



# IAVI Report

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## Conference Coverage

### Down, but not out

*At the first Keystone meeting on HIV vaccines following the STEP trial, researchers discuss recalibrating research and development priorities*

**by Kristen Jill Kresge and Simon Noble**

Just a few days after many of the leading researchers in the AIDS vaccine field gathered for an HIV Vaccine Summit sponsored by the National Institute of Allergy and Infectious Diseases (NIAID) to discuss US government spending and the research priorities for the field (see *Balancing AIDS vaccine research*, below), they reconvened in vastly different environs for the annual Keystone Symposium on HIV Vaccines: Progress and Prospects. This year's meeting took place from March 26 to April 1 in Banff, Canada, and like the field itself, was much more focused on fundamental immunology and discovery research than the clinical pipeline of vaccine candidates.

Many speakers remarked in some way on the results of the STEP trial and its repercussions. Updates on the ongoing analysis were also scattered throughout the conference. Larry Corey of the University of Washington said during his opening keynote presentation that the STEP trial has "recalibrated" the HIV vaccine field, but he dismissed the notion that nothing positive has come out of it. "We should still have optimism," he said, making it clear that in his estimation, "T-cell vaccines are not dead," because there is still no clear explanation for the failure of Merck's adenovirus serotype 5 (Ad5)-based candidate, known as MRKAd5. "The uncertainty in the mechanism suggests we should not give up on T cell-based vaccines," added Corey. "The ability to make such vaccines may be more approachable than getting effective neutralizing antibody vaccines."

Alan Bernstein, executive director of the Global HIV Vaccine Enterprise, said there is currently a lot of "navel gazing" going on in the wake of the STEP trial as researchers try to figure out where to go next. The way forward, for now, seems to include figuring out some basic science questions. Efforts to more fully understand mucosal immunology, the types

*continued on page 2* 

## Balancing AIDS vaccine research

*Leading AIDS vaccine researchers gather to discuss balancing AIDS vaccine funding*


**by Kristen Jill Kresge**

The US National Institute of Allergy and Infectious Diseases (NIAID), one of the major financial supporters of AIDS vaccine research and development, is reevaluating its funding allocations in light of the recent failure of Merck's vaccine candidate in the Phase IIb test-of-concept trial, known as STEP, as well as pressure from scientists.

Without more money, which remains unlikely given the NIAID budget has remained flat for five years, the question is whether available funds should be shifted away from clinical development and directed toward basic discovery research grants. "I think the answer is an overwhelming yes," said Anthony Fauci, director of NIAID, at the

conclusion of a day-long summit on HIV Vaccine Research and Development held March 25 in Bethesda, Maryland. "We will make adjustments to existing resources."

Fauci said he would likely start by moving US\$10 million over to discovery research in 2009 to fund a request for research proposals with the goal of stimulating broad

*continued on page 8* 

of T-cell responses a vaccine should induce, the still mostly uncharacterized role of innate immunity in HIV infection, the mechanism of protection for live-attenuated vaccines in nonhuman primates (NHPs), and the mysteries of long-term nonprogressors figured prominently at this meeting and remain clear priorities for the field. Researchers also presented novel strategies for combining microbicides and partially-effective vaccines to try to prevent HIV infection. “There isn’t one way forward or one simple way forward,” said Bernstein. “If anyone says there is they’ve got a crystal ball that I don’t have.”

### Side-stepping

Corey presented what he referred to as a “glimmer of data” from the STEP trial. In a very small number of vaccinated individuals who had no pre-existing Ad5 immunity and who mounted high levels of immune responses to HIV Gag, there was evidence of lower viral loads. But he also acknowledged some caveats, including that this was only discovered in a post-hoc exploratory analysis and that the sample size is small. Corey said an understanding of the way immune responses against HIV are related to disease progression “is just beginning to emerge.”

Human leukocyte antigen (HLA) typing for individuals in the STEP trial and epitope mapping of the infecting viruses is still ongoing. Results from these analyses might shed light on the reason the candidate failed to provide any protection, but Corey suggested the “character of the T-cell response with three sequential doses of Ad5 vector may not be optimal.”

Corey also addressed possible explanations for why the HIV incidence increased with a higher frequency of anti-Ad5 antibodies. Susan Buchbinder of the San Francisco Department of Public Health, and a principle investigator on the STEP trial, said there was a two- to three-and-a-half-fold increase in risk of infection in the vaccine group as the Ad5 antibody titers increased. The majority of volunteers in the trial were men who have sex with men and so one possible mechanism is that more Ad5-specific CD4<sup>+</sup> T cells were present in the rectal mucosa, creating more targets for HIV, according to Corey. He also said an indirect biological mechanism could be at play and that perhaps MRKAd5 interfered with innate immune responses against HIV.

A Phase I trial conducted by the Collaboration for AIDS Vaccine Discovery (CAVD), an initiative funded by the Bill & Melinda Gates Foundation, was analyzing the effect of MRKAd5 on innate immune responses until it was halted following the results of the STEP trial. Julie McElrath of the Fred Hutchinson Cancer Research Center said that in this study the innate immune responses appeared different in individuals with high pre-existing immunity, but she didn’t specify how. She did suggest results from this prematurely-halted trial might help elucidate the role of Ad5 immunity in increasing susceptibility to HIV.

Danny Casimiro of Merck said that his company has committed support for conducting in-depth analyses of MRKAd5 in NHPs to see if they can duplicate the results of the STEP trial and

answer some of these lingering questions. But Corey said conducting a prospective clinical trial, in which biopsies are taken from trial volunteers, is the only way to know if and how this vaccine enhanced susceptibility to HIV infection, and he said he hopes that the US Food and Drug Administration will approve such a trial.

Buchbinder said analyses of other potential confounding factors that may also help explain this observation are still ongoing. These factors include host genetics, sexual networks, clusters of HIV infections at certain sites, and sexual risk behavior; there is some evidence that risk behaviors increased among vaccine recipients during the course of the study, indicating that perhaps these volunteers suspected they had received the vaccine candidate and not placebo, according to Buchbinder.

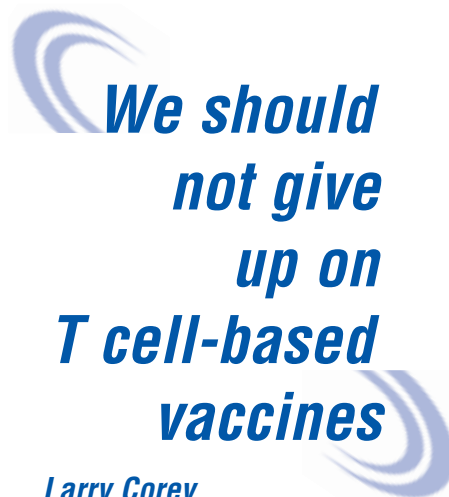
McElrath also presented data from the ongoing analysis of the immune responses induced by MRKAd5. Only 15% of vaccine recipients who became HIV infected did not develop CD4<sup>+</sup> or CD8<sup>+</sup> T-cell responses against HIV. While the magnitude of CD4<sup>+</sup> T-cell responses was similar in vaccinated individuals regardless of their Ad5 serostatus, the magnitude of CD8<sup>+</sup> T-cell responses was lower in vaccinated individuals with pre-existing Ad5 immunity. However, even in these individuals there was no correlation between the magnitude of response and whether they became HIV infected. Most of the CD8<sup>+</sup> T cells induced by the vaccine secreted IFN- $\gamma$  and TNF- $\alpha$ , and McElrath and colleagues are now analyzing the proliferative capacity of these T cells.

In the meantime, a Phase I clinical trial with another adenovirus vector, based on adenovirus serotype 26 (Ad26), was recently initiated by Dan Barouch and colleagues at the Beth Israel Deaconess Medical Center. This is the first time an Ad26-based vaccine candidate is being analyzed in human volunteers and Barouch said it shows that regulatory authorities are comfortable allowing trials with other Ad vectors to proceed.

In NHP studies Barouch found that immunizing macaques with an Ad26 vector-based candidate followed by an Ad5 candidate afforded a 1.4 log decrease in peak viral loads and a statistically significant improved survival ratio after simian immunodeficiency virus (SIV)mac251 challenge. This Ad26 vector “outperforms Ad5 vectors in rhesus macaques,” said Barouch.

### Pushing forward

One of the burning questions in the field since the MRKAd5 candidate showed no efficacy in the STEP trial has been how to determine which vaccine candidates should be advanced to clinical, and particularly efficacy, trials. Gary Nabel, director of the Vaccine Research Center (VRC) at NIAID gave a presentation on the ‘Criteria for advancement of novel vaccine candidates.’ He said the highest priority now is to develop vaccines that induce neutralizing antibodies (Nab) against HIV, not only for direct neutralization but also antibody-dependent cell-mediated cytotoxicity (ADCC), induction of complement, or other innate immune responses to virus-infected



Larry Corey

cells. For these vaccines, Nabel said, the advancement criteria are more straightforward.

But what comes next for an optimal T-cell vaccine is trickier. Nabel said the field needs to identify the characteristics of a protective T-cell response, increase the breadth of coverage against natural HIV isolates, enhance the magnitude of the relevant responses, and address the potential mechanisms of enhancement of susceptibility to HIV infection seen in the STEP trial.

To the first point, Nabel said defining the immune correlates of protection in human and NHP studies was a priority, and that this was the best rationale for going ahead with clinical testing of the VRC's DNA and Ad5 candidates in the planned PAVE 100 trial since this heterologous prime-boost approach induces immune responses qualitatively different to those induced by MRKAd5.

