaboration is most urgently needed to increase the investment and effort of the private sector in this important area of technological development. Public-private sector partnership could range from joint needs assessments, through collaboration in laboratory, clinical and field testing, to risk sharing through public sector underwriting of research or liability insurance costs [72,73].

In all these partnerships both sides must have a clear understanding of each other's motivations and expectations. It is important for public sector organizations to recognize the commercial interests of industry and understand that 'profit' at times has many dimensions. For example, industry will at times pursue 'less profitable' technologies for public relations purposes, to establish corporate goodwill, or to complement other products within sales and distribution networks. The commercial sector will not pursue technologies that are frankly unprofitable. Simultaneously, the mechanisms for protecting public sector investments of scarce research and development funds must be clear and specified in advance. Insuring the public sector's investment goal — the availability of affordable technology for resource poor environments — is facilitated by establishing clear ownership of intellectual property and by the pursuit of licensing strategies that discourage monopolies, encourage dual pricing and exploit economies of scale within the commercial sector [72].

The absence of monopolies encourages price competition, while public-sector pricing agreements help guarantee the affordability of technology for use in resource-poor environments. In such agreements, industry acquires technology, intellectual property, or the facility for clinical evaluation in return for assurances that the product will be sold to public sector organizations at a price that is a small, fixed percentage above manufacturing and distribution costs. Meanwhile, industry enjoys freedom from price regulation in commercial sales. One reason companies agree to such public-sector pricing arrangements is that large-volume production for public-sector markets provides a significant economy of scale in manufacturing and distribution, thereby increasing the profit margins of commercial sector sales.

Conclusion

The development and testing of microbicidal compounds will require advancing our basic understanding of human reproductive biology. It will rely on careful attention to the ethics of clinical trial design and require a genuine, lasting dialogue between scientists and advocates. It will also require the collaboration of professionals from many academic disciplines, as well as the co-ordination of efforts among private industry and various governmental, non-governmental, and intergovernmental organizations in both developed and developing countries. Most importantly, it will require a renewed commitment within public-sector organizations to provide the requisite leadership to champion the development of safe, effective and affordable microbicidal products that will be accessible by all women at risk of HIV infection.

It must be stressed, however, that the complexities of AIDS will not be met adequately with a 'technological fix'. Efforts to develop female-controlled prevention options must be complemented by more concerted attempts to address the underlying gender power imbalances that shape women's risk of STD, as well as their ability to protect themselves using the existing range of prevention strategies. Until women share power more equally with men — in both the public and the private sphere — they will remain at heightened risk of AIDS.

References

- STEIN ZA: HIV prevention: the need for methods women can use. *Am J Public Health* 1990, 80:460-462.
- ELIAS CJ: *Sexually Transmitted Diseases and the Reproductive Health of Women in Developing Countries. Programs Division Working Paper No. 5*. New York: The Population Council, 1991.
- GERMAIN A: Introduction. In *Reproductive Tract Infections: Global Impact and Priorities for Women's Reproductive Health*. Edited by Germain A, Holmes KK, Piot P, Wasserheit JN. New York: Plenum Press, 1992:1-6.
- CATES W, STONE K: Family planning: the responsibility to prevent both pregnancy and reproductive tract infections. In *Reproductive Tract Infections: Global Impact and Priorities for Women's Reproductive Health*. Edited by Germain A, Holmes KK, Piot P, Wasserheit JN. New York: Plenum Press, 1992:93-129.
- WORTH D: Sexual decision-making and AIDS: why condom promotion among vulnerable women is likely to fail. *Stud Fam Plann* 1989, 10:297-307.
- UUN PR: African women and AIDS: negotiating behavioral change. *Soc Sci Med* 1992, 34:63-73.
- ALEXANDER NJ: Sexual transmission of human immunodeficiency virus: virus entry into the male and female genital tract. *Fertil Steril* 1990, 54:1-18.
- ALEXANDER NJ, GABELNICK HL, SPIELER JM: *Heterosexual Transmission of AIDS*. New York: Alan R. Liss, Inc., 1990.
- ANDERSON DJ: Mechanisms of HIV-1 transmission via semen. *J NIH Res* 1992, 4:104-108.
- WOLFF H, ANDERSON DJ: Male genital tract inflammation associated with increased numbers of potential human immunodeficiency virus host cells in semen. *Andrologia* 1988, 20:297-307.
- BORZY MS, CONNELL RS, KIESSUNG AA: Detection of human immunodeficiency virus in cell-free seminal fluid. *J Acquir Immune Defic Syndr* 1988, 1:419-424.
- KRIEGER JN, COOMBS RW, COLLIER AC, ET AL: Recovery of human immunodeficiency virus type 1 from semen: minimal impact of stage of infection and current antiviral chemotherapy. *J Infect Dis* 1991, 163:477-482.
- ANDERSON DJ, O'BRIEN TR, POUTCH JA, ET AL: Effects of disease stage and zidovudine therapy on the detection of human immunodeficiency virus type 1 in semen. *JAMA* 1992, 267:2769-2774.
- BOURINBAIR AS, PHILLIPS DM: Transmission of human immunodeficiency virus from monocytes to epithelia. *J Acquir Immune Defic Syndr* 1991, 4:56-63.
- PHILLIPS DM, BOURINBAIR BS: Mechanism of HIV spread from lymphocytes to epithelia. *Virology* 1992, 186:261-273.
- PHILLIPS DM, TAN X: HIV-1 infection of the trophoblast cell line BeWo: a study of virus uptake. *AIDS Res Hum Retroviruses* 1992, 8:1683-1691.
- HUSSAIN LA, KELLY CG, FELLOWES R, ET AL: Expression and gene transcript of Fc receptors for IgG, HLA Class II antigens and Langerhans cells in human cervico-vaginal epithelium. *Clin Exp Immunol* 1992, 90:530-538.
- EDWARDS JN, MORRIS HB: Langerhans cells and lymphoid subsets in the female genital tract. *Br J Obstet Gynecol* 1985, 92:974-982.
- PETERMAN TA, ET AL: Risk of human immunodeficiency virus transmission from heterosexual adults with transfusion-associated infections. *JAMA* 1988, 259:55-58.
- EUROPEAN STUDY GROUP ON HETEROSEXUAL TRANSMISSION OF HIV: Comparison of female-to-male and male-to-female transmission of HIV in 563 stable couples. *BMJ* 1992, 304:809-813.
- BIRD KD: The use of spermicide containing nonoxonyl-9 in the prevention of HIV infection. *AIDS* 1991, 5:791-796.
- ROSENBERG M, GOLLUB E: Methods women can use that may prevent sexually transmitted disease, including HIV. *Am J Public Health* 1992, 11:1473-1478.
- ZEKENG L, FELDBLUM PJ, OLIVER RM, KAPTUE L: Barrier contraceptive use and HIV infection among high-risk women in Cameroon. *AIDS* 1993, 7:725-731.
- FELDBLUM P: Efficacy of spermicide use and condom use by HIV-discordant couples in Zambia. *VIII International Conference on AIDS/III STD World Congress*. Amsterdam, July 1992 [abstract WeC1085].
- KREISS J, NGUGI E, HOLMES KK, ET AL: Efficacy of nonoxonyl-9 contraceptive sponge use in preventing heterosexual acquisition of HIV in Nairobi prostitutes. *JAMA* 1992, 268:477-482.
- VOELLER B: Spermicides for controlling the spread of HIV [letter]. *AIDS* 1992, 6:341-342.
- REKART ML: The toxicity and local effects of the spermicide nonoxonyl-9. *J Acquir Immune Defic Syndr* 1992, 5:425-426.
- CENTERS FOR DISEASE CONTROL: Update: barrier protection against HIV infection and other sexually transmitted diseases. *MMWR* 42:30.
- NIRUTHISARD S, RODDY RE, CHUTIVONGSE S: The effects of frequent nonoxonyl-9 use on the vaginal and cervical mucosa. *Sex Trans Dis* 1991, 18:176-179.
- NIRUTHISARD S, RODDY RE, FRIED DB, CHUTIVONGSE S: A randomized trial of nonoxonyl-9 and placebo for the prevention of gonococcal and chlamydial cervicitis. *International Society for Sexually Transmitted Disease Research*. Banff, October 1991, [abstract C-22-028].
- RODDY RE, CORDERO M, CORDERO C, IRSULA B, GREY TW: Dosing study of nonoxonyl-9 and genital irritation. *VIII International Conference on AIDS/III STD World Congress*. Amsterdam, July 1992 [abstract PoB].
- BAGASRA O, LISCHNER HW: Activity of dextran sulfate and other polyanionic polysaccharides against human immunodeficiency virus. *J Infect Dis* 1988, 158:1084.
- BAGASRA O, FREUND M, WEIDMANN J, HARLEY G: Interaction of human immunodeficiency virus with human sperm *in vitro*. *J Acquir Immune Defic Syndr* 1988, 1:431-435.

