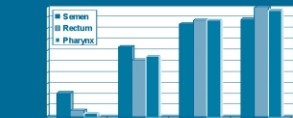
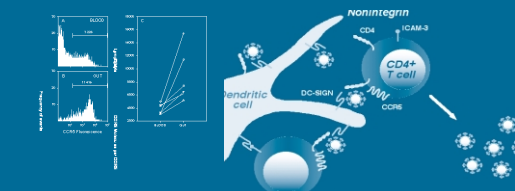
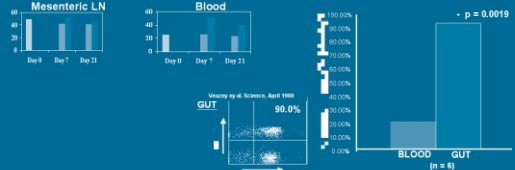
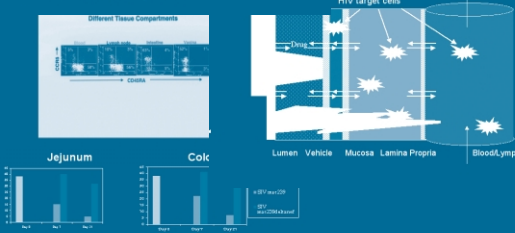


Controlling for each variable may improve the predictive value of controlled clinical trial



N-9 tolerability among receptive men

Symptoms	SIV-1 N=10	SIV-2 N=9	SIV-3 N=9
Rectal ulcers	2 (20%)	7 (78%)	4 (44%)
Rectal discomfort	1 (10%)	5 (56%)	2 (22%)
Lumbar aches	1 (10%)	7 (78%)	7 (78%)
Rectal bleeding	0 (0%)	0 (0%)	7 (78%)

Creating a Research and Development Agenda for

Rectal Microbicides that Protect Against HIV Infection

Report from the Workshop:
Baltimore, Maryland, June 7-8, 2001

This report was published by the American Foundation for AIDS Research.



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This summary report was
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Michael Gross, PhD
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The global AIDS pandemic continues to spread rapidly, with more than 5 million people becoming newly infected in the past year. Projections foresee accelerated growth of HIV infections in the absence of effective, widely adopted biological and behavioral interventions. One of the missions of the National Institute of Allergy and Infectious Diseases (NIAID) is to end this epidemic.

Most HIV transmission occurs through sex. Microbicides are any topical agent that inactivates or kills the pathogen, physically blocks its attachment to susceptible target cells, supports natural defenses, or prevents its more widespread dissemination from target cells. Advocates view microbicides as a potential complement to condoms, with the hope that eventually a highly effective and widely acceptable microbicide might become a suitable substitute for condoms.

Effective microbicides offer a potential means to control and even end the growing HIV epidemic. Vaginal microbicides have gained increasing attention because of their potential as a woman-controlled method of HIV prevention. There is evidence suggesting that within the United States, seven times as many women engage in anal intercourse as do gay men. And the rate of female anal intercourse likely is even higher in societies where the practice is used as a method of preserving virginity and preventing pregnancy.

However, transmission of HIV has been shown to be 2-3 times more efficient through anal intercourse than through vaginal intercourse. In some populations at high risk, heterosexual as well as homosexual, anal sex is a common practice, but with condoms even less readily used than during vaginal intercourse. Microbicides suitable for rectal use could be an important adjunct to condoms, but the feasibility of rectal microbicides and the requirements for a safe and effective product have yet to be completely elucidated.

The Workshop summarized in this report was initiated by the HIV Prevention Trials Network (HPTN), a multidisciplinary, multi-site global research network supported by the Division of AIDS (DAIDS), National Institute of Allergy and Infectious Diseases (NIAID), National Institute of Child Health and Human Development (NICHD), National Institute on Drug Abuse (NIDA), National Institute of Mental Health (NIMH), NIH Fogarty International Center, and the NIH Office of AIDS Research (OAR) to conduct definitive clinical trials of promising biomedical and behavioral interventions to prevent the spread of HIV and contain this epidemic.

HPTN investigators reasoned that any microbicide developed for vaginal use very likely also would be used in anal sex by consumers. Given known differences between vaginal and rectal tissue and microenvironments, the organizers understood that a microbicidal product shown to be safe in the vagina may not necessarily be safe in the rectum. Therefore, establishing appropriate safety criteria for rectal exposure to such products and assessing the rectal safety of candidate vaginal microbicides would become an important consideration in their clinical development.

To develop effective rectal microbicides, the unique challenges of conferring rectal protection need to be characterized and understood. Information gained by developing an effective rectal microbicide may be valuable in the development of an effective vaginal microbicide.

To obtain guidance on appropriate safety criteria for assessing the risk of rectal exposure, and to address the longer term goal of identifying biological considerations relevant to the design of effective rectal microbicides, HPTN investigators chose the commonly used mechanism of a workshop that would focus on discovery and development of rectal microbicides and would clarify research needs in this area. The workshop sought to:

- Establish the current state of knowledge across those disciplines that bear on rectal microbicide research and development;
- Identify biomedical and behavioral research priorities for advancing an understanding of rectal physiology, particularly in the context of sexual acts, mechanisms of HIV transmission, and the potential role of topical agents in conferring protection against rectal HIV infection;
- Outline key considerations associated with studies of product acceptability, including applicator design; and
- Propose an appropriate clinical development strategy for rectal microbicides.

The multidisciplinary organizing committee that planned the meeting included investigators working on vaginal microbicides as well as experts in gastroenterology, microbiology, virology, immunology, infectious diseases, clinical trial design, social and behavioral sciences, regulatory affairs, and policy.

This document summarizes and compiles Workshop presentations and discussions. In some cases, the sequence of material presented during the meeting has been altered to afford better continuity or to reduce duplication and overlap. It is intended to offer a state-of-the-art summary of the field and to outline priorities for future research. The Workshop and this summary cover the following areas:

- **The prevalence of anal intercourse** describes patterns of initiation and frequency of heterosexual, bisexual, and homosexual anal intercourse, use of lubricant and condoms, and the very limited availability of information about their behavior in communities most burdened by HIV.
- **Anatomy, physiology, immunology of the anorectal mucosa** offers an overview of the tissues, immune responses, and processes by which HIV infection becomes established in the gut. It offers research targets and processes that microbicial products might affect in order to prevent infection.
- **Models and tools** discusses existing *in vitro*, animal, and *in vivo* human systems for better understanding the pathophysiology of HIV infection in the gut and measuring the effects of proposed microbicial products.
- **Formulating rectal products** moves from basic to applied research, drawing upon experience with reproductive health products in the vagina and rectally administered pharmaceutical products.
- **Listening to the market** taps survey data from at home and abroad to alert researchers to product characteristics that would affect consumer acceptance of microbicial products used in the rectum.
- **Workshop conclusions and recommendations** outlines where further basic and behavioral research is needed, and issues that may be of major significance in developing commercial rectal microbicial products that will be embraced by the consumer, to reduce the spread of HIV infection.

The Prevalence and Risks of Anal Intercourse

The National Health and Social Life Survey (NHSLs) conducted in 1992 is the largest profile of sexual behavioral patterns in the United States. It is a nationally representative probability sample of 1511 men and 1921 women between the ages of 18 and 59 that offers a comprehensive inventory of population subtypes. Its methodology was an interview of approximately 90 minutes conducted by experienced interviewers and it had a remarkably high completion rate of 79%; of those, only 2% declined to answer explicit sexual questions, while 12% declined to answer questions relating to household income. Its focus was opposite sex partners.

Oral sex has become a rather common practice, whereas anal sex has not entered into the repertoire of regular sexual practices among most heterosexuals. Nearly a quarter of all Americans report having tried anal sex at least once during their lifetime and 1 in 12 have made it an occasional or episodic part of their sexual activity.

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Distributions of the prevalence of anal sex practices

Variables	Men	Women
Lifetime experience with anal sex	26%	21%
Anal sex in the last 12 months	8%	8%
Anal sex during the last event	2%	1%

When anal intercourse does occur it is most likely to be in the context of an established ongoing relationship rather than a “pick-up” situation. Males have a significantly greater interest in the practice than do females. Experience with anal sex is greater with age. It is unclear whether this is a product of aging or of the experience of that population cohort. It may reflect a combination of factors including the sexual revolution of the 1960s, fear of HIV among the younger cohort, a correlation with established relationships, or other influences. There are no strongly explanatory correlations that predict the likelihood of engaging in anal sex.

Researchers who conduct these types of surveys say that the greatest impediment to their work is political opposition to conducting surveys on sexual behavior, which in turn inhibits government administrators from funding and supporting such work.

Higher Risk Groups

The National Sexual Health Survey (NSHS, 1996) and the Gay Urban Men’s Health Study (GUMS, 1996 - 1997) examined the prevalence of anal intercourse (AI) among heterosexuals and men who have sex with men (MSM) in the U.S. These household probability surveys were conducted in Spanish and English using telephone-administered questionnaires with participants 18 years or older. The NSHS includes data for nearly 8000 adults from the 48 contiguous US states and the GUMS database includes data for 2881 MSM from four major U.S. cities — San Francisco, Los Angeles, Chicago, and New York. They are available on CD-ROM from the UCSF Center for AIDS Prevention Studies [<http://hsru.ucsf.edu/hsru/DataSets.htm>].

Findings from these surveys support four observations. First, there is evidence that the developmental onset of AI occurs in early adulthood for both MSM and heterosexuals. On average, MSM initiate sexual activity in their teens with oral sex and introduce anal practices several years later, in their early 20s. The NSHS data indicate a similar pattern among heterosexuals, with AI becoming part of the sexual repertoires several years after the onset of vaginal and oral sex.

Second, prevalence of anal sex among heterosexuals is low but the behavior is most common among young persons, ethnic minorities, and people with lower educational attainment. Only 6 – 8% of heterosexuals reported engaging in AI in the past year and, of those reporting the behavior, nearly 80% did not use condoms.

Heterosexual men and women aged 18 – 29 reported the highest prevalence of AI, with steady declines in the behavior observed in subsequent age groups. Hispanics, African-Americans, and Asian-Americans reported AI more commonly than did other ethnic groups. Also, those reporting lower levels of educational attainment were more likely to report AI than their more educated peers. The latter finding may be attributed in part to confusion about terminology used in the surveys.

Third, prevalence of AI among MSM is higher than it is among heterosexuals and it is associated with age and ethnicity. More than half of surveyed MSM reported engaging in AI in the past year, but almost one-third reported practicing the behavior without the use of condoms. Moreover, the majority of unprotected AI reported by these men occurred within seroconcordant sexual dyads. Only 6 – 8% of men practice unprotected AI conferring the highest risk of HIV transmission — namely, unprotected AI in which the insertive partner was HIV-infected. It is noteworthy that HIV incidence at the time these data were collected was estimated at 1 to 2 percent.

