



## Spotlight

### Exploring mucosal protection for a mucosal virus

*Understanding mucosal immune responses is critical to developing effective AIDS vaccines, but progress has been slow*

HIV is primarily a mucosal infection (see *Primer*, this issue). The virus is transmitted most often through the mucosal tissues of the genitals or rectum. And although the progression of HIV infection is tracked by measuring the number of CD4<sup>+</sup> T cells (a subset of immune cells) in the blood, most of the havoc wreaked by HIV is in the moist mucosal surfaces that line several of the body's internal cavities. Understanding immune responses against HIV at these mucosal surfaces is therefore important in designing AIDS vaccine candidates capable of preventing HIV transmission or in controlling infection once it occurs.

Only a few research groups are currently studying HIV infection at the mucosal level, says Lucia Lopalco of the San Raffaele Scientific Institute in Milan, Italy. "This is a huge gap," Lopalco says. "We need more scientists who study mucosal immunity." Lopalco also points out that researchers are getting a late start at studying the types of immune responses that could effectively block HIV at mucosal sites. "We should have started 20 years ago," she adds.

Progress in studying mucosal immunity and HIV infection has been slow, in part, because measuring mucosal immune responses is much more complicated than measuring those produced systemically. While systemic immune

responses can be measured with a simple blood test, measuring mucosal immune responses requires taking tissue samples or collecting secretions from these sites. It is also more difficult to deliver a vaccine directly at mucosal tissues.

Still, over the past several years, researchers have gained some important insights into the role of mucosal immune responses in HIV infection that could contribute to the development of an AIDS vaccine candidate that stimulates mucosal immunity against the virus.

#### Measure for measure

Measuring immune responses in mucosal tissues can be difficult, especially within the context of an AIDS vaccine clinical trial. The major type of antibody in most mucosal secretions is known as immunoglobulin A or IgA, according to Jiri Mestecky of the University of Alabama at Birmingham. But there is often inconsistency in the measurement of IgA levels in human secretions, depending on where the laboratory tests were conducted. This makes it more difficult to interpret and compare findings from different studies.

Collecting secretions from mucosal tissues, such as the vagina or the rectum, is not easy. One method called lavage involves washing the mucosal surfaces with a salt solution and then collecting the liquid for analysis. But Pam Kozlowski of Louisiana State University has found that this approach often dilutes the secretions too much, making it hard to detect antibodies.

Instead, she has developed a method that uses an absorbent sponge to obtain vaginal and rectal secretions. This sponge method can be used in both

animal studies and in human volunteers and causes very little discomfort. It is also less intrusive than lavage methods. "It's only in for ten minutes at most," Kozlowski says, adding that it would be much easier to use in clinical trials. Also lavages require immediate processing, while the sponges can simply be frozen after collection and analyzed later, making them more practical.

While this sponge method may help researchers get around some of the problems with collecting mucosal antibodies, measuring cellular mucosal immune responses at these sites is still challenging. Isolating cellular immune responses in the rectum requires collecting a tissue sample by biopsy, according to Julie McElrath of the University of Washington. This is a much riskier and more invasive medical procedure than a lavage or blood test. If it is not done properly, the colon can be perforated, which results in a condition called peritonitis. To collect vaginal cells, some researchers use a cytobrush, a small brush-like device that is inserted into the cervix and rotated.

But even when mucosal tissue samples are properly collected, the number of cells that can be analyzed is often much smaller than in blood samples. This drastically limits the type of immune responses that can be measured, says Robin Shattock of the University of London. These samples must also be analyzed within a few hours of collection, which requires

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researchers to have a lab available at the same site where samples are obtained, McElrath says, which is not always feasible during clinical trials that are conducted in developing countries.

Together, these limitations are part of the reason why mucosal immune responses are not frequently measured during clinical trials. McElrath says that in AIDS vaccine trials, mucosal tissue samples are usually only taken from a subset of volunteers. “We wouldn’t do it in all people,” she says. “It’s just an amazing amount of work technically.” For example, in the recently conducted STEP trial with Merck’s AIDS vaccine candidate MRKAd5, mucosal samples were only collected in about 20 of the 3,000 total volunteers. These samples were analyzed in McElrath’s laboratory.