34. PEARCE-PRAATT R, PHILLIPS DM: Studies on adhesion of lymphocytic cells: implications for sexual transmission of HIV. *Biol Reprod* 1993, 48:431-445.
35. MILLER CJ, ET AL: Genital mucosal transmission of simian immunodeficiency virus: animal model for heterosexual transmission of the human immunodeficiency virus. *J Virol* 1989, 63:4277-4284.
36. STEWART GJ, CUNNINGHAM AL, DRISCOLL GL, ET AL: Transmission of human T-cell lymphotropic virus III (HTLV-III) by artificial insemination by donor. *Lancet* 1985, ii:581-584.
37. ALEXANDER NJ: Comments at the *Biology of Heterosexual Transmission of HIV Workshop*. National Institute of Allergy and Infectious Diseases; 1991. Bethesda, May 1991.
38. SCOFIELD VL: Sperm as vectors and cofactors for HIV-1 transmission. *J NIH Res* 1992, 4:105-111.
39. ANDERSON DJ: Comments at the *Biology of Heterosexual Transmission of HIV Workshop*. National Institute of Allergy and Infectious Disease, Bethesda, May, 1991.
40. VOELLER B, ANDERSON DJ: Heterosexual transmission of HIV. *JAMA* 1992, 267:1917-1918.
41. VOELLER B, ANDERSON DJ: *pH and Related Factors in the Urogenital Tract and Rectum that Affect HIV-1 Transmission*. Mariposa Occasional Paper No. 16. Topanga: The Mariposa Education and Research Foundation; 1992.
42. WASSERHEIT JN: Epidemiological synergy: interrelationships between HIV infection and other STDs. In *AIDS and Women's Reproductive Health: Science for Policy and Action*. Edited by Chen L, Sepulveda J, Segal S. New York: Plenum Press; 1991:47-72.
43. WASSERHEIT JN: The significance and scope of reproductive tract infections among third world women. *Int J Obstet Gynecol* 1989, (suppl 3):S145-S168.
44. HILLIER S, HOLMES KK: Bacterial vaginosis. In *Sexually Transmitted Diseases*. Edited by Holmes KK, Mardh P, Sparling PF, Wiesner PJ, Cates W, Lemon SM, Stamm WE. New York: McGraw-Hill; 1989:547-559.
45. REIN MF, MULLER M: Trichomonas vaginalis and trichomoniasis. In *Sexually Transmitted Diseases*. Edited by Holmes KK, Mardh P, Sparling PF, Wiesner PJ, Cates W, Lemon SM, Stamm WE. New York: McGraw-Hill; 1989:481-492.
46. ANDERSON DJ, HILL JA: Cellular and soluble factors in semen and the vaginal environment that may influence the heterosexual transmission of HIV type 1. In *Vaginitis and Vaginosis*. New York: Wiley-Liss; 1991:69-76.
47. MCGROARTY JA, ET AL: Influence of the spermicidal compound nonoxonyl-9 on the growth and adhesion of urogenital bacteria *in vitro*. *Current Microbiol* 1990, 21:219-223.
48. MCGROARTY JA, CHONG S, REID G, BRUCE AW: The spermicidal compound nonoxonyl-9 increases adhesion of *Candida* species to human epithelial cells *in vitro*. *Infection Immun* 1990, 58:2005-2007.
49. FORREST BD: Women, HIV, and mucosal immunity. *Lancet* 1991, 337:835-836.
50. ANDERSON DJ, PUDNEY J: 1992. Mucosal immune defense against HIV-1 in the male urogenital tract. *Vaccine Res* 1992, 3:143-150.
51. REBELLO R, GREEN FHY, FOX H: A study of the secretory immune system of the female genital tract. *Br J Obstet Gynecol* 1975, 82:812-816.
52. UNDERDOWN BJ, SCHIFF JM: Immunoglobulin A: strategic defense initiative at the mucosal surface. *Annu Rev Immunol* 1986, 4:389-417.
53. ZATUCHNI GI, SOBRERO AJ, SPEIDEL JJ, SCIARRA JJ: Vaginal contraception: new developments. New York: Harper and Row; 1979.
54. CHYAPIL M, DROEGEMUELLER W, OWEN JA, ESKELSON CD, BETTS K: Studies of nonoxonyl-9. I. The effect on the vaginas of rabbits and rats. *Fertil Steril* 1980, 33:445-450.
55. MILLER CJ, ALEXANDER NJ, GETTIE A, HENDRICKX AG, MARX PA: The effect of contraceptives containing nonoxonyl-9 on the genital transmission of simian immunodeficiency virus in rhesus macaques. *Fertil Steril* 1992, 57:1126-1128.
56. FOLDESZ RG, FARR MG, THOMPSON DC, CATOTTI DN: Design of clinical studies of spermicides for prophylaxis against sexually transmitted diseases. In *Heterosexual Transmission of AIDS*. Edited by Alexander NJ, Gabelnick HL, Spieler JM. New York: Alan R. Liss, Inc.; 1990: 291-301.
57. LEVINE C, DUBLER NN, LEVINE RJ: Building a new consensus: ethical principles and policies for clinical research on HIV/AIDS. *Institutional Rev Board* 1991, 13:194-210.
58. UNITED STATES PUBLIC HEALTH SERVICE: Consultation on international collaborative human immunodeficiency virus (HIV) research. *Law Med Health Care* 1991, 19:259-263.
59. COUNCIL FOR INTERNATIONAL ORGANIZATIONS OF MEDICAL SCIENCES: International Guidelines for Ethical Review of Epidemiological Studies (Appendix I). *Law Med Health Care* 1991, 19:5-30.
60. FREEDMAN B: AIDS and the ethics of clinical trials: learning the right lessons. *Cont Clin Trials* 1992, 13:1-5.
61. NDIANYA-ACHOLA JO: A review of ethical issues in AIDS research. *East Afr Med J* 1991, 68:735-740.
62. INTERNATIONAL WOMEN'S HEALTH COALITION: *Creating Common Ground: Women's Perceptions on the Selection and Introduction of Fertility Regulation Technologies*. Geneva: WHO; 1991 (WHO/HRP/ITT).
63. SCRIMSHAW SCM: The cultural acceptability of vaginal contraceptives. In *Vaginal Contraception: New Developments*. Edited by Zatuchni GI, Sobrero AJ, Speidel JJ, Sciarra JJ. New York: Harper and Row; 1979:282-293.
64. EHRHARDT AA, YINGLING S, ZAWADZKI R, MARTINEZ-RAMIREZ M: Prevention of heterosexual transmission of HIV: barriers for women. New York: Hayworth Press; 1992:37-67.
65. DALLABETTA G, MIOTTI P, CHIPHANGWI J, LIOMBA G, SAAH A: Vaginal tightening agents as risk factors for acquisition of HIV. *VI International Conference on AIDS*. San Francisco, June, 1990 (abstract THC574).
66. HEISE L: Violence against women: the missing agenda. In *Women's Health: A Global Perspective*. Edited by Koblinsky M, Timyan J, Gay J. Boulder: Westview Press; 1993.
67. PICK DE WEISS S, ATKIN LC, GRIBBLE JN, ANDRADE-PALOS P: Sex, contraception, and pregnancy among adolescents in Mexico City. *Stud Fam Plann* 1991, 22:74-82.
68. POPULATION SERVICES INTERNATIONAL *The Zaire Mass Media Project: A Model AIDS Prevention and Motivation Project*. Report No. 1. Washington, DC: Population Services International; 1992.
69. BARDIN CW: Public sector contraceptive development: history, problems, and prospects for the future. *Technol Soc* 1992, 9:289-305.
70. MASTROIANNI L, DONALDSON PJ, KANE TT: Special report: development of contraceptives — obstacles and opportunities. *N Engl J Med* 1990, 322:482-484.
71. HARR JE, JOHNSON PJ: *The Rockefeller Conscience*. New York: Scribners; 1991:31-45, 395-442.
72. FREE M: Health technologies for the developing world: addressing the unmet needs. *Int J Tech Assessment Health Care* 1992, 8:623-634.
73. WORLD HEALTH ORGANIZATION/GLOBAL PROGRAMME ON AIDS: *Potential for WHO-Industry Collaboration on Drug and Vaccine Development for HIV/AIDS*. Geneva: WHO; 1993.
74. COHEN J: Differences between proportions. In *Statistical Power Analysis for the Behavioral Sciences*. New York: Academic Press; 1977.

Vaginal microbicides for preventing the sexual transmission of HIV

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The place of vaginal microbicides in prevention strategies

Globally, over 80% of HIV infections are acquired heterosexually; current projections suggest that by the year 2000 some 25 million individuals worldwide will have been infected by heterosexual transmission. In parts of Africa, Asia and Latin America the scale and rate of progression of the epidemic give rise to the gravest concern. Projections beyond the year 2000 are even more pessimistic.

What measures are available or in development for preventing the spread of HIV? Prophylactic vaccination is, and must remain, a key objective. If long-term immunity to HIV can be induced on a large scale by vaccination, the epidemic could be brought under a considerable measure of control. A number of candidate vaccines, based on inactivated virus particles, attenuated HIV strains or specific viral components (on their own or integrated within viral and microbial vectors) have been developed and some are undergoing preliminary clinical evaluation [1]. However, there are few, if any, precedents for sterilizing immunity caused by vaccination, so the prevention of HIV infection may be difficult to achieve with a vaccine. Nevertheless, a vaccine that would suppress subsequent rounds of viral replication and prevent HIV disease/AIDS might be a more achievable target and would be extremely important for the prevention of the morbidity and mortality currently associated with this fatal sexually transmitted disease (STD). Unfortunately, preliminary data suggest that HIV vaccines of proven safety and efficacy for use in large-scale inoculation programmes are unlikely to be available until the turn of the century at the earliest.

Even when a vaccine does become available, efforts to decrease risky sexual behaviour will remain the fundamental component of intervention strategies. 'Safer sex' practices include (1) delaying coital debut, (2) having fewer sexual partners, and (3) using condoms consistently and correctly. The active promotion of condom use is the primary public-health strategy in many countries, although, unfortunately, persuading people to use condoms consistently and correctly is difficult to achieve. Research in this area is extremely important. Some progress is being made in understanding the sexual attitudes and behavioural factors that influence HIV transmission, and support for such studies needs to be expanded, with special emphasis on the sustainability of those behavioural changes that decrease transmission risk.

It is now clear that the risk of becoming infected or of infecting others with HIV is substantially increased by the presence of an STD such as chancroid, genital herpes, syphilis, trichomoniasis, gonorrhoea or chlamydial infection. Both ulcerative and non-ulcerative STDs have been shown to be associated with an increased risk of HIV infection. The increased risk associated with genital ulcer diseases appears to be higher (up to 10-fold) compared with discharge diseases (between three- and fivefold), although the higher prevalence of discharge diseases results in a much higher population-attributable risk overall.

Conversely, there is evidence to suggest that HIV infection may increase the prevalence of some STDs (for example genital ulcers) at a community level. Thus, HIV and other STDs may amplify one another. This 'epidemiological synergy' may fuel the explosive growth of the HIV pandemic in some populations [2]. Therefore, strategies to reduce in-

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section with other STDs should also have an impact on HIV infection.

The need for female-controlled methods

In the context of the recommendations from four recent international conferences on microbicide development, a consensus has emerged that safe, effective, female-controlled chemical barriers are urgently needed to prevent sexually transmitted HIV infection and other STDs. The mechanical and chemical barriers currently available have many limitations. Although the male condom can provide a very effective barrier against the transmission of HIV and discharge diseases, its use requires the active cooperation of the male partner and therefore cannot be independently implemented at the discretion of the female partner. In contrast, the female condom requires only the male partner's passive consent, but little is known about its efficacy in preventing viral and bacterial STDs. Existing chemical spermicides have activity *in vitro* against most STD pathogens, including HIV; however, no clinical studies have yet been performed that demonstrate unequivocally that spermicides prevent HIV infection. Moreover, several studies have revealed that spermicides can cause mucosal erosions and ulcers; whether these lesions increase the risk of HIV infection is presently unknown.

In addition to the inherent limitations of these existing methods, there are many situations in which personal, social or cultural attitudes interfere with a woman's ability to negotiate and implement the use of barriers that could decrease the risk of infection. Specifically, the need for a method that can be implemented by women is based on high prevalences of sex without condom use, risky sexual behaviour on the part of one partner without the knowledge of the other, and non-consensual sex [3].