Regarding the breadth of the immune response, Nabel discussed at length the informatics approach to genetic diversity that Bette Korber of Los Alamos National Laboratory and colleagues are pursuing. Her group is using computational optimization methods to design polyvalent vaccine antigens from sets of mosaic proteins that maximize the representation of potential T-cell epitopes for a viral population (*Nature Medicine* **13**, 100, 2007). The optimization offers the advantage that, at least in computational models, mosaic coverage exceeds any equivalent-sized natural sequence cocktail, and the immune evaluation of the mosaic concept in mice has indicated that vaccine-induced CD8<sup>+</sup> T-cell responses to different antigen designs is much broader using mosaic antigens.

#### **A leaky gut**

One of the distinguishing features of HIV infection is the chronic immune activation that occurs, leading to immunodeficiency and eventually progression to AIDS. This is in marked contrast to the nonpathogenic immunodeficiency virus infection in some NHP species, notably sooty mangabeys and African green monkeys, which have negligible immune activation and few detectable consequences of SIV infection.

In contrast, immune activation in HIV infection has many deleterious consequences; it results in a higher frequency of activated T cells that are the preferred targets for HIV, leading to the selective destruction of CD4<sup>+</sup> T cells and the maintenance of virus replication. Daniel Douek of the VRC and his group have been looking into why HIV disease is progressive and what drives the immune activation in chronic HIV infection that is the strongest predictor of disease progression. "The extent of CD4<sup>+</sup> T-cell loss in the acute phase is not the sole determinant of progression rate in the chronic phase," said Douek.

Previous studies from as early as the mid-1980s documented enteropathy in HIV-infected individuals and observed that gut permeability can be increased up to 10-fold. These and other findings led Douek to postulate that microbial translocation—the transfer of microbes and/or microbial products across the gastrointestinal bar-

rier and into systemic circulation without overt bacteremia—across the compromised gut mucosal surface contributes to immune activation. "You have more bacterial cells in your gut than you have human cells in your body," said Douek.

As a marker of microbial translocation, Douek's group measured the amount of lipopolysaccharide (LPS)—an immunostimulatory signature component of Gram negative bacterial cell walls—in the plasma of HIV-infected and uninfected individuals, and found levels of plasma LPS correlate with HIV disease progression (*Nature Medicine* **12**, 1365, 2006). As a more direct measure of microbial translocation, they have also measured the amount of 16S ribosomal RNA (seen only in bacteria) in plasma and again found that significantly higher levels are seen in HIV-infected individuals as compared to uninfected controls.

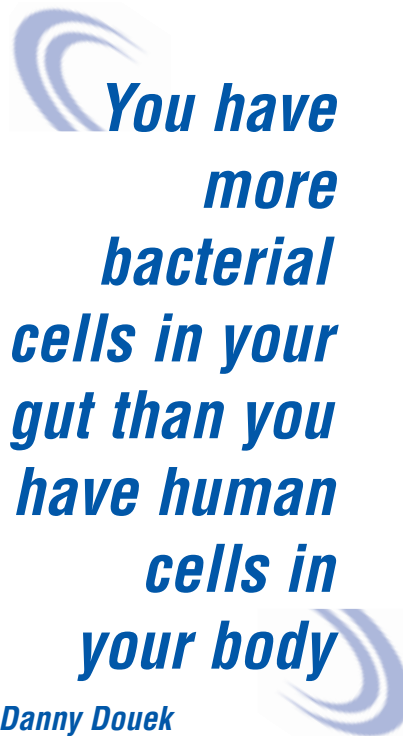
CD14<sup>+</sup> monocytes and macrophages are known to secrete soluble CD14 (sCD14) and proinflammatory cytokines after LPS stimulation. Douek's group has demonstrated that the elevated levels of LPS in HIV-infected individuals correlates with significantly higher levels of plasma sCD14, suggesting chronic stimulation of monocytes *in vivo*, as well as a number of other measures of innate and adaptive immune activation. They have also documented differences in microbial translocation between progressive and non-progressive HIV infection in humans, seeing less elevated levels of plasma LPS and sCD14 in long-term nonprogressors or elite controllers, as compared to chronic progressors. Moreover, plasma LPS levels decreased after initiation of highly-active antiretroviral therapy (HAART) in HIV-infected individuals, suggesting that reduction in viral load might allow immunological and structural reconstitution of the gastrointestinal barrier. The reduced LPS levels in these individuals after starting HAART also inversely correlated with the number of blood CD4<sup>+</sup> T cells.

Douek and his group have also compared natural SIV infection in sooty mangabeys—which is nonpathogenic, with low immune activation despite a high viral load—to pathogenic SIV infection in rhesus macaques. They saw no evidence for microbial translocation in the sooty mangabeys, whereas SIV-infected rhesus macaques had elevated plasma LPS levels compared to uninfected macaques.

Taken together, this evidence indicates that the immune activation seen in HIV disease is at least partly due to insult at the gut mucosa, which enables microbial flora to cross this barrier, and further reinforces the idea that HIV is primarily a disease of the gut mucosa. "We've got a leaky gut," said Douek.

#### **At the front lines**

HIV is not unique in causing the most substantial damage within the first days and weeks of infection; all lentivirus infections begin with a fast and furious phase of infection, followed by a slowly progressive stage, said Ashley Haase of the University of Minnesota. It is the very earliest stages of HIV infection, within 10 days of transmission, which is the subject of his research. His laboratory is study-



**You have  
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have human  
cells in  
your body**

**Danny Douek**



## **The basis of elite control remains undefined from a genomic standpoint**

**Bruce Walker**

ing early events in transmission of SIV because acute HIV infection is discovered too late to study the earliest interactions between the virus and the immune system. Studying SIV transmission and acute infection offers many clues about critical immunologic time points at which a vaccine could act.

In the female genital tract, which Haase called the “active front line of the mucosal immune system,” the naturally-occurring cellular immune responses against HIV are actually quite robust (see *The great barrier*, page 10). Four days after rhesus macaques are challenged mucosally with a high dose of SIV, only concentrated foci of virus-infected cells can be found after exhaustive examination of several layers of cervical tissues, illustrating the success of the mucosal barrier. Haase said that a vaccine capable of focusing and augmenting these natural cellular immune responses could prevent establishment of an HIV infection. It could also provide an opportunity for microbicides or early antiretroviral treatment to limit this founder population of HIV-infected cells even further, said Haase.

Part of the reason for the small initial founder population is that there are limited target cells for HIV in vaginal mucosal tissues. Most of the target cells for the virus are on the other side of the mucosal barrier, said Tom Hope of Northwestern University. To overcome this, Haase speculated that the virus induces an influx of plasmacytoid dendritic cells (pDCs) through a mucosal outside-in signaling mechanism. An influx of inflammatory cells creates a favorable environment for expansion of the infection. The pDCs secrete chemokines that stimulate the expression of the CCR5 receptor on mucosal cells, providing “fuel” for HIV, Haase said. This allows the limited founder population of HIV-infected cells to expand massively and, once this occurs, the virus is then “broadcast” distally to other sites. HIV then quickly establishes an infection in lymphoid tissues and in the epithelial cells of the intestine, where it wreaks havoc on the critical cells of the immune system.

But before this happens microbicides and/or vaccines working in concert could subjugate the virus. Haase said a microbicide could potentially interfere with this proposed signaling mechanism, and a vaccine that induces a potent T-cell response at mucosal sites could help snuff out the virus before a systemic infection occurs.

Recent studies with live-attenuated SIV vaccines, cited by Haase, show a T-cell response could be enough to stop infection, especially if these responses are present at mucosal sites. Vaccinated rhesus macaques that are exposed to a high-dose mucosal SIV challenge develop a “very robust” CD8<sup>+</sup> T-cell response in cervical vaginal tissues within three weeks after challenge, said Haase. There is also a correlation between this CD8<sup>+</sup> T-cell response and the reduction in the size of the founder population of virus-infected cells. If a microbicide could help reduce the founder population even further, it would increase the likelihood of success for a vaccine.

A study by Meritxell Genesca of the University of California in Davis corroborated the idea that mucosally-available CD8<sup>+</sup> T cells may play a role in protection. Genesca presented results from a study in which 12 rhesus macaques immunized with a hybrid simian-human immunodeficiency virus (SHIV)89.6 live-attenuated vaccine were challenged intravaginally with SIVmac239. The majority (60%) of vaccinated macaques controlled viral replication. However when the CD8<sup>+</sup> T cells were completely depleted, the partial protection afforded by the live-attenuated vaccine was eliminated, leading Genesca to conclude that this protection is at least in part mediated by CD8<sup>+</sup> T-cell responses. Gag-specific T-cell responses were present on the day of challenge at the site of inoculation and, after challenge, vaccinated macaques have more SIV-specific T cells secreting multiple cytokines and chemokines in their vaginal tissues.

In an impromptu talk, John Moore of Cornell University also presented a rationale for combining vaccines and microbicides in an effort to bolster immune defenses against HIV. Moore suggested that a vaginal microbicide that could deliver monoclonal antibodies against HIV might be a substitute for the mucosally available broadly-neutralizing antibodies that current AIDS vaccine candidates can not, as of yet, induce. “Science suggests these fields could actually work together,” said Moore.

A microbicide that does not block infection would be considered a failure, but if it could lower the levels of viral inoculum in the genital mucosa and therefore increase the likelihood that a vaccine would be effective, it would be a great success, added Moore. To test this hypothesis he is preparing to conduct

a combination microbicide/vaccine study in 20 rhesus macaques in collaboration with Barouch. This study will evaluate the efficacy of the vaginal microbicide T-1249—a gel formulation of an entry inhibitor developed by Roche Pharmaceuticals but never licensed for the treatment of HIV infection—in combination with a prime-boost vaccine regimen using an Ad26 vector-based vaccine followed by a chimeric Ad5/Ad48 vector-based candidate, both developed by Barouch, against a vaginal SIVmac251 challenge.