To get around these limitations, some researchers have proposed a way to measure mucosal immune responses using a blood sample. Immune cells that are headed for mucosal tissues can be identified by a molecule known as a receptor on their outer surface, which acts like a tag that shows where the cell is going. If these tagged cells can be detected in a blood sample, it could provide a rough estimate of the quantity of immune cells that will end up at mucosal tissues. But this model is not perfect. These tag-like receptors are only known for a few specific mucosal sites. And even though a cell may be headed for mucosal tissues, detecting its presence in the blood does not show whether or not it will actually arrive there. Mestecky compares it to a letter with an address. “Whether it will actually get there and have its effect is unknown,” he says.

### **Mucosal protection?**

Researchers have also been trying to find out if mucosal immunity could, in part, explain why some individuals, called exposed or highly-exposed seronegatives (ESNs), remain HIV uninfected despite repeated exposure to the virus (see *VAX* March 2007 *Primer on Understanding Why an Effective Vaccine is Feasible*). Researchers are closely studying ESNs and are exploring many different hypotheses for their apparent immunity to HIV. Some studies have investigated if mucosal antibodies, like

IgA, are responsible for protection. But these have led to contradictory results, according to Mestecky.

Other studies have focused on characterizing the mucosal immune responses in long-term nonprogressors (LTNPs)—people who are HIV infected but don’t develop AIDS within the typical time frame. It is still unclear if mucosal antibodies detected in ESNs or LTNPs are actually playing a protective role. To find out, researchers are creating models of mucosal tissue in the lab using actual human cells, which simulate the mucosal tissue barrier, and running

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experiments to see if antibodies isolated from LTNPs or ESNs can effectively block HIV. Indeed some studies show that such antibodies can keep HIV from crossing these tissues in the laboratory. However, not everyone is convinced that these observations are meaningful because the model is not foolproof.

### **Routes of delivery**

Delivering vaccines in a way that will induce mucosal immune responses is another challenge for AIDS vaccine researchers. Often systemic immunizations, by intramuscular injection for example, are not sufficient to induce immunity at all mucosal tissues, says Mestecky. “Antibodies from the blood may protect the genital tract but probably not the intestinal tract,” he says.

Instead, studies suggest that the strongest mucosal immune responses would be expected when a vaccine is administered directly at a mucosal surface. Researchers have learned in recent

years that mucosal antibody responses are often local and restricted to the sites where they are first induced. Kozlowski’s lab, for instance, has found mostly localized mucosal immune responses when comparing different mucosal delivery routes in women. An exception was nasal immunization, which generates responses in both the rectum and the female genital tract. Because of these results, Kozlowski is now conducting nonhuman primate studies with intranasally-administered vaccine candidates. Shattock’s group has initiated studies in both nonhuman primates and human volunteers to evaluate a vaccine that is applied vaginally, with the hope of inducing vaginal immune responses against HIV.

Others are trying oral vaccinations, which are best for induction of immune responses in the intestine or gut, says Kozlowski. The gut is a critical site for HIV infection. Gary Nabel’s group at the Vaccine Research Center, part of the US National Institute of Allergy and Infectious Diseases (NIAID), is investigating oral administration of adenovirus serotype 41 (Ad41) as a vector for a mucosal AIDS vaccine candidate because of its tendency to travel to intestinal tissues.

Still others are evaluating new routes such as applying a vaccine candidate directly onto the skin, under the tongue, or onto the tonsils. Researchers are also looking for substances called adjuvants that, they hope, can augment mucosal immune responses (see *VAX* December 2005 *Primer on Understanding Mucosal Immunity*).

Although it is clear that understanding mucosal immunity is important, it remains an open question as to whether a mucosal immune response will be sufficient to prevent HIV infection. Still that doesn’t mean it is not important to induce mucosal immune responses, says Barbara Shacklett of the University of California, Davis. “Even if we can’t prevent the initial infection, we may be able to limit viral replication and dissemination,” she adds.

Given what is now known about mucosal immunity, some researchers say that inducing a combination of mucosal and systemic immune responses is the ideal goal for future AIDS vaccine candidates.