Just as oral contraceptives have dramatically increased the ability of women to avoid unwanted pregnancy, effective vaginal microbicides would enhance their ability to avoid sexually transmitted infections. Moreover, chemical barriers that inactivate pathogens in vaginal/cervical secretions as well as in the ejaculate could reduce both female-to-male and male-to-female transmission. There is an urgent need for such products, in both developed and developing societies, to provide an alternative for those who either cannot or will not use condoms.

Because of the influence of STD co-infection on the risk of HIV transmission (see above), microbicides that inactivate *Neisseria gonorrhoeae*, *Chlamydia trachomatis* and the agents of genital ulcer disease as well as HIV would substantially enhance their po-

tential to make an impact on the HIV epidemic. It is possible to demonstrate mathematically that a microbicide with 90% efficacy against HIV without any spectrum of activity against other sexually transmitted pathogens would be less effective than a product with 50% efficacy against HIV and a similar efficacy against other sexually transmitted pathogens [4].

Furthermore, STDs cause significant morbidity and mortality *per se* and contribute greatly to the increasing costs of health care. They disproportionately affect the woman, the fetus and the newborn. Gonococcal and chlamydial infections can cause pelvic inflammatory disease, infertility and ectopic pregnancy. Several common STDs adversely affect pregnancy and result in spontaneous abortion, stillbirth and chorioamnionitis, premature rupture of membranes, preterm delivery and postpartum endometritis. Neonatal infections include congenital syphilis, gonococcal conjunctivitis, chlamydial pneumonia and herpes encephalitis. Moreover, genital infections due to human papillomavirus are causally associated with cervical cancer, one of the most common cancers in women worldwide [5].

It should be recognized at the outset that although the principle of the microbicide approach is straightforward, research in this area faces some difficult challenges. For example, the aim in HIV microbicide development is to inactivate the infectious agent in male and female genital fluids. Unfortunately, there is a lack of quantitative information about the content of free virus, cell-associated virus and infectious nucleic acid in semen and cervico-vaginal secretions, and it is not known how these vary within and between individuals (for example, at different stages of disease), or what their relative contributions are to male or female infectivity. Above all, the central challenge is the development of a microbicide that is effective without causing unacceptable levels of inflammation to the exposed tissue.

However, the microbicide approach does avoid one of the major problems complicating the design of antiretroviral drugs and vaccines against HIV: genetic variability. A successful microbicide that disrupts the envelope of the virus, or which prevents its initial attachment, is likely to be unaffected by genetic variability and can therefore be expected to be effective against all HIV strains, present and future.

While this report focuses on the vaginal use of microbicides, their possible use rectally is not excluded. However, there are many significant differences between the two situations and it cannot be assumed that a product shown to be effective and safe for vaginal use will be so if used rectally; specific tests would need to be undertaken to determine this.

A balanced, multifaceted approach to prevention

There have been fears that the availability of microbicides might detract from the adoption of other 'safer sex' behaviours, such as condom use. Moreover, it is argued that if this happens, and if the microbicide is less than 100% effective, the spread of HIV could be accelerated. A similar argument can of course be made about anti-HIV vaccines. The essential point is that no single approach to HIV/STD prevention should be regarded as sufficient. The efficacy of vaccines, condoms or microbicides could be affected by a variety of factors, including the frequency of sex, the frequency of product use and whether it is used correctly, and the nature of any other infections present. Moreover, vaccines effective against today's strains may not protect against tomorrow's. Safe, responsible sexual behaviour should start with the assumption that both partners may be infected with one or more transmissible agents and consideration should be given to the use of physical and chemical barrier methods, early diagnosis and treatment of STDs, and the use of available vaccines as appropriate.

Efficacy versus toxicity

There is no shortage of agents capable of inactivating HIV and HIV-infected cells in genital secretions. Many detergents, for example, some of which have long been used as spermicides, can do so effectively if used in sufficient concentration. The central problem is to identify substances that can achieve this under conditions (concentration of agent, duration of exposure and pH, for example) where they do not cause significant damage to the vaginal and cervical epithelia. This is a crucial factor in the context of AIDS prevention because of the serious concern that inflammation of the mucosa may, in principle, increase the risk of HIV transmission [6,7].

The non-ionic surfactant nonoxynol-9 (N-9) is used in various types of contraceptive product: for example, in foams and pessaries, and as an external coating on some brands of condom. N-9 has been shown to be capable of inactivating HIV *in vitro* [8,9]; this is also true of some other surfactant substances (see below). Detergent spermicides have also been shown to have activity *in vitro* against herpes simplex viruses, *N. gonorrhoeae*, *C. trachomatis*, *Candida albicans* and other pathogens [10-13], which can cause inflammatory lesions. There is little reliable information, however, about spermicide efficacy against HIV infection *in vivo*.

Clinical studies

There have been some clinical studies of microbicide efficacy and rather more of toxicity. Unfortunately, differences in study design, small sample sizes, high frequencies of sexual activity, compliance problems (including the measurement of compliance) and questions about the validity of self-reported data limit both the extent to which firm conclusions can be drawn and the reliability of extrapolating the findings to different populations. However, there are trends that suggest that spermicides may reduce the risk of gonorrhoea, chlamydial infection and HIV infection.

A commercial contraceptive sponge containing 1000 mg of N-9 was the subject of a randomized, placebo-controlled trial in 128 prostitutes in Nairobi. There was no evidence that this was effective in reducing the risk of HIV infection among these highly exposed women. During the follow-up period, the seroconversion rate in the N-9 group was not statistically different from that in the placebo group (45 versus 36%). The frequency of genital ulceration and vulvitis was greater in the N-9 group [14].

This might suggest that N-9 is unsuitable as a vaginal microbicide. However, the significance of these findings is unclear. First, the amount of N-9 in the product used was high compared with that in many other spermicidal preparations. Second, it is not known whether the surfactant, the sponge itself, or a combination of the two was responsible for the pathological findings, because the placebo group, instead of an N-9-free sponge, used an N-9-free vaginal cream. Furthermore, the extent to which the observed pathology may have been causally related to the infection rates is not known. Nor is it clear whether the findings in these subjects, who used the sponge 14 times a week on average, can be extrapolated to individuals who might use it less frequently. Finally, the women's compliance is not certain, since only self-reported data were collected.

Findings from certain other studies are more encouraging. A recent study conducted in sex workers in the Cameroon demonstrated that vaginal suppositories containing N-9 can reduce the incidence of HIV infection [15]. Similarly, there is evidence that the consistent use of N-9 spermicide reduced the rate of HIV infection in a group of Zambian women with discordant partners [16]. However, the size and design of both of these studies limit the significance that can be attached to the results.

A number of other clinical investigations have provided toxicity data on surfactants used intravaginally. In a Phase I study conducted in Thailand, women agreed to use a preparation containing 150 mg of N-9 four times a day for 14 days. Epithelial disruption was observed in the cervix and

vagina in 43% of the women. These symptoms resolved within 1 week of discontinuing N-9 use [17]. In a larger study, 343 Thai women were randomized to use either 72 mg of N-9 or placebo at a rate of up to 22 insertions per week. The use of N-9 was found to be associated with a slightly higher rate of vaginal irritation, but not with increased genital ulceration [18].

These two investigations were followed up by a dosing study of N-9 and genital irritation in women attending a family planning clinic in the Dominican Republic [19]. Participants were randomized to receive vaginal suppositories (each containing 150 mg of N-9) at a rate of either one every other day or once, twice or four times a day for a 2-week period. There were 35 women in each group. Another group of 35 women received placebo suppositories at a rate of four a day over the same period. The rates of reported symptoms (vaginal itching and burning) for N-9 users were not significantly different from those of the placebo users. However, colposcopic examination revealed rates of epithelial disruption (vaginal and cervical) that increased with more frequent N-9 use. The rate for N-9 use once every other day was no different from that of the placebo group; rates for N-9 use once or twice a day and four times a day were 2.5 and five times greater, respectively. This suggests that the safety of such products could depend very much on frequency of use. Furthermore, it underlines the fact that self-reporting of irritation and discomfort with product use may not be a reliable indicator of mucosal disruption.

A different surfactant spermicide, menfegol, was the subject of a safety study in Senegal (J. Goeman, I. Ndoye, L.M. Sakho, *et al.*, submitted for publication). Women were randomized to receive menfegol foaming tablets or placebo tablets at different frequencies of intravaginal insertion. About one-fifth of the 125 participants developed cervical lesions, with or without concomitant vaginal lesions, the incidence increasing with the higher frequencies of product use. The incidence was similar for menfegol and placebo use, indicating that the vehicle contributed significantly to toxicity. As in the Dominican Republic study, reported symptoms were poor predictors of colposcopic findings.

The clinical significance of chemically induced irritation, inflammation and ulceration of the genital mucosa is not clear, but these could have serious consequences, as discussed above. Nevertheless, it would be unfortunate if potentially valuable microbicides were excluded on the basis of irritation that did not affect the risk of infection. Clearly, studies that facilitate the interpretation of the clinical significance of these lesions are needed.

Animal models for studies of toxicity and efficacy

Well-characterized animal models of vaginal irritation and local toxicity, developed for studies with vaginal contraceptives, are suitable for studies with microbicides. There are also animal systems suitable for evaluating the impact of vaginal agents on fertility and embryogenesis.

There is a need for further work on the development of animal models for studies of microbicidal efficacy. Some such studies have already been performed in female macaques, where the vaginal administration of commercial spermicidal products containing N-9 was shown to reduce the number of animals infected after vaginal challenge with STV [20]. Analogous results have been obtained in the cat, in which it was demonstrated that N-9 contraceptive gel prevented infection by vaginal challenge with cells infected with the feline immunodeficiency virus [21]. The latter study also showed that N-9 gel provided protection against a rectally administered inoculum. The suitability of these models for evaluating microbicides against other STDs is somewhat limited. The well-defined models for genital herpes and chlamydial infection may be adaptable for this purpose.

The vaginal ecosystem

The vaginal environment is highly complex and there are many aspects about which our understanding is limited. Relatively little is known about mucosal cell biology, about the early steps in host-pathogen interaction, or about the processes involved in infection with HIV or other sexually transmitted pathogens via the different types and regions of genital epithelia. More work is needed on the role of the vaginal flora in various states of normality and disease. This lack of knowledge makes it difficult to predict how a particular microbicide might enhance resistance to infection in the vagina and also how its use might disrupt the vaginal ecology, thereby decreasing resistance to infection.

