Exploring the Potential of HIV Microbicides

As the number of HIV-infected women escalates worldwide, vaginal microbicides may help slow the spread of AIDS.

The face of the AIDS epidemic has changed considerably in the last quarter of a century. Although the disease was first identified in homosexual men, today women comprise half of the world's nearly 40 million HIV-infected individuals. Most of these women became infected through heterosexual contact.

Women are particularly vulnerable to HIV infection because their mucosal exposure to the virus during intercourse is greater than men's. Condoms can help prevent HIV transmission, but their use is not under a woman's control. Because of these and other vulnerabilities, new HIV infections now arise more rapidly among women than among men in many parts of the world.

Public health officials have long called for new HIV prevention methods for women—methods that are inexpensive, easy-to-deliver, and under their control. This need is especially urgent, given that a cure for AIDS and development of a safe and effective HIV vaccine has proven elusive.

Microbicides are substances, typically formulated as gels or creams, that can be applied topically to the vagina to prevent sexual transmission of HIV or other pathogens. The products, now being tested in preclinical and clinical studies, may offer women the protection they need. Mathematical models predict that if only 20 percent of women in the developing world used a microbicide in just half of their sexual encounters, 2.5 million HIV infections could be prevented over a three-year period.

NCRR-funded resource centers are helping scientists to pursue

■ Christopher Miller has found that microbicides, if applied soon after intercourse, may prevent a widespread HIV infection. His research suggests that HIV infection can remain limited to the vagina and cervix for about four days prior to systemic infection.

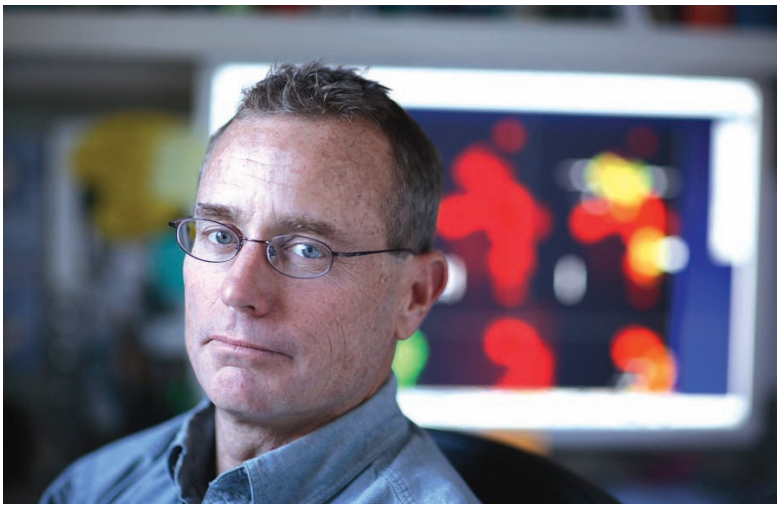


PHOTO BY TIMOTHY ARCHIBALD

■ Women of Galufu, Malawi, a small village in southern Africa, prepare food after the funeral of a female villager who died of AIDS a few hours earlier. Poverty in the village has increased dramatically in the last few years because of drought and AIDS.

novel approaches to microbicides on several fronts. Investigators supported by NCRR's Research Centers in Minority Institutions (RCMI) Program are conducting preclinical studies of an effective yet inexpensive microbicide based on a common product additive. An NCRR-supported General Clinical Research Center (GCRC) is helping efforts to examine both how microbicides react to the human vaginal environment and how the vagina reacts to the compounds. And studies with macaque monkeys at the NCRR's National Primate Research Centers (NPRCs) are uncovering new roles for microbicides and developing approaches that target the molecular interactions between HIV and the immune cells it invades.

In the 1980s, scientists working at NCRR-funded NPRCs laid the groundwork for much of today's HIV research when they discovered the simian immunodeficiency virus (SIV), a close relative of HIV that infects nonhuman primates. SIV infection in macaque monkeys has since become a vital animal model for the study of HIV infection, treatment, and prevention.