Fourth, there is evidence that unprotected AI among MSM increased in the mid-1990s, following documented declines in the late 1980s and early 1990s. Two studies examining changes in unprotected oral and anal sex indicate that unprotected AI reported by MSM in San Francisco declined in the mid-1980s but began to increase in the mid-1990s. By 1999, nearly 50% of MSM reported unprotected AI. As the GUMS data indicate, as recently as 1997, the bulk of unprotected AI occurred within seroconcordant sexual dyads. An important research question is whether this remains the case.

Some 14% of the GUMS respondents indicated that they had sex with both males and females within the last five years, as compared with less than 2% of the NSHS survey who reported same sex partners. While only 6-8% of the NSHS group engaged in anal intercourse, the vast majority of those heterosexuals did not use condoms with that activity. In contrast, 52% of the receptive and 64% of the insertive GUMS responders said they used condoms when engaging in anal intercourse. That practice carries a two- to threefold higher risk of transmission of HIV to the receptive partner than does unprotected vaginal sex.

Among high-risk women (those who use drugs and/or are sex workers), about 10% reported engaging in anal sex when asked the question in face-to-face interviews, but 32% answered affirmatively when queried through a self-administered computer-assisted survey. A subset of 68 women were first asked the questions directly and six months later self-administered the same set of questions using a computer. They were nine times more likely to report engaging in anal sex with the self-administered survey. Perhaps the most likely explanation of this huge differential is the social stigma attached to anal sex, which played out in face-to-face interviews but not with the anonymity of the computer.

American Youth

Data on the sexual practices of teens comes from the National Longitudinal Study of Adolescent Health, a school-based study that led to selection of a representative sample of those 12-19 years of age for home interview. It began in the 1994/5 school year, with questions on anal sex added only in the second year (1996) of follow-up interviews. The sample is being re-interviewed in 2001.

Some 15% have experienced anal sex by the time they graduate from high school, though it is not widely or regularly practiced. This reflects data gathered from their elder cohorts that the practice is not widespread but raises questions as to whether it is acquired relatively later in the repertoire of sexual practices. These conflicting findings reinforce the need for further research.

Parents and public school administrators often are reticent to discuss matters of sexuality with teens or include them in the curriculum. That may be especially true of anal intercourse, where adults may be particularly ill-informed, and which carries added burdens of stigma. If the introduction of anal intercourse is in fact delayed, this may provide an additional window for the effective delivery of prevention messages and interventions in the post-high-school period when there are fewer parental and societal barriers to such activities.

While only a small percentage of heterosexuals engage in anal sex, they seldom use condoms when performing that act. Some have suggested that the gross number of unprotected anal sex acts by heterosexuals is five-fold that of MSM. This is because the sheer number of acts, combined with very limited use of condoms, and the several-fold increased risk of transmission of HIV as compared with unprotected vaginal sex, multiplies out to a significant number of opportunities for transmission of disease. Anal sex among heterosexuals has the potential, if perhaps not yet the actuality, of becoming the route of more new HIV infections than similar acts between MSM.

Anthropologic studies from Brazil indicate that couples substitute anal for vaginal sex to preserve virginity during courtship, but upon marriage the practice becomes taboo and is largely dropped. Some have speculated that young Hispanic women pursue that same strategy in the US, but the NHLS survey offered little evidence to substantiate that hypothesis as the incidence of reported anal sex was similarly low among all population subsets. However, as pointed out above, the stigma of anal sex and the use of face-to-face interviews may bias the survey to underreport such activity. Those few who do report engaging in anal sex are likely to do so with partners of the same sex or partners of both sexes. There is a low reported incidence of anal sex only with a partner of the opposite sex.

Thai Data

In Thailand, approximately 1 in 10 21-year-olds are chosen by lottery to serve in the Royal Thai Army (RTA). Sexual orientation, HIV status, and drug use are not grounds for exemption, so the sample provides a good cross-section of that nation's age cohort. Periodically throughout the 1990s, the RTA recruits were interviewed for lifetime and recent (past 6 months) sexual behavior. The prevalence of MSM in the population was low, but showed a doubling in the early 1990s, then an even more rapid decline in subsequent years. The meaning of this is not understood; it may reflect less reluctance to conceal such behavior, or a tendency for same-sex behavior to have become more normative.

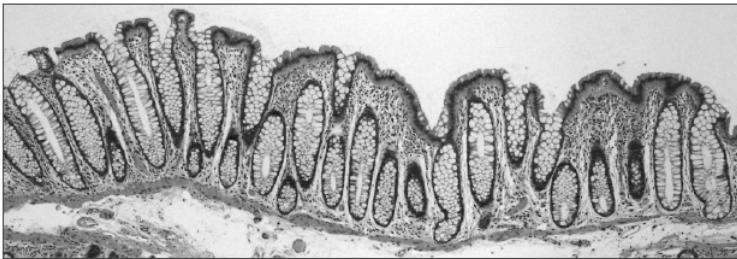
Sexual practices have changed dramatically in Thailand with implementation of the government's HIV education/prevention efforts to contain the epidemic. Over the course of the last decade, the percentage of those who have visited commercial sex workers within the last six months has declined by half, while condom use has increased to greater than 95% during those visits. This demonstrates the potential to change sexual practices to curb the spread of HIV in a large population.

Male sex workers interviewed in Chiang Mai, Thailand, during the period 1989-1994, were young men who tended to identify as heterosexual and practiced the trade for about four months in order to earn money. They averaged only two partners a week and seldom used condoms. In comparison, brothel-based female sex workers averaged four clients per night and condom use is near universal. This near universal use of condoms in commercial sex with females reflects the government's "100 Percent Condom Campaign" that began in 1991. HIV seroprevalence among this group of young men was 18.6%, compared with 12% among similar aged RTA recruits. They are a potential bridge of infection to heterosexual Thai women who generally are considered to be at low risk for infection.

Anatomy, Physiology, Immunology of the Anorectal Mucosa

Maria T. Abreu, MD, UCLA Inflammatory Bowel Disease Center; Peter Anton, MD, UCLA AIDS Institute; Robert Coombs, MD, PhD, University of Washington; Galen Cortina, MD, PhD, UCLA School of Medicine; Joel Palefsky, MD, University of California at San Francisco.

Anatomy of Rectal Mucosa



Gastrointestinal research and training focuses upon digestive disease, with an almost complete avoidance of the sexual function and pathophysiology of the rectum. The upper GI tract is comparably more rugged as it is equipped to handle the wide variety of foods and substances ingested through the mouth. The colon/rectum is more fragile as it receives processed foods that have been broken down into simpler, more standardized components. It is a poor barrier to pathogens, while at the same time it hosts a variety of friendly bacteria that aid in the processes of digestion and maintenance of colonic health.

The layers of the colorectal wall, proceeding inward from the exterior, are a perirectal adipose (fatty) tissue supported by pelvic fascia, muscular propria, submucosa, and mucosa. The rectum is a reservoir 8–13 cm in length that ends with the voluntary muscles of the sphincter. It is a sensate organ that perceives its solid, liquid, and gaseous content and adjusts appropriately. Muscular control is both visceral and somatic. Signaling pathways of comfort and discomfort are closely associated with those of the genital and urinary muscular system, explaining the pleasure that many experience with anal sex.

The submucosa can be compared to nylon; it is a web-like loose connective tissue capable of a great deal of stretch. The mucosa, comprising about 10% of the thickness of the rectal wall, is more like an accordion, expanding and contracting along its many folds.

The mucosa in turn is divided into the epithelium, lamina propria, and muscularis mucosa. The muscularis mucosa (not to be confused with the muscular propria) is smooth muscle tissue a few cell layers thick that lies at the base of the mucosa. The lamina propria is the structural network that connects the folds and crypts of the epithelium to the muscularis mucosa.

The single-cell thick rectal epithelium consists primarily of goblet cells and absorptive cells. Goblet cells contain mucus granules that are secreted as mucin to coat the tissue surface. Absorptive cells are cone-like arrangements of microvilli that absorb water and some electrolytes. Epithelial crypts are tubular glands with a generative zone at the base; as cells mature they migrate to the surface. Thin strands of muscle help to provide structure to the crypts.

The lamina propria is a structure of stromal cells that contain blood, lymphatic and nerve networks, as well as a resident population of inflammatory cells that include macrophages, lymphocytes, plasma cells and eosinophils.

The real barrier to the outside world is a single layer of epithelial cells attached to a collagenous foundation. There may be spots in the rectum where the muscular mucosa is not complete, resulting in direct interaction between the crypt epithelium and lymphoid tissue in the submucosa. These may be particularly vulnerable points for the entry of pathogens.

The mucosa is a delicate tissue that can be damaged by such relatively benign action as oral cathartics, enemas, or hard stools. Thus endoscopic examination and anal sex are likely to cause some degree of trauma. With trauma the secreted layer of mucin is depleted, as are the tall columnar cells of the crypts, and blood cells proliferate. Damage may consist of congestion and hemorrhage in the lamina propria, and may sometimes include detached epithelium and inflammatory cell infiltration.

The ano-rectal juncture brings a transition from glandular to squamous mucosal cell structure and a transition from smooth to voluntary muscles. Stratified squamous

Sampling of luminal contents by M cells and inter-epithelial dendritic cells

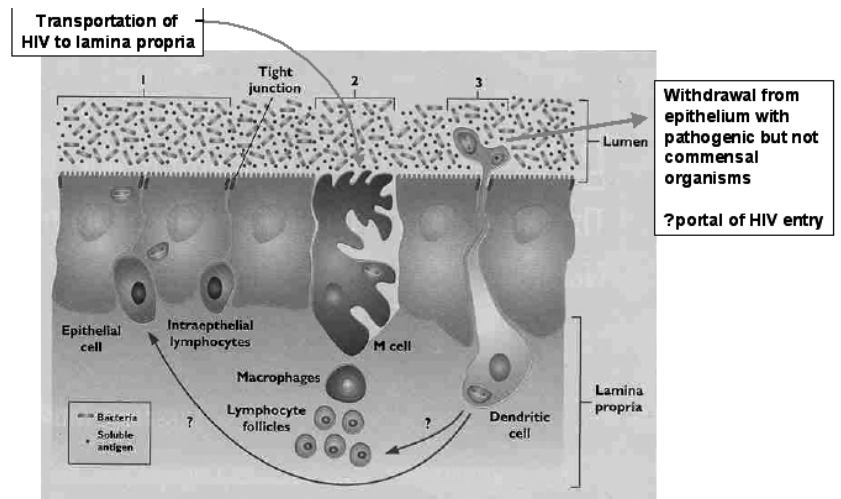
mucosa is present in the oral cavity, vagina, anus and skin. It is a tough multilayered tissue containing keratin, the stuff of hair and nails. The superficial layer of cells is dead, not capable of being infected, and is constantly sloughed off.

The gut is the largest immune organ, some 20+ feet in length with a huge surface area. It is unlike any other organ in that 10^{14} bacteria reside in the gut, in contact with the epithelium. Macrophages and leukocytes constantly sample the luminal content for both friendly bacterium and pathogens. M cells and inter-epithelial dendritic cells are a link from the lamina propria through the epithelium to the lumen that likely are a route for HIV transmission to lymphoid tissue.