We do know that some of the lactobacilli that colonize the healthy vagina produce hydrogen peroxide and there is evidence that this can restrict colonization by a number of pathogenic organisms [22]. Other lactobacilli produce lactic acid, and the resultant low pH (within the range 3.4–6.0 in healthy women of reproductive age [23]) also limits infection by pathogens. *In vitro*, hydrogen peroxide-generating lactobacilli have been shown to reduce the viability of HIV [24], and low pH can completely inactivate cell-free HIV and reversibly inactivate cell-associated virus [25]. Thus, the presence of lactobacilli in the vagina may influence the survival and infec-

tiousness of HIV and other pathogens in that environment. However, matters are complicated by the fact that semen has a considerable buffering capacity at slightly alkaline pH (7.0–8.4) [26], and after ejaculation the pH of the vaginal content increases to neutral or higher [27].

It is conceivable that a woman's repeated use of a microbicide could disrupt vaginal ecology by affecting lactobacilli, either by killing them or by disrupting their attachment sites on the mucosal surface. The consequent reduction in levels of hydrogen peroxide and lactic acid could increase the chances of HIV and other pathogens surviving in the genital tract. Loss of the normal flora might also contribute to overgrowth by pathogens such as *Candida* spp. and *Escherichia coli*.

It is important to appreciate that the lack of definitive information on such issues can be overcome and is not a permanent impediment to microbicide development. Research to improve our understanding of the complex phenomena involved in the natural defences against infection can proceed in parallel with work to evaluate empirically the benefits and side-effects of promising candidate microbicides.

Desirable characteristics of microbicidal products for intravaginal use

The ideal vaginal microbicide should be colourless, odourless, tasteless (physically 'invisible'), stable, easy to store, fast-acting for an appropriate duration after insertion, effective pre- and postcoitally, inexpensive, available without a prescription, and safe for use at least once or twice daily. Candidate classes of microbicidal compounds include, but are not limited to, detergents, chemicals such as iodophores, carbohydrates, antibodies, antiviral drugs, defensins and pyocins.

Ideally, vaginal microbicides should not be inherently spermicidal but should be capable of formulation with or without spermicidal activity. While a woman's contraceptive choices may change over a lifetime, so long as she is sexually active she will always require/desire protection from sexually transmitted infections. Non-contraceptive microbicides would clearly be extremely valuable for use by women who wish to become pregnant. They would of course need to be carefully tested for any impact on embryogenesis, including teratogenicity.

Some potentially useful intravaginal agents

A wide range of substances have properties consistent with what is required of a vaginal microbicide.

Moreover, it is conceivable that the use of two or more agents in combination, if chosen judiciously, will prove more effective and less toxic than one agent alone. The inclusion of substances to control the vaginal pH could also be important in view of the above evidence that HIV can be inactivated *in vitro* by low pH.

Several surfactants are already licensed for intravaginal use as contraceptive agents in one country or another. N-9 and menfegol have already been discussed; octoxynol and benzalkonium chloride are other examples. All disrupt lipid membranes and are able to inactivate HIV and other STD pathogens *in vitro*, with minimum inhibitory concentrations of 0.025–0.05% and exposure times of a few seconds to a few minutes.

Some surfactants that are not licensed as spermicides also exhibit anti-HIV and anti-STD effects *in vitro*. This applies, for example, to various bile salts [28] and to chlorhexidine [29]. Interestingly the latter has been used, without adverse effects, to disinfect the vagina during labour as a means of reducing neonatal morbidity associated with group B streptococcal infections [30]. Certain more novel mild detergents also merit investigation as anti-HIV agents. Octyl-glucopyranoside for example is a non-ionic surfactant of low toxicity that can solubilize membrane proteins—for example, the glycoprotein of vesicular stomatitis virus—without denaturing them [31].

If it were necessary to use surfactants to inactivate cell-associated pathogens, whether HIV, herpesviruses or chlamydial elementary bodies, the margin between safety and efficacy would be narrow. There is nothing unique about the membrane of the infected cell that might enable the surfactant to distinguish it from that of a healthy cell (although the cells of the intact genital mucosa may be protected to some extent by a coating of mucus). However, it is noteworthy that the bacterial cell membrane contains unique lipids that can be specifically solubilized by certain detergents that do not seriously affect the membranes of eukaryotic cells. Such detergents may thus be interesting candidates for use as vaginal microbicides. Their primary action would be against bacterial pathogens; at first sight, therefore, they may fall short of expectations for microbicides active against HIV. However, they may work effectively and safely against HIV infection, including cell-associated virus, albeit secondarily, by combating STDs that increase the risk of HIV transmission.

Diverse non-surfactant substances appear to be plausible candidates as intravaginal agents. Some sulphated polysaccharides, although not virucidal, are of considerable interest because they have been shown to block an early stage of HIV infection (either the virus's interaction with its cell-surface receptor or its entry into the cell) and also to pre-

vent syncytia formation between HIV-infected and uninfected cells [32-34]. Generally, toxicity is unlikely to be a serious problem with these substances. However, it is important that candidate microbicides that act by such means are evaluated against both HIV and other sexually transmitted pathogens. This would address the possibility that while an agent might block the attachment of HIV to its vaginal/cervical target cells, it might at the same time increase the attachment of other pathogens to their host cells; this could ultimately exacerbate HIV infection rates by increasing the risk or severity of STDs.

There is evidence that myeloperoxidase can kill HIV [35], and a commercial product containing both this enzyme and eosinophil peroxidase is available. Whether this approach will be effective in preventing the sexual transmission of HIV is likely to depend upon whether such enzymes can be made available in a formulation in which they will be non-toxic and active in genital secretions.

Povidone-iodine (Betadine), which is frequently used as a hospital disinfectant, can also inactivate HIV [29].

As already mentioned, HIV is unstable at low pH. Acidic preparations are available commercially as pH adjusters for vaginal use (for example, a gel containing acetic acid buffered at pH 4-4.5), but their value in preventing HIV infection has not been determined.

The vaginal administration of specific antibodies to block HIV transmission is a theoretical possibility but this approach has not been tested experimentally.

The vaginal administration of antiretroviral drugs, including nucleoside and non-nucleoside inhibitors of viral reverse transcriptase, could in theory be useful in preventing HIV replication. The limited value of some of these drugs when used as systemic agents, because of their toxicity, poor bio-availability or the emergence of drug-resistant HIV strains, may not be experienced to the same degree if they are used as vaginal agents.

Formulation of products; delivery systems

The manner in which a product is formulated (for example, dose, pH, nature of excipient) is likely to have a significant influence on its behaviour in the vagina. The importance of microbicide dose and pH has already been discussed. With regard to the excipient, some considerations are straightforward. For example, microbicides may sometimes be used in combination with physical barrier methods, such as condoms or diaphragms, so the vehicle should not attack latex-based products. Other aspects are

more speculative. It is possible, for instance, that a biphasic vehicle could be devised that coats the genital epithelium and thus to some extent protects it from the active anti-HIV ingredient. This would enhance the efficacy:toxicity ratio and might permit the incorporation of agents that otherwise appear unacceptably irritant. (Conversely, if this approach resulted in the agent being held in closer contact with the epithelium for longer it might increase the risk of inflammatory reactions.)

The delivery system for a vaginal product—that is, whether it is a foam, foaming tablet, cream, gel, pessary, impregnated sponge or film—is likely to have a major impact on its acceptability to the potential user. A considerable body of relevant information, including preferences in different societies, has been gained from long experience of contraceptive product acceptability in the area of family planning; however, since the objective of contraception is to prevent pregnancy, as opposed to disease, the factors influencing microbicide use may be different.

Research priorities

An effective research agenda requires the immediate implementation of three critical, complementary strategies covering basic research, product development and clinical evaluation. The productivity of basic research will be maximized if it is pursued in a multidisciplinary context. Product development, in terms of microbiological efficacy and preclinical safety testing, needs to be expedited. Clinical evaluation should include safety assessment as well as large efficacy trials. It is important to evaluate a product's safety in women at both high and low risk of infection. Concurrent microbiological studies are critical in order to interpret gross and microscopic pathological findings.

To have the greatest impact on the HIV/AIDS epidemic, it is important to take the shortest route to achieving a safe and adequately effective product, even if this is later superseded by more effective versions. Therefore, some priority should be given to the study of the safety and efficacy of surfactants already licensed for use as vaginal spermicides. Their evaluation as candidate microbicides would permit the development of protocols for safety and efficacy assessment. This would allow the implementation of relevant and effective regulatory guidelines for the approval of vaginal microbicides.

At the same time, there should be research aimed at developing and testing novel agents, and combinations of agents, with greater efficacy and specificity. This would be a more lengthy undertaking, particularly because extensive animal toxicology is likely to be required. It will be necessary to conduct Phase I

human studies on vaginal/cervical irritation and on the agents' systemic absorption before carrying out efficacy studies.

Specialized *in vitro* test systems need to be developed so that substances can be evaluated in the laboratory for activity against HIV and HIV-infected cells, as well as other sexually transmitted pathogens, under conditions that mimic as closely as possible the physical parameters of the vaginal environment. Clearly, the findings will need to be interpreted with caution, but they will provide some basis for selecting agents for further study. The *in vitro* work will provide data on the effects of such variables as concentration of agent and pH on microbicidal activity and cytotoxicity. In terms of measuring virucidal activity, these systems must be able to distinguish the agent's direct effect on the infective inoculum from its effect on the tissue culture cells used to assay surviving virus.

It would be useful to compare the findings from standardized *in vitro* test systems with the effects of such agents on pathogens in samples of cervico-vaginal secretion and semen obtained from infected individuals. However, the methodology for carrying out this kind of investigation needs to be developed, refined and validated. Techniques are available for quantifying HIV in plasma and peripheral blood mononuclear cells (PBMC), by limiting dilution followed by cocultivation with donor PBMC, for example [36], and by quantitative polymerase chain reaction measurements of HIV RNA and DNA [37,38]. Adapting such methods for use with genital secretions will require overcoming difficulties relating to the viscosity of the samples and to the presence of substances that interfere with the assays—both natural constituents of the secretions and residual microbicide. Some promising work has been carried out in this area [39–41] and its further development deserves to be given a high priority. Infectivity assays are preferable to methods based on nucleic acid detection for estimating microbicidal efficacy.

Predclinical studies to assess vaginal irritation and inflammation can be carried out in an established rabbit model, but clinical studies will also be necessary. Such investigations, conducted in healthy, uninfected, volunteer women, should include examination of the genital epithelium following repeated use of the agent. Macroscopic changes to the vaginal mucosa can be observed by colposcopy and cervicography. More subtle changes can be studied by appropriate histological and immunocytochemical techniques. Effects on the vaginal flora also need to be documented. The findings should be related to the concentration of agent, duration in the vagina and frequency of use.

Since it is probable that any effective microbicide will have an effect on the genital mucosa, it will

be important to document what degree of inflammation is clinically significant. Clearly, frank ulceration would be unacceptable, but the significance of more subtle changes is likely to be elucidated only when the results of efficacy studies are available.