### **Quantity vs. quality**

Rafi Ahmed's laboratory at Emory University is looking at various vaccine vectors to see if they induce T-cell responses of differing qualities. His studies suggest that the most important marker of T-cell function is their proliferative capacity. In all other disease models, "expansion potential *in vivo* is the function that gives the best protection," he said. In a study in mice, Ahmed compared an Ad5 vector expressing the full glycoprotein of lymphocytic choriomeningitis virus (LCMV) with a *Listeria monocytogenes* (LM) vector expressing the gp33 epitope of the LCMV glycoprotein. The control group included mice infected with LCMV, which is rapidly cleared and induces a long-lasting memory T-cell response in the animals.

T-cell responses were analyzed in the three groups of mice at various time points over a period of 60 days. In the mice challenged with the LM vector, the CD8<sup>+</sup> T-cell responses peaked after eight days at about 8-9% of cells measured in peripheral blood, and leveled off at a markedly lower 2%. Comparatively, the Ad5 vector was highly immunogenic. In the Ad5 mice the levels of CD8<sup>+</sup> T cells hovered around 8%. "Things look extremely good in terms of numbers," said Ahmed. "Levels were as high as we've seen with LCMV and we did not see much of a contraction."


When memory responses were analyzed in tissues, the quantity of Ad5-induced cells was also much higher—6.3% of cells in the spleen recognized gp33 in the mice given Ad5, compared to 2.5% in the control group. The absolute number of CD8<sup>+</sup> T cells was also higher in the mice that received Ad5 both in the lungs and liver. "It's hard to trump LCMV in numbers," said Ahmed, yet Ad5 did.

But when the Ad5-induced memory cells in peripheral blood were analyzed for CD127


and interleukin (IL)-7 expression—markers of healthy memory cells—the levels of CD127 declined and did not recover, as they did in the other two groups. And when researchers honed in on the quality of these responses at other sites, they found that fewer of the Ad5-induced CD8<sup>+</sup> T-cell responses were secreting interferon (IFN)- $\gamma$  or IL-2.

To evaluate the recall potential—the proliferative capacity of memory T cells upon re-exposure to antigen—of the Ad5-induced CD8<sup>+</sup> T cells, memory cells from the spleen or liver of all three groups of mice were transferred into naïve mice, which were subsequently challenged with vaccinia virus expressing gp33. This produced the most striking data, according to Ahmed. In response to viral challenge there was a dramatically lower expansion of the Ad5 memory T cells in the spleen, as compared to both the LM or control mice. When liver cells were transferred from the Ad5 group of mice, they did not proliferate at all. "Even though there was a very high frequency in the liver, when you look at their capacity to proliferate, they are very much compromised," said Ahmed. Even at varying doses of Ad5, the cytokine expression profile and proliferative capacity, in both lymphoid and non-lymphoid compartments, of the CD8<sup>+</sup> T cells were impaired, as compared to LCMV and LM. This led Ahmed to conclude that, at least in mouse experiments, the T-cell phenotype induced by the Ad5 vector "is not matching what a good memory T-cell phenotype should look like." This raises questions about the value of the T-cell responses induced by the Ad5 vector in humans. As to how these findings relate to the human system, Ahmed said jokingly, "I'll leave that to the experts to figure out."


But in NHP studies conducted by Louis Picker of the Oregon Health & Science University, optimal protection from a live attenuated SIV vaccine does not seem to depend on the recall potential of SIV-specific CD4<sup>+</sup> or CD8<sup>+</sup> T cells. He suggests other possibilities for protection, including the qualitative aspects of the T-cell response, still unidentified non-classically neutralizing antibodies, or innate immune responses such as natural killer cells. Picker is currently utilizing a genomic approach to try to elucidate the mechanism for protection from live-attenuated SIV vaccines. He emphasized that intrinsic "innate" mechanisms may play a key role and he is hopeful that a large study involving



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***Rafi Ahmed***



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**Andrew McMichael**



120 rhesus macaques—a collaborative experiment with IAVI and the CAVD—will be able to determine just what is responsible for the partial protection mediated by live-attenuated SIV vaccines.

Andrew McMichael is looking at human T-cell immune escape in very early acute HIV infection using samples from plasma donors in the US, some of whom donate as much as once or twice a week. If one of these donors becomes infected with HIV, researchers have a series of samples that can be analyzed for extremely early time points in their infection. McMichael reported that in several individuals there is evidence of HIV escaping T-cell immune responses within 16 days. At the same time there is a rapid loss in the quantity of CD4<sup>+</sup> T cells, suggesting that early cytotoxic T lymphocyte (CTL) responses quickly decay once virus mutation occurs. This led McMichael to conclude that many of the CTL responses may be of limited utility. “It’s a lot like the neutralizing antibody problem,” he said. “A lot of the T-cell responses are probably useless.”

#### **Taking control**

Bruce Walker of Massachusetts General Hospital is also studying the role of T-cell responses, among other factors, in HIV-infected individuals who are dubbed elite controllers because they effectively control viral replication without the aid of antiretroviral therapy. Most elite controllers have some measurable level of ongoing viral replication by ultra-sensitive assays. In some, the viral load is as low as 4 copies/ml of blood. “Do we have a model for a successful vaccination in people who control the virus at those levels?” asked Walker.

He is seeking an explanation for this control by studying the T-cell and neutralizing antibody responses in these individuals, as well as the characteristics of the infecting virus and the genetic makeup of the individuals. So far, neither T cells nor neutralizing antibodies seem to be the key to control. In the elite controllers Walker is studying, in cooperation with Steve Deeks at the University of California in San Francisco, the magnitude of T-cell immune responses are actually lower than those seen in chronic progressors. These individuals can control virus replication without any remarkable T-cell responses, at least by the way they are currently being measured, said Walker. The same is true for

neutralizing antibodies. “Neutralizing antibody responses are much, much weaker in these individuals.” He said some of the elite controllers would not even test positive for HIV infection by Western blot methods because their HIV-specific antibodies target so few epitopes. Uniquely, the T-cell responses in elite and viremic controllers (individuals who control the virus but at higher levels of viral replication than elite controllers) primarily target HIV’s Gag protein.

So far there does not appear to be an explanation in studying the properties of the infecting virus either—no genetic defects in the viruses of elite controllers have been identified that contribute to decreased replication capacity or reduced viral fitness. Only three out of a group of 63 elite controllers have any deletions in HIV’s *nef* gene, which are known to be associated with weakened viral replication capacity. Some viral polymorphisms have been identified in the HIV Gag protein that are strongly associated with the elite controller phenotype, but when a virus is constructed in the laboratory with these same mutations, researchers observe no effect on viral replication capacity. However, viruses isolated from some elite controllers do seem to have a lower replicative capacity, suggesting something else may be contributing to their reduced fitness.

Genome-wide association scans, using a single nucleotide polymorphism (SNP) platform, are ongoing in Walker’s elite controller cohort (see *HIV Controllers: Can the Human Genome Project advance AIDS vaccine development?*, *IAVI Report*, May-June 2007). More than 600 elite controllers have been recruited and so far three SNP associations have been identified in these individuals, said Walker. For now, “the basis of elite control remains undefined from a genomic standpoint,” he added.

Studies by April Ferre of the University of California in Davis, suggest that strong, polyfunctional CD8<sup>+</sup> T-cell responses at mucosal sites might contribute to the control of viral replication in elite controllers. In a cohort of 28 elite controllers, she reported that these individuals have stronger, more polyfunctional (as defined by secretion of INF- $\gamma$ , TNF- $\alpha$ , IL-2, and MIP-1 $\beta$ ) mucosal immune responses, as compared to HIV-infected individuals on HAART. Elite controllers also have more CD4<sup>+</sup> T cells and more polyfunctional

CD8<sup>+</sup> T cells in the rectal mucosal tissues than either chronic progressors or individuals on HAART.

### **Regulating responses**

Regulatory T cells (Tregs), which were given a bad name in the past, have had their immunological status restored in recent years, including within HIV pathogenesis studies (see *Balancing Act*, *LAVI Report*, July-August 2007). Tregs are a specialized subset of CD4<sup>+</sup> T cells that are still somewhat uncharacterized, but their signature markers include constitutive expression of CD25, low levels of CD127, and, most specifically, Foxp3.

Claire Chougnnet and colleagues at Cincinnati Children's Hospital Medical Center are investigating the role of Tregs in HIV infection. Chronic progressive HIV infection is usually characterized by weak HIV-specific T-cell responses and a multitude of studies published in the past 2-3 years have indicated that Tregs could play some part in this ineffective immune control. For instance, depletion of CD4<sup>+</sup>CD25<sup>+</sup> T cells *in vitro* during chronic HIV infection has been shown to increase HIV-specific T-cell responses, chronic progressive HIV disease correlates with increased numbers of Tregs in lymphoid tissues compared to non-progressors or uninfected individuals, and Foxp3 levels correlate closely with HIV and SIV viral loads during infection of humans and macaques, respectively.

However, Chougnnet's group now has *in vitro* data indicating that Tregs can also provide some benefit by inhibiting HIV replication in effector T cells in a dose-dependent manner. Whether these data have *in vivo* relevance must now be determined but Chougnnet thinks Tregs may be "double-edged swords" in HIV infection. She also noted that almost all of the published studies describe chronic infection and there is clear variation in the function and dynamics of Tregs depending on the model studied; therefore further studies are required to elucidate the precise role of Tregs during HIV infection.


### **Enterprising strategy**

Bernstein gave a special lecture entitled 'HIV Vaccines: Progress and Prospects' which he said represented "preliminary, and therefore mutable, thoughts" about the state of the field. He began by listing what he called structural challenges, including insufficient scale of some projects to solve the

major scientific problems, the need for more coordination and information sharing, challenges in manufacturing and clinical trials capacity, and the need for new approaches and innovation. He emphasized that the Enterprise is an alliance of organizations that would serve as a "convener and honest broker."