Animals modified to have weakened gut epithelium, that cannot provide a shield of protection from luminal bacteria, exhibit chronic inflammation of that tissue similar to chronic inflammatory bowel disease seen in humans. When pathogens breach the epithelium they stimulate increased production of pro-inflammatory cytokines $\text{TNF}\alpha$, $\text{IL-1}\beta$, IL-2 , IL-18 , and $\text{INF}\gamma$ to fight the invaders. This upsets the natural balance with anti-inflammatory cytokines that is seen in healthy tissue.

In humans, inflammation of colon mucosa literally decreases capacity of the colon to handle volume. It produces a sense of rectal fullness, a feeling of urgency in the need to defecate.

Normal, healthy colon epithelial cells closely abut each other, essentially glued together by a series of tight junctures, and carry an electrical charge that makes them resistant to penetration. When inflammation kills off epithelial cells, the remaining cells flatten, polymerize their actin filaments and expand to try to maintain complete surface coverage. The epithelium can lose up to half of its cell population and still maintain physically intact coverage, but the quality of that coverage deteriorates, making cellular communications more vulnerable to disruption by bacterial toxins.

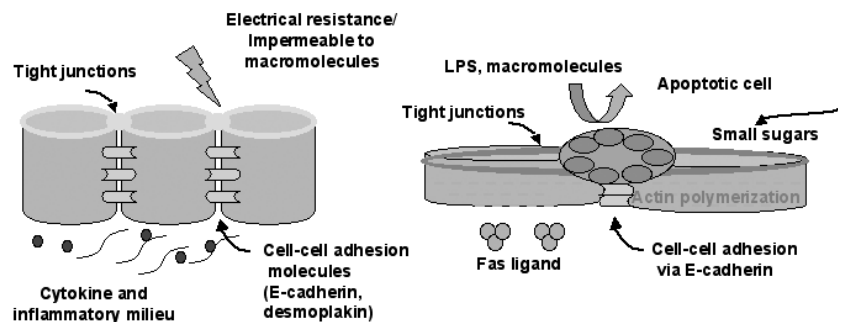


Model of intestinal epithelium and effect of apoptosis on barrier function

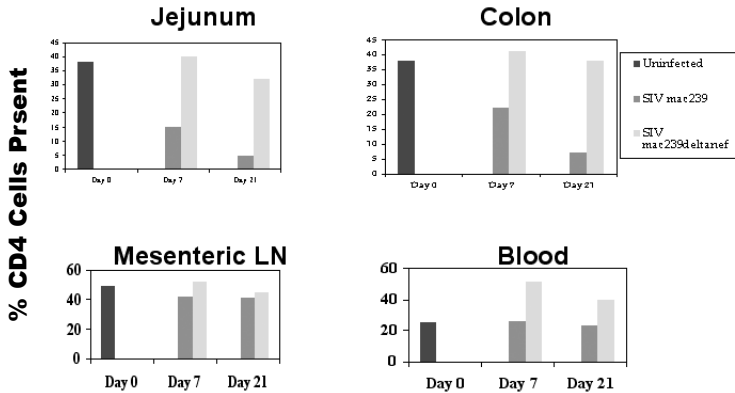
Gut Immune Function

Some have called HIV primarily a mucosal disease, with the gut being its chief reservoir of infection. It holds the body's greatest reservoir of CD4^+ and other immune cells vulnerable to HIV entry. It is primed for early infection and rapid spread when these target cells are activated and produce large amounts of virus. HIV infection triggers an inflammatory state that is above and beyond what already exists. Sexually exposed mucosa is a particularly vulnerable target for infection.

The intestinal immune system is organized in three layers. Beginning with the interior, the first is composed of intra-epithelial lymphocytes (IELs) at a ratio of 1:6-to-10 epithelial cells. They are primarily activated CD8^+ cells, though some CD4^+ cells also



Deletion of CD4 Cells in Primary Infection



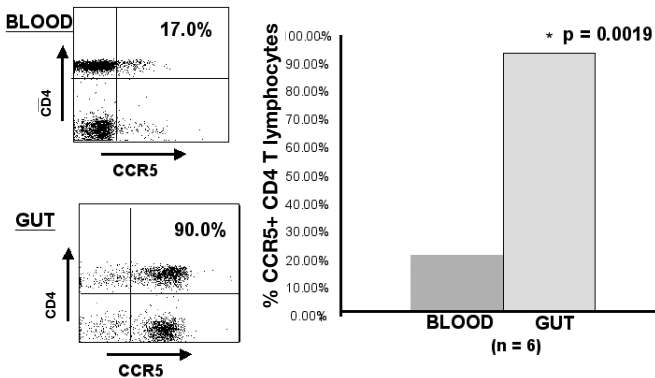
are present. The second layer consists of lamina propria mononuclear cells and lamina propria lymphocytes (LPLs), primarily CD4+ cells found in proportions similar to those found in peripheral blood. The third layer consists of lymphoid cells aggregated into tissue that includes dendritic cells, Peyer's patches in the small bowel, lymphoid nodules/aggregates in the colon, and mesenteric lymph nodes.

A profound depletion of CD4+ cells occurs in the colon when macaques are infected with SIV, regardless of the route of infection. That depletion occurs during the first three weeks of infection and is not mirrored in other tissue or blood compartments.

There is thought to be regional variation in the immune population of the colon. UCLA researchers take biopsies at about 30 cm up from the sphincter, in order to standardize samples and avoid areas of traumatic or infectious proctitis. In healthy tissue there is sufficient vascular constriction that even 20 biopsies taken within that site result in little if any bleeding.

Most biopsies down to the lamina propria heal within three days and the sites are barely identifiable within a week. Epithelial cells damaged by trauma are replaced within a few hours, up to a day. The window of greatest vulnerability likely lasts an hour or two. However, physical appearance may not be the criterion most clinically relevant to vulnerability to transmission of HIV; other indicators need to be examined. A key research question is: what markers of epithelial destruction are clinically meaningful?

% CCR5 Receptor Expression is Increased on Mucosal CD4 T cells (Healthy, HIV- Controls)



Biopsy of healthy HIV-negative individuals has shown that 90% of colon CD4+ cells express CCR5, the major coreceptor for HIV. In comparison, only 17% of CD4+ cells taken from blood express CCR5. Not only are more colon cells expressing CCR5, each cell expresses significantly higher numbers of those receptors on their surfaces. Similar patterns are seen with cells expressing both the CCR5 and the secondary CXCR4 receptor. Heightened expression of the CCR5 coreceptor is associated with greater vulnerability to infection. Thus larger pools of activated CD4+ cells likely to become productively infected are located in the gut than in other regions of the body. The issue goes beyond simply the number of cells making virus. It appears that each of the gut CD4+ cells also produces more virus than do their PBMC brethren. *In vitro* challenge of tissue and blood of healthy and infected patients with the same viral isolates suggests that inflammation is responsible for the differences in viral production.

The pro-inflammatory β -chemokines MIP-1 α , MIP-1 β , and RANTES recruit additional CD4+ cells to the area, amplifying and fueling the cascade of infection. *In vitro*, high levels of beta chemokines favor HIV access to cell surfaces and binding, but they also play a role in downregulation of the CCR5 coreceptor. It is unclear how this balancing act plays out *in vivo*.

Trauma associated with anal sex likely increases inflammation; that includes trauma that would be associated with use of a preparatory enema. Semen contains inflammatory cytokines and has natural mucalytic properties that likely favor transmission of virus through the epithelial barrier.

DC-SIGN

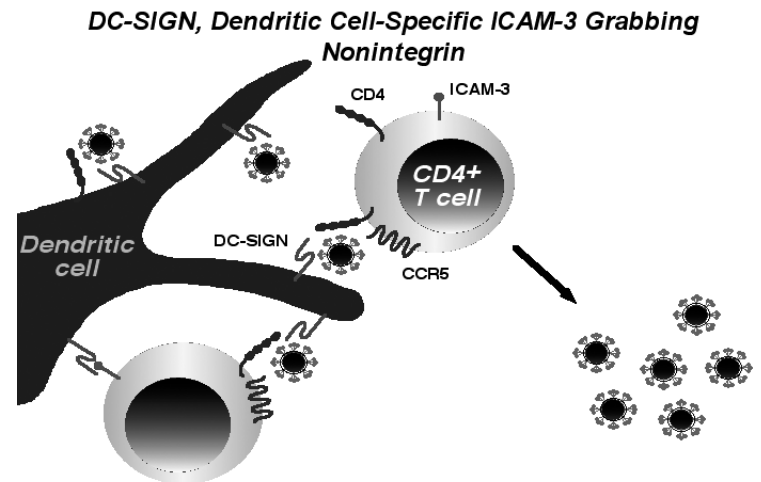
Vineet N. KewalRamani, PhD, National Cancer Institute

DC-SIGN is a type II membrane protein in humans that appears to be specific to dendritic cells (DC), and is found in higher concentrations on immature dendritic cells. Its normal function is to interact with ICAM-2 and ICAM-3 molecules and to facilitate DC and T-cell interactions. It also captures HIV-1 through a high affinity interaction with Env, without the DC becoming infected, and transports it to draining lymph nodes. There the dendritic cell surface bound HIV binds to CCR5 and CXCR4 receptors on CD4+ cells.

The ICAM family of molecules is known to endocytose upon encountering antigen. In binding with HIV, DC-SIGN internalizes soluble gp120 and can maintain the virus in a stable and infectious state for days on end. Binding of DC-SIGN with ICAM-3 and gp120 is calcium dependent and can be inhibited with mannan. Antibody to DC-SIGN inhibits its functionality.

DC-SIGN appears to be highly species specific. The macaque version of DC-SIGN contains 92% of the same amino acid sequences that are found in humans. It captures and transmits SIV, and can be neutralized by cross-reactive SIGN antibodies. But there is little expression on macaque dendritic cells.

This raises a series of questions. Is SIV transmitted with the assistance of other macaque SIGN molecules? And if so, are they analogous to human molecules not yet understood? Does HIV utilize other helper molecules to infect other types of cells in the immune system? Do primate lentivirus simply target a general property of dendritic cells, such as antigen acquisition, in order to reach CD4+ cells? And if so, how do they escape antigen processing?