If HIV-infected women volunteers can be recruited, there would be an opportunity to investigate an agent's anti-HIV activity in *ex vivo* studies. It should be possible to assess viral load in cervico-vaginal fluid samples taken immediately before, and at various times after, the insertion of an intravaginal virucide. The design of such studies would be assisted by the availability of data obtained from investigations of the kind already described.

Sociological studies on the acceptability of promising formulations and delivery systems, in various target populations, would provide valuable information for product design and for planning efficacy field trials. Although many lessons can be borrowed from experience in contraception, the behavioural issues surrounding acceptance and compliance with vaginal microbicides may be significantly different. It may be desirable, in some circumstances, to develop the behavioural observations within the context of spermicide evaluation; the basic science that pertains to theoretical constructs, and the development of interventions in relation to microbicide use, should be initiated as soon as feasible. Clearly, some of this work is dependent on the availability of a safe and effective microbicide.

The clinical evaluation of microbicidal products: do they protect individuals against HIV infection?

It would be a mistake to produce, distribute and recommend the use of a microbicide merely on the basis of its characteristics as defined in the studies described above. Clear evidence must be obtained that it really protects people from HIV infection. While animal toxicity and challenge models may be useful in the initial *in vivo* evaluation of new products [20,21], it will be necessary to conduct large-scale efficacy trials.

Microbicide efficacy trials have certain features in common with Phase III trials of prophylactic vaccines as far as logistic, statistical and ethical considerations are concerned. The number of subjects required depends on the anticipated incidence of new infections in the trial participants, on the reduction in incidence (attributed to microbicide use) predicted, and on factors such as the retention and compliance of those recruited. In terms of overall trial design, since it is clear that the correct and consistent use of condoms decreases the risk of infection with HIV and some STDs, it would be unethical to randomize

subjects between microbicide and no microbicide unless all participants were given adequate counselling on measures to avoid acquiring HIV and were encouraged to use condoms; condoms would have to be made freely available. Counselling and condom use can be expected to reduce the incidence of new infections in the population concerned, and the extent to which this is the case would need to be piloted before calculating the sample size.

An understanding should be reached with the community at the outset about the prospects for providing the microbicide on a wider scale should it be shown to have significant benefits in the trial population.

In summary, there are a number of possible designs for efficacy trials, but it is crucial that they are able to distinguish the positive and negative effects of the microbicide from those of the vehicle and delivery system used. The main endpoints of the trial will be microbiological (or seroconversion in the case of HIV infection), but it is also necessary to study side effects (genital irritation, inflammation and ulceration), effects on the vaginal flora, and aspects of acceptability within the population concerned, to ascertain the significance of the microbiological findings.

Ideally, trials should be conducted in high-frequency users of the product (for example, sex workers) as well as in low-frequency users.

International coordination of microbicide research

The need for safe, effective, inexpensive and acceptable microbicides against HIV is urgent. In view of the wide scope and multidisciplinary nature of research in this area, not to mention the broad range of potential agents, combinations and conditions that merit investigation, it would be highly desirable to coordinate efforts both nationally and internationally. This should occur at the strategic planning level and, where appropriate, at the level of specific projects. It could include sharing expertise and scarce reagents and developing standardized methodologies (for example, for quantifying HIV and for assessing agents' anti-HIV efficacy and toxicity).

New research findings should be widely shared as soon as possible. Plans for laboratory studies should take into account data arising from more empirical approaches (including clinical trials), while the design of new clinical studies could benefit from a knowledge of the latest laboratory findings. The aim should be to reach the stage, as quickly as progress allows, where it is possible to mount rational and

definitive intervention studies designed on the basis of sound scientific data.

The development of effective barrier products is a global priority of the highest order. Government agencies, non-governmental organizations, industry and research institutions must work together in the support and coordination of individual and collective efforts. It is clear that both a scientific rationale and a research strategy exist for the development of vaginal microbicides that will neutralize viruses, bacteria and protozoa. Although the task is difficult, the rewards will be immense. The eventual implementation of an HIV/STD prevention strategy based on female-controlled barrier methods is a logical step towards curtailing the spread of HIV. The question is not whether the microbicide approach will prevent HIV infection but, rather, what proportion of HIV infections it will prevent [42].

References

1. Fast PE, Walker MC: Human trials of experimental AIDS vaccines. *AIDS* 1993, 7 (suppl 1):S147-S159.
2. Wasserheit JN: Epidemiological synergy: inter-relationships between HIV infection and other STDs. In *AIDS and Women's Health: Science for Policy and Action*. Edited by Chen L, Sepulveda J, Segal S. New York: Plenum Press; 1991:47-72.
3. Hitchcock P, Claypool L: HIV and Other STDs Working Group Report. In *Barrier Contraceptives*. Edited by Mauck C, Cordero M, Gabelnick H, Spieler JM, Rivera R. New York: Wiley-Liss; 1994:353-362.
4. Hitchcock PJ, Wasserheit JN, Harris JR, Holmes KK: Sexually transmitted diseases in the AIDS era: development of STD diagnostics for resource-limited settings is a global priority. *Sex Transm Dis* 1991, 18:133-135.
5. Botly MC, Brunham RC: The impact of HIV and other STDs on human populations. In *Infectious Diseases Clinics of North America: Sexually Transmitted Diseases in the AIDS Era. Part I*. Edited by Cohen MS, Hook EW, Hitchcock P. Philadelphia: W.B. Saunders; 1993:771-792.
6. Bird KD: The use of spermicide containing nonoxonyl-9 in the prevention of HIV infection. *AIDS* 1991, 5:791-796.
7. Jeffries DJ: Nonoxonyl-9 and HIV infection (letter). *BMJ* 1988, 296:1798.
8. Malkovsky N, Newell A, Dalgleish AG: Inactivation of HIV by nonoxonyl-9 (letter). *Lancet* 1988, i:645.
9. Polsky B, Baron PA, Gold JWM, Smith JL, Jensen RH, Armstrong D: *In vitro* inactivation of HIV-1 by contraceptive sponge containing nonoxonyl-9. *Lancet* 1988, i:1456.
10. Stone KM, Grimes DA, Magder LS: Personal protection against sexually transmitted diseases. *Am J Obstet Gynecol* 1986, 155:180-188.
11. Asculai SS, Weis MT, Rancourt MW, et al.: Inactivation of herpes simplex viruses by nonionic surfactants. *Antimicrob Agents Chemother* 1978, 13:686-690.
12. Kelly JP, Reynolds RB, Stagno S, et al.: *In vitro* activity of the spermicide nonoxonyl-9 against *Chlamydia trachomatis*. *Antimicrob Agents Chemother* 1985, 27:760-762.
13. Rosenberg MJ, Rojanapithayakorn WM, Feldblum PJ, Higgins JE: Effect of the contraceptive sponge on chlamydial infection, gonorrhea and candidiasis. *JAMA* 1987, 257:2308-2312.
14. Kretz J, Nguji E, Holmes K, et al.: Efficacy of nonoxonyl-9 contraceptive sponge use in preventing heterosexual acquisition of HIV in Nairobi prostitutes. *JAMA* 1992, 268:477-482.

- Zeheng L, Feldblum PJ, Oliver RM, Kaprue L: Barrier contraceptive use and HIV infection among high-risk women in Cameroon. *AIDS* 1993, 7:725-731.
16. Feldblum P, Hira S, Goodwin S, Kamanga J, Mukelabale G: Efficacy of spermicide use and condom use by HIV-discordant couples in Zambia. *VIII International Conference on AIDS/STD World Congress*. Amsterdam, July 1992 (abstract WeC1085).
17. Nrusimhan S, Roddy RE, Chuthongse S: The effects of frequent nonoxonyl-9 use on the vaginal and cervical mucosa. *Sex Transm Dis* 1991, 18:176-179.
18. Nrusimhan S, Roddy RE, Chuthongse S: Use of nonoxonyl-9 and reduction in rate of gonococcal and chlamydial cervical infections. *Lancet* 1992, 339:1571-1575.
19. Roddy RE, Cordero M, Cordero C, Fortney JA: A dosing study of nonoxonyl-9 and genital irritation. *Am J STD AIDS* 1993, 4:165-170.
20. Miller CJ, Alexander NJ, Gettle A, Hendricks AG, Marx PA: The effect of contraceptives containing nonoxonyl-9 on the genital transmission of simian immunodeficiency virus in rhesus macaques. *Fertil Steril* 1993, 57:1126-1128.
21. Muench TR, Whaley KJ, Mandrell TD, Bishop BD, Win CJ, Coe BA: The cat/feline immunodeficiency virus model for transcutaneous transmission of AIDS: nonoxonyl-9 contraceptive jelly blocks transmission by an infected cell inoculum. *AIDS* 1993, 7:797-802.
22. Klebanoff SJ, Hillier SL, Eschenbach DA, Wakenndorph AM: Control of the microbial flora of the vagina by H₂O₂-generating lactobacilli. *J Infect Dis* 1991, 164:94-100.
23. Moller BR, Kasperen P: The acidity of the vagina. In *Vaginitis and Vaginosis*. Edited by Hurowitz B, Mark PA. New York: Wiley-Liss, 1991:63-68.
24. Klebanoff SJ, Coombs RW: Viricidal effect of *Lactobacillus acidophilus* on human immunodeficiency virus type 1: possible role in heterosexual transmission. *J Exp Med* 1991, 174:289-292.
25. Ongstad J, Ceccherini-Nelli L, Pistell M, Spector S, Bendinelli M: Acid sensitivity of cell-free and cell-associated HIV-1: clinical implications. *AIDS Res Hum Retroviruses* 1990, 6:1433-1436.
26. Wolters-Everhard E, Duxy JMJ, Lemmens WAJG, Doesberg WH, De Pont J-JHM: Buffering capacity of human semen. *Fertil Steril* 1986, 46:114-119.
27. Fox CA, Meldrum SJ, Watson BV: Continuous measurement by radiotelemetry of vaginal pH during human coitus. *J Reprod Fertil* 1973, 33:69-75.
28. Lloyd G, Atkinson T, Sutton PM: Effect of bile salts and of fusidic acid on HIV-1 infection of cultured cells. *Lancet* 1988, i:1418-1421.
29. Harblson MA, Hammer SM: Inactivation of human immunodeficiency virus by Betadine products and chlorhexidine. *J Acquir Immune Defic Syndr* 1989, 2:16-20.
30. Burnan LG, Christensen P, Christensen K, et al: Prevention of excess neonatal morbidity associated with group B streptococci by vaginal chlorhexidine disinfection during labour. *Lancet* 1992, 340:65-69.
31. Petri WA Jr, Wagner RR: Reconstitution into liposomes of the glycoprotein of vesicular stomatitis virus by detergent dialysis. *J Biol Chem* 1979, 254:4315-4316.
32. Babu M, Nakajima M, Schols D, Pauwels R, Balzarini J, De Clercq E: Pentosan polysulfate, a sulfated oligosaccharide, is a potent and selective anti-HIV agent *in vitro*. *Antiviral Res* 1988, 9:335-343.
33. Bagdasarian O, Lischner HW: Activity of dextran sulfate and other polyanionic polysaccharides against human immunodeficiency virus. *J Infect Dis* 1988, 158:1084-1087.
34. McClure MO, Moore JP, Blanc DF, et al: Investigations into the mechanism by which sulphated polysaccharides inhibit HIV infection *in vitro*. *AIDS Res Hum Retroviruses* 1992, 8:19-25.
35. Chase MJ, Klebanoff SJ: Viricidal effect of stimulated human mononuclear phagocytes on human immunodeficiency virus type 1. *Proc Natl Acad Sci USA* 1992, 89:5582-5585.
36. Ariyoshi K, Bloor S, Bieniasz PD, et al: Development of rapid quantitative assay for HIV-1 plasma infectious virus: culture-PCR (CPD). *J Med Virol* 1994, in press.
37. Semple MG, Kaye S, Loveday C, Tedder RS: HIV-1 plasma viraemia quantification: a non-culture measurement needed for therapeutic trials. *J Virol Methods* 1993, 41:167-179.
38. Bieniasz PD, Ariyoshi K, Housley MAD, et al: Variable relationship between proviral DNA load and infectious virus titre in the peripheral blood mononuclear cells of HIV-1-infected individuals. *AIDS* 1993, 7:803-806.
39. Wainberg MA, Spira H, Bleau G, Thomas R: Inactivation of human immunodeficiency virus type 1 in tissue culture fluid and in genital secretions by the spermicide benzalkonium chloride. *J Clin Microbiol* 1990, 28:156-158.
40. Henin Y, Mandelbrot L, Herrion R, Pradinaud R, Coulaud JP, Montagnier L: Virus excretion in the cervicovaginal secretions of pregnant and nonpregnant HIV-infected women. *J Acquir Immune Defic Syndr* 1993, 6:72-75.
41. Clemenson DBA, Moss GB, Willerford DM, et al: Detection of HIV DNA in cervical and vaginal secretions: prevalence and correlates among women in Nairobi, Kenya. *JAMA* 1993, 269:2860-2864.
42. Hitchcock P: Barrier methods for the prevention of gonococcal and chlamydial infection. In *Barrier Contraceptives*. Edited by Mauck C, Cordero M, Gabelnick H, Spicler JM, Rivera R. New York: Wiley-Liss, 1994:225-241.