### Cutting progress?

In December 2006, the US National Institutes of Health halted two clinical trials in Kenya and Uganda after study results indicated that male circumcision cut a man's risk of contracting HIV by more than half (see *VAX* December 2006 *Global News*). These studies confirmed results from a previous randomized, controlled trial of adult male circumcision conducted in South Africa. Soon after, the World Health Organization (WHO) issued guidelines urging countries to consider adding male circumcision to their existing HIV/AIDS prevention strategies (see *VAX* April 2007 *Global News*). Last year, the US-based news magazine *Time* ranked circumcision as the number one medical breakthrough of 2007 because of its potential to slow the spread of HIV. But to date, only a handful of health ministries in sub-Saharan Africa, the region most severely affected by HIV/AIDS, have started developing national policies on circumcision, and even fewer have established actual programs. This has spurred some public health officials to question the delay.

In an editorial published in the January issue of the journal *Future HIV Therapy*, Daniel Halperin, senior research scientist at Harvard University, and colleagues emphasized the benefits of male circumcision and called upon countries, international leaders, and donor agencies to introduce safe circumcision practices. Halperin says that in response to the WHO guidelines, approximately nine African governments conducted consultations with the Joint United Nations Programme on HIV/AIDS (UNAIDS) and the WHO. "I think in every case, after doing the consultation, they decided that they wanted to have a circumcision program or circumcision policy," he says. But so far few policies have been enacted. "A lot of these countries are on their way, but only Kenya and Rwanda have actual policies as far as I know."

This month Rwanda launched a voluntary national circumcision campaign aimed at reducing the risk of HIV

transmission. The campaign prioritizes circumcision for male soldiers, policemen, and students. In September of 2007, Kenya's Ministry of Health published its national policy on male circumcision. The Kenyan policy stipulates that safe, voluntary male circumcision should be promoted in conjunction with other HIV prevention strategies, and delineates the roles of the Ministry of Health, the National AIDS Control Council, and other partner organizations in coordinating these programs. But there is no indication when circumcision programs will be implemented.

Providing circumcision services in areas with high HIV prevalence could have a considerable effect on reducing the number of new infections. Surgically removing the foreskin eliminates a site with a high concentration of cells that are targets for HIV (see *Primer*, this issue). Computer modeling studies conducted by the WHO and other health agencies to determine the impact circumcision could have on the course of the HIV epidemic suggest that if all males in sub-Saharan Africa were circumcised, two million HIV infections could be averted over the next 10 years. Using this same model, an additional 3.7 million new infections could be prevented over the following 10 years.

Evidence for the potential impact of circumcision programs can already be seen on the population level, says Halperin. "It's not just about modeling. We can actually see the real-world impact." For example, in Cameroon, a country where male circumcision is common practice, the adult HIV prevalence rate is only 5%, whereas in Botswana and Swaziland, countries where the majority of men are uncircumcised, adult HIV prevalence rates are up to five times higher.

If more males were circumcised there would also be a herd immunity effect—although only men directly benefit from the procedure, reducing the level of HIV in the population would also result in fewer new infections among women.

Many challenges have contributed to delays in introducing male circumcision programs, including cultural hurdles, a shortage of trained professionals, and

financial constraints. While the US President's Emergency Plan for AIDS Relief (PEPFAR) has agreed to fund circumcision programs, the governments and health ministries need to specifically request this support. "Once they ask for it, it's like anything else, it takes a while for the money to come down," says Halperin. "It's going to vary in different places but I'm sure there will be a lag before things really get going."



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## What events lead to the sexual transmission of HIV and how can mucosal immune responses protect against infection?

Most infectious agents, including viruses and bacteria, enter the body through mucosal surfaces. These surfaces, or membranes, are the moist tissues that line the body's internal cavities, such as the lungs, nose, intestine, and genitals. HIV is most commonly transmitted from person to person through sexual contact—researchers estimate that 85% of infections are sexually transmitted. HIV transmission can also occur from direct blood-to-blood contact, which occurs primarily when injection-drug users share needles, or through mother-to-child transmission, either during delivery or from tainted breast milk.