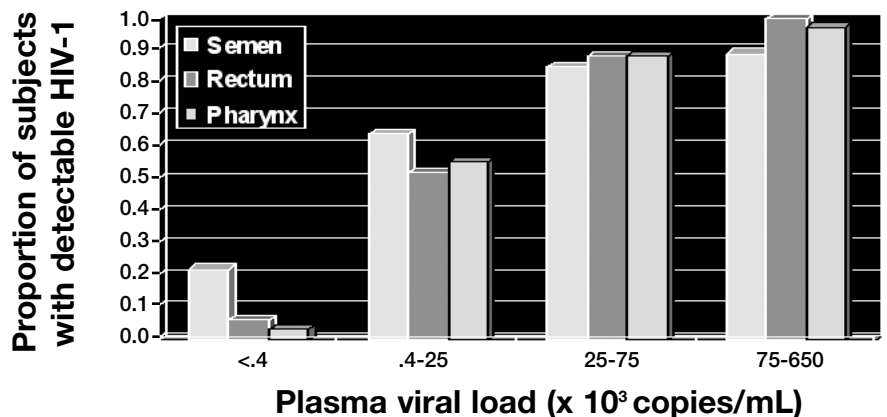
Patterns of Rectal HIV Shedding

Rectal shedding of HIV virus correlates with high plasma RNA and inflammation of the anorectal canal as assessed by rectal Pap, but not with low CD4+ count. Effective antiretroviral therapy decreases viral shedding. Among men with <50 HIV RNA copies/mL in plasma, rectal samples detected HIV RNA in 2% and HIV DNA in 28% of the men examined.

HIV shedding in three compartments—rectum, pharynx, semen—was compared with plasma RNA over three successive visits (0, 2, 4 weeks) in MSM who were free of STDs, in a study conducted in Seattle and Lima, Peru. Rectal secretions were gathered with Sno-strips™ and pharynx samples by swab.

Intermittent detection of HIV was more common in the rectum (13%), pharynx (13%), and semen (23%) than in blood (6%). Only semen exhibited significant variability from plasma in terms of shedding RNA, while

Detection of HIV shedding at mucosal sites by quartiles of plasma viral load



rectal shedding tended to be at higher levels of RNA than plasma. Five (38%) of 13 men with persistently undetectable plasma HIV (<400 copies) had detectable HIV RNA from at least one mucosal site.

It is clear that plasma RNA levels cannot be used as a surrogate for viral shedding in other compartments. The ability of Sno-strips™ to sample the rectal compartment, and the low variability of HIV RNA shedding between specimens taken from the same patient, supports the viability of this approach in evaluating the impact of potential microbicides on rectal shedding of HIV RNA. However, the significance of rectal shedding of RNA in the transmission of new infection has yet to be determined definitively.

HPV as a Cofactor

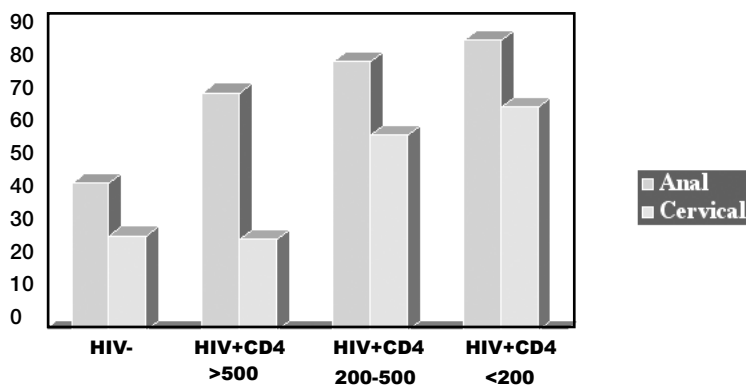
Human papilloma virus (HPV) infection and anal intraepithelial neoplasia (AIN) is an STD cofactor that must be considered in developing a rectal microbicide. AIN may be associated with inflammatory infiltrate that may have implications for both the transmission and acquisition of HIV. HPV-associated lesions also may bleed more easily than normal epithelium when traumatized.

HPV is the most common STD. More than 40% of women and 60% of MSM who are HIV-negative test positive for anal HPV. Those numbers swell to nearly universal infection among those who are HIV-positive. Surprisingly, HIV-positive women are more likely to be anally than cervically infected with HPV.

The anus and cervix are homologues in that both have transition zones where cell organization and structure changes. HPV seems to target the transition zones.

Microbicides will need to be evaluated for their effects upon AIN, the penis, and synergistic action with regard to both HIV and HPV infection. The experience with HPV may offer some guidance toward development of microbicides.

Anal and cervical HPV infection in HIV-positive women



HIV can infect rectal tissue through a variety of mechanisms and pathways.

In vitro models strongly suggest that HIV may be able to infect rectal epithelial cells, though this has not been established *in vivo*. Interaction with infected lymphocytes may induce endocytosis of virus through epithelial cells, allowing them to come in contact with lymphocytes, macrophages, and dendritic cells residing immediately below the epithelium.

Trauma may remove the epithelial layer of protection and allow for direct exposure of these cells. Effective lubrication itself may provide some degree of protection by reducing physical trauma and by offering physical barrier protection simply by lining the mucosal epithelium. Reducing other bacterial and viral pathogens will reduce inflammation and may have some protective effect. Co-use of prescription and non-prescription substances likely are variable cofactors of vulnerability to infection by HIV and STDs. Steroids decrease beta chemokines and by removing this negative inhibition, may increase expression of CCR5. Alcohol use increases permeability of tissue, while nitrites (poppers) debilitate phagocytosis.

Disrupting HIV interaction with cells in a manner that does not prove harmful to those cells may prove to be difficult. Other strategies employed in developing therapy for established infections, such as entry and RT inhibitors, may prove useful in a microbicidal product.

Commercial products are likely to offer a layered approach to protection by combining a number of compounds that perform several of these functions.

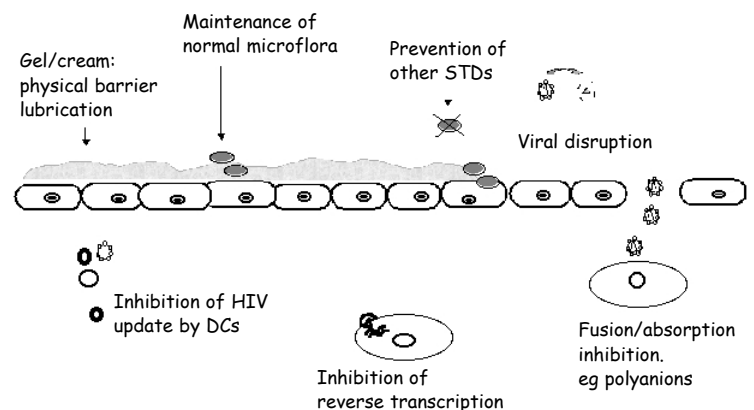
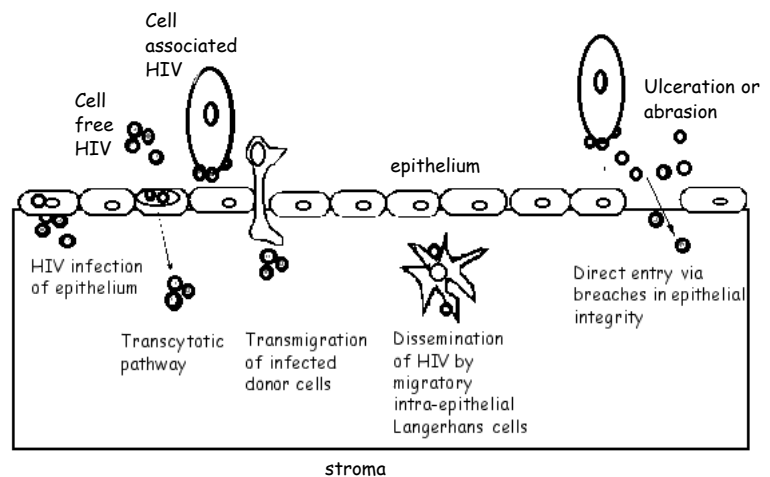
Assessment criteria adapted from developmental work with vaginal microbicides may provide a framework for evaluating and perhaps prioritizing developmental work on potential compounds.

- No cytotoxic or inflammatory potential
- Inhibitory activity against the primary isolates of HIV
- Continued activity in the presence of blood, semen, and fecal material and the changes in pH they may induce
- Activity against cell-associated virus
- Understanding of activity (ideally synergy) and antagonism in the presence of other microbicidal agents targeting the same and other steps in the infection process [with combined use and with potential sequential use by the consumer]

High throughput screening can rapidly winnow out compounds with little anti-viral activity, while cell cultures can screen out major toxicity among the surviving compounds. Fluorescence technology can now yield a reading of antiviral activity within 48 hours. Using panels of isolates, primers, and cell lines one can evaluate patterns of antiviral activity of specific compounds and combinations of compounds.

Rectal explant tissue, while labor intensive to procure and prepare, can be used to evaluate the effects of a compound on crypt structure, epithelium and other cells. The tissue is taken from surgical resection specimens and is cultured on stainless steel grids

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using a liquid air interface at 95% oxygen and 37°C. It can be maintained for at least two weeks. The culture has been used to determine cytotoxicity of compounds at varying concentrations and over time.

Gene expression microarrays indicate what genes are being switched on and off. Initial work focusing on 270 cytokines, chemokines, cell surface receptors, transcription regulators and apoptotic genes gives a sense of what the compound is doing and suggests directions to modify the compound to achieve greater and more targeted efficacy.

In vitro it appears that topical products are able to down regulate CCR5 and CXCR4 receptors, which may offer another avenue for protection. As discussed below, it strongly suggests the need to identify the cells that become infected with HIV, and the dissemination and timing of the steps in that infection, so as to design and evaluate interventions for their efficacy, durability, and timeliness in reducing the risk that exposure to the virus becomes an active infection. Immediate and downstream targets of infection likely will require different interventions, which may be combined in a layered defense.

Animal Models

The mouse model with herpes simplex virus-2 (HSV-2) can be used to evaluate the effectiveness of antiviral agents for general as opposed to HIV-specific antiretroviral activity, using either vaginal or rectal challenge. HSV-2 kills mice within a few days of challenge. Herpes virus, like HIV, is an enveloped virus. Nonoxynol-9 (N-9), the only spermicide commercially available in the US, acts on viral envelope, so the challenge model may have some relevance in developing an anti-HIV microbicide. The model has been used to evaluate a number of candidate compounds.

In the mouse model, rectal challenge with HSV-2 requires a higher dose of viral inoculum than does vaginal challenge. And even though the rectal mucosal tissue appears similar in males and females, females are more likely to become infected by a sub-lethal dose.

In a vaginal challenge, over the counter products containing N-9 protected most of the mice from HSV-2, while Carraguard (PC-515) protected all of the mice. Carraguard is a variation of carageenan that has strong antimicrobial properties; carageenan is a polymer derived from red seaweed. It is used commercially in a variety of ways including as a food thickener and as a carrier for pharmaceuticals.

In a rectal challenge, using male mice to avoid possible vaginal contamination, HSV-2 proved lethal, while Carraguard protected about half of them. Speculation is that Carraguard's incomplete protection was not because of failure of the compound but perhaps because the rectum is an open system and the challenge reached beyond the zone of protection offered by the gel that was administered.

When N-9 products were applied prior to challenge, not only did they not offer protection, the mice sickened and died more rapidly. They continued to do so even when the challenge load of HSV-2 was reduced to the point where ordinarily fewer than 10% of control mice would become infected.