HIV AND OTHER STDs WORKING GROUP REPORT

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Blocking transmission is the ideal way to prevent and control sexually transmitted diseases (STDs), and interest in developing more effective mechanical and chemical barrier methods for this purpose is intense. The barrier methods that are currently available, however, have limitations. Most require male partner consent, if not active cooperation, and therefore cannot be independently implemented at the discretion of the female partner. Although epidemiological studies have demonstrated that the use of condoms or spermicides can reduce the risk of acquiring some STDs, there are many situations in which personal, social, or cultural barriers interfere with a woman's ability to successfully negotiate and implement their use. Specifically, the need for a method which can be implemented by women is grounded in the high prevalence of: 1) non-consensual sex; 2) sex without condom use; and 3) risky behaviors that occur without partner knowledge.

Therefore, just as oral contraceptives dramatically enhanced the ability of women to avoid unwanted pregnancy, effective female-controlled mechanical and chemical barriers are urgently needed to enhance the ability of women to avoid sexually transmitted infections. Ideally, topical microbicides could be developed with or without contraceptive efficacy; these would be responsive to women who wish to become pregnant while avoiding disease, who use contraceptive methods which either have no effect on or increase risk of STD transmission, or who wish to prevent STDs during their non-reproductive years. Furthermore, chemical barriers that inactivate pathogens in

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vaginal/cervical secretions as well as in the ejaculate could reduce female-to-male as well as male-to-female transmission.

In order to develop products and the necessary behavioral interventions, many questions regarding the biological, biochemical, anatomical, microbiological and behavioral aspects of the problem must be addressed. Among the relevant issues are: 1) the acceptability of barrier methods that prevent infection alone or also prevent pregnancy; 2) the early steps in pathogenesis; 3) the mechanisms of action; and 4) the associated inflammation, irritation, and toxicity.

ACCEPTABILITY OF BARRIER METHODS FOR STD PREVENTION

Important acceptability factors should be addressed for methods that are only antimicrobial as well as for those that are both antimicrobial and contraceptive. The product should be invisible (i.e., colorless, odorless, and tasteless); stable, with a long shelf life; convenient to carry; easy to use; available over the counter; inexpensive; rapidly effective for the appropriate duration; and safe for daily single use (however, daily multiple use is desirable).

It is recognized that potential users of topical microbicides are socially, economically and culturally heterogeneous, therefore, product characteristics should be modifiable based on specific user needs. For example, the invisible nature of the product might not meet the needs of some users - visible assurance of product use, medication-like qualities, or erotic features might be preferred. Unique packaging and distribution systems may also be needed to reach particular user groups such as adolescents, post-menopausal women, and sex workers, as well as non-monogamous and monogamous women. Behavioral research on acceptance and compliance issues must be undertaken. This agenda should include basic as well as intervention research.

MECHANISM OF ACTION FOR BARRIER METHODS

Identification/characterization of new microbicidal compounds is likely to be essential to develop chemical barrier methods for STD prevention. A number of potential compounds have been suggested including sulfated polysaccharides such as the dextran sulfates and carageenins [Ueno and Kuno, 1987; Busso and Resnick, 1990; Pearce-Pratt and Phillips, 1993] or plant products such as gossypol [Polisky et al., 1989] and neem tree derivatives [Riar et al., 1990]. After efficacy has been demonstrated in screening tests, the effects of active ingredients on normal flora and on the female and male reproductive tract should be thoroughly investigated. The effect of microbicides on yeast infections and bacterial vaginosis should also be characterized. It is presumed that microbicides will need to be fast acting in order to block infection, although our understanding of the early steps and chronology of establishing infection is still very limited. Mechanism of action studies should be undertaken on promising compounds.

The use of agents that might prevent infection by maintaining the acidic pH (4.0-

Moller and Kasperaon, 1991] of the vagina certainly merits investigation. Nuclear cells and free HIV have been shown to be very labile at pH 4.0-4.5, (Dugradi et al., 1990; Voeller and Anderson, 1992). Whether acidic pH could be maintained in the vagina given the effects of the alkaline ejaculate (Fox et al., 1973) is unknown. The dynamics and distribution of pH changes relative to tissue surfaces and vaginal lumen also needs to be further clarified.

The role of H_2O_2 -producing lactobacilli, and the possibility of developing better barriers for explant requires further study. Whether the lactobacilli contribute to disease resistance merits additional attention, as well. Relevant research is already underway in the rhesus monkey to determine the effects of seminal plasma and pH on infectivity of human immunodeficiency virus (HIV). It may also be possible to consider systemic approaches to acidify the ejaculate by systemic therapy such that infected mononuclear cells and pathogens would not survive. Associated effects on spermatozoa and fertility would be characterized.

The microbial spectrum of activity within the genital environment must be established. Bacterial STDs, such as chlamydial infection and gonorrhea occur at very high incidence rates and are typically established within the columnar epithelium of the cervix; these organisms may ascend to the upper tract causing pelvic inflammatory disease (PID). Chronic sequelae include tubal scarring, which leads to tubal infertility, ectopic pregnancy, and pelvic pain (Westrom, 1980; Wasserheit, 1989). Furthermore, human papilloma virus infection of the cervix may lead to cervical dysplasia or cervical neoplasia. It may therefore be prudent to prioritize sites in the lower tract which should be protected, placing cervix first, vagina second, and vulva third. This likely represents the order in which protection is most feasible through currently available barrier contraceptives (Cates and Stone, 1992).

The role of spermatozoa in transporting pathogens or otherwise facilitating movement up the female reproductive tract should be clarified by further research [for HIV, see Anderson, 1992; Scofield, 1992]. Whether microbicides could target pathogens on the surface of, or within, spermatozoa without being spermicidal is unknown.

Once efficacy has been determined, the potential impact of all products on public health should be assessed. From the perspective of the individual, the ideal method is 100% efficacious. However, from a public health perspective, there is merit in considering the usefulness of approaches that are less effective. These may, with correct and consistent use, significantly reduce disease transmission and prevalence of infections. The problems associated with developing and licensing such partially or modestly effective products are formidable, however.

The efficacy and safety of barrier methods that are used by men to prevent sexually transmitted diseases should be viewed in the context of a variety of sexual behaviors. This includes condoms made of latex or of other materials. The development of behavioral interventions that target increased and correct use of condoms are needed, as well as new and better condom designs. Additional research to clearly establish the effects of vasectomy on the transmission of HIV and other pathogens is also warranted.

PATHOGENESIS

A more complete understanding of the early steps in infection, pathogen survival, and the kinetics of infection is critical. These features of infection are microbe-specific and relevant to the feasibility of both precoital and postcoital microbicides. Currently, the only postcoital practices that are purported to influence infection rates are urination in males and douching in females; neither have been demonstrated to reduce infection rates, however. In fact, douching has been associated with increased risk of PID [Wolner-Hanssen et al., 1990; Phillips et al., 1992]. Interestingly, douching is very widely practiced by large numbers of women in different cultures, who use a variety of techniques in doing so. The effects of different douching techniques on infection rates is not known, but interventions based on microbicide use may need to take into account the fact that women may be very reluctant to abandon this practice. This is especially likely where it is a traditional custom, or when associated with hygienic standards, e.g., when intercourse with different partners occurs within a relatively short interval of time.

The feasibility of targeted microbicidal agents is also related to the specific pathogens. Among the approaches that may be useful are: 1) immunoglobulins directed against conserved antigens on pathogens; 2) compounds that block receptor function; or 3) antibiotic peptides such as magainins or defensins. Other biological agents, such as plant products, may be useful, although much more knowledge regarding efficacy, safety, and their mechanisms of action is necessary. Specificity of action, however, is a very important property of effective, safe products, especially if deleterious effects on the natural flora and on the genital epithelium can be avoided. Achieving a high degree of specificity may also be a key element in developing products that are active against pathogens, especially HIV, without being spermicidal.