Bernstein gave a brief overview of how the Enterprise is, and will be, structured. The secretariat that he heads is now located in New York City, and that body will work with three other arms: the Enterprise Council and an associated board of directors, a scientific stewardship committee, and stakeholder assemblies. In addition, working groups will be convened to share ideas and advise; Bernstein has already asked Bob Seder of NIAID and Rafick Sekaly of the University of Montreal to organize the first of these regarding the immune correlates of protection, a key question that he was surprised the field doesn't have a better handle on.

The original scientific strategic plan that the Enterprise coordinated for the HIV vaccine field was published in 2005 and one of Bernstein's first priorities will be to ask whether it should be amended, or if a new plan is required in light of progress in the intervening years. He also said that "we need to reorient our thinking from product development to interrogations of the human immune response to HIV," and that the field was pinning its hopes on a home run, which doesn't often happen. He also asked if there was a need to establish transparent criteria to decide on which candidates should progress to efficacy trials.

Bernstein finished with some parallels from his previous career in cancer research. Much like cancer therapy, he said, there is no clear path to an HIV vaccine. Childhood leukemia used to be fatal in the majority of cases but is now treatable about 80% of the time, and yet we still don't have a clear idea of precisely how the therapy works. Improvements in this field have come from incremental, empirical advances over the past 50 years or so. In contrast, some of the new anti-cancer drugs like Gleevec and Herceptin, which are also extremely effective, were developed only after the culmination of 40 years of fundamental research into the molecular mechanisms of cancer and combinatorial chemistry, as well as successful clinical trials. The lesson, Bernstein said, is that a multitude of approaches are needed. 



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**Alan Bernstein**

new approaches. “There are so many things we do not know in this field of HIV vaccines,” he said.

The US government is the largest financial backer of AIDS vaccine research and the majority of this funding is funneled through the National Institutes of Health to NIAID. Last year NIAID spent \$1.5 billion on all areas of AIDS-related research (see Figure 1). Of this amount, \$497 million funded AIDS-vaccine research and development—47% went to basic or discovery research, and 38% funded clinical development. NIAID is providing an additional \$300 million over seven years, through a separate funding mechanism, to the Center for HIV/AIDS Vaccine Immunology (CHAVI), a virtual consortium of AIDS vaccine researchers.

More funding for discovery research could also be freed up if NIAID chooses to move forward with a scaled-down version of the PAVE 100 trial—a planned Phase IIb test-of-concept trial with a combination regimen of DNA and adenovirus serotype-5 (Ad5) candidates (see *What Next?*, *LAVI Report*, September-December 2007). “Trials cost more money than grants,” Fauci said, adding that conducting that trial in 3,000 volunteers, instead of the 8,000 originally planned for, would save between \$35 million and \$60 million over seven years.

The start of the PAVE 100 trial was postponed following the results of the STEP trial and discussions are still ongoing. “Everything is going to be looked at,” Fauci said. “We need to look much more carefully at these clinical trials, both in their design and their scope.”

Responding to a call from one group to cut government-sponsored funding of AIDS vaccine research all together, Fauci and the more than 200 researchers who gathered for the summit remained steadfast in their commitment to discovering an AIDS vaccine. “Under no circumstances will we stop AIDS vaccine research,” Fauci said. “I’m going to keep fighting like crazy for *more* money.”

Several researchers echoed these sentiments. “There’s no better health impact on prevention and disease control than vaccines,” said Adel Mahmoud of Princeton University and summit co-chair.

### Stepping back

The allocation of funding between discovery and clinical research pillars was called into question recently by a group of outspoken researchers; first in a letter to NIAID and later publicly at the Conference on Retroviruses and Opportunistic Infections (see *Clues from CROI, LAVI Report*, January-February 2008). This cadre of scientists urged NIAID to place a higher priority on basic discovery research because of the outstanding questions about how best to develop a vaccine against HIV/AIDS.

Some of these questions surfaced when Merck’s vaccine candidate showed no efficacy in either preventing HIV infection or modulating peak viral replication in individuals who became HIV infected despite vaccination (see *A STEP back?*, *LAVI Report*, September-December 2007). Things went from bad to worse when researchers later reported that among certain sub-groups of individuals—mainly uncircumcised men with pre-existing immunity to the Ad5 vector—there

was a trend toward a higher susceptibility to HIV among vaccine recipients (see *Clues from CROI, LAVI Report*, January-February 2008).

An explanation for the candidate’s failure or the potential effect vaccination had on HIV acquisition remains elusive. Yet, in light of these results, researchers in the field began looking critically at the current clinical pipeline and the strategies to stimulate protective immunity against HIV. “The field is clearly at a critical crossroads,” said Warner Greene, director of the Gladstone Institute of Virology and Immunology and co-chair of the summit.

Throughout the summit researchers discussed several of the still largely uncharted territories in AIDS vaccine discovery. Among them were the need to more fully understand mucosal immunity and its role in protecting against HIV infection (see *The great barrier*, page 10); the ability of certain nonhuman primate species, including sooty mangabeys and African green monkeys to control simian immunodeficiency virus (SIV) infection; the early events in HIV/SIV transmission and infection; the validation and utilization of the nonhuman primate model; and how to induce broadly neutralizing antibodies against HIV.

“The biggest challenge is what is a promising vaccine,” said Rafi Ahmed, an immunologist from Emory University. He emphasized the importance of research into developing vaccine candidates that can stimulate neutralizing antibodies, a task that has stumped the field for many years. “I know something about T cells,” he said, “CD4<sup>+</sup> T cells on their own have great limitations. Vaccine concepts that test only one arm of the immune system are doomed for failure,” added Ahmed. “Let’s remember what we learned in immunology 101.” This idea was repeated a week later when many of the same researchers gathered for the annual Keystone Symposium on HIV Vaccines:


Progress and Prospects (see *Down, but not out*, page 1).

A recurring theme at the summit was the need for more checks and balances before candidates advance into clinical trials. Ahmed proposed a clear, yet stringent, requirement for late-stage trials, saying that a vaccine candidate should not enter efficacy trials unless it induces responses from both arms of the immune system.


But this does not mean that clinical development should be stopped entirely. Almost everyone agreed that clinical research, in the form of Phase I and II trials, was still necessary. “We have a lot to learn from clinical investigation,” said Alan Bernstein, who was recently appointed executive director of the Global HIV Vaccine Enterprise. Several participants spoke instead about more carefully bridging discovery and clinical research to ensure that each was informing the other. To achieve this, Scott Hammer of Columbia University, said a “nimble, collaborative clinical trial system” is required. Others proposed using the already-established clinical trial infrastructure to explore research questions that could inform the design of future vaccine candidates. “There needs to be more emphasis on discovery,” said Ahmed, but “this should not come at the expense of jeopardizing the clinical infrastructure.”

### Between mice and men

In a session devoted to the strength and limitations of the current animal models for HIV infection and their role in vaccine discovery,

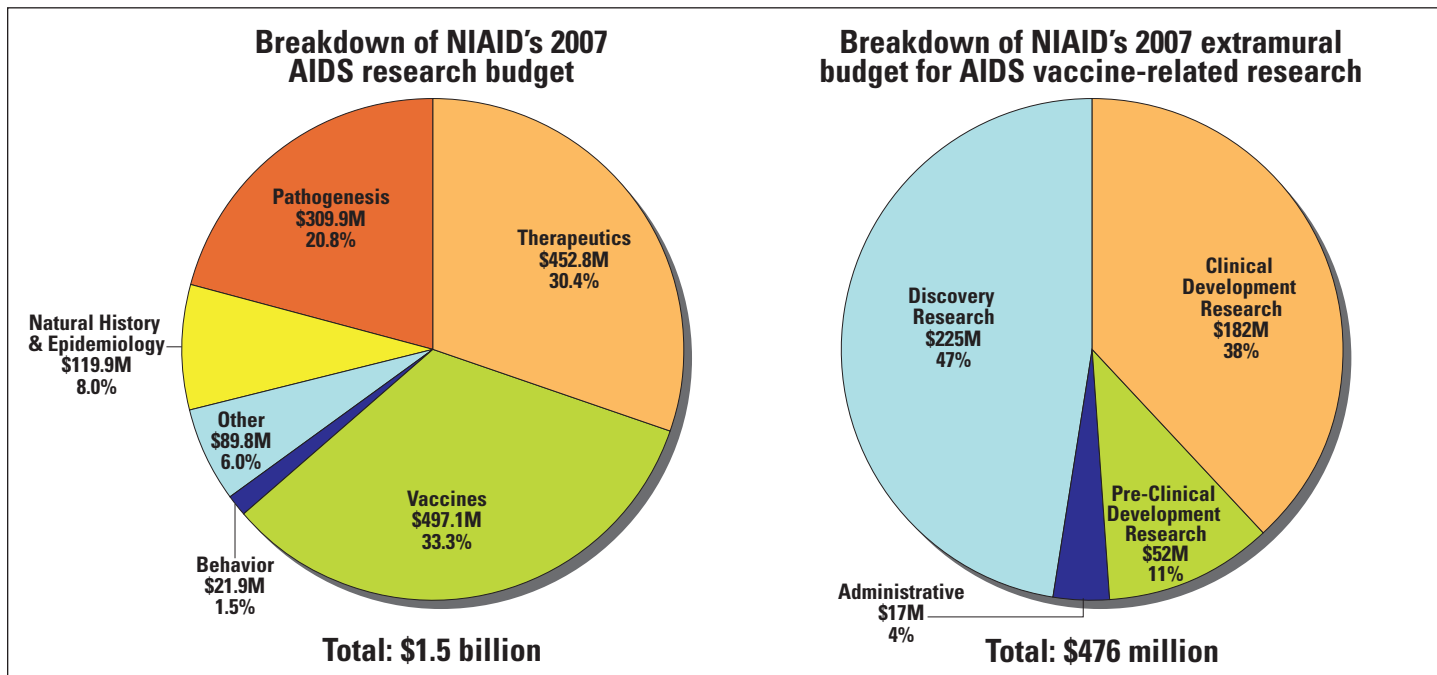


**I'm going  
to keep  
fighting  
like crazy  
for more  
money**



**Anthony Fauci**





**Figure 1.** The total AIDS research budget for the US National Institute of Allergy and Infectious Diseases (NIAID) was \$1.5 billion in 2007. Of this, 33.3% (\$497 million) was spent on all vaccine research. The vast majority of vaccine-related funding (\$476 million) was spent on AIDS vaccine research and development. A breakdown of this spending is illustrated in the second pie chart.