Rectal lavage of the N-9 treated animals not challenged with HSV revealed sheets of epithelial cells stripped from the tissue wall and floating free, exposing the underlying connective tissue. That exposure likely explained why infection occurred so readily and quickly in those mice. However, an hour later, the epithelium surface appeared to be largely restored as remaining cells had flattened and spread out and new cells slide up from the mucosal crypts.

None of the over-the-counter spermicides provided protection from HSV in rectal challenge trials. A long-lasting bioadhesive suppository (LARS), a product under development, supplemented with N-9 at 1% or 2% concentrations showed some limited initial protection that quickly faded.

Detergents and surfactants that destroy the HIV envelope also are likely to destroy cell membranes. The best approach might be a fusion inhibitor because it does not induce the inflammation that draws CD4+ target cells.

The polystyrene sulfonate gel T-PSS, synthesized by free radical polymerization of sodium styrene sulfonate in water, acts by blocking pathogen attachment to a host cell. It showed a greater degree of protection but then a decline in a vaginal trial in mice. Rectal challenge after application of a 10% formulation of T-PSS protected about half of the animals. This work was confirmed with rectal challenge in the guinea pig model.

The work demonstrates that both bioadhesive gels and attachment blockers are viable approaches in developing effective microbicides.

Macaques are used as a model for the study of reproductive health issues, including the development of microbicides. The vaginal and rectal tissue and environments are similar, though not exact, and this may reduce the need for testing in both compartments if adequate markers are identified and validated in each compartment.

N-9 was tested against placebo in rectal challenges over multiple days in 24 animals. Post-application lavage showed increased bleeding, and discharge, including large sheets of epithelial cells that correlated with increased use of N-9. This likely increases vulnerability to STD infection. Repeated use of N-9 also significantly reduced the population of peroxide-producing organisms *Lactobacillus* and *Viridans streptococcus* in the microenvironment of the rectum, resulting in a more hospitable milieu for pathogens. This has been demonstrated in the rectum as well as in the vagina.

The macaque model has been used to glean much of what is known about viral transmission in the rectal compartment. The intestinal immune system is separate and distinct from the cellular immune system. Cells that are stimulated in the intestine will migrate to mucosal surfaces as determined by leukocytes and endothelial adhesion molecules and chemokines. This is largely regulated in the intestine by the MAdCAM-1 (mucosal addressin cell adhesion molecule-1) on postcapillary venules in intestinal mucosa, and by a4b7 and aEb7 on lymphocytes.

Upon rectal challenge with SIV, CD4+ cells are rapidly depleted in the intestine but remain relatively stable in peripheral tissues. If this were simply the product of CD4+ antigen downregulation or masking, then we should see an increase in CD4neg, CD8neg, and double negative cells when gating through total T-cells (CD3+), but this does not occur. Therefore, this indicates that a bona fide loss of intestinal CD4+ T-cells occurs in early SIV infection.

An assumption, not yet conclusively proven, is that the route of infection—rectal, vaginal, blood—has little effect on the dynamics of CD4+ depletion in the intestine.

The biggest challenges in developing a microbicide are in understanding the physical and immunological mechanisms by which the virus crosses the mucosa and which cells are the first to become infected, and hence must be protected. Current technology cannot distinguish the first cells infected in situ and thus are insufficient to answer those questions.

Ideal microbicidal products should maintain activity in the rectum for at least as long as the virus may persist in an infective state. That is at least 24 hours.

Human Trials

The HIVNET conducted a trial on the rectal safety and behavioral acceptability of nonoxynol-9 (N-9) in a bioadhesive gel expelled from a single-use applicator (Advantage-S) as a potential rectal microbicide. It used Advantage-S because the product comes in a single dose applicator of 52.5 mg N-9/1.5 mL designed to deliver a consistent volume of N-9 in this safety study. The trial enrolled 25 HIV-negative and 10 HIV-positive male couples in a 10-week dose escalation trial that required monogamy, daily application of the product, intention to have anal sex on average three times a week, and restricted each partner to an insertive or receptive role for sex in association with use of the test product only during the trial period.

The first cohort of 5 study couples used 1 applicator of the product daily for five weeks followed by 1 week of two applicators daily. After assessing safety outcomes in those 5 couples, the next 30 couples used 2 applicators daily (applying the product on the glans by insertive partners and above the anal verge by receptive partners) increasing to 4 applicators during the last week of the trial.

Only one couple dropped out of the trial, but the study was demanding given the daily use of product and the expected frequency of sexual activity. These factors should be considered in constructing future trials and in selecting participants.

Safety in the designated receptive partner was assessed by anoscopy and rectal biopsies for rectal inflammation and epithelial disruption at baseline, 5 and 6 weeks after N-9 use. Biopsy conducted about 8 hours after use of the product concluded that there was no problem with toxicity of N-9. Safety in the designated insertive partner was assessed by urethral exam and first-void urine leukocyte esterase testing at the same time points. Visible mucosal abnormalities were rarely observed by anoscopy (2 men with erosions and 4 with friability). However, the majority (69%) of the receptive partners had baseline focal, mild inflammation on rectal biopsies and 89% had slightly abnormal or abnormal histology (i.e., focal inflammation) after use of N-9. There was mild rectal inflammation at baseline, perhaps related to the practice of anal sex. This points to the need to better understand the relation of anal sex and inflammation as a baseline, absent use of a microbicide.

Great variability in rectal viral shedding among HIV-seropositive participants, as measured with Sno-strips™ for specimen collection, correlated with lower CD4+ count and higher plasma viral load. But viral shedding by individual participants changed only marginally from baseline over the course of the trial.

The most common complaint among receptive partners was rectal fullness reported by 74% of men during the N-9 phase. The sensation was associated with application of the product and seemed to dissipate within a half hour, regardless of sexual activity. There was some association of the sensation of “rectal fullness” with frequency escalation to twice the number of applicators, which doubled the volume of the applied gel.

The study also verified that N-9 has an anesthetic effect on the tongue, producing numbness. It is possible that the anesthetic property of N-9 is responsible for this feeling of “rectal fullness.”

While men in this study were evaluated eight hours after product use, subsequent work conducted by the Population Council in a small number of volunteers has demonstrated short-term (<1 hour) epithelial disruption associated with the use of N-9.

The observation in mice of rapid shedding of large sheets of rectal epithelial cells after N-9 products were applied, and subsequent rapid repair of that tissue, was the basis for a small study conducted by the Population Council to see if similar action

N-9 tolerability among receptive men

Symptoms	HIV+ N=10		HIV- N=25	
	Placebo	N-9	Placebo	N-9
Rectal fullness	3 (33%)	7 (70%)	6 (24%)	19 (76%)
Rectal discomfort	1 (11)	5 (50)	2 (8)	16 (64)
Loose stools	1 (11)	7 (70)	2 (8)	14 (56)
Rectal bleeding	0 (0)	2 (20)	2 (8)	8 (32)

occurs in humans. Lavage rather than biopsy was employed because it was relatively non-invasive and it could be self-administered by volunteers, thus lowering the cost of this unfunded, double-blinded study.

It used four agents: the over-the-counter lubricants ForPlay (1% N-9) and KY Plus (2% N-9), Carraguard (PC-515) a non-surfactant microbicide in development, and, as a non-toxic control, methylcellulose, which has the same viscosity as the other products. Each of the four volunteers provided a baseline lavage and subsequently used each of the products, with each use separated by a washout period of at least three days. The procedure was that the product was inserted; 15 minutes later a lavage was performed; 8-10 hours later a second lavage was performed. Each lavage was saved in fixative for later analysis.

Light and electron microscope examination found typical bacteria but few cells in the baseline and second lavages, or in the primary lavages using Carraguard or methylcellulose. However, all of the lavages taken 15-minutes post-application of products containing N-9 showed sheets of exfoliated tissue containing hundreds of epithelial cells that included columnar and goblet cells, and varieties of other epithelial cells typical of rectal epithelial morphology.

Rapid exfoliation of rectal epithelium correlated with exposure to an increased concentration of N-9. This is highly likely to leave the rectum significantly more vulnerable to infection. The mucosa appears able to repair itself, or at least stop shedding additional epithelial cells, within eight hours. This observation points to the importance of timing in conducting such examinations, as rectal mucosa is a very quickly repairing tissue. It is unclear how rapidly the epithelium is able to repair itself, and that should be the subject of further research.

Measuring Volume, Mixing, and Coverage

The mouse model has been used to assess volume and distribution of a typical microbicidal vehicle in the rectum and compare it with coverage in the vagina. A 1% gentian violet dye was added to the gel, it was administered, and left undisturbed for 10 minutes before being removed with a generous saline lavage. The animal was sacrificed, dissected, an image of the coverage was digitally recorded, and calculations were made as to the extent of coverage.

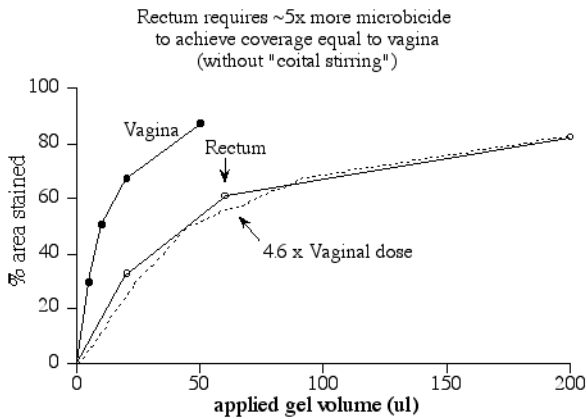
The model is useful for determining the fractional area of microbicidal coverage, patterns of coverage, and the degree to which it does or does not penetrate tissue folds. It is relatively quick, easy, and affordable, allowing sufficient sampling for statistical confidence. Limitations include little information on the proportional thickness of the gel in particular regions, which may affect its role as a physical barrier, differences between the model and humans, natural acts such as defecation, passing gas, applicator properties, and sexual acts, all of which likely affect coverage.

In humans, relatively small 10-15 mL enemas can backtrack all the way over to the right side of the colon, absent any gymnastics that may be part of sex. The implication for protective coverage of a microbicide is to think in terms of the need for greater rather than less coverage. As the real world use of a microbicide will involve sex, it is important that pre-clinical testing mimics that activity in order to realistically evaluate coverage of a product in development.

Greater volume improves coverage in both the vagina and the rectum, however, the rectum requires at least three-fold greater volume (with stirring) in order to achieve the same degree of coverage as in the vagina. This is primarily because the vagina is a closed pouch while the rectum is an open-ended system. However, a “fecal plug” (or a

physical obstruction created by a large volume of microbicide) may at least partially close the system. Either may be breached by intestinal gas, the act of sex, or ejaculation going around the barrier.

The typical 1.5-5 ml of lubricant used in human vaginal and anal sex is not likely to afford sufficient volume for a microbicide to provide reliable coverage and protection in the rectum. Estimates are that 50 ml of product would be adequate to provide rectal coverage and create a “gel plug.” Initial study has demonstrated that the human rectum can easily accommodate 50-100 ml of volume of product, while very preliminary and limited anecdotal use of 50-75 ml of volume of lubricant prior to and during sex was found to be “acceptable.”