IRRITATION, INFLAMMATION, AND TOXICITY

An ideal microbicide must be *safe* as well as effective. In its most concentrated form, it must be non-inflammatory but potent enough to retain sufficient efficacy after being diluted with semen and vaginal secretions. The extent and effect of this dilution may vary greatly between individuals or within the same individual over time. With active agents that are more specific than the detergent compounds currently used in spermicides, the window between safe, efficacious concentrations and toxic concentrations may be much wider. In any case, more dose-response data is needed for current products as well as for any new agents and formulations. For dose-response data, it will also be necessary to identify parameters to be used in preclinical animal studies and to define their relevance as predictors of safety and efficacy in human clinical trials.

It is also desirable that the vehicles be neutral or synergistic with regard to antimicrobial activity, and neutral or ameliorative with regard to inflammatory effects. Better vehicles might promote more efficient coating of the vaginal, ectocervical, and penile tissues, and thereby contribute to preventing infection. Vehicles might also reduce the inflammatory effects of active ingredients.

or existing products and future candidates, it is necessary to define the frequency parameters for which the microbicide is safe. For example, if nonoxonyl-9-*ox* spermicides cause genital ulceration with high-frequency use [Kreiss et al., 1992; Roddy et al., 1993], it is important to determine whether there is a frequency of use at which they are completely safe. In addition to frequency, there may be other patterns of use that would contribute to decreased safety. These include pre-existing inflammation or traumatic injury. If so, these need to be identified and safety limits defined.

The vaginal mucosal surface might be altered by microbicide-induced inflammation, thereby increasing infection risk by decreasing the infectious dose. The microbicide might affect tissue integrity (increased friability), vaginal pH, presence of target cells, mucus coat, endogenous normal flora and other more subtle properties of the normal reproductive tract. In other words, it is desirable that a normal healthy vaginal environment be maintained - changes induced by the use of microbicides should be characterized. Qualitative and quantitative effects and their duration should be evaluated.

In order to address these questions, the development of new methods/models as well as the adaptation of those that have been developed for other purposes may be necessary. Preclinically appropriate standardized methods for quantifying and documenting effects need to be defined and implemented. Parameters observed in non-clinical animal studies, such as cytokine release, changes in tissue permeability, and cell mobilization, may differ greatly from those in human clinical studies, so the correlates of toxicity in humans will have to be established.

Standardized and improved clinical methods are also necessary for evaluating inflammatory responses and associated reports of irritation. In this regard, characteristics of the normal or typical reproductive tract tissues should be better defined at the level of the individual and the population. Tissue changes that occur due to the hormonal changes of the menstrual cycle, hormonal contraceptive use, and many other factors need to be investigated. Clinically, the correlation between symptoms and signs of inflammation is poorly defined. Furthermore, it is unclear what degree of inflammation is significant with respect to alteration of infection risk or comfort. Inflammatory responses may also be very site specific; the variability and significance of this response would be investigated.

Appropriate regulatory guidelines must be defined to expedite the development of safe and effective microbicides or mechanical barrier methods. Adequate regulatory guidelines already exist for estimating the toxicity and teratogenicity of new drugs (e.g., United States Department of Health and Human Services, Federal Register 45:82014-2077, 1980). Unfortunately, most existing barrier methods were marketed prior to the development of these FDA regulations, consequently safety and efficacy data for most barrier methods have never been gathered. Ideally, compounds will not be absorbed systemically and will be safe for use during pregnancy. Products which may be highly efficacious in preventing disease, but damage spermatozoa without being completely spermicidal, will present special regulatory problems.

The potential for antagonistic or synergistic interactions with combination products using both chemical and barrier approaches needs to be examined. Use of methods

combining a chemical barrier with a mechanical barrier, or a chemical barrier with a delivery device, may be associated with increased or decreased inflammatory effects, and should be addressed experimentally. If mechanical barriers are used, it is desirable that sustained pressure on tissues be minimized. Ease of insertion is also relevant for reducing trauma and irritation during product use.

MAJOR QUESTIONS TO ADDRESS

In order to facilitate development of effective barrier methods, three major questions should be addressed:

- 1 What is the safety and efficacy of currently available barrier methods for preventing sexually transmitted infections?
- 2 What new strategies and new methods are feasible to use as protective barriers against sexually transmitted infections without partner knowledge or cooperation?
- 3 What are the general and culturally specific factors that influence compliance with barrier method use? Based on knowledge of these factors, what interventions will result in sustained, correct and consistent use of barrier methods to prevent sexually transmitted infections?

MAJOR ACHIEVEMENTS FOR THE COMING DECADE

Progress in this critical area will require the establishment of international, multidisciplinary, and coordinated efforts that include:

- 1 A significant increase in funding for research on barrier methods for STD prevention, with special emphasis on topical microbicides.
- 2 The establishment of a global clinical trials network to test the safety and efficacy of barrier methods for STD prevention.
- 3 The establishment of cooperative working relationships with regulatory agencies to define and implement an expeditious and responsible approval process for barrier methods for STD prevention.
- 4 The establishment of a creative and rational resolution of associated liability issues, perhaps including the establishment of a "no-fault" international compensation program based on pooled resources from manufacturers.

REFERENCES

- de la DJ (1992): Mechanisms of HIV-1 transmission via semen. *Journal of NIH Research* 4:104-108.
- de la JE, Resnick L (1990): Anti-human immunodeficiency virus effects of dextran sulfate are strain dependent and synergistic or antagonistic when dextran sulfate is given in combination with dideoxynucleosides. *Antimicrob Agents Chemother* 34:1991-1995.
- de la J Jr., Stone KM (1992): Family planning, sexually transmitted diseases and contraceptive choice: a literature update - Part I. *Family Planning Perspectives* 24:75-84.
- de la C., Meldrum SJ, Watson BW (1973): Continuous measurement by radio-telemetry of vaginal pH during human coitus. *J Reprod Fertil* 33:69-75.
- de la J., Ngugi E, Holmes K, Ndinya-Achola J, Waiyaki P, Roberts PL, Ruminjo I, Otieno R, Kimani J, Fleming TR, Anzala A, Holton D, Plummer F (1992): Efficacy of nonoxynol-9 contraceptive sponge use in preventing heterosexual acquisition of HIV in prostitutes. *JAMA* 268:477-482.
- de la BR, Kasperian P (1991): The acidity of the vagina. In Horowitz B, Marc PA eds. "Vaginitis and Vaginosis." New York: Wiley-Liss, Inc., pp 63-68.
- de la J, Ceccherini-Nelli L, Pistelli M, Spector S, Bendinelli M (1990): Acid sensitivity of cell-free and cell-associated HIV-1: clinical implications. *AIDS Res Hum* 6:1433-1436.
- de la R, Phillips DM (1993): Sexual Transmission of HIV. *Biol Reprod* 48:431-445.
- de la RS, Tuomala RE, Feldblum PJ, Schachter J (1992): The effect of cigarette smoking, *Chlamydia trachomatis* infection, and vaginal douching in ectopic pregnancy. *Obstet Gynecol* 79:85-90.
- de la B, Segal SJ, Baron PA, Gold JWM, Ueno H, Armstrong D (1992): Inactivation of human immunodeficiency virus *in vitro* by glycyrrhizin. *Contraception* 39:579-589.
- de la S., Devakumar C, Ilavazhagan G, Bardhan J, Kain AK, Thomas P, Singh R, Singh I (1990): Volatile fraction of neem oil as a spermicide. *Contraception* 42:479-487.
- de la JE, Cordero M, Cordero C, Fortney JA (1993): A dosing study of nonoxynol-9 and genital irritation. *AIDS*, in press.

Scofield VL (1992): Sperm as vectors and cofactors for HIV-1 transmission. *Journal of NIH Research* 4:104-111.

Ueno R, Kuno S (1987): Dextran sulfate, a potent anti-HIV agent *in vitro* having synergism with zidovudine. *Lancet* 1987i:1379.

Voeller B, Anderson DJ (1992): Heterosexual transmission of HIV. *JAMA* 267:1917-1918.

Westrom L (1980): Incidence, prevalence, and trends of acute pelvic inflammatory disease and its consequences in industrialized countries. *Am J Obstet Gynecol* 138:880-892.

Wasserheit JN (1989): The significance and scope of reproductive tract infections among third world women. *Int J Gynaecol Obstet, Supplement* 3:145-168.

Wolner-Hanssen P, Eschenbach DA, Paavonen AA, Stevens CE, Kiviat NB, Critchlow C, Derouen T, Koutsky L, Holmes KK (1990): Association between vaginal douching and pelvic inflammatory disease. *JAMA* 263:1936-1937.

DISCUSSION

Gabriel Bialy: The assumptions are correct that there is indeed a crisis situation and that in some way NIH will have to get away from its rigid and parochial method of reviewing applications. I think the system as it currently exists was fine for basic research, but we need to develop strategies with room for at least some initial investment in innovative approaches that are not extensively developed with a lot of data, so that the investigator can establish applicability, effectiveness, etc. Our current approach is stifling. While I do not necessarily take issue with the great value of the peer review system, I think we need to alter our criteria. How we do this remains a question, but we need to work together and create a new forum for the review and implementation of critical projects.

Clark Taylor: It was immensely exciting to work with this particular committee. In addition to the ground we've covered here, we also need to develop a new paradigm for looking at what we're doing with barrier techniques. We need to clarify our understanding and language about sex. We mustn't confuse sexual orientation and psychological issues with sexual techniques. We mustn't confuse what causes conception with what transmits disease. This means thinking about things in a different way. Something we haven't talked about in this meeting is the immense suffering, such as the blindness, the pain, and the death, that are caused by sexually transmitted cofactors like cytomegalovirus, Epstein Barr virus, and herpes, for people who are already HIV-infected. This raises issues, such as kissing, oral sex, anal sex, etc., that we haven't thought about when we limit our focus to preventing conception with STD prevention as

secondary benefit. What we really have to do is re-think our goals as we incorporate STD prevention as a major focus of our activity.

Enelope Hitchcock: I think that all of us want to take action and we should recognize that this meeting represents a very substantial step in this process. This meeting has taken an interdisciplinary approach, looking at things from different perspectives, and talking about public and private sector interaction. It is not easy to develop a new discipline. It is not easy to address emerging problems. I think this meeting, certainly from the perspective of my own education and understanding of the problem, and the opportunity to network with all of you, has been very successful. I want to thank the organizers for having the insight to do this, and everybody in this room for their contribution and commitment. I think it's been a very productive and important meeting.

James Koch: Following on the comments that were just made, I'd like to know if anyone has plans for carrying on the tremendous impetus that was generated by this conference. If I were among those who planned and organized this I would be very proud, but I'm not close enough to these organizations to know what might be feasible to carry forward this impetus and not let it simply die after this conference is over.

Henry Gabelnick: As I said at the beginning of the Workshop, and repeated this morning, one of the primary objectives of the working groups was to develop action plans. Although not all of the items can be implemented with specific actions, there are certainly aspects which can be, and we intend to do so, whether it's those things that are directly within our power, such as putting together interagency groups, or things that are indirectly within our power, such as establishing public sector/private sector collaborations. There are many other issues that will come forward. As you know, the proceedings will be published and the statements and recommendations from the working groups included. We intend to prioritize the most urgent recommendations and see that they are implemented.