Louis Picker of Oregon Health and Sciences University said any rational approach to AIDS vaccine development would have to involve full exploitation of the nonhuman primate model.

But to fully exploit this model, more consistency is required. Jeffrey Lifson of the National Cancer Institute called for more standardization among nonhuman primate models, including the strains of challenge viruses used when evaluating candidates. The failure of MRKAd5 ignited some discussion among researchers about the utility of the SHIV model, a hybrid simian/human immunodeficiency virus (see *Getting it right early*, *LAVI Report*, September-December 2007). Talk about the utility of the SHIV model continued, but many researchers overwhelmingly spoke in favor of using the SIV challenge model to prioritize pre-clinical vaccine candidates. “The SIV system has many problems, but there are many aspects of it that are highly reminiscent of HIV,” said Malcolm Martin of NIAID.

Ronald Desrosiers of New England Primate Research Center called for extensive pre-clinical testing of viral vector-based vaccine candidates in nonhuman primates, although he admitted he had a hard time imagining any viral vector used to deliver HIV immunogens being successful against HIV, “without some breakthrough discovery that I just don’t see coming right now.” Instead, he touted more creative approaches, including the use of viral vectors to deliver HIV monoclonal antibodies.

Overall there was little dissent about the role of nonhuman primate models in preclinical development, but some participants were reluctant to endorse the model as the “gatekeeper” by which decisions are made about which vaccine candidates should be advanced into clinical trials. Julie Overbaugh of the Fred Hutchinson Cancer Research Center argued that none of the nonhuman primate models have been validated in their ability to predict vaccine efficacy in humans. “It [the


nonhuman primate model] shouldn’t be used solely as a go no-go,” said Seth Berkley, president and chief executive officer of IAVI.

### Influx of ideas

If there was one point where there was almost unanimous agreement, it was on the need for more creative approaches to vaccine discovery. Carl Dieffenbach, director of the Division of AIDS at NIAID, said that in 2007, NIAID funded all “meritorious” discovery grants on HIV vaccine research that were solicited. He said this was not a comment on the amount of funding available, but rather the “dearth of ideas.”

“The easy things have been done,” said James Hoxie of the University of Pennsylvania. There are several innovation programs currently operating in the field, including those from IAVI and the Bill & Melinda Gates Foundation, but other mechanisms for supporting novel research are still required, according to many summit attendees. Bruce Walker of Harvard University said coming up with innovative ideas isn’t the problem, it is actually having the money to test them.

Some ideas for encouraging innovation were recruiting young researchers into the AIDS vaccine field and also collaborating with scientists from outside, but related, disciplines. The hope is that young scientists would bring fresh perspective to this now 25-year-old problem. “The real next step is going to come from outside this room,” said Mahmoud.

And although this point was mentioned repeatedly throughout the day, the question of just how to recruit young researchers remained largely unanswered. “We have to find mechanisms to recruit young people into the field and not just talk about it,” said Dennis Burton of the Scripps Research Institute. More guidance on this issue may come from future sessions—Fauci said this meeting was just the initial step and that finding the right balance in AIDS vaccine research would be an iterative process. “We’re just getting started,” added Hoxie. 

# The great barrier

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

by **Andreas von Bubnoff**

**H**IV is primarily a mucosal infection—about 85% of transmissions occur at the mucosal surfaces of the genitals or rectum and most of the target cells necessary for HIV replication are found in mucosal tissues.

The gut mucosa is also a critical battleground during acute HIV infection (see *Beast in the belly*, *IAVI Report*, March-April 2006). Just two weeks after initial infection is established, 70% of T cells in the gut are depleted. In the blood, perhaps 30% to 40% fewer T cells are observed one or two months after HIV infection, says Jiri Mestecky of the University of Alabama at Birmingham.

For these reasons, understanding mucosal immunity is important in preventing HIV transmission, and also in controlling infection. Bolstering immune responses at the mucosa during transmission may make it even harder for the virus to gain an initial foothold, and in the early stages of infection (see Figure 2), mucosal immune responses play an important role in limiting the depletion of T cells in mucosal tissues, averting permanent damage to the immune system. “There are lots of antiretroviral drugs [that] can partially restore CD4<sup>+</sup> T cells in the blood,” Mestecky says, “but so far you cannot fully restore them in the mucosal tissues.” Satya Dandekar’s lab at the University of California in Davis has found that to restore CD4<sup>+</sup> T-cell levels in mucosal tissues of rhesus macaques, antiretrovirals must be given within days or, at most, a few weeks after infection, a largely impractical time frame in human infection.

Despite its perceived importance, only a few research groups study 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. But we are late because we should have started 20 years ago.”

The understanding of mucosal immunity in HIV infection is hampered by the difficulty of studying these types of immune responses in humans. Currently, AIDS vaccine clinical trials are not designed to systematically look for mucosal antibodies in external secretions. “[It] is a terrible mistake,” Mestecky says. “Everybody measures serum antibodies, which is fine, but it’s a mucosal disease after all.”

Measuring mucosal immune responses is difficult and, Lopalco says, it is also much harder to come up with an *in vitro* model for mucosal tissues because they harbor many kinds of cells in a specific arrangement. Even when mucosal responses are assessed,

measurements are often not standardized, leading to contradictory results.

Despite these difficulties, researchers have gained important insights over the past several years into mucosal immune responses in HIV infection. But they are just beginning to understand their role.


## **Mechanism of protection**

Researchers are using many different models to help determine the role of mucosal immune responses in protection against HIV. One involves challenge studies in nonhuman primates with live-attenuated simian immunodeficiency virus (SIV) vaccines.


Researchers attenuate SIV by deleting the virus’s *nef* gene. Such studies have shown that vaccines containing this live-attenuated version of SIV can protect rhesus macaques from subsequent SIV infection (*Science* **258**, 1938, 1992), however the mechanism of this protection is still unknown. Ashley Haase’s laboratory at the University of Minnesota, in collaboration with Paul Johnson of Harvard Medical School and IAVI’s Live-Attenuated Consortium, is studying this model to garner clues about how this vaccination strategy works and whether or not it involves mucosal immunity.

Researchers have also been studying individuals who remain HIV uninfected despite repeat exposure to the virus, a group known as exposed seronegatives (ESNs), to mine for clues about a possible role of mucosal immunity. Several explanations have already been used to explain this phenomenon in cohorts of sex workers and discordant couples, but studies that looked specifically

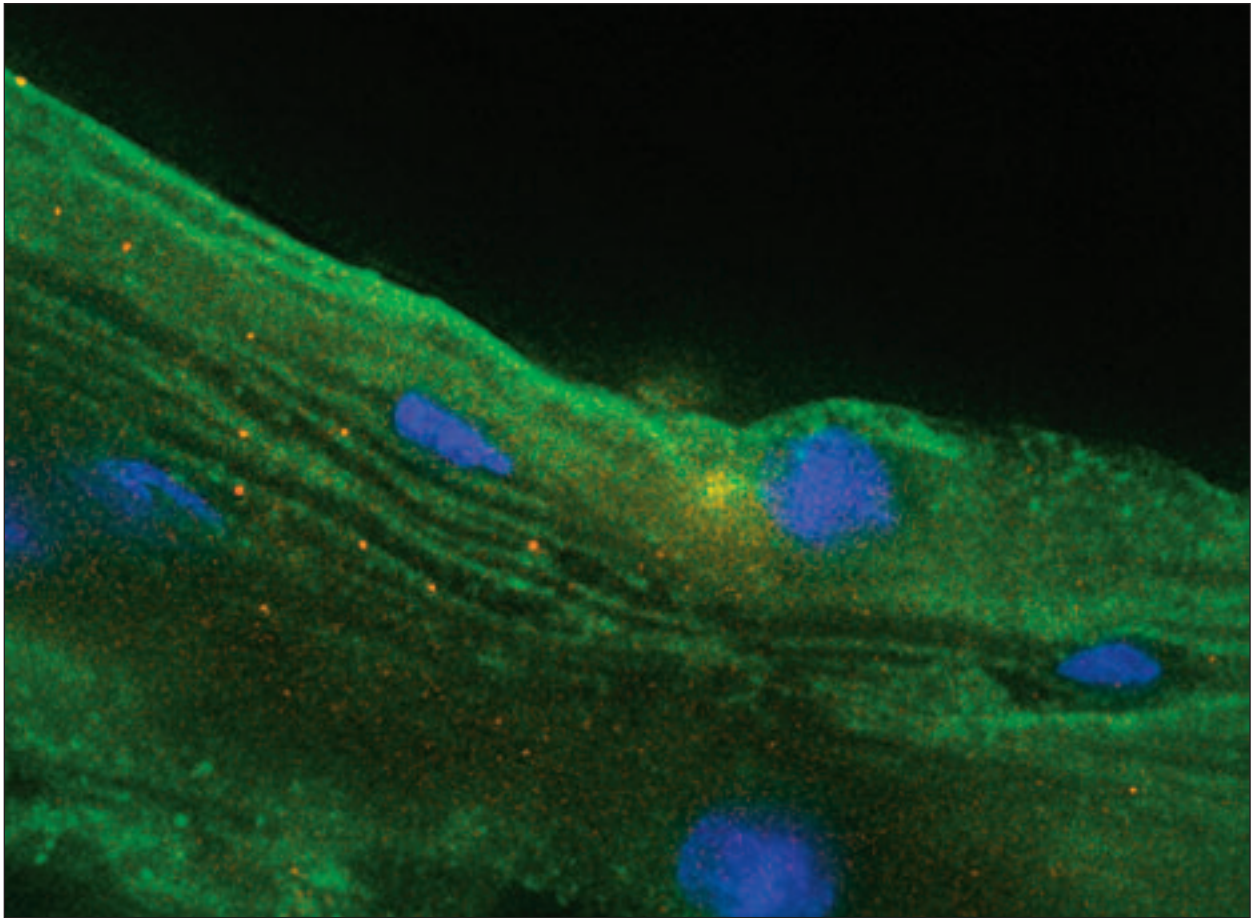
at the presence of Immunoglobulin A (IgA) antibodies—the major type of antibody response in most human secretions—have led to contradictory results, according to Mestecky. Some studies suggest that HIV-uninfected partners in discordant couples have high levels of HIV-specific IgA antibodies in vaginal or urine samples (*Nature Medicine* **3**, 1250, 1997). Additional studies showed that some of these IgA antibodies were directed against the coiled-coil pocket region of the gp41 part of HIV’s Env protein (*AIDS* **16**, 1731, 2002). However, Mestecky says that other labs could not reproduce these results. Recently, samples from 70 women were sent to six different labs and the results still could not be confirmed. “[The uninfected women] have protection by some other mechanism, but not mucosal antibodies,” concludes Mestecky.