Functional Assays in Vivo

Tools such as sigmoidoscopy and colonoscopy offer visual and even sampling access to much of the gut. However, they do little in terms of evaluating functionality. Most biological testing, such as those for iron or bleeding, will give a read of the entire gut and are not specific to a particular region of it.

A functional test can evaluate gut permeability *in vivo*. Increased permeability is associated with increased inflammation and disease. The test uses two orally ingested agents [lactulose and L-rhamnose], one that is absorbed through normal gut tissue, the second that cannot permeate the epithelium but can be absorbed where there are breaks in the epithelium. By measuring the ratios of these two agents excreted in urine, one can determine the extent of inflammation and damage to the epithelium.

Healthy HIV-positive individuals register in the normal range on this assay. But as CD4+ cell counts decline and among patients with advanced HIV disease characterized by diarrhea and wasting, the assay indicates increased intestinal permeability (paracellular permeation) with malabsorption (decreased transcellular permeation). Antiretroviral therapy that suppresses HIV results in increased absorption and normalization of the permeability findings in patients both with and without diarrhea. This is likely because of the increased presence of CD4+ cells in the epithelium, which help control inflammation. Malabsorption or increased intestinal permeability does not correlate with the presence of any particular pathogen but with CD4+ count.

A fecal calprotectin assay measures inflammation in the entire gut by counting the mg/ml of a neutrophil-selective protein in a single stool. It has been validated with Crohn’s disease and in patients on nonsteroidal anti-inflammatory drugs and other therapies.

Colon permeability can be measured by simultaneously administering lactulose and sucralose or 51CrEDTA orally. The former, excreted in urine, represents permeation through only the small intestine, while the latter represents permeation through both large and small intestine. Subtracting the former from the latter yields colonic absorption. A variation of the assay rectally administers 20-50 micro Curies of 51CrEDTA in a 20-30 ml solution. This has been validated in patients with ulcerated proctitis.

Transepithelial electrical resistance can be used as a surrogate marker for intestinal epithelial integrity. By measuring the change from baseline one can evaluate both the direct effect that a microbicidal product has on epithelium, as well as epithelial recovery and repair.

Formulating Rectal Products

Criteria and processes used *in vitro* and *in vivo* to develop vaginal microbicides may be relevant to developing rectal products. General principles for an effective formulation include:

- Microbicidal potency is good
- Disturbance of the vaginal/rectal tissue and microenvironment is bad
- Spreading and retention in the orifice is good
- Cosmetic acceptability by the user is good

Deployment is the term for physical distribution and retention of the product. Delivery is the release and availability of the active ingredients to target regions, tissues, and fluids. Bioactivity is the action of the ingredients within the milieu. The microbicide carrier and active ingredients may also perform a physical barrier function while in use.

Ideally products will be developed from the molecular structure of an active ingredient, formulated to elicit those properties, and tested *in vitro* and *in vivo* to see if the theoretical premise of the compound translates into protection in a real-world setting. That requires close interaction between the development teams that often differ at each stage of the process, so that lessons learned by one group are effectively shared with the others.

Microbicidal products are distributed within the rectal compartment through a combination of the physical forces of squeezing, shearing, sliding, and seeping that characterize use. Evaluation must contend with issues of product adhesion with rectal and penile tissue and condom surfaces, and with miscibility and mixability with rectal and seminal fluids.

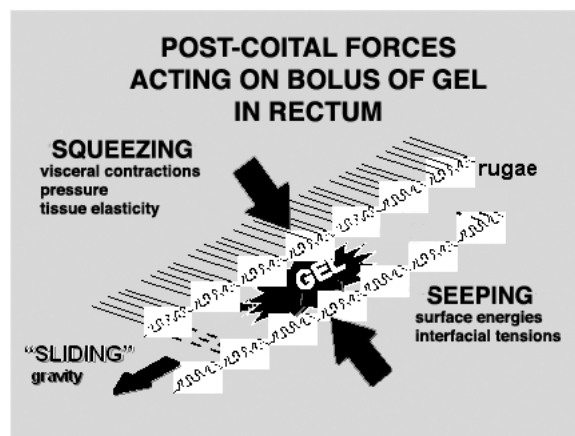
Mechanical squeezing devices have been developed to evaluate that property, and from that data a complex algebraic equation has been developed that predicts coverage of gels. It also allows for comparisons of gel volumes and formulations.

In situ distribution can be measured using a number of techniques. Remote sensing technology such as gamma scintigraphy and MRI is one option. Duke University researchers have measured vaginal coverage by placing a fiberoptic endoscopic device inside what is essentially a transparent dildo in order to observe and measure the thickness of gels labeled with fluorescent material. The intensity of fluorescence correlates with the thickness of the gel, so researchers are able to measure distribution, plot it, and compare products. This technique can be used to measure rectal coverage in the rectum, though it does not extend far into the colon.

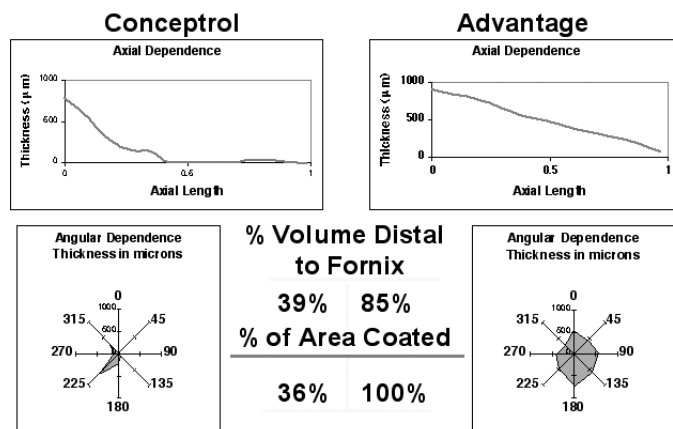
It is crucial to understand the distribution and clearance of the active component, virus, and cellular targets of the rectum over space and time. Formulation, patient adherence, pharmacokinetics, and pharmacodynamics each contribute greatly to response and each should be evaluated separately and in combination in rational drug development.

Active ingredients may have varying action and efficacy depending upon the character of the vehicle or carrier. Those effects also may vary within separate tissue layers or compartments. That raises consideration of a combination of agents within a microbicidal product to maximize killing or blocking efficacy in each environment.

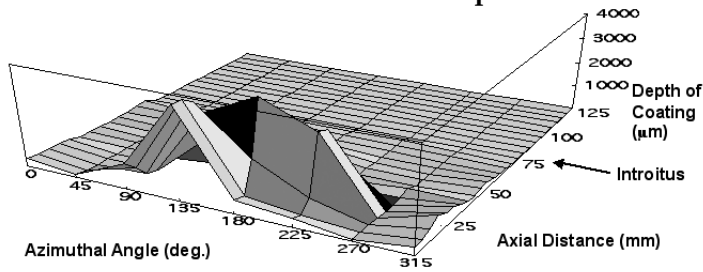
Craig Hendrix, MD, Johns Hopkins Hospital School of Medicine; David F. Katz, PhD, Department of Biomedical Engineering, Duke University; A. Garnell Rogers, PhD, SOLVAY Pharmaceuticals (Marietta, Georgia).



Axial and Angular Dependence of Coating Thickness Distribution



Vaginal Coating Thickness Distribution of Conceptrol



Volume Inserted (ml):	3.25
Percentage of volume distal to fornix:	21.2%
Percentage of area covered:	69.0%
Overall average coating thickness (mm):	0.160
Position of center of mass of coating (mm):	14.93
Coefficient of variation of distribution thickness	1.88

Among approved antiretroviral drugs, AZT has relatively poor rectal absorption compared with oral absorption, while ddI has no absorption through rectal mucosal tissue.

Scintigraphic mapping of the rectum over time indicates that volume can influence distribution, and distribution can shift over time, resulting in highly variable product coverage and protection.

Learning from Rectal Drugs

Rectal administration of drugs is used as an alternative when the compounds are poorly absorbed by the upper gastrointestinal tract, unstable to proteolytic enzymes, irritating to the stomach or upper GI tract, or difficult to administer orally, such as with the very young or old. Depending upon formulation characteristics, suppositories or other rectal products can be used for localized or systemic drug delivery. Rectal dosage forms can deliver drug quantities greater than oral dosing allows in some cases.

Of the types of rectal dosage forms, suppositories constitute approximately 98% of current products administered rectally. The vehicle may be composed of one of the following: fatty bases with a low melting point; water-soluble (dissolving) components or glycerinated gelatin; or hydrogels (controlled release). The suppository can weigh up to 1 gram in pediatric and 2.5 grams in adult formulations. They are made to dissolve or melt at approximately 36–37° C.

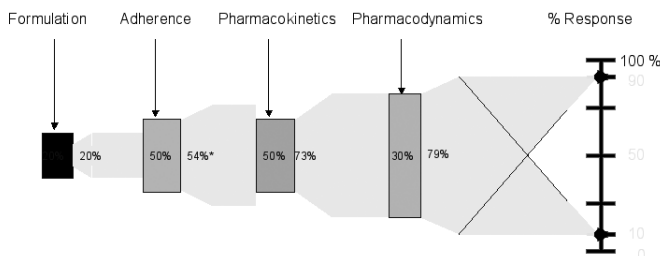
Solutions and suspensions are less frequently used because of inconvenience with administration, leakage, and in some instances, difficulties in long-term stability. Product spreading can be affected by viscosity and volume characteristics of the formulation. Drug absorption from solutions and suspensions can exceed that from suppositories in some cases.

Semi-solids—gels, foams, ointments—currently are used most often for local indications such as proctitis. Reported to exhibit better patient compliance, a variety of these formulation systems are available for possible adaptation for use with microbicidal products.

Controlled release suppositories can allow up to three times the dosing of oral delivery. Hydrogels are macromolar networks that do not dissolve in water but swell because of hydrophilic functional groups attached to a polymeric network. A possible application in the context of rectal microbicides is to extend the length of microbicide residence time in the rectal area by slow release, thereby either increasing antiviral contact time locally or increasing absorption systemically. Such a release pattern may not be considered an effective design for application immediately prior to anal intercourse.

Anatomical considerations in designing products include the length of the rectum (about 150 mm) and its surface area (200–400 cm²). The average rectal internal temperature of a healthy individual is 36.9° C, with a range of 36.2–37.6° C. Often there is a small amount of fluid ranging from 1 to 3 ml in the rectal area, with a pH of about 7.2 (vaginal pH is 4–5) and very little buffering capacity. However, the important consideration for product development may be the pH at the epithelial membrane surface below the mucosal tissue, which is 6.0–6.5. This is reported to remain constant and may influence product absorption. Some microbicidal products may be designed to maximize absorption while others may seek to minimize it for localized or topical effects with minimal systemic absorption.