Soon after the discovery of SIV, Christopher Miller, a researcher at the California NPRC at the University of California, Davis, and his colleagues showed that they could infect monkeys in a way that mimics sexual transmission of HIV in humans by applying SIV to the genital mucous membranes of macaques. Studies with these monkeys have shown how SIV spreads systemically from genital mucosal sites.

"This is a particularly good model for understanding what happens in human HIV infections, because these nonhuman primates have the same sort of anatomy and physiology as do humans, and they also have 28-day menstrual cycles, just as women do," says Miller. "Especially for studies that hopefully will be predictive of how an intervention affects human patients, we want the animal model to be as close as possible to the real thing."

Miller and his colleagues recently found that new SIV infec-

WOMEN AND AIDS

The number of women with HIV infection and AIDS has increased steadily. By the end of 2005, of the 38.6 million people living with HIV, almost half (17.3 million) were women, according to the World Health Organization. The vast majority (76%) of HIV-infected women live in sub-Saharan Africa. In the United States, the proportion of adult HIV cases among women has more than tripled in the past two decades—from 8% in 1985 to 25% today, according to United Nations and U.S. Centers for Disease Control and Prevention statistics. HIV infection disproportionately affects the nation's African-American and Hispanic women. Together, these two ethnic groups represent less than 25% of all U.S. women, yet they account for 79% of women with HIV nationally.

PHOTO BY PER-ANDERS PETTERSSON/GETTY IMAGES

tions in female macaques remain limited for about four days to a relatively small number of cells, primarily in the vagina and cervix. "There's something of a delay between the time that the virus comes in contact with the genital tract and the time that full-fledged systemic replication of the virus occurs," says Miller. This finding suggests that microbicides might do more than just block sexual transmission of HIV. If a woman has already become infected through sexual contact, a microbicide, if administered soon enough, might prevent or limit a wider systemic HIV infection. "It gives people hope and a rational basis to keep exploring interventions that are aimed at an early timepoint," he says.

At the Tulane NPRC, another NCRR-funded primate center, Ronald Veazey and his colleagues are testing a promising new type of microbicide called a fusion inhibitor. These agents inhibit infection in a specific and targeted way by preventing the binding, or fusion, between glycoprotein molecules on the outer coat of HIV particles and the receptors for those glycoproteins on the surface of immune cells.

Veazey studies fusion inhibitors that target a type of cellular receptor called CCR5. As one of the main receptors that HIV uses to infect cells, CCR5 appears to play a major role in HIV transmission across mucosal surfaces, like those in the vagina. When these fusion inhibitors bind to a cell's CCR5 receptors, they block viral access to the receptors and in some cases also trigger cellular changes that reduce the number of receptors on the cell's surface. These mechanisms greatly limit viral entry points into the cell.

Veazey and his colleagues have found that both vaginal and oral administration of CCR5-based fusion inhibitors protects macaques against infection. "We've shown tremendous proof of concepts in blocking this receptor," says Veazey. "Blocking CCR5 seems to be all that is necessary to prevent transmission of the AIDS virus, at least in the monkey model."

In one study, the researchers administered three experimental microbicide gels, alone and in combination, to female macaques and found that all three were protective against vaginal infection with simian HIV. In addition, significant protection was achieved when two of the agents—known as Compound 167 and BMS-378806—were administered in combination. The combination gel was protective even when applied up to six hours before viral exposure. In a separate study, Veazey and colleagues found that orally administered formulations of Compound 167 can prevent vaginal infections of simian HIV in macaques.

Scientists are now working to develop more cost-effective molecular preparations of fusion inhibitors. "Clearly, the CCR5 point of attack is extremely effective. Our major obstacle now is to develop a fusion inhibitor that can be produced economically," says Veazey. Clinical trials of the fusion inhibitor gels are now being planned.