**We need more  
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years ago**



**Lucia Lopalco**



**Figure 2. HIV infecting a mucosal site.** HIV particles (red) can penetrate the epithelium of the female genital tract as early as a few hours after infection. This microscopic image shows a section of human ectocervical tissue from routine hysterectomy surgery that was exposed to HIV *in vitro* for four hours. The cell nuclei are shown in blue and the cell surfaces in green. This image was provided by the laboratory of Tom Hope at Northwestern University.

Researchers are also studying long-term nonprogressors (LTNPs)—people who are HIV infected but do not progress to AIDS within the typical time frame—to unlock potential clues about the role of mucosal immunity. Some studies have found mucosal antibodies directed not toward parts of HIV, but to the CCR5 coreceptor that HIV uses to infect T cells. Lopalco's group has found that mucosal secretions from LTNPs contain CCR5-specific IgA and Immunoglobulin G (IgG) antibodies and follow-up studies suggest that loss of these antibody responses is associated with the development of AIDS. One possible mechanism is that such antibodies cause the CCR5 receptors to disappear from the surface of T cells because they are internalized (*Blood* **107**, 4825, 2006).

In addition to antibodies, LTNPs may also have higher levels of cellular mucosal immune responses. Some LTNPs have a higher CD8<sup>+</sup> T-cell response in the rectal mucosa than people who progress normally, according to studies from Barbara Shacklett's lab at the University of California in Davis.

Researchers have also started to use *in vitro* models of mucosal tissue to see if antibodies can inhibit transmission of HIV. Lopalco's group showed that anti-CCR5 antibodies can block transcytosis of HIV through a cultured monolayer of human epithelial cells, which is one way HIV is thought to enter the body through mucosal tissues (*AIDS* **21**, 13, 2007). But Pam Kozlowski of Louisiana State

University cautions that the transcytosis assay is very difficult to reproduce because the epithelial cells grown *in vitro* must form a really tight layer.

#### **Measure for measure**

To better understand mucosal immunity in humans, researchers must collect samples, but collecting secretions from mucosal tissues in the vagina or rectum can be difficult. One method, called lavage, involves washing the mucosal surfaces with a buffer solution and then collecting the liquid for analysis, but this approach can often dilute the secretions too much, making it hard to detect antibodies, according to Kozlowski. This is problematic because antibodies in mucosal secretions are already more diluted than in blood, according to Morgane Bomsel of the Institute Cochin in Paris.

An alternative approach developed by Kozlowski for use in both humans and nonhuman primates involves using an absorbent sponge called Weck-Cel to obtain vaginal and rectal secretions. She says it should be more acceptable to volunteers in clinical trials because it causes very little discomfort and is only in place for 10 minutes, at most. And unlike rectal washes, which require immediate processing, the sponge can simply be frozen after collection. Robin Shattock of St George's, University of London will use the sponge method to sample vaginal fluids in a clinical trial he has initiated.

Measuring cellular immune responses in mucosal tissues is even more complicated. For colorectal tissues, a biopsy is needed to collect tissue samples, according to Julie McElrath of the University of Washington. This is an invasive and somewhat risky procedure. One possible complication is peritonitis, which occurs when the colon gets perforated. "If it's only for research and not for the patient's benefit, it makes it hard to convince people to do it," says Jay Berzofsky of the National Cancer Institute (NCI).

To assess cervical cellular responses, researchers like Shattock use a small brushlike device called a cytobrush that is inserted into the cervix and rotated 360 degrees. The number of T cells collected in mucosal samples is usually much smaller than in blood—vaginal samples from a cytobrush contain only about 1,000 lymphocytes, Shattock says, much fewer than the 10 million obtained from a typical 10 ml blood sample. This drastically limits the assays that can be conducted, especially when researchers are interested in multiparameter analysis using flow cytometry. "That becomes almost undoable with those types of samples," Shattock says.

It is also important to have fresh cells and to measure cellular immune responses in the samples within a few hours after collection, which requires having a laboratory available at the same site where samples are obtained. And while blood samples can be put directly into cytokine assays, mucosal tissue samples from rectal biopsies must first be run through a series of enzymatic digests to release the lymphocytes from the collagen matrix, Shacklett says, which is a time-consuming process.

Together, these limitations are part of the reason why mucosal immune responses are not routinely measured in clinical trials. McElrath says that in AIDS vaccine trials, mucosal 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." In the STEP trial, cellular immune responses were measured in the semen of only about 20 of the 3,000 volunteers, who were primarily men who have sex with men. Analysis of these samples is still ongoing.

Even when human samples are collected, there is inconsistency in the measurement of mucosal antibodies that can make it difficult to compare results. "Most of the data in the literature is really meaningless because people don't know how to measure IgA responses in secretions," says Kozlowski. Few labs have been able to accurately detect and quantitate anti-HIV IgA in clinical samples, according to a 2002 study (*AIDS Res. Hum. Retroviruses* **18**, 1291, 2002).

Given the inconsistencies in measuring mucosal immune responses, the US National Institutes of Health (NIH) has formed a group called the Mucosal Immunity Discovery Team chaired by Johnson that will try to standardize the measurement of cellular mucosal immune responses, according to Ronald Veazey of Tulane

University, who is the group's co-chair. A similar effort at the Center for HIV-AIDS Vaccine immunology (CHAVI) is trying to standardize measuring humoral mucosal immune responses, Veazey says.

### **Mucosal homing pigeons**

To get around these limitations, some researchers have proposed a shortcut. Instead of directly measuring mucosal T cells, researchers could identify T cells from blood samples that are heading for mucosal sites. T cells that traffic to mucosal tissues express specific homing receptors on their surface. Antibody-secreting cells in the mucosal tissues have been shown to express such homing receptors three weeks after intranasal immunization (*J. Clin. Invest.* **99**, 1281, 1997). "If you had a marker on cells that allowed you to purify them from peripheral blood," Shattock says, "it would really change the number of parameters that we could assess in terms of mucosal immune responses."

The two most well-known markers are the  $\alpha 4\beta 7$  receptor, which seems to be associated with trafficking to the gastrointestinal (GI) tract, and cutaneous lymphocyte antigen (CLA), which is associated with cells homing to skin tissues, Shattock says. But there are no receptors that have been specifically identified for homing to genital sites or the colorectum, he adds. "It's definitely an important priority for the scientific community to start trying to address."

Another problem is that one can never be certain that cells that express such a homing receptor will actually reach the desired mucosal tissues, Mestecky says. He compares it to a letter with an address. "Whether it will actually get there and have its effect is unknown," he says. There is also limited time for measuring the cells that express such homing receptors in blood before they enter their target tissues, according to Marianne Neutra of Harvard University.