Sources of Variability in Drug Response



Controlling for each variable may improve the predictive value of controlled clinical trial

Lack of motility in the anorectal area allows the maintenance of drug concentrations locally for extended periods after intrarectal administration. In some cases, systemic drug absorption by the rectal route provides reduced drug metabolism facilitated by the fact that the lower and middle hemorrhoidal veins partially bypass the hepatic portal circulation during first-pass absorption. An extensive lymphatic network also facilitates systemic circulation. Intracellular junctional complexes are tighter in rectal tissue than elsewhere in the gut, thus decreasing absorption of smaller, water-soluble drugs. The spreading of rectal formulations is facilitated by intraluminal rectal pressure. The result is a balance of factors affecting drug absorption. Lack of motility and increased spreading enhance drug absorption, and intracellular junction complex density reduces drug absorption rates.

The cellular metabolism of drugs absorbed in the rectum is similar to that seen in the small intestine. However, the colon/rectum does not serve a digestive function and therefore lacks enzymatic metabolic activity. Thus, it is a stable environment for proteolytically labile drugs such as peptides and proteins. Carrier systems for amino acids, sugars, and certain vitamins are not present, which inhibits absorption of drugs that use those carrier systems.

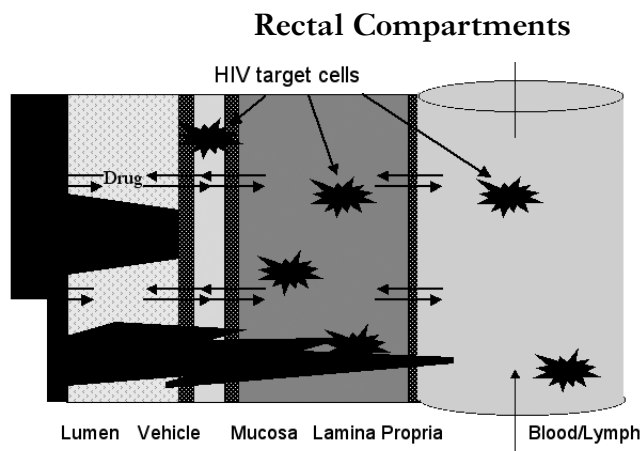
Semen and sperm may decrease fluid absorption and increase the permeability of the mucosal barrier. It has been reported that semen/sperm cause acute damage to mucosa and may elicit leucocytic infiltration. In studies of rat colonic mucosa exposure to human semen, it has been proposed that seminal collagenase is present in sufficient amounts to cause acute damage to the colonic mucosa, and that this could be a factor in facilitating viral transmission across the colonic wall.

Suppository carrier formulations take too long to dissolve to be used for a fast-acting microbicide that would be applied close to initiating sexual activity. In theory, they could be used to deliver protective agents meant for local intracellular absorption in the submucosa and other tissue.

Desirable general characteristics of a vehicle for a rectal dosage are that it is non-toxic and nonirritating to the mucus membrane; compatible with a variety of drugs, not binding with them on the shelf or during use; and able to melt or dissolve in the rectum at body temperature. Some initially promising compounds will have insufficient stability on long-term storage to be developed into commercially viable products.

Bioavailability of the drug depends upon the physiochemical properties of both the drug and the carrier base. Drug dissolution character and rate should be known, as well as the particle size distribution of the drug in the dosage form. The hydrophilic/lipophilic characteristics of the drug can be defined by determination of the partition coefficient, which will assist in understanding passive absorption behavior for the drug.

The major absorption mechanism in the anorectal area is passive diffusion through cell membranes in accordance with the pH-partition hypothesis. Typically drugs are absorbed in non-ionized or neutral form at physiological pH in the rectal area. However, some may be ionized, which makes them more soluble but also less efficiently absorbed. Ion pairs may be used to neutralize the charge. In general, drugs with a high partition coefficient, that is, more lipophilic, are better absorbed. Drugs must be dispersed from the dosage form and dissolved to be absorbed. Consequently, solubility must be balanced with the fact that non-ionized species tend to be better absorbed by passive diffusion.



Formulation factors: The drug and carrier are matched to maximize separation once the vehicle melts or dissolves. Water-soluble drugs typically release better from lipophilic or fatty bases, while lipophilic drugs release better from water-soluble bases.

Small drug molecules are more readily absorbed while large drug molecules may require facilitated transport or penetration enhancers. Enhancers with surface active properties may also irritate the mucous membrane at certain levels of concentration, as is the case with nonoxynol-9 (N-9), making the tissue more vulnerable to microbial invasion. While N-9 appears to degrade the cell surface, the mechanism of action in all cases has not been fully defined.

Additional formulation factors include:

- Melting/liquefaction character of the vehicle
- Particle size of the drug
- Vehicle spreading capacity
- Viscosity and volume of product at rectal temperature
- Drug binding by excipients
- Drug stability, i.e. oxidation, hydrolysis character
- pK of the drug
- pH of rectal fluid
- Considerations for use of prodrugs or different salt forms
- Storage temperatures, time effects (polymorphic changes)
- Microbial burden in product, preservation
- Expense: Rectal forms are more expensive than tablets, in general

Drugs that may prove useful in extemporaneous preparations used over short periods may fail under real world conditions because they fall short on one or more of these formulation criteria on long-term storage.

Models: Tissue-diffusion cell systems and cultured colon cell lines may be used for testing formulations *in vitro*. The rat is often the first *in vivo* animal model used, though its small size often requires the preparation of micro doses of a product. The rabbit offers larger volume but its more permeable colon sometimes overestimates absorption parameters. The beagle dog is the best choice, being anatomically similar to humans, while trials in macaques may be necessary for IND approval for human trials.

The most efficacious product will have little value if not used. It is therefore prudent to understand the qualities that consumers seek in an anal product and develop a microbicide with those characteristics in mind.

The Puerto Rican Men's Study (PRMS) of men who have sex with men (MSM) indicated that 91% engaged in anal sex during the previous year, 84% as the insertive partner and 64% as the receptive partner. About half claimed to use condoms all of the time and about 10% claimed to never use them, while the remainder did so intermittently. There was little difference in the interview response patterns of the insertive and receptive participants. Dislike of condoms was the principal reason they were not used more frequently.

When a microbicial product was described to them, 89% in the PRMS study said they would be likely to use the product. This suggests that those engaging in anal sex would readily embrace a lubricant containing a microbicide if it afforded protection from HIV.

HIVNET studies found that among a large six-city sample of sexually active MSM engaging in receptive anal sex, 79% used lubricant more than 80% of the time, while only 59% used condoms at least 80% of the time.

In their quest for protection, 41% of the men looked for products containing nonoxynol-9 (N-9), even though only 5% thought that N-9 by itself would offer protection from HIV. The implication is that they seek additional protection beyond condoms.

Another study indicated that 82% of the MSM surveyed are purchasing lube to use with anal sex; they are not getting it for free. Lubricant is used significantly more often than condoms, in part because the former enhances sexual pleasure while the latter typically is thought to detract from it.

A preliminary survey indicates American MSM seem to prefer a lubricant that is colorless, odorless, lubricious, long-lasting, and tasteless. Gender specific packaging is advisable. Thai survey data on lubricants support those preferences and strongly suggest that any microbicide must function as a lubricant, come in single-use applicators, and be disposable if it is to be widely adopted by that culture. The product should have a long stable shelf life, be available over the counter, and be inexpensive. Similar preferences have been expressed in surveys of men and women in Africa.

A HIVNET trial in Seattle using Advantage-S applicators with a fixed volume of 52.5 ml of lubricant gel found complaints of "rectal fullness," or a bloated gaseous feeling, perhaps associated with the volume of the lubricant applied, the location the gel was deposited, and/or air in the applicator. However, anecdotal evidence indicates other carbomer-based formulations similar to BufferGel can be used in very large volumes without causing "rectal fullness" and can be formulated to be both adhesive and lubricating. People engaging in anal sex with current lubricants typically use 1-5 ml of product. The adhesive quality of the gel also drew some complaints. Issues of slipperiness, ease of use, and packaging will affect consumer acceptance of any microbicial product.

Attitudes about adopting use of a hypothetical microbicide and possibly using it in lieu of condoms were gathered through a self-administered survey of 385 men in West Hollywood, California who had engaged in anal intercourse within the last year. Respondents showed no attitudinal difference whether playing insertive or receptive roles with a partner of unknown HIV serostatus. The men required an average 84% killing efficacy from a microbicide before they would be willing to forego use of a condom in anal intercourse, with 53% of the men requiring at least 95% efficacy in a microbicide.

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Preferred efficacy of microbicides by serostatus

Men	Preferred efficacy	% who wanted 95%+ efficacy
HIV-positive	71%	26%
HIV-negative	85%	55%
HIV-unknown	91%	65%

Multivariate analysis indicates that the most important factor in deciding to use only a microbicide is the efficacy of that product (.33), with the second most important factor being a negative attitude toward condoms (.18). Some 77% said they would use a condom and microbicide together, especially if the microbicide had an efficacy of less than 95%.

If 50-100 ml volume were required for a rectal microbicide, both portability and acceptance would be uncertain. An education campaign probably would be necessary to support acceptance and to change practices from current tendencies to minimize the volume of lubricant used. However, this should not deter researchers and developers from using large volumes if necessary. The experience with N-9 lubricants has demonstrated that gay men will use a product, even when it is unpalatable, numbs oral mucosa, suppresses the gag reflex, and causes rectal rashes — if they believe it confers some protection from disease.

The sensory experience of a product is important for consumer acceptance. Pharmaceutical manufacturers have little experience dealing with this concept. As a rule, absorption would be minimized so as to maintain the tactile quality of the microbicide. Resistance to putting large volumes of lubricant into the rectum may have a lot to do with the adhesive properties of a product. It needs to stay where it is placed and not run out. Silicon reduces friction. While some users may find that desirable, others may desire a sense of friction. Carbomer products may offer greater flexibility for creating a range of sensate qualities that appeal to different segments of consumers.

Because anal sex often is part of a repertoire that also includes oral sex or oral-anal sex, taste is an important factor to consider.

It is important that sexologists and other sexually knowledgeable and experienced consumers be brought into the process early so that resources are not wasted developing products that will meet significant consumer resistance. A product that is not widely embraced in the marketplace will do little good

Workshop Conclusions and Recommendations

Behavioral Issues

Improved methodological tools and data are needed in order to shape more effective education and intervention programs that complement the physical development of microbicidal products. That is particularly true in developing nations where knowledge is scant.

Some of the greatest rates of increase in HIV infection are occurring in the non-urban southern United States, while the most detailed behavioral data has been gathered in the urban epicenters of the epidemic. The assumption is that because urban sites contain significant numbers of migrants from non-urban areas, and their responses do not vary significantly from urban natives, the sample reflects the broader population. However, this should be substantiated through study of non-urban areas to determine if those who migrate differ from those who remain in non-urban areas.

With whom are MSM and heterosexuals most likely to practice AI (casual vs committed partners? both?), under what circumstances and in what settings?