Another novel approach to microbicide development is being pursued in preclinical studies at the NCRR-funded RCMI at Meharry Medical College in Nashville. James Hildreth, director of Meharry's Center for AIDS Health Disparities Research, is investigating an agent called beta-cyclodextrin. Cyclodextrins are simple polymer sugars that are already widely used in a variety of products, including mouthwash, topical creams, food flavorings, and some intravenous medications.

"Cyclodextrin is easy to synthesize, very inexpensive to pro-

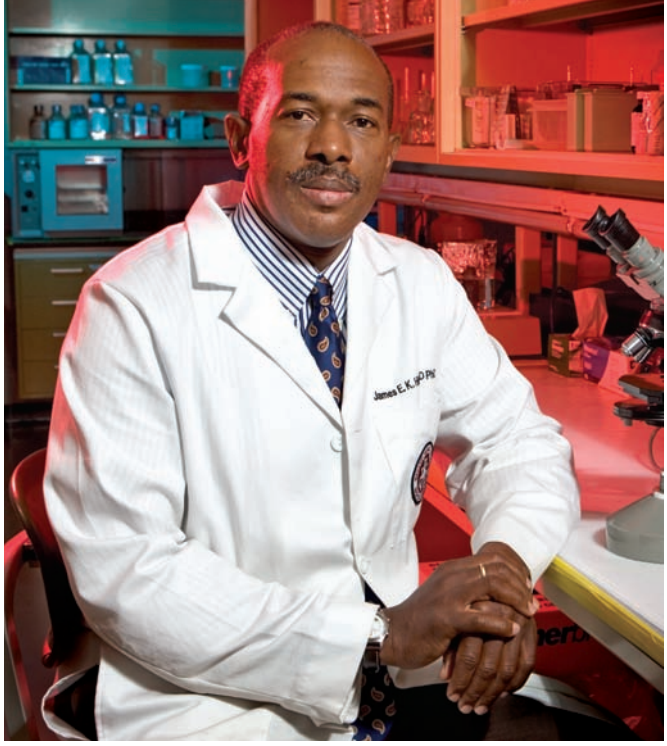
NIH-FUNDED MICROBICIDE CLINICAL TRIALS

With funding from several NIH institutes, two multisite clinical trials are now under way to evaluate the safety and efficacy of the potential vaginal HIV microbicides described below. Although called "microbicides," most of these agents do not actually kill the virus, but instead simply impede its ability to infect host cells.

Tenofovir/PMPA A Phase II clinical study, expected to involve 200 women, will be evaluating the safety and acceptability of a microbicide gel formulation of tenofovir (PMPA). Tenofovir works by inhibiting enzymes that are needed for HIV replication. An orally administered tablet form of tenofovir was approved several years ago by the U.S. Food and Drug Administration for the treatment of HIV infection in combination with other anti-HIV medicines.

BufferGel and PRO 2000/5 A Phase II/IIb clinical trial, with an anticipated enrollment of 3,220 women, will be evaluating the safety and effectiveness of two vaginal microbicides, each administered alone to different volunteer participants. BufferGel is expected to help maintain the normal acidity of the vagina even in the presence of ejaculate, which has a higher pH; studies have shown that HIV may be inactivated at an acidic pH of less than 4.5. The other microbicide, PRO 2000/5, inhibits viral entry into susceptible cells. Both microbicides are being evaluated for their potential in blocking HIV and also other sexually transmitted agents. Study results are expected in 2009.

Primary funding for the clinical trials described above comes from the National Institute of Allergy and Infectious Diseases. Additional NIH sponsors and collaborators include the National Institute of Child Health and Human Development, the National Institute on Drug Abuse, and the National Institute of Mental Health.



■ Physician James Hildreth is studying beta-cyclodextrin, an agent widely used in mouthwash, topical creams, and food flavorings. The compound can prevent cell-to-cell transfer of HIV, an important route of infection for sexually transmitted viruses.

duce, and if it works it will cost about 7 cents per application," says Hildreth. "For developing countries, where the annual income is often only a few hundred dollars per year, we have to produce something that is very inexpensive."