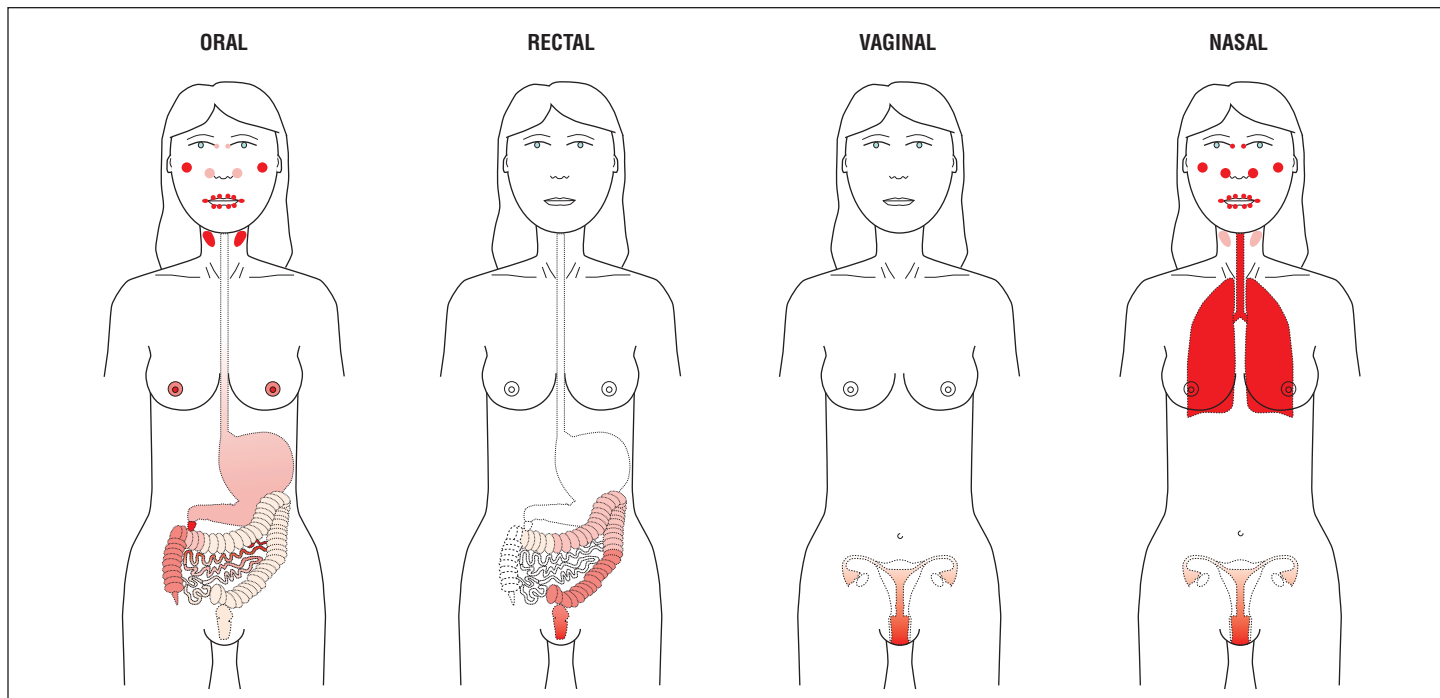
### **Routes of delivery**

There are several ways to administer a vaccine to induce mucosal immune responses. Often a vaccine has to be delivered to mucosal tissues, for example intranasally, to induce mucosal immune responses, although systemic immunizations can also induce mucosal immunity. The company Mymetics is developing a mucosal vaccine using gp41-derived antigen, which is fused to a lipid anchor that is inserted into the membrane of a virosome, a stripped down version of the flu virus. Researchers at Mymetics have found that intranasal, as well as intramuscular, immunizations of the virosome-based vaccine can induce vaginal and intestinal mucosal IgA antibodies in rabbits and macaques, says Sylvain Fleury, the company's chief scientific officer. "People believe that the classical route of administration which is intramuscular or intraperitoneal does not work for mucosal [immunity]," he says. "In our case [it] can trigger vaginal and intestinal [IgA] antibodies." Mymetics plans to initiate a Phase I trial of this vaccine candidate later this year.



**You can get  
mucosal T-cells  
by systemic  
immunization  
[as well], but  
you don't have  
the same level  
or the same  
quality**

**Jay Berzofsky**



**Figure 3. Routes of immunization.** Different vaccination routes elicit different mucosal IgA responses. In general, the ‘common mucosal immune’ system is more restricted than previously thought—the strongest response takes place at the vaccine-exposed mucosa and the second best response at adjacent mucosae. Oral immunization leads to an immune response in parts of the gut, as well as mammary and salivary glands; rectal immunization induces immune responses in the rectum; and vaginal immunization induces a vaginal immune response. A notable exception is nasal immunization, which not only stimulates an immune response in saliva, nasal secretions, and in the respiratory tract, but can also elicit a strong vaginal mucosal immune response. In the diagram, the red shading indicates the strength of the response.

Similarly, a recent study has shown that intramuscular immunization alone or combined with intranasal immunization can protect rhesus macaques against a vaginal SHIV challenge (*AIDS* **22**, 339, 2008).

Still, the strongest mucosal responses would be expected after mucosal immunization, Neutra says. Berzofsky’s lab showed that applying a vaccine rectally in rhesus macaques leads to a better mucosal cellular CD8<sup>+</sup> T-cell immune response and better protection against a rectal challenge than subcutaneous administration with the same vaccine (*Nature Medicine* **7**, 1320, 2001; *J. Immunol.* **178**, 7211, 2007). “You can get mucosal T cells by systemic immunization [as well],” Berzofsky says, “but you don’t have the same level or the same quality.” Berzofsky’s lab also showed that the presence of mucosal CD8<sup>+</sup> T cells in monkeys delayed the appearance of the virus in the blood (*Blood* **107**, 3258, 2006).


Researchers have also learned in recent years that mucosal antibody responses are more localized than previously thought, according to Shattock. Previously researchers thought that there was a common mucosal immune system, meaning that if an immune response is induced at one mucosal site, induction also occurs at other sites. This may not be entirely true. “There are links between mucosal sites, but they are not universal,” Shattock says. For example, oral vaccination will give an immune response in the GI tract and in breast milk, but little vaginal response, while nasal immunization will promote strong vaginal responses but poor GI tract responses (see Figure 3).

Kozlowski’s lab has found mostly localized responses when using a strong immunogen—cholera toxin B subunit—to compare different mucosal delivery routes in women. After applying it orally, rectally, vaginally, and nasally, and measuring mucosal IgA antibodies


in saliva, rectal, and vaginal secretions, she found that often the responses were observed at the site of vaccination. Only the nasal immunization route generated IgA responses in both the female genital tract and rectum (*J. Immunol.* **169**, 566, 2002). Kozlowski says she was surprised to see such a distal response. “The female reproductive tract is pretty far away [from the nose].” Based on these results, Kozlowski is now working on intranasally-administered vaccine candidates.

But nasal immunizations could be risky if they involve viral vectors, which could migrate to the central nervous system, according to experiments in mice (*J. Virol.* **77**, 10078, 2003). Shattock has chosen instead to explore vaginal vaccination to induce mucosal immune responses against HIV. He has started both nonhuman primate and human studies simultaneously to evaluate a trimeric gp140 clade C protein vaccine candidate using a gel that is applied vaginally. The plan is to apply nine doses of the gel over a month-long menstrual cycle. “Our approach is to maintain high levels of mucosal immune responses,” he says. Memory cells generated by conventional vaccinations take three to five days to become activated and that may be too slow to provide sterilizing immunity at mucosal surfaces, since it only takes up to three days for a localized HIV infection to occur. In the vagina, there is a window of opportunity of three days after initial exposure to keep HIV from spreading systemically.

Others are trying oral vaccinations, which is best for induction of immune responses in the gut. “Oral immunization is the best way to generate an immune response in the small intestine where HIV wants to live,” says Kozlowski. Currently several groups are exploring this approach. Gary Nabel’s lab at the Vaccine Research Center, part of the NIH, is investigating oral administration of an adenovirus serotype 41



**Exposure at  
a mucosal  
site might  
bypass the  
mucosal  
immune  
system  
entirely if  
the mucosal  
surface is  
breached**



**Barbara Shacklett**

(Ad41) vector-based vaccine candidate to see if it can elicit mucosal immune responses in the gut. Marjorie Robert-Guroff at the NCI is planning a Phase I safety trial of an orally-administered tablet containing a replicating Ad4 vector encoding HIV clade C Env. An oral, replicating Ad4 vaccine was shown to be safe and effective during 25 years of use protecting American soldiers against acute respiratory disease, Robert-Guroff says. She later plans to follow the oral vaccination with an Env protein boost injected intramuscularly. She says similar oral vaccinations with a replicating Ad5 vector in rhesus macaques followed by a protein boost resulted in mucosal immunity and protected from a rectal challenge with SIVmac251 (*Vaccine* **25**, 8021, 2007).

Others are looking for new routes to induce mucosal immune responses. For example, a recent study has shown in mice that sublingual immunization induces mucosal responses in the respiratory and genital mucosa, as well as the GI tract (*Vaccine* **25**, 8598, 2007).

Another study found that spraying adenoviral vector vaccines onto the tonsils can elicit both cellular and humoral immune responses, and spraying a combination of different vectors provides similar protection from an SIV challenge as systemic immunization with the same vaccine (*J. Virol.* **81**, 13180, 2007). Another good route might be application through the skin, perhaps like a nicotine patch, Kozlowski says, adding that there is some evidence that it may generate mucosal IgA immune responses in humans.

Researchers are also looking for adjuvants that will make it possible to get mucosal immune responses without having to deliver the vaccines directly to mucosal tissues. The hope is to change immune cells so they will go to mucosal tissues. “We could continue to administer vaccines intramuscularly if an adjuvant is identified that targets the immune cells that are activated to go to the mucosa,” Kozlowski says.


For example, one recent study by Harriet Robinson’s group at Emory University in Atlanta found that in macaques, intramuscular injection of DNA encoding Granulocyte Macrophage Colony Stimulating Factor (GM-CSF) together with the DNA prime for a DNA-MVA prime-boost regimen results in increased IgA antibody response in rectal secretions (*Virology* **369**, 153, 2007).

Another possible adjuvant is retinoic acid (RA). J. Rodrigo Mora’s lab at Harvard and others have found that dendritic cells in the gut-associated lymphoid tissue can secrete RA, which induces T and B cell migration to the

gut mucosa. But delivering RA could be a challenge because it is not soluble in water and would have to be administered in a solvent like dimethyl sulphoxide (DMSO), which could have side effects. Therefore, Mora’s lab is working on methods to “teach” dendritic cells to make RA and to “pack” RA in dendritic cells so that dendritic cells loaded with RA (and not soluble RA) can be used to immunize and induce T- and B-cell mucosal immune responses. In this way, the effect of RA will be restricted to the site of immunization, thus eliminating potential systemic effects, Mora says. However, RA only appears to be relevant to immune responses in the upper GI tract and not to other relevant mucosal areas like the colorectum or genital mucosal surfaces, says Shattock. Nonetheless, Mora says, it might be possible to make B cells that migrate to the small bowel using RA. The resulting antibody-secreting cells would then make protective IgA antibodies, which can potentially be transported to, and protect, the colon mucosa—similar to what happens with maternal antibodies in breast milk.