Since the majority of infections are due to sexual transmission, immune responses induced at these mucosal surfaces are the first line of defense against HIV and are critical to stopping the virus in its tracks. Researchers have attempted to develop HIV prevention methods, including microbicides, which could block the virus at the mucosal surfaces of the genitals or rectum, where exposure to the virus first occurs.

In recent years researchers have also focused on developing AIDS vaccine candidates capable of inducing potent immune responses at mucosal surfaces (see *Spotlight* article, this issue). Scientists are studying different types of immunization strategies, as well as ways to measure mucosal immune responses in clinical trials (see *VAX* December 2005 *Primer* on *Understanding Mucosal Immunity*). They have also been closely studying the events leading up to sexual transmission using animal models to better understand the type of mucosal immune responses that would be necessary to thwart HIV infection in humans.

### Crossing the barrier

HIV preferentially infects CD4<sup>+</sup> T cells, which are a subset of immune cells that are vital to the functioning of the human immune system. These cells

are located throughout the body but certain compartments, such as the mucosal tissues lining the intestine or gut, contain the greatest number of CD4<sup>+</sup> T cells. For this reason, the intestine is one of the main breeding grounds for HIV during the early stages of infection (see *VAX* April 2006 *Primer* on *Understanding the Early Stages of HIV Infection*).

But before HIV reaches cells in the intestine or other areas of the body, it must first get beyond the genital or rectal mucosal tissues. These tissues are often referred to as the mucosal barrier because viruses have difficulty penetrating the outer layer of cells, known as the epithelium. This is one method the immune system uses to fend off infectious agents.

The mucosal barrier is actually quite effective at blocking HIV—researchers estimate that only one successful HIV infection occurs for every 1,000 times a person is exposed to the virus during vaginal intercourse. There are relatively few target cells in the vaginal mucosal tissues that are susceptible to HIV, which makes it more difficult for HIV to reproduce. If a vaccine or microbicide induces potent immune responses against HIV at these surfaces, it may make it even more of a struggle for HIV to establish an infection, especially in the vaginal tissues. Researchers have observed that in studies with non-human primates, transmission of a related monkey virus known as simian immunodeficiency virus (SIV) occurs more easily rectally than vaginally, and therefore HIV infection may also be more difficult to block at rectal tissues.

It is much easier for HIV to establish an infection at mucosal sites if these tissues are damaged in some way. For example, other sexually-transmitted infections can cause inflammation and irritation, or even ulceration, of the mucosal tissues. This attracts more immune cells to the site, increasing the total number of target cells for HIV and making it easier for the virus to establish an infection. For this reason, infection with other STIs is thought to increase an individual's risk of contracting HIV. During sexual intercourse,

small tears or scrapes are often created in the mucosal surface and researchers think this too can compromise the mucosal barrier effect.

### Timing

The speed with which HIV spreads makes it impractical to study transmission and the earliest stages of infection in human volunteers. Instead, researchers conduct studies with nonhuman primates, mostly rhesus macaques, using SIV. After SIV crosses the mucosal barrier, research suggests that the virus first establishes a small, localized infection in the genital mucosal tissues. After that, it begins to spread rapidly and is quickly dispersed to other areas of the body through the blood. Results from some animal studies suggest that within one week, and sometimes even within a day, SIV can spread to and be detected at different sites throughout the animal's body.

If HIV is disseminated as quickly, mucosal immune responses (antibodies and/or cellular immune responses) induced by an AIDS vaccine candidate would have to be induced very rapidly to completely prevent an HIV infection. Typically, immune responses elicited by vaccines take between three and five days to become active. Once HIV begins spreading to other regions of the body that are rich with target cells, such as the mucosal lining of the intestine, research has shown that the virus reproduces explosively and destroys large populations of CD4<sup>+</sup> T cells. This is referred to as the acute stage of HIV infection. The quick and often irreversible loss of these immune cells further weakens the immune system's ability to fight the virus.

Even at this stage, mucosal immune responses can play an important role in helping to limit or control HIV infection. An AIDS vaccine candidate capable of inducing immune responses at mucosal sites, including the intestine, could help prevent some damage to CD4<sup>+</sup> T cells and therefore preserve the immune system's defenses. Systemic or more generalized immune responses would also help control the progression of HIV infection at this stage.