Do heterosexuals use lubricant with anal sex? What are their reasons for using/not using lubricant? Do they differ from reasons given by MSM? Are heterosexuals avoiding the use of water-based lubricants?

Do heterosexuals have networks of those who engage in anal sex? If so, can they be tapped for the dissemination of education programs?

There is no consensus on whether there is sufficient knowledge for the rapid initiation of an education program that targets women concerning the benefits (reduced trauma) of using lubricants in anal sex.

Can the sex industry, particularly the segment that focuses upon anal sex, help disseminate information and educate the public?

Tactile and oral properties of candidate products should be evaluated early in the development process so as not to lead to resistance or rejection in the marketplace. Often sex is multifaceted, involving body parts and sequences not originally anticipated when the product was developed.

The applicator and packaging should be considered early in the development process and perhaps be customized for gender and culture. Market research will be required to shape these products to the needs and desires of target audiences.

Molecular and Cellular Research Issues

Which cells in the rectum become infected and when? What are the initial target cells? What are the course and all types of cells implicit in the path from local to systemic infection?

What is the nature of local immune response in affording some protection from HIV infection? Do microbicidal products have the capacity to degrade or enhance these protections?

What non-visible evidence, markers such as localized CD4+ count, cytokine levels, etc., should we be looking for? What is clinically relevant to transmission?

What are the effects of douching and anal sex upon rectal epithelium (e.g. trauma, inflammation), and how long do they persist?

How far up the colon does semen travel and hence require protection from infection? Does that change with various sexual positions?

What are the effects of inflammation, sloughing, fissures, lesions, and hemorrhoids on susceptibility to HIV and performance of potential rectal microbicides?

Does defecation remove the microbicide and/or mucosal epithelial tissue?

How long does mucosal epithelium take to repair itself to adequately provide a physical barrier to HIV?

Are there clinically significant gender differences in rectal tissue? In viral shedding? In impact of STD coinfection?

There is a need to develop standardized criteria to:

- Evaluate inflammation that is clinically significant
- Identify where rectal biopsy should be taken and how it is evaluated

Are particular physical points in the rectum (e.g. transition zone) more vulnerable to trauma and/or infection, and hence in greater need of protection?

What is the impact of pH in a resting state and how may that change in the presence of semen? (e.g., does ejaculate raise the pH? Is that salient?) Does the greater or lesser presence of feces affect the microenvironment and product efficacy?

Can a topical microbicide downregulate CCR5 receptors on CD4+ cells in the gut? Does that matter?

What is the impact of viral load, CD4+ count (particularly in the rectal mucosa), and individual and multiple anal STD infections in terms of viral shedding?

What existing animal models can be adapted for rectal microbicide research?

Pre-Clinical Product Development

Factors affecting rectal microbicide use include:

- Impact of mucosal secretion and product adhesion (mucolated vs. non-mucolated)
- Sites where semen might be deposited
- Impact of flatulence and defecation
- Product distribution with differing formulations and volumes
- Extent and sites of systemic absorption
- Lubricating properties that may differ from vaginal use
- Post-coital behavior
- Acceptability of a large volume of product, if necessary for adequate coverage

Necessary characteristics of a microbicide include: low toxicity; stability; compatibility with latex; activity in the presence of semen, blood, feces, gas, and varying pH (ideally 4-8 for use in both vagina and rectum).

This may not require creation of an *in vitro* model to simulate them, but these factors should be carefully evaluated in phase I/II trials.

It is crucial to consider the microenvironment of the rectal compartment if, as with vaginal products, a high priority is to not disturb the normal flora. It is too early to say if there are similar concerns with a product in the rectum.

Different microbicidal formulations may be required to prevent infection of an uninfected person through receptive anal intercourse and to prevent viral shedding from rectal tissue to protect an insertive partner.

Does product efficacy vary with viral load (e.g., will it be less effective with a high viral load in semen or greater shedding of RNA from rectal mucosal tissue)?

Is primate data crucial for advancing to human trials?

Can small, elegantly designed Phase I/II human “micro-studies” be used in conjunction with animal studies to test safety, dispersion, and sexological qualities in a cost-effective manner?

Phase I/II Trials

Safety studies should involve HIV-negative and positive MSM, and high-risk women, to evaluate differences, if any, in the rectal tissue of males and females, and also to evaluate safety through vaginal exposure.

Are vaginal safety criteria adequate for rectal trials? It probably depends on the mode of action and absorption profile of the compound. A new pK and pH profile will almost certainly be required for each component.

Natural variations in the rectum as well as variations in sex acts and associated trauma require observational controls in Phase I trials to better evaluate the product. Technical standards and expertise in making these evaluations is scanty and should be standardized. Realistic baseline data should be established for those engaging in anal sex.

Bleeding, inflammation, ulceration, erosion, sloughing, and absorption are major safety coordinates to evaluate. Additional useful measures may include itching, fullness, bloating, numbness, and diarrhea. Differentiating the separate contributions of the product and the act of anal sex may be difficult.

Viral shedding may be a useful marker but the elusive character of HIV probably requires biopsy to fully evaluate presence of the virus. Biopsy protocols should be at a standard site; multiple and averaged; read by more than one expert; and be taken at multiple time points, including soon after application of the product.

Permeability studies are useful to determine if there are non-inflammatory factors associated with the product that may affect viral entry.

Both applicator and digital application of product needs to be evaluated.

Pre-IND meetings with the FDA are essential to shaping trials and gaining market approval. They also may yield information about how others have gone about developing products.

Phase III Trials

The initial Phase III trial population is likely to be in high risk HIV-negative MSM who practice both receptive and insertive roles. Women who do engage in anal sex also engage in unprotected vaginal sex, confounding the interpretation of incident infection among women who have both vaginal and anal sex (no population of women has been observed to engage in anal sex to the exclusion of vaginal sex). HIV-positive MSM will likely be ruled out because of the considerable methodological difficulty of determining whether a test product also reduces the likelihood of transmission of new or “super” infection to casual sexual partners.

The FDA will have to be consulted about accepting rectal trial data conducted solely in MSM for a labeling indication that would also include women.

The primary endpoint of an efficacy trial will be incidence of HIV infection. A secondary endpoint may be a lower viral set point among those who do become infected. The impact on acquisition of other STDs should also be considered as a secondary endpoint.

The effect size should be 33% efficacy, the same as proposed for large scale proof of concept trials of new vaginal products. Products of moderate efficacy may provide critical insight into highly salient product development considerations.

There is a difference between establishing proof of concept and documenting efficacy that would gain market approval in the United States. Less efficacious products may have a significant public health impact in regions of high prevalence of HIV, but may not be approved in the US and/or may not gain market acceptance in the US.

Counseling safer sex practices, including the use of condoms, must be included in any protocol, even if it implies a larger number of trial participants and a longer duration of the trial.

Ideally a microbicide would function equally well in the vagina and rectum. But that does not preclude development of a rectal-only microbicide.

Academia and Industry

Microbicide development needs to attract more researchers to the field, particularly those skilled in applying sexological, biophysical, and engineering principles to product formulation.

Smaller and start up companies, and nonprofit organizations such as the Population Council that interact with industry, are likely to be the initial players in developing microbicidal products. Big pharmaceutical companies may enter the picture later through purchase of a company or signing joint development agreements around compounds that show promise, but it is unlikely to initiate work in the field.

One tactic to foster greater industry involvement might be to focus on products that have a tangential or associated relationship with microbicides, such as development of anti-inflammatory drugs, and share information and expertise with industry.

No good market analysis or model for microbicides has been published, nor has the sexual products industry been tapped for its expertise. These analyses and sources should be developed. Use of lubricants for anal sex may be a useful starting point in developing marketing and distribution networks.

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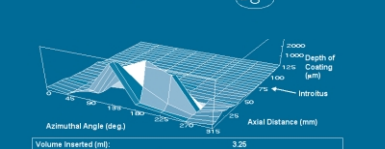
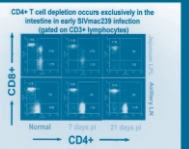
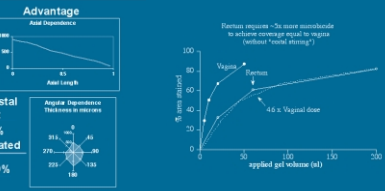
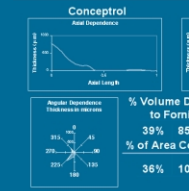
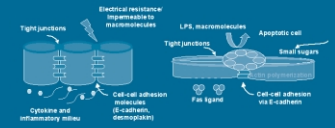
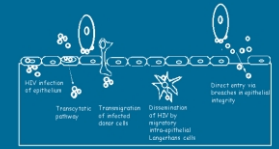
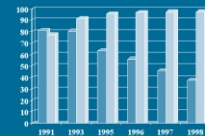
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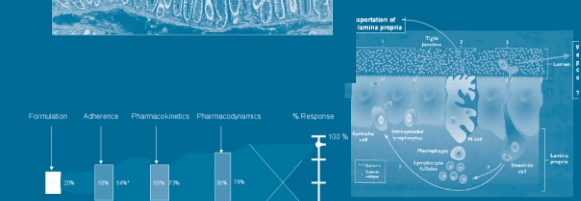
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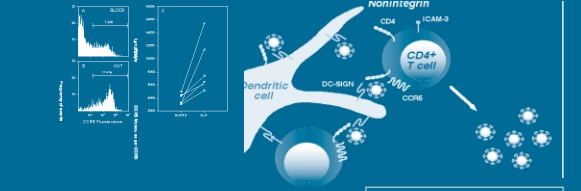
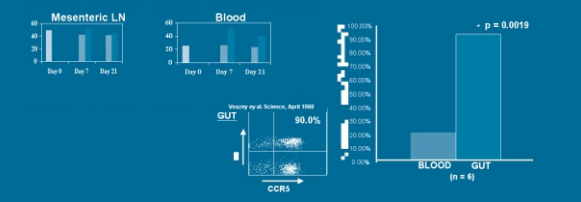
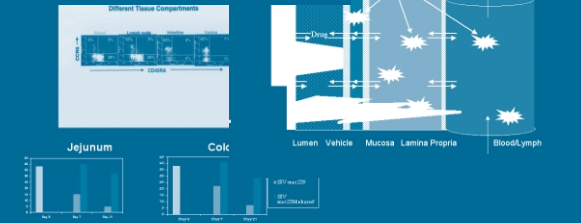
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Parameter	Value
Volume Inserted (ml)	3.25
Percentage of volume distal to fornix	21.2%
Percentage of area covered	69.0%
Overall average coating thickness (mm)	0.160
Position of center of mass of coating (mm)	14.93
Coefficient of variation of distribution thickness	1.88



Controlling for each variable may improve the predictive value of controlled clinical trial



Symptoms	N-9 tolerability among receptive men	
	HIV+ N=10	HIV- N=5
Rectal ulcers	1 (10%)	4 (80%)
Rectal discomfort	1 (10%)	2 (40%)
Lower stools	1 (10%)	2 (40%)
Rectal bleeding	0 (0%)	1 (20%)

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