Despite all of these efforts to study different routes to induce mucosal immune responses, what is needed and what has been lacking in the field is the systematic comparison of the same immunogen using various routes of administration in nonhuman primate SIV challenge studies, says Wayne Koff of IAVI. “IAVI is in the preliminary stage of developing plans to do such studies,” adds Koff.

So given what is now known about mucosal immunity, what should future AIDS vaccine candidates look like? Some researchers say that inducing a combination of mucosal and systemic immune responses is the goal. Still, it remains an open question as to whether mucosal immunity can actually prevent HIV infection, Shacklett says. “Exposure at a mucosal site might bypass the mucosal immune system entirely if the mucosal surface is breached,” she says. “It’s easy to see how this might happen in the context of sexual intercourse.” Shattock agrees. “If you look at women after intercourse, in 60% of them you can visually identify microabrasions.”

But that does not mean inducing mucosal immune responses is not important, says Shacklett. “Even if we can’t prevent the initial infection,” she says, “we may be able to limit viral replication and dissemination, and lower the total amount of replicating virus by arming effector cells that are able to localize to tissues.” 

### Some candidate microbicides can damage epithelia

The candidate microbicide cellulose sulfate (CS) can disrupt epithelial integrity and make it easier for HIV particles to cross epithelial barriers, according to a study by researchers at Albert Einstein College of Medicine in New York City. The results, still unpublished but presented earlier this year at the Conference on Retroviruses and Opportunistic Infections in Boston and at the Microbicides 2008 conference in New Delhi, offer a possible explanation for the association between use of CS and increased HIV acquisition in a recent clinical trial. Two trials testing the ability of CS gels to prevent HIV transmission were halted last year because one trial showed a trend toward higher HIV infections in some women who received CS, as compared to placebo.

The study, led by Betsy Herold of Albert Einstein College of Medicine, found that CS can damage epithelial layers. Herold and colleagues also analyzed three other compounds. The spermicide Nonoxynol-9 (N-9) showed even greater epithelial disruption than CS, consistent with clinical trial results that found N-9 also tends to increase susceptibility to HIV infection in women (*Lancet* **360**, 971, 2002). Two other microbicide candidates, PRO 2000 and the antiretroviral tenofovir, did not show the same disruptive effects when applied in the same concentrations.

The researchers measured the effects of these compounds on an *in vitro* cultured epithelial cell layer—made of either uterine epithelial cells or of reconstituted vaginal tissue—by measuring the transepithelial resistance to an electric current. As long as the integrity of these epithelia remains intact, transepithelial resistance should remain high. The researchers found that an 18-hour exposure to either N-9 or CS led to a drop in resistance, while PRO 2000 and tenofovir had little effect. CS is a polymer composed of sugar molecules with sulfate groups. It is unclear why PRO 2000, which is structurally similar to CS, does not have the same detrimental effects on epithelia. However, Herold says, preliminary data from her lab suggest that CS and PRO 2000 might activate different signaling pathways. Use of N-9 and CS also made the cultured epithelium leaky, allowing HIV particles to more easily cross the epithelium.

Confocal microscopy of the cultured reconstituted vaginal tissue showed that CS treatment led

to a marked loss of syndecan and desmoglein, proteins that are important for epithelial integrity. There was also downregulation of the RNA expression of the genes encoding such proteins.

CS also increased the expression of interleukin (IL)-6 in the cultured epithelial cells and induced a three-fold increase in activation of the transcription factor NF- $\kappa$ B in peripheral blood mononuclear cells (PBMCs), suggesting that it caused an inflammatory response.

The researchers also administered the microbicide gels to mice daily for one week and then analyzed their genital tracts. Both N-9 and CS caused mice to be more susceptible to HSV infection.

Preclinical and Phase I assessments of CS did not uncover its detrimental effects on epithelia. Currently, the FDA recommends microbicide candidates be tested preclinically in rabbits, Herold says. In this model, 10 daily doses of a candidate microbicide are applied vaginally followed by histological analysis of the vaginal tissues. Phase I trials assess subjective symptoms, such as whether a microbicide burns, and use colposcopy, which looks at visible disruptions of the cervix, Herold says. These assessments, however, missed the effects observed in this study.

“We propose that epithelial integrity, inflammatory response, and susceptibility to infection are included in preclinical testing of candidate microbicides,” concludes Pedro Mesquita, who was also involved in the study.

The other two microbicide candidates evaluated in this study, PRO 2000 and tenofovir, are currently in clinical trials. A Phase III trial in women in South Africa, Tanzania, Uganda, and Zambia is testing safety and effectiveness of PRO 2000 in preventing HIV infection in women and is sponsored by the UK’s Medical Research Council. That trial’s independent data monitoring committee recommended discontinuing the high-dose arm (testing a 2% dose of PRO 2000) earlier this year due to lack of efficacy, but the low-dose arm (using an 0.5% dose of PRO 2000) is ongoing. The National Institute of Allergy and Infectious Diseases is also testing safety and effectiveness of the low dose of PRO 2000 in a Phase II/IIIb trial called HPTN 035 in Malawi, South Africa, Zambia, Zimbabwe, and the US. A microbicide gel formulation of tenofovir is also currently being tested in a Phase II trial (HPTN 059).

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## IOM report addresses the challenges of HIV prevention trials

The prestigious US Institute of Medicine (IOM), an independent advisory group on public health policy, convened a series of meetings last year on the methodological challenges of conducting non-vaccine HIV prevention trials (see *Advisory Panel considers complexities of HIV prevention trials, LAVI Report*, January-February 2007 and *Optimizing HIV prevention research, LAVI Report*, March-April 2007). The final report based on these proceedings, as well as site visits by IOM committee members to clinical trial sites in Uganda and South Africa, was issued in February ([www.nap.edu/catalog/12056.html](http://www.nap.edu/catalog/12056.html)). The report outlines the recent spate of late-stage clinical trials in the HIV prevention field that have failed to provide any benefit in reducing the risk of HIV infection, leading the authors to conclude that, "A near-perfect biomedical intervention for preventing HIV infection is unlikely to be available in the near future."

The IOM committee was organized at the request of the Bill & Melinda Gates Foundation and was to provide recommendations on how future trials of microbicides and pre-exposure prophylaxis (see *Treatment as Prevention, LAVI Report*, May-June 2006) could be conducted in a way that could increase the likelihood of success and enable donors to optimally invest their limited financial resources. Stephen Lagakos, director of the Center for Biostatistics at Harvard University, chaired this committee and co-authored the report with Alicia Gable of the IOM. The two also recently wrote a perspective article in the *New*

*England Journal of Medicine* summarizing the report's main conclusions (*N. Engl. J. Med.* **358**, 1543, 2008). "Shortcomings in research design have inhibited progress in identifying effective HIV-prevention interventions," the authors said.

One shortcoming the report focused on in particular was the way HIV incidence has been estimated in advance of some efficacy trials (see *Moving Target, LAVI Report*, May-June 2007). The IOM committee recommended that all late-stage trials be designed based on incidence estimates collected through traditional cohort follow-up studies in the communities where the trial will occur, and that these estimates should be corroborated by at least one other source.

High pregnancy rates during HIV prevention trials, and the impact on retention of female volunteers, was another critical issue addressed in the report. Female volunteers are typically not allowed to receive the experimental intervention—microbicide gel or antiretroviral drug—during pregnancy because of potential safety risks to the fetus, but their exclusion can confound results. On this issue, the authors suggested researchers should try to determine the safety of the intervention in pregnant women as best as possible before initiating the trial to determine circumstances in which women could potentially continue to participate in HIV prevention trials even during pregnancy.

The report also outlines several other ways that trials can be designed to determine the influence individual behavior and adherence have on the final results, which is critical in non-vaccine HIV prevention trials.

## Microbicide trials update

In February the Population Council announced that the microbicide gel Carraguard, which was tested in a randomized, double-blind, placebo-controlled Phase III trial, had no effect on HIV infection rates in women. The trial was conducted at three sites in South Africa and involved 6,202 women between the ages of 16 and 72. Final results showed that 134 women who received Carraguard became HIV infected, compared to 151 placebo recipients.

Carraguard contains the compound carrageenan, a seaweed derivative commonly used as a stabilizer and thickening agent in food and cosmetics. Prior to initiating the Phase III trial, the Population Council conducted two Phase II safety studies of Carraguard in South Africa and Thailand, involving a total of 565 HIV-uninfected women.

A critical aspect of the Carraguard trial was adherence. Women were counseled to apply the microbicide before every sex act, and although the self-reported adherence rates were 96%, researchers estimate that the actual adherence was much lower. "It's possible that low levels of adherence in the trial were responsible for why the product didn't show an effect," says Barbara Friedland of the Population Council.

To measure adherence, researchers collected behavioral information directly from participants and also treated the microbicide applicator with a compound that, after analysis, indicated whether or not

the applicator had been in contact with vaginal mucous. The results of these tests showed women used the gel in only 44% of sex acts, and only 10% were estimated to have used it during every sex act.

An applicator test is one method researchers are using to better estimate adherence, but even this approach is complicated. "All we can tell is whether the applicator was inserted in the vagina or not," Friedland says. "We don't know when in relation to the sex act the applicator was inserted."

Other updates on microbicide trials occurred during the Microbicide 2008 Conference, which was held in New Delhi from February 24-27. Researchers presented data from a Phase II safety study, known as HPTN 059, which tested a gel formulation of tenofovir, an antiretroviral (ARV) commonly used in the treatment of HIV infection. The findings, presented by Sharon Hillier of the Microbicide Trials Network, revealed that the product was safe for daily use in sexually-active, HIV-uninfected women. This trial evaluated both daily use of the microbicide and a sex-dependent regimen, in which women were instructed to only apply the microbicide just prior to sex. Adherence was based on self-reported behavior and researchers estimated that 80% of the women in the sex-dependent group adhered to the regimen, compared to 83% of the women in the daily-use group. This candidate is one of several ARV-based microbicides that are currently being tested.