In 2001, when Hildreth was an associate professor at Johns Hopkins University School of Medicine, he and his colleagues discovered that cholesterol plays a critical role in HIV fusion and entry into cells. In later studies, the scientists showed that HIV buds from infected cells at cholesterol-rich regions known as lipid rafts, small fatty globules scattered throughout cellular membranes. "HIV is a thief. It steals proteins and other molecules from the host cell when it buds from that cell," says Hildreth. "In 2003 we showed that HIV particles themselves also appear to have lipid rafts, probably picked up from cellular membranes as the virus exits the cell."

Further studies revealed that beta-cyclodextrin drains cholesterol from both HIV and the host cell membrane. "As you remove cholesterol from HIV particles, you can unplug that lipid raft, and the virus will lose its essential components and become noninfectious," Hildreth says. The compound also enables healthy cells to resist HIV and makes infected cells less able to spread the virus.

By studying mice that contain transplanted human cells, Hildreth and his colleagues demonstrated that beta-cyclodextrin can prevent cell-to-cell transfer of HIV, an important route of infection for sexually transmitted virus. In 24 out of 27 mice, vaginal administration of beta-cyclodextrin blocked the passage of HIV from infected cells in the vagina to uninfected cells in the body.

Encouraged by his mouse research, Hildreth is planning a small clinical trial at Meharry's RCMI Clinical Research Center to assess the safety of a beta-cyclodextrin microbicide in women. Beta-cyclodextrin has already been found safe for human use in toxicity studies related to its various product applications.

Some microbicide candidates have already advanced to clinical trials, although these generally have a less specific and targeted mechanism of action than the CCR5 fusion inhibitors and other agents now being investigated in preclinical studies involving animals. Among the compounds currently in clinical trials, substances called sulfated polyanions, which bind proteins HIV uses to enter human cells, appear particularly promising. The National Institute of Allergy and Infectious Diseases is currently funding a multicenter clinical trial—involving more than 3,000 women in the United States and Africa—examining both a sulfated polyanion called PRO 2000/5 and a gel designed to lower the pH of the vagina (BufferGel) to evaluate their safety and ability to prevent HIV infection in at-risk women. Other NIH-funded microbicide clinical trials are also under way. (See box on page 6.)

Along with their colleagues, Marla Keller and Betsy Herold at the NCRR-supported GCRC at Mount Sinai School of Medicine in New York recently showed that the environment of the human vagina does not lessen the potency of PRO 2000/5. In fact, they demonstrated for the first time that a microbicide can remain highly effective after contact with the human vagina.

The researchers placed PRO 2000/5 into the vaginas of 10 women and then collected cervicovaginal fluid samples. The samples were mixed independently with HIV and herpes simplex virus type 2 (HSV-2)—the pathogen responsible for genital herpes, which is known to increase a person's risk for HIV. When human cells were inoculated with the cervicovaginal samples, the PRO 2000/5, still present in the samples, inhibited both HIV and HSV infection at least 1,000-fold. In a follow-up study, involving 24 healthy women, Herold and Keller determined that daily applications of PRO 2000/5 does not trigger an inflammatory response in cervicovaginal secretions, suggesting that repeated use of this microbicide is safe.

By supporting this type of clinical research, as well as preclinical animal-based studies, NCRR is helping to translate basic research findings into potential AIDS-prevention strategies for women. While NCRR's nonhuman primate resources are helping to identify and evaluate promising microbicides, the RCMI and patient-oriented resources stand ready to facilitate clinical investigations. Through these efforts, NCRR is assisting NIH's broad efforts to provide women with an effective agent that they can easily and safely use to achieve protection from HIV infection. ■

PHOTO COURTESY OF MEHARRY MEDICAL COLLEGE