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PART V
PART

Points to Consider in the Nonclinical Pharmacology/Toxicology Development of Topical Drugs Intended to Prevent the Transmission of Sexually Transmitted Diseases (STD) and/or for the Development of Drugs Intended to Act as Vaginal Contraceptives¹

Division of Reproductive and Urologic Drug Products
Office of Drug Evaluation II

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and
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1. This document is an informal communication under 21 CFR 10.90 (b) (9) that represents the best judgement of the Divisions of Reproductive and Urologic Drug Products, the Division of Antiinfective Drug Products and the Division of Antiviral Drug Products at this time. This document does not necessarily represent the formal position of the Center for Drug Evaluation and Research or the Food and Drug Administration, and it does not bind or otherwise obligate the Center or Agency to the views expressed.

Introduction

This document discusses several nonclinical pharmacology/toxicology issues that are inherent with the development of topical drugs that are intended to prevent sexually transmitted diseases (STD) or with the development of drugs proposed to act as vaginal contraceptives. It is not meant to replace existing guidance concerning the nonclinical pharmacology/toxicology safety data required to support the submission of Investigational New Drug (IND) applications. Rather, it offers insight into some aspects of these classes of drugs that make them unique from systemically administered drugs, namely, the topical sites of their administration and action and the prophylactic nature of the indications. The publication of this document is a joint effort of three independent review divisions in the Center for Drug Evaluation and Research of the FDA, and represents their best harmonized view of the issues at this time. However, all the recommendations contained herein may be subject to modification and sponsors are encouraged to discuss the content of their nonclinical submissions with the review division appropriate to each individual application

Toxicology Studies

To support the safety of the individual phases of clinical development, studies in at least two animal species (one being a non-rodent) should be used to assess acute, subchronic and chronic toxicity at the proposed site of exposure. Potential sites of exposure to topical microbicides for the prevention of STDs include vaginal, cervical, penile, oral and rectal mucosal areas. For the purpose of this document, contraceptives are considered to expose vaginal and penile tissue. All toxicology studies should use at least three dose levels of the drug and an appropriate control, with the high dose showing frank toxicity (it is understood that there may be limits to the ability to achieve a toxic dose), and the low dose showing little or no toxicity. With the exception of the varying concentrations of the active product, the drug to be applied should be in its final formulation and the study duration should be equal to, or longer than, the proposed duration of treatment in the clinical trial. However, if in an early trial, the duration of treatment is for less than two weeks, a nonclinical study duration of two weeks should generally be used. All nonclinical studies carried out to assess safety of a drug should be performed according to Good Laboratory Practices as outlined in 21 CFR. 58. Sponsors should consult with the FDA reviewing pharmacologist concerning approaches and protocols regarding these issues.

Because the topical microbicides are intended for intermittent use during periods in which the individual will be at risk of contracting a STD and contraceptives are intended for intermittent use over long periods of reproductive competence, they are, for regulatory purposes, considered to be chronically administered drugs. Thus, in the latter stages of clinical development, six month studies in a rodent and one year toxicity studies in a non-rodent will be necessary to support phase 2/3 human trials. Carcinogenicity studies in rats and mice should be performed during the late stages of the drug development program.

Pharmacokinetic/Toxicokinetic Studies

During the conduct of subchronic and chronic toxicity studies, concurrent pharmacokinetic/toxicokinetic analyses should be performed to evaluate the drug's ADME (absorption, disposition, metabolism and excretion) profile and to determine if the drug is systemically absorbed. Appropriate analytical methodology should be established as early as possible to provide for precise, consistent and reliable pharmacokinetic data. Major pharmacokinetic endpoints such as maximum plasma concentration, time to maximum plasma concentration, area under the concentration time curve, volume of distribution, bioavailability and clearance should be determined. To better assess margins of safety, the animal pharmacokinetic data should be compared with data in humans. For instance, a drug might be absorbed in humans and the vaginal route of delivery in animals cannot achieve much higher systemic drug levels than those seen in humans. In that case, a one to three-month toxicology study in one species, with parenteral or oral administration of the drug, may be requested to produce sufficiently high blood levels to identify all potential toxicities. If the drug is absorbed, histopathology should be carried out on the full spectrum of organs and tissues.

Irritation

The likelihood of drug-induced irritation (adverse reactions such as inflammatory responses) to drug exposed tissues should be evaluated in animals at both the macroscopic and microscopic levels. Vaginal irritation tests should be carried out in rabbits with daily applications for ten days. If rabbits are adequately tested by the vaginal route for adverse effects in toxicology studies, no separate vaginal irritation study is required.

If the drug is found to be irritating to mucosal tissues and the sponsor decides to pursue further development, additional pharmacokinetics should be carried out to compare the extent of penetration of the drug across the inflamed tissue with that occurring in intact tissue. These data will help to determine whether the absorption kinetics of the drug is facilitated when the mucosa is compromised.

Hypersensitivity and Photosensitivity

If appropriate, the potential to produce hypersensitivity and photosensitivity of the drug should be evaluated. Examples of animal models and protocols for tests related to these toxicities can be found in references such as Marzulli and Maibach's "Dermatotoxicology" or Burleson, Dean and Munson's "Methods in Immunotoxicology."

Genotoxicity

A standard battery of tests should be carried out to evaluate all new molecular entities for the potential to induce genotoxic effects. A drug should be evaluated for potential genetic toxicity prior to the submission of the IND. The standard battery consists of the three following tests:

- 1) A gene mutation test in bacteria;
- 2) An in vitro mouse lymphoma tk assay or a mammalian cell in vitro cytogenetic test for chromosomal aberrations;
- 3) An in vivo test for chromosomal damage to rodent bone marrow cells or a mouse micronucleus test.

If the three tests chosen indicate that the drug is devoid of genetic toxicity, no additional studies need to be carried out. If one or more of the battery is positive, the sponsor will be expected to carry out additional genotoxicity tests in consultation with the appropriate review division. For detailed information regarding genotoxicity, sponsors should refer to the document entitled "Guidance on Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals." Copies of the Guideline are available from the CDER Consumer Affairs Branch, HFD-210, Center for Drug Evaluation and Research, 5600 Fishers Lane, Rockville, MD 20857.

Reproductive Toxicology

Reproductive toxicology studies should be carried out to explore the possible effects of the drug on fertility and reproductive performance. Additional studies should be performed to examine whether the drug is teratogenic or has an effect on perinatal/postnatal development. Studies designed to assess the teratogenic potential should be carried out in two species, usually in rats and rabbits. It is expected that reproductive toxicology studies will be completed prior to Phase 2/3 trials. For detailed information regarding reproductive toxicology, sponsors should refer to the document entitled, "Guideline for Industry, Detection of Toxicity to Reproduction for Medicinal Products." Copies of the Guideline are available from the CDER Consumer Affairs Branch, HFD-210, Center for Drug Evaluation and Research, 5600 Fishers Lane, Rockville, MD 20857.

Carcinogenicity

Carcinogenicity information is usually obtained during the final phase of the clinical development program. For a vaginal contraceptive, carcinogenicity tests in rats and mice should be submitted with the NDA. For a microbicide, carcinogenicity studies are also required, but the expected date of completion of the studies in relation to the filing of the NDA may vary. The drug should be administered for two years and the doses used should be chosen according to the principles outlined in the document entitled, "Guideline for Industry, Dose Selection for Carcinogenicity Studies of Pharmaceuticals." Copies of the Guideline are available from the CDER Consumer Affairs Branch, HFD-210, Center for Drug Evaluation and Research, 5600 Fishers Lane, Rockville, MD 20857. Sponsors should consult with the appropriate reviewing division for the current position and strategic approaches to these issues. Currently, it is recommended that sponsors request that the Executive Committee of the Carcinogenicity Assessment Committee of the Center for Drug Evaluation and Research review the protocols for the carcinogenicity studies

prior to their initiation and concur on their content.

Already Marketed Drugs

On 2/3/95, a notice of proposed rulemaking from the Food and Drug Administration entitled, "Vaginal Contraceptive Drug Products for Over-the-Counter Human Use" appeared in the Federal Register (Vol. 60, No. 23, pp. 6892-6903). The proposed rule stated that manufacturers of over-the-counter (OTC) vaginal contraceptive drug products would be required to obtain approved applications for marketing of their products. The agency took this action because the effectiveness of these products is dependent upon the final formulation. Therefore, each product must be tested in appropriate clinical trials under actual conditions of use. The proposed rulemaking does not affect the current marketing status of OTC vaginal contraceptives. However, on the effective date of a final regulation, an OTC vaginal contraceptive drug product that is not the subject of an approved application would be regarded as a new drug and be subjected to regulatory action regardless of its status prior to the publishing of the final regulation.

The agency has determined that nonoxynol 9 and octoxynol 9 would be appropriate ingredients for an approved application and has concluded that although nonoxynol 9 and octoxynol 9 kill sperm in vitro and in vivo, the spermicidal activity and resulting effectiveness of these contraceptive active ingredients cannot be considered separately from a product's vehicle. These active ingredients lose some of their effectiveness in humans when the spermicide in final formulation is diluted by various amounts of genital secretions during coitus. Thus, clinical studies are necessary to establish effectiveness of the spermicide's final formulation when used in humans. However, applications for currently marketed OTC products containing these ingredients require no additional nonclinical data, but, instead, may refer to (1) the recommendations of the Advisory Review Panel on OTC Contraceptives and Other Vaginal Drug Products that appeared in an advanced notice of proposed rulemaking in the Federal Register (12/12/1980, Vol. 45, No.241, pp 82014-82049), entitled, "Vaginal Contraceptive Drug Products for Over-the-Counter Human Use; Establishment of a Monograph; Proposed Rulemaking," and (2) the history of use of approved drug products containing nonoxynol 9.

The quest to identify safe and effective products for the prevention of HIV and other STDs is a high priority public health concern. Sponsors are strongly encouraged to evaluate OTC contraceptive products towards this use. However it should be recognized that studies designed to assess the safety and effectiveness of a formulated drug to prevent pregnancy and to prevent the transmission of an STD may be different. Manufacturers wishing to develop a formulation towards one or the other are encouraged to enter into a dialog with the appropriate reviewing divisions concerning the content of their applications.

References

Dermatotoxicology. Ed., Marzulli, F.N. and H.I. Maibach. New York: Hemisphere Publishing, 1991.

Methods in Immunotoxicology. Ed., Burleson, G.R., J.H. Dean and A.E. Munson. New York: Wiley-Liss, Inc., 1995.